External Scientific Report

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Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs)

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Abstract

This tender, developed under a self-task mandate from the BIOHAZ Panel, analysed the characteristics of the water and the practices followed by the European food business operators (FBOs) to maintain process water quality used during the post-harvest handling and processing operations for fresh and frozen fruits, vegetables and herbs (ffFVHs) using: information and data obtained from FBOs, experimental data extracted from literature and dynamic mass balance modelling. Quantitative data were obtained from 61 FBO scenarios (29 from the fresh-whole sector, 19 from the fresh-cut sector, and 13 from the frozen sector). The impact of no water treatment was evaluated in 17 scenarios, while in 44, the challenges of maintaining the microbiological quality with water disinfection agents were examined, including chlorine, peroxyacetic acid, and hydrogen peroxide. The findings highlighted that when no water disinfection treatment was used *Listeria monocytogenes* was detected in some scenarios of the fresh-whole and frozen FVH sectors as well as *Salmonella*, pathogenic *Escherichia coli* and norovirus in the fresh-cut and frozen FVH sector. Additionally, inadequate or improper monitoring systems resulted in either excessively high or insufficient disinfectant concentrations in the water, which, when too low, failed to sufficiently reduce the microbial load. The literature review revealed a tendency to: focus on leafy greens, use chlorine-based disinfectants, and employ chemical oxygen demand (COD) as the primary physico-chemical parameter, with total dissolved solids (TDS) and turbidity considered to a lesser extent, as indicators of water quality. Additionally, dynamic mass balance modelling was used to interpret experimental data from literature and FBOs. The model was fundamental to estimate key unknown parameters, predict the microbial contamination and accumulation of organic matter and allowing to simulate "what-if scenarios."

Keywords: fruit, food safety, foodborne pathogens, dynamic mass balance modelling, process water, vegetables, water disinfection,

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Summary

In this external scientific report of the tender, developed under a self-task mandate from the BIOHAZ Panel, the characteristics of the process water and practices followed by the European food business operators (FBOs) to maintain water quality used during the post-harvest handling and processing operations for fresh and frozen fruits, vegetables and herbs (ffFVHs) are described. Data on current production practices, physico-chemical parameters, and microbiological quality were obtained from 61 industrial case scenarios selected from which 29 were from the fresh-whole sector, 19 from the fresh-cut sector, and 13 from the frozen sector. The impact of no water disinfection treatment was evaluated in 17 scenarios, while in 44 the challenges of maintaining the microbiological quality with water disinfection agents, including chlorine, peroxyacetic acid (PAA), and hydrogen peroxide (H_2O_2), were examined. The study included in most cases two sampling visits to each FBO, where data were collected and compiled in an Excel file. This Excel file contained 3 sheets, namely: (1) 'Coversheet data' that lists and describes all the variables, andalsoindicates their possible values, (2) 'scenario characterization' that gives an overview of all FBO scenarios based on the food category, food group, processing operation, use or no use of disinfectant, disinfectant type, and disinfectant agent and (3) 'data' including all collected data from FBOs. For each sampling visit and sampling time point, the processing practices were reported (e.g. product water contact time, water agitation, tank water volume, date and time for tank fill and tank empty, water replenishment, process start, and the amount of product processed). Physico-chemical parameters of the process water (e.g. residual disinfectant concentration, pH, chemical oxygen demand (COD) and temperature) were included. Microbiological analyses were performed according to harmonised analytical protocols for the enumeration of moulds, yeasts, total bacterial, coliforms, generic *Escherichia coli* and *Listeria* spp., as well as the detection of a range of pathogens such as *Salmonella* spp., *Listeria monocytogenes*, pathogenic *E. coli,* norovirus and *Cryptosporidium* spp.

The most relevant information that allowed the characterization of each scenario was combined and presented in this report as individual datasheet including flowcharts, photos and summary tables. Information on the processing operation, sampling dates, water volumes, and additional details, such as water source and water treatment were included. Data collected per food sector is represented in boxplots combining the 'ID code - specific FVH – operation' for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs and per type of water disinfection treatment. Overall, the boxplots provide a visual representation of the data and the dispersion that allowed the comparison across different scenarios and water disinfection treatments for each food sector. For those scenarios with the use of chemical disinfectants, notable discrepancies in residual concentrations suggest inadequate water management practices. Inadequate or improper monitoring systems resulted in either excessively high or insufficient disinfectant concentrations in the process water, which, when too low, failed to sufficiently reduce the microbial load.

The microbiological results revealed significant insights into the differences in the post-harvest handling and processing operations of ffFVHs. Variation in mould and yeast counts across different food sectors was noted, with chlorine and PAA generally reducing counts compared to scenarios with no water treatment. Total bacterial counts decreased with chlorine treatment but remained high without water treatment, highlighting the importance of the addition of a disinfectant. Coliform and *E. coli* counts, which are indicator groups of faecal contamination, were effectively reduced by chlorine, whereas PAA showed inconsistent results due to improper concentration levels. Norovirus counts were found in untreated scenarios as well as in some with water disinfection treatments, emphasizing the need for thorough disinfection practices. *Cryptosporidium* was never detected, but c*rAssphage* presence suggested potential human faecal

contamination. These findings stress the critical role of effective water disinfection practices. Moreover, the detection of *Salmonella* spp., *L. monocytogenes*, and pathogenic *E. coli* across sectors highlights the importance of comprehensive disinfection strategies to minimize the occurrence of microbial hazards. Additionally, findings regarding viable but non-culturable bacteria (VBNC) and spores of *Clostridium perfringens* underscore the need for continual monitoring and improvement of disinfection measures. By implementing monitoring and control systems for residual disinfectant concentration e.g., for chlorine adjustments can be made in real-time to optimize dosing rates and prevent situations of over or under-chlorination.

A systematic literature review was performed with predefined keywords in Scopus and Web of Science. The searches were performed to answer the following research questions: RQ1: which data and models are available that can quantify the microbiological contamination of the water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches and RQ2: which microbiological and physico-chemical parameters or methods and models are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs. A two-tier approach was used in which titles, keywords and abstracts were screened for relevance in Tier 1 and full texts in Tier 2. Furthermore, a Google Advanced search was performed to search for relevant reports published on the websites of AESAN, ANSES, UK, FSA, BfR, WHO, FAO and US FDA. However, this latter search did not result in additional data or models that could be extracted for further analysis. The papers identified as relevant for modelling (RQ1b and RQ2b) were used as input for model development. Full-text screening of the Tier 1 relevant papers for RQ1a and RQ2a showed that 123 references were considered relevant on the topic of which 105 contained relevant data. The EFSA WG selected a total of 69 papers for which data were extracted according to the EFSA Excel template format. The literature review revealed that most studies were performed in an experimental setting and primarily focused on lettuce or berries. The most frequently studied microorganisms were *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* followed by hygiene indicators. The most frequently studied disinfection methods for treating process water were chlorine-based disinfectants and UV applied as a single technique or in combination with other techniques. Other methods studied entail the use of acids such as PAA, H_2O_2, ultrasound and pulsed light. Most studies were performed at lab scale. The efficacy of chlorine on the inactivation of pathogens in water used for processing lettuce decreases with increasing COD and ranges between 0 and 7 log reductions. Based on the lab scale results from the literature search, chlorine, UV and PAA can all thus reduce bacterial load in the water where their efficacy depends both on the dose and on physico-chemical properties of the process water, such as pH and COD. The most suitable physico-chemical parameters as indicators for water quality are COD and to a lesser extent TDS and turbidity. Parameters such as ORP, pH, and temperature are important to maintain proper disinfectant efficacy. A limited number of papers were found on inline/online monitoring. These showed that UV absorbance seems a promising technique to assess process water quality and predict chlorine demand. Chronoamperometric sensor seems to be another promising method for calibration of an online detector for monitoring the residual disinfectant, such as for PAA.

Modelling the behaviour of microbiological hazards in process water required different steps, including a literature review for available modelling approaches relevant to the scope of this mandate, proper adjustments and modifications of existing dynamic modelling approaches, fitting of updated models, analysis of estimated relevant parameters and model simulations to find best disinfectant dosages. First, the review analysis of the available models in the literature revealed relevant mechanisms, assumptions and critical model parameters. The selection was based on two criteria: the model should include either microbiological dynamics or the interaction of these dynamics with relevant physico-chemical indicators. All the retrieved dynamic models were analysed and mathematically rewritten to allow direct comparisons, for

example in terms of the considered mechanisms or equations used for each mechanism. Secondly, a general framework based on mass balance conservation was proposed to include all critical influential factors for the microbial, disinfectant and COD dynamics, identified in the literature and the associated experimental data. This general framework allows to model diverse experimental conditions (of lab and industrial scale) by simulating the case-specific relevant dynamics. Mass action law, the standard theory of chemical reactors, was employed for most parts of the model. The only exception was the use of Hill kinetics to model the inactivation of microbiological contamination with the disinfectant, which was dependent on organic matter in a non-linear form. This general model was simplified to analyse industrial data from two studies in the literature. In both cases, the model was able to reproduce the experimental data after estimation of the relevant parameters. Nevertheless, a deep analysis of the results demonstrated that a simplified fit-for-purpose model was needed for the simulation of industrial data where measured data is more limited. Therefore, a refined model was finally proposed to understand the microbiological and physico-chemical interplay in industrial washing operations, being able to reproduce major trends for the dynamics after unknown parameters were estimated from measurements. Modelling the operations without water disinfection was a powerful tool to understand contamination: transfer rates from product to water were confidently estimated allowing us to analyse their variability between similar visits, or among different operations or food matrixes. On the other hand, operations with water disinfectants were analysed to understand the inactivation dynamics of total bacterial and *Listeria* spp. counts with chlorine-based disinfectants. Two open-source codes are shared with this external scientific report of the tender to: (1) simulate the modelled FBO cases and (2) simulate what-if scenarios of relevance for the FVHs handling and processing industry. The last code allows to understand and validate alternative water management practices that can be tailored to different products by adjusting the parameters specific to different product-hazard combinations considering two types of intervention measures, i.e., water disinfection and water replenishment.

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1 Introduction

1.1 Background and terms of reference as provided by the requestor

Large volumes of water are used during harvest and post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs) as well as during fresh-cut/freeze value-added operations, distribution and end-user handling of ffFVHs. The quality of the water used in post-harvest handling and processing operations of ffFVHs should be monitored and controlled to prevent the accumulation of microbiological hazards. To avoid cross-contamination of the product due to the use of contaminated water, water disinfection treatments are needed to eliminate, or reduce to an acceptable level, microorganisms of public health concern but these treatments should not have an adverse effect on the quality and safety of the produce.

Water quality and use in post-harvest handling and processing operations of ffFVHs is an increasing concern at the EU and global level, mostly because there is an expected decline of the availability of water of drinking quality due to climate change. Recent FAO/WHO JEMRA MRA series reports address the: (i) safety and quality of water used in food production and processing as well as (ii) microbiological hazards in fresh leafy vegetables and herbs. In 2017, the European Commission developed a Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (OJ 2017/C 163/01), including good hygiene practices related to the use of irrigation water and water used in on-farm post-harvest associated operations. During the 43th session of the Codex Alimentarius Commission on the Joint FAO/WHO Food Standards Programme in Autumn 2020, the development of guidelines for the safe use and reuse of water in food production was approved. These guidelines will contain a specific Annex on the use and reuse of water in fresh produce production.

This tender has been developed in the context of an ongoing self-task mandate from the BIOHAZ Panel on the microbiological hazards associated with the use of water in the post-harvest handling and processing operations of ffFVHs[1]. This self-task mandate is related to all past scientific opinions from the BIOHAZ Panel on food of non-animal origin (FoNAO). Due to the expected scarcity of available data and information on the topics listed above, this tender is envisaged to generate specific deliverables (e.g. external scientific report, summary tables/figures) containing data representative of the industry settings and of relevance to address some of the specific assessment questions in the self-task mandate. This tender will also facilitate the review of available scientific literature/information to support the self-task mandate.

The aim of this procurement procedure is to conclude a direct contract for the execution of specific tasks over a clearly defined period as defined in these tender specifications.

The main objective of this procurement procedure is to **gain insights on the characteristics of the water and practices followed by the industry to maintain water quality used during the post-harvest handling and processing operations for ffFVHs**. In the packinghouses and processing facilities, large volumes of water are used, and, in many cases, the same water is used in the same unit operation (e.g., washing or cooling) for many kilograms of product. Under these conditions, it is difficult to maintain properly the microbiological quality of the processing water because of the accumulation of organic matter and the lack of operational limits and test methods to monitor the processing operation. Water disinfection treatment is one of the most critical processing steps in the production of ffFVHs aimed

[1] https://open.efsa.europa.eu/questions/EFSA-Q-2021-00374

at preventing cross-contamination. The information obtained via this tender should allow to have an overview of different water quality scenarios in industry settings.

Specific objectives

The specific objectives of the contract resulting from the present procurement procedure are as follows:

- **Objective 1: to characterize the water used in different post-harvest handling and processing operations of ffFVHs where processing water is not subject to any water disinfection treatment**

The first objective is to carry out the characterisation of water used in different post-harvest handling and processing operations of ffFVHs with the aim to evaluate the microbiological and physico-chemical quality of the processing water during the working day in industry settings where processing water is not subject to any water disinfection treatment. Three food categories should be considered in this study, namely: (i) fresh-whole FVHs, (ii) fresh-cut FVHs and (iii) frozen FVHs, including at least two and ideally three different food products per food category. At least one post-harvest handling or processing operation, but ideally two processing operations should be assessed for each food product (e.g., cooling, pre-washing, washing, rinsing, glazing or blanching). Experiments are expected to be repeated at least twice on different processing days. Different types of industry settings should be included e.g., small, medium-sized and large enterprises. At least two different dimensions (industry size) should be included. The selection of the different food product/food handling or processing operation/industry size combinations should be well justified and aim as much as possible at covering the relevant variability in food products/processing operations/industry size.

Examples of scenarios (food product, industry size, and handling and processing operation combinations) that could be included in the study performed for this objective (see also **Table 1**) are as follows:

- Fresh-whole FVHs: cooling and washing of peppers, apples or pears
- Fresh-cut FVHs: pre-washing and washing of leafy greens, fresh herbs as well as cut vegetables such as peppers, carrots or onions
- Frozen FVHs: washing and cooling of peppers, onions, leek or sweet corn

The selected contractor is expected, as part of the project, to take processing water samples at different time points during post-harvest handling and processing operations in the industry settings starting at the beginning of the working day and covering a large part of the processing time for one single day. The handling and processing operations should be characterised with at least three sampling points distributed along the working day (e.g. beginning, middle and end of the working day) to have insight on the variability of the processing. Ideally four to five sampling points distributed along the working day should be assessed.

The aim is to characterize the evolution of the physico-chemical and microbial quality of the processing water in each scenario during the working day. This characterization should allow the investigation of the contamination rate of the processing water in different situations. Efforts should be made to ensure that the characterized processing water comes from industry settings where different ffFVHs are being processed. The microbial characterization of the processing water should include the occurrence and concentration of foodborne pathogens including several pathogenic microorganisms (e.g. *Salmonella* spp., STEC, *Listeria monocytogenes*, norovirus, parasites) as well as microbial indicators (e.g. non-pathogenic *E. coli*, *Listeria* spp., coliphages, *CrAssphage*) justifying all the selected microbial parameters. The physico-chemical characterization of the processing

water should include at least the chemical oxygen demand (COD), the turbidity, the redox potential and the UV-absorbance. The proposed food categories/food products/handling and processing operations/microbiological and physico-chemical characterisation will be discussed, fine-tuned and agreed upon during the kick-off meeting.

The selected contractor is expected, as part of the project, to determine the potential contamination rate between different batches of product that are processed in the same volume of water. The potential contamination rate could be determined based on the evaluation of pathogenic and/or indicator microorganisms previously mentioned along processing time.

Establishment of potential correlations between the occurrence/concentration of foodborne pathogens and microbial indicators present in processing water and ffFVHs should be also attempted.

Table 1. Examples of possible scenarios that could be included in the assessment of the processing water performed for objective 1. (Note: these are provided only as examples, scenarios selected by the tenderers could be different from the ones shown in the table)

Food category	Food product	Processing operations	Foodborne pathogens based on ISO methods[a]	Microbial indicators in processing water based on ISO methods[a]	Physico-chemical analyses in processing water
Fresh-whole FVHs	Melons	Cooling Washing	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Redox potential pH
	Pepper	Cooling Washing	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Redox potential pH
Fresh-cut FVHs	Leafy greens Fresh herbs	Pre-washing Washing	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Redox potential pH
	Onion	Pre-washing Washing	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Redox potential pH
Frozen FVHs	Peppers Peas	Washing Cooling	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Redox potential pH
	Sweet corn	Washing Cooling	*L. monocytogenes* *Salmonella* spp.	*E. coli* *Listeria spp.*	Turbidity COD

Food category	Food product	Processing operations	Foodborne pathogens based on ISO methods[a]	Microbial indicators in processing water based on ISO methods[a]	Physico-chemical analyses in processing water
			STEC norovirus parasites	coliphages	UV-absorbance Redox potential pH

[a] Other testing procedures may be used, if it can be demonstrated that these procedures provide at least equivalent guarantees

- **Objective 2: to develop case-studies in industry settings to provide evidence of the efficacy of different intervention strategies during the post-harvest handling and processing operations of ffFVHs**

The second objective is to develop specific case-studies in industry settings for the post-harvest handling and processing operations of ffFVHs. These case studies should be designed to provide evidence of the efficacy of different intervention strategies used in the post-harvest handling and processing operations of ffFVHs to maintain the microbiological quality of processing water in industry settings. Ideally, the study should include at least two different food products per each food category, namely: (i) fresh-whole FVHs, (ii) fresh-cut FVHs and (iii) frozen FVHs in at least one processing operation using different intervention strategies, i.e. different water disinfection treatments or water replenishment rates or their combination (see **Table 2**). Different types of industry settings should be included such as small, medium-sized and large enterprises. At least two different dimensions (industry size) should be included. Experiments are expected to be repeated at least twice, allowing one to sample different batches. The selection of different food product/food handling or processing operation/industry size/water disinfection treatment or water replenishment rate or their combination should be well justified and aim as much as possible at covering the relevant variability in food products/processing operations/industry size/water disinfection treatment or water replenishment rate or their combination.

Water samples should be taken at different time points during post-harvest handling and processing in the industry settings starting at the beginning of the working day and covering a large part of the processing time for one single day. A minimum of three sampling points distributed along the working day (e.g. beginning, middle and end of the working day) should be assessed. Ideally, four to five sampling points distributed along the working day should be assessed.

Ideally, the selected contractor should assess different water disinfection treatments (e.g. chlorine, peroxyacetic acid, chlorine dioxide, UV-light, filtration, combinations of different treatments) as well as several water replenishment rates (e.g. 100 L/h versus 500 L/h) and their combinations.

Table 2. Examples of possible scenarios that could be included in the assessment performed for objective 2. (Note: These are provided only as examples, scenarios selected by the tenderers could be different from the ones shown in the table)

Food category	Food product	Water disinfection treatment or replenishment rate or their combination	Foodborne pathogens based on ISO methods[a]	Microbial indicators in processing water based on ISO methods[a]	Physico-chemical analyses in processing water
Fresh-whole FVHs	Apples Pears	Peroxyacetic acid	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Disinfectant residual Redox potential pH
	Pepper	100 L/h replenishment rate	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Disinfectant residual Redox potential pH
Fresh-cut FVHs	Leafy greens	Chlorine	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Disinfectant residual Redox potential pH
	Onion	Chlorine	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Disinfectant residual Redox potential pH
Frozen FVHs	Strawberry	Chlorine dioxide	*L. monocytogenes* *Salmonella* spp. STEC	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance

Food category	Food product	Water disinfection treatment or replenishment rate or their combination	Foodborne pathogens based on ISO methods[a]	Microbial indicators in processing water based on ISO methods[a]	Physico-chemical analyses in processing water
			norovirus parasites		Disinfectant residual Redox potential
	Sweet corn	500 L/h replenishment rate	*L. monocytogenes* *Salmonella* spp. STEC norovirus parasites	*E. coli* *Listeria spp.* coliphages	Turbidity COD UV-absorbance Disinfectant residual Redox potential pH

[a] Other testing procedures may be used if it can be demonstrated that these procedures provide at least equivalent guarantees.

The physico-chemical characterization should include at least the residual concentration of the water disinfection treatment, if any, the chemical oxygen demand (COD), the turbidity, the redox potential and the UV-absorbance. The tenderer is expected to determine the potential contamination rate between different batches of products that are processed in the same volume of water. The microbial characterization should include the occurrence and concentration of foodborne pathogens, including several pathogenic microorganisms (e.g. *Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes*, norovirus, parasites) as well as microbial indicators (e.g. non-pathogenic *E. coli*, *Listeria* spp., coliphages) providing justification for all the selected microbial parameters. The potential contamination rate could be determined based on the evaluation of the pathogenic and/or indicator microorganisms previously mentioned. Establishment of potential correlations between the occurrence/concentration of foodborne pathogens and microbial indicators present in processing water and ffFVHs should be attempted.

The proposed food categories/food products/food handling or processing operation/industry size/ water disinfection treatment or water replenishment rate or their combination/microbiological and physico-chemical characterisation will be discussed, fine-tuned and agreed upon during the kick-off meeting.

The selected contractor is also expected, as part of this tender, to characterize the physiological state of the bacteria present in the processing water as well as in the studied ffFVHs, including culturable and 'viable but non-culturable' (VBNC) pathogenic bacteria and indicator microorganisms. The techniques implemented by the contractor to distinguish between culturable and VBNC cells should have been optimized for its use in processing water, due to the complexity of the matrix, such as the viability PCR using propidium monoazide and ethidium monoazide (e.g. PMA-EMA qPCR or digital PCR (v-dPCR).

- **Objective 3: to search quantitative data and mathematical models for the microbial contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches**

The third objective is to search (identify, describe and validate) the available quantitative data and mathematical models for the microbial contamination of water used in different post-harvest handling and processing operations of ffFVHs and between batches of ffFVHs including fresh whole, fresh-cut and frozen FVHs. The literature search must be comprehensive, structured and transparent e.g., following the EFSA guidance for those carrying out systematic reviews[2]. The methodological quality of included studies must be appraised with a special focus on those studies performed under pilot and industry settings. The relevant data extracted from selected studies should be summarized in tables and figures, which should be used to interpret the results and draw conclusions.

The tenderer is expected to consider the most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations of ffFVHs in different scenarios, including fresh-whole, fresh-cut and frozen FVHs. The most suitable mathematical models used to characterize the contamination of the water used in different post-harvest handling and processing operations of ffFVHs selected from the literature search should be validated with the quantitative data **obtained from Objectives 1 and 2**. This will allow the identification of the

[2] https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2010.1637

most adequate models based on data obtained from the industry settings in the context of objectives 1 and 2 of this tender.

- **Objective 4: to explore quantitative data and mathematical models on parameters that can be used to validate/verify and/or monitor the microbiological quality of the water used for post-harvest handling and processing operations of ffFVHs**

The last objective is to explore (search, select, investigate) the available quantitative data and mathematical models on microbiological and physico-chemical parameters, which are used to validate/verify and/or monitor the microbiological quality of the water used for different post-harvest handling and processing operations of ffFVHs. The microbiological parameters should also cover viruses and parasites in addition to indicator and pathogenic bacteria. The literature search must be comprehensive, structured and transparent e.g. following the EFSA guidance for those carrying out systematic reviews[3]. The methodological quality of included studies must be appraised with a special focus on those studies performed under pilot and industry settings. The relevant data extracted from selected studies should be summarized in tables and figures, which should be used to interpret the results and draw conclusions, including correlations between microbiological and non-microbiological parameters which can be used to validate/verify and/or monitor the microbiological quality of the water used for different post-harvest handling and processing operations of ffFVHs.

The awarded contractor is expected to also present a summary of available inline systems/methodologies to validate/verify and/or monitor the relevant parameters needed to maintain the microbiological quality of water used for post-harvest handling and processing operations of ffFVHs. The awarded contractor is expected to provide information on suitable validation/verification and /or monitoring systems that can be used in different scenarios, including fresh-whole, fresh-cut and frozen FVHs. The selected inline validation/verification and /or monitoring systems should be tested under industry conditions.

This call is based on EFSA's 2021 Work Programme for grants and operational procurements as presented in Annex XIa of the Programming Document 2021 – 2023, available on the EFSA's website[4].

This contract was awarded by EFSA to:

Contractors:

Leading partner: Spanish National Research Council (Agencia Estatal Consejo Superior de Investigaciones Científicas, CSIC)

Partner 2: Universiteit Gent (UGent)

Partner 3: Stichting Wageningen Research (Wageningen Food Safety Research, WUR)

Partner 4: Institut de Recerca i Tecnologies Agroalimentaries (IRTA)

Contract title: Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs).

[3] https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2010.1637

Contract number: OC/EFSA/BIOCONTAM/2021/02

1.2 Additional information

Other additional documents/files that complement this interim report (Deliverable 5) are:

1.2.1 Supplementary files related with datasets

One Excel file named 'data.xlsx' with the sheets:

- 'Coversheet data' that lists and describes all the variables, including the possible values that each of these may acquire;
- 'Scenario characterization' that gives an overview of all FBO scenarios based on the: food category, food group, processing operation, use or no use of disinfectant, disinfectant type and disinfectant agent and
- 'data' that includes all the data obtained in the samplings from the selected FBOs collaborating with this tender.

1.2.2 Supplementary files related to modelling

Two sets of codes are shared with the tender external scientific report (available at https://doi.org/10.5281/zenodo.12759499):

- R code scripts simulating industrial cases from this tender. The files are
  - Readme.md file with information about versioning and with a flowchart to understand the structure of the code that includes
  - R script "main_industrial_cases.R", principal script to be run in R that needs and calls to the following files
    - R script "model_industrial_cases.R" with the equations of the model
    - Excel file with information about relevant measurements for modelling the industrial cases without disinfectant ("data_industrial_cases_without disinfectant.xlsx")
    - Excel file with information about relevant measurements for modelling the industrial cases with free chlorine disinfectant ("data_industrial_cases_with FC_disinfectant.xlsx")
    - Excel file with information about the estimated parameters for the industrial cases without disinfectant "Estimated_parameters_cases_WITHOUT_disinfectant.xlxs"
    - Excel file with information about the estimated parameters for the industrial cases with free chlorine disinfectant "Estimated_parameters_cases_WITH_FC_disinfectant.xlxs"

- R codes to simulate more general industrial scenarios (see comments in code for details) such as "What if" scenarios, consisting in:
  - Readme.md file with information about versioning and with a code_flowchart to understand the structure of the code
  - R script "main_what_if_scenarios.R", principal script to be run in R that needs and calls to the following files
    - R script "model_what_if_scenarios.R" with the equations of the model
    - InjectionFunction.R with the simulation of the additions of FC at different times
    - Excel file with information about relevant measurements for modelling such industrial scenarios ("input_data_what_if_scenarios.xlsx")

2 Data and methodologies

2.1 Data

2.1.1 Description of criteria and justification for the selection of case scenarios included in objectives 1, 2 and 4

The criteria followed for the selection of the case scenarios were based on:

a. Selection of **FVHs** included in the tender specifications regarded:

a.1 Food category that included fresh-whole, fresh-cut, and frozen FVHs,

a.2 Industry size that included small, medium-sized, and large processing operation enterprises.

b. Selection of the main **post-harvest handling and processing operations** of FVHs where water was involved. Thus, in the handling operations of fresh-whole FVHs, water from the: 1) hydro-cooling, 2) the dumping tank, 3) pre-sorting lines, 4) pre-washing and 5) washing was characterized for different case scenarios (**Figure 1**).

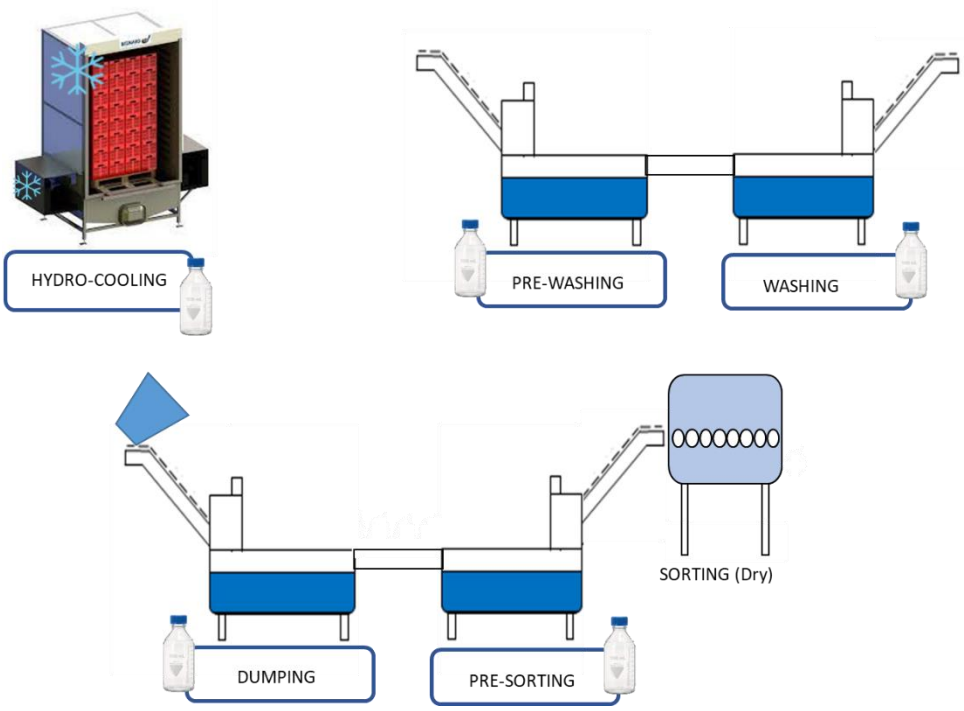


Figure 1. Processing operations for handling fresh-whole FVHs where process water was sampled

In the processing of fresh-cut FVHs after cutting, process water from the: 1) pre-washing and 2) washing operations was characterized (**Figure 2**).

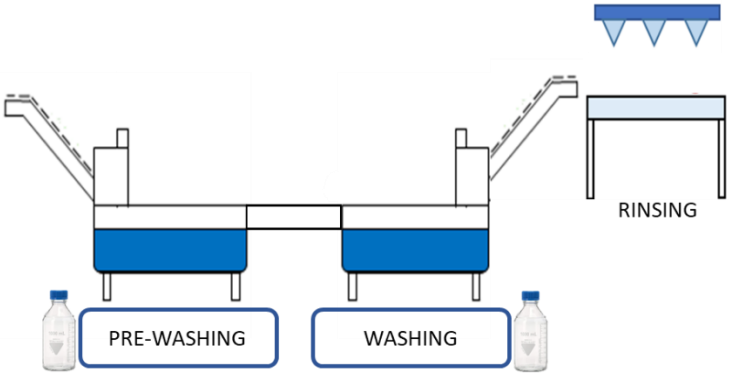


Figure 2. Processing operations for processing fresh-cut FVHs where process water was sampled

Regarding frozen FVHs, the process water used in: 1) washing, 2) cooling and 3) transporting was sampled (**Figure 3)**.

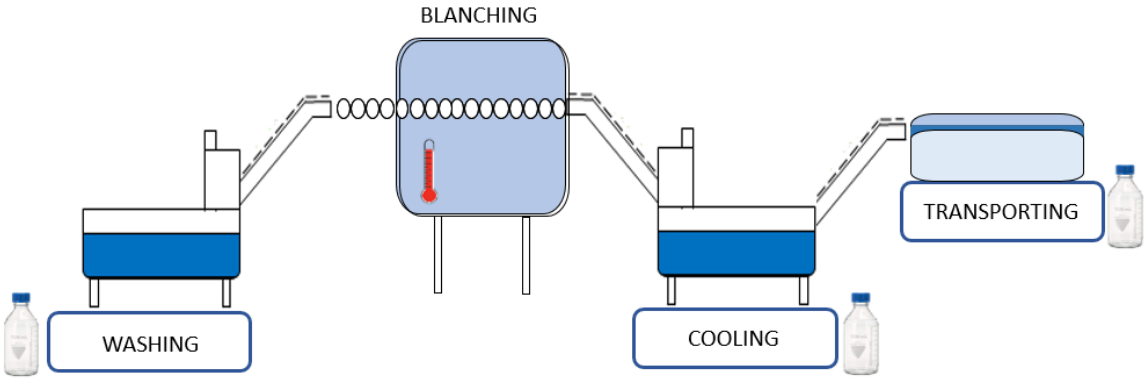


Figure 3. Processing operations for processing frozen FVHs where process water was sampled

In the case of frozen products, the general overall description of the process is that the ones that usually undergo blanching go after a cooling operation in their process. Other frozen products, however, also go to a cooling process although they are not blanched.

c. Selection of **scenarios by location**. The selection of food business operators (FBO) was done by trying to represent different handling and processing practices that FBOs of ffFVHs conduct in Europe. Thus, case scenarios in Belgium, France and Spain were selected. In Belgium (Flanders), ffFVHs such as lettuce, baby leaves, endive, radicchio, carrots, cabbages, spinach and onion are grown and processed. Due to the production volumes, many fresh-whole FVHs are also imported or exported intra and extra in some EU countries such as Spain, France, or the Netherlands. Belgian FBO have production houses spread over Europe, such as France (e.g. Arras, Bretagne, Provence), where some herbs, such as parsley are grown and processed. In Spain, the Ebro Valley is the highest growth area for pome fruits (apples and pears) and stone fruits (peaches, nectarines and cherries). The East area of Spain (Alicante, Almeria and Murcia) is the most relevant area in Europe for growing vegetables, including lettuce, baby leaves, peppers, and tomatoes. The Southeast of Spain (Malaga) is the area for growing subtropical fruits (avocados and mangoes). A representation of the selected areas distributed by location in Europe is shown in **Figure 4**.

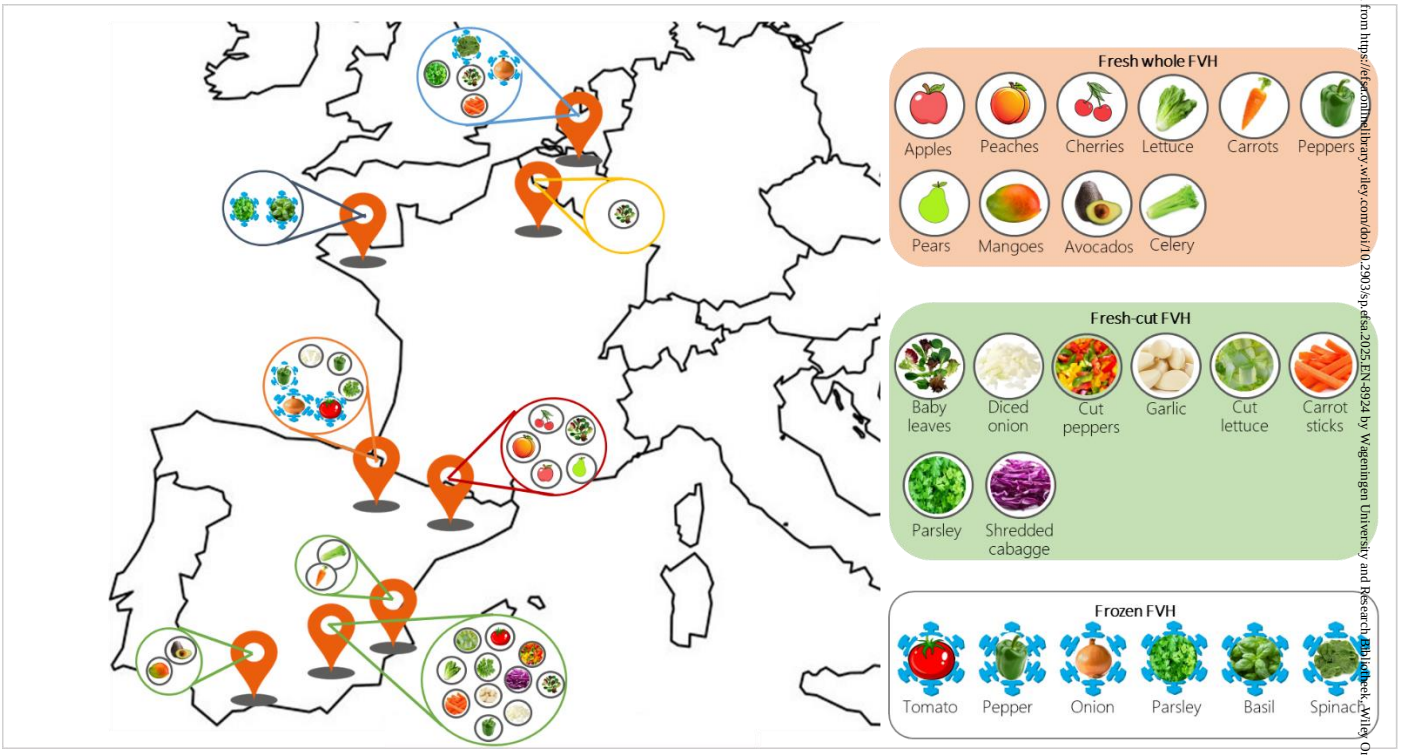


Figure 4. Overview of the case scenarios represented by item, category and location

Other criteria followed for the selection of the case scenarios where water is in contact during handling and processing were:

· Production volume of ffFVHs (Fruit Logistica, 2021),

· Volume of ffFVHs imported intra and extra the EU and volume of ffFVHs exported intra and extra the EU (Fruit Logistica, 2021),

· ffFVHs associated with outbreaks and foodborne alerts (CDC, 2022),

· Selection of leafy vegetables because of a large surface-to-volume ratio (Gil et al., 2012) and of root vegetables as underground vegetables (Brecht, 2003) and

· ffFVHs associated with changes in the water quality, such as an increase in the organic matter (López-Gálvez et al., 2019).

The rationale for the criteria followed for the selection of each case scenario is included in **Tables 3 to 5**.

2.1.2 Selection of case scenarios for objective 1

Characterisation of process water used in post-harvest handling operations of **fresh-whole FVHs** such as:

- apples
- pears
- peaches/nectarines

- peppers
- carrots
- vegetable mix for 'gazpacho' soup (tomatoes, cucumbers, peppers, and onions)
- celery

Water from the pre-sorting, dumping, pre-washing and washing were sampled. For fresh-whole FVH scenarios, different combinations of FVHs (peaches/nectarines, apple peppers, vegetable mix for 'gazpacho' soup, carrots and celery) were selected, including FBOs of three dimensions (large, medium-sized and small), with a total of 8 different cases covering the relevant variability and the criteria for the selection of cases (**Table 3**). These fruits and vegetables were selected because of their production volume, being underground vegetables or having a large surface-to-volume ratio. Peaches and nectarines were considered as one unique fruit as the selected FBOs process on the same day, one after another, using the same water. Regarding apples, the pre-sorting water was changed every 3 to 6 days. For peppers, carrots, celery and the vegetable mix for 'gazpacho' soup that includes tomatoes, cucumbers, peppers and onions, the same water was used for several days and an unknown volume of replenished was added to maintain the tank volume.

Characterisation of process water used in processing operations of fresh-cut FVHs included water from the pre-washing and washing operations of **fresh-cut FVHs** such as:

- shredded vegetables (carrots, cabbages)
- mix of curly endive and radicchio
- baby leaves
- herbs (parsley)
- salad mix (endive, curled endive, radicchio and carrots)

Case scenarios of fresh-cut FBOs in Belgium and France were examined (**Table 3**). Selected FVHs were baby leaves, carrots/shredded vegetables, salad mix that includes a combination of endive, curled endive, radicchio and carrots, and parsley. These commodities were selected because of the large surface-to-volume ratio, being underground vegetables and their association with outbreaks and foodborne alerts. Process water was sampled where FVHs were washed by immersion in a continued washing line or in batches, which could be risky. These case scenarios corresponded to large and medium-sized FBOs from Belgium and France, representing a total of 5 different scenarios.

Characterisation of process water used in the processing operations of frozen FVHs included water from the washing and transporting of FVHs such as:

- onions
- spinach

Frozen FBOs for this objective were sampled in Belgium (**Table 3**). FVHs were selected based on large surface-to-volume ratio, association with outbreaks and foodborne alerts and being underground vegetables. Process water was sampled from two different FBO sizes (large and medium-sized). The three case scenarios selected for frozen spinach were different processes in which they used recycled process water. In one scenario, water was treated with PAA, in another case water was treated with chlorine while in another water was not treated. All commercial solutions of PAA contain also hydrogen peroxide (H2O2),

acetic acid, and water in an equilibrium. However, the abbreviation PAA is used when referring to these commercial solutions. FBOs do not know the proportion of PAA in these commercial solutions. To avoid confusion with the scenarios using specifically hydrogen peroxide, the designation of peroxyacetic acid as PAA is maintained throughout the report.

Table 3. Selected case scenarios of fffvhs included in the assessment of process water for objective 1

Scenario ID	Food category	FVHs	Processing operations	FBO size	Country (WASHTOP Team)	Rationale (criteria considered for the selection of scenario)
01	Fresh-whole FVHs	Apples	Dumping	Small	Spain (IRTA)	Production volume (Top 1)
02	Fresh-whole FVHs	Apples	Pre-sorting	Large	Spain (IRTA)	Production volume (Top 1)
03	Fresh-whole FVHs	Peaches/Nectarines	Dumping	Large	Spain (IRTA)	Production volume (Top 10)
04	Fresh-whole FVHs	Peppers	Pre-washing	Medium	Spain (CEBAS-CSIC)	Production volume (Top 5)
05	Fresh-whole FVHs	Carrots	Hydro-cooling	Medium	Spain (CEBAS-CSIC)	Production volume ((Top 5)/Underground vegetables
06	Fresh-whole FVHs	Carrots	Washing	Medium	Spain (CEBAS-CSIC)	Production volume ((Top 5)/Underground vegetables
07	Fresh-whole FVHs	Vegetable mix	Pre- washing	Medium	Spain (CEBAS-CSIC)	Production volume (Top 1)
08	Fresh-whole FVHs	Celery	Washing	Medium	Spain (CEBAS-CSIC)	Large surface-to-volume ratio
30	Fresh-cut FVHs	Shredded carrots	Washing	Medium	Belgium (UGent)	Production volume ((Top 5)/Underground vegetables/Association

Scenario ID	Food category	FVHs	Processing operations	FBO size	Country (WASHTOP Team)	Rationale (criteria considered for the selection of scenario)
						with outbreaks and foodborne alerts (Top 5)
31	Fresh-cut FVHs	Curly endive and radicchio	Washing	Medium	France (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)
32	Fresh-cut FVHs	Baby leaves	Washing	Large	Belgium (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)
33	Fresh-cut FVHs	Parsley	Washing	Medium	Belgium (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)
34	Fresh-cut FVHs	Salad mix with carrots	Washing	Medium	Belgium (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)
49	Frozen FVHs	Onions	Water transport	Medium	Belgium (UGent)	Production volume ((Top 5)/Underground vegetables
50	Frozen FVHs	Spinach	Washing	Large	Belgium (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)

Scenario ID	Food category	FVHs	Processing operations	FBO size	Country (WASHTOP Team)	Rationale (criteria considered for the selection of scenario)
51	Frozen FVHs	Spinach	Washing	Large	Belgium (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)
52	Frozen FVHs	Spinach	Washing	Large	Belgium (UGent)	Large surface-to-volume ratio/Association with outbreaks and foodborne alerts (Top 5)

2.1.3 Selection of case scenarios for objective 2

In addition to the criteria for the selection of case scenarios described in Objective 1, a representative selection of different water disinfection systems was included for objective 2.

Like in objective 1, ffFVHs were selected according to their production volume, the volume of imports and exports intra and extra the EU (Fruit Logistica, 2021), or because they were underground vegetables. The fresh-whole FVHs included in the case scenarios were:

- apples
- carrots
- pears
- peaches/nectarines
- cherries
- avocado
- mango
- peppers
- a consecutive wash of fruit and vegetables (apples, tomatoes and carrots)

The selection of post-harvest handling or processing operations/FBO sizes/water disinfection treatments and water replenishment rate or their combination aimed to cover the relevant variability in ffFVHs/processing operations/FBO sizes/water disinfection treatments or their combination.

For fresh-whole FVH scenarios, a great variety of disinfectants were examined, including ozone, sodium hypochlorite and calcium hypochlorite (chlorine), hydrogen peroxide, peroxyacetic acid and PAA (**Table 4**). The targeted fresh-whole FVH were apples, pears, peaches and nectarines, cherries, carrots, avocado, mango, peppers and a consecutive wash of fruit and vegetables (apples, tomatoes and carrots); these were selected according to their production volume, and volume of imports intra/extra the EU (Fruit Logistica, 2021), covering a diversity of commercial disinfectants applied to ffFVHs. Water from the dumping, hydro-cooling, pre-sorting, pre-washing and washing operations were evaluated in small, medium-sized and large FBO sizes in a total of 22 case scenarios, all located in Spain.

Characterisation of process water treated with different intervention strategies in the processing operations of fresh-cut FVHs included the following commodities:

- tomatoes/cucumbers
- diced onions
- carrot sticks
- fresh-cut lettuce
- shredded lettuce
- baby leaves

Process water from pre-washing and washing operations of tomatoes/cucumbers, diced onion, carrot sticks, cut lettuce, shredded lettuce and baby leaves were selected due to either their production volume, their large surface-to-volume ratio, being underground vegetables, import intra/extra the EU, being FVHs

associated with outbreaks and foodborne alerts/ and/or FVHs associated with changes in the water quality (**Table 4**). The process water of shredded lettuce, diced onion, and cut peppers were affected by the high organic load that exudates from the cut edges, which was another critical factor that was considered for the selection of these scenarios. Four disinfectants (electrolysed water, sodium hypochlorite, calcium hypochlorite and their combination) from pre-washing and washing operations in small, medium and large FBOs in Spain were evaluated, with a total of 10 scenarios. In the case of electrolysed water, tomatoes and cucumbers were washed by a shower in a pre-washing and washing steps before cutting to avoid accumulation of organic matter in the process water. The same water was used for two days, washing the tomatoes on the first day and the cucumbers on the second day. This is the reason why both products were included in the same scenario. No cases from Belgium were included as processors do not use disinfectants in fresh-cut operations in this country.

The characterisation of process water treated with different intervention strategies in the processing operations of frozen FVH was carried out analysing process water from pre-washing, washing and cooling operations. The criteria for the selection of case scenarios were the large surface-to-volume ratio, being FVHs associated with outbreaks and foodborne alerts, underground vegetables, production volume as well as the diversity of the type of disinfectant treatments used. In the frozen FVH scenarios, some products were blanched such as diced peppers while others as diced onions were not (**Table 4**). Generally, if the processing operation is a cooling process is because there has been a blanching operation before. This is different to what happens when there is a washing operation in which there is no need to cool down the product temperature as there has not been any previous blanching. Different selected disinfectants such as hydrogen peroxide, and PAA were included. Medium-sized and large FBOs in Spain and Belgium were sampled. The frozen FVHs included the following (**Table 4**):

- diced peppers
- onions
- diced onions
- spinach
- parsley
- chives

Table 4. Selected case scenarios of ffFVHs included in the assessment of the processing water for objective 2

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
09	Fresh-whole FVHs	Chlorine: NaClO	Apples	Dumping	Small	Spain (IRTA)	Production volume (Top 1)
10	Fresh-whole FVHs	Chlorine: NaClO	Apples	Pre-sorting	Large	Spain (IRTA)	Production volume (Top 1)
11	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Apples	Dumping	Large	Spain (IRTA)	Production volume (Top 1)
12	Fresh-whole FVHs	Hydrogen peroxide	Apples	Dumping	Small	Spain (IRTA)	Production volume (Top 1)
13	Fresh-whole FVHs	Hydrogen peroxide	Apples	Dumping	Small	Spain (IRTA)	Production volume (Top 1)
14	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Pears	Dumping	Small	Spain (IRTA)	Production volume (Top 10)
15	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Pears	Pre-sorting	Medium	Spain (IRTA)	Production volume (Top 5)

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
16	Fresh-whole FVHs	Chlorine: NaClO	Peaches/ Nectarines	Dumping	Large	Spain (IRTA)	Production volume (Top 10)
17	Fresh-whole FVHs	Chlorine: NaClO	Peaches/ Nectarines	Dumping	Medium	Spain (IRTA)	Production volume (Top 10)
18	Fresh-whole FVHs	Chlorine: NaClO	Peaches/ Nectarines	Dumping	Small	Spain (IRTA)	Production volume (Top 10)
19	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Peaches/ Nectarines	Dumping	Large	Spain (IRTA)	Production volume (Top 10)
20	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Peaches/ Nectarines	Dumping	Small	Spain (IRTA)	Production volume (Top 10)
21	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Peaches/ Nectarines	Dumping	Medium	Spain (IRTA)	Production volume (Top 10)
22	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Peaches/ Nectarines	Pre-sorting	Medium	Spain (IRTA)	Production volume (Top 10)

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
23	Fresh-whole FVHs	Hydrogen peroxide	Peaches/ Nectarines	Dumping	Small	Spain (IRTA)	Production volume (Top 10)
24	Fresh-whole FVHs	Chlorine: NaClO	Cherries	Hydro-cooling	Medium	Spain (IRTA)	Export intra/extra the EU (Top 5)
25	Fresh-whole FVHs	Chlorine: $Ca(ClO)_2$	Cherries	Dumping	Medium	Spain (IRTA)	Export intra/extra the EU (Top 5)
26	Fresh-whole FVHs	PAA	Avocado	Pre-washing	Medium	Spain (CEBAS-CSIC)	Import intra/extra the EU (Top 5)
27	Fresh-whole FVHs	PAA	Mango	Pre-washing	Medium	Spain (CEBAS-CSIC)	Import intra/extra the EU
28	Fresh-whole FVHs	PAA	Peppers	Washing	Medium	Spain (CEBAS-CSIC)	Production volume (top 5)
29	Fresh-whole FVHs	Chlorine: NaClO	Fruit mix	Washing	Large	Spain (CEBAS-CSIC)	Import extra the EU (Top 5)
35	Fresh-cut FVHs	Chlorine:	Tomatoes/ Cucumbers	Pre-washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
		Electrolysed water					
36	Fresh-cut FVHs	Chlorine: Electrolysed water	Tomatoes/ Cucumbers	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)
37	Fresh-cut FVHs	Chlorine: NaClO	Diced onions	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Underground vegetables/Associated with changes in the water quality
38	Fresh-cut FVHs	PAA	Diced onions	Washing	Large	Spain (CEBAS-CSIC)	Underground vegetables/Associated with changes in the water quality
39	Fresh-cut FVHs	Chlorine: NaClO	Carrot sticks	Washing	Medium	Spain (CEBAS-CSIC)	Underground organ/Associated with changes in the water quality
40	Fresh-cut FVHs	Chlorine: $Ca(ClO)_2$	Fresh-cut lettuce	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Large surface-to-volume ratio/Associated with

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
							outbreaks and foodborne alerts (Top 5)/Associated with changes in the water quality
41	Fresh-cut FVHs	Chlorine: $Ca(ClO_2) + NaClO$	Fresh-cut lettuce	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Large surface-to-volume ratio/ Associated with outbreaks and foodborne alerts (Top 5)/ Associated with changes in the water quality
42	Fresh-cut FVHs	Chlorine: $Cl_2 + NaClO$	Fresh-cut lettuce	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)/Associated with

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
							changes in the water quality
43	Fresh-cut FVHs	Chlorine: NaClO	Shredded lettuce	Pre-washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)/Associated with changes in the water quality
44	Fresh-cut FVHs	Chlorine: NaClO	Shredded lettuce	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Large surface-to-volume ratio/ Associated with outbreaks and foodborne alerts (Top 5)/ Associated with changes in the water quality

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
45	Fresh-cut FVHs	Chlorine: NaClO	Baby leaves	Washing	Large	Spain (CEBAS-CSIC)	Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)
46	Fresh-cut FVHs	Chlorine: $Ca(ClO)_2$	Baby leaves	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)/Associated with changes in the water quality
47	Fresh-cut FVHs	Chlorine: $Ca(ClO_2)$ + NaClO	Baby leaves	Washing	Large	Spain (CEBAS-CSIC)	Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
48	Fresh-cut FVHs	Chlorine: NaClO	Salad mix	Washing	Medium	Spain (IRTA)	Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)
53	Frozen FVHs	Hydrogen peroxide	Diced peppers	Pre-washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)
54	Frozen FVHs	Hydrogen peroxide	Diced peppers	Cooling after blanching	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)
55	Frozen FVHs	PAA	Diced onions	Cooling no blanching	Medium	Belgium (UGent)	Underground vegetable
56	Frozen FVHs	Hydrogen peroxide	Diced onions	Pre-washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Underground vegetable
57	Frozen FVHs	Hydrogen peroxide	Diced onions	Washing	Large	Spain (CEBAS-CSIC)	Production volume (Top 5)/Underground vegetable

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (Team)	Rationale (criteria considered for the selection of scenario)
58	Frozen FVHs	PAA	Spinach	Cooling after blanching	Large	Belgium (UGent)	Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)
59	Frozen FVHs	PAA	Spinach	Cooling after blanching	Large	Belgium (UGent)	Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)
60	Frozen FVHs	PAA	Parsley	Washing	Large	Belgium (UGent)	Large surface-to-volume ratio
61	Frozen FVHs	PAA	Chives	Washing	Small	Belgium (UGent)	Large surface-to-volume ratio

2.1.4 Selection of case scenarios for objective 4

Four different scenarios, including diced onions, fresh-cut lettuce, and baby leaves washed with three disinfectants (PAA, Ca $(ClO)_2$, and Cl_2 plus NaClO) were initially selected for collecting data on suitable validation/verification and/or monitoring systems to measure non-microbiological parameters using online monitoring (OM) systems to correlate with the microbiological quality of process water. These online sensors are installed separately from the main process stream, unlike inline systems. The water sample in the 'inline' systems is taken from the main flow to the sensors for analysis. In the planned scenarios, process water treated with PAA for washing diced onion and with chlorine (Ca $(ClO)_2$, and Cl_2 plus NaClO) for baby leaves and cut lettuce were selected as representative case scenarios. The rationale for the selection of the case scenarios considered FVHs that are underground vegetables or associated with changes in the water quality, production volume, large surface-to-volume ratio or outbreaks and foodborne alerts. However, due to unexpected issues with the calibration of the detectors used in the selected industrial settings, the data generated could not be used for the WASHTOP tender. Alternatively, a "historical dataset" from a single non-WASHTOP scenario provided by the CEBAS-CSIC group (ID CEBAS-OM) is included (**Table 5**). The description of the process is mentioned in the datasheet (ID CEBAS-OM) and the description of the results is in Figure 75.

Table 5. Selected "historical" case scenario of ffFVHs included in the assessment of process water for objective 4

Scenario ID	Food category	Water disinfection treatment	FVHs	Processing operations	FBO size	Country (WASHTOP Team)	Rationale (criteria considered for the selection of scenario)
CEBAS-OM	Fresh-cut FVHs	Chlorine: NaClO	Baby leaves and fresh-cut iceberg lettuce	Prewashing and washing	Large	Spain (CEBAS-CSIC)	Large surface-to-volume ratio/Associated with outbreaks and foodborne alerts (Top 5)

As a summary, **Table 6** shows the total number of scenarios studied to obtain data that represented a wide range of situations to respond to the Tender specifications in objectives 1, 2 and 4.

Table 6: The number of scenarios that will be performed to assess objectives 1, 2 and 4

	Objective 1	Objective 2	Objective 4
Fresh-whole FVHs	8	21	0
Fresh-cut FVHs	5	14	0
Frozen FVHs	4	9	0
Total per objective	**17**	**44**	
Total scenarios	**61**		

The number of scenarios conducted by CEBAS-CSIC team is shown in **Table 7**, by IRTA team is shown in **Table 8** and in **Table 9** for UGent.

Table 7. The number of scenarios performed by CEBAS-CSIC team

	Objective 1	Objective 2	Objective 4
Fresh-whole FVHs	5	4	0
Fresh-cut FVHs	0	13	0
Frozen FVHs	0	4	0
Total per objective	**5**	**21**	
Total scenarios	**26**		

Table 8. The number of scenarios performed by IRTA team

	Objective 1	Objective 2	Objective 4
Fresh-whole FVHs	3	17	0
Fresh-cut FVHs	0	1	0
Frozen FVHs	0	0	0
Total per objective	**3**	**18**	**0**
Total scenarios	**21**		

Table 9. The number of scenarios performed by UGent team

	Objective 1	Objective 2	Objective 4
Fresh-whole FVHs	0	0	0
Fresh-cut FVHs	5	0	0

	Objective 1	Objective 2	Objective 4
Frozen FVHs	4	5	0
Total per objective	**9**	**5**	**0**
Total scenarios	**14**		

2.1.5 Sampling information

Before and after each visit, the contact person in each FBO was contacted to collect information about the processing operation. The requested information before the sampling was:

- start time of the process operation,
- type of water source (surface water, municipal + well water, municipal tap water, recycled water, well water, and municipal + recycled water),
- contact time between the product and the water,
- agitation type of the water in the tank/deposit,
- water volume of the tank/deposit,
- filling and empty time of the tank/deposit,
- water volume that is replaced between filling and emptying of the tank/reservoir,
- type of water disinfectant,
- concentration of the disinfectant used,
- monitoring of residual disinfectant concentration (automatic/manual dosing),
- residual concentration of the disinfectant in the processing operation
- ratio water/product.

After each visit, the contact person was contacted again to collect the information about the processing operation, in particular:

- total amount of product processed from start until the moment of sampling,
- total amount of disinfectant added from the start to the end of the specific sampling period for each FVH in each processing line.

In addition, most of the visited FBOs included in the case studies, supported by WASHTOP team members answered the EU survey questionnaire, characterising their sampled processing lines.

Each case scenario was sampled twice on different days/seasons/months (e.g. at the highest peak of the season and the end of the season). There were 6 sampling times distributed during the whole production process and at each one, water samples were taken in duplicate. As a result, a total of 12 water samples per visit and two visits per case scenario were examined representing 24 samples in total. There were only two exceptions: 1. the case scenario ID 35 mango was visited only once due to the end of the season. 2. the extra scenario ID 59 apples was visited once but it was not planned in the submitted proposal.

Four bottles (2 L each) per sampling time were taken (two for the physico-chemical analyses and two that contained the neutraliser (when required) for the microbiological analyses). At time 6, another bottle of 20 L was taken for norovirus, coliphages and *Cryptosporidium* analyses plus one bottle of 10 L for STEC.

A specific neutralizer per disinfectant was used (e.g. sodium thiosulfate for chlorine, sodium thiosulfate and catalase for PAA, and catalase for H_2O_2). The amount needed to be added depended on the residual concentration and it is indicated in the 'Microbiological analyses' section.

One Excel file was generated, named 'data', which contained the information requested related to each processing operation and the results of the analyses. It also included a numerical code for each FBO to keep the confidentiality of the data provided and the results obtained. Data in rows corresponded to each sampling time (1, 2, 3, 4, 5, and 6) and replicate (1, 2), having a total of 12 rows per each of the two visits for each processing operation. Some of the data in columns were completed with the sampling information requested to the quality manager person plus the results of the analytical measurements.

2.1.6 Detailed setup of sample analysis

The setup conducted for each objective has been planned as follows:

2.1.6.1 Objective 1

The first objective was to carry out the characterisation of water used in different post-harvest handling and processing operations of ffFVHs to evaluate the microbiological and physico-chemical quality of the processing water during the working day in FBO settings where processing water was not subjected to any water disinfection treatment. Water samples were evaluated for their microbiological and physico-chemical quality.

The physico-chemical characterization of process water included: pH, temperature (T), redox potential (ORP), electrical conductivity (EC), turbidity (TUR), Total Dissolved Solids (TDS), Total Soluble Solids (TSS), chemical oxygen demand (COD), UV-absorbance at 254 nm (UV-Abs) unfiltered and filtered and residual disinfectant (free chlorine, total chlorine, PAA and H_2O_2). The methodologies used are described in the Methodology section (2.2).

The microbial characterization of process water comprised the enumeration or occurrence of foodborne pathogens including several pathogenic microorganisms (*Salmonella* spp., STEC and *E. coli* O157:H7, *L. monocytogenes*) as well as microbial indicators (non-pathogenic *E. coli* and coliforms, *Listeria* spp.). In addition, total bacterial count (TBC) and moulds and yeasts were determined. Norovirus, *Cryptosporidium*, coliphages and *CrAssphage* were determined in samples collected at the end of the sampling time, as indicated in **Table 10**. The methodologies used are described in the Methodology section (2.2).

The potential contamination rate was determined based on the evaluation of pathogenic and/or indicator microorganisms between different batches of FVHs that are processed in the same volume of water along the operational time.

Table 10. Physico-chemical, microbial and other specific foodborne pathogens' analysis performed in the process water of fresh-whole, fresh-cut and frozen FVHs conducted for objective 1

Case scenarios	Physico-chemical analysis[a]	Microbiological analysis[a]	Other specific microorganisms[b]
Fresh-whole FVHs	pH, T, ORP, EC, TUR, TDS, TSS, COD, UVabs	TBC, COL, EC, SAL, LIS, LM, STEC	Norovirus, *Cryptosporidium*,

Case scenarios	Physico-chemical analysis[a]	Microbiological analysis[a]	Other specific microorganisms[b]
			CrAssphage, coliphages
Fresh-cut FVHs	pH, T, ORP, EC, TUR, TDS, TSS, COD, UVabs	TBC, COL, EC, SAL, LIS, LM, STEC	Norovirus, *Cryptosporidium*, *CrAssphage*, coliphages
Frozen FVHs	pH, T, ORP, EC, TUR, TDS, TSS, COD, UVabs	TBC, COL, EC, SAL, LIS, LM, STEC	Norovirus, *Cryptosporidium*, *CrAssphage*, coliphages

[a] According to the Methodology described in Section 2.2. TBC: Total Bacterial Count, COL: Coliforms, EC: *E. coli*, SAL: *Salmonella*, LIS: *Listeria* spp., LM: *L. monocytogenes*, STEC: Shiga-toxin producer *E. coli* and *E. coli* O157

[b] These were only determined at the final sampling time

2.1.6.2 Objective 2

The second objective was to examine the specific case scenarios selected in FBO settings of post-harvest handling and processing operations of ffFVHs that provided evidence of the efficacy of disinfection strategies to maintain the microbiological quality of process water. Water disinfection treatments included sodium and calcium hypochlorite, electrolyzed water, PAA, hydrogen peroxide and ozone, with or without water replenishment. The physico-chemical characterization of process water included the same parameters as those indicated in objective 1 plus the residual concentration of the disinfectants. The microbiological characterization of process water comprised total bacterial counts (TBC) and the enumeration or occurrence of foodborne pathogens including several pathogenic microorganisms (*Salmonella* spp., STEC, *L. monocytogenes*) as well as microbial indicators (non-pathogenic *E. coli*, coliforms, *Listeria* spp.). Norovirus, *Cryptosporidium*, coliphages and *CrAssphage* were also determined in samples collected at the end of the working period, as mentioned for objective 1 (section 2.1.7.1). The methodologies used are described in the Methodology section (2.2).

Some disinfectants could induce the formation of 'viable but non-culturable' (VBNC) cells. Nine case scenarios were selected to compare the induction of VBNC bacterial cells in process water treated with chlorine, PAA, and hydrogen peroxide (**Table 11**). According to the results obtained for the analysis of culturable data, VBNC analysis of *E. coli*, coliforms, and total bacterial counts was done. Levels were calculated as: total bacteria by qPCR, viable bacteria by EMA + PMAxx-qPCR, culturable bacteria by plate count, and VBNC by the differences between viable and culturable bacteria indicated in the methodology (section 2.2) following the protocol optimized by Truchado et al. (2020).

Spores of *Clostridium perfringens* were examined in three selected case scenarios of two underground vegetables (carrots and onions) and one leafy vegetable (baby leaves) with large surface-to-volume ratio. Thus, whole carrots (ID 05), diced onions (ID 37), and baby leaves (ID 47) were selected for these analyses (**Table 11**). No water disinfectant was added to the process water of carrots, sodium hypochlorite was used for diced onions and compared with calcium and sodium hypochlorite in baby leaves to examine its effectiveness against the presence of spores of *C. perfringens*. Process water at time point 6 was analysed as described in methodology section 2.2.

Table 11. Selected case scenarios for VBNC and spores of *Clostridium perfringens* analyses in process water treated with disinfectant included in objective 2

Scenario ID	Food category (FVHs)	Disinfection treatments	Processing operation	FBO size	Country (WASHTOP Team)	Microbial analyses[c]	Rationale (criteria considered for the selection of scenario)
05	Fresh-whole FVHs (Carrots)	No water disinfection	Hydro-cooling	Large	Spain (CEBAS-CSIC)	VBNC[b] and *C. perfringens*	Type of disinfectant/ Production volume (Top 5)/Underground vegetable
26	Fresh-whole FVHs (Avocado)	PAA	Pre-washing	Large	Spain (CEBAS-CSIC)	VBNC[b]	Type of disinfectant/Import intra/extra the EU (Top 5)
28	Fresh-whole FVHs (Pepper)	PAA	Washing	Large	Spain (CEBAS-CSIC)	VBNC[b]	Type of disinfectant/Production volume (Top 5)
37	Fresh-cut FVHs (Diced onions)	Chlorine: NaClO	Washing	Large	Spain (CEBAS-CSIC)	VBNC[b] and *C. perfringens*	Type of disinfectant/Underground vegetable
43	Fresh-cut FVHs (cut lettuce)	Chlorine: NaClO	Pre-washing	Large	Spain (CEBAS-CSIC)	VBNC[b]	Type of disinfectant/ Large surface-to-volume ratio
44	Fresh-cut FVHs (cut lettuce)	Chlorine: NaClO	Washing	Large	Spain (CEBAS-CSIC)	VBNC[b]	Type of disinfectant/Large surface-to-volume ratio

Scenario ID	Food category (FVHs)	Disinfection treatments	Processing operation	FBO size	Country (WASHTOP Team)	Microbial analyses[c]	Rationale (criteria considered for the selection of scenario)
47	Fresh-cut FVHs (Baby leaves)	Chlorine: $Ca(ClO_2)$ + NaClO	Washing	Large	Spain (CEBAS-CSIC)	VBNC[b] and *C. perfringens*	Type of disinfectant/Large surface-to-volume ratio
56	Frozen FVHs (Diced onion)	Hydrogen peroxide	Pre-washing	Large	Spain (CEBAS-CSIC)	VBNC[b]	Type of disinfectant/Underground vegetable
57	Frozen FVHs (Diced onion)	Hydrogen peroxide	Washing	Large	Spain (CEBAS-CSIC)	VBNC[b]	Type of disinfectant/Underground vegetable

[a] According to the Methodology described in Section 2.2. [b] According to the results obtained for the analysis of culturable data, VBNC (viable but non culturable) of *E. coli*, coliforms, and total bacterial counts were done. [c] These were only determined at the final sampling time

2.2 Methodologies

2.2.1 Analytical methodology

The methodologies are described according to each specific objective.

For Objectives 1, 2 and 4, the analytical procedures followed the standard methods or other testing methodologies with equivalent results. The goal was that the methodology for each parameter was specific to the parameter measured and that the methods and protocols were harmonised among the consortium members to be able to process the data together independently of who obtained it.

Some pre-process protocols such as pre-filtration/ dilutions for sample analyses were arranged as needed for the accuracy of the results as the water quality changed due to the presence or not of the disinfectant and/or differences in quality characteristics over the six-point times. A decision about these pre-process protocols was made by the expert team who processed the samples. Serial dilutions with a single plate per dilution were prepared.

Several methods were used to assess the physico-chemical and microbiological quality of water samples:

2.2.2 Physico-chemical analyses

To characterize the evolution of the physico-chemical quality of the processing water, pH, temperature, oxidation reduction potential (ORP), electrical conductivity (EC), total dissolved solids (TDS), turbidity, and the residual concentration of the disinfectant were measured 'in situ'. When the water samples in refrigerated transport arrive at the laboratory, chemical oxygen demand (COD), UV-absorbance, and total suspended solids (TSS) were determined. A scheme representing the physico-chemical parameter measured in situ and offline in the laboratory is shown in **Figure 5.**

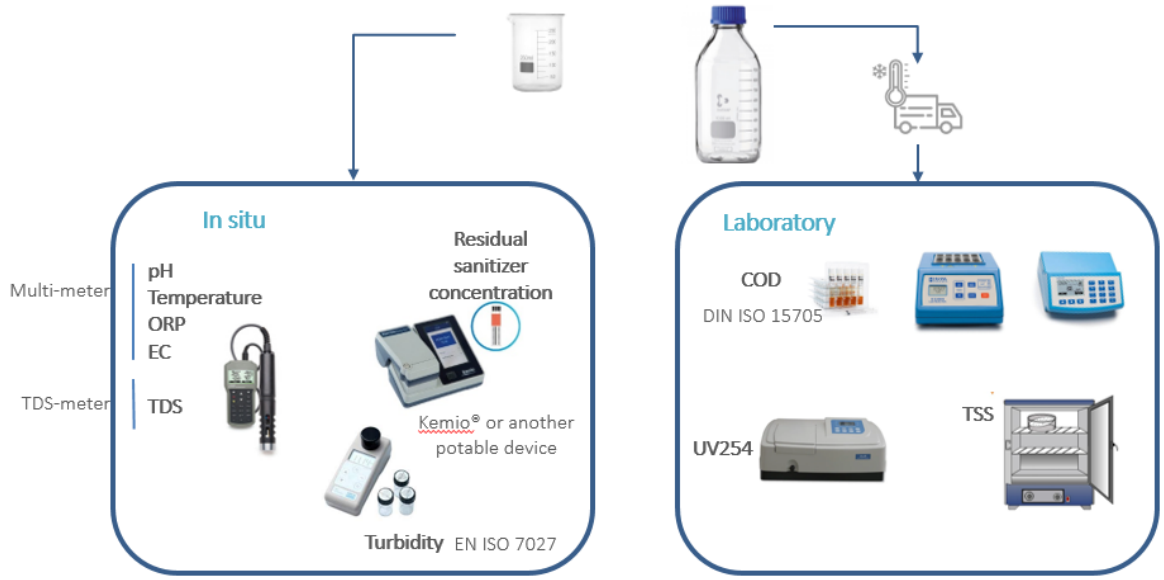


Figure 5. Schematic representation of the physico-chemical analyses conducted in situ and in the laboratory

A brief description of the harmonized protocols is described below. Some of the parameters were measured in the industrial setting whereas others were conducted after transporting the samples to the lab. The parameters listed below were performed in situ at the industrial settings:

1. pH: It was determined using a portable multi-meter (e.g. sensION+ MM150, Hach, Loveland, Colorado, USA).

2. Temperature: It was measured using the same multi-meter probe.

3. Oxidation-reduction potential (ORP) or Redox potential: ORP was determined using the same portable multi-meter.

4. Electrical conductivity (EC): EC was determined using the same portable multi-meter probe described for ORP and pH.

5. Total dissolved solids (TDS): It was analysed directly using a TDS-meter (e.g. s SDT Pocket Pro from Hach).

6. Turbidity: Turbidity was measured using a turbidimeter (e.g. Turbiquant 3000 IR, Merck).

7. Disinfectant agent for process water samples included in Objective 2: The consortium teams involved in the sampling measured the concentration of disinfectants. For chlorine, **residual chlorine** also known as **free chlorine** as well as **total chlorine** were measured using the Kemio (Palintest, Gateshead, UK) device and the corresponding test kits. For PAA and hydrogen peroxide, the Kemio (Palintest, Gateshead, UK), and in some scenarios for hydrogen peroxide the Reflectoquant® (RQflex® 10, Merck, Darmstadt, Germany) were used. For ozone, a portable device (dissolved ozone tester, DOZ-30, Twinno) was used for the measurements. In the case of chlorine, total chlorine refers to the sum of both free available chlorine (residual chlorine) and combined chlorine. Free chlorine is the chlorine that is available for disinfection and has not yet reacted with contaminants, while combined chlorine forms when free chlorine reacts with organic matter in the water. Measuring total chlorine gives an overview of all chlorine forms present.

The remaining parameters that were measured in the lab included:

8. Chemical oxygen demand (COD): COD was determined by the method DIN ISO 15705, which is analogous to EPA 410.4, APHA 5220 D, and ASTM D1252-06 B by measuring the dichromate reduction after digestion using a photometer (e.g. Spectroquant NOVA 60, Merck).

9. UV-absorbance: Water samples were shaken and after, un-filtered and filtered samples through 0.45-µm hydrophilic polyethersulfone filters were taken and the absorbance at 254 nm was measured with a UV-VIS spectrophotometer and quartz cuvettes with a 1-cm path length (e.g. Hellma, Müllheim, Germany) (**Figure 6**).

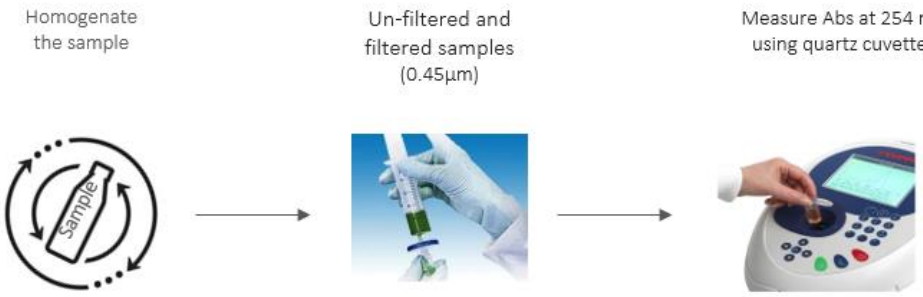
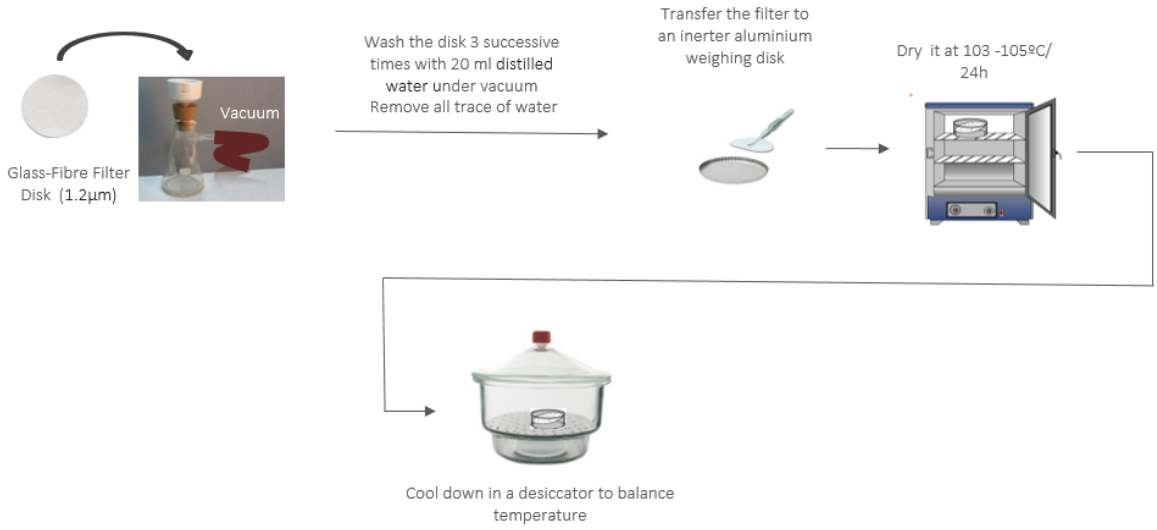


Figure 6. Protocol for measuring the UV Absorbance of unfiltered and filtered water samples at 254 nm

10. Total suspended solids (TSS): It was analysed following the protocol represented in **Figure 7** where the filter was prepared and conditioned (step A) and then the sample measured (e.g. by Standard methods 2540 C and 2540 D respectively, APHA, 2017).

A) Filter preparation



B) Sample analysis

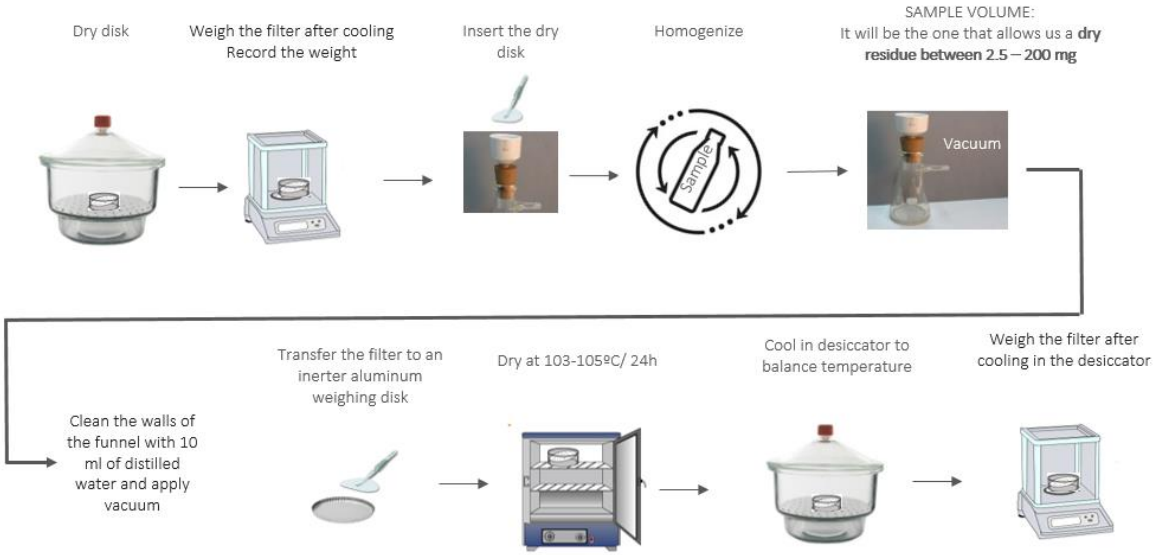


Figure 7. Scheme for total suspended solids analyses: (A) filter preparation and (B) sample analysis

2.2.3 Microbiological analyses

To characterize the microbiological quality of the process water, pathogenic and indicator microorganisms were examined either following their corresponding ISO as the standard method or using other testing

procedures with demonstrated equivalent results to increase the sensitivity for pathogen detection using selective media that support the growth of the microorganism of interest by supplying nutrients and reducing microbial competition. Schematic diagrams of the methodologies are shown for each specific group of microorganisms. There were differences in the limit of detection (LoD) because of different filtration volumes used as the filters collapsed. The LoD of each microbial group is indicated in each datasheet. In general, LoD for IRTA and CEBAS-CSIC were the ones described in the microbiological protocols. In the case of UGent, LoDs were higher for scenarios where there was high turbidity and as such a lower volume of water could be filtered or LoDs were lower where the samples were directly streaked on the plate as 1 mL was used over several plates instead of 100 µl. To stop the disinfectant influence during transport and storage before the analyses, different neutralizers were added (e.g. sodium thiosulfate for chlorine, sodium thiosulfate and catalase for PAA, and catalase for H_2O_2). After measuring the residual disinfectant concentration in each replicate at each sampling point, the volume of neutralizers needed was calculated. The amount of sodium thiosulphate (0.5 M) needed to neutralize chlorine (20 mg/L) was 1 µL/mL, and for PAA (80 mg/mL) was 2.6 µL/mL. The catalase (2000 U/mg) added for PAA (80 mg/L) was 0.8 µL/mL (Falcó et al., 2023).

1. Total yeast and moulds and total bacterial counts (TBC): The enumeration of total bacterial counts was done according to ISO 6222:1999 for the enumeration of culturable microorganisms in water with some modification for surface plating (López-Gálvez et al., 2020). Serial dilutions of the water samples in buffered peptone water were performed. Selective media for yeast and moulds such as Dichloran Rose Bengal Chloramphenicol Agar (DRBC) and Plate Count Agar (PCA) for TBC were used as more specific for each group under the test conditions described in **Figure 8**. PCA plates were incubated at 3 °C for 44 h and DRBC at 22-25 °C for 72 h. In the case of the UGent scenarios for total yeast and moulds, 1 mL of the undiluted sample (instead of 100 µL) was streaked on several plates to obtain an increased detection, resulting in a lower LoD.

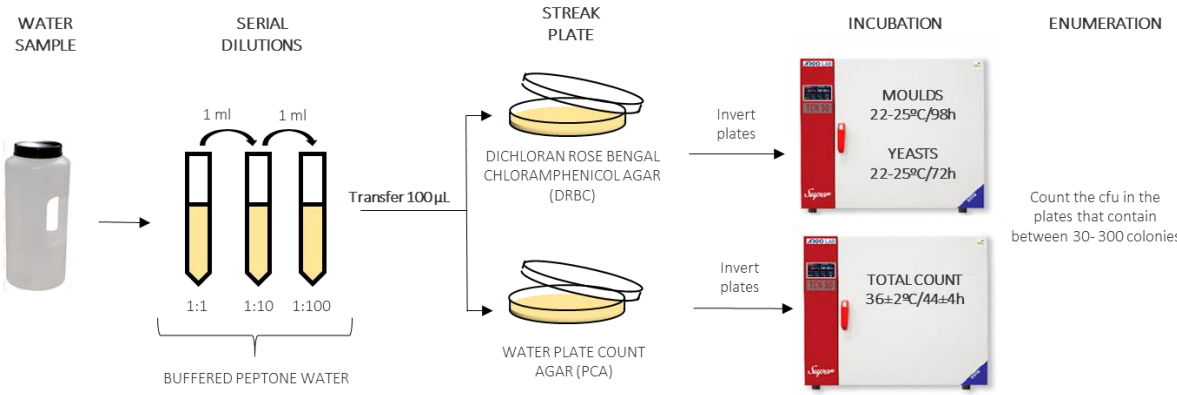


Figure 8. Scheme for the enumeration of moulds and yeast and total bacterial counts following the ISO 6222:1999 with some modifications (López-Gálvez et al., 2020)

2. Total coliforms and E. coli: Total coliforms and culturable *E. coli* were analysed according to ISO 9308-1:2014/A1:2017. Depending on the case scenario if the water was treated or not with a disinfectant, both serial dilutions and membrane filtration were made as shown schematically in **Figure 9**. In the case of the UGent scenarios one milliliter of the undiluted sample (instead of 100 µL) was streaked on several plates to obtain an increased detection, resulting in a lower LoD. Water samples (100, 50 or 10 mL) were filtered through a 0.45 µm membrane filter using a filter holder manifold. The filter was plated using a selective chromogenic coliform agar (e.g., Chromocult agar) followed by incubation for 24 h at 37 °C. For

water samples with high turbidity or high content of organic matter, a pre-filtration step was done according to ISO specifications. For *E. coli*, an extra sample was incubated at 44 °C to favour the growth and avoid interferences with coliforms. As indicated in **Figure 9**, a filtration volume of 100 mL was performed. The filter was incubated in a selective chromogenic coliform agar and the incubation was done at 44 ºC for 24 h before the colony count.

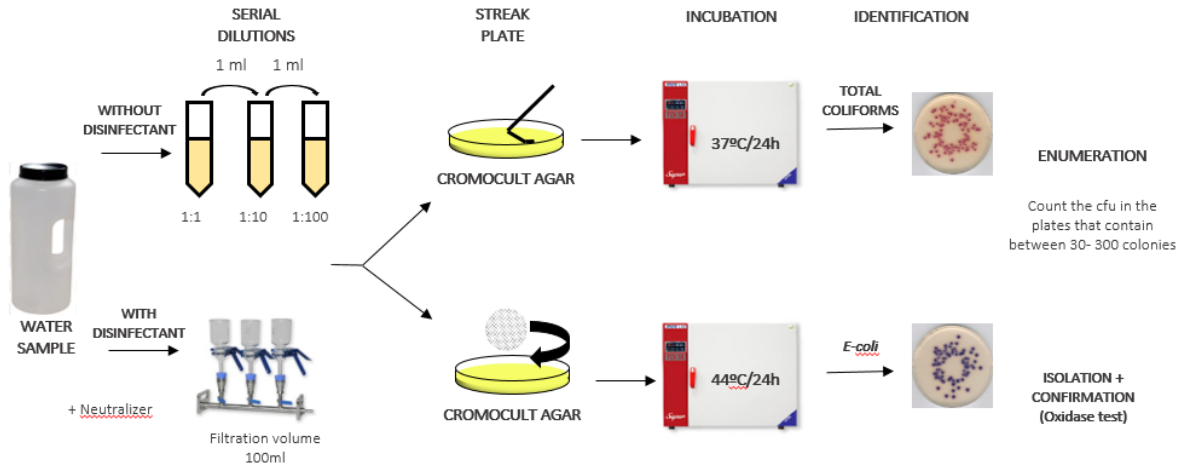


Figure 9. Scheme for the detection and enumeration of total coliform and *E. coli* following the ISO 9308-1:2014 modified protocol

***3. Salmonella* spp.:** For the detection of *Salmonella* spp., the EN ISO 19250:2010 was followed (**Figure 10**). Briefly, 100 mL of water was filtered throughout a 0.45 µm membrane filter using a filter holder manifold. For water samples with high turbidity or organic load, a pre-filtration step was performed according to ISO specifications. However, if different filtration volumes were used because the filters collapsed, influencing the detection limit, in the comments section of each datasheet, this is indicated when differed from this protocol. The filter was pre-enriched in Buffered Peptone Water (BPA) at 37 ± 1 °C for 18 ± 2 h, followed by enrichment in selective Rappaport-Vassiliades Soja Peptone Broth (RVS broth) incubated at 41.5 ± 1 °C for 24±3 h and in Muller-Kauffmann Tetrathionate Novobiocin Broth incubated at 37 ± 1 °C for 24 ± 3 h. Afterward, the samples from both selective enrichments will be streaked onto Xylose-Lysine-Desoxycholate Agar (XLD) followed by incubation at 37 ± 2 °C for 24 ± 3 h performed for colony isolation. Presumptive colonies were isolated and confirmed using a serological test for those case scenarios conducted by UGent and using a PCR for those scenarios of IRTA and CEBAS-CSIC. Confirmation of *Salmonella* spp. was done by the agglutination test using a Latex Agglutination test kit (https://www.thermofisher.com/order/catalog/product/DR1108A), which is a serological test. The presence of *Salmonella* O-, Vi, and H-antigens detected by agglutination with the specific sera as in the test kit, from pure colonies from TSA, give positive serological reactions to confirm as *Salmonella* spp. as described in the ISO 19250 (2010) and validated at UGent. In the case of CEBAS-CSIC and IRTA, confirmation by PCR was performed with the primers indicated in **Table 12**. Detection/non detection of *Salmonella* spp. is referred to 100 mL.

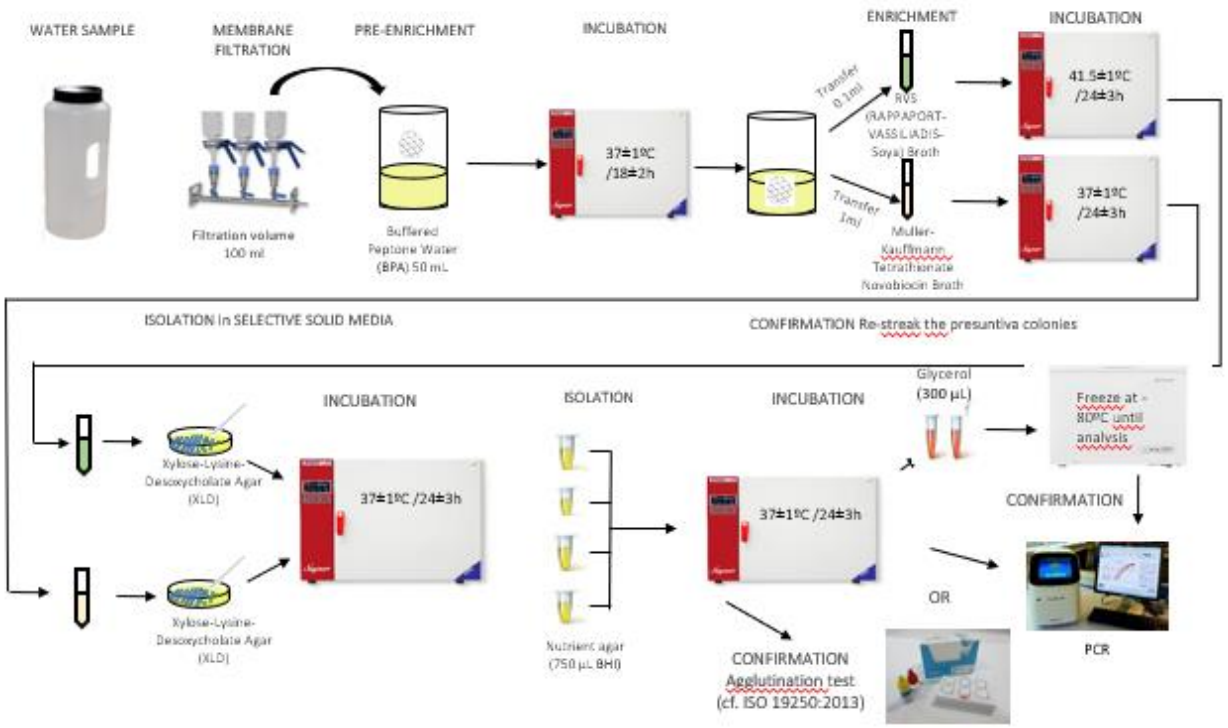


Figure 10. Scheme for the detection of *Salmonella* spp. following the ISO 19250:2010: Water quality, modified protocol.

4. Listeria* spp. and *L. monocytogenes: For the detection of *L. monocytogenes* and enumeration of *Listeria* spp. the ISO 11290-1:2017 and ISO 11290-2:2017, respectively, were followed. These methods apply to FVHs and environmental samples, and for water samples, some modifications were needed.

Detection of *L. monocytogenes* in water samples was assessed after filtration, as indicated by EFSA (2018) (**Figure 11**). Briefly, volumes of 100 mL were filtered through 0.45 µm membrane filters using a filter holder manifold (e.g., Millipore). Different filtration volumes were used when the filters collapsed, influencing the detection limit. In the comments section of each datasheet, this is indicated when it differed from this protocol. Detection was performed after the first enrichment step introduced the filter in Half Fraser Broth (e.g., Scharlau, Barcelona, Spain), incubated at 30 °C for 25 ± 1 h. Subsequently, a second selective enrichment was performed by transferring 100 µL into 10 mL of Fraser broth, followed by incubation at 37 °C for 24 h. The enriched samples were streaked onto ALOA/OCLA Listeria selective agar. The plates were incubated for 24-48 h at 37 °C before the isolation of *L. monocytogenes* as blue-green colonies with an opaque halo. Presumptive *L. monocytogenes* isolates were confirmed by UGent using the carbohydrate test according to ISO 11290-1:2017 and by IRTA and CEBAS-CSIC by using conventional PCR (e.g., Bio rad® thermal cycler system) with the primers described in **Table 12**. In the case of PCR, strains confirmed the presence of *hly* and *iap* genes for *L. monocytogenes*. Positive (e.g., *L. monocytogenes* CECT 5672 from the Spanish Culture Collection, CECT) and negative (sterile distilled water) controls were included for each PCR. Template DNA for PCR was prepared by the boiling method. The PCR products were analysed by agarose gel electrophoresis at 80 V70 min and Red-dye staining (Biotium Inc. USA). UV fluorescence emission was recorded (e.g., using ImageQuant™ LAS 500, GE Healthcare Bio-Sciences AB). Detection/non detection of *L. monocytogenes* was referred to 100 mL.

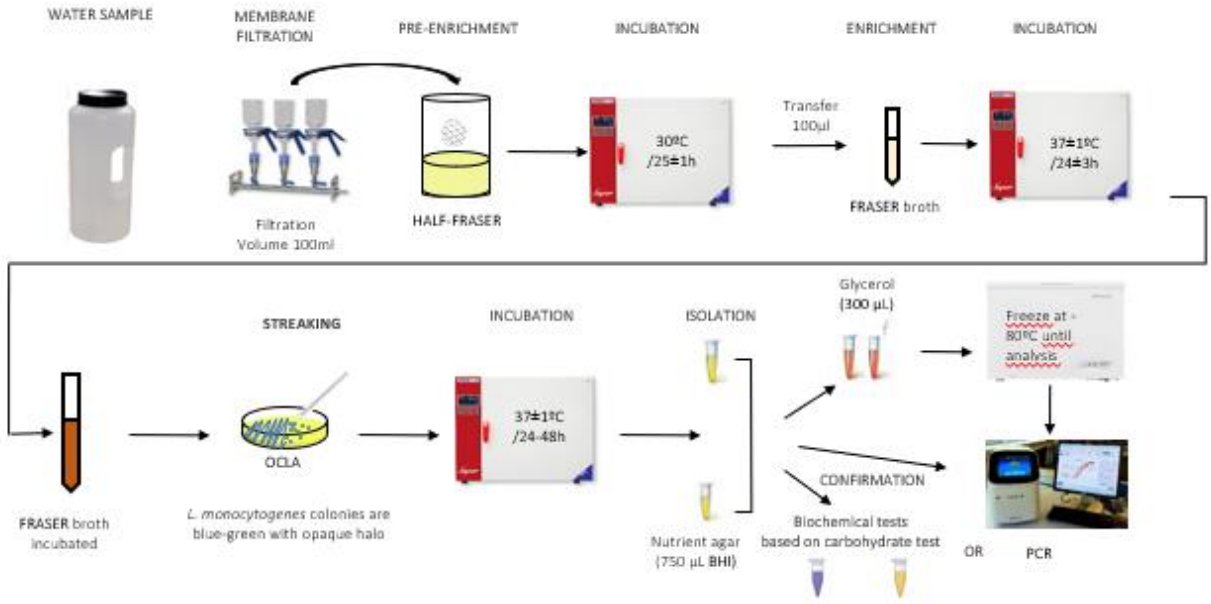


Figure 11. Scheme for the detection of *Listeria monocytogenes* following the ISO 11290-1:2017 (adapted for water samples-filter analysis) Horizontal method for the detection and enumeration of *L. monocytogenes* and *Listeria* spp. Part 1: Detection method

For the enumeration of *Listeria* spp., both the membrane filtration and the serial dilutions of water samples were conducted as indicated in **Figure 12**. One milliliter of the undiluted sample (instead of 100 µL) was streaked on several plates to obtain an increased detection, resulting in a lower LoD in the case of the UGent scenarios.

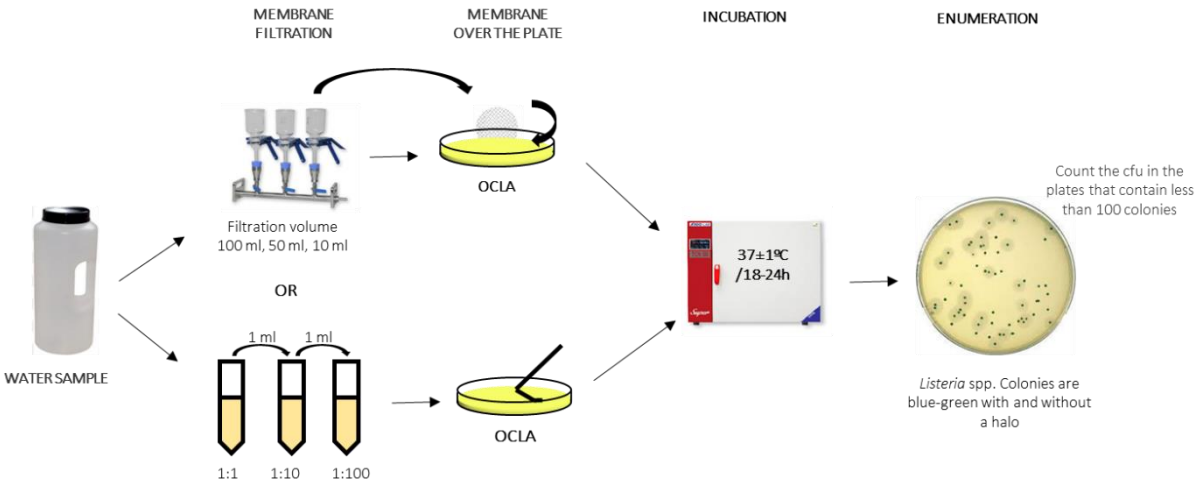


Figure 12. Scheme for the enumeration of *Listeria* spp. following the ISO 11290-2:2017 (adapted for water samples-filter analysis)

5. **Shigatoxin-producing *E. coli* and *E. coli* O157:H7:** STEC and serogroup O157 were determined following the ISO 13136:2012 with some modifications (**Figure 13**). Water samples (2 L) taken at the first five sampling times (1, 2, 3, 4, and 5) were assessed after filtration of 100 mL volumes through 0.45 µm membrane filters. At sampling point 6, a sample of 10 L was taken (twice in the case of UGent scenarios)

and filtered using the modified Moore swab (MMS) method previously validated for testing large volumes of water (Truchado et al., 2016) (**Figure 14**). As mentioned before, when different filtration volumes were used because the filters collapsed, this is indicated in the comments section of each individual datasheet. Process water was filtered through the MMS cassette containing a 91 x 95 cm^2 cheesecloth grade #90 folded as indicated by Sbodio et al. (2013). Briefly, an enrichment step was carried out by immersing either the cheesecloth or the filters in 200 mL of modified Tryptone-Soya broth supplemented with novobiocin (mTSB+N) at 37 ºC for 18 to 24 h. This medium contains several anti-microbial reagents that effectively suppress contaminating microbiota growth and non-target competitors, allowing the growth of viable O157:H7 cells (including other STEC). Selective media such as Chromagar STEC and Chromoagar O157 was used for the growth and presumptive identification of STEC and *E. coli* O157:H7, respectively. Afterward, five colonies were isolated and further confirmed. A Real-time PCR-based method for the detection of Shiga toxin producing *E. coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups was performed. DNA extraction was carried out using commercial extraction kits (e.g., Nucleo Spin Tissue. Marcherey – Nagel, Germany) following manufacturing instructions. To detect the presence of *E. coli* O157:H7 and STEC in water samples, a conventional multiplex PCR assay, targeting the five virulence factors stx1, stx2, eae and ehxA, plus the O157:H7 specific +93 uidA single nucleotide polymorphism was performed using a PCR System (e.g., Bio rad® thermal cycler) (Son et al., 2014). PCR products were separated by agarose gel electrophoresis at 85 V for 60 min. Gels were stained with 1 µg/mL red dye (Biotium Inc. USA) and visualized on a UV transilluminator (e.g., using ImageQuant™ LAS 500, GE Healthcare Bio-Sciences AB). For confirmation, if five bands in the multiplex PCR were amplified, the sample was considered positive for *E. coli* O157:H7 (**Table 12**). If positive bands were only present for stx1 or/and stx2 as well as eae genes, the sample was considered positive for STEC (Son et al., 2014).

Table 12. Detection by multiplex PCR of *Salmonella*, *L. monocytogenes* and STEC and *E. coli* O157:H7 genes, primers, sequences and size of the PCR amplicon.

Gene	Primer	Sequence	Size of PCR amplicon (bp)
Salmonella			
invA	InvA-F	ACAGTGCTCGTTTACGACCTGAAT	244
	InvA-R	AGACGACTGGTACTGATCGATAAT	
L. monocytogenes			
hly	hly-F	TAA CGA CGA TAA AGG GAC AGC AGG AC	512
	hly-R	AAT GAA TCA CGT TTT ACA GGG AGA A	
iap	iap-F	TAA AGG GAC TAC TGT TGA CG	660
	iap-R	GCT TCT GTT GGT GCT TTA GGT GCT GTT	
STEC and *E. coli* O157:H7			
stx1	stx1-F	GACTTCTCGACTGCAAAGAC	306
	stx1-R	TGTAACCGCTGTTGTACCTG	
stx2	stx2-F	CCCGGGAGTTTACGATAGAC	482
	stx2-R	ACGCAGAACTGCTCTGGATG	
eae	eae-F	GCGCGTTACATTGACTCCCG	245
	eae-R	CCATTTGCTGGGCGCTCAT	
ehxA	ehxA-F	TCTGTATCTGCGGGAGTTAG	136
	ehxA-R	CAACGTGCTCAAACATAGCC	
+93 *uidA*	uidA-F	GCGAAAACTGTGGAATTGGG	382

Gene	Primer	Sequence	Size of PCR amplicon (bp)
Salmonella			
invA	InvA-F	ACAGTGCTCGTTTACGACCTGAAT	244
	InvA-R	AGACGACTGGTACTGATCGATAAT	
L. monocytogenes			
hly	hly-F	TAA CGA CGA TAA AGG GAC AGC AGG AC	512
	hly-R	AAT GAA TCA CGT TTT ACA GGG AGA A	
iap	iap-F	TAA AGG GAC TAC TGT TGA CG	660
	iap-R	GCT TCT GTT GGT GCT TTA GGT GCT GTT	
STEC and *E. coli* O157:H7			
	uidA-R	TCGTCGGTAATCACCATTCC	

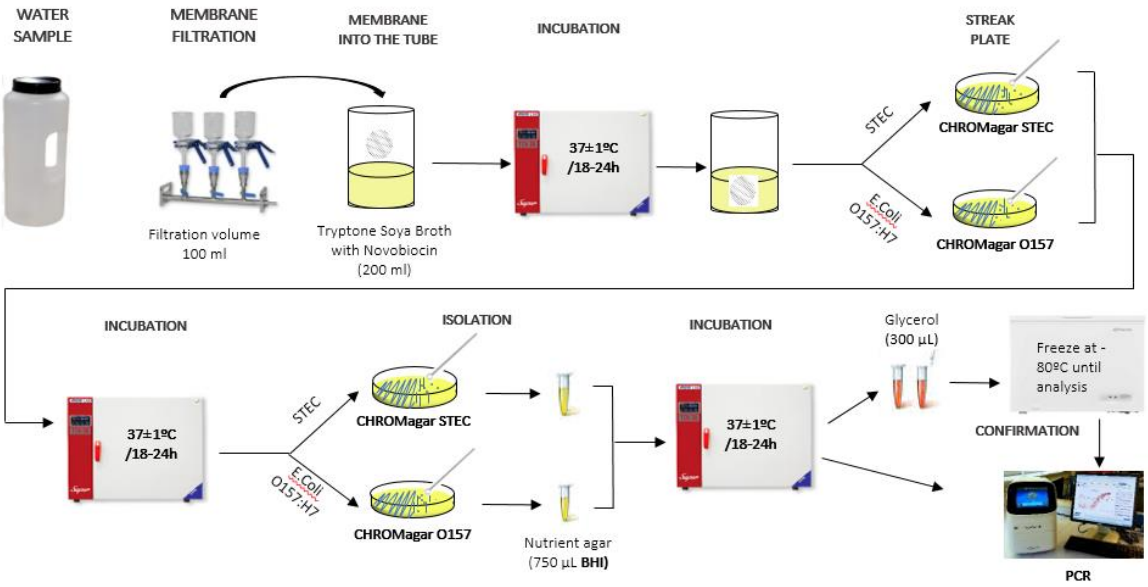


Figure 13. Scheme for the detection of STEC and *E. coli* O157:H7, following the ISO 13136:2012 (Adapted for water samples-filter analysis) for water samples taken at the sample point 1, 2, 3, 4 and 5. Detection/non detection of STEC and *E. coli* O157:H7 was referred to 100 mL

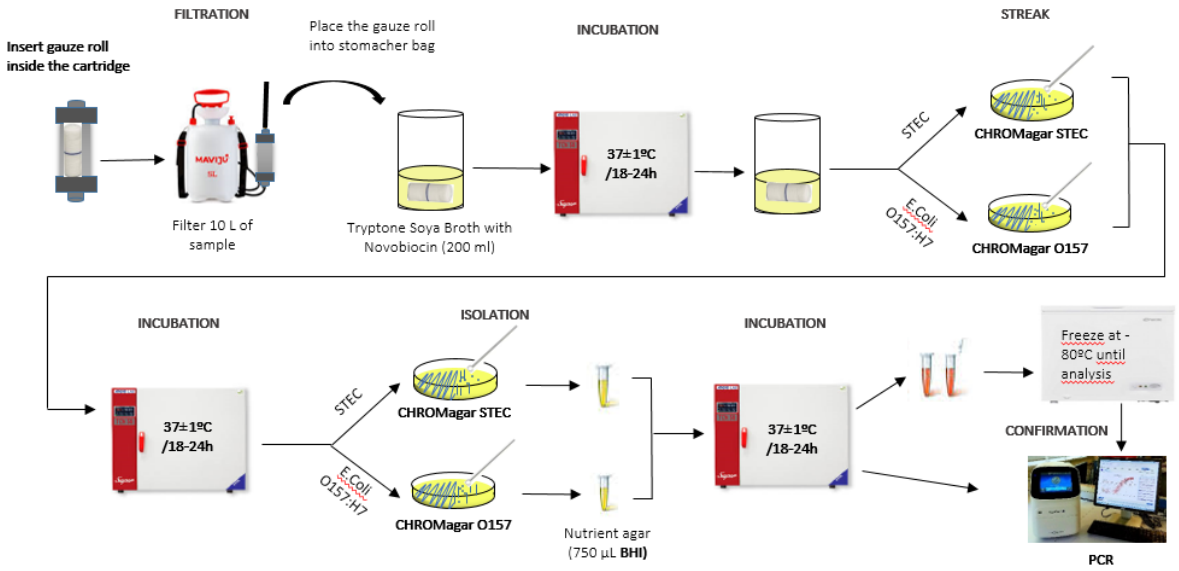


Figure 14. Scheme for the detection of STEC and *E. coli* O157, following the ISO 13136:2012 (adapted for water samples-filter analysis) for water samples taken at sample point 6. Detection/Non detection of STEC and *E. coli* O157:H7 is referred to 10 L

Detection of microbial pathogens such as noroviruses genogroup I (GI), GII and *Cryptosporidium*, as well as microbial indicators such as coliphages and *CrAssphage* was determined only at the sample point 6 in the case scenarios included in objectives 1 and 2, for which a 20 L sample was taken (twice in the case of UGent scenarios). The reason was due to the higher probability of microbiological and pathogen load over time when using the same water. The optimized protocol for water sample concentration was developed and adjusted in the framework of a project "Occurrence and accumulation of potentially infectious viruses in process water and impact of water disinfection practices to minimize viral cross-contamination" (Cuevas-Ferrando et al., 2021). Briefly, the concentration of the water sample followed a pre-treatment of the filter (step 1), the dead-end filtration (step 2), back-flush elution (step 3) and the secondary concentration (step 4) (**Figure 15** and **Figure 16**).

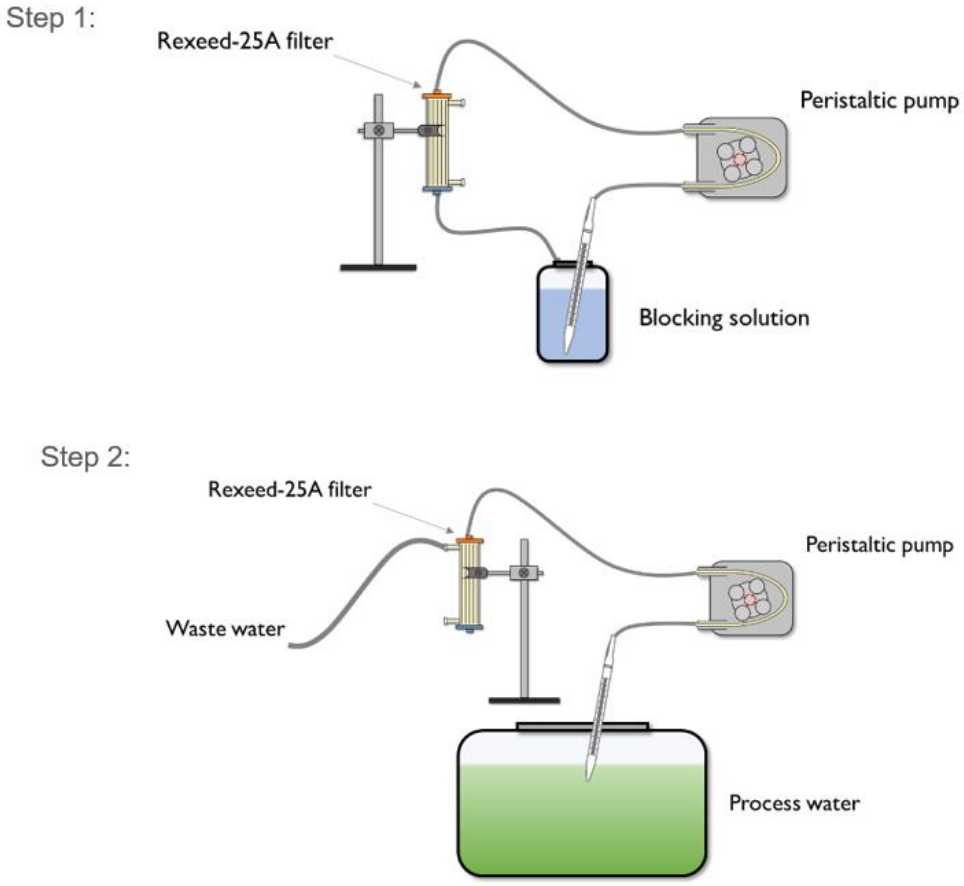


Figure 15. Pre-treatment of the Rexeed-25A filter (step 1) and dead-end filtration (step 2)

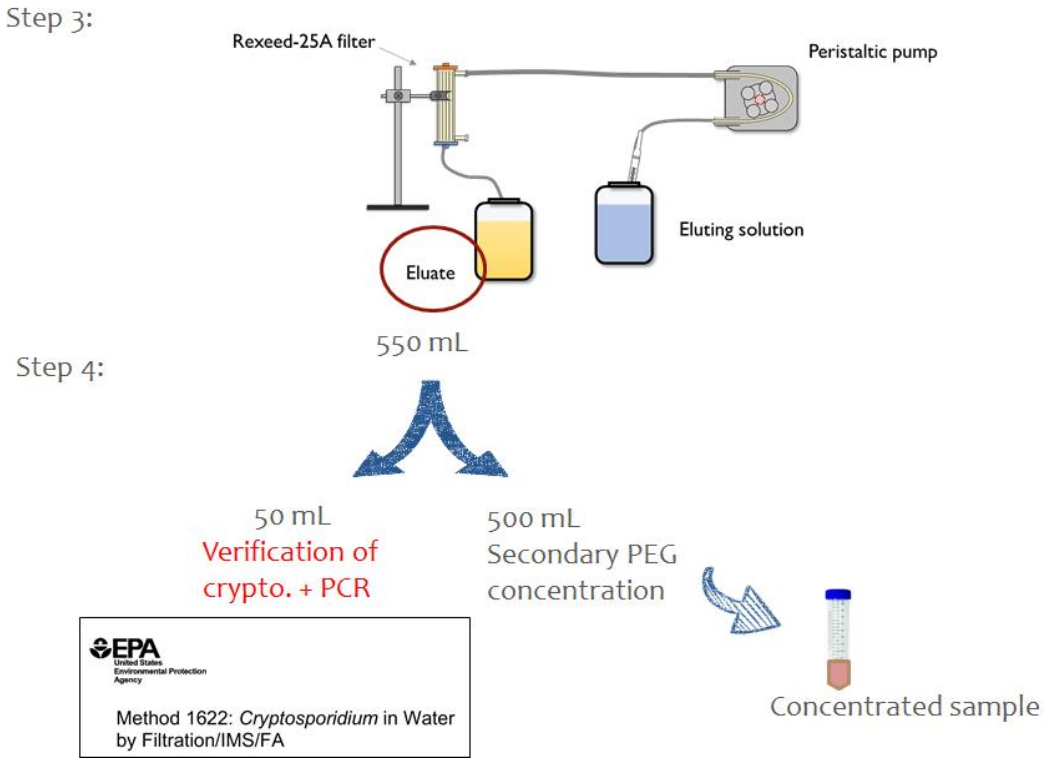


Figure 16. Back-flush elution (step 3) and secondary concentration (step 4)

Once the water sample was concentrated and eluted through the cartridge (Rexeed-25A filter), the eluted sample was precipitated with polyethylene glycol (PEG) following the protocol shown in **Figure 17**. An aliquot (1 mL) of the concentrated sample was sent to IATA-CSIC team for the analysis of norovirus, *CrAssphage* and *Cryptosporidium* and the other aliquot was cleaned for the analysis of coliphages.

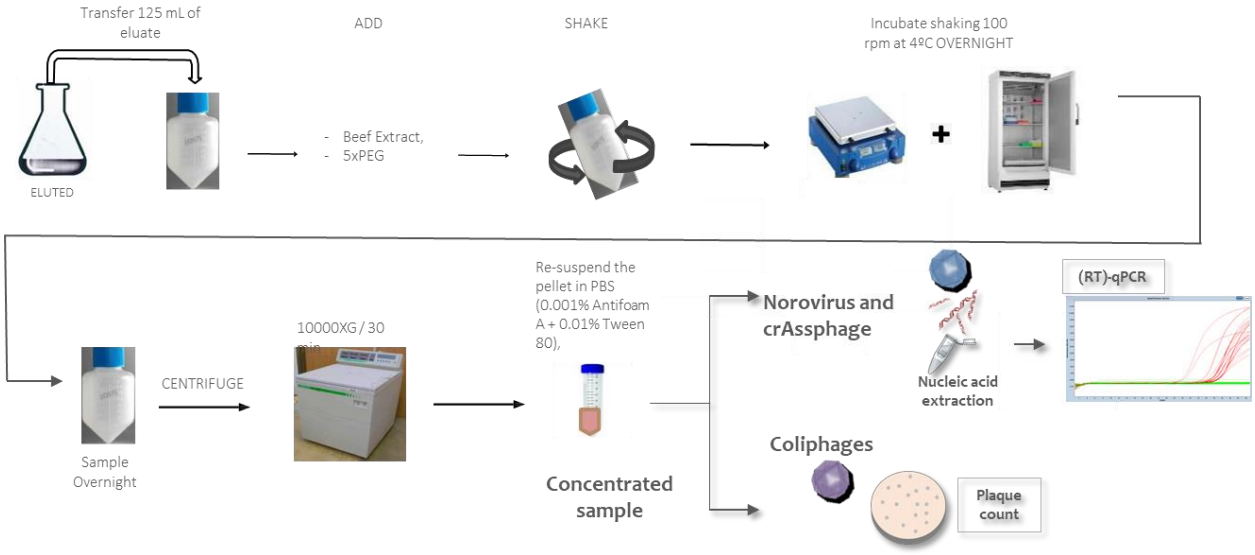


Figure 17. Protocol for the precipitation of the eluted water sample after concentration and analyses performed with the concentrated sample

The concentrated samples were received at IATA-CSIC from CEBAS-CSIC, IRTA and UGent that prepared and analysed as represented in **Figure 18**.

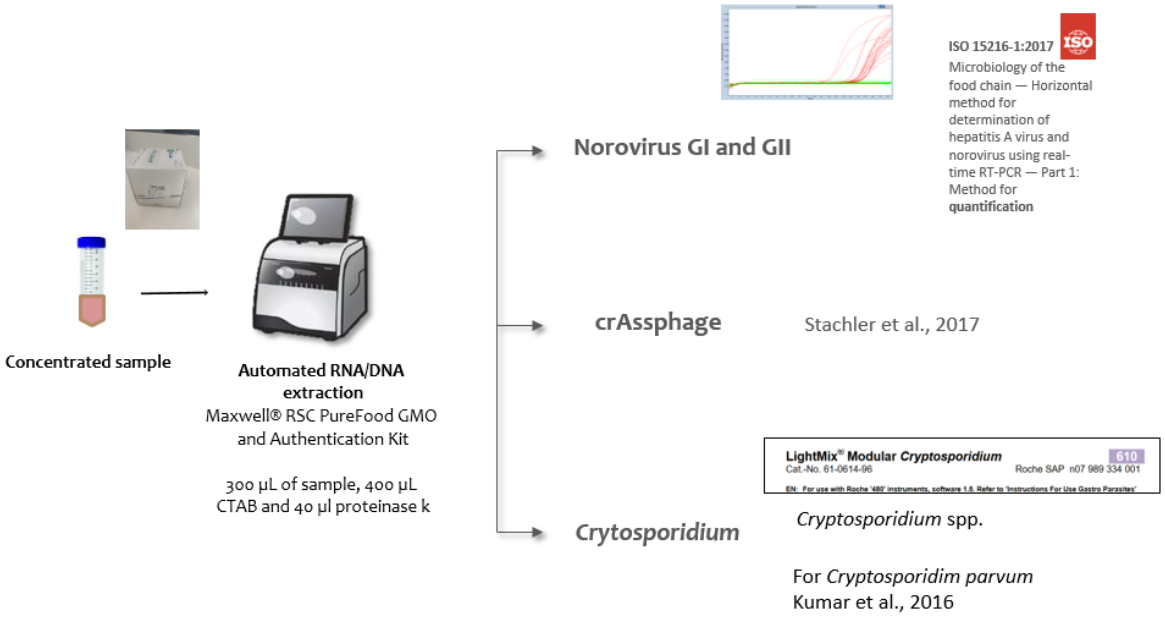


Figure 18. A summary of the extraction of RNA/DNA protocol (Cuevas-Ferrando et al., 2021) and detection and quantification methods that were followed for norovirus GI, GII, *CrAssphage* and *Cryptosporidium*

6. Norovirus: For the detection and enumeration of noroviruses GI, and GII the concentration protocol established by Cuevas-Ferrando et al. (2021) was performed combined with the RT-qPCR protocol described in ISO 15216-1:2017. Briefly, a concentration process of water samples (most of the times of 20 L) was carried out initially by a Dead End Hollow Fiber Ultrafiltration (DEUF) using single-use Asahi Kasi REXEED 25A ultrafilters (Aquavalens) (Liu et al., 2012). To recover the viruses, the filter was back flushed using 500 mL of backflush solution (0.01% Tween 80, 0.01% sodium polyphosphate, and 0.001% antifoam). The backflush volume was then concentrated using polyethylene glycol (PEG) precipitation and the final concentrate was used for the extraction of nucleic acids. The nucleic acid extraction was performed using the Maxwell® RSC PureFood GMO and Authentication Kit and the Maxwell RSC equipment (Promega). In brief, 300 µL of concentrated sample was added with 400 µL CTAB and 40 µl proteinase k (provided with the kit) and subjected to pulse-vortexing. Then, the homogenate was incubated 10 min at 60 ºC, and centrifuged for 10 min at 16000 x g. The supernatant was subsequently processed according to the manufacturer's instructions.

The presence of norovirus GI and GII was detected by RT-qPCR using the RNA UltraSense One-Step kit (Invitrogen SA) on the LightCycler® 480 instrument (e.g., Roche Diagnostics, Germany) using the primers and RT-qPCR conditions described in the ISO 15216-1:2017. Different controls were used in all assays, including negative process control consisting of PBS, whole process control to monitor the process efficiency of each sample (spiked mengovirus), and positive and negative RT-qPCR controls (Cuevas-Ferrando et al., 2021). Standard curves were determined according to the Public Health England (PHE) Reference Materials

for Microbiology for norovirus GI (batch number 0122-17), and norovirus GII (batch number 0247-17) and reported as genomic copies (GC).

Molecular detection of water-borne viruses has been utilized in the past for those viruses where a suitable culture method was not available, such as noroviruses. Quantitative PCR for DNA-based viruses (adenovirus) or RT-PCR for RNA-based viruses (is now widely available for both culturable and non-culturable viruses as an alternative to the cell culture detection methods, and these have been investigated by some researchers. The method involves the recovery of the viral particles from a water sample, extraction of the nucleic acid (DNA or RNA) and quantification of the virus present using an internal standard with PCR and specific primers to allow detection of the viruses of interest. The methods are reliant on the selection of specific primers for the virus(es) of interest, adequate optimization of the assay, and determination of non-specific amplification of other closely related viruses. The sensitivity of individual assays also requires determination to ensure low levels of viruses are detectable.

The limitation of the method is that both infectious and non-infectious virus particles are detected, possibly providing an overestimate of the public health risk. In the case of positive samples, to assess the integrity of viral capsids in norovirus GI-positive samples, a protocol based on capsid integrity with PMAxx was run in parallel. Briefly, concentrated samples were placed in DNA LoBind 1.5 mL tubes (Eppendorf, Germany), and the photoactivatable dye PMAxx™ (Biotium, USA) was added to 300 µL of each sample at a final concentration of 100 µM, along with 0.5% Triton X-100 (Thermo Fisher Scientific, Spain), and then incubated in the dark at room temperature for 10 minutes at 150 rpm. Subsequently, the samples were photoactivated for 15 minutes using a Led-Active Blue system (GenIUL, Spain), followed by a second round of incubation and photoactivation. Finally, RNA extraction and RT-qPCR detection were carried out as described above. The levels of intact capsid norovirus GI were determined by RT-qPCR after PMAxx pretreatment and RNA extraction, as described above, and reported as GC/L, indicating the levels of norovirus GI with an intact capsid.

7. Parasites: Among parasites that can cause disease in humans, protozoan parasites are the most relevant. Most studies on enteric protozoans in water focus on *Cryptosporidium* and Giardia. While Giardia is more numerous in wastewater, *Cryptosporidium* represents a greater challenge being smaller and resistant to chlorine-based disinfection. Therefore, *Cryptosporidium* was studied as the most practical pathogen to use and representative of the enteric protozoans. A concentration method as described before was carried out for water analysis. The concentrated sample was subjected to nucleic acid extraction method using the Maxwell® RSC PureFood GMO and Authentication Kit and the Maxwell RSC equipment (Promega). Generic detection of *Cryptosporidium* (*C. hominis, C. parvum, C. meleagridis, C. tyzzeri, C. wrai, C. erinace, C. cuniculus, C. ferret* and *C. viatorum*) was performed with the LightMix modular crytosporidiumkit (Roche). For positive samples, primers and probe sequences for *C. parvum* (AF188110; CrF: 5′-CGC TTC TCT AGC CTT TCA TGA-3′, CrR: 5′-CTT CAC GTG TGT TTG CCA AT-3′, *Cryptosporidium*: FAM-5′ CCA ATC ACA GAA TCA TCA GAA TCG ACT GGT ATC 3′-BHQ2) were used (Kumar et al., 2016). In the case of positive PCR, verification by immunomagnetic separation was applied to the eluted 20 L sample for *Cryptosporidium* oocyst concentration and then the oocyst counts were provided using the EPA Method 1622.

8. CrAssphage: The occurrence of *CrAssphage* as an indicator of the presence of human enteric viruses was determined by molecular techniques through qPCR Premix Ex Taq™ kit (Takara Bio Inc). Standard DNA material for *CrAssphage* standard curve generation relied on a customized gBlock gene fragment containing target sequences for *CrAssphage* (Integrated DNA Technologies, Coralville, IA). Standard DNA material for *CrAssphage* standard curve generation relied on a customized gBlock gene fragment

(Integrated DNA Technologies, Coralville, IA) containing the target sequence for CPQ_064 *CrAssphage* primers set (Stachler et al., 2017).

9. Coliphages: The occurrence of coliphages and their relationship with human enteric viruses were determined. They are split into two categories based on the route of bacterial host infection: **total coliphages** and **male-specific (F+) coliphages (F-RNA and F-DNA)**.

The analysis of total coliphages and F-specific coliphages was performed by using the host strain *E. coli* DSM 9198 (**Figure 19**) and *E. coli* CECT 5695 (**Figure 20**), respectively (Spanish Type Culture Collection) and the double-layer agar method. Levels of total coliphages, and the detection of F+ coliphages were quantified following previously published methods (Guzmán et al., 2008) with some modifications. The *E. coli* strains were grown in Luria Bertani Broth (LB, Scharlau, Barcelona, Spain) supplemented with ampicillin (100 µg/mL) at 37 °C until the log phase (optical density, OD=0.3). One mL per final concentrate obtained from each water sample (20 L) (see point 6 for noroviruses) was treated with (10 %) chloroform to disrupt all the bacteria cells and release the coliphages. Water samples were centrifuged at 2,500 × g for 10 min at 4 °C and the supernatant was filtered (0.45 µM) to remove the presence of any bacteria. For total coliphages the lower and upper layers in the double agar test using tryptone-yeast-extract glucose (TYG) agar and TYG semisolid agar were inoculated with 100 µL of supernatant from the water sample and 100 µL of *E. coli* 9198 in the log phase (OD=0.3) and supplemented with ampicillin (100 µg/mL) and calcium glucose solution according to ISO 10705-1. For F-specific coliphages, *E. coli* CECT 5695 was grown in NZCYM broth (Sigma–Aldrich, Saint Louis, MO, USA), supplemented with streptomycin (2 mg/mL; Sigma–Aldrich) at 37 °C for 18 h. As in the case of total coliphages, 1 mL of concentrate was extracted with chloroform as indicated above. For the lower and upper layers in the double agar test L-agar and Top-agar were supplemented with 1 mL of supernatant from the water sample and 500 µL of *E. coli* 5695. Plates were incubated at 37 °C for 24h and the levels of total coliphages were expressed in plaque-forming units per 100 mL (PFU/100 mL).

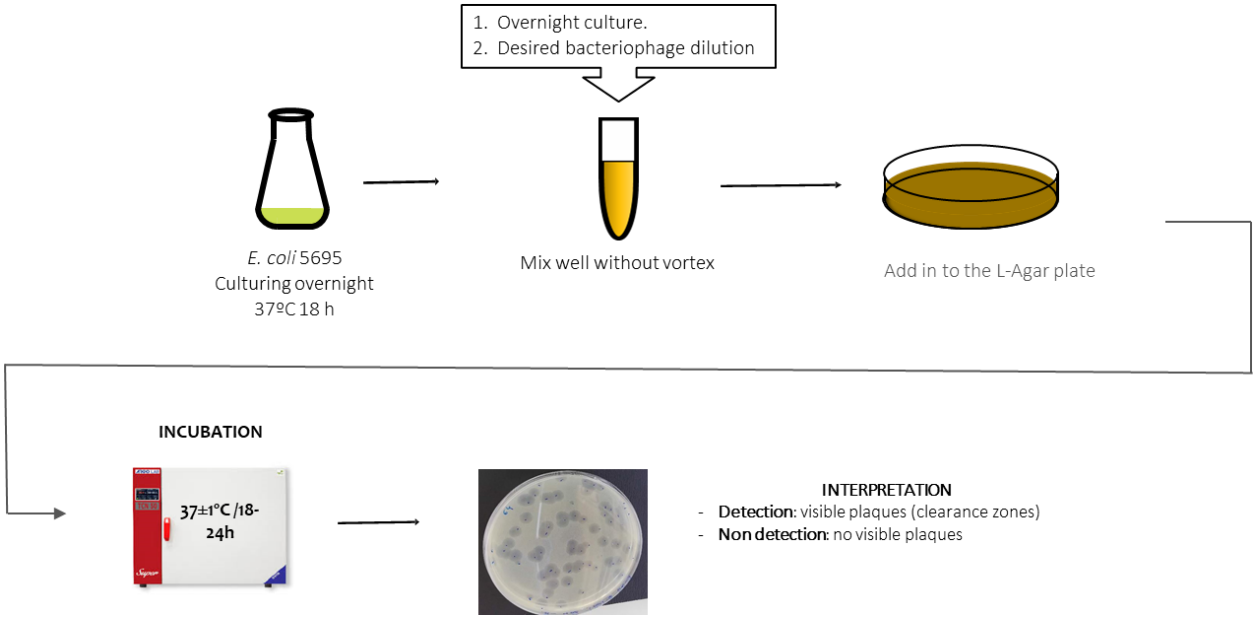


Figure 19. Detection and enumeration of F-specific coliphages with *E. coli* 5695

CEBAS-CSIC and IRTA calculated LoD from the volume of concentrate (generally 10 mL) obtained from the 20 L of process water filtered. Thus, for total coliphages, LoD was 5 PFU/L and for F-specific coliphages was 0.5 PFU/L. UGent calculated the LoD on 5 mL of concentrated coming from 500 mL of filtrate that corresponded to 8.33 PFU/L for total coliphages and 0.833 PFU/L for F-specific coliphages.

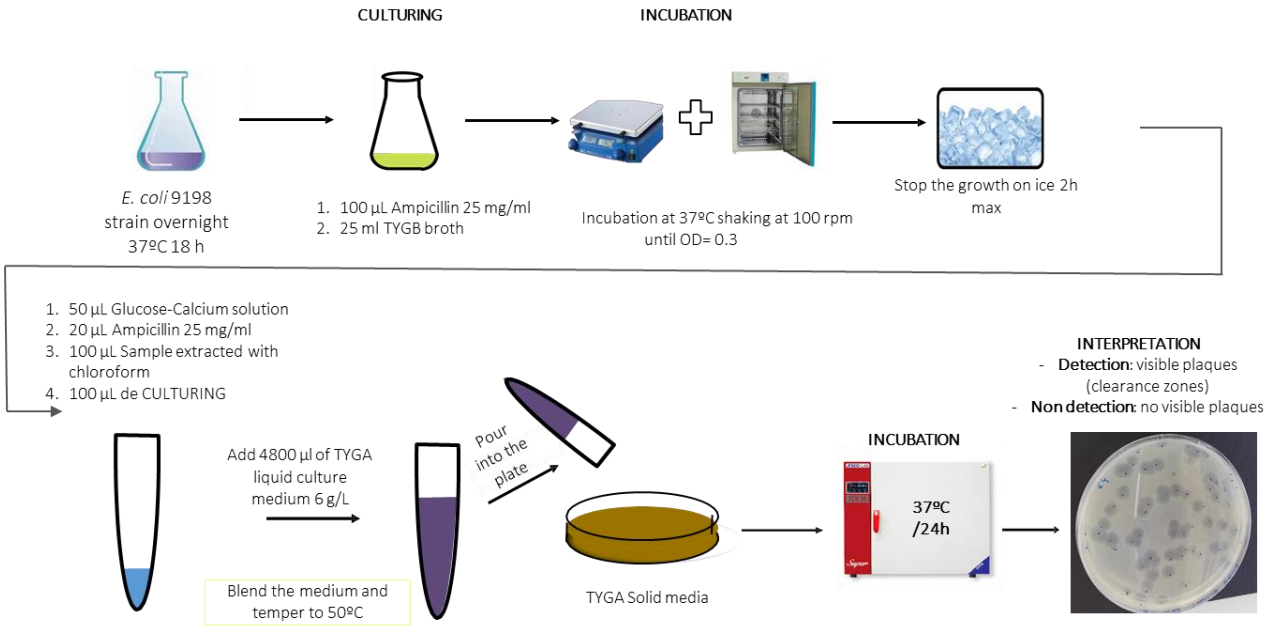


Figure 20. Detection and enumeration of total coliphages with *E. coli* 9198

An enrichment protocol was also carried out to confirm the detection/non detection of total coliphages following the protocol described in **Figure 21** (Guzmán et al., 2008).

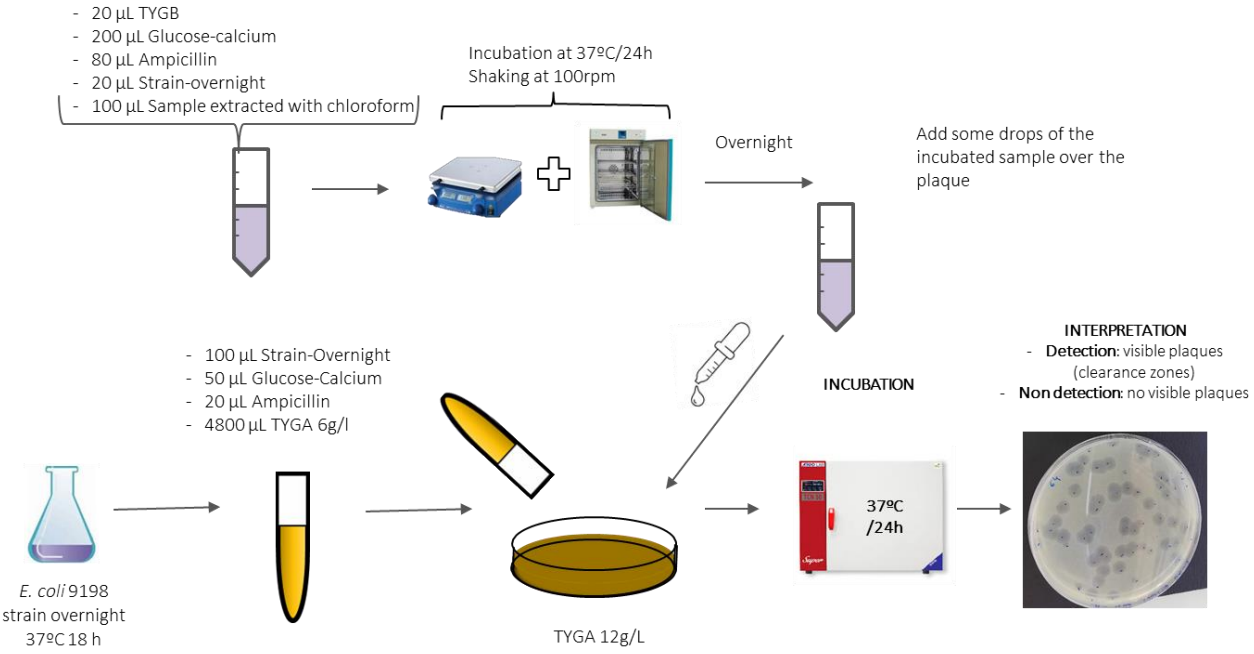


Figure 21. Enrichment protocol for the detection of total bacteriophages with *E. coli* 9198 (Guzmán et al., 2008)

10. Culturable and 'viable but non-culturable' (VBNC): VBNC bacteria were determined following the protocol optimized by Truchado et al. (2020) for processing water using viability PCR and a combination of propidium monoazide and ethidium monoazide (e.g., PMA-EMA qPCR) (**Figure 22**). These authors observed that due to the complex composition of process wash water with high organic matter content and interfering compounds, fluorescent dyes combined with flow cytometry cannot differentiate among the physiological stages of the different bacteria species. Moreover, cytometry is not a suitable methodology to distinguish between viable and dead cells in the process wash water. However, the combination of two photoreactive dyes (PMAxx and EMA) reduces the amplification of dead cells after disinfection treatments. When the results of culturable media were positive for *E. coli*, *coliforms*, and total bacteria, VBNC analyses were conducted for these microorganisms in the case scenarios mentioned in **Table 11** for water samples collected at sampling time 6.

Levels of TBC were examined as previously indicated (**Figure 8**). Viable bacteria were quantified using qPCR. Forty-five mL of water was centrifuged (3000 g, 4 ºC, 10 min). The supernatant was discarded, and the remained pellet was kept at - 20 °C until DNA genomic extraction. Levels of viable bacteria were determined using qPCR combining the two photoreactive dyes, EMA (Biotium, Hayward, CA, USA) and PMAxx™ (Biotium), an improved version of the PMA, followed by incubation at 40 °C as previously described (Truchado et al., 2020a). Briefly, both dyes were dissolved in sterile water to obtain 2 mM stock solution and stored at - 20 °C in the dark until use. Volumes of 45 mL of water were centrifuged at 3000 g for 10 min at 4 °C. After centrifugation, the supernatant was removed and the remained pellet was resuspended in phosphate-buffered saline (PBS, Sigma-Aldrich, Saint Louis, USA) at a final volume of 1000 µL supplemented with 10 µM EMA and 75 µM PMAxx. Samples were then incubated with a shaker at 200 rpm in the dark for 40 min at 40 °C. Stained samples were exposed to the blue light PMA-Lite LED photolysis (Interchim, Montluçon, France) for 15 min. Bacteria cells were concentrated by centrifugation (9000 g, 4 °C, 10 min). The supernatant was discarded and the EMA + PMAxx treated pellet kept at - 20 °C until DNA genomic extraction.

For the DNA extraction and qPCR procedure, genomic DNA was extracted using the Maxwell® RSC PureFood GMO and Authentication Kit and the Maxwell RSC equipment (Promega). The quality and concentration of DNA extracts were determined by spectrophotometric measurement at 260/ 280 nm and 260/230 nm using a NanoDrop®ND-1000 UV–Vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). qPCR and data analysis were performed using a qPCR system (e.g. QuantStudio 5, Applied Biosystems, Madrid, Spain). Primers and cycling parameters, and detection conditions were as previously described (Truchado et al., 2017).

Levels of culturable *E. coli*, *coliforms*, and total bacterial counts were evaluated by plate count as indicated previously in the protocols described. The levels of VBNC were calculated as: VBNC = viable bacteria-culturable bacteria and then Log transformed. To determine the qPCR limit of detection (LOD), standard curves of 10-fold serial dilution of DNA were examined in triplicate. The LOD was determined based on Cq of the last detectable standard. When NTC showed a signal in amplification, the calculation of LOD was performed according to the formula Cq (LOD) = Cq (NTC) – 3 (Gensberger et al., 2013). The samples with Cq values higher than Cq (LOD) were classified as non-determined and Cq values lower were classified as detected. For TBC, the limit of detection (LOD) was 1 CFU/100 mL. For total bacterial (culturable and viable) and total viable bacteria, the limit of detection was Ct = 488 CFU/100 mL.

Nine case scenarios described in **Table 11** included in Objective 2 were evaluated. One sample at point time 6 was analysed following the described protocol.

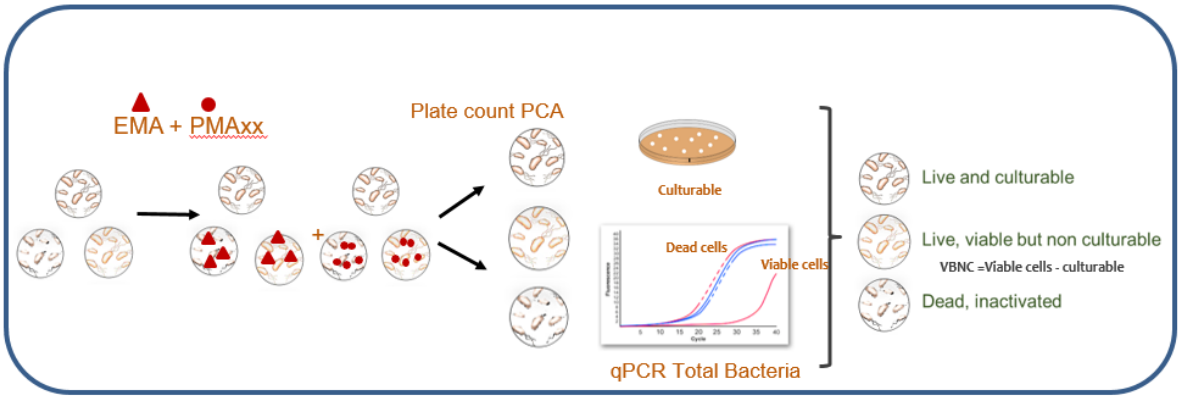


Figure 22. Diagram for the detection and enumeration of VBNC for total bacterial counts

11. *Clostridium perfringens* spores: *C. perfringens* spores were examined following the protocol established in the ISO 14189: 2013 (reviewed and confirmed in 2019) standard, with some modifications (Truchado et al., 2021a). Tryptose Sulfite Cyclosserine (TSC; Oxoid, Basingstoke, UK) agar and fluorescent supplement (TSCF; Oxoid) were used according to the manufacturer's instructions. To enumerate the spores, aliquots (100 mL) of water samples were heated at 60 °C ± 2 for 15 min. Afterward, samples were filtered through 0.45 µm membrane filters (Sartorius) using a filter holder manifold (Millipore). The filters were placed on the TSCF agar plate. The plates were anaerobically incubated under a CO_2 atmosphere (Anaero- Pack® system, Oxoid.) in anaerobic jars at 44 °C for 24 h. After incubation, plates were examined under a UV light lamp. Black and light brown colonies were counted as positive colonies when emitted fluorescence. Results were expressed in CFU/100 mL. In all water samples, the limit of detection for *C. perfringens* spores was 1 CFU/100 mL and the minimum enumeration was 10 CFU/plate.

2.2.4 Literature review

The steps followed to obtain relevant papers for objectives 3 and 4 are indicated in the sections below.

2.2.4.1 Documenting the methodology used

The following research questions (RQs) were defined:

- RQ1. For objective 3.1: which data and models are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches.
  - RQ1a: which data are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches.
  - RQ1b: which models are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches.
- RQ2. for objective 4.1: which microbiological and physico-chemical parameters or methods and models are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs?
  - RQ2a: which data on microbiological and physico-chemical parameters and methods are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs?
  - RQ 2b: which models on microbiological and physico-chemical parameters are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs?
  - RQ2c. which inline/online monitoring systems are available to validate/verify and/or monitor relevant parameters related to the microbiological quality of the process water used for ffFVHs?

Inclusion criteria were defined for each research question to narrow down the focus of our literature search:

- RQ1. papers should include information (data or models) on **microbial load** in **processing water** used for **ffFVHs**.
- RQ2. papers should include information (data, methods or models) on a combination of **microbial load and physico-chemical parameters** in **processing water** used for **ffFVHs**.

Exclusion criteria for Tier 1 (see 2.2.2.3) on Title/Keywords/Abstract:

RQ1:

- papers describing only chemical or physical hazards,
- papers describing only microbial contamination in FVHs (not in the process water),
- papers not written in English,
- papers not focusing on fresh or frozen FVHs, e.g. canned FVHs,
- papers not describing fruits, vegetables or herbs.

RQ2. Additional to the exclusion criteria mentioned for RQ1:

- papers describing detection methods that are only tested at lab scale.
- Exclusion criteria for Tier 2 (see 2.2.2.3) on full texts:

- papers discussing irrigation water or drinking water/tap water.

Demarcation:

Only papers published after 2009 were included. In order to answer RQ2c, inline systems/methodologies are defined as systems or methodologies that can be placed in a process vessel or flowing material to conduct the analysis. For RQ2c, also online methodologies are included which are defined as devices that can be connected to a process and conduct automatic sampling, for example, via a bypass. Offline and at-line methodologies are characterized by manual sampling followed by discontinuous analysis. The latter two methodologies were out of scope for this literature review.

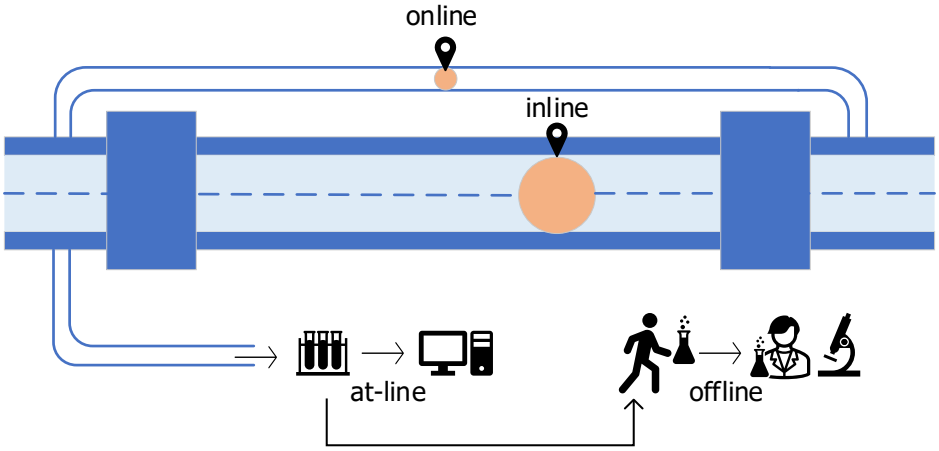


Figure 23. Visualization of the differences between inline, online, offline and at-line systems/methodologies for monitoring

Differences between the location of the monitoring systems/methodologies are shown in **Figure 23**, in which both the inline and online systems are included in the process control. The inline monitoring system is placed in a process vessel or stream of water flow to conduct the analysis while the online monitoring systems are connected to a process and conduct automatic sampling. On the contrary, the offline and at-line systems are the ones characterized by manual sampling and measurement, where samples are taken at the line and manually measured in the vicinity of the line (at-line) or taken to the lab for further analysis (offline).

2.2.4.2 Search terms to obtain relevant papers

For each of the research questions, search terms were derived to describe microbial hazards (#1), process water (#2), water activities (#2a), ffFVHs (#3), mathematical models (#4), non-microbiological parameters or methods (#5) Online and/or inline methods (#6):

1. Microbiological hazards:

pathogen* OR "microbi* hazard*" OR bacteria* OR microbial* OR pathogen* OR total bacterial counts* or TC* or "viable but non-culturable*" or VBNC OR streptococcus OR "listeria monocytogenes" OR "l. monocytogenes" OR *virus* OR bacillus OR salmonella OR clostridium OR staphylococ* OR campylobacter

OR "Escherichia coli" OR "E. coli" OR STEC OR yersinia OR shigella OR viral or surrogate* or NoV or HAV or HEV or MuNoV or MNV or Tulane* or MS2 or Mengo* OR FCV OR *calici* OR "microbial load" OR "microorganism count" OR *phage* OR O157 OR O104 OR "O:157" OR "O:104" OR "Shiga toxin*" OR Enterococ* OR VTEC OR EHEC OR Enterobacteriaceae OR coliform* OR EPEC OR parasite* or cryptosporidium or giardia or Cyclospora or C*rAssphage*

2. Process water:

"wash water" OR "wash-water" OR *washing OR "proces* water" OR "water quality" OR "wash* process" OR "tap water" OR "municipal water" OR "wash solution" OR "industrial water"

2a. Water activities:

post-harvest OR processing OR "wash* tank" OR cooling OR hydrocooling OR hydro-cooling OR blanching OR *sorting OR "dump* tank" OR "Water transport" OR drencher OR reused OR recirculated OR "flume tank" OR "produce wash*"

3. Fruits/vegetables/herbs (FVHs):

"mixed fruit*" OR "mixed vegetable*" OR "fresh produce" OR "fresh-cut produce" OR *fruit OR *berry OR *berries OR açai OR currant* OR grape OR citrus OR citron OR grapefruit OR lemon OR lime OR mandarin* OR orange OR tangerine OR *apple OR hawthorn OR loquat OR medlar OR pear OR quince OR apricot OR plum OR prune OR cherr* OR nectarine OR peach OR "Asian palmyra palm" OR avocado OR bael OR canistel OR coconut OR durian OR guava OR fig OR jujube OR kiwi OR langsat OR longan OR longkong OR lychee OR mafai OR mango* OR maprang OR papaya OR persimmon OR pitaya OR pomegranate OR rambutan OR roselle OR santol OR sapodilla OR soursop OR tamarind OR *melon OR cantaloupe OR honeydew OR galia OR "fruit* vegetable*" OR tomato* OR aubergine* OR eggplant* OR egg*plant OR pepper* OR courgette* OR zucchini* OR cucumber* OR cucurbit* OR gourd* OR pumpkin* OR squash* OR kabocha OR hokkaido OR tinda OR chilli* OR chili* OR okra OR *bean* OR *pea* OR "sweet corn" OR "leafy vegetable*" OR "green vegetable*" OR "mixed vegetable*" OR salad* OR arugula OR rucola OR "rocket lea*" OR "garden rocket" OR bitterleaf OR choy OR choi OR cabbage OR celery OR celtuce OR escarole* OR spinach OR chard OR chicory OR "mustard green*" OR "leafy green*" OR "collard green*" OR "beet green*" OR "microgreen*" OR "turnip green*" OR *cress OR endive OR epazote OR kale OR komatsuna OR lettuce OR mizuna OR mustard OR radicchio OR rapini OR tatsoi OR "turnip top*" OR "Chinese mallow" OR chickweed OR chaya OR "chrysanthemum green*" OR "fat hen" OR "fluted pumpkin" OR samphire OR "Greater plantain" OR "broadleaf plantain" OR "jute plant" OR karkalla OR "Lagos bologi" OR orache OR purslane OR sculpit OR stridolo OR soko OR "spleen amaranth" OR "brussel sprout*" OR carrot* OR arracacha OR "bamboo shoot*" OR beet* OR burdock OR chufa OR daikon OR *radish OR ginger OR turmeric OR gobo OR "hamburg parsley" OR horseradish OR *artichoke OR jicama OR mooli OR parsnip OR turnip OR salsify OR scorzonera OR skirret OR swede OR rutabaga OR "tiger nut*" OR tigernut OR ulluc* OR "water chesnut" OR wasabi OR yacón OR yacon OR asparagus OR cardoon OR celer* OR garlic OR kohlrabi OR kurrat OR keek OR "lotus root" OR nopal OR onion OR shallot OR *onion OR rhubarb OR "pie plant" OR samphire OR "bulb vegetable*" OR "stem vegetable*" OR "tuber vegetable*" OR "root vegetable*" OR "underground vegetable*" OR brocco* OR cauliflower* OR salad OR choi OR choy OR artichoke OR "courgette flower" OR "squash blossom" OR sprout* OR alfalfa OR "basil cress" OR "borage cress" OR mushroom* OR agaricus OR agrimonia OR agrocybe OR auricularia OR boletus OR clitocybe OR coprinus OR cortinarius OR craterellus OR flammulina OR ganoderma OR grifola OR gyromitra OR hericium OR hydnum OR hypsizygus OR lactarius OR lentinula OR lentinus OR lepista OR morchella OR pholiota OR pleurotus OR rhizopus OR sparassis OR stropharia OR terfezia OR tremella OR tricholoma OR tuber OR

ustilago OR volvariella OR agaric OR agarikusutake OR "Callampa Agaricus" OR champignon* OR "Cogumelo do Sol" OR kawariharatake OR himematsutake OR cremini* OR portobello* OR matsutake OR "velvet pipoppini" OR "jew's ear*" OR "jelly ear*" OR porcini OR cèpe* OR "shaggy mane*" OR "lawyer's wig*" OR "cortinar webcap*" OR "trompette du mort" OR enoki OR lingzhi OR "hen-of-the-woods" OR maitake* OR "monkey's head*" OR "lion's mane*" OR "bear's head*" OR "hedgehog mushroom*" OR shimeji OR "indigo milk cap*" OR "candy cap*" OR "saffron milk cap" OR shiitake* OR "wood blewit*" OR morel* OR nameko OR "oyster mushroom*" OR "cauliflower mushroom*" OR roundhead* OR truffle* OR "paddy straw mushroom*" OR chanterelle* OR basil OR chervil OR chives OR cilantro OR coriander OR dill OR "lemon verbena" OR marjoram OR *mint OR oregano OR parsley OR rosemary OR sage OR savoury OR savory OR sorrel OR tarragon OR thyme OR "bay lea*" OR "Ocimum basilicum" OR "Anthriscus cerefolium" OR "Coriandrum sativum" OR "Anethum graveolens" OR "Aloysia citrodora" OR "Origanum majorana" OR "Mentha spicata" OR "Mentha piperita" OR "Origanum vulgare" OR "Petroselinum crispum" OR "Salvia rosmarinus" OR "Salvia officinalis" OR "Satureja hortensis" OR "Rumex acetosa" OR "Artemisia dracunculus" OR "Thymus vulgaris" OR "Laurus nobilis"

3a. Type of ffFVHs:

fresh OR frozen OR whole OR fresh-cut OR ready-to-eat OR cut OR diced OR sliced OR chopped OR shredded OR "minimally processed"

4. Mathematical models:

"math* model" OR "mathematical description" OR dynamic* OR "kinetic model*"OR model* OR "model-based" OR "primary model" OR "secondary model" OR "equation*"OR "function*" OR "predictive microbiology" OR predict* OR regression OR correlation OR simulat* OR relationship OR distribution OR fitting OR calibration OR "Risk Assessment" OR "differential equation" OR EasyFit OR MicroHibro OR Combase OR Matlab OR Comsol OR Octave OR python OR Julia OR "R software" or "R package" or "Rstudio" or "package of R" or "R Core Team" OR NetLogo OR Bioinactivation OR "Microsoft excel" OR code OR "rate valu" OR "rate constant" OR "transfer constant" OR "inactivation*"

5. Non-microbiological parameters or methods:

"physicochemical" OR acidity OR "chloride ion concentration" OR COD OR "chemical oxygen demand" OR "dissolved oxygen" OR "electrical conductivity" OR "five-day biochemical oxygen" OR "oxidation reduction potential" OR ORP OR "redox potential" OR pH OR salinity OR turbidity OR "total alkalinity" OR "total dissolved solid*" OR TDS OR "total suspended solid*" OR TSS OR "UV absorbance" OR "water temperature" OR disinfectant* OR sanitizer* OR residue* OR "peracetic acid*" OR "peroxyacetic acid*" OR PAA OR chlorin* OR "hydrogen peroxide" OR "sodium hypochlorite*" OR "calcium hypochlorite*"

6. Online and/or Inline methodologies:

"in line" OR inline OR online OR "on line OR" automat* OR detection OR method* OR monitor* OR sensor* OR instrument* OR application* OR measurement OR "NIR spectroscopy" OR amperometr* OR "uv/vis spectro*" OR "Ultraviolet/visual spectro" OR "rapid monitoring" OR reflectometr* OR chronoamperometr* OR photometr* OR spectrophotometr* OR spectroscopy

In order to answer RQ1, the search terms for 1, 2 and 3 were combined to find relevant data on microbial load in process water. Additionally, search terms for 4 were added to find relevant models describing the microbial load in the processing water. In order to answer RQ2, the search terms for 1, 2, 3 and 5 were combined such to find relevant data on relationships between physico-chemical properties and microbial load in processing water as well as methods for verifying or validating the microbial quality of the water.

Additionally, search terms for 4 were added to find relevant models describing this relationship. Finally, for RQ2c, the search terms for 1 or 5 were combined with the search terms for 2, 3 and 6 to find online or inline methods available to determine the microbial load in the processing water. Benchmark papers were used to check whether the combinations of search terms could retrieve relevant papers (Appendix A).

The final combinations of search terms are included in Appendix B.

Two databases were used to obtain relevant papers, namely Scopus and Web of Science. Apart from the scientific literature, grey literature using Google advanced search was searched on the websites from AESAN (selected as Spain is the largest fruit and vegetable producer in Europe) and ANSES (selected as France is the second largest vegetable producer). Additionally, the websites of UK FSA, BfR, WHO, FAO and US FDA were explored since these websites usually include relevant reports on food safety. Since only a limited number of search terms can be used in Google Advanced Search, the following terms were defined per RQ:

RQ1: ("microbial hazards" pathogen) AND ("processing water" OR "wash water") AND (fruit OR vegetable OR herb OR "fresh produce") site:<website>

RQ2: ("microbial hazards" OR pathogen) AND ("processing water" OR "wash water") AND (fruit OR vegetable OR herb OR "fresh produce") AND (physicochemical OR "physico-chemical") site:<website>

RQ2a: ("microbial hazards" OR pathogen) AND ("processing water" OR "wash water") AND (fruit OR vegetable OR herb OR "fresh produce") AND (physicochemical OR "physico-chemical") AND ("verification" OR "validation" OR "monitoring")

RQ2c: ("microbial hazards" OR pathogen) AND ("processing water" OR "wash water") AND (fruit OR vegetable OR herb OR "fresh produce") AND ("line monitoring") site:<website>

2.2.4.3 Selecting relevant papers in Endnote

All papers obtained in Scopus and Web of Science were included in Endnote files. Separate Endnote files were used for the literature searches described in section 2.2.2.2. Duplicate references were removed, after which a two-tier approach was applied to select relevant studies for this research:

1. Screening on title, keywords and abstract (Tier 1)

Using the inclusion and exclusion criteria as indicated in section 2.2.2.1, papers were screened for their relevance for this research based on title, keywords and abstract. Papers were then classified as: relevant and non-relevant. 'Relevant' papers underwent full text screening in Tier 2.

Ten percent of the classifications performed in the Endnote files were checked by a second reviewer. A minimum agreement of 70% is required to proceed to tier 2. Discrepancies in outcomes were discussed between the two reviewers and the evaluation adapted accordingly.

2. Screening on full texts (Tier 2)

Papers classified as relevant, were read in full. Based on the exclusion criteria indicated in section 2.2.2.1 papers were selected for data collection.

2.2.4.4 Collecting data from included studies

An Excel file was drafted for each of the RQs separately. Data included in each of the Excel files for the different RQs are indicated in Appendix C. A two-step approach was used in which information of the relevant papers obtained in tier 2 of the literature screening (see section 2.2.2.3) was summarized using

the columns indicated in each of the Excel files created for each RQs (Appendix C). For each paper, 1 row of information was added. The sheet was evaluated internally and discussed with IMM-CSIC to derive papers that were relevant to include in the modelling. Those papers were compared by the EFSA WG to the papers selected by the EFSA WG members. The EFSA WG made a selection considering possible duplicates as well as the relevance of the modelling. The relevant information was extracted from selected papers and included using the EFSA WG overall template Excel file (up till column BJ as indicated in Annex C) in a second sheet. This second sheet contained several rows per paper to include all relevant information (e.g. pathogen sampled, time step, concentration etc). Data from the figures were extracted using a specific tool, i.e. WebPlotDigitizer (Rohatgi, 2021). For the data extraction from literature, we used CFU/ml as unit for bacterial counts. As specified in the contract, the maximum number of papers used for evaluation did not exceed the number of 300 for each of objectives 3 and 4 included in the literature research.

Information related to modelling the microbial load was extracted separately due to the difficulties including many equations in Word. Gathered models were included in tables using latex and the final tables were included in the document as images.

2.2.5 Modelling

In this tender, we define "model" as any mathematical expression providing as output a dynamic (time dependent) simulation of, at least, one variable describing the microbiological contamination. Possible outputs are, for example, numerical values of concentrations of total bacterial counts (TBC) or *E. coli* at different times.

The most interesting models are those relating microbial contamination with physico-chemical properties that can normally be measured inline. Some examples are the concentration of disinfectant, TDS, turbidity, TSS, COD, UV absorbance, EC, ORP, pH or water temperature.

Model building requires several analyses, not only to reproduce the behaviour of the variables of interest but to infer interesting parameters with low uncertainty (confident/reliable parameters), and to reproduce experimental data not used for the calibration of the model (confident model predictions). For this aim, the IIM-CSIC partner has developed an identifiability protocol for models in the food industry (García 2008, Balsa et al., 2016a, Vilas et al., 2018) that requires several steps and the use of analytical and numerical methods implemented in freely available toolboxes in Matlab: GenSII for detecting models that are not structurally identifiable (Ligon et al., 2018) and AMIGO2 for other analysis requiring numerical calculations (Balsa et al., 2016b). The used methods in this study can be outlined in the next steps:

1. Find a general model to derive the different alternative equations depending on the considered assumptions and experiments. The models are considered relevant if there are relationships, mathematical formulae, between the inputs (physico-chemical properties) and the model outputs (microbial contamination). When the mechanisms of the process are known, models can be obtained based on first principles such as mass balance conservation and are usually expressed in Ordinary Differential Equations (ODEs). These mechanistic models are preferred over empirical models because are valid even when changing the operational conditions (for example changing the amount of process product) or even assumptions without any further re-calibration. Only when mechanisms are not fully quantitatively known, empirical equations are used. An example is to use a linear interpolation between different measured free chlorine concentrations at different times, because these dynamics cannot be estimated without knowing the amount of Total Chorine added or the chlorine demand. To find the resulting mathematical relationship based on the considered assumption there are some sub-steps:
    a. Find available mathematical models: Retrieve and study of available models in the literature describing relevant dynamics such as the model simulating pathogens concentration during washing by Abnavi et. al. (2021). Articles with models with at least one of the outputs of microbial contamination, previously described, will be read and examined to build a battery of models. Analyse the literature models to find the formulas to simulate each of the relevant mechanisms, such as microbial inactivation with disinfectants.
    b. Analyse the usual included mechanisms and find a general framework where all these relationships can be included. The term general is used as the framework allows describing the mechanisms without necessarily describing any specific formula for each term.
2. Test the different alternative models using data in the literature to find which are the relevant mechanisms. Whereas in previous point the purpose was to build a very general model including any possible mechanism in the literature, the objective now is to find which of the possible alternatives are relevant depending on the purpose of the model. To test the models there is the need to estimate those parameters that are unknown (parameter estimation/model calibration)

from literature data. An example is to use microbial contamination data to infer the inactivation rate constant for certain microorganism and disinfectant. These parameters may have different values depending on the washing operation (design and control), the washed product (type and industrial sector) or the pathogen. Several non-sequential sub-steps for this parameter estimation were used (see García (2008), Balsa et al., (2016a), Vilas et al., (2018), for details and references for the different methods):

a. Analyse if the model is structurally identifiable, i.e. assuming there is non-noise and rich experimental information, there is only one possible value for the parameters. In other words, the analysis detects if using one set of parameters or another would result in the same result but provide different predictions (simulations outside of the experimental data used). For example, it was detected that only a relationship between dilution rate and contamination rate is identifiably when experiments (measured contamination, product mass and tank volume) are dynamic. Thus, the models will be reformulated or reduced, or some parameters assumed to avoid these problems.
b. Estimate the parameter values from either data from the literature or measured in FBOs by maximising the log-likelihood (equivalent to minimising the least squares error when variance/uncertainty of the data is constant). The problem to be solved to estimate the parameters was complex with several possible local solutions, and therefore the Enhanced Scatter Search optimiser (Egea, 2007) (combining local and global estimations) was selected and run several hours for each of the visits and cases, repeating the procedure to assure that the global optimum was obtained.
c. Different practical identifiability analysis such as:
    i. Calculate the parameter confidence (uncertainty) using Cramér-Rao and assuming a measured error of log (CFU/100mL)±0.5 for bacteria and (ppm)±10 for COD. The confidence intervals represent the uncertainty due to data error. When the intervals are too uncertain there is what is named a "practical identifiability" problem, requiring for example the reformulation of the model or to assume values for some of the parameters.
    ii. Calculate the Akaike Information Criteria (AIC) to discern among different models based on the balance between calibration performance and model complexity.

3. Based on the relevant mechanisms found in point 1 and 2, build a simple model useful to infer relevant parameters in different industrial cases. After the model is derived, the methodology is similar to the steps described in point 2.

3 Results

3.1 Summary description of case scenarios

The results of the analyses obtained for each case scenario during the two visits to each FBO are included in the Excel file 'data' considered the master file that contains all the data collected. The data are presented in the Excel file classified per: (i) food category (fresh-whole FVHs, fresh-cut FVHs and frozen FVHs), (ii) with or without disinfectant, (iii) food group (fruits, vegetable fruits, bulbs and roots, leafy greens, and fruits/vegetables/root/bulbs), (iv) specific food product (apples, pears, peaches/nectarines, cherries, avocado, mango, tomatoes/cucumbers, peppers, diced peppers, onions, diced onions, carrots, carrot sticks, shredded carrots, fruit mix, vegetable mix, celery, fresh-cut lettuce, shredded lettuce, curly endive and radicchio, baby leaves, spinach, parsley, chives, salad mix, salad mix with carrots), (v) disinfectant agent

(no water treatment, sodium hypochlorite, calcium hypochlorite, calcium and sodium_hypochlorite, chlorine gas and sodium hypochlorite, electrolysed water, PAA, and hydrogen peroxide), and (vi) processing operation (dumping, pre-sorting, pre-washing, cooling, hydro-cooling, water transport, and washing).

The results of the case scenarios mentioned in Tables 3, 4 and 5 are presented in this section in individual datasheets that are classified per food sector including fresh-whole FVHs (case scenarios 01 to 29), fresh-cut FVHs (case scenarios 30 to 48) and frozen FVHs (case scenarios 49 to 61). In the datasheets, the characteristics of the operation in which the process water was sampled for each processing line are detailed. The datasheets include a description of the process and the production flow. The processing operation where the water was sampled is marked with an asterisk (*) in the flow chart describing the process and a picture of the process operation where the water was sampled is included. In some scenarios, the FBO did not allow the inclusion of photos. Additionally, per scenario, three tables with the summary of the microbiological results are included. The first table shows the microbiological results per sampling visit (visit 1 and visit 2) for the enumeration of moulds, yeast, total bacteria, coliforms, *E. coli* and *Listeria* spp. For the enumeration results, the average counts presented are the geometric means of the positive enumeration results. The mean values were calculated by transforming the enumeration data in the Excel file 'data' from CFU/mL to CFU/100 mL, removing results below LoD, converting the remaining results to log CFU/100 mL (as $\log_{10}$) and calculating the average log for per sampling visit in each scenario. The LoD for each microbiological parameter is included in each scenario datasheet providing all details about differences due to specific a) consortium partner, b) microbiological parameter, or c) sample volume filtered. In the same table, another column indicates the occurrence results calculated as the number of samples presenting countable results out of the total number of samples analysed per sampling visit. When the occurrence is equal to 0/12, then the results of the average count are indicated as < LoD.

The second table shows the average counts for total coliphages, f-specific coliphages, norovirus G I and G II, *Cryptosporidium* spp. and CrAssphage expressed in Log PFU for coliphages and Log GC for the other groups per L. For the enumeration results, the same procedure as described before was followed. The LoDs may differ depending on the filtration volume and are mentioned in the datasheets. For scenarios 30 to 34, 49 to 52 and 55 to 61, there were 4 replicate results presented (i.e., 2 samples per visit at sampling time point 6) whereas for all the other scenarios only 2 replicate results were presented (i.e., 1 sample per visit for sampling time point 6).

Results of the occurrence of pathogenic enteric bacteria (*Salmonella* spp., STEC and *E. coli* O157:H7), as well as *L. monocytogenes* are shown in a third table in which their occurrence is included considering the number of positive samples out of the total number of samples analysed per sampling visit. The volumes of the water sample taken for the analyses are indicated in each case scenario.

The datasheet also includes the type of processing operation, sampling dates, the volume of water in the specific handling or processing operation, and the total volume of water in the entire processing line when it is known indicated in brackets. Moreover, the total volume of the product processed during the sampling period is included as well as the number of hours that the water is used per day. Additional information is included such as the water source, the water source treatment, and the water disinfection treatment when applicable. As indicated before, six different types of water sources were used (surface water, municipal + well water, municipal tap water, recycled water, well water, and municipal + recycled water). Recycled water is considered as water, other than first-use or reclaimed water, which has been obtained from a processing operation, or water that is reused in the same operation after reconditioning (EFSA BIOHAZ Panel, 2023). The data sheets also included the residual concentration of the disinfectant and the pH that was calculated as the interquartile range (IQR) with two values that represent 25% and 75% of the data points found. Regarding water replenishment, the term 'full replenishment' was used when all the water in

the tank was changed and 'partial replenishment' when only part of the water was added without knowing the rates. For the partial replenishment of the water tank, in most cases, it was not possible to obtain information from the company about the rate of water added to the tanks. When a dilution in COD was noticed, it occurred probably by water replenishment as the contamination rate did not increase constantly as it was shown for microbiological load and COD. When this information was known, it was included in the datasheets. There are other terminologies and classifications for replenishment or refilling, namely: continuous, on-demand or unknown. In most of the scenarios, this aspect of the process was difficult to examine except if in the industrial settings, there was a flowmeter to measure the volume of water entering the tank. However, this can be recommended to control the expenses of water to improve the sustainability of the system.

Other information included is the type of water agitation (air bubbling, centrifugal pump, flotation, none, paddle, water jet and the combination water jet + ait bubbling). The date and time of start of the handling or processing operation for all scenarios were indicated as 'process_start_dd_mm_yy_hh_mm'., Sampling at sampling timepoint 0 was performed only for some scenarios when the process operation duration was very short allowing for sampling at the start (e.g. 2 hours for ID 30). Information about the sampling time points, the product water contact time, and any comments that were needed to understand the process and if there were some problems encountered were also added. It should be noted that in most cases when FBOs apply a partial replenishment, the washing tanks are replenished when the level drops below a limit, generally using a water level buoy.

The preliminary planning of scenarios included in **Table 3** for objective 1 suffered some changes. There were 2 scenarios that changed the water management of the operational processes analysed between the two sampling visits. Thus, they were split into 2 scenarios with only one sample visit each. The scenarios involved were the following:

- ID 02. The scenario belonged to Objective 1. In the first visit, they did not add any disinfectant, but when IRTA team came there for the second visit, they had added sodium hypochlorite at the beginning of the process; no more disinfectant was added. Therefore, this second visit was included in objective 2 as Scenario ID 10, with only one sampling visit.
- ID 17. Due to the low production in 2022, the second visit was done in 2023. In 2023, they changed from sodium hypochlorite to calcium hypochlorite. Thus, the second visit was included as a new scenario ID 21, both scenarios with only one sampling, included in objective 2.
- Moreover, the second visit of scenario ID 14 was cancelled due to the low production as there was a huge frost during flowering in 2022 and more than 70% of stone and pome fruit crop was lost.
- In scenario ID 27, when the CEBAS-CSIC team was at the FBO for sampling on the second visit, the washing process was cancelled because the client cancelled the requested products.

3.1.1 Fresh-whole FVH case scenarios

Scenario ID 01: Process water used in the dumping operation of apples and pears

Description of the process	Apples (ca. 60 %) and pears (ca. 40 %) of different varieties are sorted and graded by size, and packaged in bags or cardboard boxes before marketing.
Storage ↓ Dumping* ↓ Rinsing (ozonated water) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.19	10/12	5.07	12/12
Yeasts	3.20	7/12	4.41	12/12
Total bacterial counts	4.51	12/12	5.74	12/12
Coliforms	2.61	8/12	4.30	12/12
E. coli	1.37	7/12	1.42	12/12
Listeria spp.	2.64	12/12	3.13	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	1.82
F-specific coliphages	< LoD	1.76
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 31/01/23, 01/02/23, 02/02/23, 06/02/23, and 07/02/23, 08/02/23	Visit 2: 06/03/23, 07/03/23, 08/03/23, 13/03/23, and 14/03/23, 15/03/23
Volume of water	3,000 L	
Total volume processed during the sampling period	Visit 1: 37,823 kg	Visit 2: 38,521 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Municipal tap water	
Water source treatment	None	

Water disinfection treatment	None (ozonated water is used in the final rinse, not in the tank)	None (ozonated water is used in the final rinse, not in the tank)
Water replenishment	Full replenishment: 14 days	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 9:00	Visit 2: 9:00
Sampling points (min)	Visit 1: 30, 1490, 2930, 8700, 10130, 11580	Visit 2: 120, 290, 425, 1635, 1830, 3000
Product water contact time (s)	400 - 600	
Comments	Ozonated water is used for the final rinse of fruit (sprayed). Ozonated rinsing water goes down the tank, but it is not enough to increase the ozone residual in the tank. Wood containers with cardboard to protect fruit came into the tank. No chlorine was added.	

Scenario ID 02: Process water used in the presorting operation of apples	
Description of the process	Apples of different varieties are sorted and graded by size (sometimes also by colour).
Storage ↓ Presorting* ↓ Air drying ↓ Storage	

Microbiological group	Sampling visit 1	
	Average count (log CFU/100 mL)	Occurrence
Moulds	3.91	11/12
Yeasts	4.80	12/12
Total bacterial counts	6.29	12/12
Coliforms	4.33	12/12
E. coli	2.74	12/12
Listeria spp.	3.81	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1
	Average count (log PFU/L or log GC/L)
Total coliphages	0.88
F-specific coliphages	< LoD
Norovirus (G I)	< LoD
Norovirus (G II)	< LoD
Cryptosporidium spp.	< LoD
CrAssphage	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1	
	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12
L. monocytogenes (100 mL)	2	12
Pathogenic *E. coli* (100 mL)	0	10
Pathogenic *E. coli* (10 L)	0	1

Type of operation	Pre-sorting
Sampling dates	Visit 1: 24/10/22, 25/10/22, 26/10/22 and 02/11/22
Volume of water	10,000 L
Total volume processed during the sampling period	Visit 1: 209,470 kg
Number of hours the water is used/day	Visit 1: 8 h
Water source	Well water
Water source treatment	Chlorination
Start of process operation	Visit 1: 9:00
Water disinfection treatment	Visit 1: None (0 mg/L)

Water replenishment	Full replenishment: 2 weeks
	Partially refilled with unknown volume
Water agitation	None
Sampling points (min)	Visit 1: 116, 380, 1800, 2990, 13010
Product water contact time (s)	240 - 300
Comments	They usually do not use disinfectant; however, water was chlorinated (sodium hypochlorite) at the beginning of the processing in the second visit, so second visit was classified as objective 2 scenario (ID10).

Scenario ID 03: Process water used in the dumping operation of peaches, flat peaches and nectarines	
Description of the process	Fruits are dumped in the dumping tank for further sorting and grading operations and classified (by size and/or colour) before marketing.
Storage ↓ Dumping* (fungicide treatment) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.13	12/12	4.22	12/12
Yeasts	5.06	12/12	4.20	12/12
Total bacterial counts	4.76	12/12	6.12	12/12
Coliforms	3.47	12/12	2.22	9/12
E. coli	1.37	6/12	2.25	12/12
Listeria spp.	1.61	12/12	2.88	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	1	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 26/07/22	Visit 2: 13/09/22
Volume of water	6,000 L	
Total volume processed during the sampling period	Visit 1: 22,929 kg	Visit 2: 23,615 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Well water	
Water source treatment	Chlorination	
Water disinfection treatment	Sodium hypochlorite (0 mg/L)	Sodium hypochlorite (0 mg/L)
Water replenishment	Full replenishment: Daily	

	Partially refilled with unknown volume	
Water agitation	None	
Start of process operation	Visit 1: 9:00	Visit 2: 9:00
Sampling points (min)	Visit 1: 40, 100, 160, 225, 285, 480	Visit 2: 40, 95, 150, 210, 330, 420
Product water contact time (s)	120 - 300	
Comments	Although there is a dosage of chlorine with a pump into the water, the residual chlorine measurements were below the detection limit of 0.02 mg/L. The fungicide Scholar (fludioxonil) was added in the water to prevent brown rot.	

Scenario ID 04: Process water used in the pre-washing operation of peppers	
Description of the process	The product is pre-washed to remove dirt and debris before being washed and then packed.
Dumping bins ↓ Pre-washing* ↓ Washing ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	5.17	12/12	5.31	12/12
Yeasts	5.63	12/12	5.57	12/12
Total bacterial counts	8.03	12/12	8.39	12/12
Coliforms	7.92	12/12	7.42	12/12
E. coli	1.28	12/12	1.40	12/12
Listeria spp.	5.52	12/12	5.07	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	2.69	2.85
F-specific coliphages	2.33	2.50
Norovirus (G I)	5.69	5.86
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	3.69	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by showers with recirculated water	
Sampling dates	Visit 1: 18/01/22	Visit 2: 24/01/22
Volume of water	500 L	
Total volume processed during the sampling period	Visit 1: 33,000 kg	Visit 2: 39,000 kg
Number of hours the water is used/day	Visit 1: 4 h and 30 min	Visit 2: 4 h and 30 min
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	None	
Water replenishment	Full replenishment: Daily	

	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 8:30	Visit 2: 8:30
Sampling points (min)	Visit 1: 105, 150, 195, 240, 265, 275	Visit 2: 90, 120, 150, 180, 220, 255
Product water contact time (s)	6-12	
Comments	The washing system was a two-step process where peppers on a conveyor belt with brushes received water through spray bars above in a cascade first in a pre-washing step (scenario 04) followed by a washing system using a disinfectant agent (PAA) (scenario 28). The data of the residual disinfectant on the first visit corresponded to the measurements of the company's amperometric probe because our equipment did not work well.	

Scenario ID 05: Process water used in the hydro-cooling operation of carrots	
Description of the process	One of the operations in the packinghouse of carrots is hydro-cooling of whole carrots by shower
Dumping bins ↓ Pre-washing ↓ Washing ↓ Hydro-cooling* ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	3.19	12/12
Yeasts	4.09	12/12	3.55	12/12
Total bacterial counts	7.43	12/12	4.76	12/12
Coliforms	7.13	12/12	3.13	12/12
E. coli	0.06	10/12	< LoD	0/12
Listeria spp.	2.61	12/12	1.64	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	2.47	< LoD
F-specific coliphages	2.21	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Hydro-cooling by shower	
Sampling dates	Visit 1: 19/10/22	Visit 2: 9/11/22
Volume of water	10,000 L	
Total volume processed during the sampling period	Visit 1: 18,200 kg	Visit 2: 14,082 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Surface water	
Water source treatment	None	
Water replenishment	Full replenishment: Daily	
	No partially re-filled	
Water agitation	Centrifugal pump	
Start of process operation	6:00	
Sampling points (min)	Visit 1: 120, 180, 240, 300, 360, 420	Visit 2: 90, 150, 210, 270, 330, 390
Product water contact time (s)	420	
Comments	Process water from the hydro-cooling is recycled during the working hours.	

Scenario ID 06: Process water used in the washing operation of carrots	
Description of the process	Washing whole carrots is one of the unit operations to remove soil for their preparation in the packinghouse before delivery to the customer. Carrots are dumped from the bins directly into the pre-washing water and then to the washing to finalise by cooling down in a hydro-cooling before packaging.
Dumping bins ↓ Pre-washing ↓ Washing* ↓ Hydro-cooling ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	3.50	12/12	3.24	4/12
Total bacterial counts	8.35	12/12	7.78	12/12
Coliforms	6.21	12/12	6.60	12/12
E. coli	2.28	12/12	< LoD	0/12
Listeria spp.	3.07	12/12	2.27	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	3.15	< LoD
F-specific coliphages	3.35	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 19/10/22	Visit 2: 9/11/22
Volume of water	50,000 L	
Total volume processed during the sampling period	Visit 1: 33,310 kg	Visit 2: 20,117 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 1: 8 h
Water source	Surface water	
Water source treatment	None	
Water disinfection treatment	None	
Water replenishment	Full replenishment: Daily	

	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 120, 180, 240, 300, 360, 420	Visit 2: 90, 150, 210, 270, 330, 390
Product water contact time (s)	900	
Comments	Carrots are first pre-washed in a water bath and then undergo thorough washing (scenario 06) before cooling in a hydro-cooling system (scenario 05) before packaging.	

Scenario ID 07: Process water used in the pre-washing operation of vegetable mix	
Description of the process	A mix of vegetables that includes tomatoes, cucumbers, peppers and onions are washed before making the 'gazpacho' soup.
Pre-washing* ↓ Rinsing ↓ Thermal process operation ↓ Cooling ↓ Aseptic filling ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	6.12	12/12	5.82	12/12
Yeasts	7.96	12/12	7.79	12/12
Total bacterial counts	8.65	12/12	8.94	12/12
Coliforms	8.05	12/12	8.42	12/12
E. coli	1.24	12/12	2.79	12/12
Listeria spp.	5.84	12/12	5.26	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	4.80	4.70
F-specific coliphages	4.77	4.53
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by immersion	
Sampling dates	Visit 1: 6/06/22 and 7/06/22	Visit 2: 11/07/22 and 12/07/22
Volume of water	2,700 L	
Total volume processed during the sampling period	Visit 1: 135,690 kg	Visit 2: 141,730 kg
Number of hours the water is used/day	Visit 1: 24 h	Visit 2: 24 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	None	
Water replenishment	Full replenishment: 2-3 days	

	Partially re-filled daily with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 255, 360, 465, 1680, 1800, 1900	Visit 2: 210, 405, 450, 1670, 1785, 1890
Product water contact time (s)	15-20	
Comments	Before preparing the 'gazpacho' soup, a variety of vegetables are pre-washed and then rinsed in tap water. The water was reused for 2 days. For each visit, 3 samplings were carried out on the same day of adding clean water to the tank and the other 3 samplings the following day.	

Scenario ID 08: Process water used in the washing operation of celery

Description of the process	Celery hearts are washed and packaged with a plastic sleeve, or bags.
Dumping bins ↓ Trimming ↓ Washing* ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	3.94	12/12	4.44	12/12
Coliforms	3.91	12/12	3.58	12/12
E. coli	0.62	9/12	0.61	11/12
Listeria spp.	0.86	4/12	0.22	9/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD

CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by a shower	
Sampling dates	Visit 1: 13/06/22	Visit 2: 20/06/22
Volume of water	Unknown as it is a shower	
Total volume processed during the sampling period	Visit 1: 6,250 kg	Visit 2: 4,160 kg
Number of hours the water is used/day	Visit 1: 8 h per day over 6 months	Visit 2: 8 h per day over 6 months
Water source	Recycled water	
Water source treatment	Chlorine to reach 1 mg/L	
Water disinfection treatment	Chlorine (sodium hypochlorite) at a concentration target of 0.2-1.0 mg/L in a separate tank of 500 L.	
Water replenishment	Full replenishment: 6 months	
	No partially re-filled	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 120, 180, 240, 300, 360, 420	Visit 2: 120, 165, 210, 285, 300, 345
Product water contact time (s)	50	

Comments	The water is recycled daily and pumped into a separate tank where it is treated with chlorine. Every day, the water needed is pumped to the celery handling operation and reused again. The same water can be used over 6 months.

Scenario ID 09: Process water used in the dumping operation of apples

Description of the process	Fruits are dumped in the dumping tank for further sorting and grading operations and classified (by size and/or colour) before marketing.
Storage ↓ Dumping* ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.83	4/12	4.08	11/12
Yeasts	< LoD	0/12	3.00	1/12
Total bacterial counts	3.23	7/12	3.27	9/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	0.67	5/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 27/02/23, and 28/02/23	Visit 2: 06/03/23, 07/03/23, and 08/07/23
Volume of water	13,000 L	
Total volume processed during the sampling period	Visit 1: 65,612 kg	Visit 2: 44,552 kg
Number of hours the water is used/day	Visit 1: 16 h	Visit 2: 8 h
Water source	Surface water	
Water source treatment	Visit 1: Chlorination (2.4-7.8 mg/L)	Visit 2: Chlorination (0.3-2.1 mg/L)
Water disinfection treatment	Oxone (potassium monopersulfate)	

pH	Visit 1: 7.67 – 7.73	Visit 2: 7.61-7.67
Water replenishment	Full replenishment: Visit 1: 2 days / Visit 2: 3 days	
	Partially refilled with unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 6:00	Visit 2: 8:00
Sampling points (min)	Visit 1: 225, 450, 645, 1625, 1860, 2100	Visit 2: 90, 330, 1500, 1770, 2955, 3205
Product water contact time (s)	180 - 300	
Comments	Surface water is chlorinated (sodium hypochlorite) in an off-processing line tank. Water is no longer chlorinated during processing, but they add OXONE (potassium monopersulfate) in the dumping tank.	

Scenario ID 10: Process water used in the presorting operation of apples

Description of the process	Apples of different varieties are sorted and graded by size (sometimes also by colour).
Storage ↓ Presorting* ↓ Air drying ↓ Storage	

Microbiological group	Sampling visit 1	
	Average count (log CFU/100 mL)	Occurrence
Moulds	3.18	6/12
Yeasts	3.57	6/12
Total bacterial counts	4.48	5/11*
Coliforms	2.42	6/12
E. coli	1.99	4/12
Listeria spp.	1.33	9/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1
	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD
F-specific coliphages	< LoD
Norovirus (G I)	< LoD
Norovirus (G II)	< LoD
Cryptosporidium spp.	< LoD
CrAssphage	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1	
	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12
L. monocytogenes (100 mL)	0	12
Pathogenic *E. coli* (100 mL)	0	10
Pathogenic *E. coli* (10 L)	0	1

Type of operation	Pre-sorting
Sampling dates	Visit 1: 12/04/23, 13/04/23, and 14/04/23
Volume of water	10,000 L
Total volume processed during the sampling period	Visit 2: 43,655 kg

Number of hours the water is used/day	Visit 1: 8 h
Water source	Well water
Water source treatment	Chlorination
Start of process operation	Visit 1: 14:00
Water disinfection treatment	Visit 1: Sodium hypochlorite (0.0-2.6 mg/L)
pH	Visit 2: 7.23 – 7.36
Water replenishment	Full replenishment: Visit 2: 3 days
	Partially refilled with an unknown volume
Water agitation	None
Sampling points (min)	Visit 2: 60, 120, 195, 1215, 2670, 2740
Product water contact time (s)	240 - 300
Comments	They usually do not use disinfectant in this operation (ID 02) but in the second visit, water was chlorinated (sodium hypochlorite) at the beginning of the process. Therefore, the second visit was classified as a new scenario (ID 10), and only one visit was reported. *Only 11 samples were considered for mean calculation as there was one uncountable plate, possibly due to external undesired contamination.

Scenario ID 11: Process water used in the dumping operation of apples	
Description of the process	Apples are sorted and graded by size, packaged in bags or carboard boxes before marketing.
Storage ↓ Dumping* ↓ Brushing ↓ Air drying ↓ Sorting by size ↓ Packaging ↓ Refrigerated storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.00	4/12	3.07	7/12
Yeasts	< LoD	0/12	3.00	1/12
Total bacterial counts	3.79	8/12	3.52	7/12
Coliforms	0.72	5/12	0.79	7/12
E. coli	0.00	1/12	0.00	1/12
Listeria spp.	1.15	6/12	0.67	7/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 15/11/22, 16/11/22, and 18/11/22	Visit 2: 28/11/22, 29/11/22, and 30/11/22
Volume of water	8,000 L	
Total volume processed during the sampling period	Visit 1: 31,299 kg	Visit 2: 45,896 kg
Number of hours the water is used/day	Visit 1: 7-8 h	Visit 2: 7-8 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Calcium hypochlorite (9.9-15.2 mg/L)	Calcium hypochlorite (11.2-16.6 mg/L)
pH	Visit 1: 7.92 – 7.99	Visit 2: 7.85 – 7.97

Water replenishment	Full replenishment: 3 days	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 8:00	Visit 2: 7:00
Sampling points (min)	Visit 1: 110, 240, 390, 1500, 1800, 4410	Visit 2: 120, 290, 425, 1635, 1830, 3000
Product water contact time (s)	180 - 300	
Comments	Visit 1: This line did not operate on the 17th, so last sampling was done on the 18th.	

Scenario ID 12: Process water used in the dumping operation of apples

Description of the process	Apples and pears of different varieties are sorted and graded by size, packaged in bags or carboard boxes before marketing.
Storage ↓ Dumping* ↓ Brushing ↓ Air drying ↓ Sorting by size ↓ Packaging ↓ Refrigerated storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.18	12/12	4.20	12/12
Yeasts	4.73	10/12	4.06	12/12
Total bacterial counts	5.06	12/12	4.90	12/12
Coliforms	3.34	12/12	3.32	12/12
E. coli	1.23	12/12	1.86	12/12
Listeria spp.	3.59	12/12	3.21	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	2.36	3.80

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	2	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 13/02/23, 14/02/23, and 15/02/23	Visit 2: 20/02/23, 21/02/23, and 22/02/23
Volume of water	5,500 L	
Total volume processed during the sampling period	Visit 1: 8,546 kg	Visit 2: 8,133 kg
Number of hours the water is used/day	Visit 1: 8.5 h	Visit 2: 8.5 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Hydrogen peroxide (13.8-19.0 mg/L)	Hydrogen peroxide (12.0-18.0 mg/L)

pH	Visit 1: 7.32 – 7.56	Visit 2: 7.46 – 7.61
Water replenishment	Full replenishment: 3 days	
	Partially refilled with an unknown volume	
Water agitation	None	
Start of process operation	Visit 1: 8:30	Visit 2: 9:00
Sampling points (min)	Visit 1: 30, 440, 1530, 1870, 2960, 3350	Visit 2: 45, 415, 1470, 1840, 2940, 3310
Product water contact time (s)	60 – 120	
Comments	No incident	

Scenario ID 13: Process water used in the dumping operation of apples	
Description of the process	Apples and pears of different varieties are sorted and graded by size and packaged in bags or cardboard boxes before marketing.
Storage ↓ Dumping* ↓ Rinsing (municipal tap water) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	5.73	12/12	5.00	12/12
Yeasts	4.10	12/12	4.60	11/12
Total bacterial counts	5.02	12/12	5.80	12/12
Coliforms	3.90	12/12	3.91	12/12
E. coli	0.97	10/12	1.42	12/12
Listeria spp.	3.02	12/12	3.69	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	1.89	1.77
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	2.65	2.31

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	1	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 13/03/23, 14/03/23, 15/03/23, and 16/03/23	Visit 2: 29/05/23, 30/05/23, 31/05/23, and 01/06/23
Volume of water	6,000 L	
Total volume processed during the sampling period	Visit 1: 52,120 kg	Visit 2: 39,847 kg
Number of hours the water is used/day	Visit 1: 9 h	Visit 2: 9 h
Water source	Surface water	
Water source treatment	None	
Water disinfection treatment	Hydrogen peroxide (20.0-102.5 mg/L)	Hydrogen peroxide (57.5-92.5 mg/L)

pH	Visit 1: 7.23 – 7.33	Visit 2: 7.14 – 7.36
Water replenishment	Full replenishment: Weekly	
	Partially refilled with a continuous unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 9:00	Visit 2: 9:00
Sampling points (min)	Visit 1: 60, 410, 1545, 2910, 3290, 4510	Visit 2: 60, 410, 1605, 3060, 3310, 4755
Product water contact time (s)	300 - 600	
Comments	No incident	

<table>
<tr><th colspan="2">Scenario ID 14: Process water used in the dumping operation of pears</th></tr>
<tr><td>Description of the process</td><td>Pears of different varieties are dumped in the tank for subsequent sorting and grading by size and packaging before marketing.</td></tr>
<tr><td>Refrigeration
↓
Dumping*
↓
Rinsing (tap water)
↓
Brushing
↓
Sorting
↓
Packaging</td><td>The FBO did not allow the publication of photos.</td></tr>
<tr><td colspan="2">
<table>
<tr><th rowspan="2">Microbiological group</th><th colspan="2">Sampling visit 1</th></tr>
<tr><th>Average count (log CFU/100 mL)</th><th>Occurrence</th></tr>
<tr><td>Moulds</td><td>< LoD</td><td>0/12</td></tr>
<tr><td>Yeasts</td><td>< LoD</td><td>0/12</td></tr>
<tr><td>Total bacterial counts</td><td>3.63</td><td>5/12</td></tr>
<tr><td>Coliforms</td><td>0.94</td><td>7/12</td></tr>
<tr><td>E. coli</td><td>< LoD</td><td>0/12</td></tr>
<tr><td>Listeria spp.</td><td>0.91</td><td>2/12</td></tr>
</table>
For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, E. coli and Listeria spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.
</td></tr>
</table>

Viruses and parasites	Sampling visit 1
	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD
F-specific coliphages	< LoD
Norovirus (G I)	< LoD
Norovirus (G II)	< LoD
Cryptosporidium spp.	< LoD
CrAssphage	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1	
	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12
L. monocytogenes (100 mL)	0	12
Pathogenic *E. coli* (100 mL)	0	10
Pathogenic *E. coli* (10 L)	0	1

Type of operation	Dumping
Sampling dates	Visit 1: 21/11/22, 22/11/22, 23/11/22, and 24/11/22
Volume of water	5,000 L
Total volume processed during the sampling period	Visit 1: 21,747 kg
Number of hours the water is used/day	Visit 1: 8 h
Water source	Well water
Water source treatment	Chlorination
Water disinfection treatment	Calcium hypochlorite (26.3 – 38.3 mg/mL)
pH	Visit 1: 7.76 – 8.09

Water replenishment	Full replenishment: Weekly
	Partially refilled with an unknown volume
Water agitation	None
Start of process operation	Visit 1: 9:00
Sampling points (min)	Visit 1: 75, 410, 1490, 1860, 3040, 4470
Product water contact time (s)	10 – 90
Comments	The FBO was selected for objective 1 as they did not use any disinfectant when the proposal was submitted. When sampling, they used calcium hypochlorite, without an operational range established. Therefore, the scenario was moved to objective 2. The second visit was cancelled for low production. They decided to market the fruit without processing it.

Scenario ID 15: Process water used in the pre-sorting operation of pears

Description of the process	Pears of different varieties are sorted before long-term storage in a modified atmosphere.
Storage ↓ Pre-sorting* ↓ Fungicide treatment (sprayed-optional) ↓ Air drying ↓ Plastic bin ↓ Refrigerated storage in a controlled atmosphere	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.00	1/12	3.71	5/12
Yeasts	< LoD	0/12	3.08	4/12
Total bacterial counts	3.51	5/12	4.69	9/12
Coliforms	< LoD	0/12	1.86	9/12
E. coli	< LoD	0/12	1.66	2/12
Listeria spp.	0.24	2/12	1.53	6/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	4.50	4.69
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-sorting	
Sampling dates	Visit 1: 01/08/22, 02/08/22, and 03/08/22	Visit 2: 19/09/22, 20/09/22, and 21/09/22
Volume of water	18,000 L	
Total volume processed during the sampling period	Visit 1: 209,457 kg	Visit 2: 94,600 kg
Number of hours the water is used/day	Visit 1: 18 h	Visit 2: 9 h
Water source	Well water	
Water source treatment	Chlorination	
Water disinfection treatment	Calcium hypochlorite (15.2-81.8 mg/L)	Calcium hypochlorite (8.5-42.4 mg/L)

pH	Visit 1: 8.01 – 8.16	Visit 2: 7.61 – 8.11
Water replenishment	Full replenishment: 3 days	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 5:00	Visit 2: 8:00
Sampling points (min)	Visit 1: 270, 550, 1650, 1980, 3100, 3440	Visit 2: 50, 470, 1550, 1905, 2940, 3340
Product water contact time (s)	105 – 120	
Comments	No incident	

Scenario ID 16: Process water used in the dumping operation of peaches, nectarines, apples, pears, and plums

Description of the process	Fruits are dumped in the dumping tank for further sorting and grading operations and classified (by size and/or colour) before marketing.
Storage ↓ Dumping* ↓ Rinsing ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.63	9/12	3.87	12/12
Yeasts	3.26	5/12	3.24	2/12
Total bacterial counts	4.01	11/12	3.55	8/12
Coliforms	1.27	5/12	0.62	8/12
E. coli	0.15	2/12	< LoD	0/12
Listeria spp.	2.24	8/12	0.76	9/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	0.74	< LoD
F-specific coliphages	< 1	< LoD
Norovirus (G I)	5.56	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 17/08/22, 22/08/22, 07/09/22, 14/09/22, 21/09/22 and 26/09/22	Visit 2: 12/04/23, 24/04/23, 02/05/23, 08/05/23, 17/05/23 and 24/05/23
Volume of water in the specific operation	5,000 L (60,000 L)	
Total volume processed during the sampling period	Visit 1: 5,746,429 kg	Visit 2: 2,589,287 kg
Number of hours the water is used/day	Visit 1: 16 h	Visit 2: 8 h

Water source	Surface water	
Water source treatment	None	
Water disinfection treatment	Water is continuously filtrated and chlorinated (1.4-2.1 mg/L)	Water is continuously filtrated and chlorinated (0.5-1.3 mg/L)
pH	Visit 1: 7.54 – 7.64	Visit 2: 7.00 – 7.31
Water replenishment	Full replenishment: 6 weeks	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 8:00	Visit 2: 8:00
Sampling points (min)	Visit 1: 1710, 9080, 31920, 41900, 52010, 59290	Visit 2: 175, 17760, 29055, 37900, 50640, 60705
Product water contact time (s)	60-120	
Comments	No incident	

Scenario ID 17: Process water used in the dumping operation of peaches and nectarines

Description of the process	Stone fruits (peaches and nectarines) are dumped, sorted and graded by size and classified before packaging
Storage ↓ Dumping* ↓ Fungicide treatment (spray, optional) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Refrigerated storage	

Microbiological group	Sampling visit 1	
	Average count (log CFU/100 mL)	Occurrence
Moulds	3.53	8/12
Yeasts	3.54	8/12
Total bacterial counts	3.82	11/12
Coliforms	1.22	4/12
E. coli	2.27	4/12
Listeria spp.	*	4/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

* Not able to calculate the average as in 4 samples the number of *Listeria* spp. colonies was uncountable.

Viruses and parasites	Sampling visit 1
	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD
F-specific coliphages	< LoD
Norovirus (G I)	< LoD
Norovirus (G II)	5.47
Cryptosporidium spp.	< LoD
CrAssphage	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1	
	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12
L. monocytogenes (100 mL)	2	12
Pathogenic *E. coli* (100 mL)	0	10
Pathogenic *E. coli* (10 L)	0	1

Type of operation	Dumping
Sampling dates	Visit 1: 18/07/22, 19/07/22, 20/07/22, and 21/07/22
Volume of water	4,000 L
Total volume processed during the sampling period	Visit 1: 82,566 kg
Number of hours the water is used/day	Visit 1: 9 h
Water source	Surface water
Water source treatment	Chlorination
Water disinfection treatment	Visit 1: Sodium hypochlorite (0.0-48.2 mg/L)

pH	Visit 1: 7.86 – 8.82
Water replenishment	Full replenishment: Weekly
	Partially refilled with an unknown volume
Water agitation	None
Start of process operation	Visit 1: 7:45
Sampling points (min)	Visit 1: 75, 305, 1515, 2940, 3180, 4395
Product water contact time (s)	180 – 300
Comments	In 2022, they used sodium hypochlorite, but they changed to calcium hypochlorite in 2023 (See results in ID 21). *Average of *Listeria* spp. could not be calculated: 4 out of 12 samples were uncountable for *Listeria* spp. due to high numbers found (>300 cfu/plate) and 8 samples had *Listeria* spp. counts <LoD.

Scenario ID 18: Process water used in the dumping operation of peaches and nectarines

Description of the process	Stone fruits (peaches and nectarines) of different varieties are sorted and graded by size and classified before packaging.
Storage ↓ Dumping* ↓ Rinsing (municipal tap water) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.00	1/12	3.29	7/12
Yeasts	3.48	2/12	3.42	2/12
Total bacterial counts	3.60	10/12	3.54	11/12
Coliforms	0.24	2/12	1.27	6/12
E. coli	1.04	2/12	< LoD	0/12
Listeria spp.	< LoD	0/12	0.00	3/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	1.95	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	5.56	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

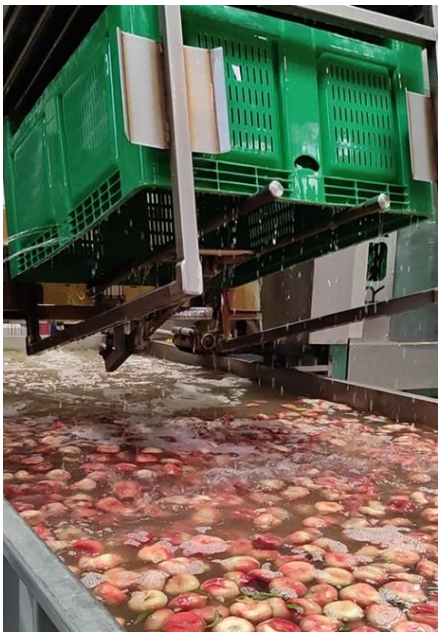
The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 04/07/22, 05/07/22, 06/07/22, and 07/07/22	Visit 2: 08/08/22, and 09/08/22
Volume of water	8,000 L	
Total volume processed during the sampling period	Visit 1: 237,763 kg	Visit 2: 123,400 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 9.5 h
Water source	Surface water	
Water source treatment	Chlorination	
Water disinfection treatment	Visit 1: Sodium hypochlorite (9.9-16.8 mg/L)	Visit 2: Sodium hypochlorite (16.9-23.6 mg/L)
pH	Visit 1: 7.72 – 7.78	Visit 2: 7.90 – 7.97

Water replenishment	Full replenishment: Visit 1: Weekly; Visit 2: Two days	
	Partially refilled with an unknown volume	
Water agitation	None	
Start of process operation	Visit 1: 8:00	Visit 2: 7:30
Sampling points (min)	Visit 1: 90, 360, 1515, 2940, 3180, 4425	Visit 2: 120, 240, 360, 1560, 1680, 1800
Product water contact time (s)	30- 90	
Comments	Full water replenishment of the tank changed between the two visits from weekly to two days.	

Scenario ID 19: Process water used in the dumping operation of peaches, flat peaches and nectarines

Description of the process	Peaches, flat peaches and nectarines are sorted and graded by size, packaged in bags or carboard boxes before marketing.
Storage ↓ Dumping* ↓ Brushing ↓ Air drying ↓ Sorting ↓ Packaging ↓ Refrigerated storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.30	3/12	3.52	3/12
Yeasts	3.30	2/12	< LoD	0/12
Total bacterial counts	3.85	11/11*	3.91	12/12
Coliforms	0.54	7/12	1.61	5/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	<LoD	0/12	0.75	6/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 11/07/22, 12/07/22, and 13/07/22	Visit 2: 29/08/22, 30/08/22, and 31/08/22
Volume of water	12,000 L	
Total volume processed during the sampling period	Visit 1: 128,147 kg	Visit 2: 155,366 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Calcium hypochlorite (7.4-11.1 mg/L)	Calcium hypochlorite (7.3-10.8 mg/L)
pH	Visit 1: 7.90 – 8.00	Visit 2: 7.80 – 7.97

Water replenishment	Full replenishment: 3 days	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 8:30	Visit 2: 8:00
Sampling points (min)	Visit 1: 30, 255, 1452, 1710, 2915, 3135	Visit 2: 60, 285, 1490, 1715, 2940, 3170
Product water contact time (s)	180 – 300	
Comments	*Only 11 samples were taken into account for mean calculation as there was one uncountable plate, possibly due to external undesired contamination.	

Scenario ID 20: Process water used in the dumping operation of peaches and nectarines	
Description of the process	Fruits are dumped in the dumping tank for further sorting and grading operations and classified (by size and/or colour) before marketing.
Storage ↓ Dumping* ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	The FBO did not allow the publication of photos.

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.00	1/12	3.00	1/12
Yeasts	3.00	1/12	< LoD	0/12
Total bacterial counts	3.40	4/12	3.29	11/12
Coliforms	0.00	2/12	1.15	6/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	0.43	6/12	0.00	2/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli,* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

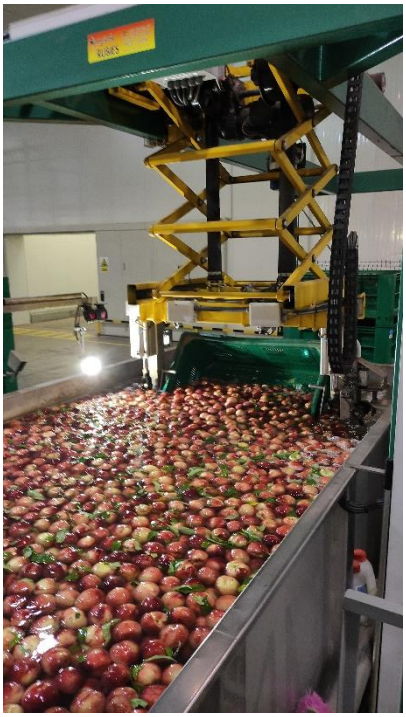
The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 19/06/23, 20/06/23, 21/06/23, and 22/06/23	Visit 2: 10/07/23, 11/07/23, 12/07/23, and 13/07/23
Volume of water	5,000 L	
Total volume processed during the sampling period	Visit 1: 48,019 kg	Visit 2: 44,702 kg
Number of hours the water is used/day	Visit 1: 10 h	Visit 2: 10 h
Water source	Surface water	
Water source treatment	Chlorination	
Water disinfection treatment	Calcium hypochlorite (21.2-45.6 mg/L)	Calcium hypochlorite (13.8-20.4 mg/L)

pH	Visit 1: 8.10 – 8.15	Visit 2: 8.05 – 8.20
Water replenishment	Full replenishment: Weekly	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 8:00	Visit 2: 8:30
Sampling points (min)	Visit 1: 90, 285, 1655, 2975, 3160, 4540	Visit 2: 80, 260, 1640, 2900, 3130, 4510
Product water contact time (s)	10 - 90	
Comments	No incident	

Scenario ID 21: Process water used in the dumping operation of peaches and nectarines

Description of the process	Stone fruits (peaches and nectarines) are dumped, sorted and graded by size and classified before packaging
Storage ↓ Dumping* ↓ Fungicide treatment (spray, optional) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Refrigerated storage	

Microbiological group	Sampling visit 1	
	Average count (log CFU/100 mL)	Occurrence
Moulds	3.88	9/12
Yeasts	4.00	6/12
Total bacterial counts	4.68	11/12
Coliforms	2.49	12/12
E. coli	2.62	2/12
Listeria spp.	1.60	11/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1
	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD
F-specific coliphages	< LoD
Norovirus (G I)	< LoD
Norovirus (G II)	< LoD
Cryptosporidium spp.	< LoD
CrAssphage	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1- See ID.17	
	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12
L. monocytogenes (100 mL)	0	12
Pathogenic *E. coli* (100 mL)	0	10
Pathogenic *E. coli* (10 L)	0	1

Type of operation	Dumping
Sampling dates	Visit 1: 24/07/23, 25/04/23, 26/04/23, and 27/04/23
Volume of water	4,000 L
Total volume processed during the sampling period	Visit 1: 102,950 kg
Number of hours the water is used/day	Visit 1: 11 h
Water source	Surface water
Water source treatment	Chlorination
Water disinfection treatment	Visit 1: Calcium hypochlorite (1.0-5.7 mg/L)
pH	Visit 1: 7.64 – 7.73

Water replenishment	Full replenishment: Weekly
	Partially refilled with an unknown volume
Water agitation	None
Start of process operation	Visit 1: 8:00
Sampling points (min)	Visit 1: 70, 285, 1650, 2930, 3160, 4440
Product water contact time (s)	180 – 300
Comments	In 2022, they used sodium hypochlorite (see ID 17), but they changed to calcium hypochlorite in 2023.

Scenario ID 22: Process water used in the pre-sorting operation of peaches, flat peaches and nectarines

Description of the process	Stone fruit of different varieties are sorted before storage.
Storage ↓ Pre-sorting* ↓ Fungicide treatment (sprayed-optional) ↓ Air drying ↓ Sorting ↓ Plastic bin ↓ Refrigerated storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.48	1/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	3.19	4/12	< LoD	0/12
Coliforms	0.85	1/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	0.24	5/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	4.26
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-sorting	
Sampling dates	Visit 1: 12/06/23, 13/06/23, and 14/06/23	Visit 2: 03/07/23, 04/07/23, and 05/07/23
Volume of water	18,000 L	
Total volume processed during the sampling period	Visit 1: 191,173 kg	Visit 2: 198,593 kg
Number of hours the water is used/day	Visit 1: 18 h	Visit 2: 18 h
Water source	Well water	
Water source treatment	Chlorination	
Water disinfection treatment	Calcium hypochlorite (45.3-73.3 mg/L)	Calcium hypochlorite (97.2-119.2 mg/L)

pH	Visit 1: 8.03 – 8.19	Visit 2: 7.96 – 8.23
Water replenishment	Full replenishment: 3 days	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 205, 570, 1650, 2070, 3030, 3440	Visit 2: 170, 500, 1610, 1935, 3035, 3370
Product water contact time (s)	120 - 240	
Comments	No incident	

Scenario ID 23: Process water used in the dumping operation of peaches and nectarines

Description of the process	Stone fruits (peaches and nectarines) of different varieties are sorted and graded by size and classified before packaging.
Storage ↓ Dumping* ↓ Rinsing (municipal tap water) ↓ Air drying ↓ Sorting ↓ Packaging ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.10	12/12	4.88	12/12
Yeasts	4.09	7/12	4.99	12/12
Total bacterial counts	6.45	12/12	6.51	12/12
Coliforms	4.60	12/12	4.79	12/12
E. coli	2.65	12/12	2.71	12/12
Listeria spp.	3.84	12/12	4.18	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	0.72	1.41
F-specific coliphages	1.61	1.39
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	3.06	2.32

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	4	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 26/06/23, 27/06/23, 28/06/23, and 29/06/23	Visit 2: 17/07/23, and 18/07/23
Volume of water	6,000 L	
Total volume processed during the sampling period	Visit 1: 61,426 kg	Visit 2: 41,234 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Surface water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Hydrogen peroxide (40.0-120.0 mg/L)	Visit 2: Hydrogen peroxide (50.0-80.0 mg/L)

pH	Visit 1: 7.34 – 7.46	Visit 2: 7.44 – 7.60
Water replenishment	Full replenishment: Visit 1: Weekly; Visit 2: Two days	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 9:00	Visit 2: 8:30
Sampling points (min)	Visit 1: 50, 220, 1610, 2925, 3105, 4500	Visit 2: 60, 165, 255, 1490, 1600, 1690
Product water contact time (s)	300 - 600	
Comments	No incident	

Scenario ID 24: Process water used in the hydro-cooling operation of cherries

Description of the process	Cherries in plastic boxes are quickly cooled in cold water in the hydro-cooler
Harvest ↓ Quick refrigeration Hydrocooling* ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.71	9/12	3.67	7/12
Yeasts	3.00	1/12	3.25	5/12
Total bacterial counts	4.17	12/12	4.35	12/12
Coliforms	0.40	3/12	1.06	10/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	0.27	4/12	0.05	6/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

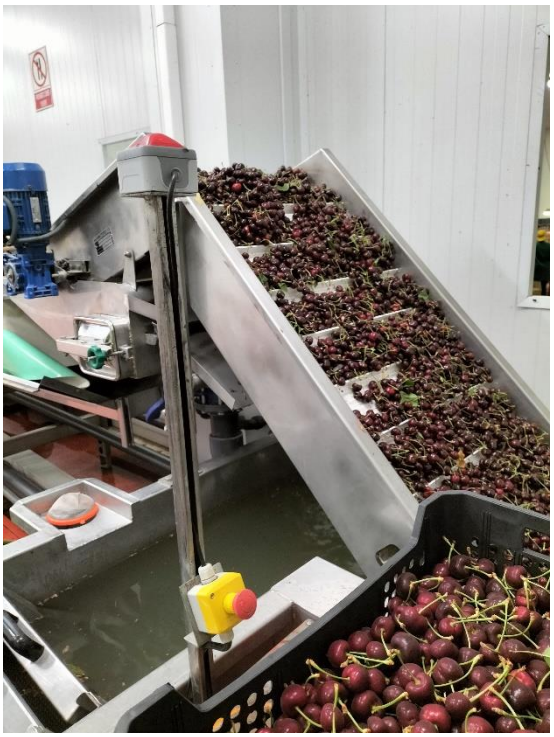
Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	3.09

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Hydro-cooling	
Sampling dates	Visit 1: 15/05/23	Visit 2: 22/05/23
Volume of water	5,500 L	
Total volume processed during the sampling period	Visit 1: 26,729 kg	Visit 2: 35,894 kg
Number of hours the water is used/day	Visit 1: 10 h	Visit 2: 10 h
Water source	Surface water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Sodium hypochlorite (3.7-15.0 mg/L)	Visit 2: Sodium hypochlorite (0.5-18.0 mg/L)
pH	Visit 1: 8.05 – 8.14	Visit 2: 8.19 – 8.24

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	None	
Start of process operation	Visit 1: 8:00	Visit 2: 7:30
Sampling points (min)	Visit 1: 30, 90, 180, 270, 360, 450	Visit 2: 75, 150, 240, 330, 420, 510
Product water contact time (s)	120 – 130	
Comments	No incident	

Scenario ID 25: Process water used in the dumping operation of cherries	
Description of the process	Cherries are dumped manually and treated with fungicide (optional), dried, sorted and packaged
Storage ↓ Dumping* ↓ De-steaming ↓ Inspection ↓ Fungicide treatment ↓ Packaging ↓ Refrigerated storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.35	9/12	3.53	10/12
Yeasts	4.01	7/12	3.24	8/12
Total bacterial counts	4.08	12/12	4.27	12/12
Coliforms	1.71	10/12	1.15	9/12
E. coli	1.43	1/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Dumping	
Sampling dates	Visit 1: 14/06/22	Visit 2: 21/06/22
Volume of water	1,600 L (7,000 L total processing line)	
Total volume processed during the sampling period	Visit 1: 20,418 kg	Visit 2: 18,944 kg
Number of hours the water is used/day	Visit 1: 10 h	Visit 2: 10 h
Water source	Municipal tap water	
Water disinfection treatment	Calcium hypochlorite (0.8-8.0 mg/L) and oxone (potassium monopersulfate)	Calcium hypochlorite (2.9-9.7 mg/L) and oxone (potassium monopersulfate)
pH	Visit 1: 7.51 – 7.77	Visit 2: 7.70 – 8.10

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	None	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 36, 94, 200, 297, 410, 475	Visit 2: 35, 131, 225, 310, 400, 485
Product water contact time (s)	60 – 120	
Comments	They used calcium hypochlorite and OXONE (potassium monopersulfate) as disinfectants in the dumping tank.	

Scenario ID 26: Process water used in the pre-washing operation of avocado

Description of the process	Ripe avocados are minimally processed for the industrial preparation of guacamole and before they are pre-washed.
Dumping bins ↓ Pre-washing* ↓ Rinsing ↓ Process operation for guacamole ↓ Cooling	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.55	12/12	3.22	4/12
Yeasts	7.44	12/12	7.57	12/12
Total bacterial counts	5.71	12/12	6.40	12/12
Coliforms	5.06	12/12	6.12	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	5.16	5.07
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	6	12	5	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by immersion	
Sampling dates	Visit 1: 15/09/22	Visit 2: 22/09/22
Volume of water	1,000 L	
Total volume processed during the sampling period	Visit 1: 15,992 kg	Visit 2: 17,700 kg
Number of hours the water is used/day	Visit 1: 4 h	Visit 2: 4 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: PAA (2.2 mg/L)	Visit 2: PAA (2.2 mg/L)
pH	Visit 1: 4.51-4.76	Visit 2: 4.47-4.79

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 30, 60, 100, 135, 195, 225	Visit 2: 0, 55, 90, 150, 180, 220
Product water contact time (s)	120	
Comments	The unexpected results, showing no enumeration of *Listeria* spp. in the 24 samples across two visits, raise questions about the limitations of the enumeration method used. A likely explanation for the confirmation of *L. monocytogenes* could be the enrichment method used. This method provides optimal nutrient conditions that may protect and promote the growth of pathogenic bacteria, potentially explaining their detection despite the negative enumeration of *Listeria* spp. In addition, the high numbers of other bacteria present could act as competing microbiota, potentially limiting the colony formation of *Listeria* spp.	

Scenario ID 27: Process water used in the pre-washing operation of mango

Description of the process	Ripe mangoes are minimally processed for the industrial preparation of mango sauce but before they are pre-washed.
Dumping bins ↓ Pre-washing* ↓ Rinsing ↓ Processing ↓ Packaging	

Microbiological group	Sampling visit 1	
	Average count (log CFU/100 mL)	Occurrence
Moulds	4.24	12/12
Yeasts	4.33	10/12
Total bacterial counts	3.59	12/12
Coliforms	1.97	12/12
E. coli	< LoD	0/12
Listeria spp.	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1
	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD
F-specific coliphages	< LoD
Norovirus (G I) Norovirus (G II)	4.92 < LoD
Cryptosporidium spp.	< LoD
CrAssphage	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1	
	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12
L. monocytogenes (100 mL)	0	12
Pathogenic *E. coli* (100 mL)	0	10
Pathogenic *E. coli* (10 L)	0	1

Type of operation	Pre-wash by immersion
Sampling dates	Visit 1: 15/09/22
Volume of water	1,350 L
Total volume processed during the sampling period	Visit 1: 692 kg
Number of hours the water is used	Visit 1: 4 h
Water source	Municipal tap water
Water source treatment	None
Water disinfection treatment	Visit 1: PAA (18.1-19.1 mg/L)
pH	5.09 - 5.18

Water replenishment	Full replenishment: Daily
	Partially refilled with an unknown volume
Water agitation	Air bubbling
Start of process operation	Visit 1: 6:00
Sampling points (min)	Visit 1: 30, 60, 100, 135, 195, 225
Product water contact time (s)	120
Comments	The second visit was cancelled at the last minute because the client cancelled the order.

Scenario ID 28: Process water used in the washing operation of peppers

Description of the process	The product is pre-washed to remove dirt and debris and then washed by a waterfall.
Dumping bins ↓ Pre-washing ↓ Washing* ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	4.59	12/12	5.75	12/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	5.35
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by showers with recirculated water	
Sampling dates	Visit 1: 18/01/23	Visit 2: 24/01/23
Volume of water	500 L	
Total volume processed during the sampling period	Visit 1: 33,000 kg	Visit 2: 39,000 kg
Number of hours the water is used/day	Visit 1: 4 h and ½ h	Visit 2: 4 h and ½ h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: PAA (477-556 mg/L)	Visit 2: PAA (351-409 mg/L)
pH	Visit 1: 3.43-3.56	Visit 2: 3.46-3.54

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 8:30	Visit 2: 8:30
Sampling points (min)	Visit 1: 105, 150, 195, 240, 265, 275	Visit 2: 90, 120, 150, 180, 220, 255
Product water contact time (s)	6-12	
Comments	The washing system was a two-step process where peppers on a conveyor belt with brushes received water through spray bars above in a cascade first in a pre-washing step (scenario 04) followed by a washing system using a disinfectant (PAA) (scenario 28).	

Scenario ID 29: Process water used in the washing operation of fruit mix	
Description of the process	Fruit mixes such as apples, tomatoes, and carrots are washed before cutting and packaging
Dumping bins ↓ Washing* ↓ Rinsing ↓ Cutting ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	< LoD	0/12	< LoD	0/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 27/02/22	Visit 2: 28/02/22
Volume of water	550 L	
Total volume processed during the sampling period	Visit 1: 1,452 kg	Visit 2: 953 kg
Number of hours the water is used/day	Visit 1: 9 h	Visit 2: 9 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Sodium hypochlorite (72-85 mg/L)	Visit 2: Sodium hypochlorite (64-88 mg/L)
pH	Visit 1: 7.94-8.48	Visit 2: 7.98-8.48

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 270, 300, 330, 360, 390, 420	Visit 2: 180, 225, 270, 315, 360, 405
Product water contact time (s)	60	
Comments	No incident	

3.1.2 Fresh-cut FVH case scenarios

Scenario ID 30: Process water used in the washing operation of shredded carrots

Description of the process	Carrots were first cut, and then shredded before being transported (in-line) to the washing bath, after which they were sorted and packed.
Storage ↓ Cutting ↓ Washing* ↓ Drying ↓ Mixing (manually) ↓ Storage ↓ Sorting ↓ Weighing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.06	12/12	3.48	12/12
Yeasts	6.43	12/12	5.96	12/12
Total bacterial counts	5.78	12/12	5.68	12/12
Coliforms	6.03	12/12	6.30	12/12
E. coli (100 mL)	<LOD	0/12	0.48	9/12
Listeria spp. (100 mL)	2.27	12/12	2.34	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LODs were 1 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds

and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages (10 L)	< LoD	< LoD
F-specific coliphages (10 L)	< LoD	< LoD
Norovirus (G I) (10 L)	< LoD	4.79
Norovirus (G II) (10 L)	< LoD	< LoD
Cryptosporidium spp. (10 L)	< LoD	< LoD
CrAssphage (10 L)	2.62	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table) due to high turbidity and COD values. LoDs were 16.67 PFU/L for total coliphages, 1.67 PFU/L for F-specific coliphages and 100 GC/L for noroviruses, *Cryptosporidium* and *CrAssphages*.

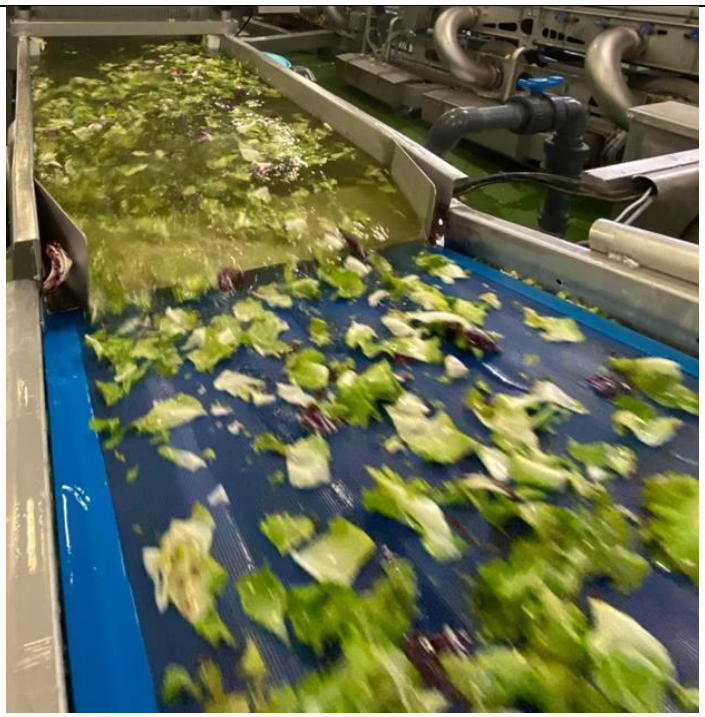
Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (50 mL)	9	12	3	12
L. monocytogenes (50 mL)	0	12	0	12
Pathogenic *E. coli* (50 mL)	0	10	0	10
Pathogenic *E. coli* (5 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Washing	
Sampling dates	Visit 1: 09/01/23	Visit 2: 30/01/23
Volume of water	2,600 L	
Total volume processed during the sampling period	Visit 1: 3,084 kg	Visit 2: 2,627 kg
Number of hours the water is used/day	Visit 1: 3 h	Visit 2: 5.5 h
Water source	Well water	

Water source treatment	Sodium hypochlorite (leading to a possible residual concentration of 0.2 ppm)	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (3 to 6 h)	
	Partially refilled: on demand	
Water agitation	Water jet	
Start of process operation	Visit 1: 4:25	Visit 2: 4:22
Sampling points (min)	Visit 1: 0, 25, 50, 75, 100, 125	Visit 2: 0, 50, 122, 194, 266, 293
Product water contact time (s)	60	
Comments	During visit 2, processing was slower due to multiple stops (meaning no fresh carrots were added to the washing bath). For both visits, filtration volumes were lowered due to high COD and turbidity.	

Scenario ID 31: Process water used in the washing operation of curly endive and radicchio

Description of the process	A mixture of curly endive and radicchio was manually cut before being washed, dried and packed.
Storage ↓ Trimming ↓ Sorting (manually) ↓ Mixing ↓ Cutting ↓ Pre-washing ↓ Washing* ↓ Rinsing ↓ Centrifugation ↓ Sorting (manually) ↓ Storage	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.61	12/12	3.48	12/12
Yeasts	6.17	12/12	5.96	12/12
Total bacterial counts	6.70	12/12	5.68	12/12
Coliforms	6.44	12/12	6.30	12/12
E. coli (100 mL)	1.94	6/12	0.48	9/12

Listeria spp. (100 mL)	2.54	12/12	2.34	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LoDs were 1 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds and coliforms and 1000 CFU/100 mL total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages (20 L)	< LoD	< LoD
F-specific coliphages (20 L)	< LoD	< LoD
Norovirus (G I) (20 L)	< LoD	4.51
Norovirus (G II) (20 L)	< LoD	< LoD
Cryptosporidium spp. (20 L)	< LoD	< LoD
CrAssphage (20 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. LoDs were 8.3 PFU/L for total coliphages, 0.83 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	7	12	4	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	1	10	0	10
Pathogenic *E. coli* (10 L)	0	2	0	2

Type of operation	Washing	
Sampling dates	Visit 1: 12/12/22	Visit 2: 23/01/22
Volume of water	2,000 L	
Total volume processed during the sampling period	Visit 1: 1,231 kg	Visit 2: 1,710 kg
Number of hours the water is used/day	Visit 1: 4 h	Visit 2: 7 h

Water source	Well water	
Water source treatment	Sodium hypochlorite (leading to a possible residual concentration of 1 ppm)	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (4 to 7 h)	
	Partially refilled: continuously	
Water agitation	Water jet	
Start of process operation	Visit 1: 11:50	Visit 2: 6:55
Sampling points (min)	Visit 1: 0, 36, 72, 108, 144, 180	Visit 2: 0, 40, 100, 144, 185, 235
Product water contact time (s)	180	
Comments	During visit 2, the washing bath was already filled upon arrival due to the washing of another product. The washing bath was filled at 4:00, hence the extra time in the number of hours the water is used (3h before + 4h production = 7h).	

Scenario ID 32: Process water used in the washing operation of baby leaves

Description of the process	A mixture of baby leaves was dumped directly into the washing bath before being dried, sorted, and packed.
Storage ↓ Visual control ↓ Mixing ↓ Washing* ↓ Drying ↓ Optical sorting ↓ Weighing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.20	12/12	2.44	6/12
Yeasts	5.05	12/12	4.38	10/12
Total bacterial counts	6.69	12/12	6.23	12/12
Coliforms	5.81	12/12	5.20	12/12
E. coli (100 mL)	1.00	7/12	0.30	1/12
Listeria spp. (10 mL)	3.76	12/12	2.02	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LoDs were 1 CFU/100 mL for *E. coli*, 10 CFU/100 mL for *Listeria* spp., 100 CFU/100 mL for yeasts, moulds, and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages (20 L)	< LoD	< LoD
F-specific coliphages (20 L)	< LoD	< LoD
Norovirus (G I) (20 L)	< LoD	4.00
Norovirus (G II) (20 L)	< LoD	< LoD
Cryptosporidium spp. (20 L)	< LoD	< LoD
CrAssphage (20 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. LoDs were 8.3 PFU/L for total coliphages, 0.83 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	3	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	1	10	0	10
Pathogenic *E. coli* (10 L)	0	2	0	2

Type of operation	Washing	
Sampling dates	Visit 1: 07/12/22	Visit 2: 18/01/23
Volume of water	1,500 L	
Total volume processed during the sampling period	Visit 1: 882.44 kg	Visit 2: 1,308.22 kg
Number of hours the water is used/day	Visit 1: 15 h	Visit 2: 15 h
Water source	Combination of municipal water and well water	
Water source treatment	None	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (15 h)	
	Partially refilled: continuously	

Water agitation	Water jet, air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:12
Sampling points (min)	Visit 1: 0, 108, 216, 324, 432, 540	Visit 2: 0, 90, 198, 303, 408, 510
Product water contact time (s)	120	
Comments	The production period was 15 h, however, samples were taken only during the first 9 hours of production.	

Scenario ID 33: Process water used in the washing operation of parsley

Description of the process	Parsley was added manually to the washing bath before being dried, weighted, and packed.

Storage
↓
Cutting
↓
Washing*
↓
Shaking
↓
Centrifugation
↓
Weighing
↓
Storage
↓
Packing

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.27	10/12	3.30	10/12
Yeasts	4.90	11/12	4.40	12/12
Total bacterial counts	4.53	12/12	4.70	11/12
Coliforms	5.02	10/12	3.44	11/12
E. coli (100 mL)	0.81	3/12	0.40	3/12
Listeria spp. (100 mL)	2.39	12/12	2.25	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LoDs were 1 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds, and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages (20 L)	< LoD	< LoD
F-specific coliphages (20 L)	< LoD	< LoD
Norovirus (G I) (20 L)	< LoD	4.27
Norovirus (G II) (20 L)	< LoD	< LoD
Cryptosporidium spp. (20 L)	< LoD	< LoD
CrAssphage (20 L)	< LoD	2.04

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. LoDs were 8.3 PFU/L for total coliphages, 0.83 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	6	12	7	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	2	0	2

Type of operation	Washing	
Sampling dates	Visit 1: 13/12/22	Visit 2: 14/02/23
Volume of water	800 L	
Total volume processed during the sampling period	Visit 1: 130 kg	Visit 2: 160 kg
Number of hours the water is used/day	Visit 1: 1.2 h	Visit 2: 2 h
Water source	Well water	
Water source treatment	None	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (1 to 2 h)	

	Partially refilled: continuously	
Water agitation	Water jet	
Start of process operation	Visit 1: 6:28	Visit 2: 6:50
Sampling points (min)	Visit 1: 0, 12, 24, 36, 48, 72	Visit 2: 0, 12, 27, 39, 51, 90
Product water contact time (s)	30	
Comments	As the parsley is added manually, and operators need to retrieve fresh baskets of parsley from a cooling cell, the addition of the product to the washing bath was temporarily discontinued. During visit 1, the stem was manually removed from the parsley before being added to the washing bath. During visit 2, the stem was not removed, and parsley as a whole was added to the washing bath.	

Scenario ID 34: Process water used in the washing operation of salad mix with carrots	
Description of the process	Salad mix (endive, curled endive, radicchio, carrots) was manually cut before being dumped into the washing baths. Carrots were also produced at the site, so they already had a washing step before being added to the mix. Afterward, the mix was dried, weighted, and packed.
Storage ↓ Cutting (manually) ↓ Washing ↓ Shaking ↓ Washing* ↓ Shaking ↓ Centrifugation ↓ Weighing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	2.47	9/12	3.11	10/12
Yeasts	3.59	11/12	5.45	12/12
Total bacterial counts	3.11	10/12	6.02	12/12
Coliforms	2.25	4/12	6.22	12/12
E. coli (100 mL)	< LoD	0/12	0.59	12/12
Listeria spp. (100 mL)	1.46	10/12	2.41	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LoDs were 1 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds, and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages (20 L)	< LoD	< LoD
F-specific coliphages (20 L)	< LoD	< LoD
Norovirus (G I) (20 L)	< LoD	< LoD
Norovirus (G II) (20 L)	< LoD	< LoD
Cryptosporidium spp. (20 L)	< LoD	< LoD
CrAssphage (20 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 8.3 PFU/L for total coliphages, 0.83 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphages*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	4	12	5	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	2	0	2

Type of operation	Washing (2nd bath)	
Sampling dates	Visit 1: 28/11/22	Visit 2: 19/12/22
Volume of water	1,315 L	
Total volume processed during the sampling period	Visit 1: 792 kg	Visit 2: 681 kg
Number of hours the water is used/day	Visit 1: 1.75 h	Visit 2: 2.5 h
Water source	Municipal tap water	
Water source treatment	None	

Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (2 to 2.5 h)	
	Partially refilled: continuously	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 13:00	Visit 2: 11:24
Sampling points (min)	Visit 1: 0, 27, 55, 75, 93, 105	Visit 2: 0, 20, 86, 111, 143, 156
Product water contact time (s)	60	
Comments	The weekend before visit 1, water tanks and pipes were disinfected. The water baths were filled with fresh municipal water; however, residues of chlorinehave been observed during sampling. During visit 2, production started at 11:24 but was paused at 12:00 due to lunch break. The water in the washing bath was not renewed and the product remained in the bath during this break (roughly an hour).	

Scenario ID 35: Process water used in the pre-washing operation of tomatoes and cucumbers	
Description of the process	Tomatoes and cucumbers are pre-washed before washing, drying and packaging.
Dumping bins ↓ Pre-washing* ↓ Washing ↓ Cutting ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	4.08	12/12	3.42	12/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by showers with recirculated water	
Sampling dates	Visit 1: 29/05/23	Visit 2: 30/05/22
Volume of water	1,500 L	
Total volume processed during the sampling period	Visit 1: 3,198 kg	Visit 2: 4,184 kg
Number of hours the water is used/day	Visit 1: 5 h and ½ h	Visit 2: 4 h
Water source	Municipal tap water	
Water source treatment	Filtration for calcium removal	
Water disinfection treatment	Visit 1: Electrolysed water (1.9-5.8 mg/L free chlorine)	Visit 2: Electrolysed water (21.3-34.1 mg/L free chlorine)

pH	Visit 1: 8.55-8.60	Visit 2: 8.31-8.36
Water replenishment	Full replenishment: 1 week	
	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 7:40	Visit 2: 7:40
Sampling points (min)	Visit 1: 20, 80, 140, 200, 260, 320	Visit 2: 65, 95, 110, 125, 160, 230
Product water contact time (s)	240	
Comments	Water used in the pre-washing operation is recycled for 2 days. Water is reused in the same operation after reconditioning. On the first visit, the company washed tomatoes and on the second visit cucumbers. For cut fruits, the pre-washing operation is a step before washing and then the fruits are cut and packed.	

Scenario ID 36: Process water used in the washing operation of tomatoes and cucumbers

Description of the process	Tomatoes and cucumbers are washed after pre-washing before drying and packaging.
Dumping bins ↓ Pre-washing ↓ Washing* ↓ Cutting ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	1.92	12/12	1.34	8/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by showers with recirculated water	
Sampling dates	Visit 1: 29/05/23	Visit 2: 30/05/22
Volume of water	1,500 L	
Total volume processed during the sampling period	Visit 1: 3,198 kg	Visit 2: 4,184 kg
Number of hours the water is used/day	Visit 1: 5 h and ½ h	Visit 2: 4 h
Water source	Municipal tap water	

Water source treatment	Filtration for calcium removal	
Water disinfection treatment	Visit 1: Electrolysed water (14.6-24.9 mg/L free chlorine)	Visit 2: Electrolysed water (44.4-61.0 mg/L free chlorine)
pH	Visit 1: 8.62-8.68	Visit 2: 8.38-8.43
Water replenishment	Full replenishment: unknown	
	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 7:40	Visit 2: 7:40
Sampling points (min)	Visit 1: 20, 80, 140, 200, 260, 320	Visit 2: 65, 95, 110, 125, 160, 230
Product water contact time (s)	240	
Comments	Water used in the washing operation is recycled for 2 days. Water is reused in the same operation after reconditioning. On the first visit, the company washed tomatoes and on the second visit cucumbers. or cut fruits, the pre-washing operation is a step before washing and then the fruits are cut and packed.	

Scenario ID 37: Process water used in the washing operation of diced onions

Description of the process	Diced onions are washed after cutting and then rinsed before drying and packaging.

Dicing
↓
Pre-washing
↓
Washing*
↓
Rinsing
↓
Drying
↓
Packaging

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.18	9/12	3.16	11/12
Yeasts	3.66	12/12	< LoD	0/12
Total bacterial counts	4.79	12/12	3.22	12/12
Coliforms	3.37	12/12	2.23	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	1.82	12/12	1.43	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	5.03
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 24/10/22	Visit 2: 14/11/22
Volume of water	1,100 L	
Total volume processed during the sampling period	Visit 1: 4,482 kg	Visit 2: 4,173 kg
Number of hours the water is used	Visit 1: 16 h	Visit 2: 16 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Chlorine (sodium hypochlorite 5.0-50.0 mg/L)	Visit 2: Chlorine (sodium hypochlorite 0.4-2.1 mg/L)
pH	Visit 1: 7.18-7.45	Visit 2: 7.33-7.72

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	6:00	
Sampling points (min)	Visit 1: 180, 225, 270, 315, 360, 405	Visit 2: 180, 225, 270, 315, 360, 405
Product water contact time (s)	60	
Comments	No incident	

Scenario ID 38: Process water used in the washing operation of diced onion

Description of the process	Diced onions are washed after cutting and then rinsed before drying and packaging.

Dicing
↓
Pre-washing
↓
Washing*
↓
Rinsing
↓
Drying
↓
Packaging

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.57	12/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	3.31	12/12	3.66	12/12
Coliforms	2.74	12/12	2.21	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

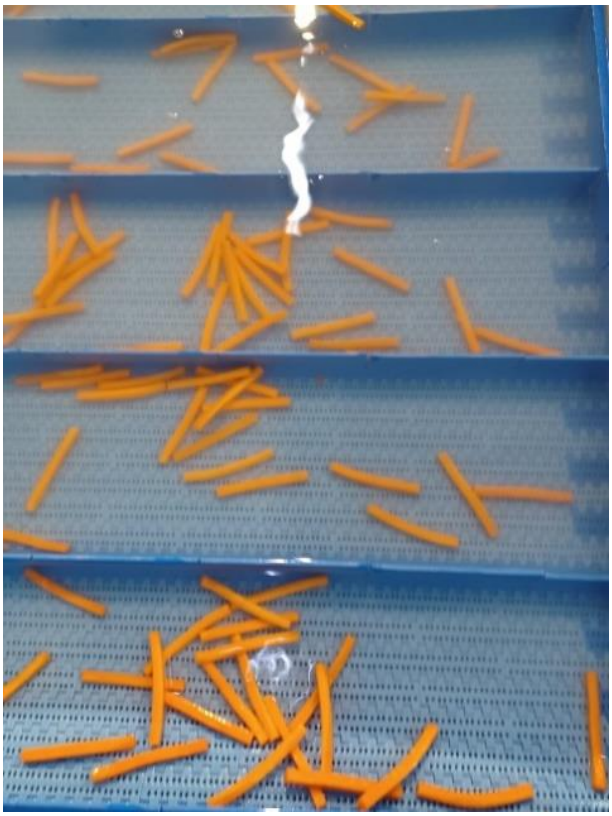
Type of operation	Washing by immersion	
Sampling dates	Visit 1: 27/03/23 and 28/03/23	Visit 2: 29/03/23
Volume of water	1,250 L	
Total volume processed during the sampling period	Visit 1: 1,240 kg	Visit 2: 1,382 kg
Number of hours the water is used/day	Visit 1: 4 h	Visit 2: 4 h
Water source	Well water	
Water source treatment		
Water disinfection treatment	Visit 1: PAA (21.8-82.5 mg/L)	Visit 2: PAA (55.5-65.0mg/L)
pH	Visit 1: 5.04-5.42	Visit 2: 5.15-5.80

Water replenishment	Full replenishment: Unknown	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 21:00	Visit 2: 21:00
Sampling points (min)	Visit 1: 75, 120, 165, 210, 285, 300	Visit 2: 435, 480, 525, 570, 615, 660
Product water contact time (s)	60	
Comments	On the first visit, 10 min before taking sampling point 2, the tank was emptied and refilled again. On the second visit, there were no incidents.	

Scenario ID 39: Process water used in the washing operation of carrot sticks

Description of the process	Peeled carrots are trimmed and washed to obtain sticks that are packaged in plastic bags or bowls.

Cutting stems and tips
↓
Peeling
↓
Cutting
↓
Manual selection
↓
Washing*
↓
Rinsing
↓
Antioxidant bath
↓
Water removal
↓
Packaging



Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	< LoD	0/12	< LoD	0/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts,

coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 13/06/22	Visit 2: 20/06/22
Volume of water	1,800 L	
Total volume of product processed during the sampling period	Visit 1: 1,900 kg	Visit 2: 2.185 kg
Number of hours the water is used/day	Visit 1: 18 h	Visit 2: 18 h
Water source	Municipal tap water	
Water source treatment	None	

Water disinfection treatment	Visit 1: Chlorine (sodium hypochlorite 56.4-72.1 mg/L)	Visit 2: Chlorine (sodium hypochlorite 68.4-75.6 mg/L)
pH	Visit 1: 8.42-8.51	Visit 2: 8.29-8.39
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 150, 240, 330, 420, 510, 630	Visit 2: 150, 240, 330, 420, 480, 600
Product water contact time (s)	30	
Comments	No incident	

Scenario ID 40: Process water used in the washing operation of fresh-cut lettuce

Description of the process	Fresh-cut iceberg lettuce is pre-washed, rinsed and then washed again before rinsing and drying and packaging.
Pre-washing ↓ Rinsing ↓ Washing* ↓ Rinsing ↓ Drying ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	3.50	11/12	3.70	12/12
Total bacterial counts	1.87	12/12	3.95	12/12
Coliforms	1.57	12/12	3.19	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1 Average count (log PFU/L or log GC/L)	Sampling visit 2 Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 24/04/23	Visit 2: 25/04/23
Volume of water	3,000 L	
Total volume processed during the sampling period	Visit 1: 1,500 kg	Visit 2: 2,000 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Municipal tap water	
Water source treatment	None	

Water disinfection treatment	Visit 1: Calcium hypochlorite (10.3-12.7 mg/L)	Visit 2: Calcium hypochlorite (1.1-12.1 mg/L)
pH	Visit 1: 7.29-7.36	Visit 2: 7.18-7.27
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 15, 30, 45, 60, 75, 90	Visit 2: 15, 30, 45, 60, 90, 135
Product water contact time (s)	60	
Comments	Cut iceberg lettuce of 6 mm piece size that was processed without any incident.	

Scenario ID 41: Process water used in the washing operation of fresh-cut lettuce	
Description of the process	Fresh-cut lettuce is washed before rinsing and drying
Pre-washing ↓ 1 st Washing* ↓ 2nd Washing ↓ Rinsing ↓ Drying ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	0.83	10/12	2.64	12/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or log GC/L)	Average count (log PFU/L or log GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	4.72	5.48
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 30/01/23	Visit 2: 06/02/23
Volume of water	2,500 L	
Total volume of product processed during the sampling period	Visit 1: 2,100 kg	Visit 2: 4,909 kg
Number of hours the water is used/day	Visit 1: 17 h	Visit 2: 17 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Chlorine (51.8-74.5 mg/L)	Visit 2: Chlorine (79.0-158.8 mg/L)

pH	Visit 1: 7.77-8.06	Visit2: 8.12-8.23
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 5:00	Visit 2: 5:00
Sampling points (min)	Visit 1: 345, 360, 435, 480, 525, 570	Visit 2: 315, 360, 405, 450, 495, 540
Product water contact time (s)	48	
Comments	The disinfection agent is calcium hypochlorite but when the residual concentration is low, sodium hypochlorite is also added.	

Scenario ID 42: Process water used in the washing operation of fresh-cut lettuce

Description of the process	Fresh-cut iceberg lettuce is pre-washed, rinsed and then washed again before rinsing and drying and packaging.

Pre-washing
↓
Rinsing
↓
Washing*
↓
Rinsing
↓
Drying
↓
Packaging

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	< LoD	0/12	< LoD	0/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1 Average count (log PFU/L or log GC/L)	Sampling visit 2 Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp. *CrAssphage*	< LoD < LoD	< LoD < LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 27 and 28/03/23	Visit 2: 28/03/23
Volume of water	3,000 L	
Total volume processed during the sampling period	Visit 1: 7,325 kg	Visit 2: 2,998 kg
Number of hours the water is used/day	Visit 1: 16 h	Visit 2: 16 h
Water source	Well water	
Water source treatment	Chlorine gas (120 mg/L)	
Water disinfection treatment	Visit 1: Chlorine gas plus sodium hypochlorite (23.8-79.0 mg/L)	Visit 2: Chlorine gas plus sodium hypochlorite (74.5-90.3 mg/L)
pH	Visit 1: 6.34-7.08	Visit 2: 6.31-6.41

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 21:00	Visit 2: 21:00
Sampling points (min)	Visit 1: 120, 180, 240, 300, 360, 420	Visit 2: 600, 645, 690, 735, 780, 810
Product water contact time (s)	60	
Comments	On visit 1, at sampling point 4, there were problems with the chlorine injection pump. In visit 2, sampling point 6 was advanced because there was an audit.	

Scenario ID 43: Process water used in the pre-washing operation of shredded lettuce	
Description of the process	After cutting, shredded lettuce is pre-washed to remove the free cellular content released after cutting, as well as dirt and debris from the product surface before washing, rinsing, drying and packaging.
Shredding ↓ Pre-washing* ↓ Rinsing ↓ Washing ↓ Rinsing ↓ Drying ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	1.56	12/12	3.26	12/12
Coliforms	< LoD	0/12	1.67	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts,

coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	5.27
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	3.45

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by immersion	
Sampling dates	Visit 1: 7/05/22	Visit 2: 21/11/22
Volume of water	2,300 L	
Total volume processed during the sampling period	Visit 1: 6,609 kg	Visit 2: 11,248 kg
Number of hours the water is used/day	Visit 1: 16.5 h	Visit 2: 16 h
Water source	Municipal tap water	
Water source treatment	None	

Water disinfection treatment	Visit 1: Chlorine (sodium hypochlorite 5.0-16.3 mg/L)	Visit 2: Chlorine (sodium hypochlorite 1.3-9.7 mg/L)
pH	Visit 1: 8.01-8.49	Visit 2: 7.52-8.21
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 195, 240, 300, 345, 390, 480	Visit 2: 165, 210, 255, 300, 345, 390
Product water contact time (s)	60	
Comments	No incident	

Scenario ID 44: Process water used in the washing operation of shredded lettuce

Description of the process	The shredded lettuce moves from the pre-washing tank to a rinse step by showers of cold water and then it enters into a second tank for washing, then rinse again before drying and packaging.
Shredding ↓ Pre-washing ↓ Rinsing ↓ Washing* ↓ Rinsing ↓ Drying ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	1.88	12/12	2.38	12/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	5.28
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 05/07/22	Visit 2: 21/11/22
Volume of water	2,300 L	
Total volume processed during the sampling period	Visit 1: 6,609 kg	Visit 2: 11,248 kg
Number of hours the water is used/day	Visit 1: 16.5 h	Visit 2: 16 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Chlorine (sodium hypochlorite 64.4-93.0 mg/L)	Visit 1: Chlorine (sodium hypochlorite 9.1-52.8 mg/L)

pH	Visit 1: 8.42-8.54	Visit 2: 7.98-8.27
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 195, 240, 300, 345, 390, 480	Visit 2: 165, 210, 255, 300, 345, 390
Product water contact time (s)	60	
Comments	No incident	

Scenario ID 45: Process water used in the washing operation of baby leaves	
Description of the process	Baby leaves are pre-washed, rinsed and then washed again before rinsing and drying.
Pre-washing ↓ Washing* ↓ Rinsing ↓ Drying ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	3.31	12/12	4.04	12/12
Coliforms	1.93	12/12	1.79	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	2.33	2.06

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

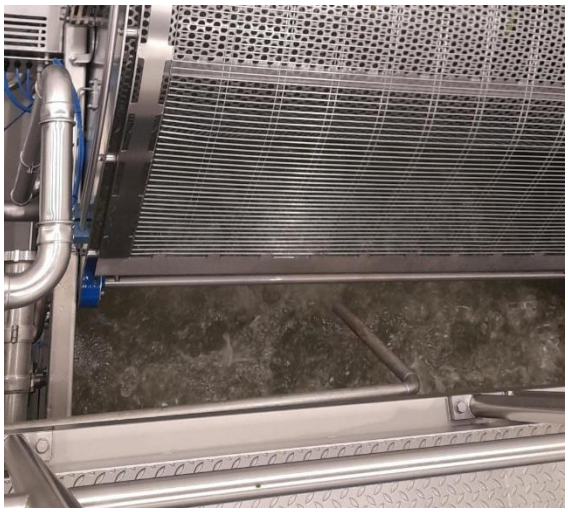
Type of operation	Washing by immersion	
Sampling dates	Visit 1: 24/10/22	Visit 2: 14/11/22
Volume of water	1,500 L	
Total volume processed during the sampling period	Visit 1: 1,823 kg	Visit 2: 2,043 kg
Number of hours the water is used/day	Visit 1: 16 h	Visit 2: 16 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Chlorine (sodium hypochlorite 40- 66 mg/L)	Visit 2: Chlorine (sodium hypochlorite 54 mg/L)
pH	Visit 1: 7.92-8.05	Visit 2: 8.32-8.67

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 180, 225, 270, 315, 360, 450	Visit 2: 180, 225, 270, 315, 360, 405
Product water contact time (s)	60	
Comments	No incident	

Scenario ID 46: Process water used in the washing operation of baby leaves

Description of the process	Baby leaves are pre-washed, rinsed and then washed again before rinsing, drying and packaging.

Pre-washing
↓
Rinsing
↓
Washing*
↓
Rinsing
↓
Drying
↓
Packaging



Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	3.91	6/12	3.69	6/12
Total bacterial counts	4.33	12/12	2.35	12/12
Coliforms	1.09	12/12	1.34	12/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 24/04/23	Visit 2: 25/04/23
Volume of water	3,000 L	
Total volume processed during the sampling period	Visit 1: 2,585 kg	Visit 2: 2,974 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Calcium hypochlorite (9.8- 11.2 mg/L)	Visit 2: Calcium hypochlorite (7.5-9.9 mg/L)
pH	Visit 1: 6.98-7.04	Visit 2: 6.96-6.99

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 21:00	Visit 2: 21:00
Sampling points (min)	Visit 1: 675, 720, 765, 810, 855, 900	Visit 2: 660, 705, 750, 795, 840, 885
Product water contact time (s)	60	
Comments	The baby leaves that were washed were: arugula, lamb's lettuce, baby lettuce and baby spinach.	

Scenario ID 47: Process water used in the washing operation of baby leaves

Description of the process	Baby leaves are pre-washed and double washed before rinsing and then drying and packaging

Pre-washing
↓
1 st Washing*
↓
2nd Washing
↓
Rinsing
↓
Drying
↓
Packaging

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	< LoD	0/12
Total bacterial counts	2.30	12/12	2.34	12/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli	< LoD	0/12	< LoD	0/12
Listeria spp.	< LoD	0/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	5.08	5.05
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 30/01/23	Visit 2: 02/06/23
Volume of water	2,500 L	
Total volume of product processed during the sampling period	Visit 1: 7,000 kg	Visit 2: 1,388 kg
Number of hours the water is used/day	Visit 1: 17 h	Visit 2: 17 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Chlorine (47.3-58.3 mg/L)	Visit 2: Chlorine (67.3-78.5mg/L)
pH	Visit 1: 7.06-8.37	Visit 2: 8.00-8.13

Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Water jet	
Start of process operation	Visit 1: 5:00	Visit 2: 5:00
Sampling points (min)	Visit 1: 345, 360, 435, 480, 525, 570	Visit 2: 315, 360, 405, 450, 495, 540
Product water contact time (s)	48	
Comments	The disinfection agent is calcium hypochlorite but when the residual concentration is low, sodium hypochlorite is also added.	

Scenario ID 48: Process water used in the washing operation of salad mix

Description of the process	Fresh-cut iceberg lettuce, carrot, and red cabbage are washed (one step), rinsed, dried, and packaged.

Dicing
↓
Mix
↓
Washing*
(one step)
↓
Rinsing
↓
Drying
↓
Packaging

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.00	1/12	< LoD	0/12
Yeasts	3.00	2/12	3.30	1/12
Total bacterial counts	3.87	9/12	3.43	3/12
Coliforms	1.68	4/12	< LoD	0/12
E. coli	0.40	1/12	< LoD	0/12
Listeria spp.	1.63	6/12	< LoD	0/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	< LoD	< LoD
F-specific coliphages	< LoD	< LoD
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing	
Sampling dates	Visit 1: 03/10/22	Visit 2: 24/04/23
Volume of water	2,000 L	
Total volume processed during the sampling period	Visit 1: 1,057 kg	Visit 2: 1,288 kg
Number of hours the water is used/day	Visit 1: 4 h	Visit 2: 4 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Sodium hypochlorite (91.6-134.3 mg/L)	Sodium hypochlorite (52.8 - 185.3 mg/L)

pH	Visit 1: 5.76 – 7.04	Visit 2: 7.40 – 7.72
Water replenishment	Full replenishment: In general, daily. However, in Visit 1, they filled the tank on Friday but the process started on Monday before our sampling took place.	
	Partially refilled with an unknown volume	
Water agitation	Air bubbling	
Start of process operation	Visit 1: 8:00	Visit 2: 8:00
Sampling points (min)	Visit 1: 50, 75, 105, 135, 175, 215	Visit 2: 50, 85, 110, 160, 190, 220
Product water contact time (s)	20 – 30	
Comments	Water is changed daily except Friday which is the day they filled the tank and maintained until Monday.	

3.1.3 Frozen FVH case scenarios

Scenario ID 49: Process water used in the water transport of peeled onions

Description of the process	Peeled onions, whether cut into small cubes or sliced, were transported through water.
Storage ↓ Washing ↓ Cutting ↓ Water transport* ↓ Cooling ↓ Draining ↓ Freezing ↓ Storage (-20 °C)	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.02	12/12	4.46	12/12
Yeasts	6.30	12/12	6.41	12/12
Total bacterial counts	6.94	12/12	7.53	12/12
Coliforms	7.99	12/12	7.76	12/12
E. coli (10 mL)	1.20	5/12	1.82	5/12
Listeria spp. (10 mL)	3.11	12/12	3.05	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts and moulds and 1000 CFU/100 mL for coliforms and total bacterial counts. The

occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	< LoD	< LoD
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

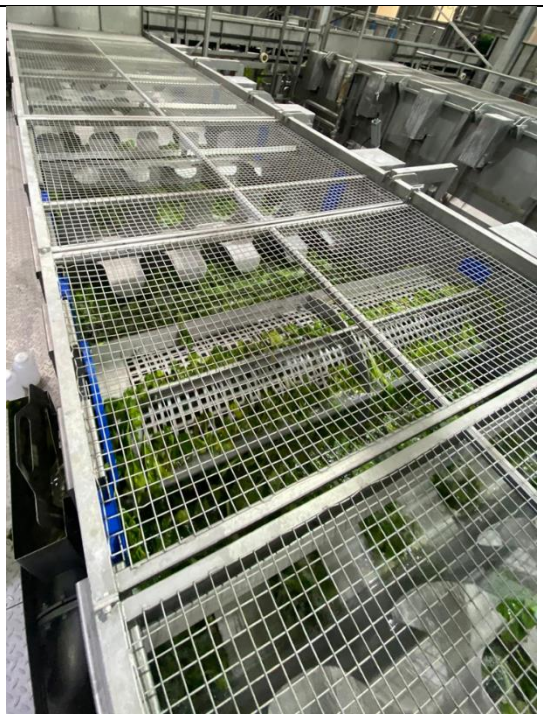
The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.33 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10/50 mL)	3	12	5	12
L. monocytogenes (10/50 mL)	0	12	0	12
Pathogenic *E. coli* (10/50 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Transport of the onions through recirculated water	
Sampling dates	Visit 1: 21/03/23, 22/03/23, 23/03/23, 24/03/23	Visit 2: 29/03/23, 30/03/23, 31/03/23
Volume of water	Not applicable (360,000 L is used per working day)	
Total volume processed during the sampling period	Visit 1: 524,421 kg	Visit 2: 329,042 kg
Number of hours the water is used/day	Visit 1: 24 h	Visit 2: 24 h
Water source	Recycled water	

Water source treatment	Process water is collected from various points in production before being recycled as a reconditioning step. This recycled water is then used for water transport.	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (very variable! Sometimes after 4 days, sometimes after 2 days)	
	Partially refilled: continuous	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 13:00	Visit 2: 02:00
Sampling points (min)	Visit 1: 15, 1155, 1395, 2595, 2805, 4210	Visit 2: 535, 800, 1835, 3215, 3380, 3550
Product water contact time (s)	30	
Comments	After the water transport, the onions go to the freezing step. However, in this production line there is a blancher in between; the onions are going through the blancher without it running. The cooling step is always working (which is spraying of cold water), so it can be seen as an extra washing step before it goes to the draining and the freezing tunnel. During visit 2, production was stopped on 30/03/23 at 12:00 due to a lack of delivery of the onions. The production line was cleaned and used for potato production instead. Onion production restarted 31/03/23 at 02:30 after cleaning of the production line and full replenishment of the water. For both visits, filtration volumes were lowered due to the high COD and turbidity. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (time points 1 to 5), 10 or 50 mL was filtered instead of 100 mL. For the enrichment of E. coli STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L.	

Scenario ID 50: Process water used in the washing operation of spinach leaves	
Description of the process	Spinach leaves were washed, blanched and frozen before being packed.
Storage ↓ Removal of insects, sand… ↓ Washing* ↓ Optical sorting ↓ Blanching ↓ Cooling ↓ Draining ↓ Freezing ↓ Storage ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.80	12/12	3.38	12/12
Yeasts	6.44	12/12	6.06	12/12
Total bacterial counts	7.20	12/12	8.75	12/12
Coliforms	6.95	12/12	8.65	12/12
E. coli (10 mL) *Listeria* spp. (10 mL)	3.34 3.26	7/12 12/12	5.90 3.98	12/12 12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts and moulds and 1000 CFU/100 mL for coliforms and total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	< LoD	< LoD
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	2.56	2.80

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.33 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10/50 mL)	2	12	5	12
L. monocytogenes (10/50 mL)	0	12	4	12
Pathogenic *E. coli* (10/50 mL)	0	10	0	10
Pathogenic *E. coli* (1/5 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Washing	
Sampling dates	Visit 1: 19/04/23, 20/04/23, 21/04/23, 23/04/23, 24/04/23	Visit 2: 12/06/23, 13/06/23, 14/06/23, 15/06/23, 16/06/23
Volume of water	15,000 L	

Total volume processed during the sampling period	Visit 1: 1,055,000 kg	Visit 2: 609,149 kg
Number of hours the water is used/day	Visit 1: 24 h	Visit 2: 24 h
Water source	Recycled water	
Water source treatment	Process water is collected from various points in production before being filtered (aerobic, anaerobic and sand filtration) and treated with PAA as a reconditioning step. This recycled water is then used in the washing bath.	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (± 5 days)	
	Partially refilled: continuously	
Water agitation	Paddles	
Start of process operation	Visit 1: 13:00	Visit 2: 17:55
Sampling points (min)	Visit 1: 0, 1130, 1650, 2820, 5950, 6900	Visit 2: 0, 905, 1390, 2345, 3785, 5200
Product water contact time (s)	600	
Comments	For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (timepoints 1 to 5), 10 or 50 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 5 L was filtered instead of 10 L for visit 1 and 1 L instead of 10 L for visit 2. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L. Visit 2 occurred at the end of the harvest season. In addition, the end of the harvest season was delayed as sowing of new spinach kept being delayed due to the rainfall at the end of April and beginning of May.	

Scenario ID 51: Process water used in the washing operation of spinach leaves	
Description of the process	Spinach leaves were washed through a paddle washer before being blanched, frozen and packed.
Raw material ↓ Removal of insects ↓ Washing* ↓ Blanching ↓ Cooling ↓ Dehydration ↓ Visual inspection ↓ Crystallization ↓ Transport (belt conveyor + separation paddles) ↓ Freezing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	2.73	12/12	3.02	11/12
Yeasts	6.09	12/12	6.16	12/12
Total bacterial counts	9.46	12/12	8.96	12/12
Coliforms *E. coli* (10 mL)	9.43 4.49	12/12 12/12	9.78 5.84	12/12 12/12
Listeria spp. (10 mL)	3.68	12/12	3.27	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts and moulds and 1000 CFU/100 mL for coliforms and total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	3.53	< LoD
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

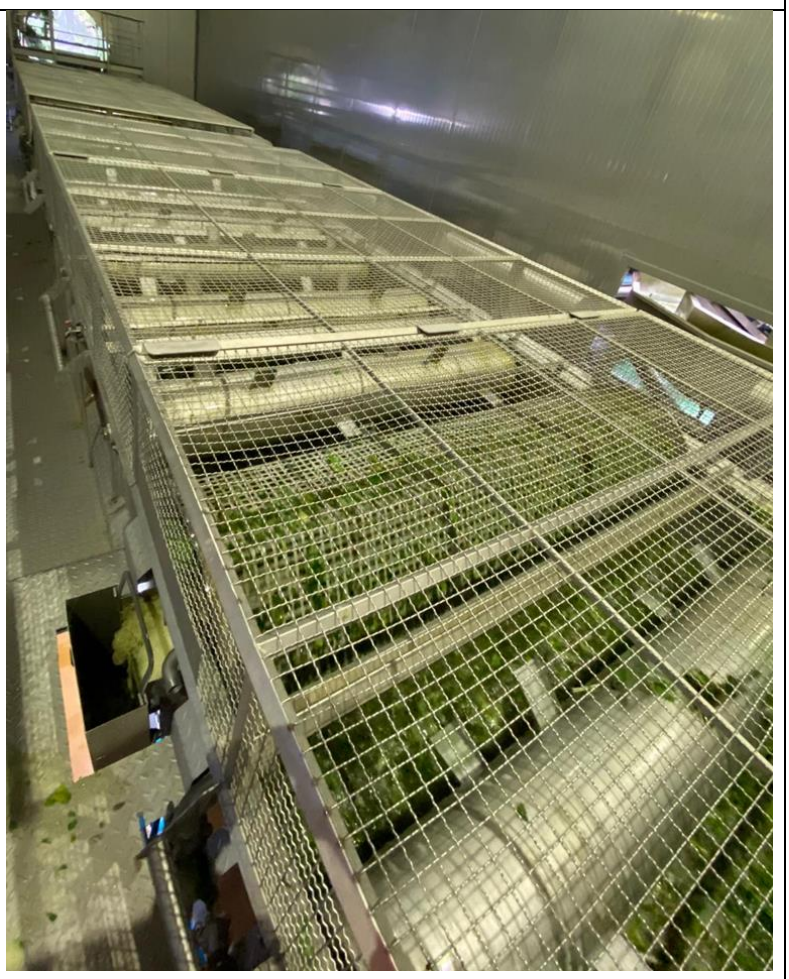
The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphages*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10 mL)	0	12	1	12
L. monocytogenes (10 mL)	7	12	12	12
Pathogenic *E. coli* (10 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Washing	
Sampling dates	Visit 1: 05/06/23, 06/06/23, 07/06/23, 08/06/23	Visit 2: 12/06/23, 13/06/23, 14/06/23, 15/06/23
Volume of water	40,000 L	
Total volume processed during the sampling period	Visit 1: 628,203 kg	Visit 2: 660,621 kg

Number of hours the water is used/day	Visit 1: 24 h	Visit 2: 24 h
Water source	Recycled water	
Water source treatment	Process water is collected from various points in production before being recycled on-site in a water treatment process. This recycled water undergoes reconditioning with hypochlorite treatment, which may result in a residual concentration of 0.25 ppm. The reconditioned water is then used in the washing bath.	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (± 3 - 4 days)	
	Partially refilled: continuously	
Water agitation	Paddles	
Start of process operation	Visit 1: 6:00	Visit 2: 4:50
Sampling points (min)	Visit 1: 85, 630, 1570, 2070, 2985, 4470	Visit 2: 185, 700, 1630, 2225, 3070, 4510
Product water contact time (s)	300	
Comments	For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (timepoints 1 to 5), 10 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L. Both visits occured at the end of spinach harvest season. In addition, the end of the harvest season was delayed as sowing of new spinach kept being delayed due to the rainfall at the end of April and beginning of May.	

Scenario ID 52: Process water used in the washing operation of spinach leaves	
Description of the process	Spinach leaves were washed through a paddle washer before being blanched, frozen and packed.
Storage ↓ Air cleaning ↓ Washing* ↓ Optical sorting ↓ Water transport ↓ Pre-heating ↓ Blanching ↓ Cooling ↓ Draining ↓ Crystallization ↓ Cutting ↓ Portioning ↓ Crystallization ↓ Freezing ↓ Glazing ↓ Weighing ↓	

Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.55	12/12	3.06	11/12
Yeasts	7.80	12/12	5.81	12/12
Total bacterial counts	7.99	12/12	9.09	12/12
Coliforms	8.58	12/12	9.17	12/12
E. coli	4.61	12/12	5.02	11/12
Listeria spp. (10 mL)	3.36	12/12	3.16	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *Listeria* spp., 100 CFU/100 mL for yeasts, moulds and *E. coli* and 1000 CFU/100 mL for coliforms and total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	< LoD	4.47
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10 mL)	4	12	2	12
L. monocytogenes (10 mL)	0	12	4	12
Pathogenic *E. coli* (10 mL)	1	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Washing	
Sampling dates	Visit 1: 01/05/23, 02/05/23, 03/05/23, 04/05/23	Visit 2: 30/05/23, 31/05/23
Volume of water	25,000 L	
Total volume processed during the sampling period	Visit 1: 431,713 kg	Visit 2: 275,944 kg
Number of hours the water is used/day	Visit 1: 24 h	Visit 2: 24 h
Water source	Recycled water	
Water source treatment	Process water is collected from various points in production before being recycled as a reconditioning step. This recycled water is then used in the washing bath.	
Water disinfection treatment	Visit 1: none	Visit 2: none
Water replenishment	Full replenishment: after production is finished (± 3 – 4 days)	
	Partially refilled: continuously	
Water agitation	Paddles	
Start of process operation	Visit 1: 17:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 15, 960, 1410, 2390, 2850, 3960	Visit 2: 110, 365, 1575, 1765, 1980, 2165
Product water contact time (s)	120	

Comments	For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (timepoints 1 to 5), 10 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L. Visit 2 occurred at the end of the harvest season. In addition, the end of the harvest season was delayed as sowing of new spinach kept being delayed due to the rainfall at the end of April and beginning of May. This also explains why production was shorter during visit 2 (2 days instead of 4 days), as there was less spinach to be harvested.

Scenario ID 53: Process water used in the pre-washing operation of diced peppers	
Description of the process	Peppers without calyx, placenta and seeds are pre-washed before blanching. After that, they are cooled, cut, and frozen before being packaged.
Pre-washing* ↓ Blanching ↓ Cooling ↓ Cutting ↓ Freezing ↓ Packaging	The FBO did not allow the publication of photos.

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.63	9/12	4.94	12/12
Yeasts	4.64	12/12	4.84	12/12
Total bacterial counts	8.21	12/12	7.63	12/12
Coliforms	7.68	12/12	6.67	12/12
E. coli	6.56	12/12	7.78	12/12
Listeria spp.	4.32	12/12	3.80	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages F-specific coliphages	3.64 3.82	3.53 3.47
Norovirus (G I)	4.93	4.93
Norovirus (G II) *Cryptosporidium* spp.	< LoD < LoD	4.77 < LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by showers with condensed water from the blanching	
Sampling dates	Visit 1: 04/10/22	Visit 2: 11/10/22
Volume of water	Unknown as it is a shower	
Total volume of product processed during the sampling period	Visit 1: 18,455 kg	Visit 2: 7,285 kg
Number of hours the water is used/day	Visit 1: Water is not reused	Visit 2: Water is not reused
Water source	Recycled water	
Water source treatment	None	

Water disinfection treatment	Visit 1: Hydrogen peroxide (50-100 mg/L)	Visit 2: Hydrogen peroxide (60-80 mg/L)
pH	Visit 1: 7.67-7.75	Visit 2: 7.70-7.86
Water replenishment	Full replenishment: Unknown	
	No re-filled	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 9:30	Visit 2: 13:00
Sampling points (min)	Visit 1: 0, 40, 75, 110, 150, 190	Visit 2: 0, 15, 30, 45, 60, 75
Product water contact time (s)	15	
Comments	No incident	

Scenario ID 54: Process water used in the cooling operation of diced peppers

Description of the process	Peppers without calyx, placenta and seeds are pre-washed before blanching. After that, they are cooled, cut, and frozen before being packaged.
Pre-washing ↓ Blanching ↓ Cooling* ↓ Cutting ↓ Freezing ↓ Packaging	The FBO did not allow the publication of photos

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	3.34	4/12
Yeasts	3.87	12/12	4.93	12/12
Total bacterial counts	7.89	12/12	8.18	12/12
Coliforms	7.81	12/12	6.95	12/12
E. coli	6.43	12/12	7.50	12/12
Listeria spp.	3.08	12/12	3.97	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	3.29	3.37
F-specific coliphages	3.28	3.29

Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Cooling by a shower	
Sampling dates	Visit 1: 04/10/22	Visit 2: 11/10/22
Volume of water	Unknown as it is a shower	
Total volume of product processed during the sampling period	Visit 1: 18,455 kg	Visit 2: 7,285 kg
Number of hours the water is used/day	Visit 1: Water is not reused	Visit 2: Water is not reused
Water source	Well water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Hydrogen peroxide (90-100 mg/L)	Visit 1: Hydrogen peroxide (120-160 mg/L)
pH	Visit 1: 7.72-7.77	Visit 2: 7.72-7.82
Water replenishment	Full replenishment: Unknown	
	No re-filled	
Water agitation	Centrifugal pump	

Start of process operation	Visit 1: 9:30	Visit 2: 13:00
Sampling points (min)	Visit 1: 0, 40, 75, 110, 150, 190	Visit 2: 0, 15, 30, 45, 60, 75
Product water contact time (s)	30	
Comments	No incident	

Scenario ID 55: Process water used in the cooling operation of peeled onions

Description of the process	Spraying cold water onto the sliced/cut onions. Onions are NOT blanched before!

Storage
↓
Washing
↓
Cutting
↓
Water transport
↓
Cooling*
↓
Draining
↓
Freezing (IQF)
↓
Storage (- 20 °C)

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.85	12/12	4.27	12/12
Yeasts	6.11	12/12	6.28	12/12
Total bacterial counts	6.36	12/12	7.41	12/12
Coliforms	7.62	12/12	7.54	12/12
E. coli (10 mL)	1.00	2/12	2.19	8/12
Listeria spp. (10 mL)	2.95	12/12	3.07	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts and moulds and 1000 CFU/100 mL for coliforms and total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	< LoD	< LoD
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphages*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10 mL)	3	12	6	12
L. monocytogenes (10 mL)	0	12	1	12
Pathogenic *E. coli* (10 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Cooling	
Sampling dates	Visit 1: 21/03/23, 22/03/23, 23/03/23, 24/03/23	Visit 2: 29/03/23, 30/03/23, 31/03/23
Volume of water	Not applicable	
Total volume processed during the sampling period	Visit 1: 524,241 kg	Visit 2: 329,042 kg
Number of hours the water is used/day	Visit 1: 24 h	Visit 2: 24 h
Water source	Municipal tap water	

Water source treatment	None	
Water disinfection treatment	Visit 1: peracetic acid (5 mg/L, 6.56 < pH < 6.98)	Visit 2: peracetic acid (5 mg/L, 6.27 < pH < 6.38)
Water replenishment	Full replenishment: not applicable	
	Partially refilled: not applicable	
Water agitation	None	
Start of process operation	Visit 1: 13:00	Visit 2: 02:00
Sampling points (min)	Visit 1: 30, 1140, 1380, 2580, 2790, 4170	Visit 2: 560, 790, 1820, 3200, 3370, 3535
Product water contact time (s)	15	
Comments	After the water transport, the onions go to the freezing step. However, in this particular production line there is a blancher in between; the onions are going through the blancher without it running. The cooling step is always working (which is spraying of cold water), so it can be seen as an extra washing step before it goes to the draining and the freezing tunnel. During visit 2, production was stopped on 30/03/23 at 12:00 due to a lack of delivery of the onions. The production line was cleaned and used for potato production instead. Onion production restarted 31/03/23 at 2:30 after cleaning of the production line. For both visits, filtration volumes were lowered due to the high COD and turbidity. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (time points 1 to 5), 10 or 50 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L.	

Scenario ID 56: Process water used in the pre-washing operation of peeled onions	
Description of the process	The peeled onions are pre-washed before washing, cutting, freezing and packaging.
Pre-washing* ↓ Washing ↓ Cutting ↓ Freezing ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	4.94	11/12	4.93	12/12
Yeasts	5.48	12/12	6.24	12/12
Total bacterial counts	8.94	12/12	8.20	12/12
Coliforms	7.89	12/12	8.13	12/12
E. coli	3.31	12/12	< LoD	0/12
Listeria spp.	4.00	12/12	4.53	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	5.09	3.34

F-specific coliphages	4.97	3.20
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD
Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Pre-washing by immersion	
Sampling dates	Visit 1: 18/07/22	Visit 2: 26/09/22
Volume of water	2,000 L	
Total volume of product processed during the sampling period	Visit 1: 33, 750 kg	Visit 2: 30,375 kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Well water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Hydrogen peroxide (100.0 mg/L)	Visit 1: Hydrogen peroxide (74-125 mg/L)
pH	Visit 1: 7.75-7.92	Visit 2: 7.77-7.82
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	

Start of process operation	Visit 1: 6:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 210, 250, 290, 330, 370, 450	Visit 2: 165, 210, 240, 300, 345, 405
Product water contact time (s)	85	
Comments	The first visit was carried out in 2 days: on the first day 3 sampling points were taken and the next day the other 3 points. On the second visit, all the points were taken on the same day. This also occurred for scenario ID 57 (washing).	

Scenario ID 57: Process water used in the washing operation of peeled onions

Description of the process	The peeled onions are washed after pre- washing. Then, they are cut, frozen and packed.
Pre-washing ↓ Washing* ↓ Cutting ↓ Freezing ↓ Packaging	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	3.21	12/12	4.24	10/12
Yeasts	6.30	12/12	6.00	12/12
Total bacterial counts	8.18	12/12	8.05	12/12
Coliforms	7.90	12/12	7.74	12/12
E. coli	2.69	11/12	< LoD	0/12
Listeria spp.	5.70	12/12	4.76	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL with LoDs of 1000 CFU/100 mL for moulds and yeast, and 1 CFU/100 mL for total bacterial counts, coliforms, *E. coli* and *Listeria* spp. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages	4.99	3.27
F-specific coliphages	4.85	3.33
Norovirus (G I)	< LoD	< LoD
Norovirus (G II)	< LoD	< LoD

Cryptosporidium spp.	< LoD	< LoD
CrAssphage	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L with LoDs of 5 PFU/L for total coliphages, 0.5 PFU/L for F-specific coliphages and 50 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (10 L)	0	1	0	1

Type of operation	Washing by immersion	
Sampling dates	Visit 1: 18/07/22	Visit 2: 26/09/22
Volume of water	5,000 L	
Total volume of product processed during the sampling period	Visit 1: 33, 750 kg	Visit 2: 30,375kg
Number of hours the water is used/day	Visit 1: 8 h	Visit 2: 8 h
Water source	Well water	
Water source treatment	None	
Water disinfection treatment	Visit 1: Hydrogen peroxide (100 mg/L)	Visit 1: Hydrogen peroxide (160-240 mg/L)
pH	Visit 1: 7.58-7.70	Visit 2: 7.66-7.73
Water replenishment	Full replenishment: Daily	
	Partially refilled with an unknown volume	
Water agitation	Centrifugal pump	
Start of process operation	Visit 1: 6:00	Visit 2: 6:00

Sampling points (min)	Visit 1: 210, 250, 290, 330, 370, 450	Visit 2: 165, 210, 240, 300, 345, 405
Product water contact time (s)	85	
Comments	The first visit was carried out in 2 days: on the first day 3 sampling points were taken and the next day the other 3 points. On the second visit, all the points were taken on the same day. This also occurred for scenario ID 56 (pre-washing).	

Scenario ID 58: Process water used in the cooling operation of spinach	
Description of the process	Spraying cold water (cooling step) on the spinach leaves after blanching.
Storage ↓ Removal of insects, sand… ↓ Washing ↓ Optical sorting ↓ Blanching ↓ Cooling* ↓ Draining ↓ Freezing ↓ Storage ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	< LoD	0/12	< LoD	0/12
Yeasts	< LoD	0/12	2.84	4/12
Total bacterial counts	3.61	12/12	4.33	12/12
Coliforms	< LoD	0/12	4.18	2/12
E. coli (10 mL)	< LoD	0/12	3.65	4/12
Listeria spp. (10 mL)	2.15	12/12	2.82	9/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	5.34	3.97
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	3.14

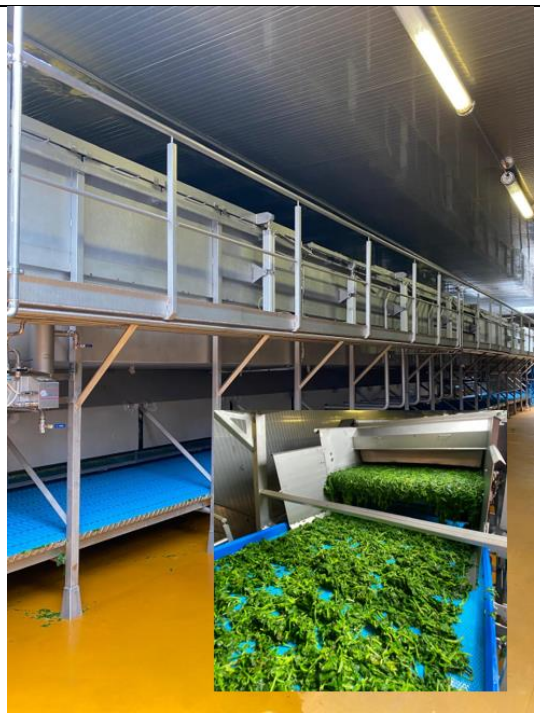
The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphages*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10 mL)	2	12	5	12
L. monocytogenes (10 mL)	0	12	4	12
Pathogenic *E. coli* (10 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Cooling	
Sampling dates	Visit 1: 19/04/23, 20/04/23, 21/04/23, 22/04/23, 23/04/23, 24/04/23	Visit 2: 12/06/23, 13/06/23, 14/06/23, 15/06/23, 16/06/23
Volume of water	Not applicable	
Total volume processed during the sampling period	Visit 1: 1,055,000 kg	Visit 2: 609,149 kg

Number of hours the water is used/day	Visit 1: not applicable	Visit 2: not applicable
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: peracetic acid (5 mg/L, 6.95 < pH < 7.12)	Visit 2: peracetic acid (5 mg/L, 7.34 < pH < 7.81)
Water replenishment	Full replenishment: not applicable	
	Partially refilled: not applicable	
Water agitation	Not applicable	
Start of process operation	Visit 1: 13:00	Visit 2: 17:55
Sampling points (min)	Visit 1: 30, 1160, 1665, 2850, 5980, 6940	Visit 2: 5, 925, 1375, 2365, 3840, 5255
Product water contact time (s)	95	
Comments	For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (timepoints 1 to 5), 10 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L. Visit 2 occurred at the end of spinach harvest season. In addition, the end of the harvest season was delayed as sowing of new spinach kept being delayed due to the rainfall at the end of April and beginning of May.	

Scenario ID 59: Process water used in the cooling operation of spinach leaves	
Description of the process	Spraying cold water (cooling step) on the spinach leaves after blanching
Storage ↓ Air cleaning ↓ Removal of insects ↓ Washing ↓ Optical sorting ↓ Water transport ↓ Pre-heating ↓ Blanching ↓ Cooling* ↓ Draining ↓ Crystallization ↓ Cutting ↓ Portioning ↓ Crystallization ↓ Freezing ↓ Glazing ↓	

Weighing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	2.48	1/12	< LoD	0/12
Yeasts	3.93	3/12	< LoD	0/12
Total bacterial counts	3.48	12/12	3.75	12/12
Coliforms	4.34	1/12	< LoD	0/12
E. coli (10 mL)	< LoD	0/12	1.15	2/12
Listeria spp. (10 mL)	2.11	9/12	2.84	12/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	4.66	3.69
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10 mL)	2	12	2	12
L. monocytogenes (10 mL)	0	12	0	12
Pathogenic *E. coli* (10 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Cooling	
Sampling dates	Visit 1: 01/05/23, 02/05/23, 04/05/23, 04/05/23	Visit 2: 30/05/23, 31/05/23
Volume of water	Not applicable	
Total volume processed during the sampling period	Visit 1: 431,713 kg	Visit 2: 275,944 kg
Number of hours the water is used/day	Visit 1: not applicable	Visit 2: not applicable
Water source	Combination of recycled water and municipal tap water	
Water source treatment	Process water is collected from various points in production. This process water is then reconditioned (=recycled water) and combined with tap water.	
Water disinfection treatment	Visit 1: peracetic acid (5 mg/L, 6.84 < pH < 7.06)	Visit 2: peracetic acid (5 mg/L, 7.15 < pH < 7.28)
Water replenishment	Full replenishment: not applicable	
	Partially refilled: not applicable	
Water agitation	Not applicable	
Start of process operation	Visit 1: 17:00	Visit 2: 6:00
Sampling points (min)	Visit 1: 0, 930, 1380, 2370, 2820, 3930	Visit 2: 95, 350, 1565, 1745, 1960, 2190
Product water contact time (s)	120	

Comments	During visit 2, production was shorter due to a lack of product (2 days instead of 4 days). For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (timepoints 1 to 5), 10 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L. Visit 2 occurred at the end of spinach harvest season. In addition, the end of the harvest season was delayed as sowing of new spinach kept being delayed due to the rainfall at the end of April and beginning of May. This also explains why production was shorter during visit 2 (2 days instead of 4 days), as there was less spinach to be harvested.

Scenario ID 60: **Process water used in the washing operation of parsley**	
Description of the process	Parsley was washed in a flotation washer after which they were frozen and packed.
Raw product ↓ Cutting ↓ Pre-washing ↓ Paddle washing ↓ Flotation washing* ↓ Rinsing ↓ Centrifugation ↓ Freezing ↓ Cutting ↓ Removal of stems ↓ Calibrating ↓ Metal detection ↓ Filling ↓ Weighing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	2.00	2/12	< LoD	0/12
Yeasts	2.87	2/12	2.95	6/12
Total bacterial counts	< LoD	0/12	< LoD	0/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli (100 mL)	< LoD	0/12	0.30	1/12
Listeria spp. (100 mL)	2.21	8/12	0.87	6/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LoDs were 1 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	< LoD	< LoD
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (100 mL)	0	12	0	12
L. monocytogenes (100 mL)	0	12	0	12
Pathogenic *E. coli* (100 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Washing	
Sampling dates	Visit 1: 29/06/23	Visit 2: 12/07/23
Volume of water	2,500 L	
Total volume processed during the sampling period	Visit 1: 12,317 kg	Visit 2: 15,546 kg
Number of hours the water is used/day	Visit 1: 9.7 h	Visit 2: 4 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: peracetic acid (479 – 1203 mg/L, 4.09 < pH < 4.40)	Visit 2: peracetic acid (128 – 491 mg/L, 4.40 < pH < 7.20)
Water replenishment	Full replenishment: in theory every 8 hours, however they visually check the water and decide whether it's necessary to replenish or not. During sampling, it was after 9 - 10 hours.	
	Partially refilled: on demand (when water level is below a certain value, fresh water is added to the bath)	
Water agitation	Flotation	
Start of process operation	Visit 1: 9:15	Visit 2: 8:45
Sampling points (min)	Visit 1: 0, 105, 195, 300, 390, 480	Visit 2: 0, 105, 195, 345, 405, 465
Product water contact time (s)	420	

Comments	During visit 1, production was cut short (about 30 min before the actual end) due to an oil leak in the washing bath. It is thus possible that there were some oil remnants in the samples of sampling point 6, however this was visually not observed. During visit 2, the water bath was emptied at 12:30 due to the product being too dirty. There was no cleaning and disinfection, but the bath was refilled with fresh water and production restarted at 13:30. For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L.

Scenario ID 61: Process water used in the washing operation of chives	
Description of the process	Chives were washed in a flotation washer after which they were frozen and packed.
Raw product ↓ Cutting ↓ Pre-washing ↓ Paddle washing ↓ Flotation washing* ↓ Rinsing ↓ Centrifugation ↓ Freezing ↓ Cutting ↓ Removal of stems ↓ Calibrating ↓ Metal detection ↓ Filling ↓ Weighing ↓ Packing	

Microbiological group	Sampling visit 1		Sampling visit 2	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Moulds	2.51	5/12	2.27	4/12
Yeasts	2.65	12/12	< LoD	0/12
Total bacterial counts	3.00	4/12	2.30	2/12
Coliforms	< LoD	0/12	< LoD	0/12
E. coli (10 mL)	< LoD	0/12	< LoD	0/12
Listeria spp. (10 mL)	1.65	10/12	1.90	7/12

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 10 CFU/100 mL for *E. coli* and *Listeria* spp., 100 CFU/100 mL for yeasts, moulds and coliforms and 1000 CFU/100 mL for total bacterial counts. The occurrence was calculated as the number of positive samples/total number of samples analysed.

Viruses and parasites	Sampling visit 1	Sampling visit 2
	Average count (log PFU/L or (GC/L)	Average count (log PFU/L or (GC/L)
Total coliphages (5 L)	< LoD	< LoD
F-specific coliphages (5 L)	< LoD	< LoD
Norovirus (G I) (5 L)	< LoD	4.75
Norovirus (G II) (5 L)	< LoD	< LoD
Cryptosporidium spp. (5 L)	< LoD	< LoD
CrAssphage (5 L)	< LoD	< LoD

The average counts for coliphages are expressed in Log PFU/L and for Norovirus, *Cryptosporidium* and *CrAssphage* in Log GC/L. Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values. LoDs were 33.3 PFU/L for total coliphages, 3.3 PFU/L for F-specific coliphages and 200 GC/L for noroviruses, *Cryptosporidium* and *CrAssphage*.

Bacterial pathogens	Sampling visit 1		Sampling visit 2	
	Number of positive samples	Number of samples analysed	Number of positive samples	Number of samples analysed
Salmonella spp. (10 mL)	0	12	0	12
L. monocytogenes (10 mL)	0	12	0	12
Pathogenic *E. coli* (10 mL)	0	10	0	10
Pathogenic *E. coli* (1 L)	0	2	0	2

Filtration volumes were lowered (as indicated in the table above) due to high turbidity and COD values.

Type of operation	Washing	
Sampling dates	Visit 1: 21/08/23	Visit 2: 22/08/23
Volume of water	2,500 L	
Total volume processed during the sampling period	Visit 1: 10,192 kg	Visit 2: 7,582 kg
Number of hours the water is used/day	Visit 1: 12.3 h	Visit 2: 12.3 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Visit 1: peracetic acid (221 – 243 mg/L, 4.44 < pH < 4.50)	Visit 2: peracetic acid (233 – 285 mg/L, 4.34 < pH < 4.39)
Water replenishment	Full replenishment: in theory every 8 hours, however they visually check the water and decide whether it's necessary to replenish or not. During sampling, it was after 12 hours.	
	Partially refilled: on demand (when water level is below a certain value, fresh water is added to the bath)	
Water agitation	Flotation	
Start of process operation	Visit 1: 8:50	Visit 2: 8:45
Sampling points (min)	Visit 1: 10, 70, 130, 190, 250, 310	Visit 2: 0, 65, 125, 185, 245, 305
Product water contact time (s)	420	

Comments	During visit 1, organic chives were produced before the production run of conventional chives. It was communicated that the water would be fully refreshed before the start of production of conventional chives, however, the operators decided the water was still clean enough and thus did not refresh the water. At the start of production of conventional chives, the water had been used for approx. 7 hours already. During visit 2, the water was once again not fully refreshed before the start of production of conventional chives, even though this was communicated differently. Up until that point, the water had been used for approx. 7.2 hours already. For both visits, due to the high COD and turbidity, filtration volumes were lowered. For the enrichment of *Salmonella* spp., *L. monocytogenes* and *E. coli* STEC/O157 (timepoints 1 to 5), 10 mL was filtered instead of 100 mL. For the enrichment of *E. coli* STEC/O157 (timepoint 6), 1 L was filtered instead of 10 L. For the detection of viruses and coliphages, 5 L was filtered instead of 20 L.

3.1.4 Historical case scenario for objective 4

<table>
<tr><th colspan="2">Scenario ID CEBAS-OM: SELECTED "HISTORICAL" CASE SCENARIO INCLUDED IN THE ASSESSMENT OF PROCESS WATER FOR OBJECTIVE 4</th></tr>
<tr><td>Description of the process</td><td>Washing operation of baby leaves and fresh-cut iceberg lettuce Baby leaves (lamb lettuce, tatsoi, red lettuce, spinach, and rocket) and fresh-cut iceberg lettuce were washed sequentially, not mixed. Only iceberg lettuce was cut into 30 mm pieces. Afterward, the washed produce was dried, weighed, and packed.</td></tr>
<tr><td>Cutting automatically only the iceberg lettuce
↓
Pre-Washing
↓
Washing*
↓
Rinsing
↓
Drying
↓
Weighing
↓
Packing</td><td>  </td></tr>
</table>

Microbiological group	Pre-washing tank		Washing tank	
	Average count (log CFU/100 mL)	Occurrence	Average count (log CFU/100 mL)	Occurrence
Total bacterial counts	3.16	10/10	2.11	10/10
Coliforms	1.27	10/10	0.52	6/10
E. coli	0.22	2/10	0.16	2/10

For the microbiological parameters, the average counts are expressed in Log CFU/100 mL. LODs were < 1 CFU/100 mL for total bacterial counts, coliforms and *E. coli*. The occurrence was calculated as the number of positive samples/total number of samples analysed.		
Type of operation	Prewashing and washing sequent in two separated tanks	
Sampling dates	Prewashing: 13/02/18	Washing: 13/02/18
Volume of water	1500 L in each tank	
Total volume processed during the sampling period	Prewashing: 4797 kg	Washing: 4797 kg
Number of hours the water is used/day	Prewashing: 8 h	Washing: 8 h
Water source	Municipal tap water	
Water source treatment	None	
Water disinfection treatment	Prewashing: Sodium hypochlorite with automatic control of free chlorine (13.3 mg/L) and pH (6.12)	Washing 2: Sodium hypochlorite with automatic control of free chlorine (15.4 mg/L) and pH (6.19)
Water replenishment	Full replenishment: after 8 h	
	Partial replenishment: water is entering continuously from the shower)	
Water agitation	Air bubbling	
Start of process operation	06:00	
Sampling points (min)	125, 200, 270, 375, 435	
Product water contact time (s)	60	
Comments	The online monitoring system that controlled free chlorine and pH was the SmartWash™ System from the company Smartwash Solutions, LLC (Salinas, CA, USA).	

3.2 Graphical presentation of the aggregated results per sector

The results are presented in box plots ordered by food category: Fresh-whole FVHs, Fresh-cut FVHs and Frozen FVHs. In each category, the case scenarios were grouped depending on the water disinfection treatment: for fresh-whole FVHS results were grouped in four areas: no water treatment, chlorine, PAA and H_2O_2. For fresh-cut FVHs results were shown in three areas: no water treatment, chlorine, and PAA. For frozen FVHs results are presented in three areas: no water treatment, PAA, and H_2O_2. For each case scenario, the interquartile range (IQR) of the parameters measured was calculated using R (Core Team, 2022). The graphical figures represent boxes as the first and the third quartiles and the horizontal line shows the median. The "whiskers" from the quartiles are the minimum and maximum values and the symbols (●) represent the outliers. Any data point in a boxplot that is more than 1.5 IQR points below the first quartile data or more than 1.5 IQR points above the third quartile data is considered an outlier (Q1-1.5 IQR and Q3 + 1.5 IQR). The reasons for outliers were included in the 'Comments' section of the data sheets and also detailed information can be found in the Excel data file. For the graph of *Listeria* spp. and *E. coli* the number of positive samples out of the total samples analysed is included per scenario. The pathogens *Salmonella*, pathogenic *E. coli,* and norovirus are indicated in the *E. coli* boxplot by different symbols. For *Listeria* spp. boxplot is indicated by another symbol when *L. monocytogenes* was detected. These are the symbols that indicate when enteric pathogens, norovirus, or *L. monocytogenes* were detected:

○ *Salmonella* spp. □ pathogenic *E. coli* △ norovirus ☆ *L. monocytogenes*

3.2.1 Fresh-whole FVHs

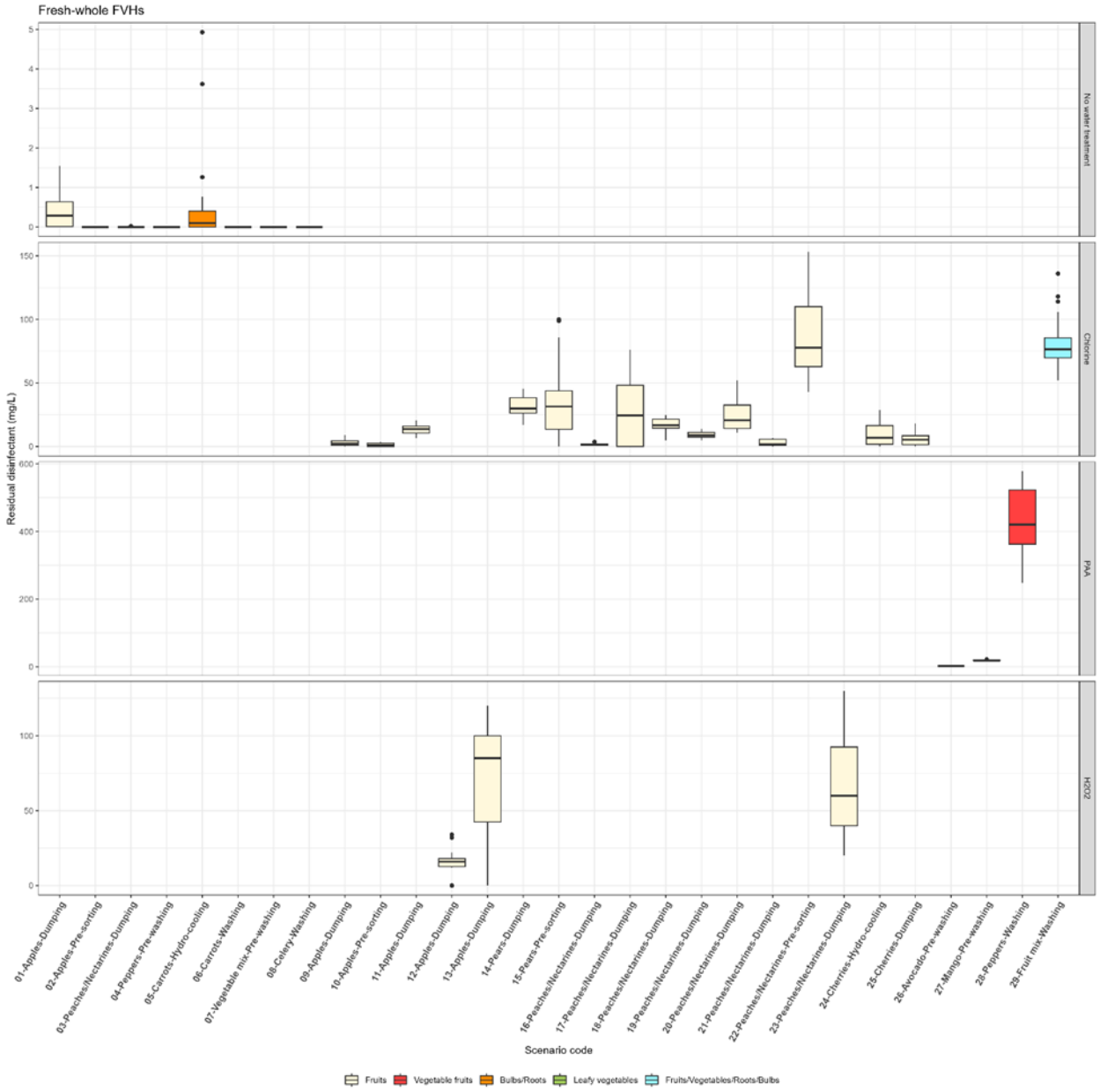


Figure 24. Boxplot graph that represents changes in residual disinfectant concentration of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

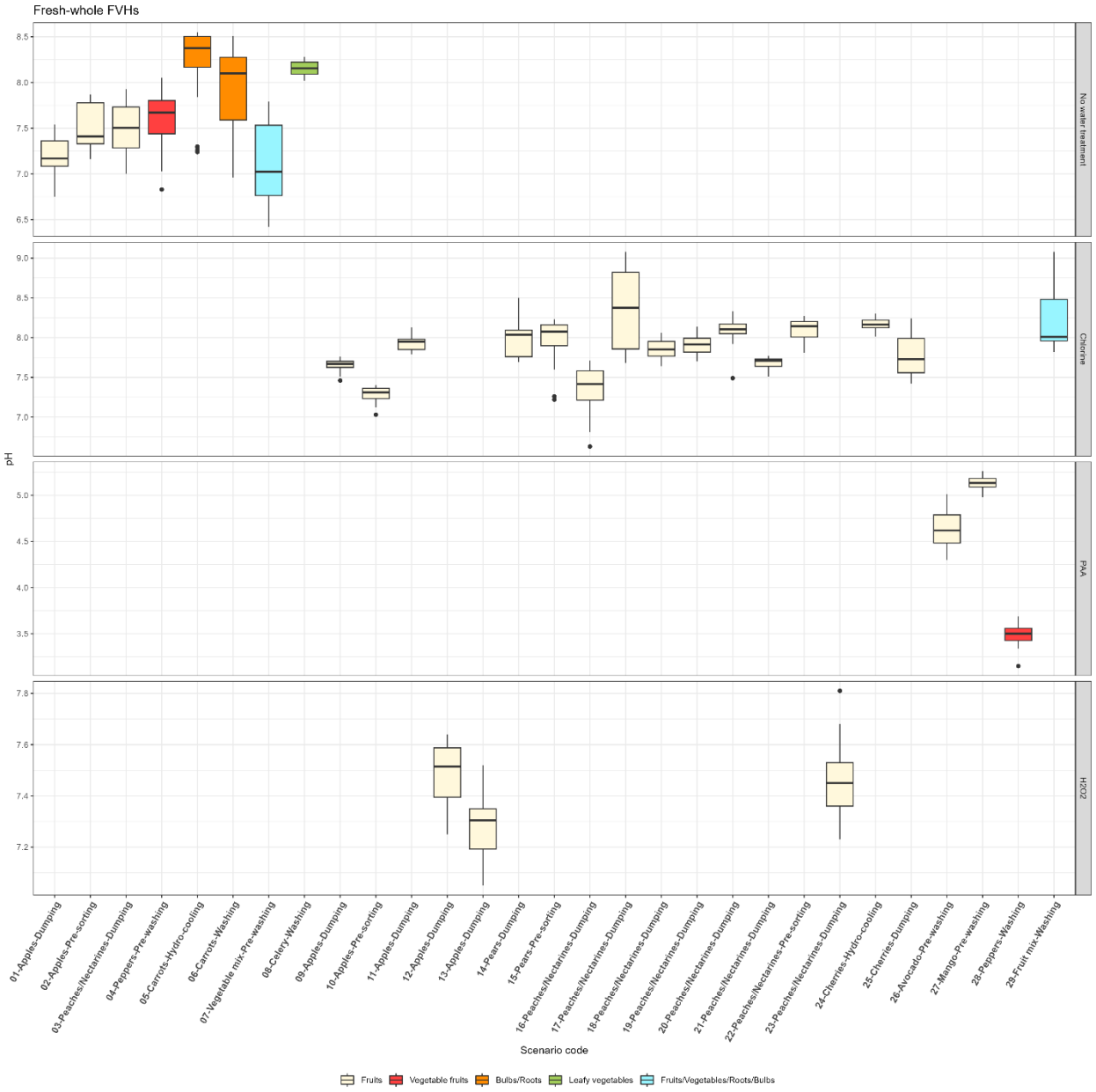


Figure 25. Boxplot graph that represents changes in pH of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

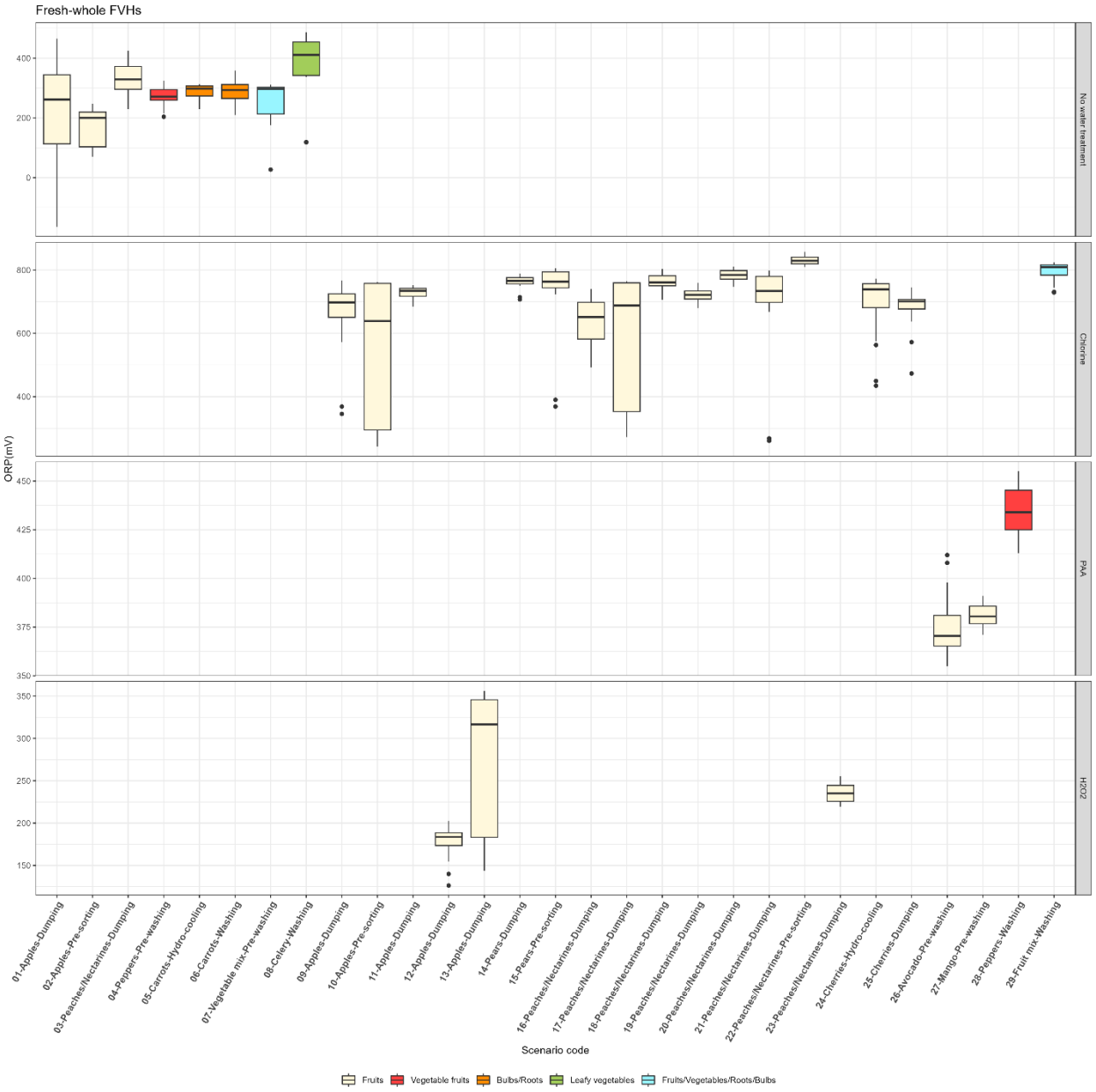


Figure 26. Boxplot graph that represents changes in oxidation-reduction potential (ORP) of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

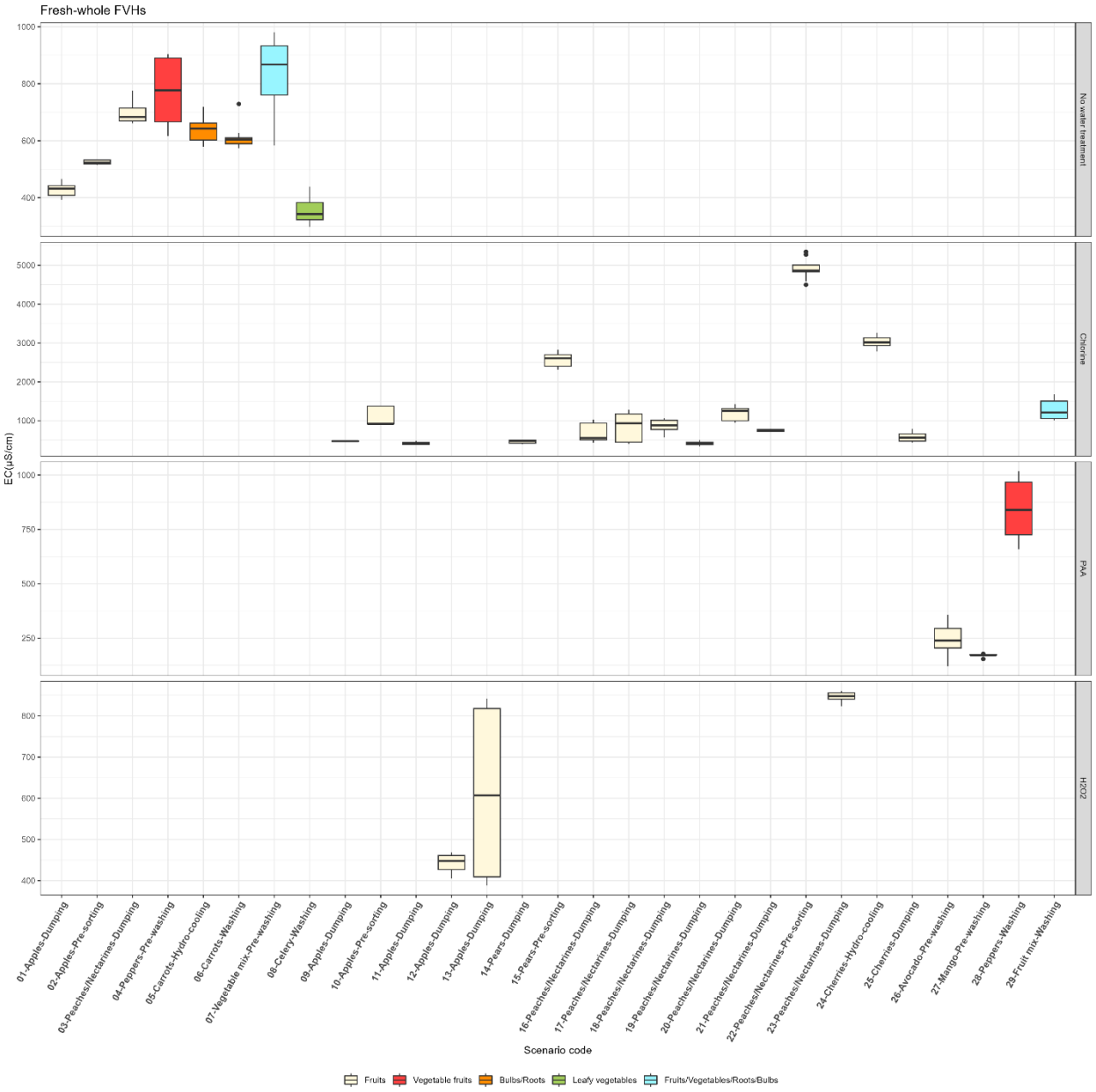


Figure 27. Boxplot graph that represents changes in electrical conductivity of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

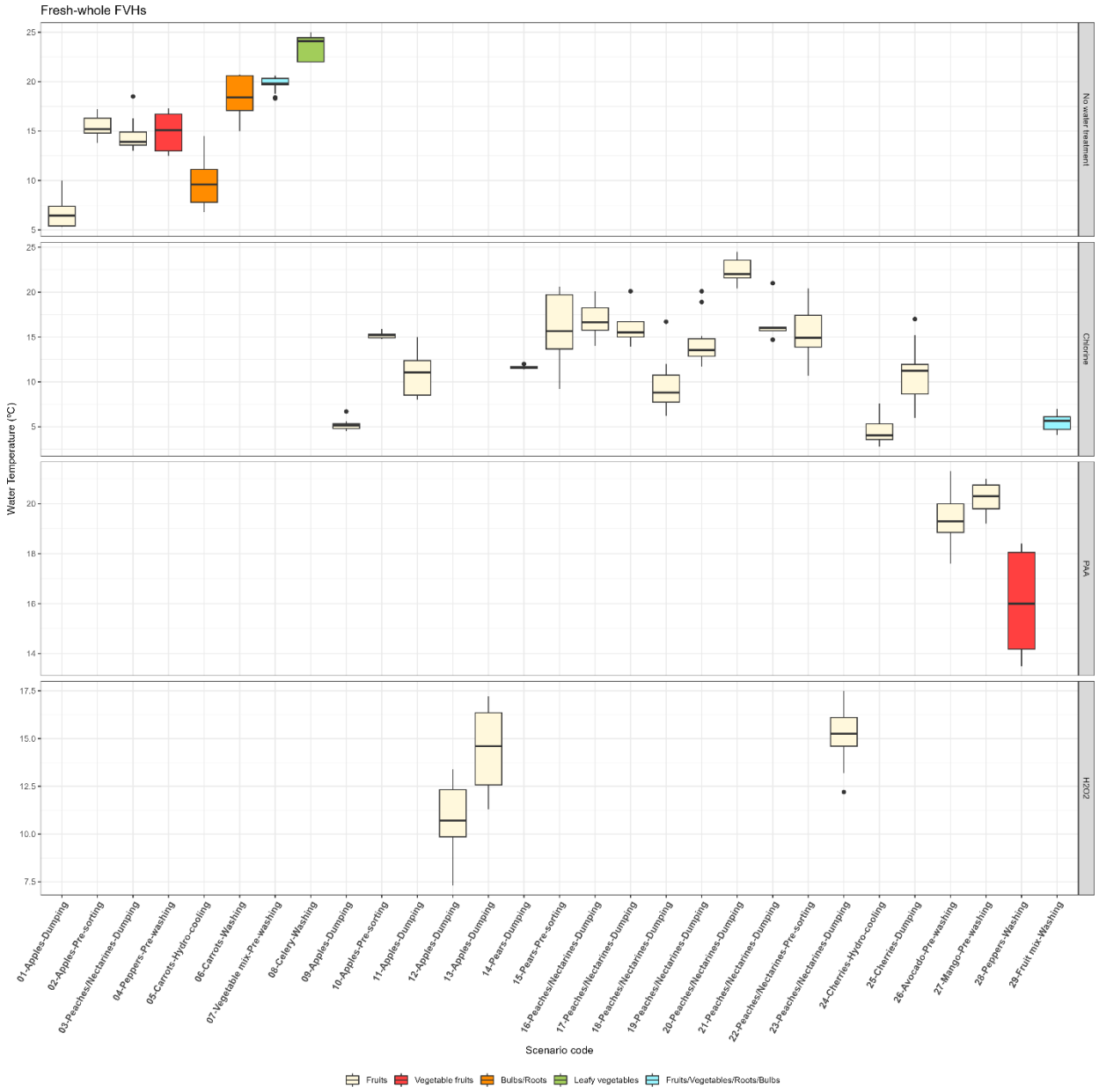


Figure 28. Boxplot graph that represents changes in temperature of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

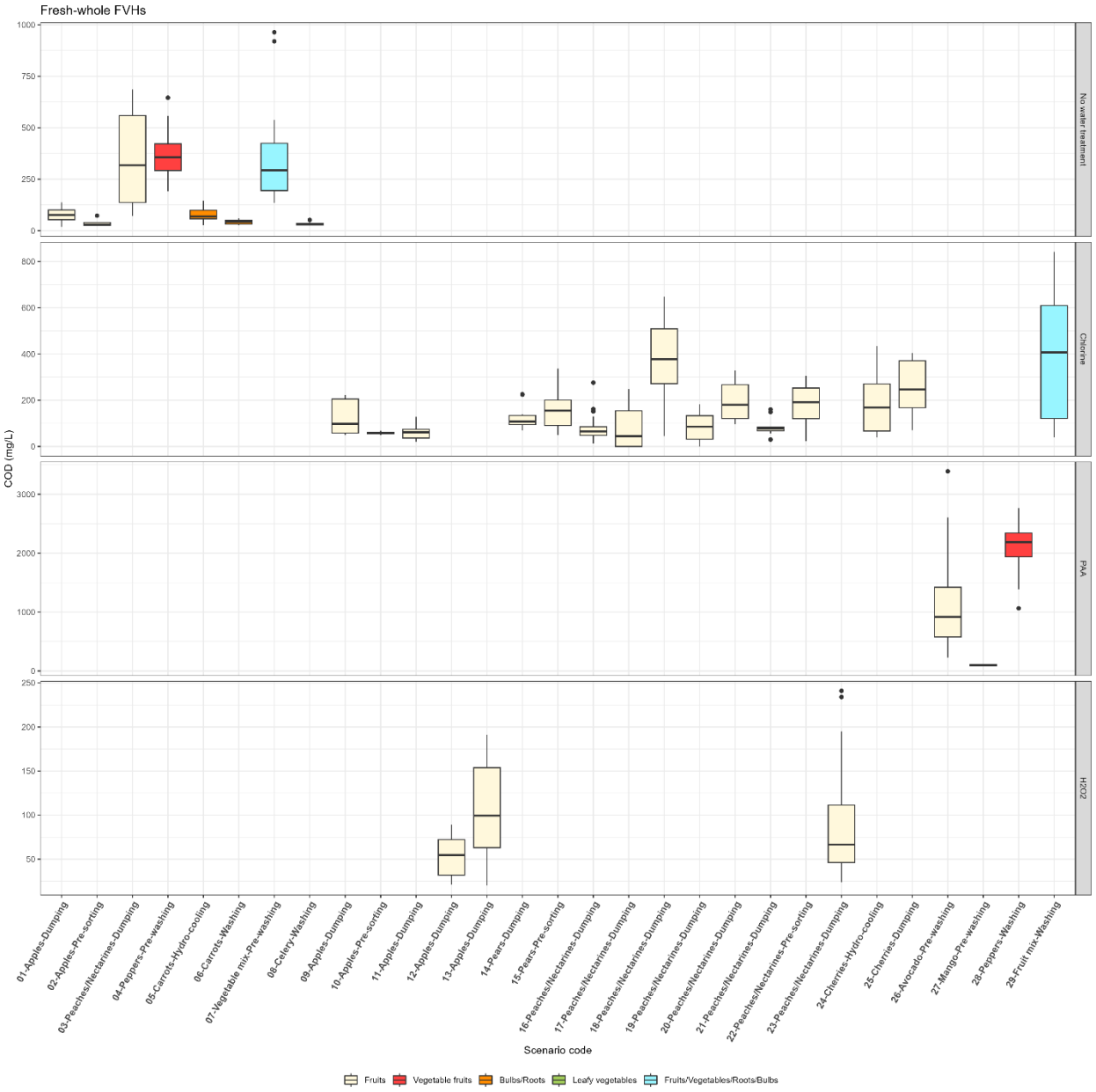


Figure 29. Boxplot graph that represents changes in chemical oxygen demand (COD) of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

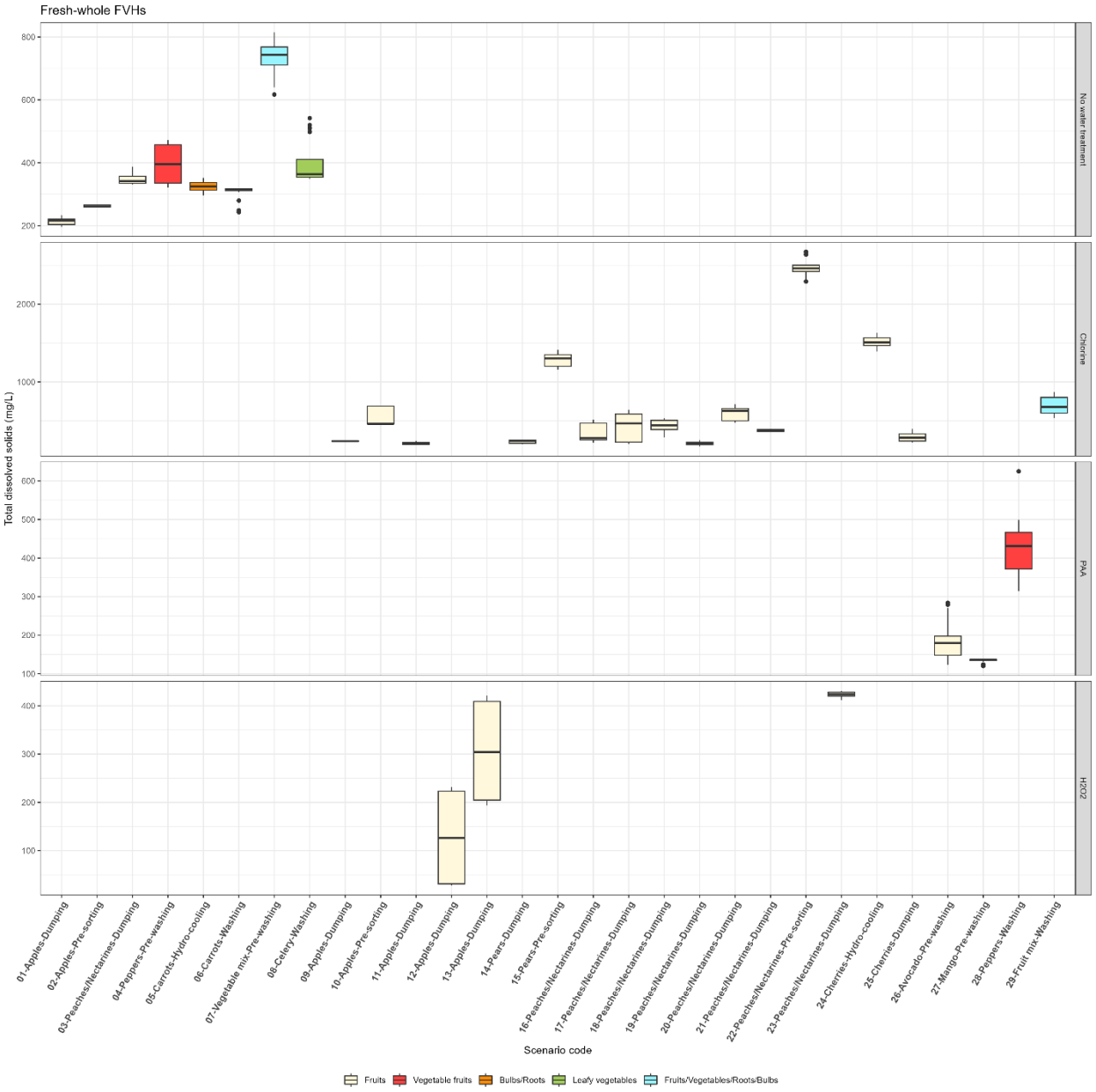


Figure 30. Boxplot graph that represents changes in total dissolved solids (TDS) of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

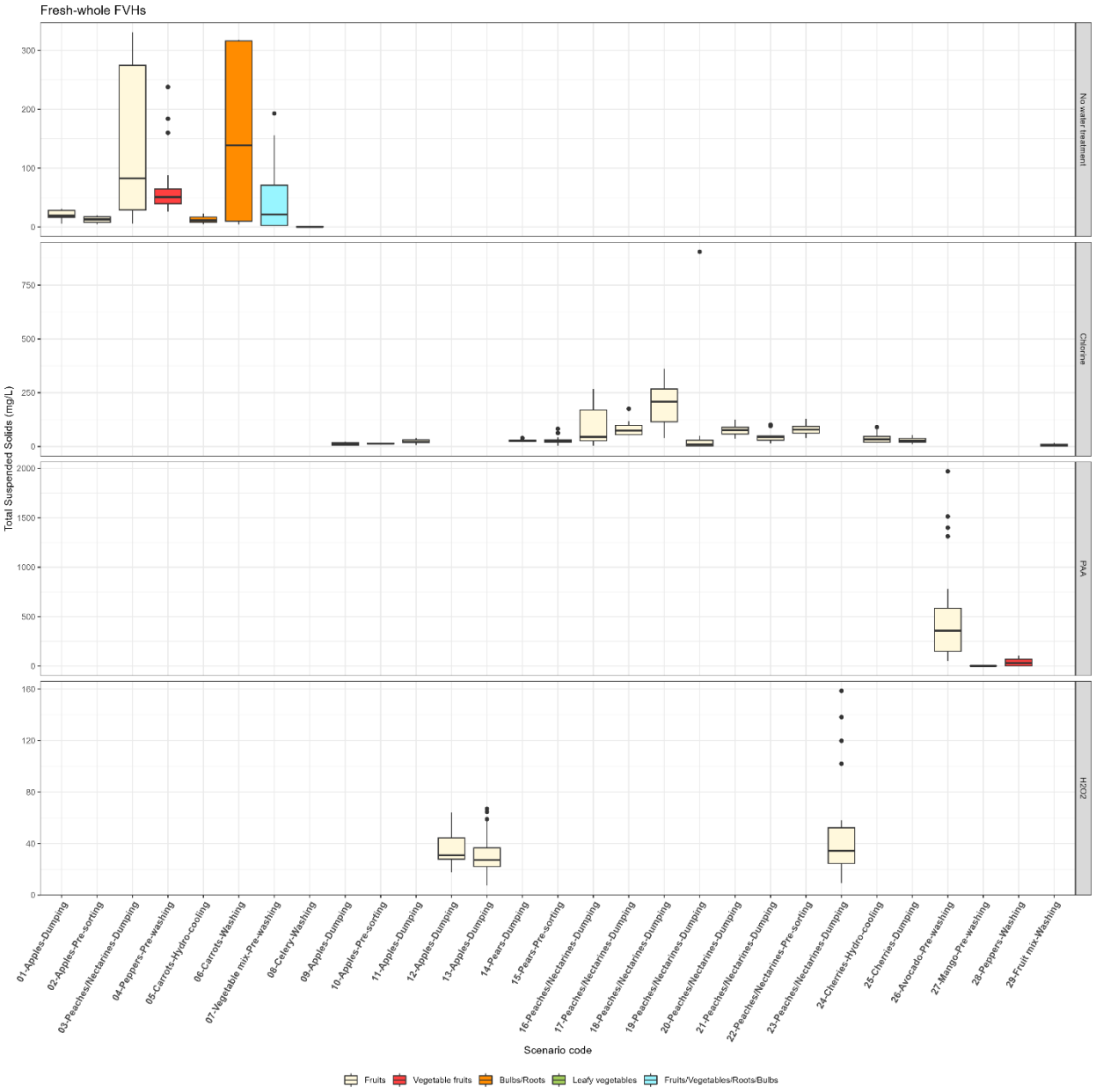


Figure 31. Boxplot graph that represents changes in total suspended solids (TSS) of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

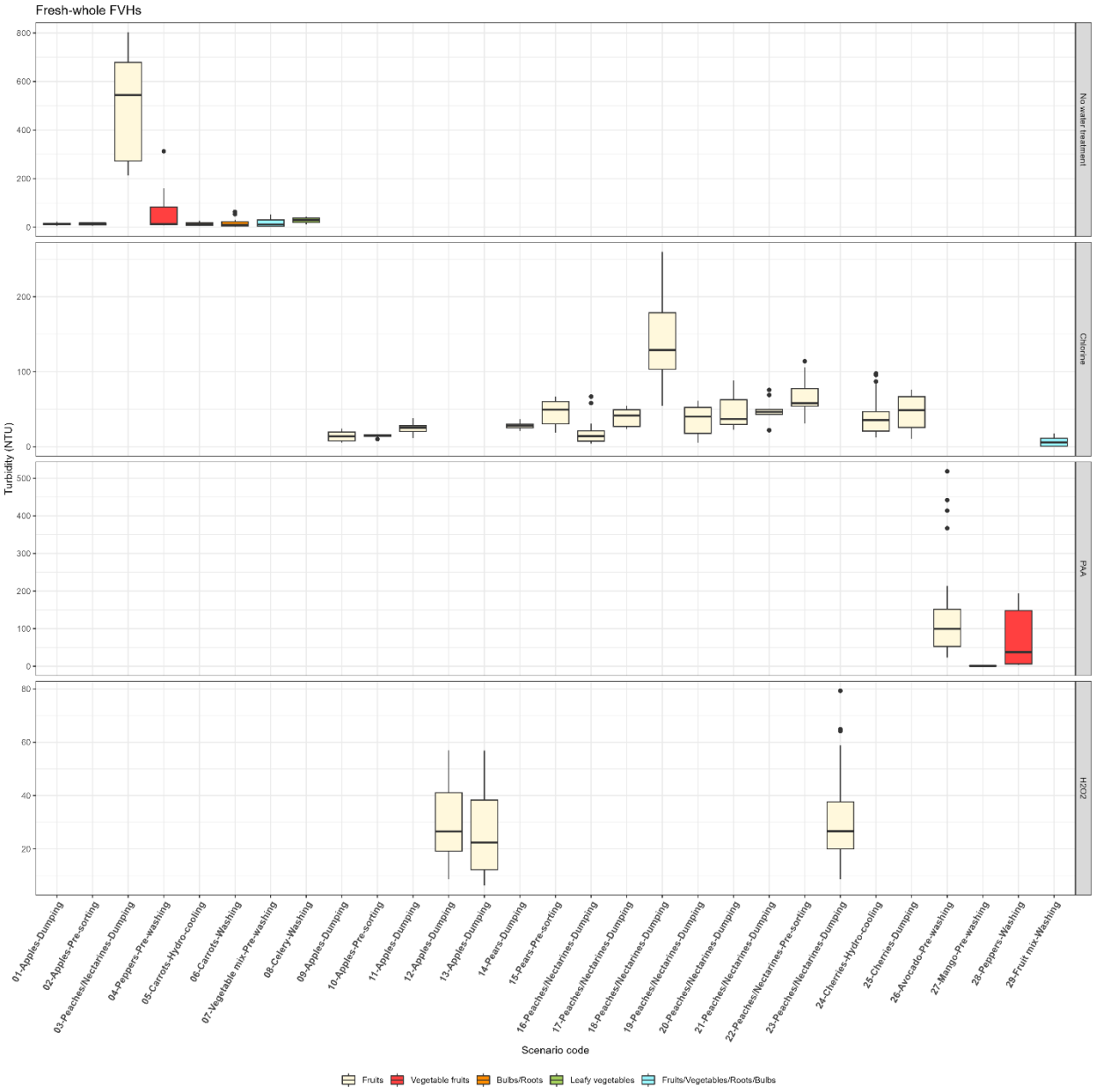


Figure 32. Boxplot graph that represents changes in turbidity of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

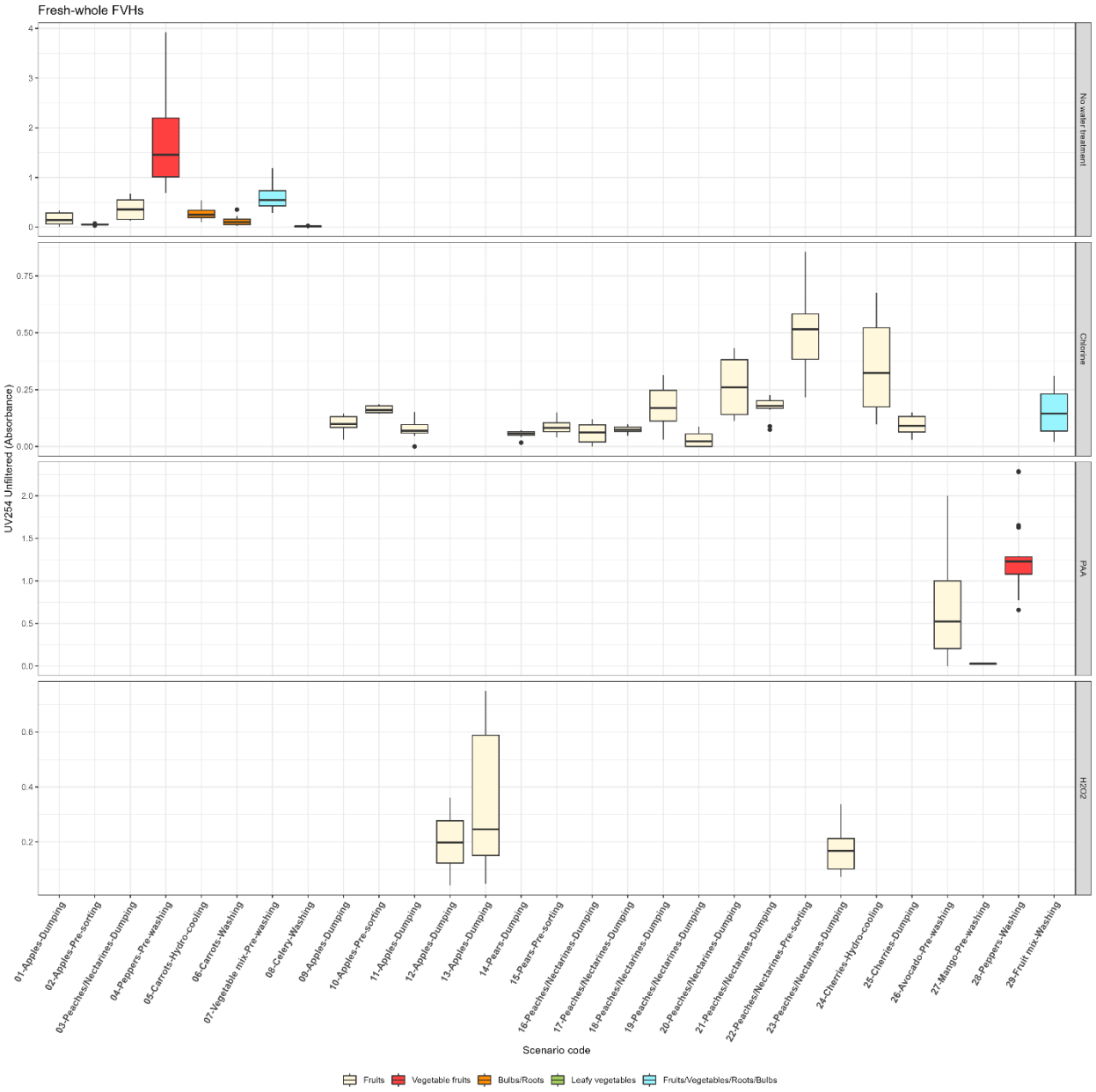


Figure 33. Boxplot graph that represents changes in UV254 unfiltered absorbance of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

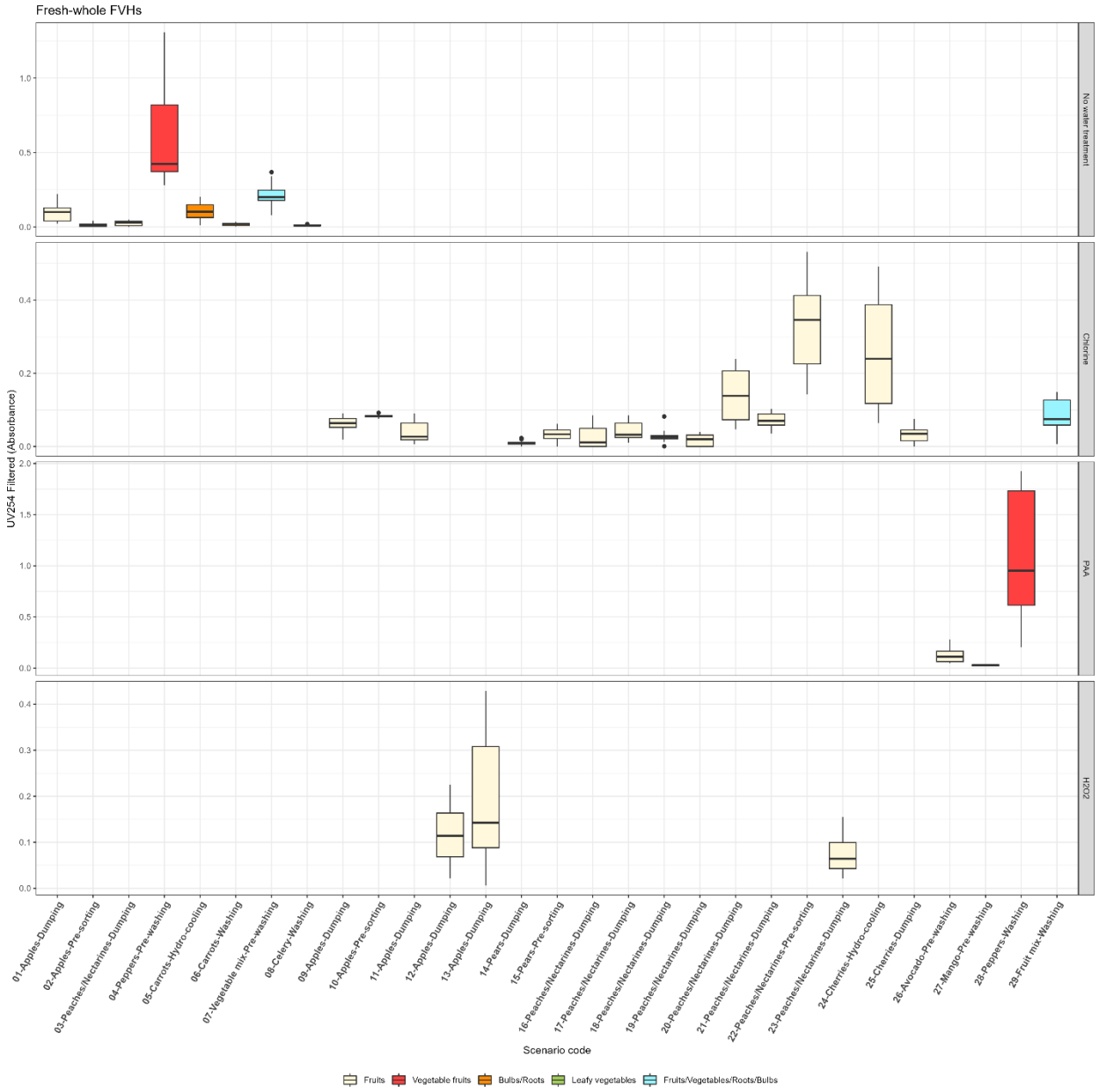


Figure 34. Boxplot graph that represents changes in UV254 filtered absorbance of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

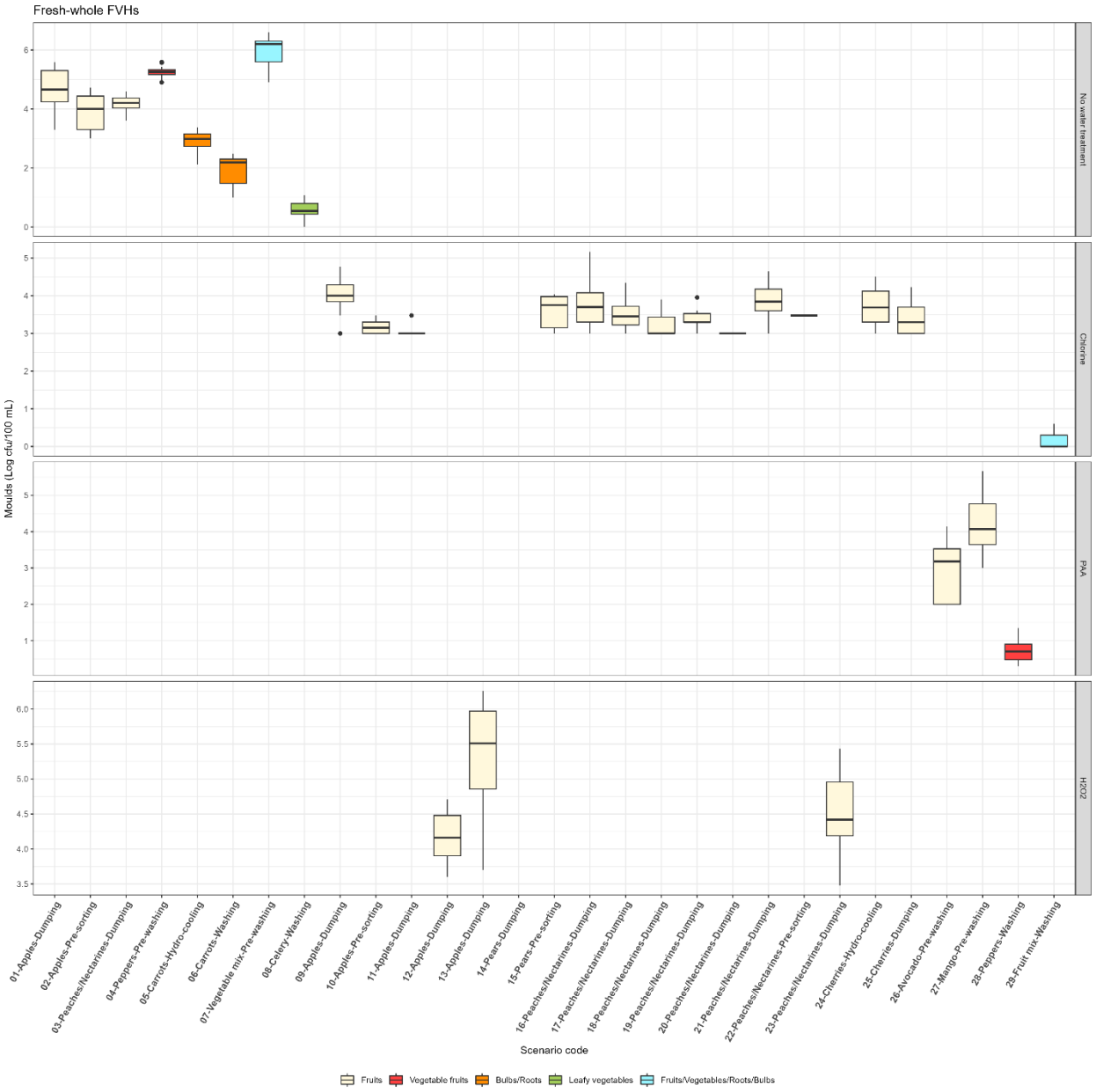


Figure 35. Boxplot graph that represents changes in total mould counts of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

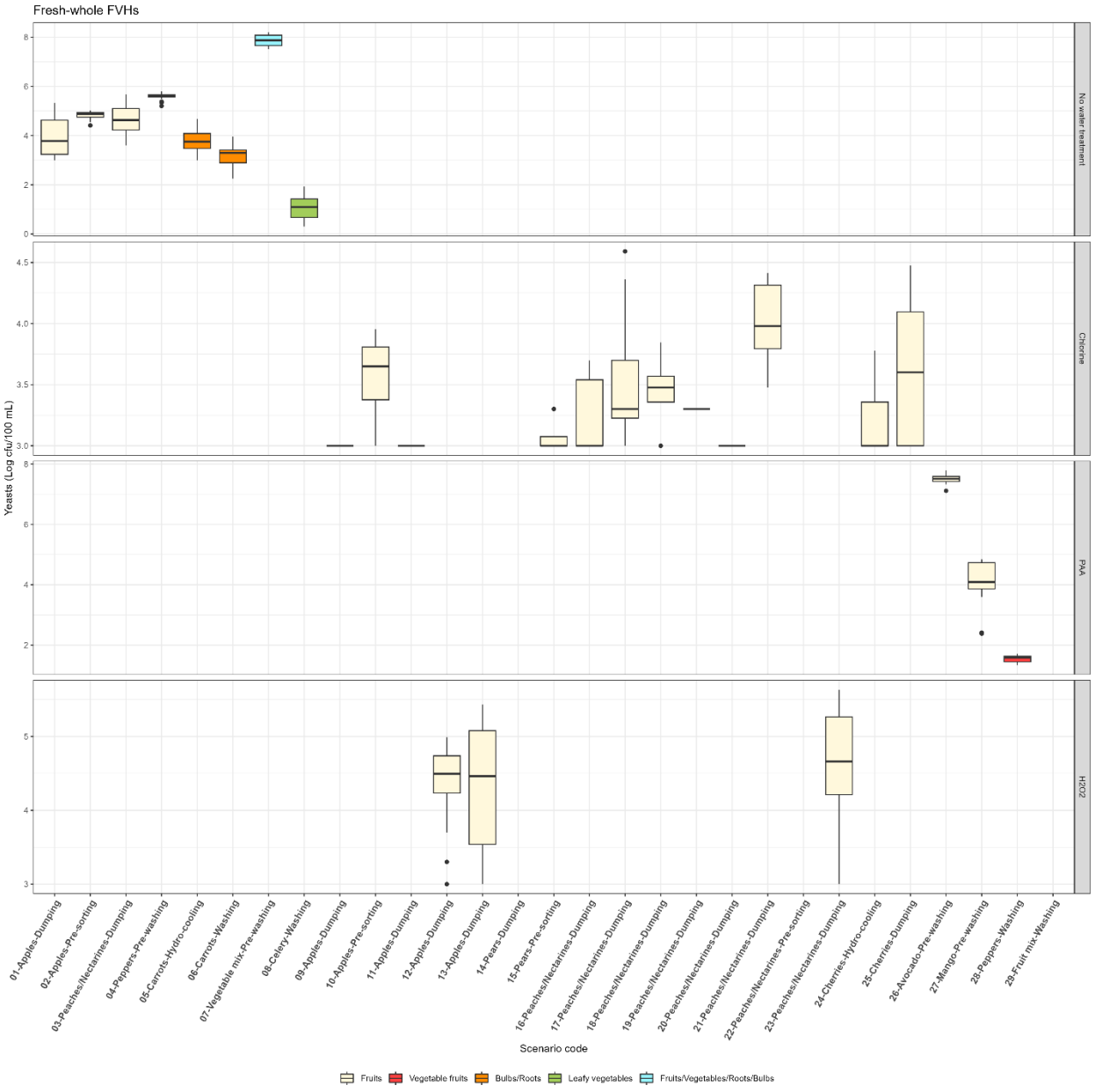


Figure 36. Boxplot graph that represents changes in total yeast counts of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

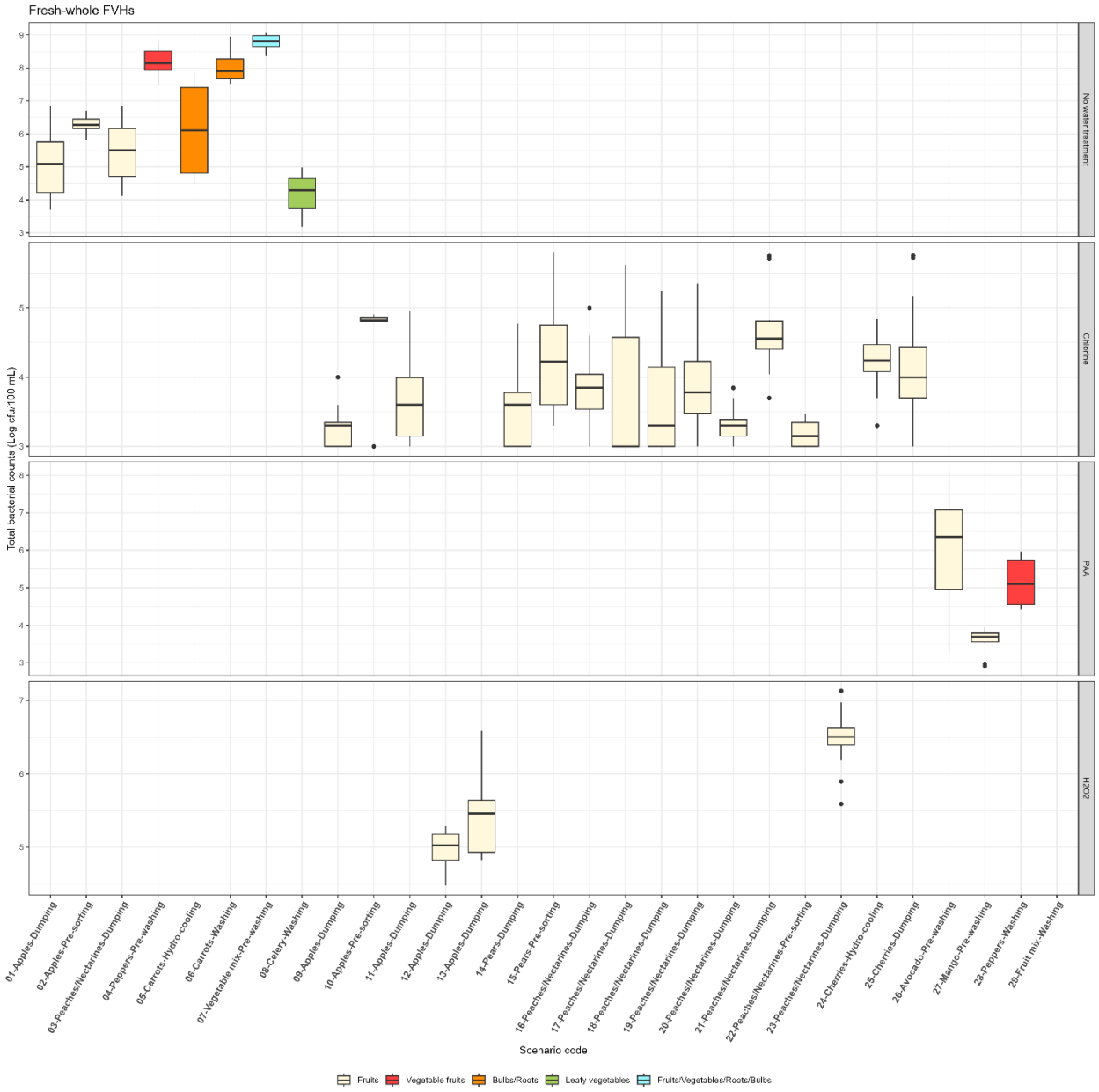


Figure 37. Boxplot graph that represents changes in total bacterial counts of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

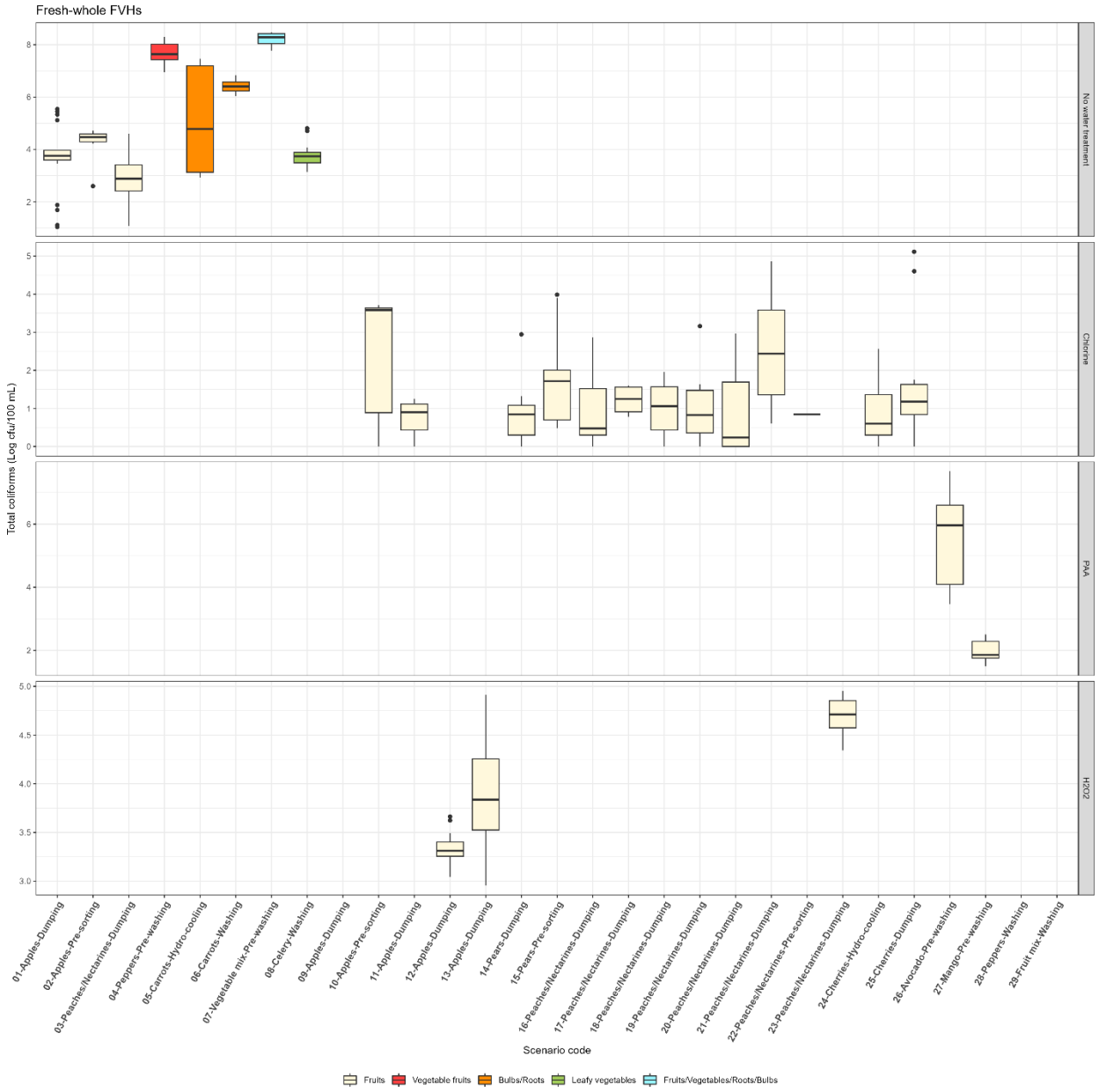


Figure 38. Boxplot graph that represents changes in total coliform counts of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

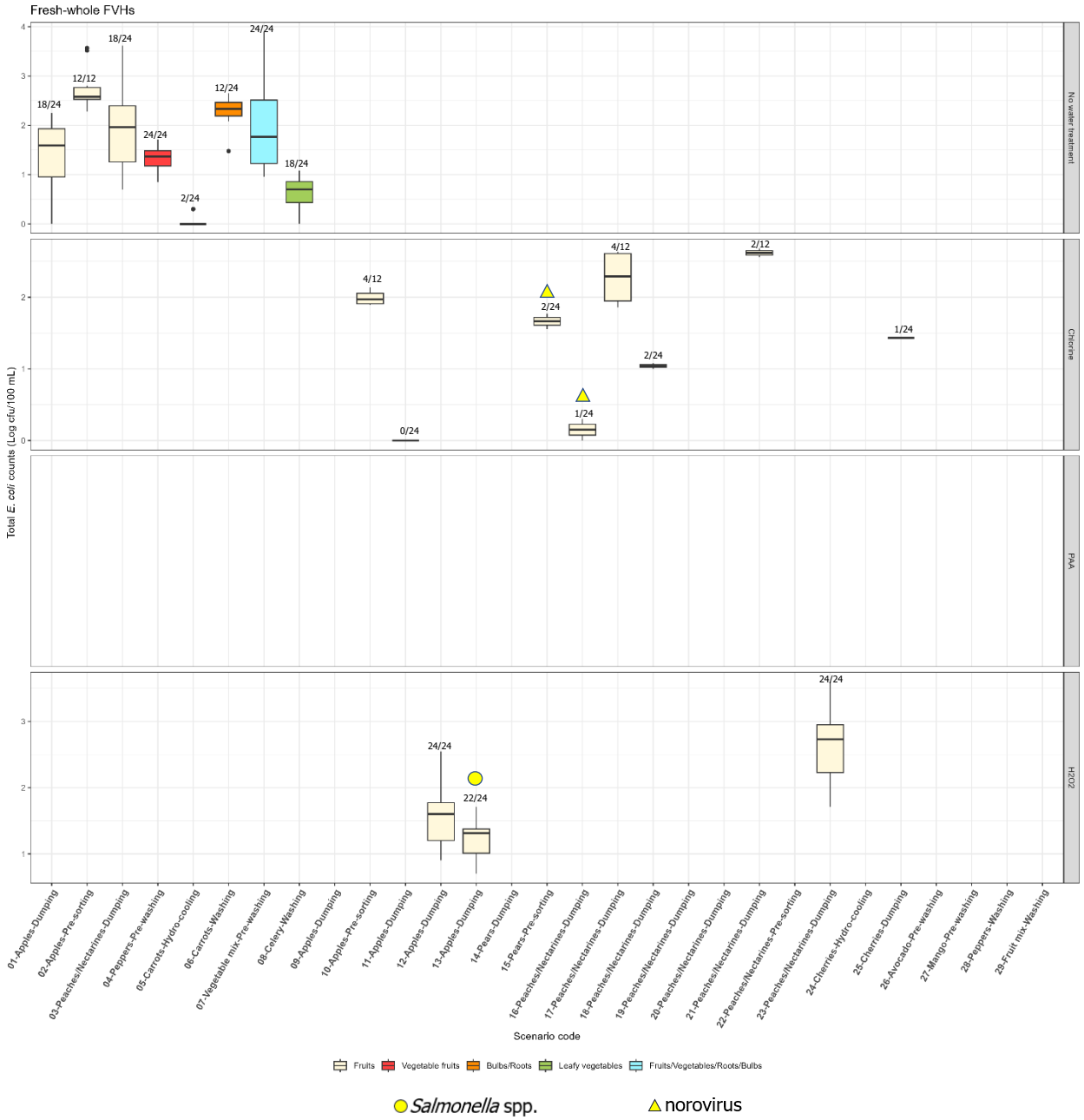


Figure 39. Boxplot graph that represents changes in the total *E. coli* counts of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

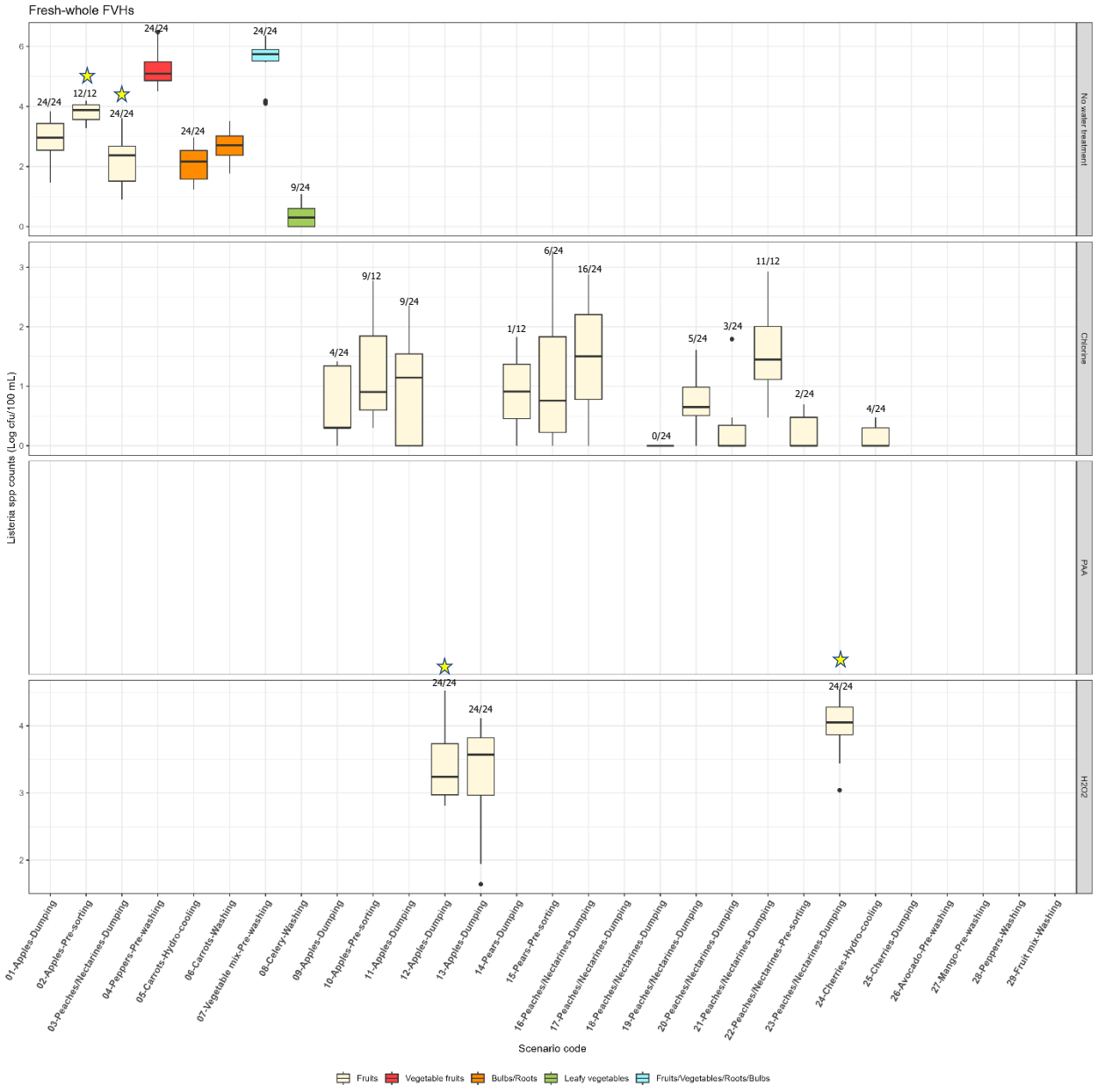


Figure 40. Boxplot graph that represents changes in *Listeria* spp. counts of process water throughout the sampling period across different scenarios of the fresh-whole FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

3.2.2 Fresh-cut FVHs

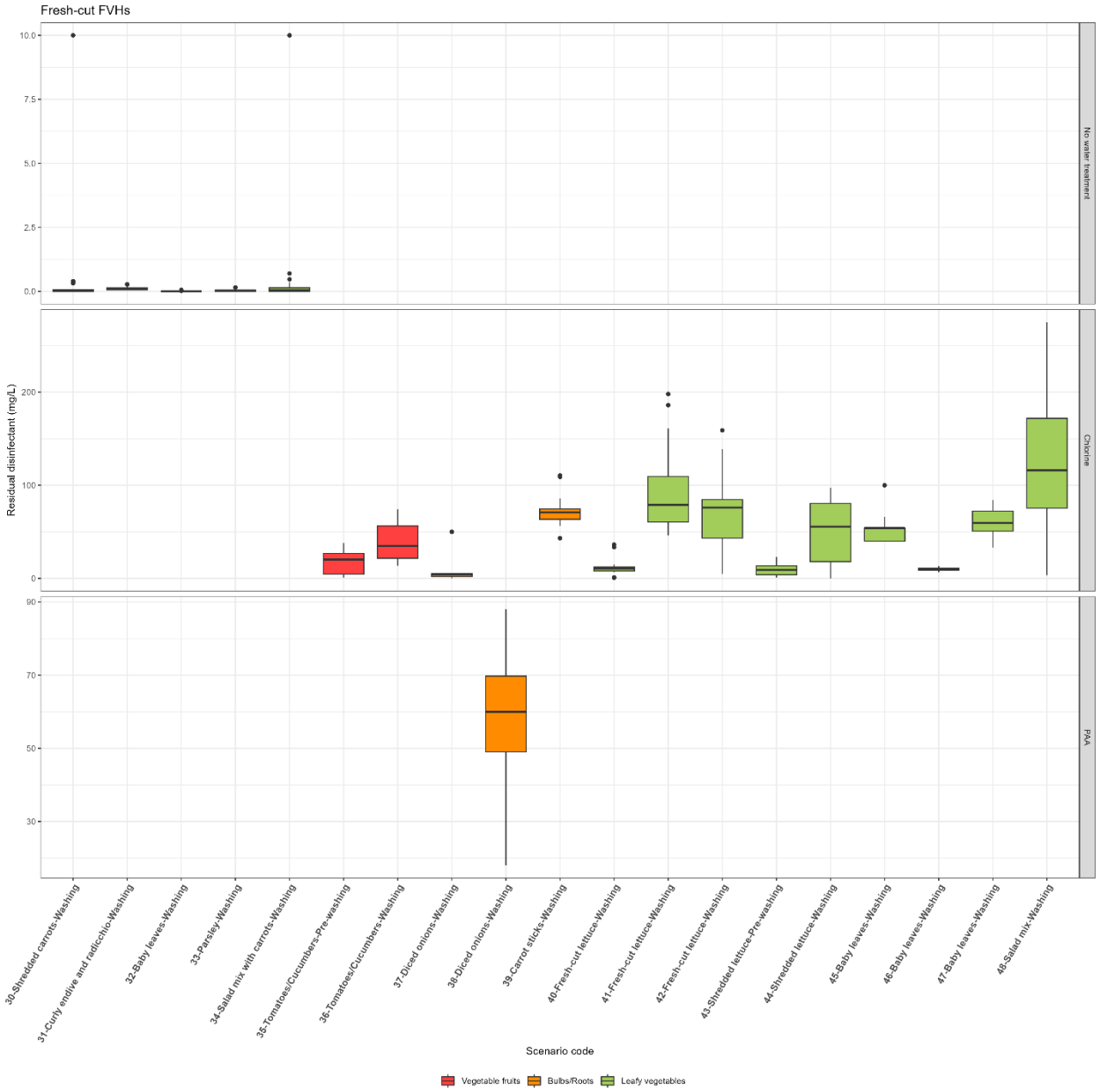


Figure 41. Boxplot graph that represents changes in residual disinfectant concentration of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

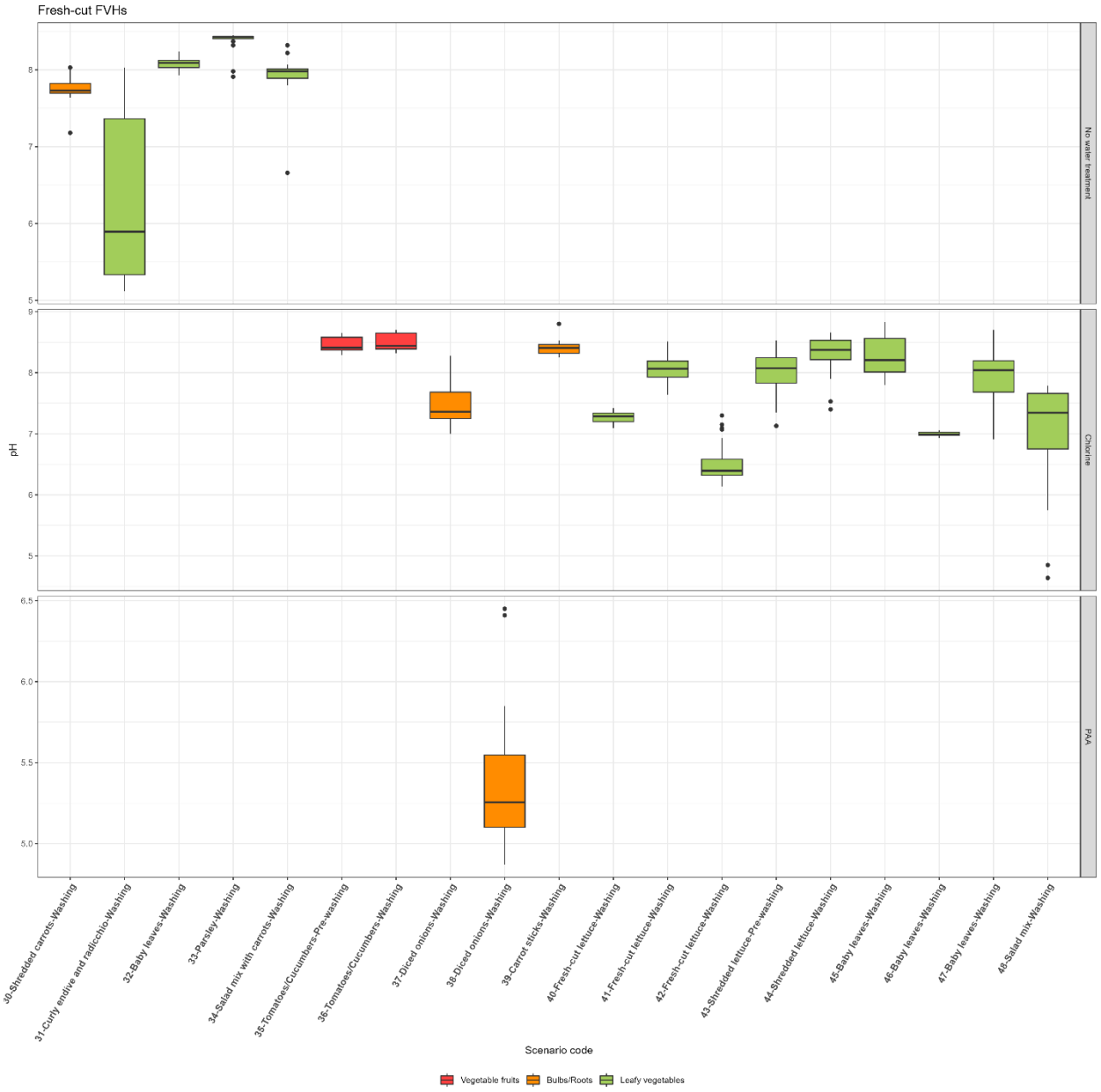


Figure 42. Boxplot graph that represents changes in pH of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

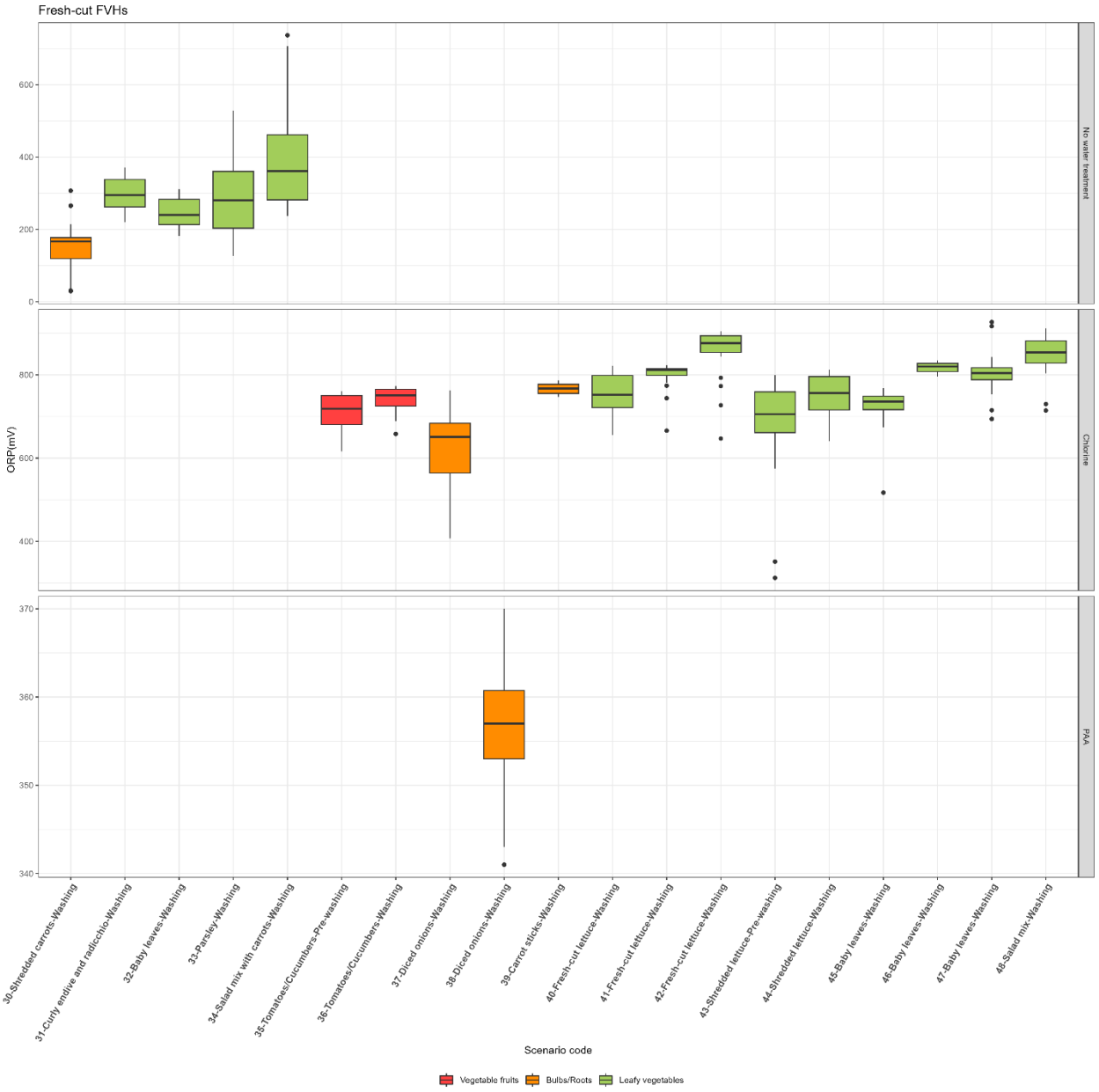


Figure 43. Boxplot graph that represents changes in oxidation-reduction potential (ORP) of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

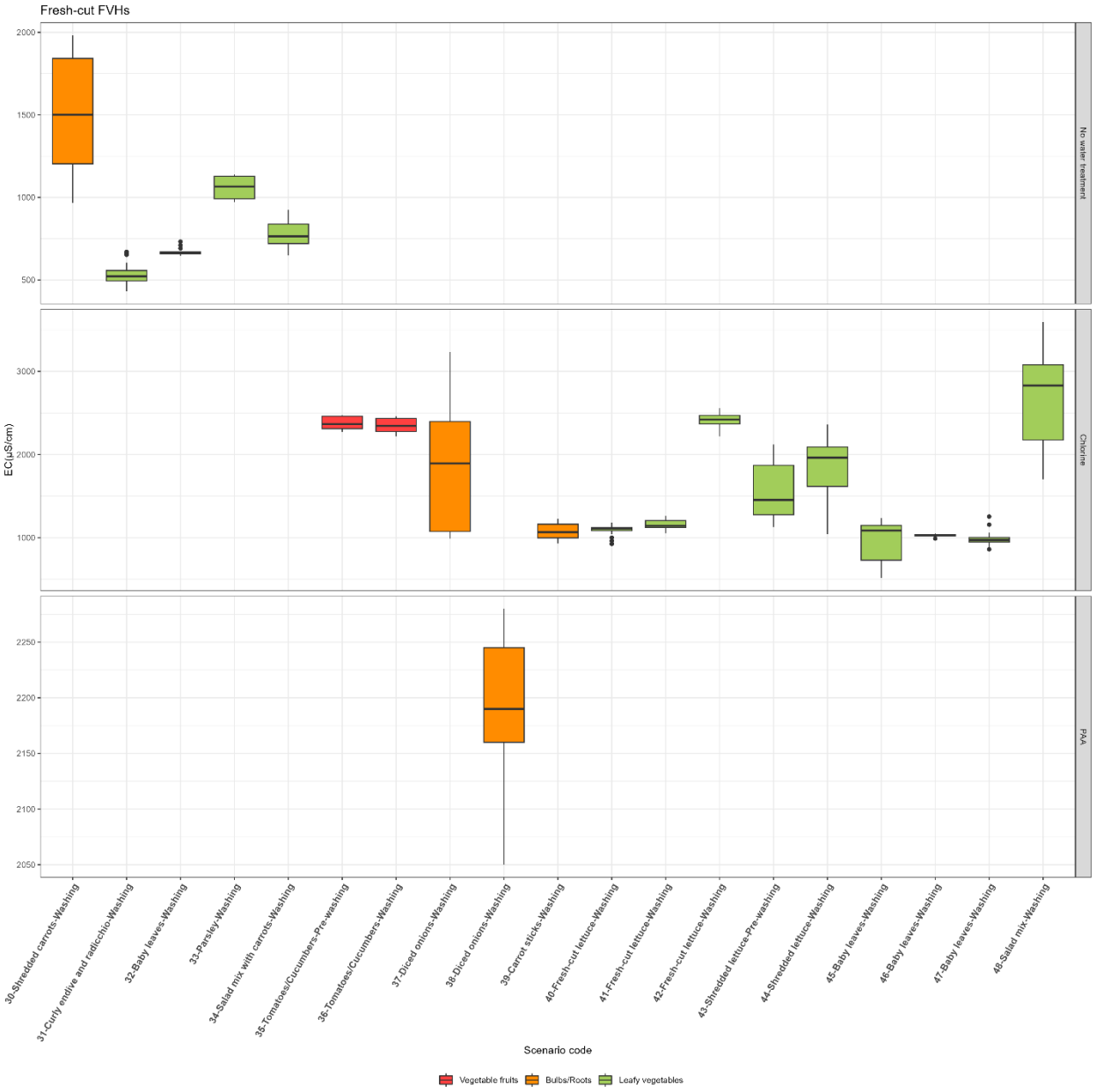


Figure 44. Boxplot graph that represents changes in electrical conductivity (EC) of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

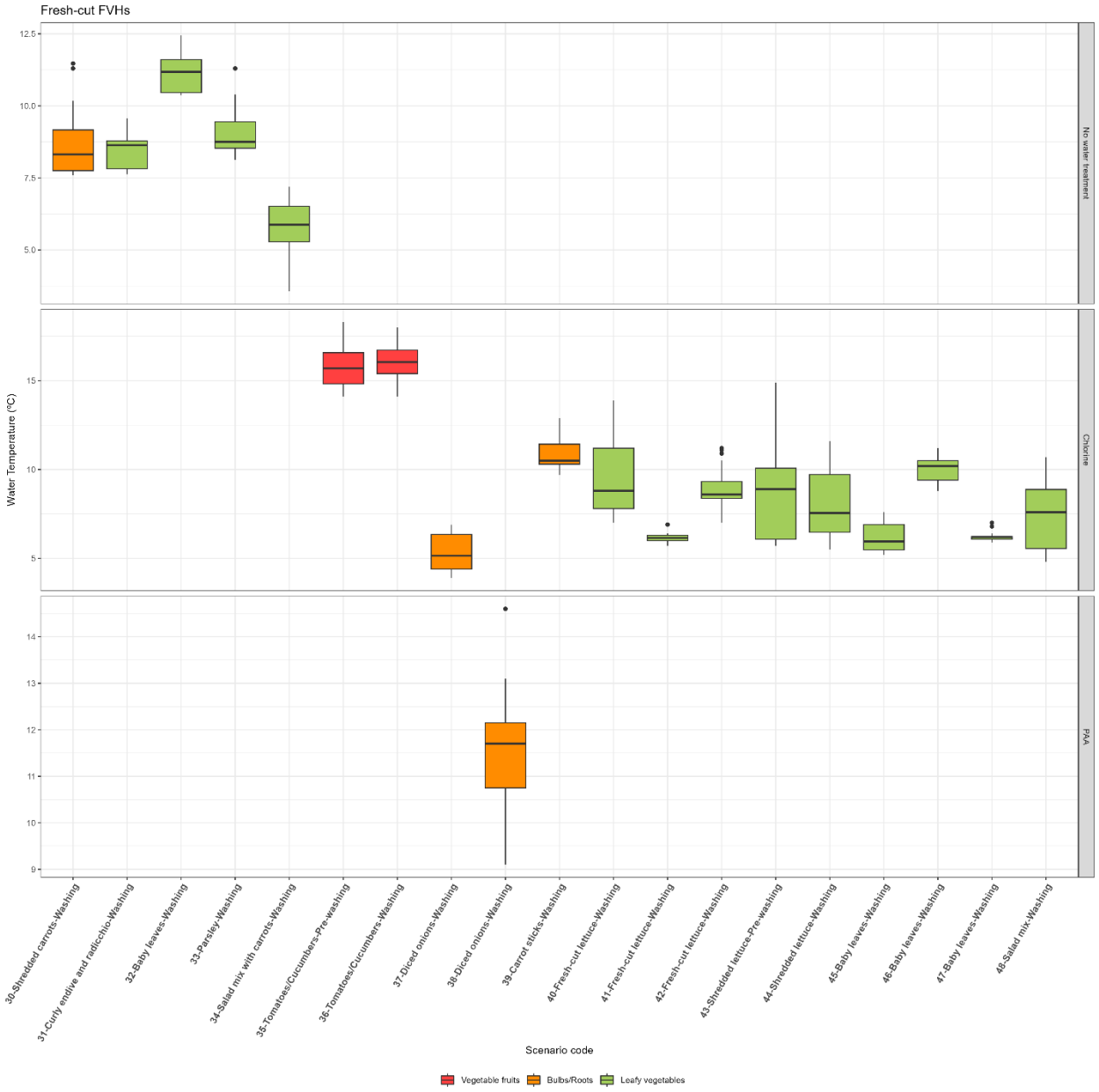


Figure 45. Boxplot graph that represents changes in temperature of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

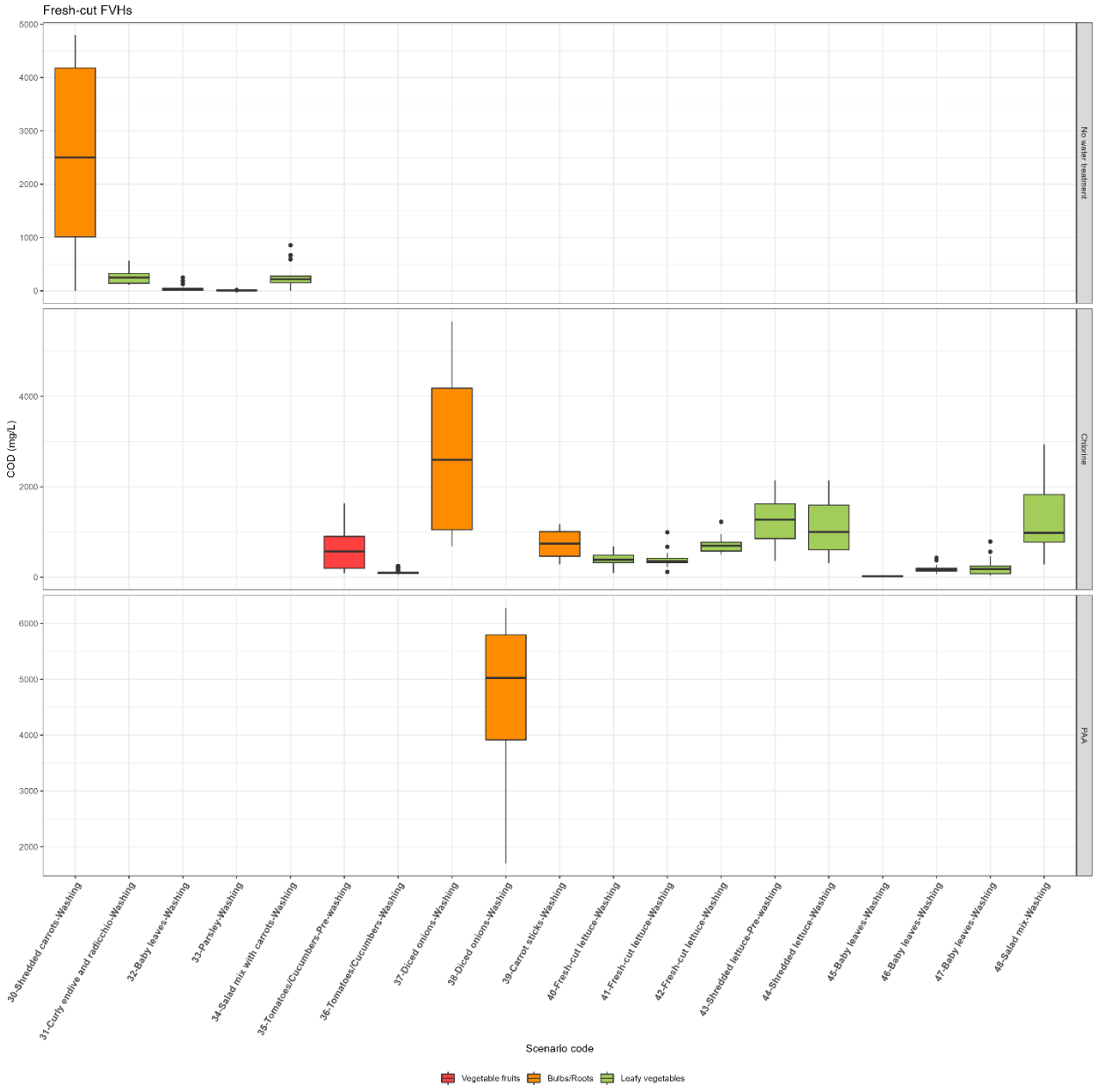


Figure 46. Boxplot graph that represents changes in chemical oxygen demand (COD) of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

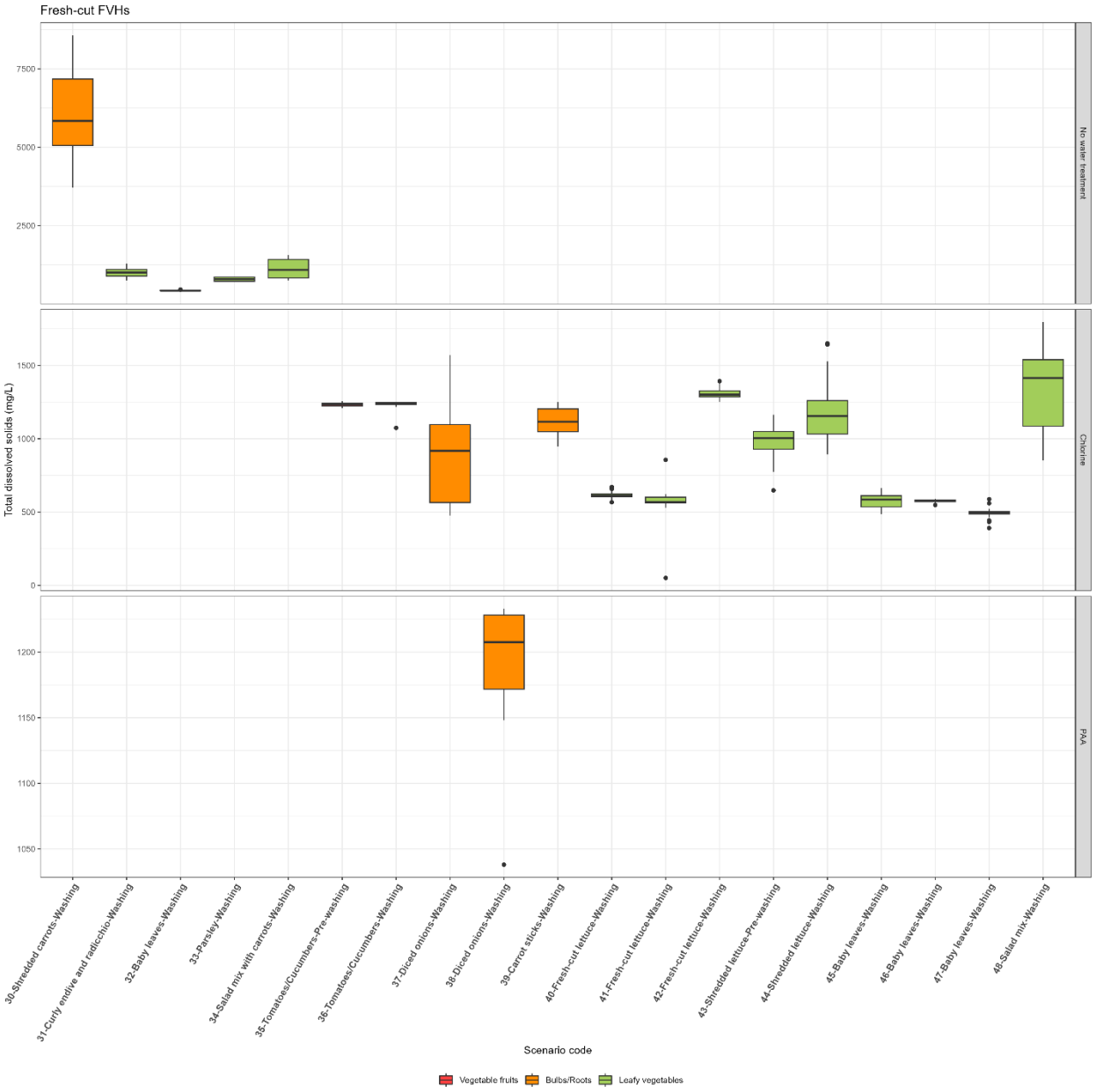


Figure 47. Boxplot graph that represents changes in total dissolved solids (TDS) of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

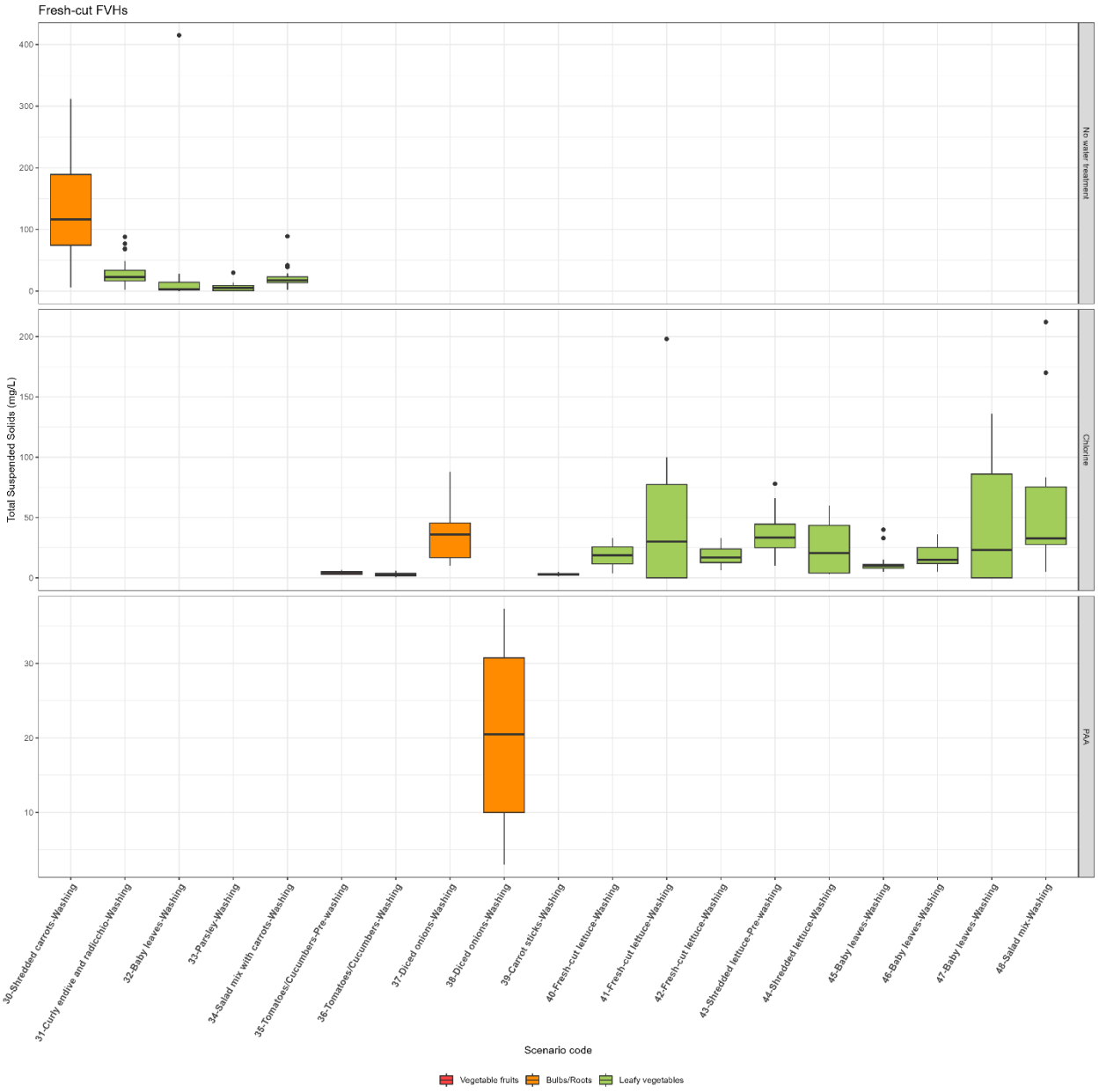


Figure 48. Boxplot graph that represents changes in total suspended solids (TSS) of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

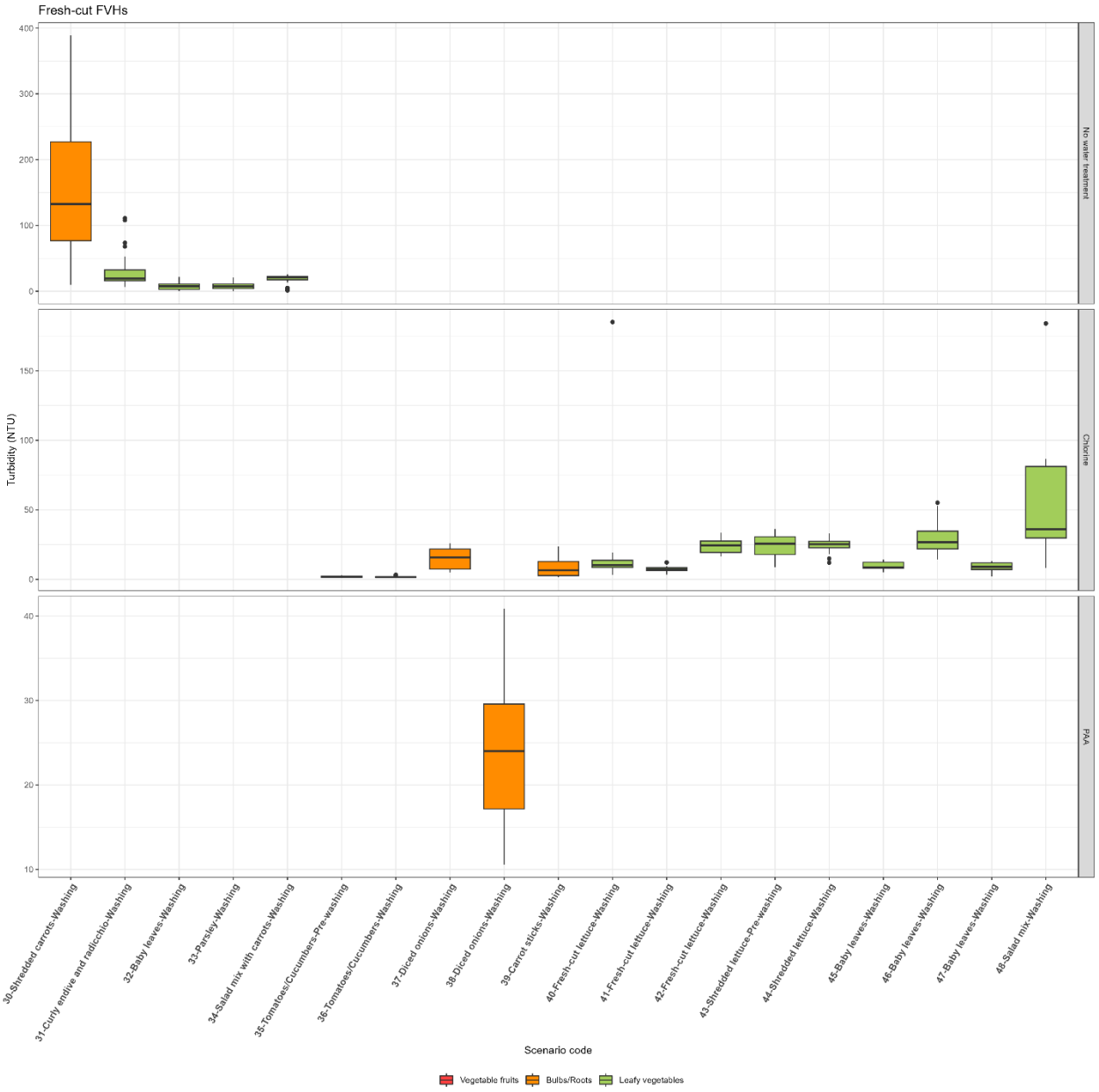


Figure 49. Boxplot graph that represents changes in turbidity of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

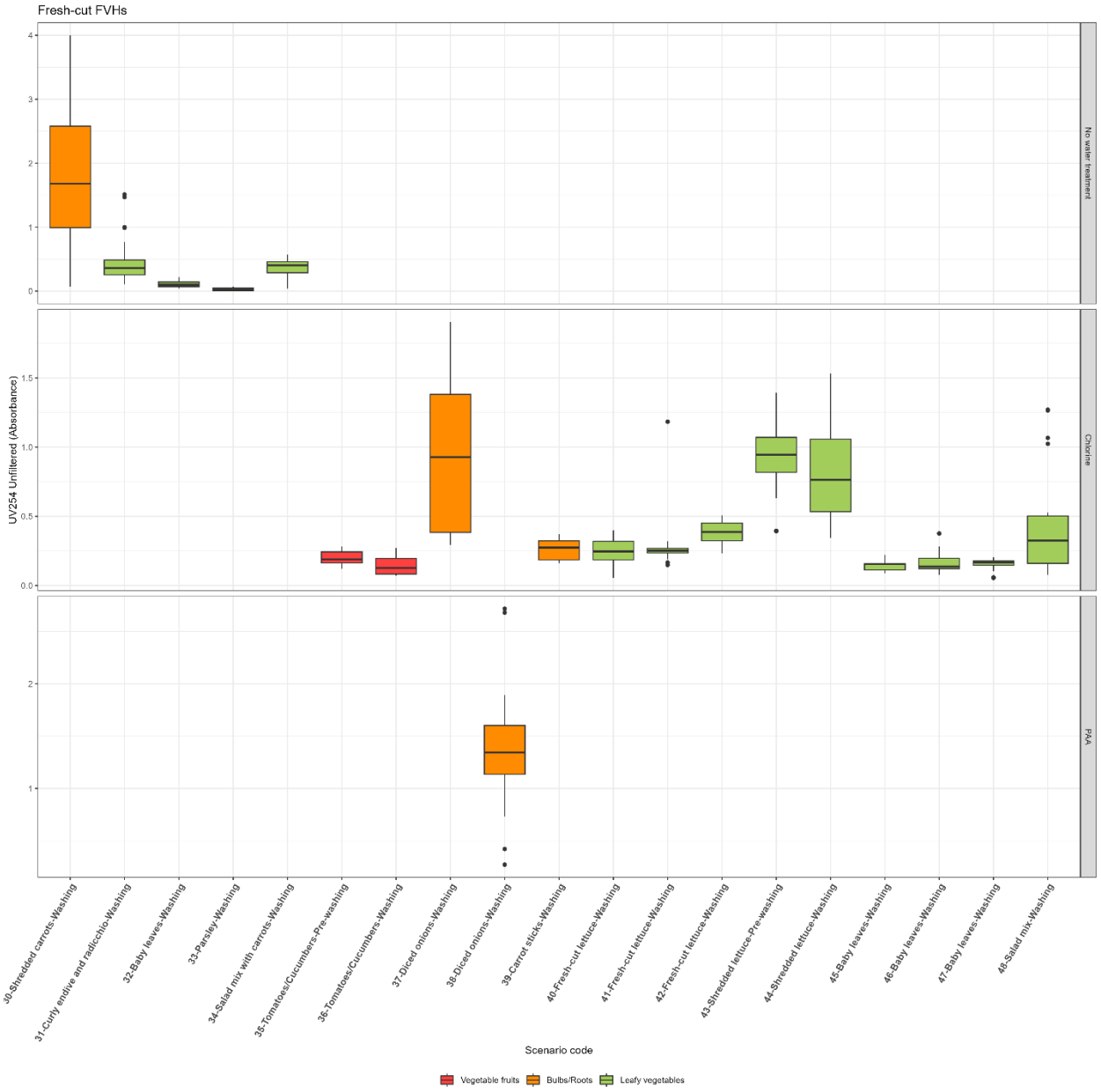


Figure 50. Boxplot graph that represents changes in UV254 unfiltered absorbance of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

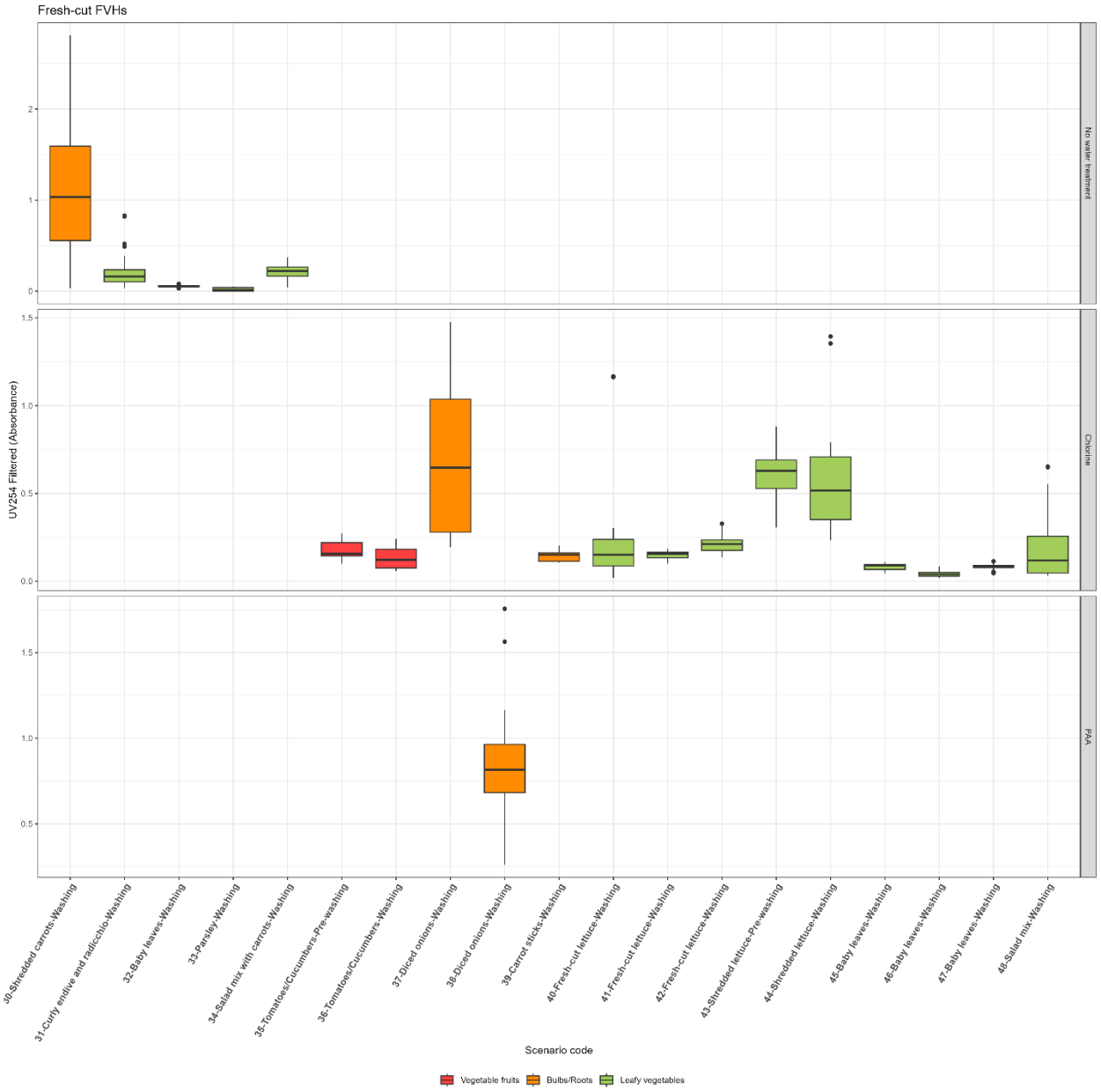


Figure 51. Boxplot graph that represents changes in UV254 filtered absorbance of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

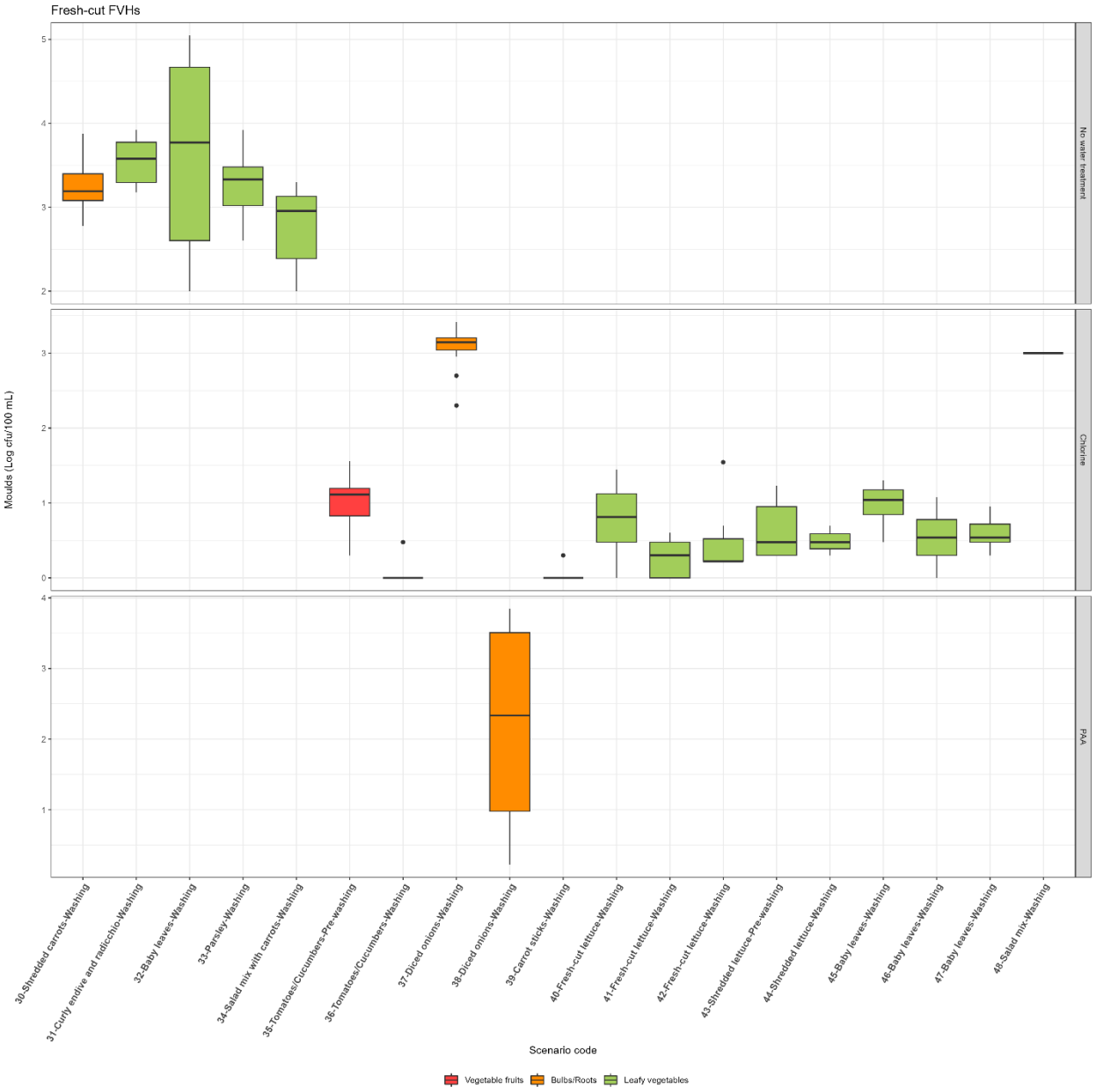


Figure 52. Boxplot graph that represents changes in total mould counts of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

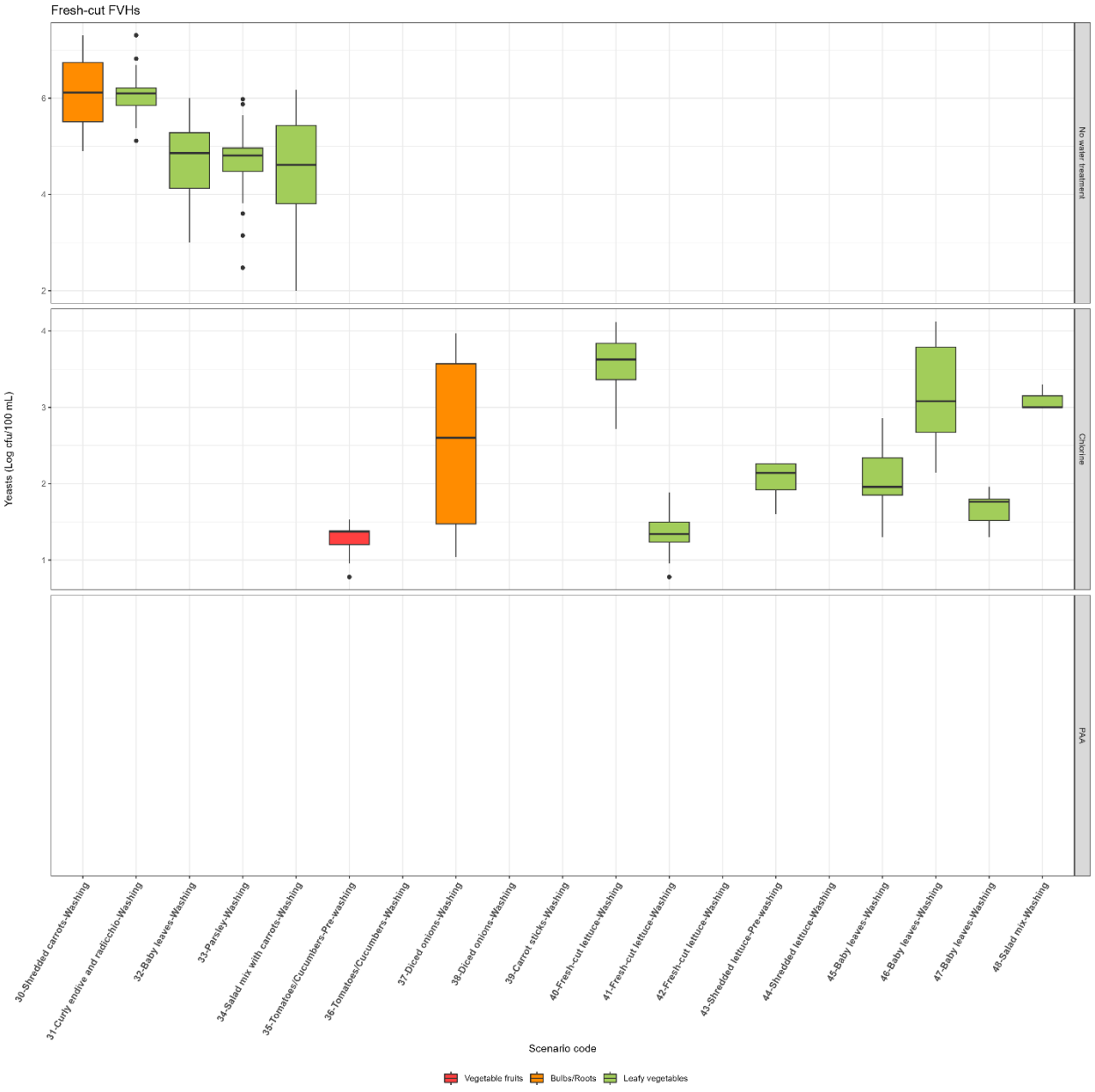


Figure 53. Boxplot graph that represents changes in total yeast counts of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

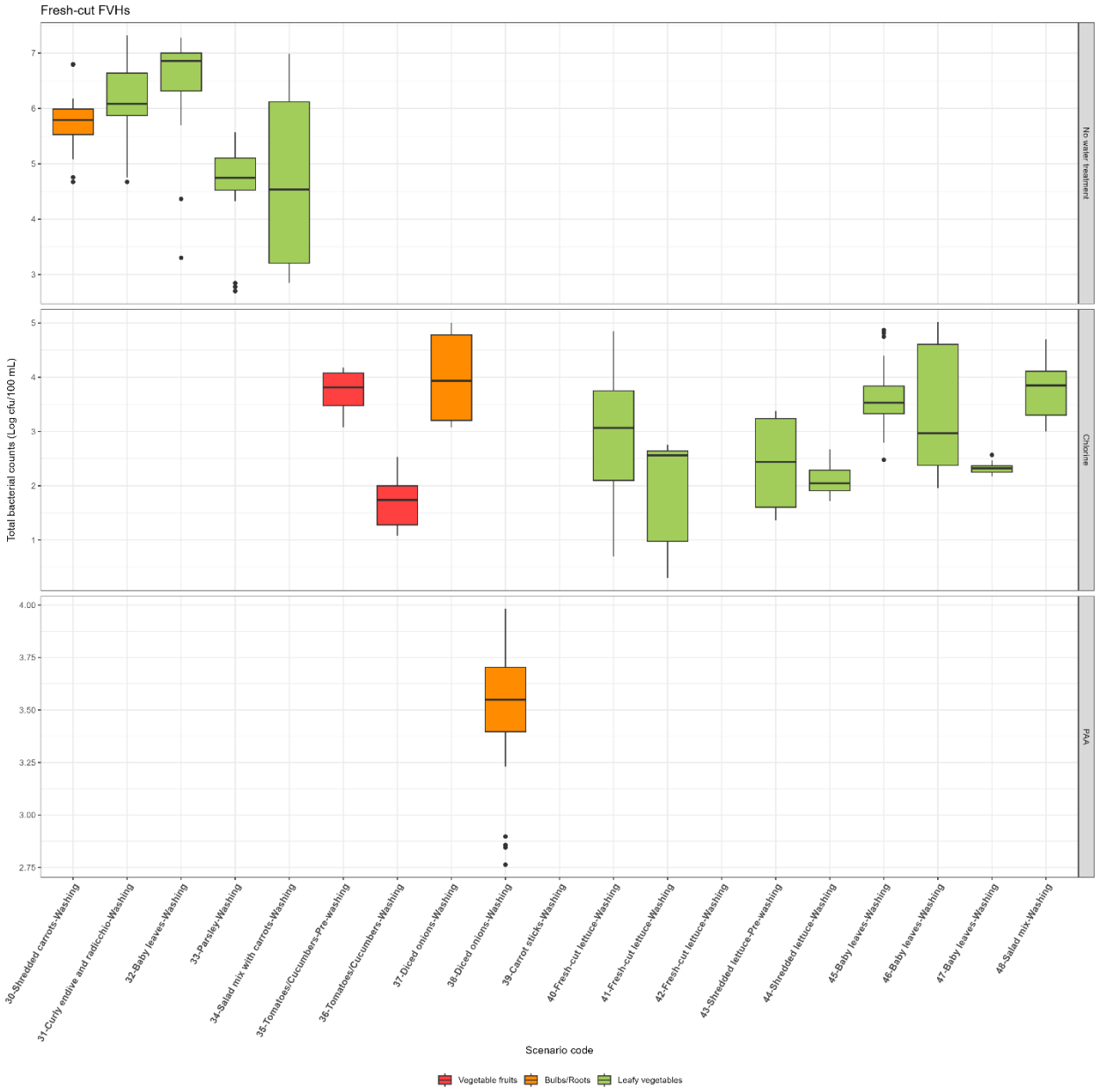


Figure 54. Boxplot graph that represents changes in total bacterial counts of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

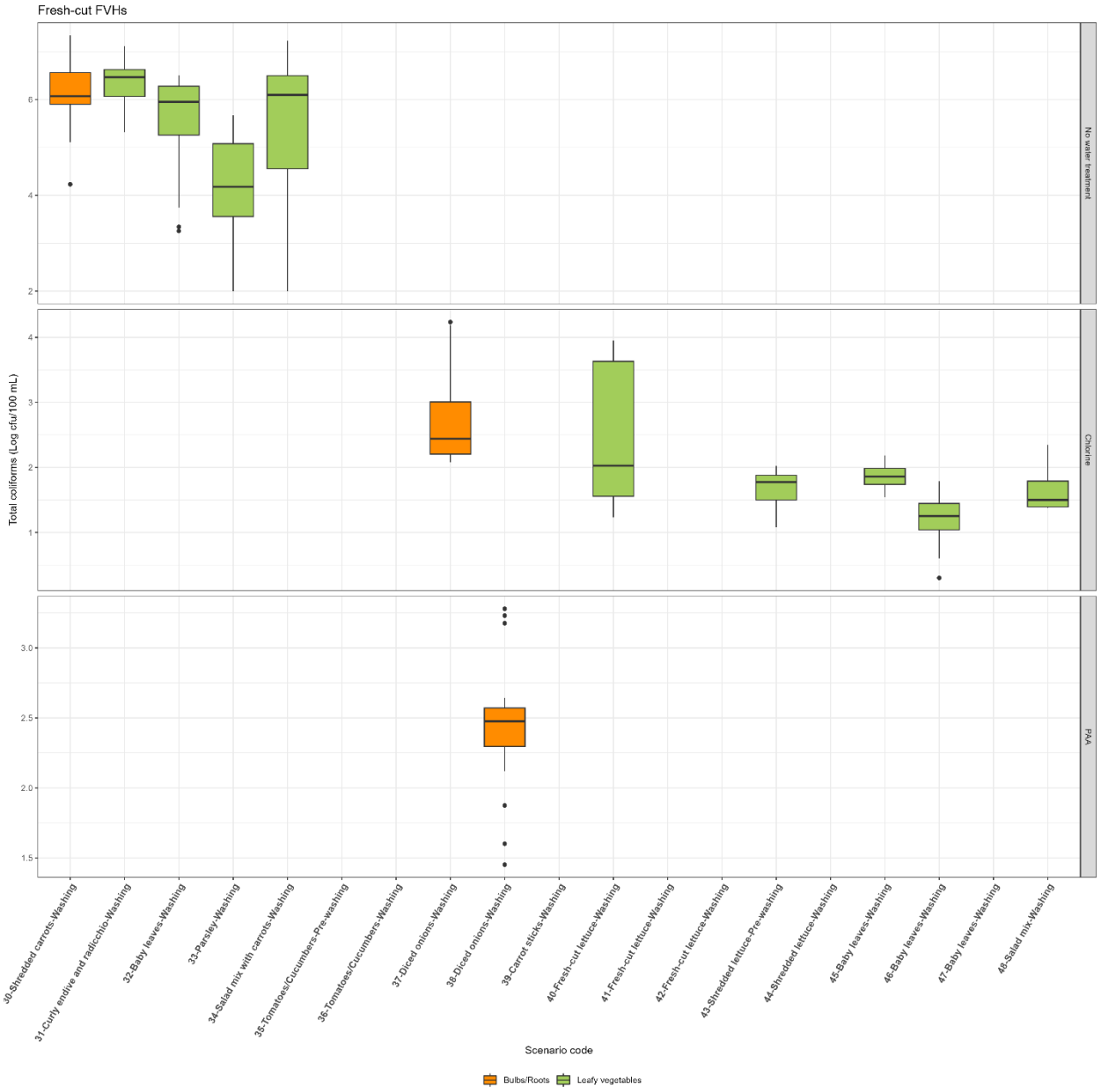


Figure 55. Boxplot graph that represents changes in total coliform counts of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

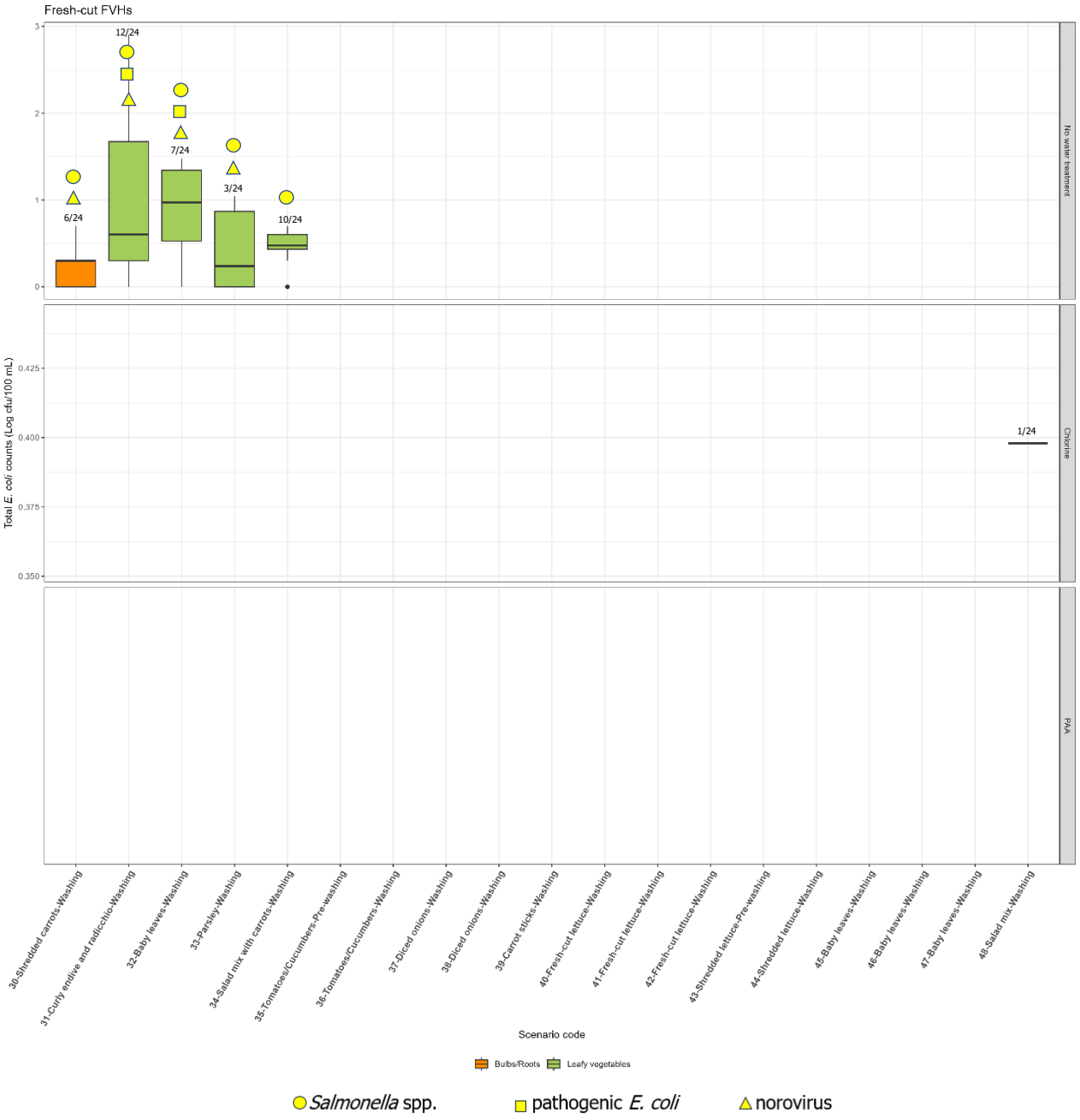


Figure 56. Boxplot graph that represents changes in total *E. coli* counts of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups:(i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

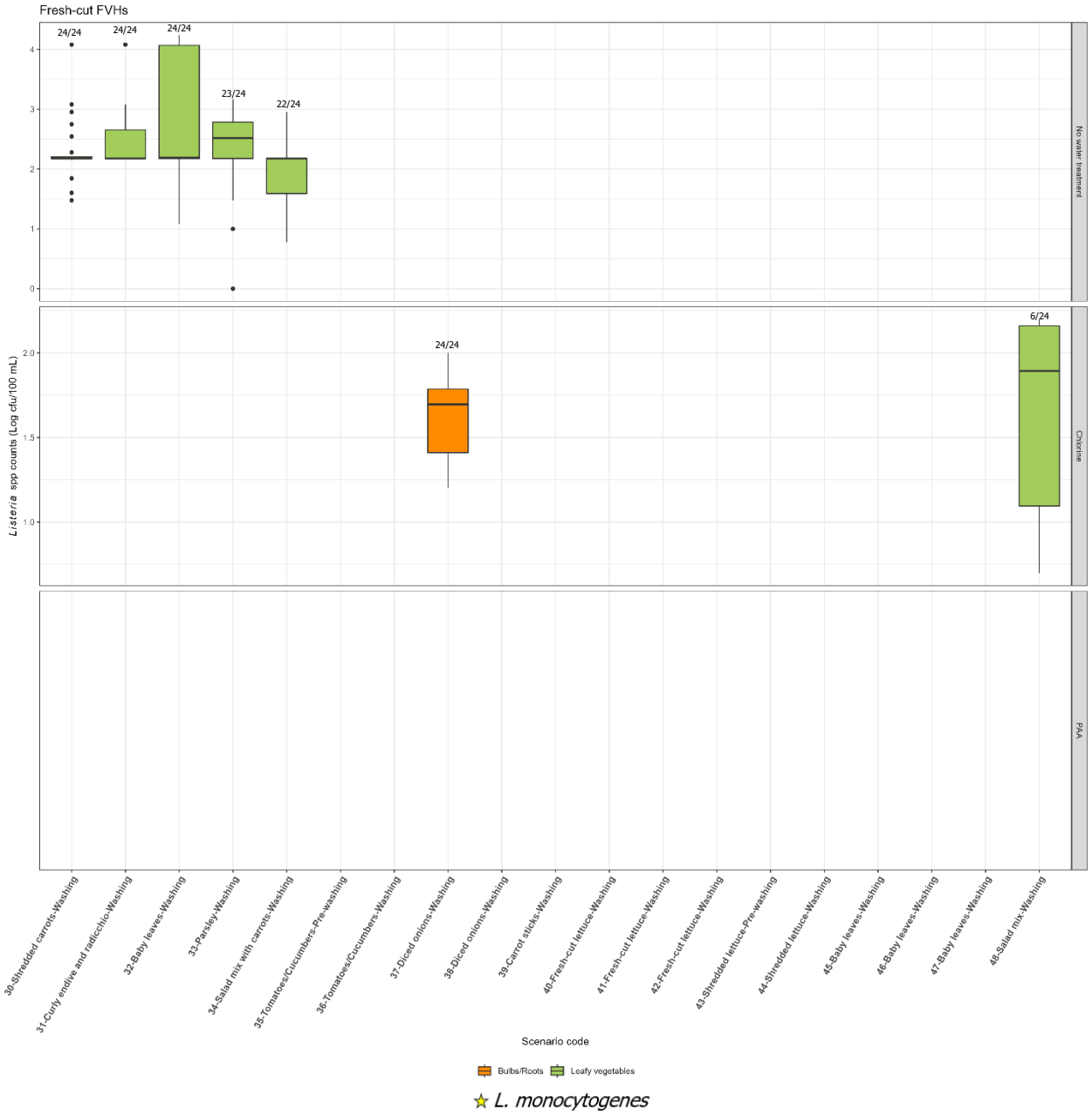


Figure 57. Boxplot graph that represents changes in *Listeria* spp. counts of process water throughout the sampling period across different scenarios of the fresh-cut FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Fruits, (ii) Vegetable Fruits, (iii) Bulbs and Roots, (iv) Leafy greens and (v) Fruits/vegetable/roots/bulbs.

3.2.3 Frozen FVHs

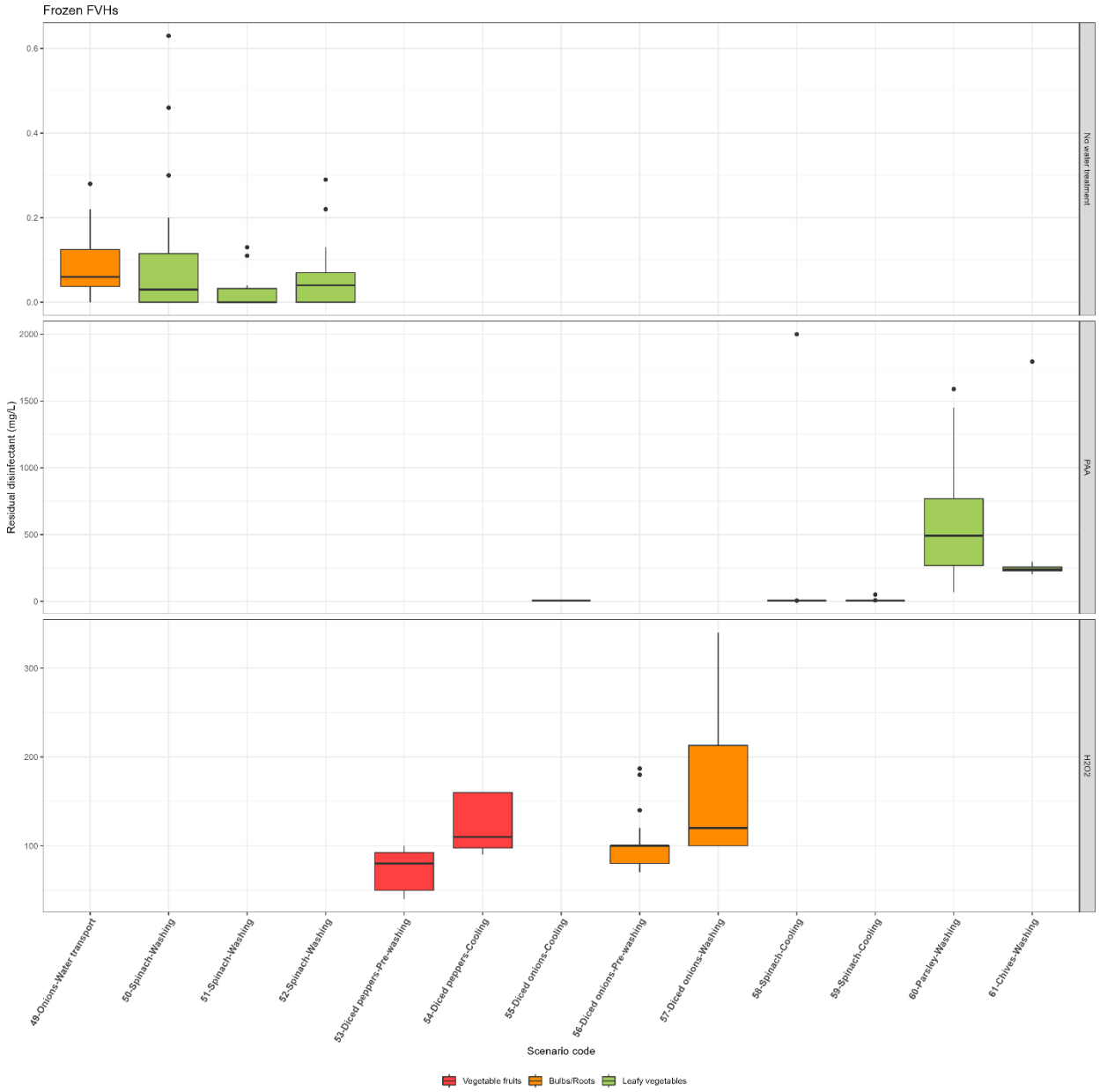


Figure 58. Boxplot graph that represents changes in residual disinfectant concentration of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

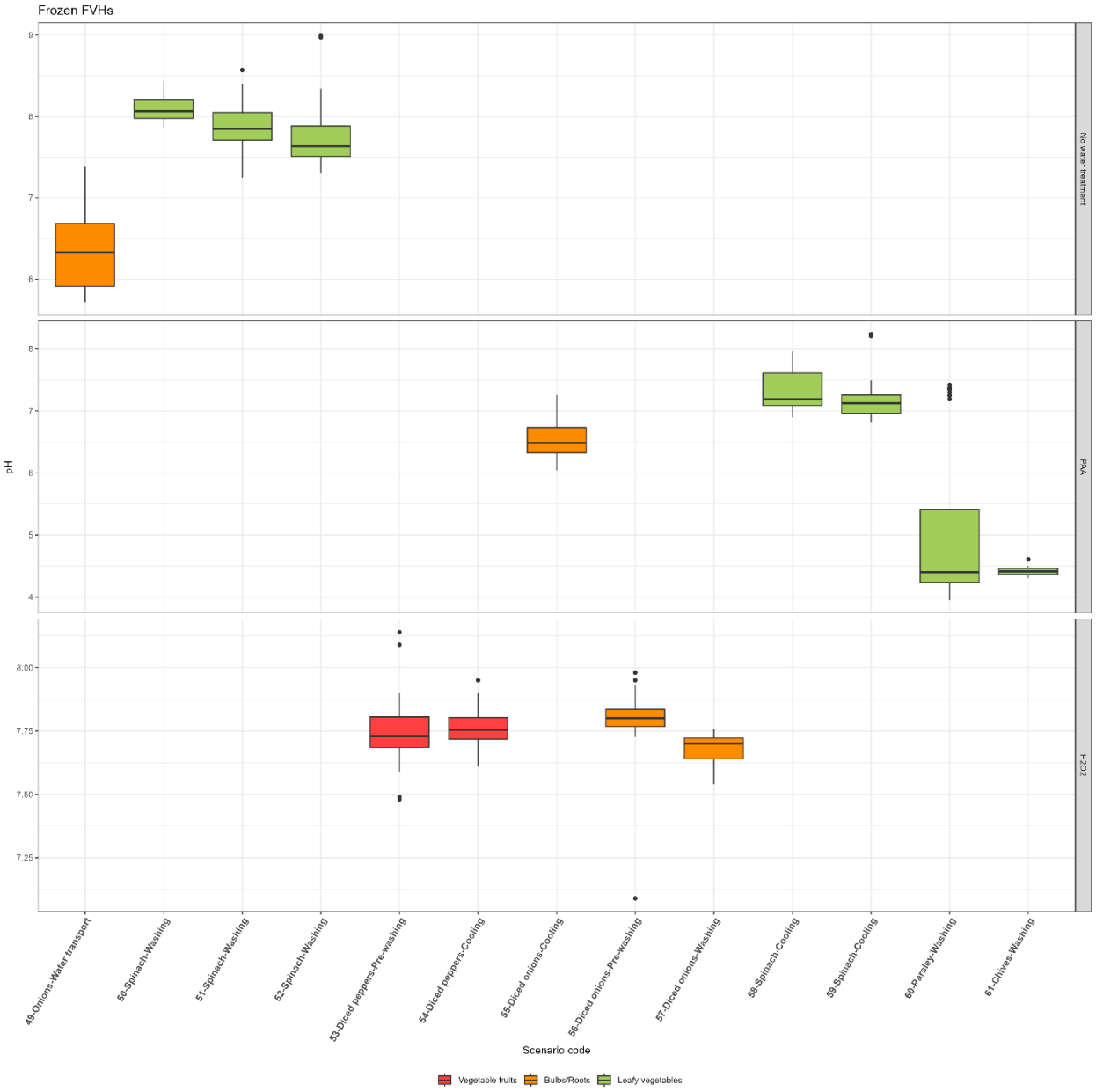


Figure 59. Boxplot graph that represents changes in pH of process water across different scenarios of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

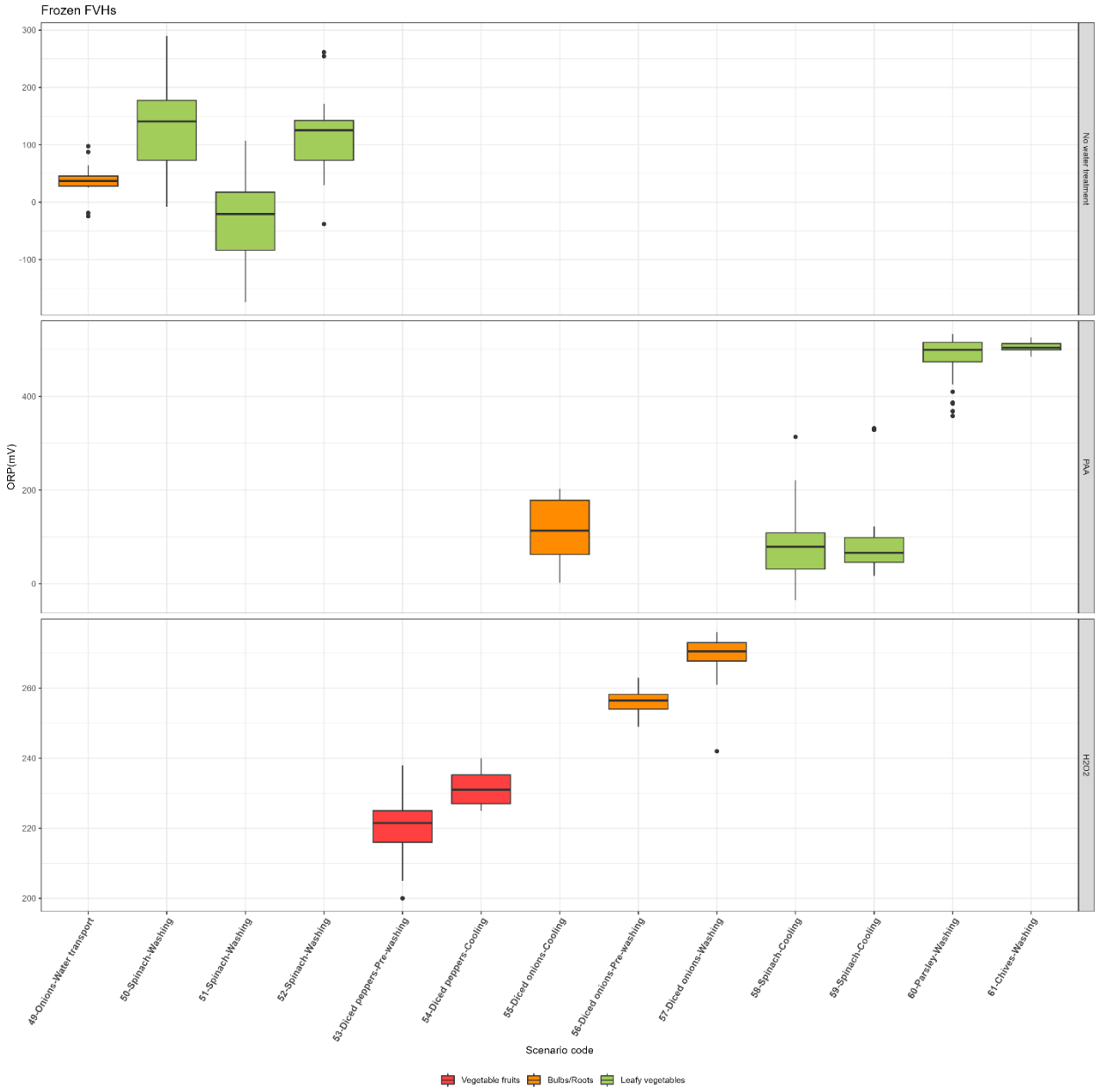


Figure 60. Boxplot graph that represents changes in oxidation-reduction potential (ORP) of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

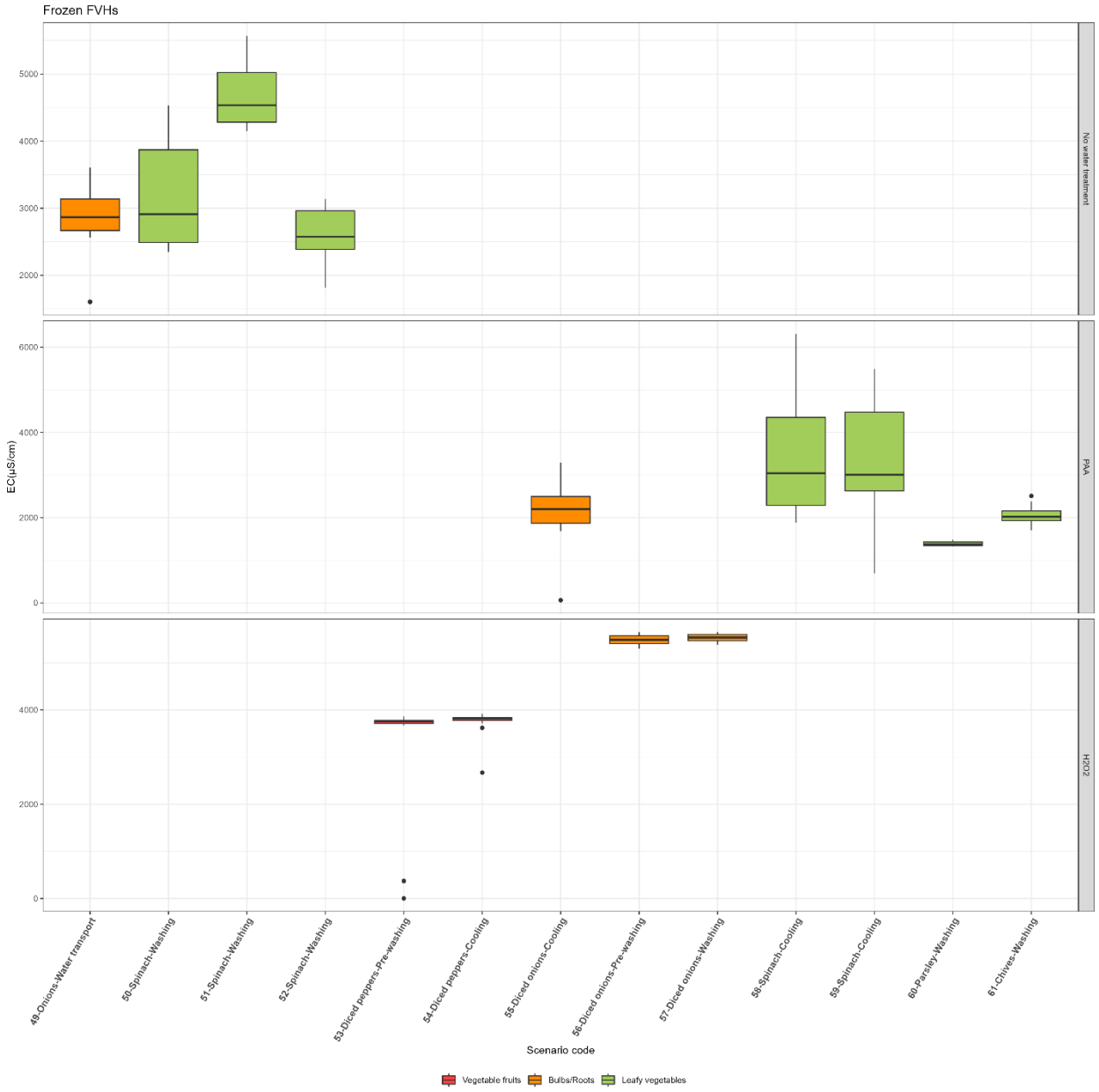


Figure 61. Boxplot graph that represents changes in electrical conductivity (EC) of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

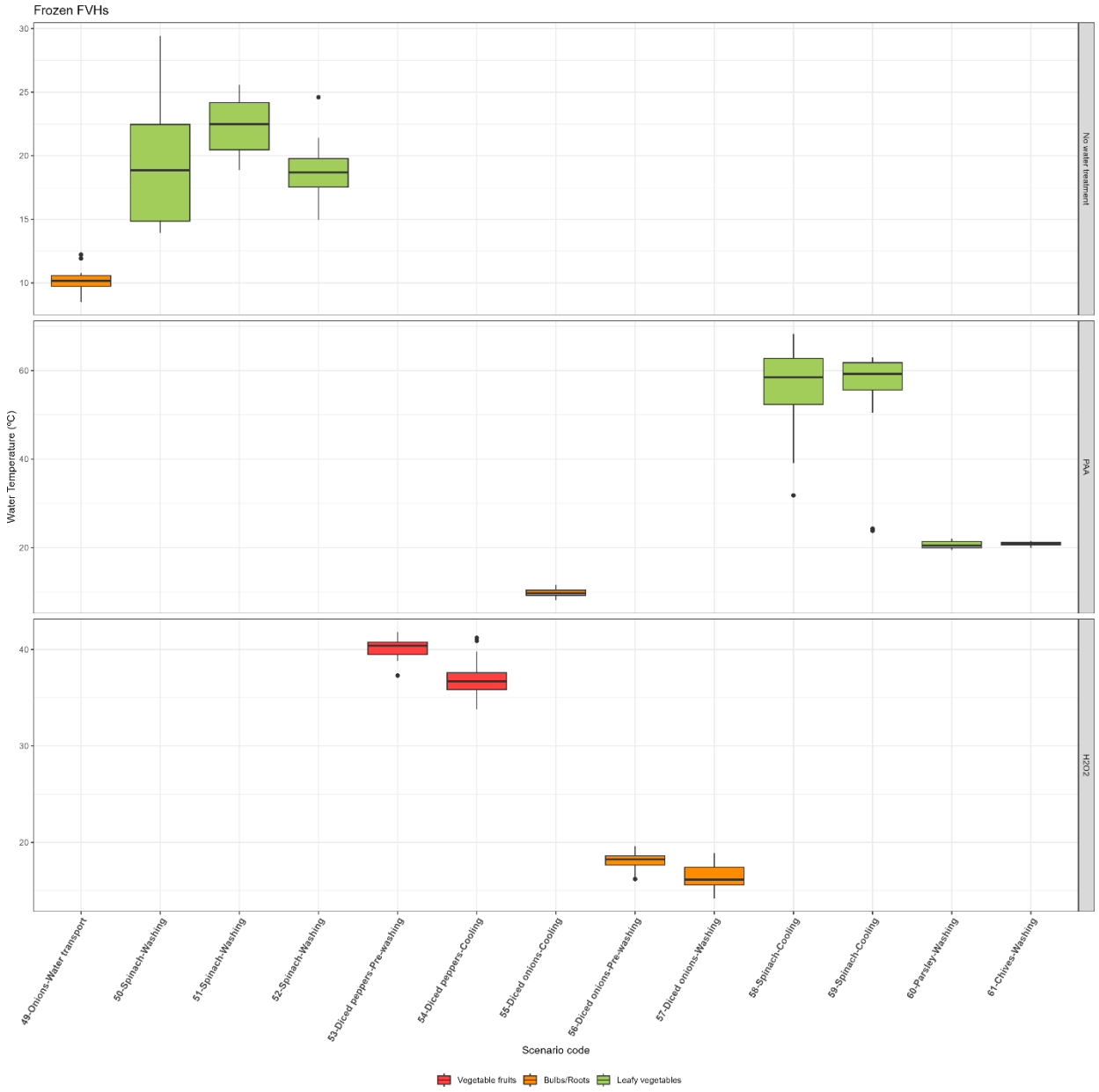


Figure 62. Boxplot graph that represents changes in temperature of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

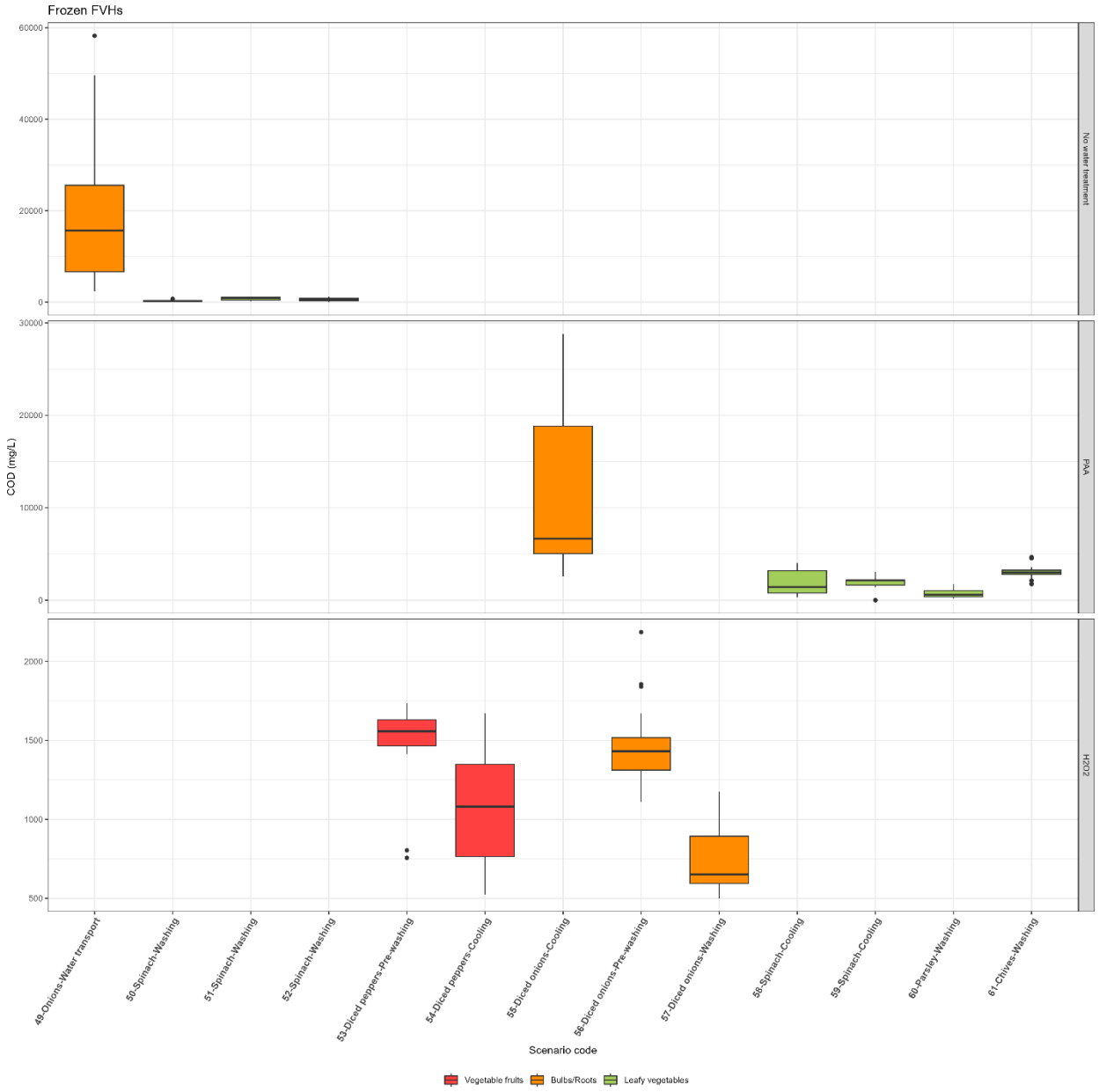


Figure 63. Boxplot graph that represents changes in chemical oxygen demand (COD) of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

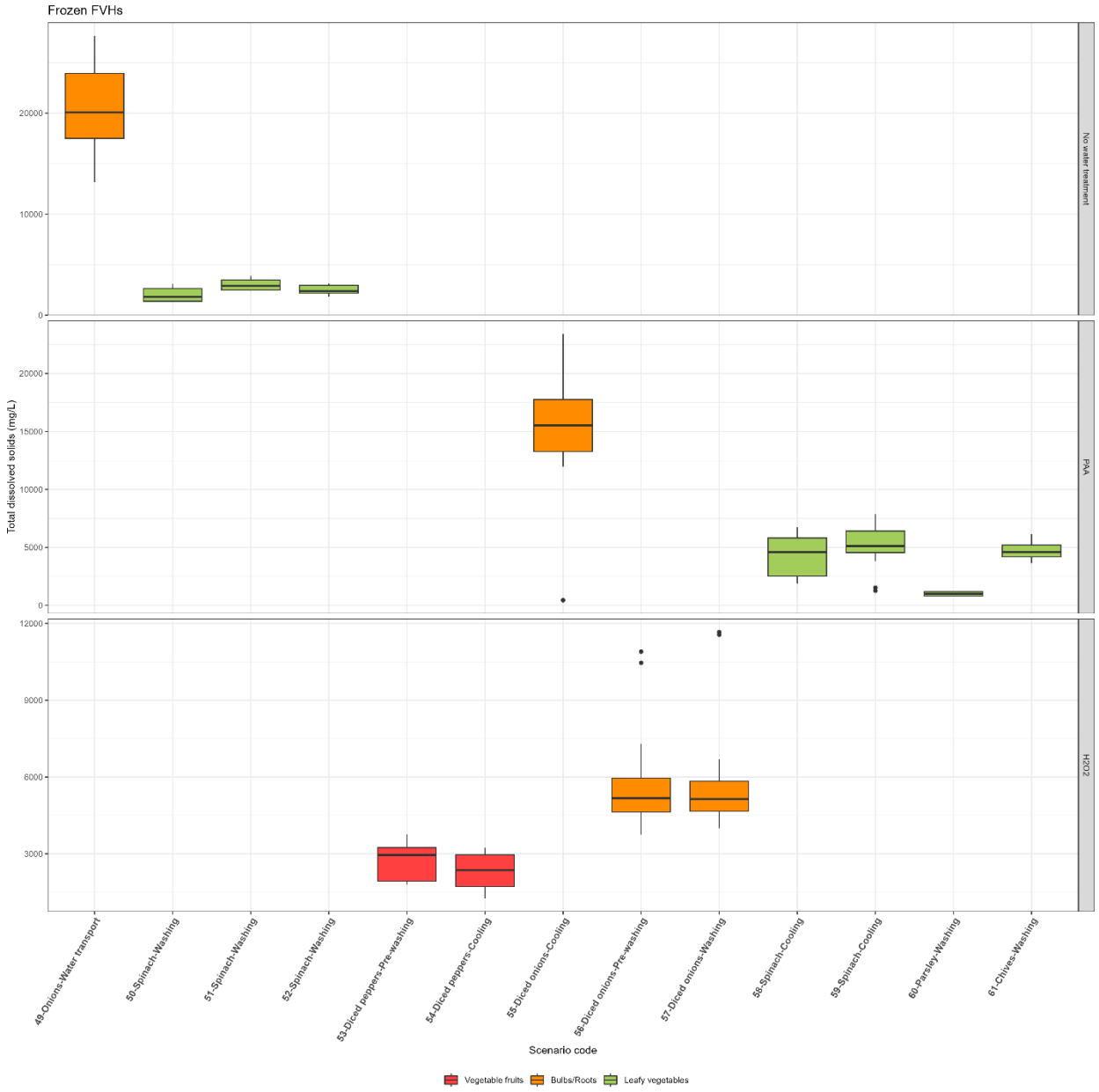


Figure 64. Boxplot graph that represents changes in total dissolved solids (TDS) of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

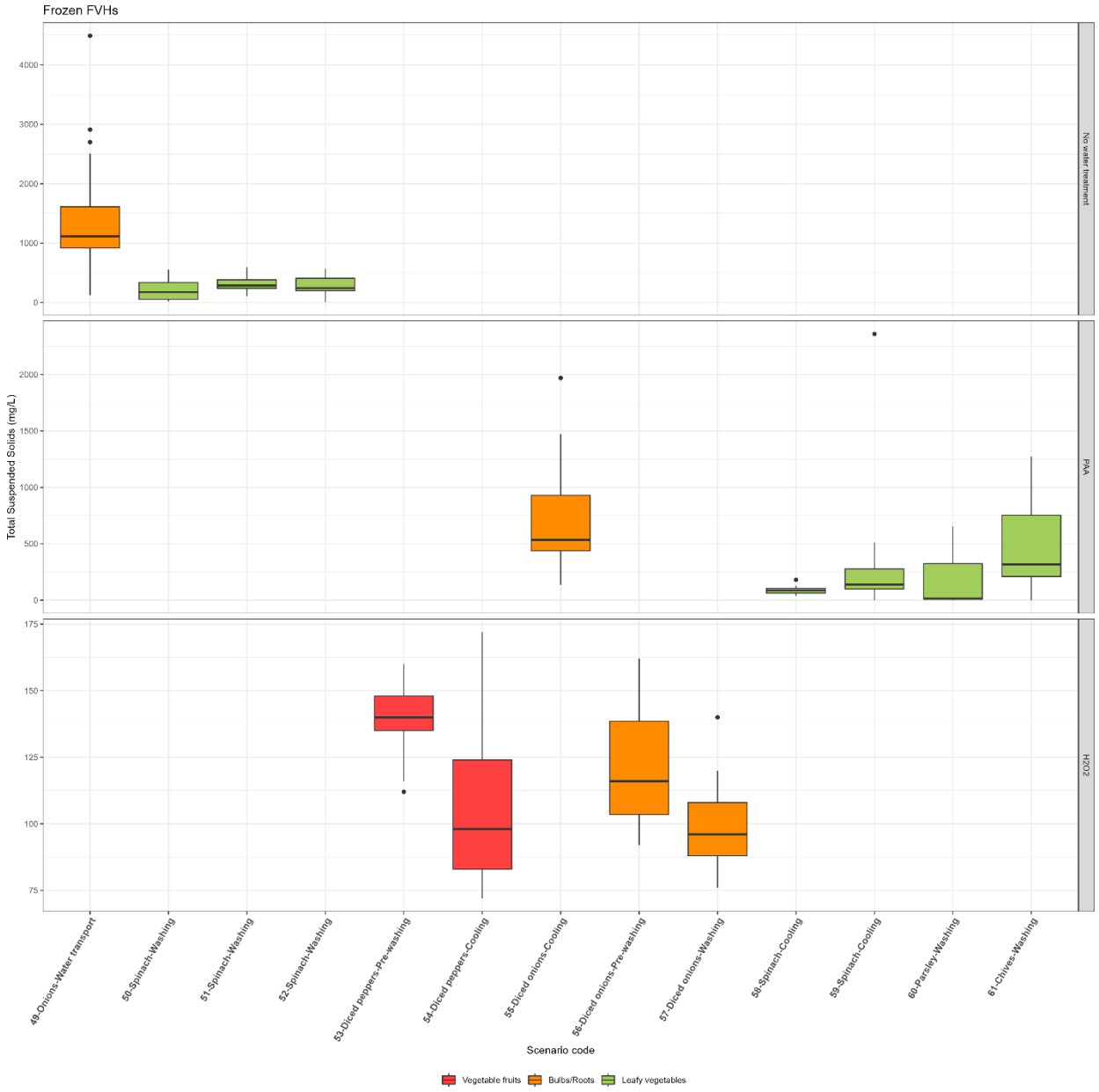


Figure 65. Boxplot graph that represents changes in total suspended solids (TSS) of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

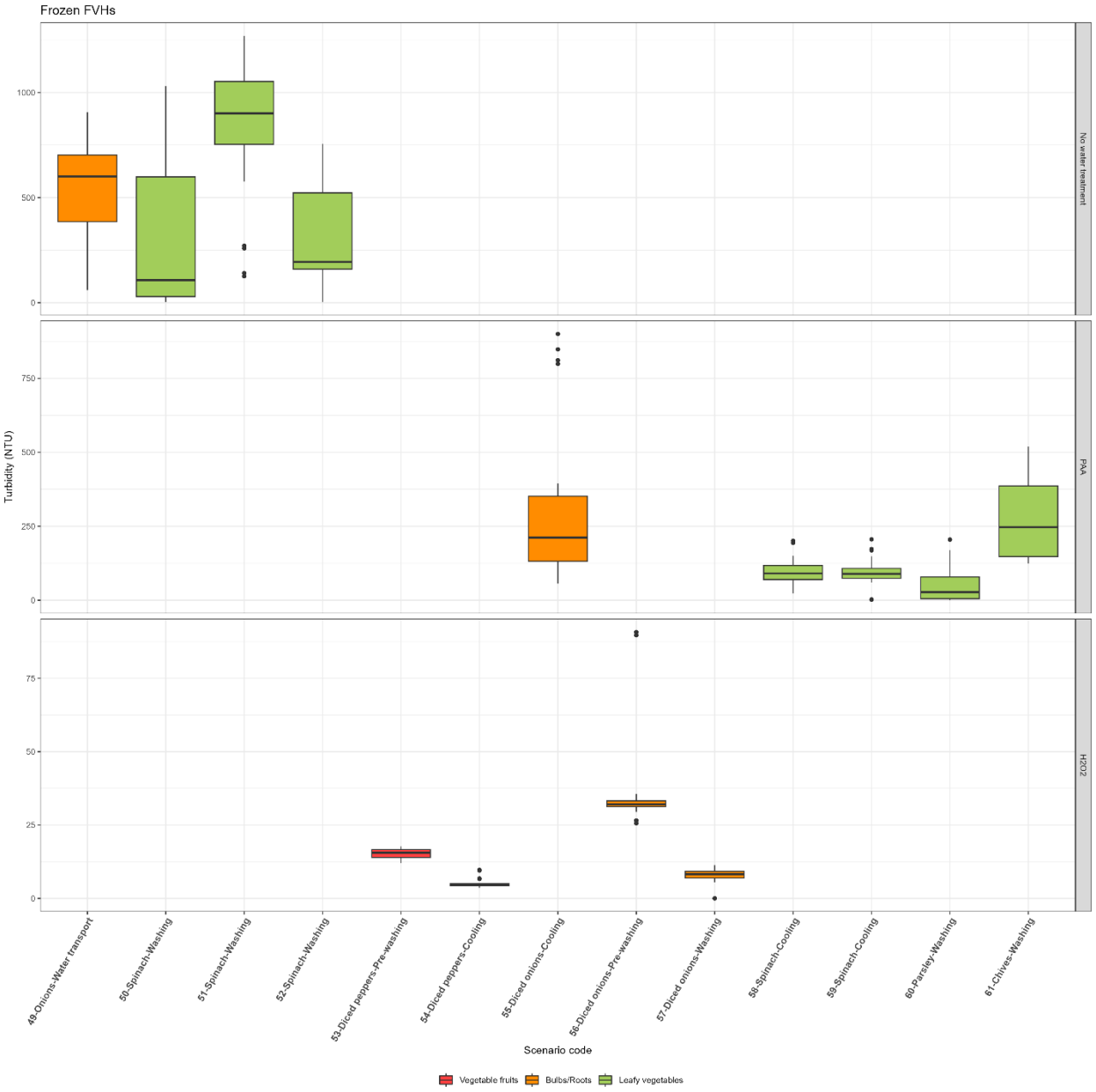


Figure 66. Boxplot graph that represents changes in turbidity of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

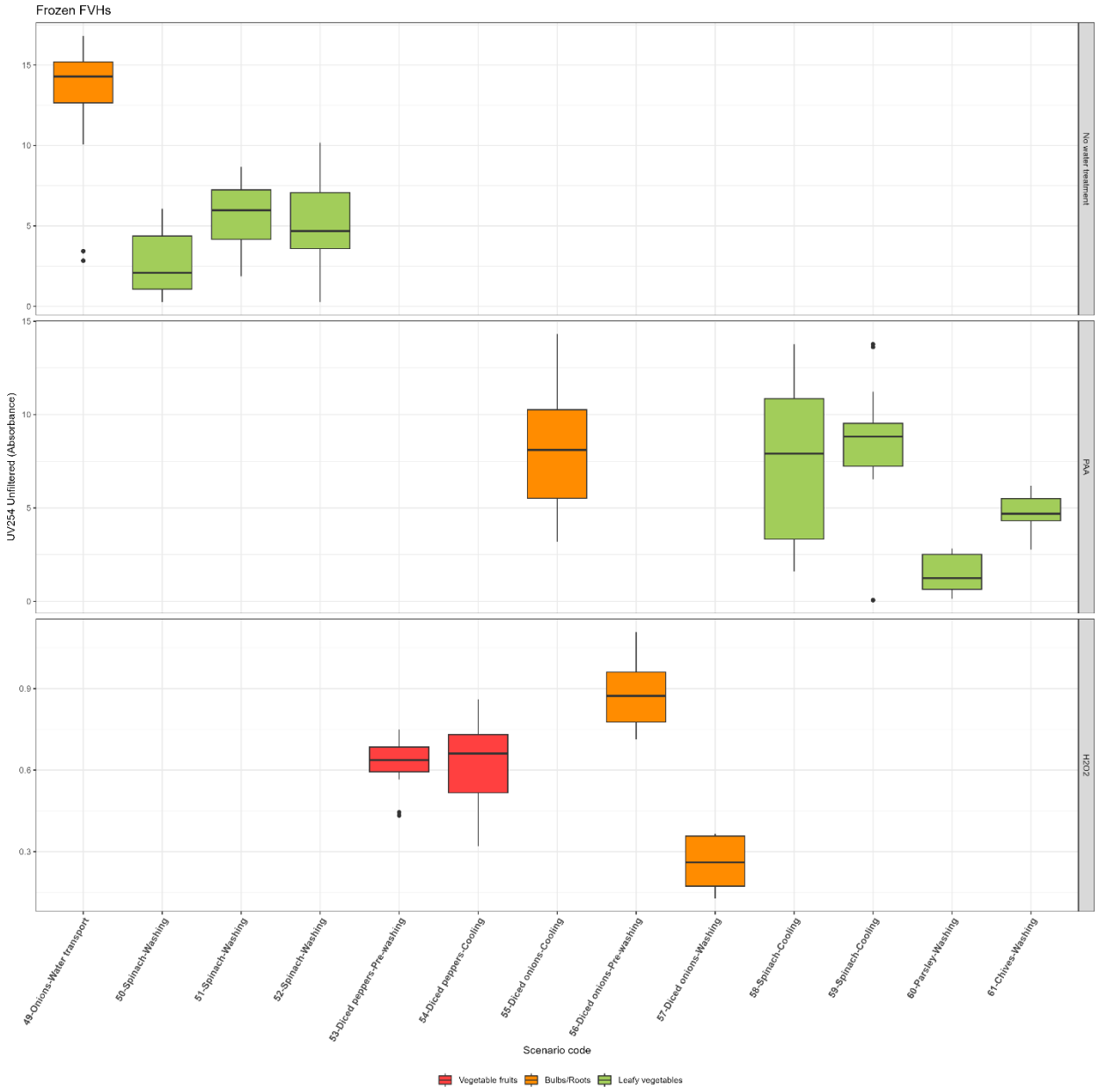


Figure 67. Boxplot graph that represents changes in UV254 unfiltered absorbance of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

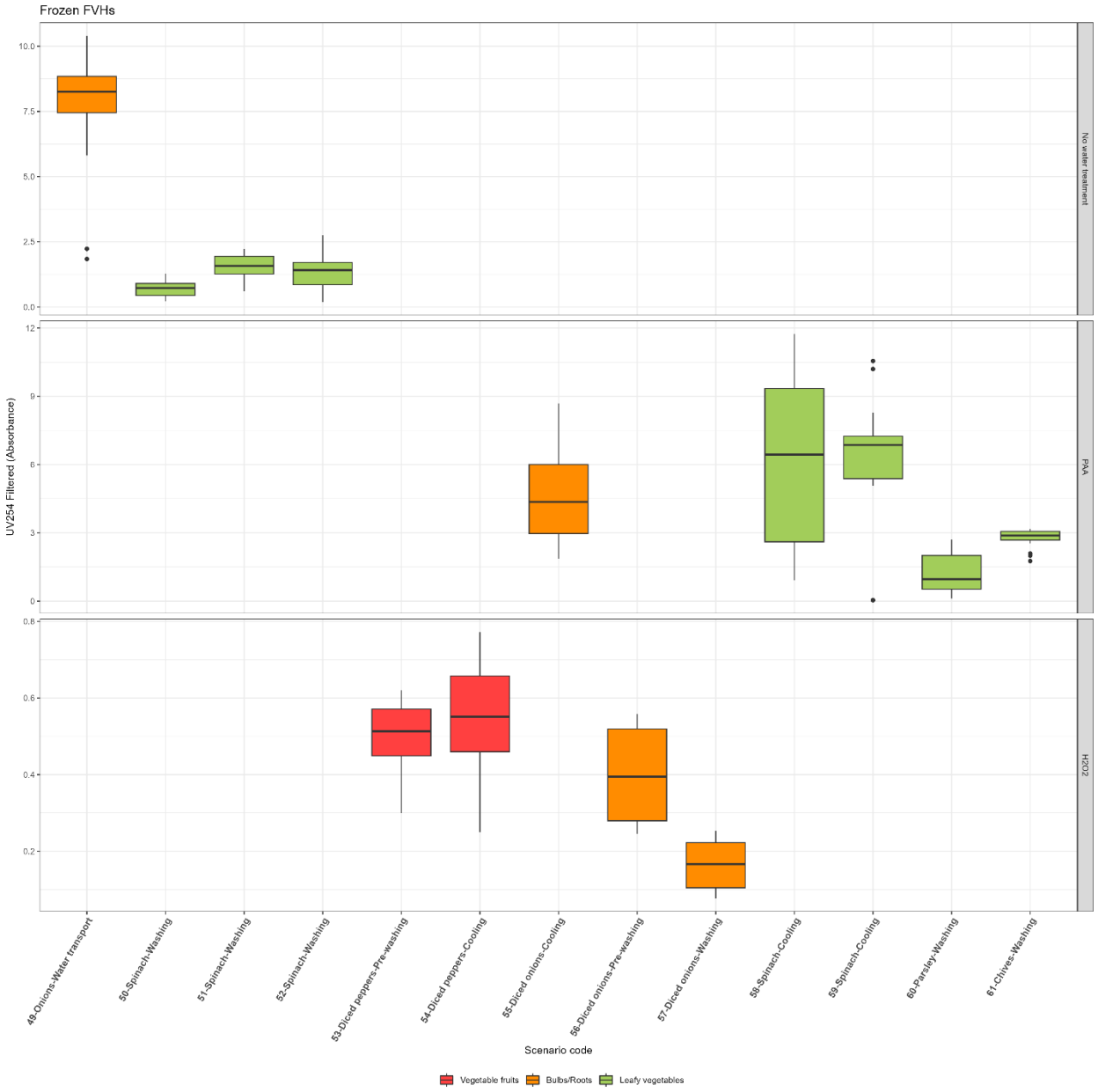


Figure 68. Boxplot graph that represents changes in UV254 filtered absorbance of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

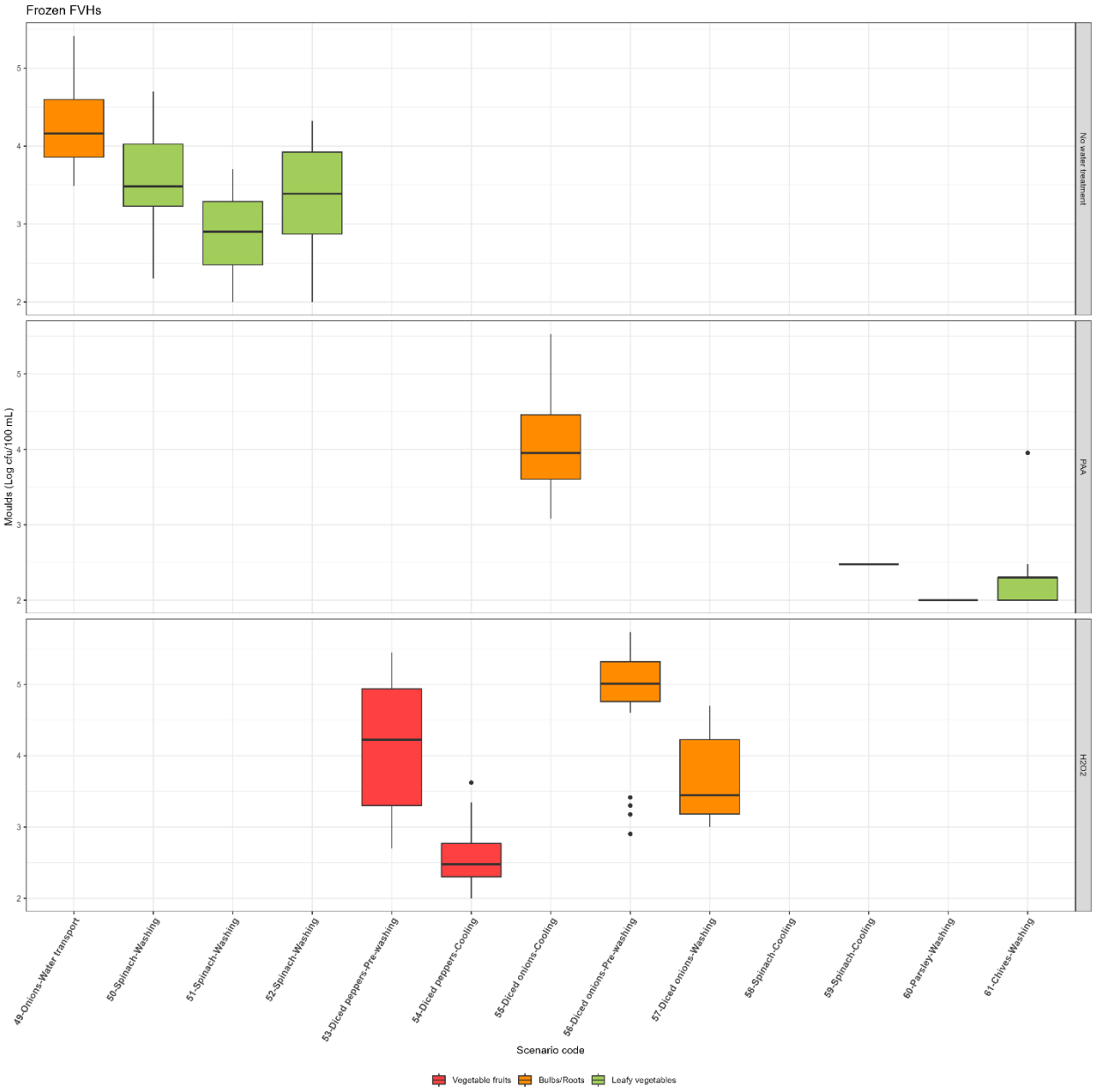


Figure 69. Boxplot graph that represents changes in total mould counts of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

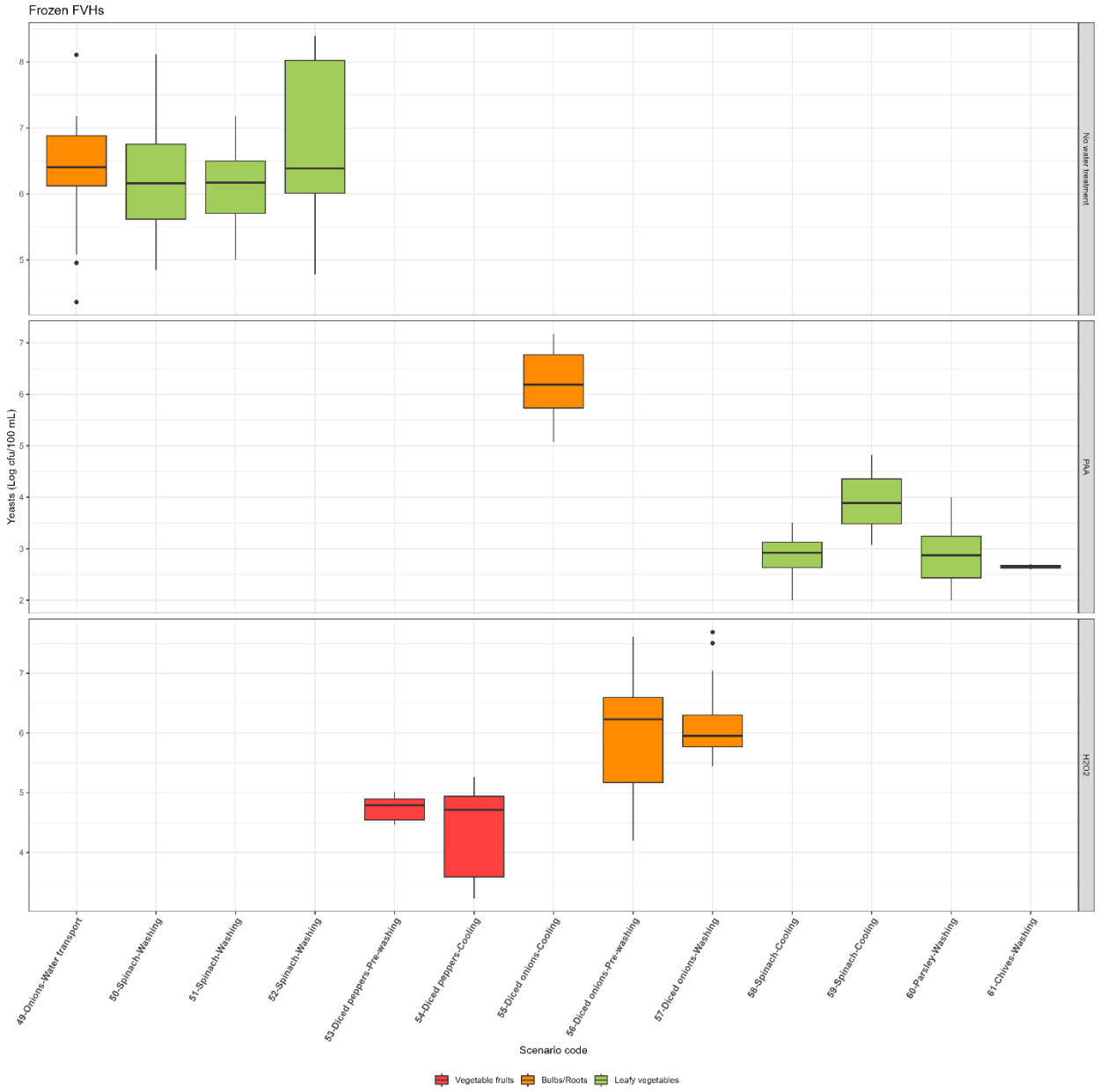


Figure 70. Boxplot graph that represents changes in total yeast counts of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

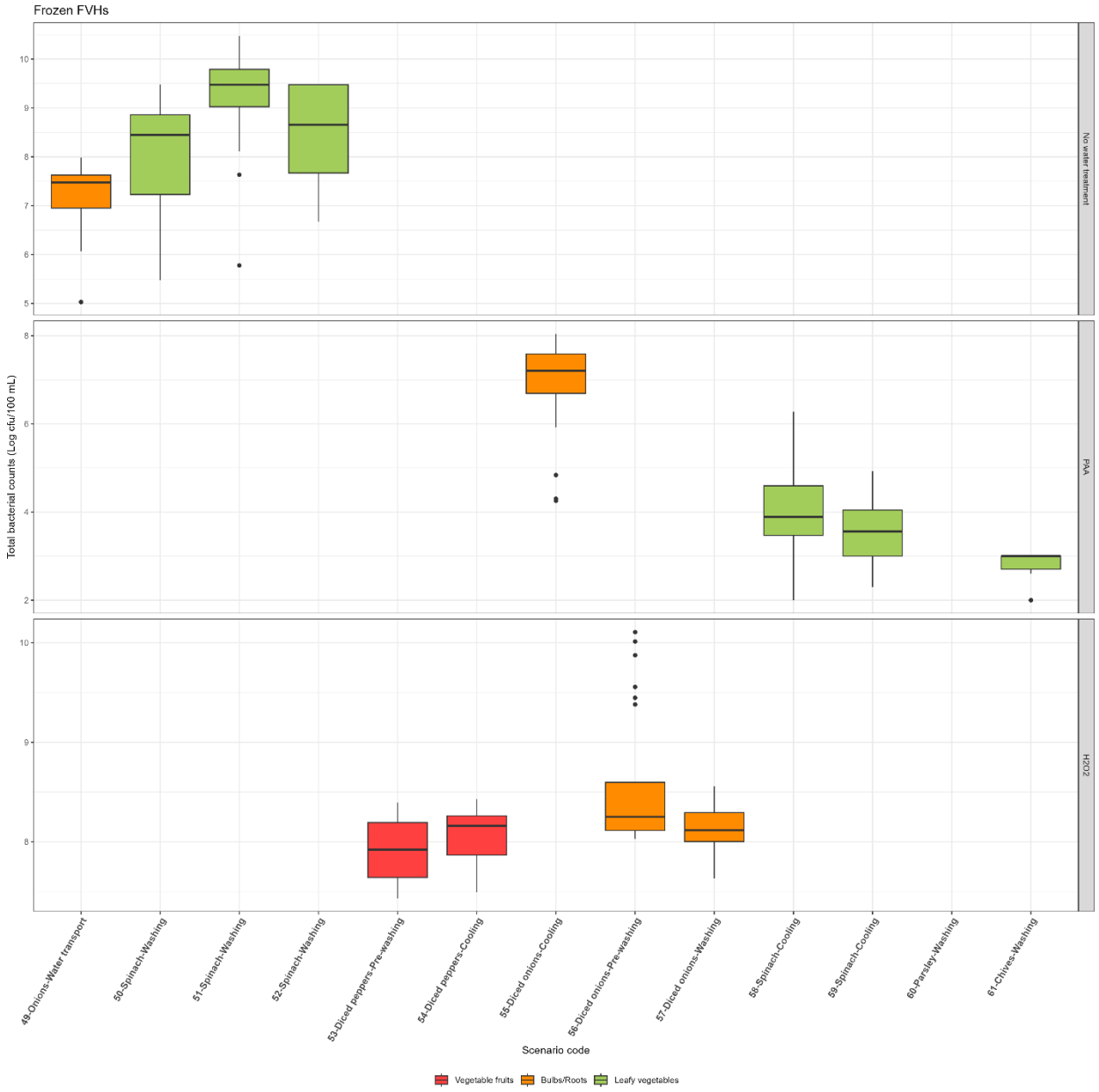


Figure 71. Boxplot graph that represents changes in total bacterial counts of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

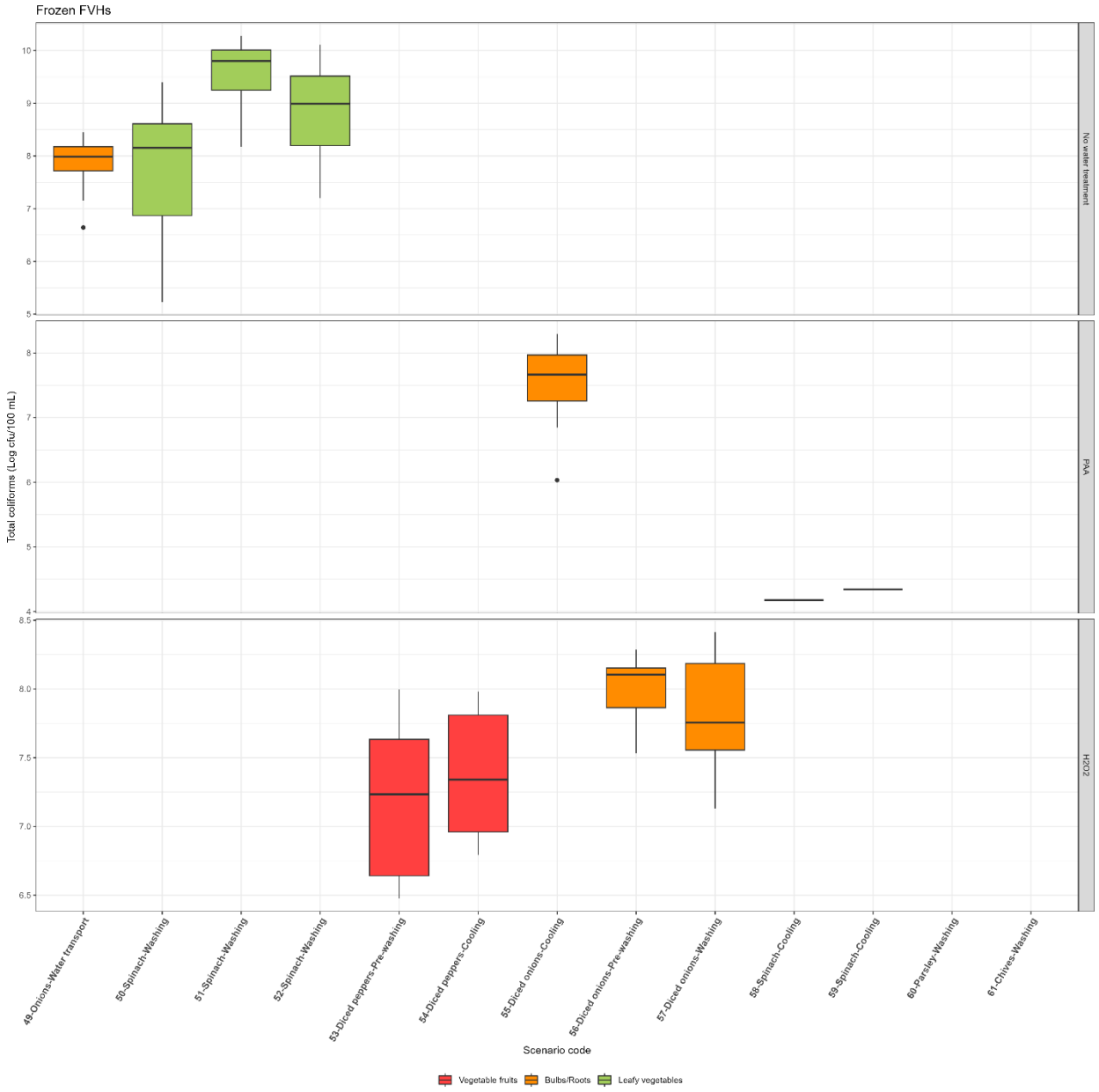


Figure 72. Boxplot graph that represents changes in total coliform counts of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

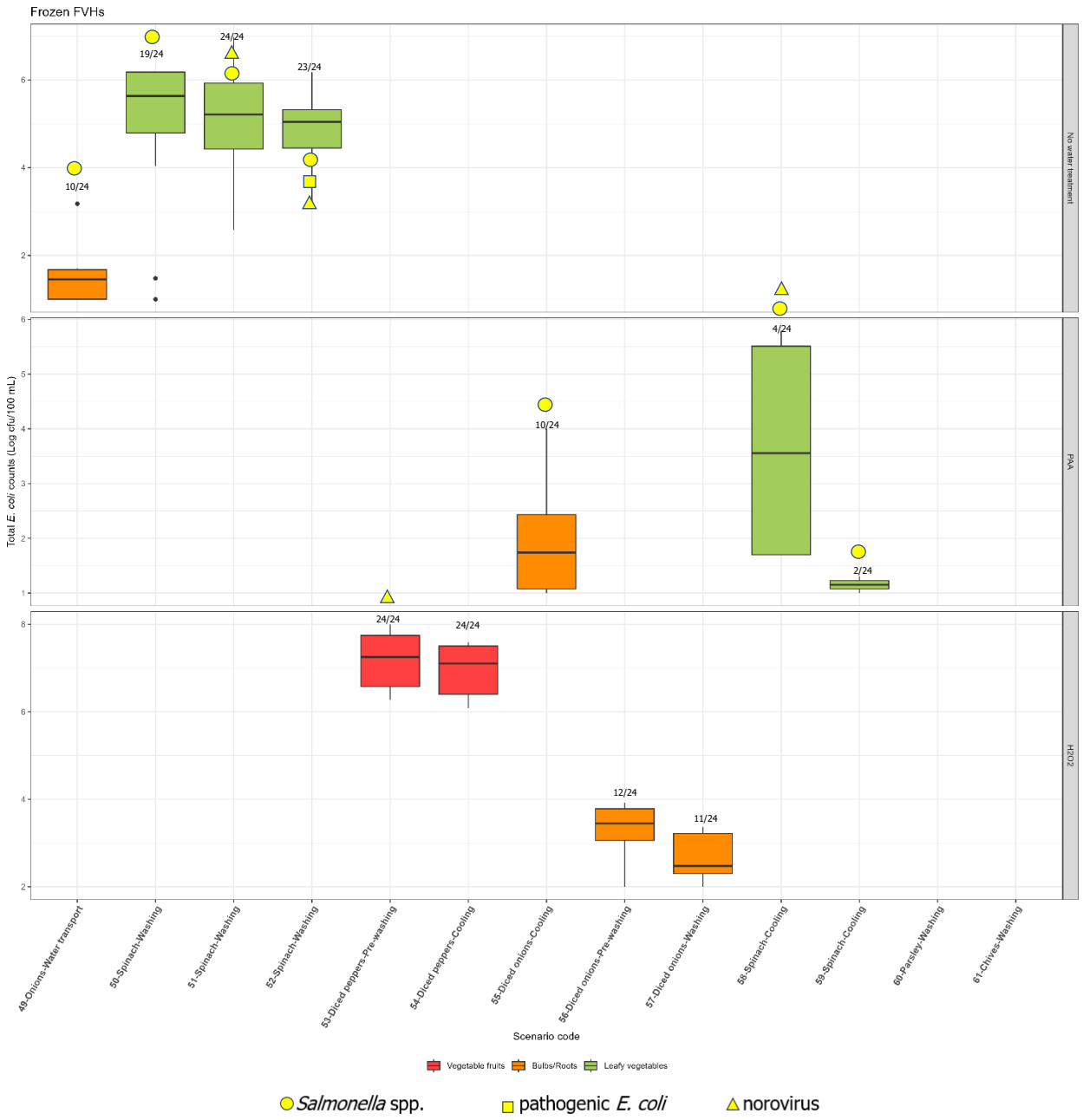


Figure 73. Boxplot graph that represents changes in total *E. coli* counts of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

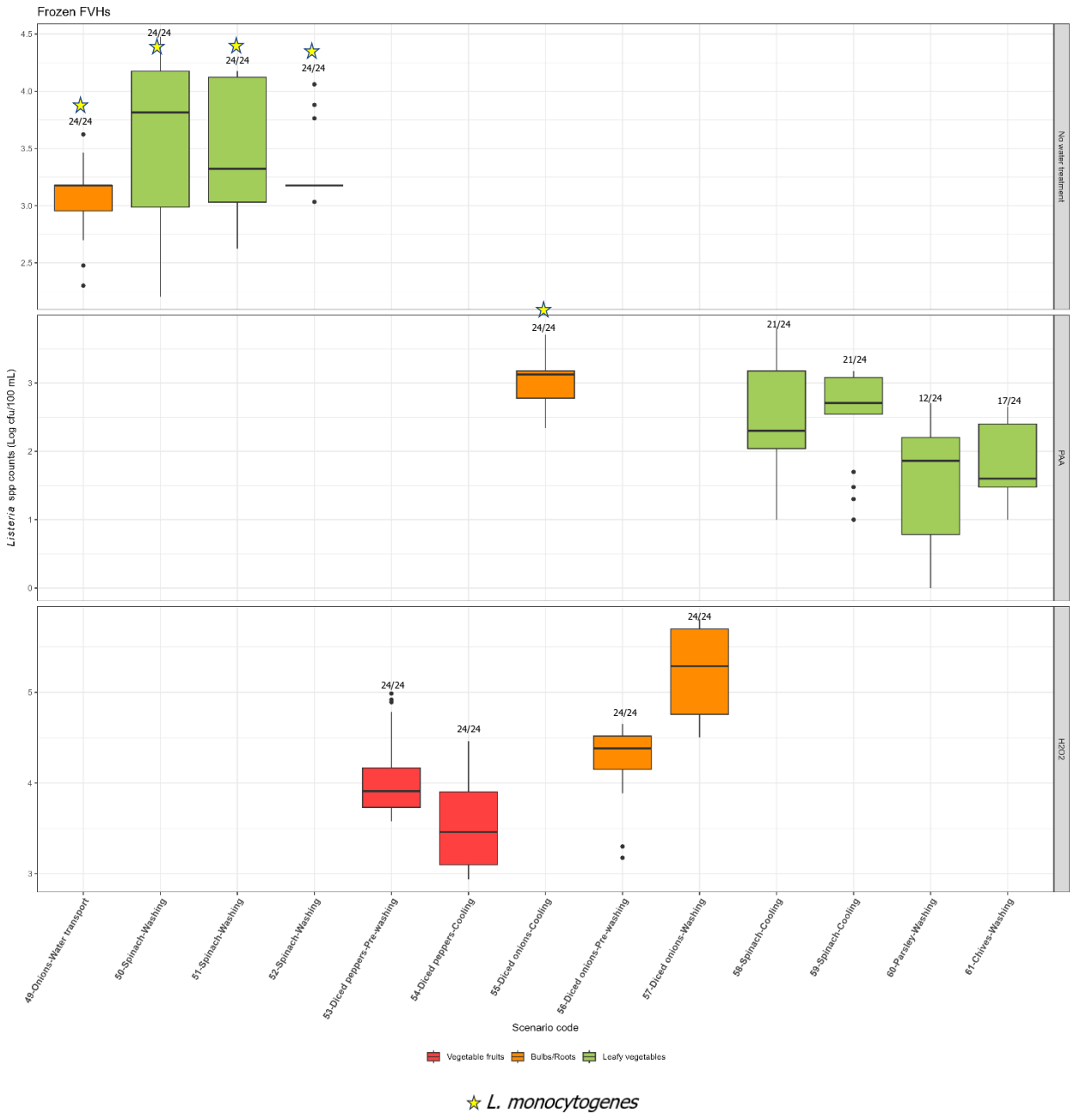


Figure 74. Boxplot graph that represents changes in *Listeria* spp. counts of process water throughout the sampling period across different scenarios of the frozen FVHs sector. The scenarios are described in the X-axis by a label combining the ID code - specific FVH - operation and have been grouped using distinct colours for the following food groups: (i) Vegetable Fruits, (ii) Bulbs and Roots, (iii) Leafy vegetables.

3.3 Summary of the aggregated results for enteric viruses, parasites, fecal indicators and VBNC

3.3.1 Results of human norovirus, viral fecal indicators, *Cryptosporidium* and spores of *Clostridium perfringens*

Process water was collected from FBOs during the two sampling visits (Visit 1 and Visit 2) from the washing tanks at different sampling times including sampling point 6 which was the time at which the process water was taken to perform the analyses of human norovirus, viral fecal indicators, *Cryptosporidium* spp. and spores of *Clostridium perfringens* in specific scenarios mentioned before. After concentration using Rexeed-25A cartridges, the levels of coliphages were determined by plaque assay, while the enumeration of human norovirus GI, GII, *Cryptosporidium*, and *CrAssphage* was determined using molecular techniques, including RT-qPCR and qPCR (**Table 13**). Spores of *Clostridium perfringens* were only examined in three scenarios: carrots (ID 05) as a root commodity in contact with the soil with higher potential for the presence of spores and process water not treated with any disinfectant; diced onions (ID 37) in which the process water was treated with sodium hypochlorite; and baby leaves (ID 47) also treated with calcium plus sodium hypochlorite to examine possible differences between distinct chlorine forms and their effectiveness against the presence of spores of *C. perfringens*.

Table 13. Counts of total and F-specific coliphages, Norovirus GI and GII, *CrAssphage*, *Cryptosporidium* and *Clostridium perfringens* in the process water (presented for Visit 1/Visit 2) of selected case scenarios from fresh-whole, fresh-cut and frozen FVHs.

Disinfectant	Food Category	Scenario ID	Total coliphages (Log PFU/L)	F-specific phages (Log PFU/L)	Norovirus GI (Log GC/L)	Norovirus GII (Log GC/L)	CrAssphage (Log GC/L)	*Cryptosporidium* (Log GC/L)	*C. perfringens* (CFU/100 mL)
None	Fresh-whole FVHs	01	<LoD/1.82	<LoD/1.76	<LoD/<LoD	<LoD/ <LoD	<LoD/ <LoD	<LoD/<LoD	NE
		02	0.88	<LoD	<LoD	<LoD	<LoD	<LoD	NE
		03	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		04	2.69/2.85	2.33/2.50	5.69/5.86	<LoD/<LoD	3.69/<LoD	<LoD/<LoD	NE
		05	2.47/<LoD	2.21/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/1
		06	3.15/<LoD	3.35/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		07	4.80/4.70	4.77/4.53	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		08	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
	Fresh-cut FVHs	30	<LoD/<LoD	<LoD/<LoD	<LoD/4.79	<LoD/<LoD	2.62/<LoD	<LoD/<LoD	NE
		31	<LoD/<LoD	<LoD/<LoD	<LoD/4.51	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		32	<LoD/<LoD	<LoD/<LoD	<LoD/4.00	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		33	<LoD/<LoD	<LoD/<LoD	<LoD /4.27	<LoD/<LoD	<LoD /2.04	<LoD/<LoD	NE
		34	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE

	Frozen FVHs	49	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		50	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	2.56/2.80	<LoD/<LoD	NE
		51	<LoD/<LoD	<LoD/<LoD	3.53/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		52	<LoD/<LoD	<LoD/<LoD	<LoD/4.47	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
Chlorine	Fresh-whole FVHs	09	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		10	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	NE
		11	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		14	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	NE
		15	<LoD/<LoD	<LoD/<LoD	4.50/4.69	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		16	0.74/<LoD	<1/<LoD	5.56/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		17	<LoD	<LoD	<LoD	5.47	<LoD	<LoD	NE
		18	1.95/<LoD	<LoD/<LoD	<LoD/<LoD	5.56/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		19	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		20	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		21	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	NE
		22	<LoD/<LoD	<LoD/<LoD	<LoD /4.26	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE

		24	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD /3.09	<LoD/<LoD	NE
		25	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		29	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
	Fresh-cut FVHs	37	<LoD/<LoD	<LoD/<LoD	<LoD /5.03	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	1/<LoD
		39	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		40	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		41	<LoD/<LoD	<LoD/<LoD	4.72/5.48	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		42	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		43	<LoD/<LoD	<LoD/<LoD	<LoD/5.27	<LoD/<LoD	<LoD /3.45	<LoD/<LoD	NE
		44	<LoD/<LoD	<LoD/<LoD	<LoD/5.28	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		45	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	2.33/2.06	<LoD/<LoD	NE
		46	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		47	<LoD/<LoD	<LoD/<LoD	5.08/5.05	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD
		48	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
Hydrogen peroxide	Fresh-whole FVHs	12	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	2.36/3.80	LoD/<LoD	NE
		13	<LoD/<LoD	1.89/1.77	<LoD/<LoD	<LoD/<LoD	2.65/2.31	LoD/<LoD	NE
		23	0.72/1.41	1.61/1.39	<LoD/<LoD	<LoD/<LoD	3.06/2.32	<LoD/<LoD	NE
	Frozen FVHs	53	3.64/3.53	3.82/3.47	4.93/4.93	<LoD/4.77	<LoD/<LoD	<LoD/<LoD	NE

		54	3.29/3.37	3.28/3.29	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		56	5.09/3.34	4.97/3.20	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		57	4.99/3.27	4.85/3.33	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
Peroxyacetic acid	Fresh-whole FVHs	26	<LoD/<LoD	<LoD/<LoD	5.16/5.07	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		27	<LoD	<LoD	4.92	<LoD	<LoD	<LoD/<LoD	NE
		28	<LoD/<LoD	<LoD/<LoD	<LoD/5.35	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
	Fresh-cut FVHs	38	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
	Frozen FVHs	55	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		58	<LoD/<LoD	<LoD/<LoD	5.34/3.97	<LoD/<LoD	<LoD/3.14	<LoD/<LoD	NE
		59	<LoD/<LoD	<LoD/<LoD	4.66/3.69	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		60	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		61	<LoD/<LoD	<LoD/<LoD	<LoD/4.75	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
Electrolysed water	Fresh-cut FVHs	35	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE
		36	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	<LoD/<LoD	NE

<LoD: Below Limit of detection, NE: Not executed
Levels of Visit 1/visit 2 of coliphages expressed as log plaque forming units (PFU/L), viruses expressed as log genomic copies (GC)/L and C. perfringens expressed as log colony forming units (CFU/100 mL)

In process water without disinfectant, total coliphages and F-specific coliphages were detected in 24.2% (8 out of 33 samples) and 21.2% (7 out of 33 samples), respectively (**Table 13**), while human norovirus GI, GII, and *CrAssphage* were detected in 24.2%, 0%, and 15.1% of the analyzed samples, respectively. As the occurrence of human norovirus GI was high (**Table 13**), a viability RT-qPCR based on PMAxx pretreatment was conducted in parallel (**Table 14**) to assess viruses with intact capsids (Leifels et al., 2020; Randazzo et al., 2016). Of the 8 norovirus GI positive samples, 4 remained positive after PMAxx pretreatment (ID30, ID31, ID32, and ID33), which were also positive for *Salmonella* (**Table 14**).

When chlorine was used as a disinfectant, coliphages were detected with occurrences of 4.1% (2/48) and 2.1% (1/48) for total coliphages and F-specific coliphages, respectively. Human norovirus GI, GII, and *CrAssphage* were detected in 16.6%, 4.1%, and 8.3% of the analysed samples, respectively. When the viability RT-qPCR based on PMAxx pretreatment was conducted in parallel (**Table 14**), out of the 11 norovirus GI positive samples, 4 remained positive after PMAxx pretreatment (ID15, ID41, and ID47).

A high occurrence of coliphages was observed on process water samples treated with hydrogen peroxide, with 71.4% and 85.7 for total coliphages and F-specific coliphages, respectively. Human norovirus GI, GII, and *CrAssphage* were detected in 14.3%, 7.1%, and 42.8% of the analysed samples, respectively. When the viability RT-qPCR based on PMAxx pretreatment was conducted in parallel (**Table 14**) the two positive samples of norovirus GI from scenario 53 remained positive.

No coliphages nor norovirus GII were detected in process water treated with peroxyacetic acid. However, 9 out of the 17 analysed samples tested positive for norovirus GI (**Table 13**). When the viability RT-qPCR based on PMAxx pretreatment was conducted in parallel (**Table 14**), out of the 9 norovirus GI positive samples, 3 remained positive after PMAxx pretreatment (ID26, ID27, and ID28). No noroviruses or fecal indicators were detected in electrolyzed process water. *Cryptosporidium* was not detected in any of the analysed samples.

Table 14. Counts of norovirus GI (Visit 1/Visit 2), in positive samples, before and after viability PCR (PMAxx)

Water disinfection treatment	Scenario ID	Norovirus GI (Log GC/L) (total viruses)	Norovirus GI + PMAxx (Log GC/L) (intact capsid viruses)
None	04	5.69/5.86	<LoD/<LoD
	30	<LoD/4.79	NE/4.56
	31	<LoD/4.51	NE/4.27
	32	<LoD/4.00	NE/4.36
	33	<LoD/4.27	NE/4.10
	51	3.53/<LoD	<LoD /NE
	52	<LoD/4.47	NE/<LoD
Chlorine	15	4.50/4.69	4.90/4.55
	16	5.56/<LoD	<LoD/NE
	22	<LoD/4.26	NE/<LoD
	37	<LoD/5.03	NE/<LoD
	41	4.72/5.48	4.41/<LoD
	43	<LoD/5.27	NE/<LoD
	44	<LoD/5.28	NE/<LoD
	47	5.08/5.05	<LoD/4.25
Peroxyacetic acid	26	5.16/5.07	<LoD/5.35
	27	4.92	4.59
	28	<LoD/5.35	NE/4.44
	58	5.34/3.97	<LoD/<LoD
	59	4.66/3.69	<LoD/<LoD
	61	<LoD/4.75	NE/<LoD

Water disinfection treatment	Scenario ID	Norovirus GI (Log GC/L) (total viruses)	Norovirus GI + PMAxx (Log GC/L) (intact capsid viruses)
Hydrogen peroxide	53	4.93/4.93	5.36/5.11

<LoD: Below Limit of detection, NE: Not executed, Levels of visit 1/visit 2 of noroviruses expressed as log genomic copies (GC)/L

3.3.2 Results of VBNC of total bacteria, coliforms and *E. coli*

Regarding VBNC, the methodology section concerning the microbiological analysis provides details about which scenarios have been selected for the assessment of VBNC bacteria (**Table 15**). The VBNC analyses were conducted on samples taken at the last sampling time point of each visit, with two replicates per sample. This approach targeted the microbial groups of total bacteria, coliforms, and *E. coli* that were previously enumerated by plate count. Results are indicated in **Table 15**.

The findings indicated that total VBNC bacteria, VBNC coliforms and VBNC *E. coli* were comparable to their respective viable groups, with minimal reduction. Notably, total VBNC bacteria, VBNC coliforms and VBNC *E. coli* closely matched or were slightly lower than the levels of viable bacteria. These results imply that environmental stress such as disinfectant exposure can trigger bacteria to enter a VBNC state where they remain metabolically active but fail to grow on conventional culture media. This phenomenon was observed across all scenarios examined including non-treated process water (ID 05), disinfectants evaluated such as chlorine (IDs 37, 43, 44, 47), PAA (IDs 26 and 28), and H_2O_2 (IDs 56 and 57). The difference between the VBNC and culturable bacteria loads indicates that a large percentage of the bacteria is still culturable. The differences between total cells and culturable bacteria ranged between 5.07-9.95 Log cells/100 mL for total bacteria, 3.43-8.27 Log cells/100 mL for total coliforms and 4.46-7.71 Log cells/100 mL for total *E. coli*.

Table 15. Total viable bacteria, total bacterial counts, viable but non-culturable (VBNC) bacteria, total viable coliforms, total coliform counts, VBNC coliforms, viable *E. coli, E. coli* counts and VBNC *E. coli* in the process water samples of the different scenarios at the last sampling timepoint (sampling time point 6). Results for viable and VBNC bacteria are expressed in log cells/100 mL and for bacterial counts in log CFU/100mL.

		Total bacteria			Coliforms			*E. coli*		
Scenario ID	**Visit**	**Viable** (log cells/100 mL)	**Counts** (log CFU/100mL)	**VBNC** (log cells/100 mL)	**Viable** (log cells/100 mL)	**Counts** (log CFU/100mL)	**VBNC** (log cells/100 mL)	**Viable** (log cells/100 mL)	**Counts** (log CFU/100mL)	**VBNC** (log cells/100 mL)
05	**1**	8.77	7.77	8.71	3.87	7.44	-	-	0.15	-
	2	6.96	4.87	6.96	4.06	3.18	4.00	-	-	-
26	**1**	7.47	6.49	7.41	6.23	6.14	5.53	-	-	-
	2	9.15	8.09	9.11	7.82	7.57	7.45	-	-	-
28	**1**	6.80	4.51	6.80	-	-	-	-	-	-
	2	8.80	5.96	8.80	-	-	-	-	-	-
37	**1**	5.23	4.97	4.88	4.64	4.17	4.45	-	-	-
	2	5.70	3.28	5.70	4.28	2.20	4.28	-	-	-
43	**1**	5.07	1.43	5.07	-	-	-	-	-	-
	2	5.41	3.31	5.41	3.45	1.71	3.44	-	-	-
44	**1**	5.70	1.75	5.70	-	-	-	7.71	-	7.71
	2	5.31	2.63	5.31	-	-	-	-	-	-
47	**1**	5.41	2.31	5.41	-	-	-	-	-	-

Scenario ID	Visit	Total bacteria			Coliforms			*E. coli*		
		Viable (log cells/100 mL)	Counts (log CFU/100mL)	VBNC (log cells/100 mL)	Viable (log cells/100 mL)	Counts (log CFU/100mL)	VBNC (log cells/100 mL)	Viable (log cells/100 mL)	Counts (log CFU/100mL)	VBNC (log cells/100 mL)
	2	5.34	2.22	5.34	-	-	-	-	-	-
56	**1**	9.97	8.21	9.96	8.45	7.65	8.37	6.04	3.15	6.04
	2	9.82	8.05	9.81	8.46	7.90	8.32	-	-	-
57	**1**	8.90	8.07	8.77	7.05	7.69	-	4.47	2.30	4.47
	2	8.73	7.80	8.67	7.17	7.78	-	-	-	-

Data are the mean of two replicates per visit at time point 6. Total viable bacteria calculated by EMA + PMAxx-qPCR. Culturable bacteria by plate count. VBNC by the difference between viable and culturable bacteria cells and then log_{10}transformed. (-) means not analysed

3.4 Case scenario included in Objective 4

In an "historical" industrial setting using SmartWash™ system the effectiveness of chlorination management through an online monitoring system was evaluated. SmartWash™ System Solutions provides continuous monitoring and control of wash line disinfectant levels with unique calibration standards for chlorine electrodes and an automatic control process that utilizes sodium hypochlorite and online amperometric and pH sensors to regulate free chlorine and pH levels during the washing process in both the pre-washing tank (tank 1) and washing tank (tank 2) (**Figure 75**). To meet chlorine demand, it was necessary to measure these parameters continuously, allowing the system to adjust the chlorine concentration to a predetermined level. The use of this automated online monitoring and control system resulted in improved regulation of free chlorine and pH. Variations in the microbiological water quality, as indicated by total bacterial and coliform counts, showed higher counts in the pre-washing stage than in the washing stage, as expected but it demonstrates how the continuous monitoring and control of free chlorine at the correct pH effectively managed bacterial buildup (**Figure 75**).

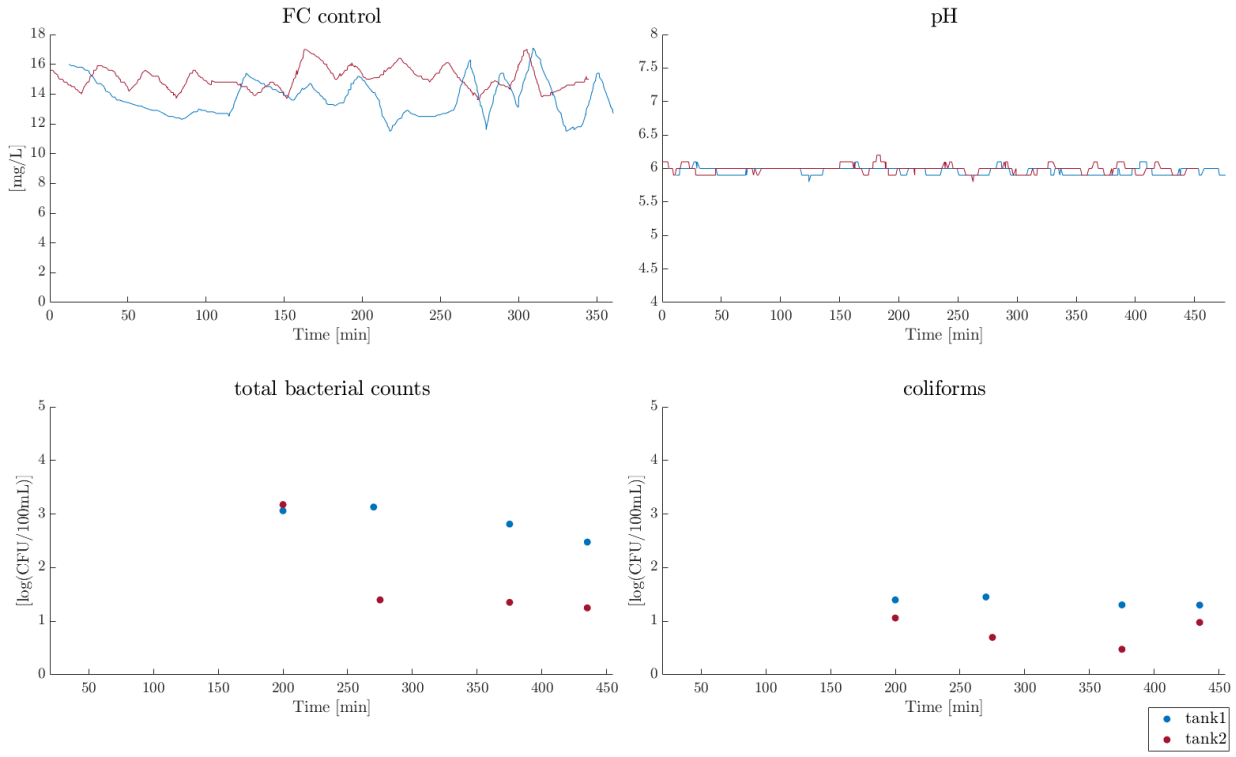


Figure 75. Chlorination management using online sensors and the changes in free chlorine, pH, total bacterial counts and coliform counts in the pre-washing (tank 1) and washing (tank 2) operations of baby leaves and cut iceberg lettuce. Values are the mean ± standard deviation of n=2 samples for each sampling time. See ID CEBAS-OM for sampling characteristics.

3.5 Results of the literature review – Tier 1

On 21 July 2022, the search terms as indicated in section 2.2.2.2 were used in both Scopus and Web of Science and resulted in a total of 1097 hits for RQ1, i.e. which data and models are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches, before deduplication. For RQ2, i.e. which microbiological and physico-chemical parameters or methods and models are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs, a total of 1676 hits were obtained in Scopus and Web of Science prior to deduplication. For each sub-question, a separate Endnote file was established and deduplication was performed according to the method published by Bramer et al. (2016). Following the method as described in section 2.2.2.3, the papers were screened for their relevance based on title, keywords and abstract in Tier 1. This selection resulted in 'Relevant' papers for which the full text was screened in Tier 2 and 'Non-relevant' papers that did not fulfil the inclusion criteria and were not evaluated further. The results of the screening performed in Tier 1 are indicated in the table below (**Table 16a**). In case papers were not relevant for the RQ at hand, but could potentially be relevant for another RQ, they were stored in a separate group and evaluated by the respective researcher for its relevancy. So, for papers about modelling, i.e. RQ1b and RQ2b, additionally 16 papers were evaluated and 4 classified as relevant and not yet included in the RQ. For RQ1a, additionally 25 papers were screened of which 1 was relevant and not yet included in the RQ. And finally, for RQ2a and RQ2c, a total of 53 papers were additionally screened of which 8 were classified as relevant and not yet included for RQ2c and 3 for RQ2a. The relevant additional papers are indicated in **Table 16b**.

Table 16a. Results of Tier 1 in the literature screening

RQ	Combination of terms	No. of hits Scopus	No. of hits WoS	Total hits (before deduplication)	Benchmark identified Scopus	Benchmark identified WoS	Duplicates[a]	Total after deduplication	% of duplicates	Relevant	Not relevant
RQ1a	title, key, abstracts(#1 AND #2) AND title (#3 AND #3a)	420	332	752	6 of 6	6 of 6	294	458	39%	91	367
RQ1b	title, key, abstracts (#1 AND #2) AND title (#3 AND #4)	175	170	345	1 of 1	1 of 1	148	197	43%	63	134
Total RQ1		595	502	1097			442	655		154	501
RQ2a	title, key, abstracts ((#1 OR #5) AND (#2 AND 2a AND #3a)) AND title #3	403	491	894	9 of 9	9 of 9	295	599	33%	130	469
RQ2b	title-key-abstract (#1 OR #5) AND #2 AND title (#3 AND #4)	328	306	634	4 of 4	4 of 4	248	386	39%	64	322
RQ2c	title-key-abstract (#1 OR #5) AND	68	80	148	1 of 1	1 of 1	47	101	32%	9	92

RQ	Combination of terms	No. of hits Scopus	No. of hits WoS	Total hits (before deduplication)	Benchmark identified Scopus	Benchmark identified WoS	Duplicates[a]	Total after deduplication	% of duplicates	Relevant	Not relevant
	title (#2 AND #3 AND #6)										
Total RQ2		799	877	1676			590	1086		203	883

a(Bramer et al., 2016)

Table 16b. Results of Tier 1 in the literature screening – additional papers found in one RQ that were indicated as potentially relevant for another RQ

Input for RQ	Identified papers	Relevant and not yet included in the RQ	Not relevant or already included in the RQ
For RQ1a	25	1	24
For RQ2a	53 (identified as relevant for RQ2a + RQ2c)	3	50
For RQ2c	53 (identified as relevant for RQ2a + RQ2c)	8	45
For RQ1b/2b	16	4	12

For convenience, the 5 Endnote files were combined into 1 summary Endnote file and duplicates removed. In total, 151 papers were classified as relevant, 130 as maybe relevant and the remaining 1031 as not relevant. Ten percent of all papers were screened by a second reviewer. Consistency between the first and second reviewer was more than 70% for all RQs, i.e. 85% for RQ1a, 71% for RQ1b, 77% for RQ2a, 90% for RQ2b and 73% for RQ2c. Discrepancies were discussed between reviewer 1 and reviewer 2 and in some cases led to a re-evaluation of the Endnote file. For example, RQ1a retrieved papers that described an artificial contamination of the produce. After discussion, it was decided to move these papers from 'not relevant' to 'maybe relevant'. Reasons for excluding papers for further evaluation were:

- Papers were about surface water, ground water, swimming pools, or irrigation water and not about process water
- Papers were exclusively about the microbial load or decontamination of the fresh produce, not about the process water
- Papers were not about microbial contamination, but about other aspects, e.g. vitamin content, flavour, chemical residues etc
- Papers were not about modelling (RQ1b and RQ2b)
- Papers were about COVID
- Papers were about modelling transfer of viruses from food handlers (RQ1b)
- Papers were about plankton or water beetles
-
- The papers that were identified as relevant will be read in full (Tier 2 of the literature review) to evaluate whether they contain narrative information on the topic and whether they contain data that can be used for the modelling. Some papers were retrieved in multiple RQs. These were evaluated only once. The final numbers are indicated in **Table 16c**.

Table 16c. Number of relevant papers to be evaluated in Tier 2

Research question (number of relevant papers)	Total number of relevant papers	Duplicates between RQ1 and RQ2	Total number of papers after deduplication
RQ1a and RQ2a			
Relevant (91) + additional RQ1a (1)	92	32	92[1]
Relevant (130) + additional RQ2a (3)	133	32	101
Total RQ1a and RQ2a	225	32	193
RQ1b and RQ2b			
Relevant RQ1b	63	33	63[1]
Relevant RQ2b	64	33	31

Research question (number of relevant papers)	Total number of relevant papers	Duplicates between RQ1 and RQ2	Total number of papers after deduplication
Additional for modelling (RQ1b/RQ2b)	4	0	4
Total RQ1b and RQ2b	131	30	98
Relevant + additional RQ2c	17	NA	17

[1]Duplicates between RQ1 and RQ2 were evaluated in RQ2 and removed from RQ2

A Google Advanced Search was performed to search for relevant reports published on the websites of AESAN, ANSES, UK FSA, BfR, WHO, FAO and US FDA as indicated in section 2.2.2.2. Initially, separate searches (RQ2a and RQ2c) were defined to look for reports focusing on verification, validation or (inline) monitoring. However, since these searches overlapped the searches RQ1 and RQ2, it was decided to perform them as such and in both searches not only look for information on microbial loads (and physico-chemical properties) but also manually go through the results and look for methods (inline or offline) to verify the microbial quality of the water.

Reasons for excluding reports for further evaluation were:

- Reports focused exclusively on food and not on process water
- Reports focused on drinking water, surface water, grey water or household water not on process water
- Reports did not focus on FFVHs but, for example, on beef, fish or pork
- Reports only described minutes of a meeting
- PDF found only included a list of participants, etc.
- Reports were on ship sanitation
- Reports were on antimicrobial resistance
- Reports were about other topics: climate change, infant feeding, medical technologies etc.
- Links were not working

Table 17 provides an overview of the number of hits found and the number of relevant reports. Appendix D gives an overview of the rationale per evaluated report in case of exclusion in Tier 1.

Table 17. Results of the Google Advanced Search as performed for RQ1 and RQ2 in Tier 1

RQ	Query	Website	Result[a]	Relevant
RQ1	("microbial hazards" pathogen) AND ("processing water" OR "wash water") AND (fruit	https://who.int	**78**	**4**
		https://aesan.gob.es	**6**	**3**

RQ	Query	Website	Result[a]	Relevant
	OR vegetable OR herb OR "fresh produce") site:**<website>** filetype:pdf	https://anses.fr	**1**	**0**
		https://food.gov.uk	**20**	**2**
		https://fda.gov	**46**	**6**
		https://bfr.bund.de	**2**	**1**
		https://fao.org	**173**	**19**
RQ2	("microbial hazards" OR pathogen) AND ("processing water" OR "wash water") AND (fruit OR vegetable OR herb OR "fresh produce") AND (physicochemical OR "physico-chemical") site:**<website>** filetype:pdf	https://who.int	**10**	**4**
		https://aesan.gob.es	**3**	**2**
		https://anses.fr	**0**	**0**
		https://food.gov.uk	**4**	**0**
		https://fda.gov	**2**	**1**
		https://bfr.bund.de	**0**	**0**
		https://fao.org	**25**	**6**

[a]Results obtained on 20 September for RQ2 and on 22 September for RQ1

For RQ1, a total of 12 relevant and 23 maybe relevant reports were found. In total, 28 unique reports were found and read in full in Tier 2. For RQ2, in total 13 relevant reports were found for which 4 were found in multiple websites resulting in a total of 9 unique reports that will be read in full in Tier 2.

3.6 Results of the literature review – Tier 2 Data extraction and inline monitoring

The Google search resulted in a total of 37 reports or papers (28 unique results for RQ1 and 9 unique results for RQ2) that were read in full to determine their relevance for this research. This showed that most reports only contained general information on FVHs processing and water quality. In total, 4 scientific papers were found in the Google search that were also found in the

search for scientific papers in Scopus and Web of Science. Overall, the Google search did not result in reports or papers that were relevant to include for data extraction.

The following part describes the results of the literature review to obtain relevant data related to microbial loads in water used for processing ffFVHs (RQ1a), data on microbial and physico-chemical parameters (RQ2a) and results related to inline/online monitoring systems (RQ2c). Results obtained on the literature review with respect to modelling RQ1b and RQ2b are described in section 3.7.1.

A total of 193 scientific papers that were evaluated as relevant for RQ1a/RQ2a were read in full. After a full-text screening, 123 references were considered relevant while the remaining 70 were not relevant. Of these 123 relevant papers, 105 contained relevant data. The EFSA WG selected a total of 69 papers for which data has been extracted according to the EFSA Excel format, which was submitted as a separate file to this report. This section provides a narrative summarising the results of these papers. Additionally, 17 papers identified as relevant for RQ2c were read in full, 8 of which contained relevant information that is also summarised in this section (section 3.6.3).

The majority of the 69 studies were performed in the USA (n=36), followed by Spain (n=10). Other countries included were Belgium, Brazil, Canada, Egypt, Finland, Ireland, Italy, Korea and New Zealand. Most papers (55 out of the 69 papers) described experiments in a lab setting. Only 14 papers described industrial or pilot scale experiments studying water used for processing spinach, lettuce, tomatoes, cabbage, bell peppers, carrots, endive, radicchio and peaches. Most papers studied vegetables or a combination of vegetables and fruits (54). Only 18 papers studied fruit or a combination of fruits and vegetables and only 2 papers were found describing herbs (cilantro and parsley). Lettuce was the most studied vegetable (35 in total) and berries were the most studied fruit (9). More papers studied fresh-cut vegetables than whole vegetables, while for fruit it was the other way around (**Figure 76**). In the category fresh-cut, all products that were not whole, i.e., cut, blended, juiced, shredded or chopped were included.

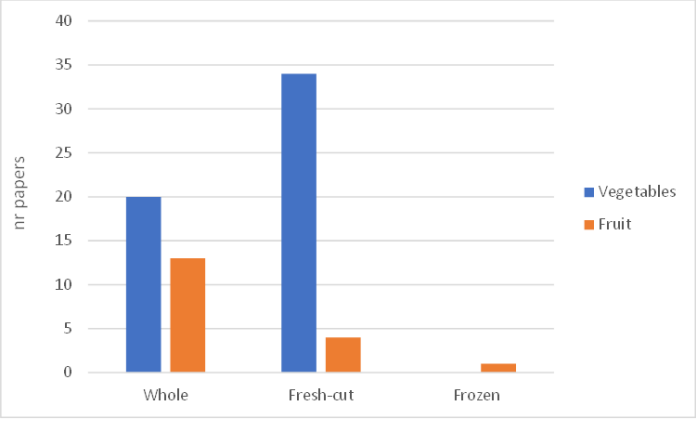


Figure 76. Number of papers describing water quality used for the handling and processing operations of fresh-whole, fresh-cut (including blended, juiced, shredded, chopped or cut) and frozen FVHs obtained for RQ1a and RQ2a.

In this literature analysis, a clear distinction was made between studies describing the detection of pathogens, hygiene indicators, or experimental indicator/index organisms. Pathogenic microorganisms most frequently studied in the collected literature included *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes*. Hygiene indicators studied included the determination of coliforms, coliphages, and total mesophilic count. Non-pathogenic *E. coli* and *Listeria innocua* were most often used as indicator organisms in experimental set-ups. The majority of the papers studied pathogens followed by hygiene indicators. Three papers studied viruses (norovirus and SARS-CoV-2) and only one paper included parasites.

3.6.1 Results RQ1a

The following sections summarise the literature found to answer RQ1a: which data are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches Section 3.3.1.1. summarises the microbial levels found in the water used for processing or handling a range of ffFVHs studied in the literature both at pilot/industrial scale and at lab scale. Section 3.3.1.2. summarises the results of lab studies performed on the effect of disinfectants on reducing pathogens in the process water. The subsequent sections (3.3.1.3-5) give a narrative summary for FVHs separately.

3.6.1.1 Microbial contamination in water used for processing ffFVHs

In order to evaluate the microbial contamination of water used for processing ffFVHs, levels found at industrial and pilot scale for all 69 papers were plotted (**Figure 77**; data from Barrera et al., 2012; Tomás-Callejas et al., 2012b; Maatta et al., 2013; Wang et al., 2013; Wulfkuehler et al., 2013; Guo et al., 2017; Bornhorst et al., 2018; López-Gálvez et al., 2020; Bertoldi et al., 2021; Cuevas-Ferrando et al., 2021). Bacteria studied were grouped as non-pathogenic bacteria, pathogenic bacteria and total bacterial counts. Total counts included total aerobic counts also mentioned as total viable counts, Enterobacteriaceae, total coliforms, total coliphages etc. Pathogenic bacteria comprised *Bacillus cereus*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* enterica and *Salmonella* Typhimurium. Non-pathogenic bacteria included other *E. coli* strains, *L. innocula* and *Pseudomonas* spp.

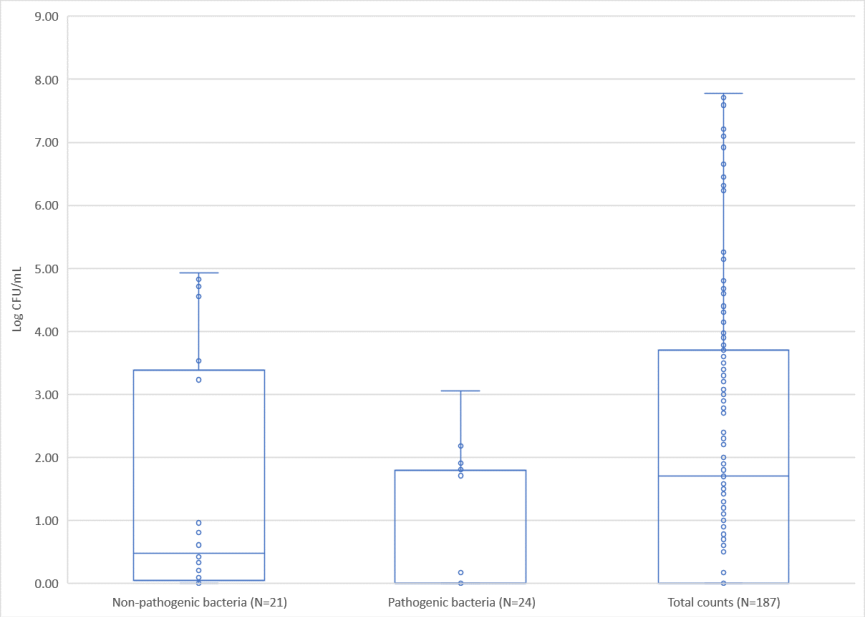


Figure 77. Boxplots representing the levels of bacteria found in the water used for processing all FFVHs studied at industrial or pilot scale grouped by non-pathogenic bacteria, pathogenic bacteria (*Salmonella enterica*) and total bacterial counts. N values represent the number of measurements(Barrera et al., 2012; Tomás-Callejas et al., 2012b; Maatta et al., 2013; Wang et al., 2013; Wulfkuehler et al., 2013; Guo et al., 2017; Bornhorst et al., 2018; López-Gálvez et al., 2020; Bertoldi et al., 2021; Cuevas-Ferrando et al., 2021), the lines are the median values and the points are the measurements (assuming <LoD = <0 log CFU/mL).

As expected, the highest bacteria levels were represented by the total bacterial counts found at a range between 0-8 log CFU/mL. Pathogenic and non-pathogenic bacteria were found at lower levels ranging between 0-3 and 0-5 log CFU/mL, respectively. It should be noted that the number of data points for the latter two groups of bacteria was much lower (around 20) than for total bacterial counts (n=187). Furthermore, the graphs represent a range of industrial processes using a variety of disinfection and no disinfection methods.

Some papers studied microbial contamination at a lab scale by collecting process water from FBOs for their experiments. **Figure 78** shows the total bacterial counts found for non-inoculated water at time point t=0 (control setting) for data obtained from Anese et al., 2015 and Borges et al., 2020. The range is comparable as found in industrial and pilot setting (**Figure 77**). It should be noted that the number of data points in Figure 78 was different than in Figure 78 with only 6 data points for total bacterial counts.

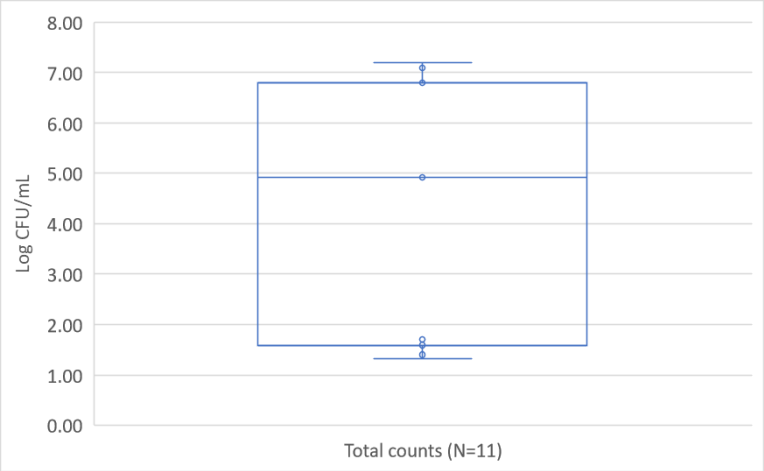


Figure 78. Box plots representing the total bacterial counts of bacteria found in the water used for processing FFVHs studied at a lab scale (t=0, control). N values represent the number of measurements (Anese et al., 2015; Borges et al., 2020), the lines are the median values and the points are the measurements in process water (assuming <LoD = 0 log CFU/mL)

3.6.1.2 Effect of disinfectants on microbial contamination in water used for processing ffFVHs

To prevent cross-contamination of pathogens from process water to produce, chemical disinfectants or physical treatments such as UV may be used to treat the water. Several papers studied the effect of disinfectants on reducing the bacterial levels in the water used for processing and handling ffFVHs. The most frequently studied disinfection methods are chlorine based disinfectants and UV applied as a single technique or in combination with other techniques. Other methods studied entail the use of acids such as peracetic acid, hydrogen peroxide (H_2O_2), ultrasound and pulsed light. These methods were studied at lab scale. At pilot scale or industrial, disinfection technologies applied were: chlorine, peracetic acid and UV. **Figure 79** shows the efficacy of chlorine on the inactivation of *Escherichia coli* O157:H7 in water used for processing lettuce expressed as log reductions depending on COD levels. The figure demonstrates that the efficacy of chlorine decreases with increasing COD and that the reductions ranged between 0 and 7 log (Davidson et al., 2014). Bertoldi et al. (2021) indeed indicated that the organic load of process water, indicated by turbidity, chemical oxygen demand (COD), and total dissolved solids (TDS), can increase over time, affecting the effectiveness of disinfection methods applied. However, a wide variability can be seen in log reductions in the water depending on the FVH processed (**Figure 80**; data from Guo et al. (2017) and Huang and Chen (2020)). This figure shows that at the same chlorine dose and comparable COD levels (around 2000 mg/L), log reductions range from 1 to 6.5 log. It should be noted that in this plot various experimental setups are combined as well as various sampling points, which may affect the log reductions found (**Figure 80**). Furthermore, differences observed may be due to different experimental setups where in some cases the wash water is inoculated and in others the produce that is subsequently washed. Settings such as pH and residual concentration are not always recorded in these experiments.

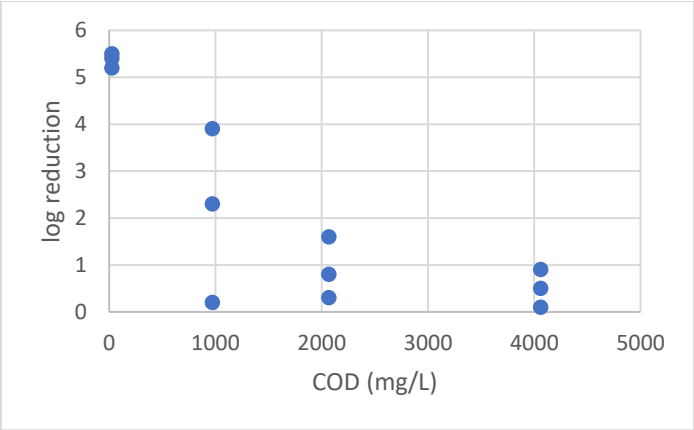


Figure 79. Log reductions of *Escherichia coli* O157:H7 in water used for processing Iceberg lettuce where the chlorine concentration was maintained at 50 mg/L chlorine as a function of COD performed at pilot scale. Data extracted from Davidson et al. (2014).

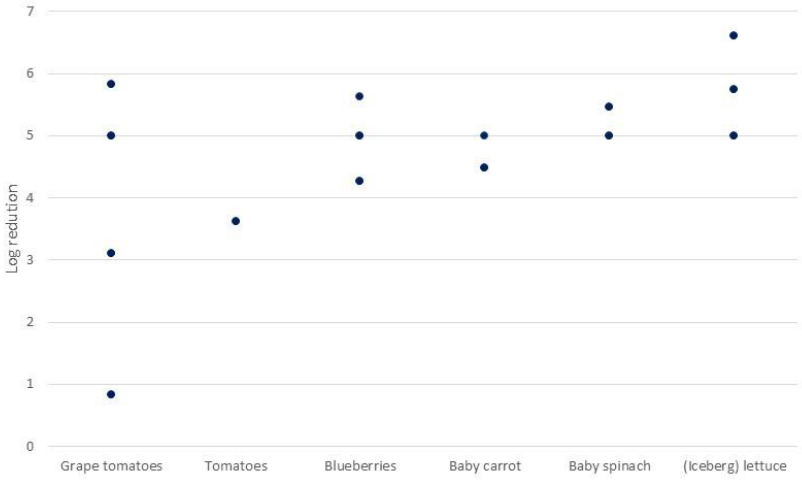


Figure 80. Log reductions of pathogens in process water when washing different FVHs with water treated with chlorine at a dose of 10 mg/L with a COD of around 2000 mg/L at lab scale (data extracted from Guo et al. (2017) and Huang and Chen (2020)).

Since the use of chlorine disinfectants may lead to the production of disinfection by-products (DBPs), alternative disinfectants are being explored in the literature. Peracetic acid (PAA) is the most frequently studied alternative. The effect of this disinfectant on log reductions in the process water is depicted in **Figure 81** in which up to 7 log reductions may be obtained depending on the dose (data extracted from Huang et al. (2018), Mathew et al. (2018) and Davidson et al. (2017)).

This figure shows large variability in log reductions between 3 and 7 log for a range of products using a dose of 80 ppm PAA. When exploring the same product, i.e., Iceberg lettuce at a dose of

50 ppm, log reductions range between 3 and 6 log. COD levels seem to affect the efficacy of PAA to a lesser extent than that of chlorine (**Figure 81**).

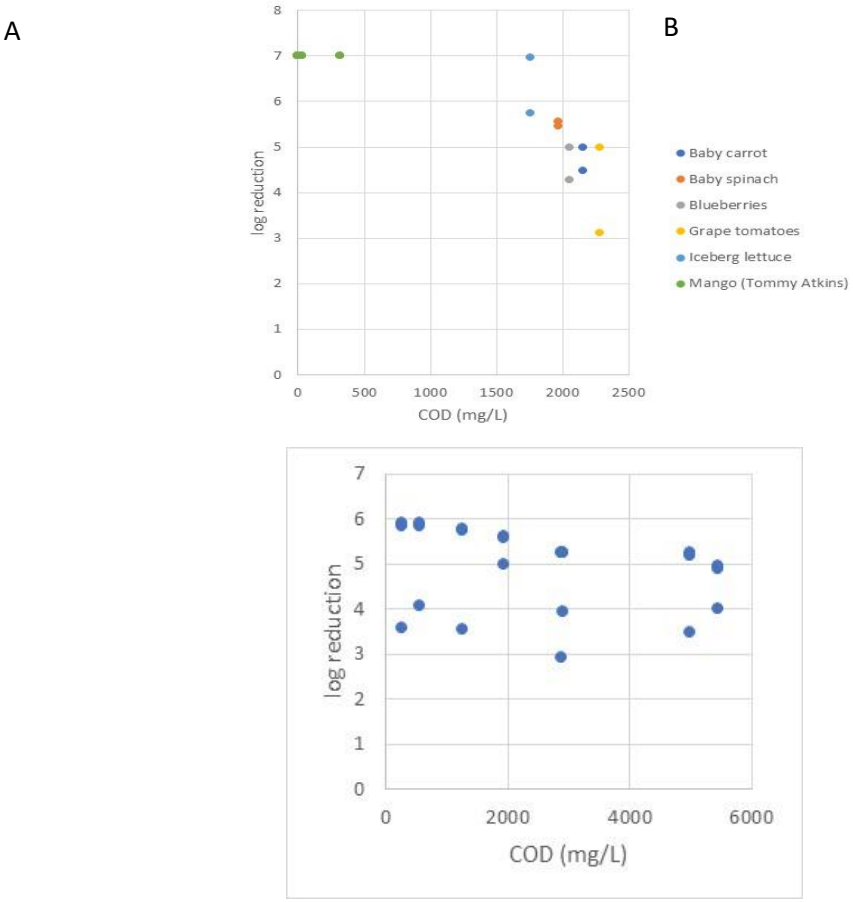


Figure 81. (A) Log reductions of *E. coli* O157:H7 in the process water from washing different FVHs with water treated with PAA at 80 mg/L at lab scale. Data extracted from Huang et al. (2018) and Mathew et al. (2018). (B) Log reductions obtained in process water from washing Iceberg lettuce at pilot scale by PAA at 50 mg/L. Data from Davidson et al. (2017)

Another frequently studied disinfection technique is UV. **Figure 82** shows that log reductions between 3 and 5 log were obtained at COD levels between 1500 and 2500 mg/L for a range of products, contrary to our expectations (data from Cossu et al. (2016), de Oliveira et al. (2018) and Huang et al., (2018)). In general, the lower log reductions were obtained when the water had higher turbidity. When a lower dose of around 3 mW/cm^2 was applied, log reductions achieved were less than 1 log (right graph). Apart from turbidity, the UV dose, thus, also influences the log reductions achieved.

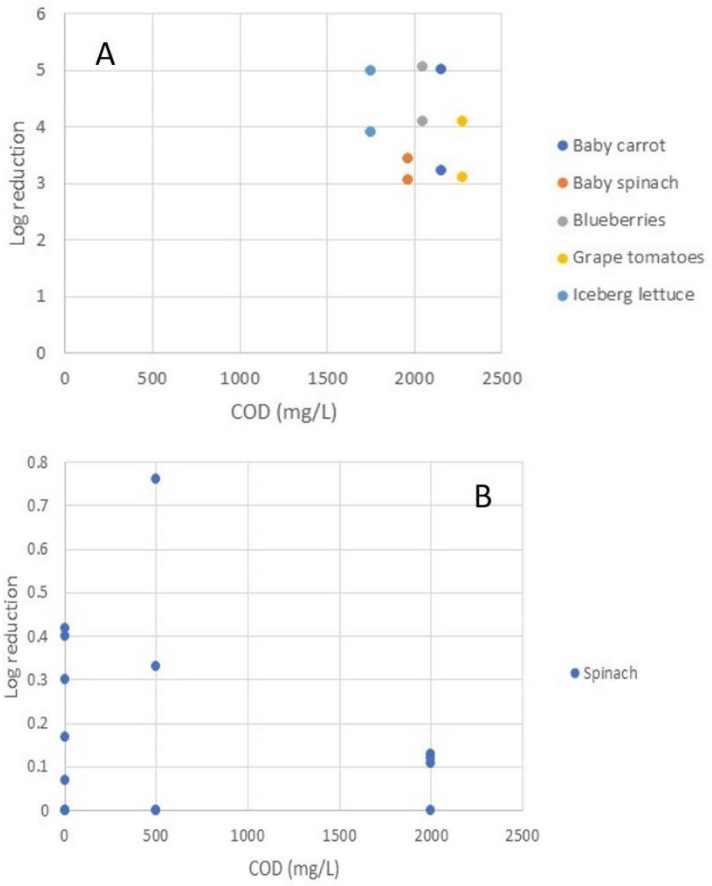


Figure 82. (A) Log reductions of *E. coli* O157:H7 in the process water from washing different FVHs by UV using a dose of 23-28 mW/cm^2 at high COD levels at lab scale. Data extracted from Cossu et al. (2016) and de Oliveira et al. (2018). (B) Log reductions of *Salmonella enterica* in the process water from washing spinach at a dose of around 3 mW/cm^2. Data extracted from Huang et al. (2018).

3.6.1.3 Data on microbial contamination of the water used for processing vegetables

In this report, we focus on summarizing studies that have investigated microbial accumulation in water used for processing vegetables, excluding studies solely on product decontamination.

Most papers on vegetables studied leafy greens and tomatoes. Microbial contamination in the water used for processing these crops is summarised in **Figure 83** (data from Barrera et al., 2012; Bornhorst et al., 2018; Bertoldi et al., 2021). This figure shows that the levels of pathogenic and non-pathogenic bacteria in water used for processing leafy greens are higher than for tomatoes. It should be noted that the number of data points for tomatoes was lower than for lettuce.

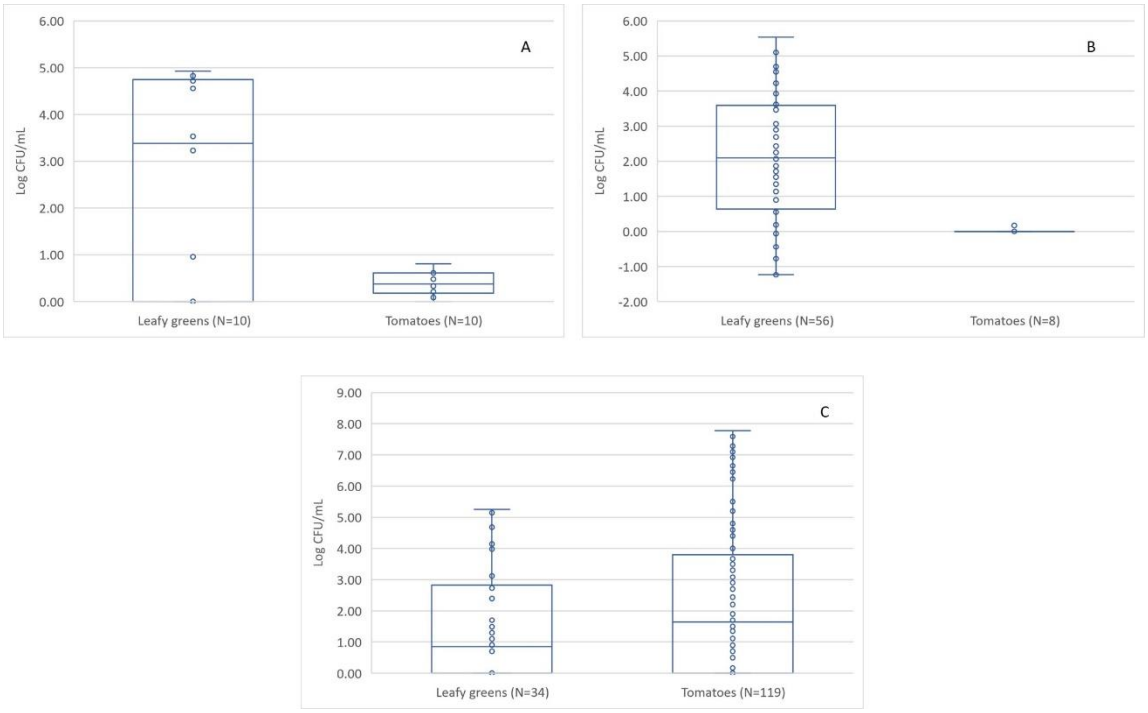


Figure 83. Box plots representing non-pathogenic (A), pathogenic (B) and total bacterial counts (C) in process water used for washing leafy greens and tomatoes at pilot or industrial scale. N values represent the number of measurements (Barrera et al., 2012; Bornhorst et al., 2018; Bertoldi et al., 2021), the lines are the median values and the points are the measurements in process water (assuming <LoD ≤0 log CFU/mL).

In conclusion for RQ1a (i.e. which data are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches?), there are data available on the microbial contamination of process water. These data showed that total bacterial counts in the water range between 0 and 8 log CFU/ml, non-pathogenic micro-organisms were found between 0 and 5 log CFU/ml and pathogenic bacteria (*Salmonella enterica*) between 0 and 3 log CFU/ml. It should be noted that the higher pathogenic levels were obtained from an experimental setup with inoculated leafy greens. Most data were found for experiments performed at lab scale and only a limited number of studies described microbial levels in process water used at pilot or industrial scale. Furthermore, papers were found that described the effect of disinfectants on microbial levels in the water. These showed that chlorine solutions and UV were the water treatments most frequently studied. Furthermore, the effect of PAA was frequently described. The studies showed that chlorine solutions, PAA and UV each have the potential to reduce the microbial load in the water depending on the dose applied, the organic load of the process water and the proper management of the water treatment. However, the effectivity was influenced by the type of FFV

processed and efficacy of chlorine solutions was influenced by COD levels. The following sections give more detailed information on the data found for the specific ffFVHs included in this study.

Fresh-whole vegetables

Most papers on fresh-whole vegetables included lettuce (n=9), tomatoes (n=8), spinach (n=2), carrots (n=1) as well as other vegetables, such as radicchio, red chard and watercress (n=1) (**Figure 84**).

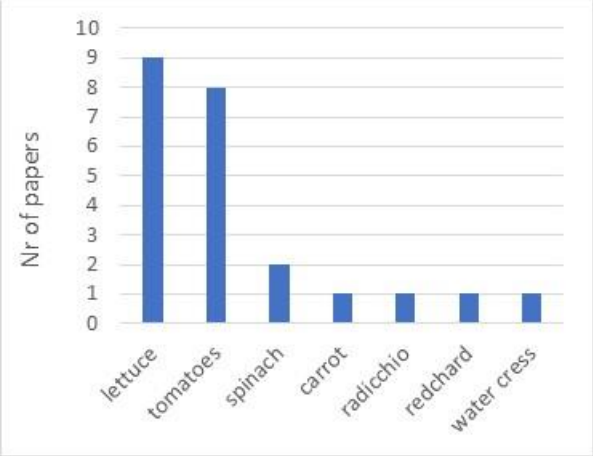


Figure 84. Number of papers studying the contamination of water used for processing and handling fresh-whole vegetables

When evaluating the most frequently studied vegetables (i.e., leafy greens and tomatoes), the majority of the papers studied washing procedures at lab scale on a variety of microorganisms (e.g., *Salmonella* spp*., E. coli, Listeria* spp., total viable counts and coliforms). Also, a wide range of water disinfection methods were studied, of which those based on exposure to chlorine-compounds and UV were used the most as both single or in combination techniques. Other methods studied were based on exposure to hydrogen peroxide, PAA, ultrasounds or cold plasma. Log reductions obtained in these lab experiments varied widely. For instance, no log reductions were obtained in a lab experiment in which blended spinach suspension was inoculated with *E. coli* at around 2 x 10^6 CFU/ml and treated by UV at 2.6 mW/cm^2 for 5 min (Cossu et al. (2016). However, in another experiment in which iceberg lettuce was dip-inoculated with 10^8-10^9 CFU/ml *Salmonella* cocktail and then transferred to clear water, around 7 log reduction was obtained in the water after 2 min UV treatment using 4 UV lamps at 265 W. **Figure 85** visualises the number of papers that studied disinfection methods in fresh-whole vegetables.

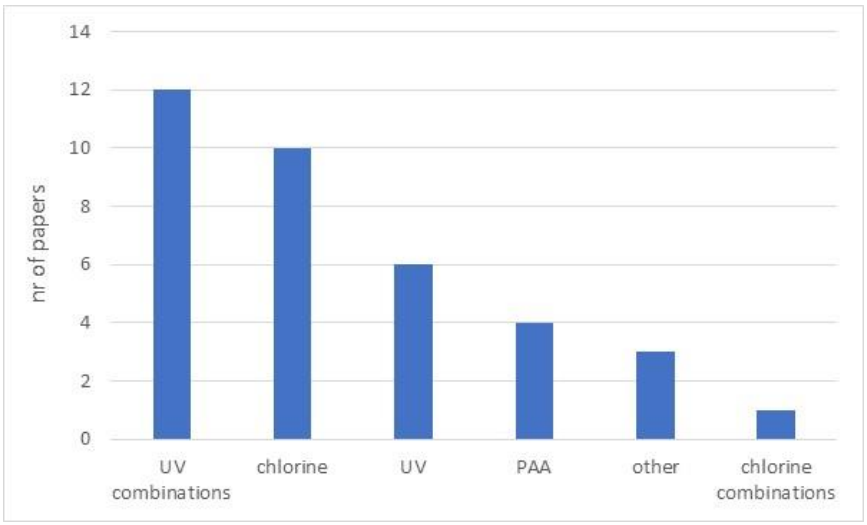


Figure 85. Number of papers studying the effect of different disinfection methods on the microbial quality of water used for processing and handling fresh-whole vegetables

A limited number of papers studied leafy greens and fresh-whole tomatoes at pilot and industrial scales. One paper that studied microbial loads in the wash water of leafy greens at a pilot scale, focused on *E. coli* O157:H7 (Buchholz et al., 2012). The applied produce was dip-inoculated with differentotal bacterial counts of *E. coli* O157:H7, i.e., 10^7, 10^5 and 10^3 CFU/mL and the transfer into water was studied. The microbial contamination ranged between 81-118% for iceberg lettuce, 49-65% for romaine lettuce and 49-147% for baby spinach, an estimation based on the total number of *E. coli* O157:H7 transferred from the inoculated leafy greens into the flume water (Buchholz et al., 2012). Percentages above 100% might be explained by experimental error.

Leafy green processing at an industrial scale was only studied in one paper (Barrera et al., 2012) while three studies on tomato processing at an industrial scale were conducted (Tomás-Callejas et al., 2012; López-Gálvez et al., 2020; Bertoldi et al., 2021). The microbial contamination found in these four papers is visualised in **Figure 86**.

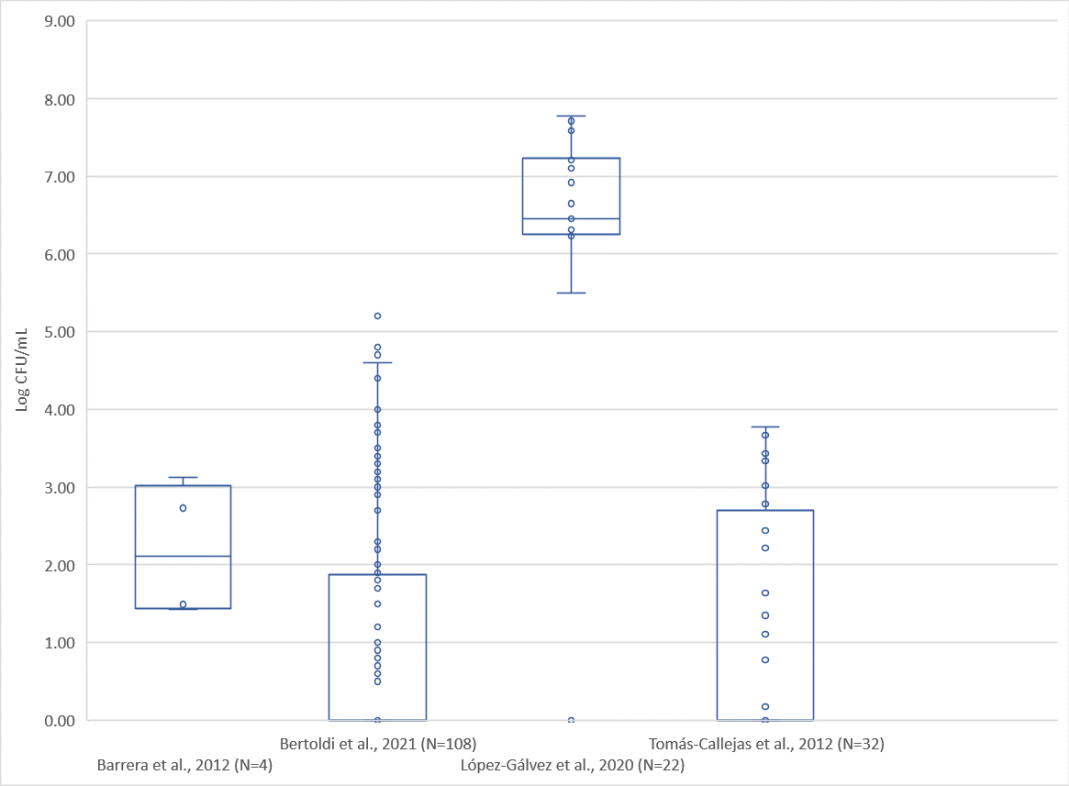


Figure 86. Box plots representing total bacteria counts in the water used for processing fresh-whole vegetables in an industrial setting as reported in leafy greens (Barrera et al., 2012) and tomatoes (Bertoldi et al., 2021; López-Gálvez et al., 2020; Tomás-Callejas et al., 2012). N values represent the number of measurements, the lines are the median values and the points are the measurements in process water (assuming <LoD = <0 log CFU/mL).

In the leafy green study, Barrera et al. (2012) examined the changes in the microbial load of wash water of a second wash tank of two spinach processors and found that *E. coli* was present in 57% of the water samples of processor A, while it was not detected in the wash water of processor B. This difference may have been due to variations in water treatment between the two processors, as processor A did not maintain a constant disinfectant concentration (i.e., PAA) or refreshed the water, whereas processor B did.

The microbial levels in water used for washing tomatoes varies widely, ranging levels from 0 to 5 log CFU/mL (Bertoldi et al., 2021; Tomás-Callejas et al., 2012), whereas higher levels between 5.5 and 7.8 log CFU/mL were detected in other cases (López-Gálvez et al., 2020) (**Figure 87**). However, it is difficult to compare these studies since the manufacturing practices, packing house, variety, amount of product washed and disinfectant differed. For example, López-Gálvez et al. (2020) studied a worst-case scenario in which recirculated process water was used for a whole working day. This may explain the higher levels found compared to the other two studies. In the study reported by Tomás-Callejas et al. (2012), sampling at three tomato packing houses in the US showed that the coliform levels in the dump tank were similar across processors, but

differences were observed in the flume system. Coliform levels in the dump tank water were significantly higher than in the flume system for all trials. These authors concluded that although the use of chlorine dioxide at the lab scale showed to be effective, its efficiency at the industrial scale was not demonstrated (Tomás-Callejas et al., 2012). Another study on tomatoes revealed that even though hypochlorite was used to treat the water in all three packing houses in Florida, microbial levels increased over time, and the levels of COD, TDS, and turbidity of the water also rose significantly over time (Bertoldi et al., 2021). Aerobic plate count and total coliform levels in water flume tanks varied between the three packing houses. According to these authors, this was due to the differences in free chlorine levels and the amount of organic load accumulating in the water (Bertoldi et al., 2021). López-Gálvez et al. (2020) found that using recirculated water treated with chlorine dioxide to wash tomatoes, levels of aerobic mesophilic bacteria, coliforms, and *E. coli* were more than 1 log lower than the untreated water.

Data on the natural occurrence of enteric viruses in industrial process water is limited due to methodological difficulties. Cuevas-Ferrando et al. (2021) reported on the occurrence of enteric viruses in water samples periodically collected from the washing tanks of commercial facilities where bell peppers, baby leaves or a veggie-fruit mix were processed (Cuevas-Ferrando et al., 2021). No human norovirus GI or GII, astrovirus, rotavirus, or hepatitis A were detected in any of the water samples except for one process water sample from peppers, in which rotavirus RNA was detected. Counts of total and F-specific RNA coliphages varied considerably ranging from non-detected to high counts (4.1 log pfu/L). DNA of *CrAssphage* was detected in 60-70% of samples. The occurrence and concentration of bacteriophages depended on the type of product washed, the product/water ratio and the residual concentrations of the disinfectant used (chlorine and PAA). The concentration of coliphages and crAssphage was the highest in process lines with a low replenishment rate and no disinfectants. The occurrence and concentration of coliphages were low when residual chlorine was constantly maintained (Cuevas-Ferrando et al., 2021).

Fresh-cut vegetables

This category includes all vegetables being processed through cutting, blending, juicing, shredding or chopping. **Figure 87** shows that the vast majority of papers studied fresh-cut lettuce (n=25) while other vegetables, i.e., carrots, broccoli, cabbage, celery, endive, mung bean sprouts, mushroom, spinach and tomato were studied in a lower number of studies.

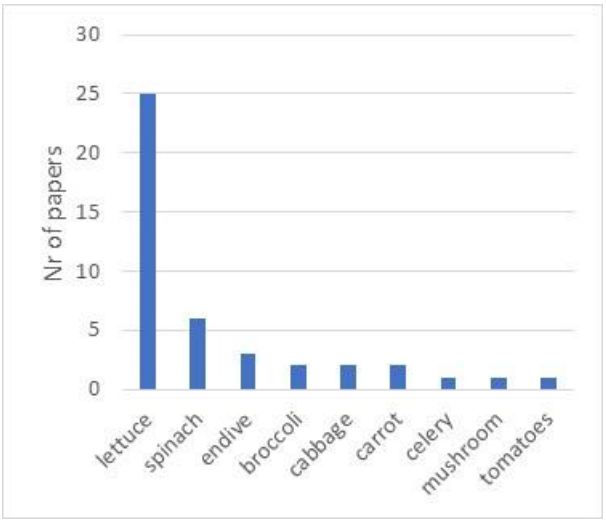


Figure 87. Number of papers studying the microbial quality of water used for processing and handling fresh-cut vegetables

For leafy greens (i.e., lettuce and endive), the majority of the papers focused on *Salmonella* spp., *E. coli* and total viable counts. Other microorganisms included in these studies were *Listeria* spp., *Pseudomonas* spp., norovirus, total coliforms and Enterobacteriaceae.

Most papers studied the process water of washing fresh-cut vegetables at a lab scale. The effect of various disinfection methods was studied either as single or combined treatments. The majority of the papers studied the effects of chlorine-based disinfectants (25 papers in total) as indicated in **Figure 88**.

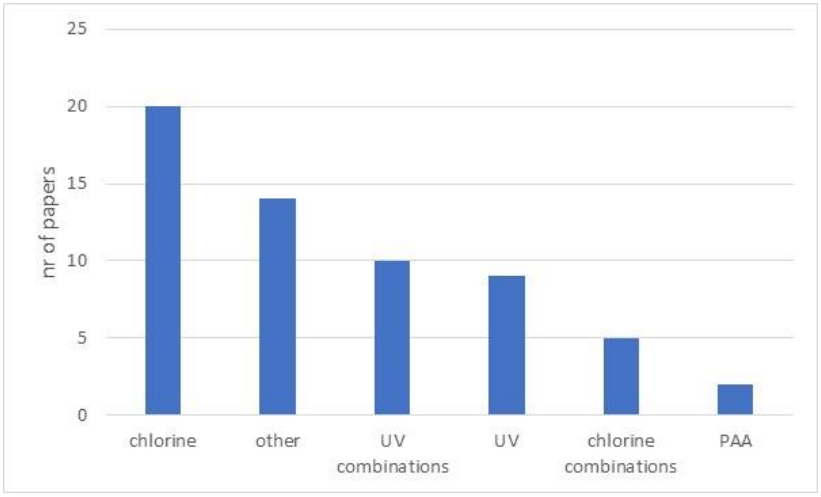


Figure 88. Number of papers studying the effect of different disinfection methods on the microbiological quality of water used for processing and handling fresh-cut vegetables at a lab scale. Other methods include electrocoagulation, organic acids, pulsed light, ultrasound, grapeseed extract, electrolysed water and benzyl isothiocyanate

Reductions obtained in process water ranged from 0.2 log after 1 min contact time for murine norovirus (MNV) treated with free chlorine (Dunkin et al., 2017) to more than 8 log for *Salmonella* in wash water treated with a combination of chlorine and UV (Guo et al., 2017). Other disinfectants studied were acids like citric acid and PAA as well as hydrogen peroxide (H_2O_2). Apart from chemical disinfectants, physical treatments for process water were also studied, such as electrocoagulation, UV, ultrasound and pulsed light.

A few papers described experiments with fresh-cut leafy greens at an industrial scale. **Figure 89** summarizes the microbial contamination found in process water used for fresh-cut vegetables (data from Barrera et al. (2012), Bornhorst et al. (2018) and Maatta et al. (2013)).

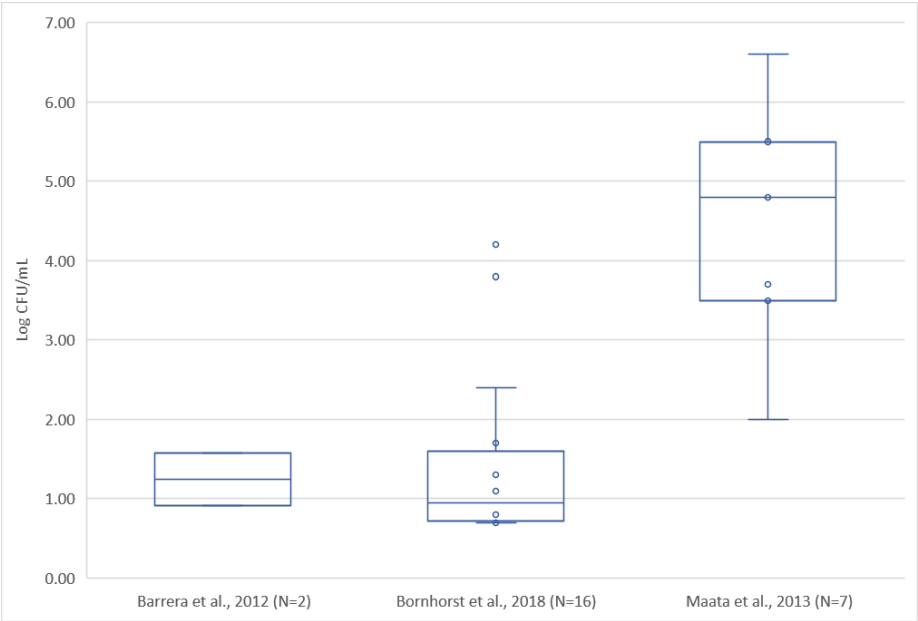


Figure 89. Box plots representing total bacterial counts in the water used for processing fresh-cut vegetables in an industrial setting as reported by Barrera et al. (2012), Bornhorst et al., (2018) and Maatta et al. (2013). N values represent the number of measurements, the lines are the median values and the points are the measurements in process water (assuming <LOD ≤0 log CFU/mL).

The study by Barrera et al., (2012) which was described in section (3.6.1.1.1) not only included fresh-whole spinach, but also shredded lettuce. A comparison of the wash water between shredded lettuce (processor C) and spinach (processor B) showed a comparable average for aerobic plate counts. Coliform levels in the wash water of shredded lettuce (processor C) were significantly lower (around 1 log CFU/ 100 mL) than those for spinach processed at processor A (around 3 log CFU/100 mL), but comparable to those for spinach processed at processor B (see also section 3.6.1.1.1 where the results for spinach are described). Like processor B, processor C continuously replenished the water, which was not the case for processor A (Barrera et al., 2012). Bornhorst et al. (2018) compared a conventional fluming system for the processing shredded iceberg lettuce and diced cabbage with an immersion free, single pass produce washing system, which consisted of a series of overhead sprayers used for pre-washing after cutting and during

tumbling. They found that the organic load in the alternative washing system was consistent over time while the organic load in the flume system increased significantly. Comparing the microbial load at the various stages of processing showed that the highest organic loads and aerobic plate counts (3.8 and 4.2 log CFU/100 mL in the wash water for cabbage and lettuce, respectively) were found at the cutting stage compared to the other stages (between <0.6-2.4 log MPN/100 mL). Aerobic plate counts in the flume system were around 1 log MPN/100 ml for both cabbage and lettuce processing (Bornhorst et al., 2018). A Finnish study showed that non-pathogenic *Yersinia enterocolitica* was found in several wash water samples of carrot processors. In general, higher heterotrophic plate counts were found in the water used for washing the carrots (4.8-6.6 log CFU/mL) than in the water used for rinsing and cooling the processed carrots (3.7-5.7 log CFU/mL). No *E. coli* was detected in any of the water samples taken and coliform levels ranged between 3.5-5.5 log CFU/100 mL for wash water and between 2.0-5.5 log CFU/100 mL in process water. No specifications of the washing procedures and/or water treatment were indicated (Maatta et al., 2013). A study in the US evaluated the water quality of a commercial lettuce processing company, where water was collected from 2 sequential wash tanks that were replenished with municipal water at a 30% recharge rate. Hypochlorite was added using an in-line system. Analysis of the water showed that both wash tanks operated below the chlorine demand resulting in fluctuating free chlorine levels. Furthermore, spent wash water was collected from the two wash tanks and at the laboratory either directly inoculated with a cocktail of *E. coli* O157:H7*, Salmonella* spp *or L. monocytogenes* (to a final concentration of around 7 log CFU/mL) or supplemented with chlorine to achieve a level of 2.5 mg/L free chlorine before inoculation with these pathogens. In general, log reductions for *E. coli* O157:H7 and *Salmonella* were lower in the water from wash tank 1 compared to wash tank 2. According to the authors, this difference may be due to the differences in disinfection byproducts found between the two tanks. The higher DBP in wash tank 1 resulted in higher antimicrobial activity compared to wash tank 2. No significant differences were observed for *L. monocytogenes*. The water supplemented with chlorine showed significantly higher log reductions for the studied pathogens (Murray et al., 2018).

Alternative washing procedures for fresh-cut iceberg lettuce and endive at pilot scale showed that although applying warm water (45 °C) with or without $CaCl_2$ improved the product quality, it was not effective in reducing microbial counts in the wash water. On the other hand, chlorinated water resulted in final levels in the wash water below the level of detection (10 CFU/mL) (Wulfkuehler et al., 2013). This study was conducted on a pre-industrial scale using chlorinated water (4 °C, 120 mg/L) as the purpose was to study the impact of such a high chlorine concentration on the quality of fresh-cut iceberg lettuce. However, a residual concentration of 10 mg/L should be enough as higher concentrations will not improve the microbiological quality of the process water.

Frozen vegetables

None of the 69 papers described the microbial load in water used for processing frozen vegetables.

3.6.1.4 Data on microbial contamination of the water used for processing fruits

Fresh-whole fruits

In total, 13 papers included fresh-whole fruits in their study of which 12 papers were performed at a lab scale. Berries were the most frequently fruits studied (n= 6), which included blueberries,

raspberries and strawberries. Other fruits included in one or two papers were apples, grapes, mangos, papayas and cantaloupes (**Figure 90**).

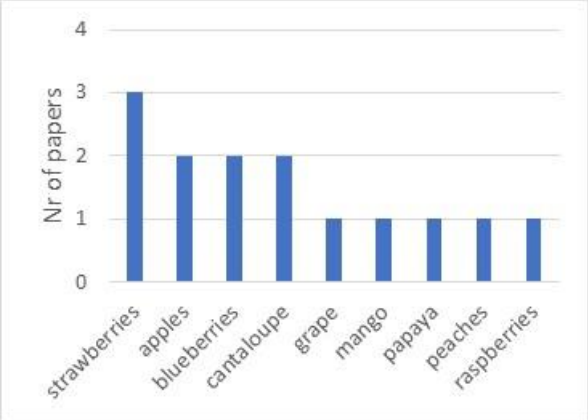


Figure 90. Number of papers studying water used for processing and handling fresh-whole fruits

The most studied pathogens were *Salmonella* spp. and *Listeria* spp. Like for the vegetable papers, most studies were conducted at a lab scale and investigated the efficacy of chlorine-based disinfectants followed by UV disinfection as a single technique or in combination. Other methods studied were PAA, H_2O_2 or pulsed light (**Figure 91**).

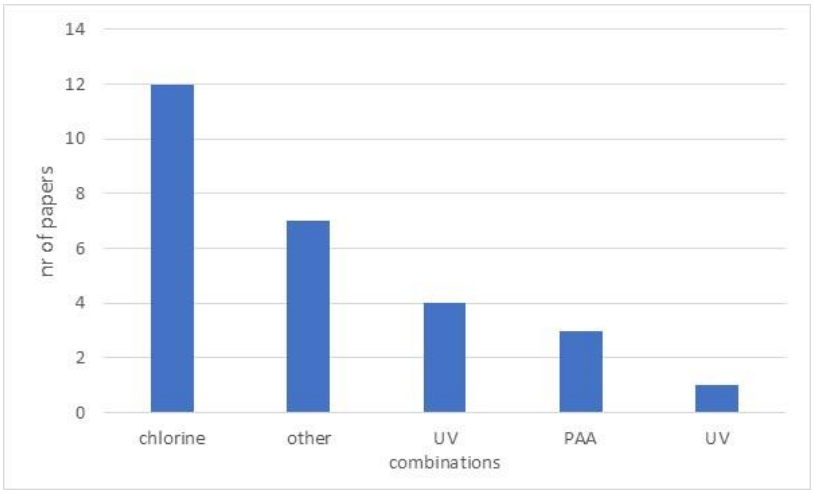


Figure 91. Number of papers studying various disinfection methods applied for water disinfection used for processing and handling of fresh-whole fruits at a lab scale

Wang et al. (2021) studied the microbial quality of the wash water used at three fresh peach packing facilities at an industrial scale. Significant differences were seen between the three processors where microbial levels of the water collected at the washing and waxing system for one of the processors were higher than for the other two. For example, total aerobes were detected at 2.7 log CFU/mL for processor 3 while it was 1.1 and 0.6 log CFU/mL for processors 1 and 2, respectively. Although the waxing system for the three processors was comparable, chlorine

levels used in the overhead spray water were different as around of 50 mg/L of chlorine was used at processors 2 and 3 and between 50 and 100 mg/L at processor 1 (Wang et al., 2021).

Fresh-cut fruits

Four studies included fresh-cut fruits that were studied at a lab scale (Abadias et al., 2011; Jung et al., 2017), blended (Huang and Chen, 2020) or shredded (Chen and Hung, 2016b). The latter study described a model to predict chlorine demand based on wash water quality parameters. No data on microbial load were described. Abadias et al. (2011) studied the efficacy of antimicrobial agents as an alternative to chlorine treatments and found that PAA, H_2O_2, citrox and sodium hypochlorite were most effective in reducing *E. coli* O157:H7*, Listeria* spp. and *Salmonella* spp*.* This study showed that PAA, H_2O_2 and Citrox could be used as alternatives to chlorinated water as these methods resulted in levels of pathogens <LoD (1.7 log CFU/ml) after treatment. Vanillin, carvacrol and N-acetyl-L-cysteine were less effective, showing a reduction between 0 and 1 log. Jung et al. (2017) performed lab experiments with fresh-cut cantaloupes showing that electrolyzed water (EW) effectively inactivated aerobic bacteria, *Salmonella* and *L. monocytogenes* in the wash water. The same results were obtained by using an acid-based disinfectants (AS, based on lactic acid and phosphoric acid). However, the latter was less effective in inactivating *L. monocytogenes*. In this study, melons were dip-inoculated with 5 log CFU/cm^2 and then washed using either EW or AS. No *L. monocytogenes* was found after treatment with EW. However, 1.30 log CFU/ml was found in the water treated with AS (Jung et al., 2017).

Frozen fruits

Only one paper focused on frozen blueberries and strawberries in a lab setting. The berries were inoculated with SARS-CoV-2 at a concentration of around 5 log $TCID_{50}$/g and washed for 10 min by immersion in sterilised distilled water. Results showed a reduction on the berries of around 1 log $TCID_{50}$/g. In the case of blueberries, the wash water contained significantly higher virus titer (around 2.5 log $TCID_{50}$/mL) than the strawberry wash water (around 0.5 log $TCID_{50}$/mL) (Esseili et al., 2022).

3.6.1.5 Data on microbial contamination of the water used for processing herbs

Limited data is available for microbiological contamination of wash water used for herbs at a lab scale as well as at pilot scale and in industrial settings. One monitoring study describes the parasitic contamination of final product samples collected in Egypt (Eraky et al., 2014). Thirty-four percent of 102 fresh parsley samples tested positive using microscope images. The percentage of positive samples for parasites tested in parsley were on average lower than for lettuce and watercress but higher than for green onion and leek that were sampled simultaneously (**Figure 92**).

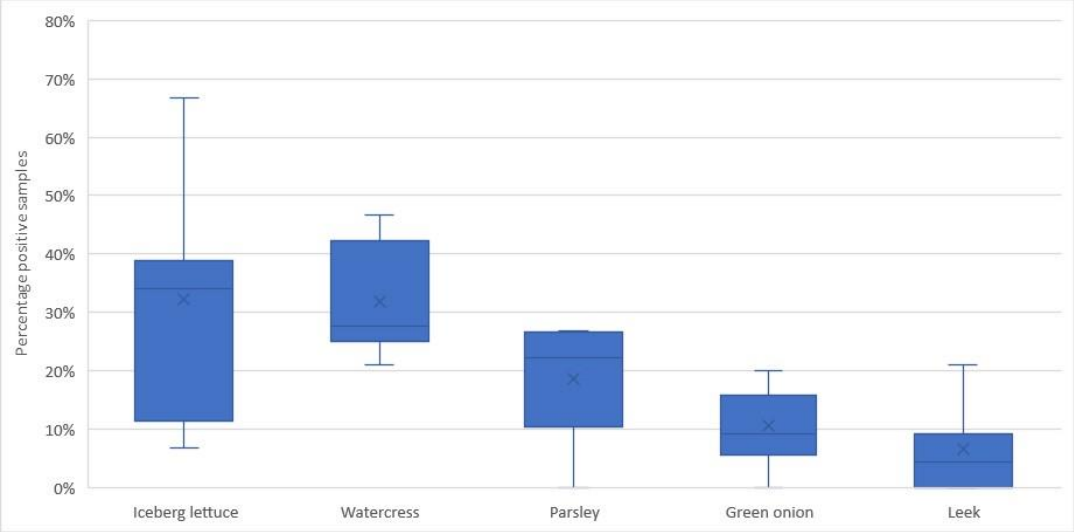


Figure 92. Percentage of samples containing parasites for different crops as well as parsley. Data obtained from Eraky et al. (2014)

Another study on bunches of cilantro (coriander) inoculated with one of three different combinations of bacterial pathogens (nalidixic acid-resistant *S. enterica, E. coli* O157:H7*, L. monocytogenes,* 5.0 log CFU/g each) studied the risk of cross-contamination to non-inoculated samples via wash water (>70 L), mimicking the soaking step during crisping at retail setting (Jung et al., 2022). The antimicrobial efficacy of electrolyzed water (60 mg/L of free chlorine), a lactic acid and phosphoric acid-based antimicrobial, and a citric acid-based antimicrobial were compared to tap water alone. The aerobic plate count results for electrolyzed water were significantly lower than for tap water, or the other two antimicrobial agents ($p < 0.05$). In soaking water with antimicrobials, none of the pathogens was detected, in contrast to tap water. Cross-contamination of foodborne pathogens from inoculated cilantro to non-inoculated cilantro was completely mitigated only by electrolyzed water during three subsequent soaking events (Jung et al., 2022).

3.6.2 Results RQ2a

The following sections summarise the results obtained for RQ2a: which data on microbiological and physico-chemical parameters and methods are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs?

Relevant parameters that can be used to estimate the microbiological quality of process water are e.g., the concentration of disinfectant (e.g., dosing, residual level), turbidity, total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), UV absorbance, electrical conductivity (EC), oxidation-reduction potential (ORP), pH, and water temperature.

A total of 14 papers mentioned the measurement of physico-chemical parameters to assess the quality of process water or the efficacy of disinfectants (Appendix E). Various factors influence the microbial load of the wash water, such as the replenishment rate of the water, maintaining the residual disinfectant level and the temperature of the water. It is important to note that microbial inactivation depends directly on the water temperature so at higher temperatures, the inactivation

is higher than at low temperatures. For example, lower temperatures result in lower log reductions (Barrera et al., 2012).

In several studies (n=5), experiments were carried out with wash water temperature exceeded 40 °C, either by design or due to technical limitations (Appendix E). For instance, in a study by Anese et al. (2015) a combination of ultrasound and heat treatment was applied to decontaminate wastewater from lettuce washing (Anese et al., 2015). The study found that both *E. coli* and *S. enterica* were readily inactivated by heat treatment (up to ±90 °C) but that more heat tolerant micro-organisms such as *L. monocytogenes* required additional treatment using ultrasound. In a study by Tomás-Callejas et al. (2012) in wash water from flume tanks from tomato processing facilities, wash water temperatures ranging between 34 and 44 °C. Additional analyses revealed that the ORP was positively correlated with temperature (Tomás-Callejas et al., 2012). In another experiment, the average temperature of the water in dump and flume tanks was 32 and 45 °C, respectively. The study found that the water temperature can impact the microbial safety and quality of tomatoes, in particular when the water was maintained at least 5.5 °C above the temperature of the incoming fruit. This elevated temperature reduces water infiltration into the tomatoes and contamination with pathogens; however, care must be taken not to impact the sensory attributes of the tomatoes. In another study, fresh-cut endive was subjected to different washing procedures, including washing with water at 45 °C with or without UV treatment (Hagele et al., 2016). The study found that treatment with warm wash water did not sufficiently reduce the microbial load (0.7 log CFU/g reduction), but this reduction was enhanced by UV-C radiation (2.1 log CFU/g reduction). Experiments by Mathew et al. (2018) with mangoes washed in warm wash water at 46 °C showed no significant effect in reducing *Salmonella* populations in either the water or on mangoes (Mathew et al., 2018). The addition of disinfectants, such as 200 mg/L chlorine and 80 mg/L PAA, did however result in a >7 log reduction of *Salmonella* in water.

In a large number of studies (Appendix E), physico-chemical parameters of process water or wash water were measured, often to establish the efficacy of conventional (e.g., chlorine, PAA) disinfectants and/or alternative/novel treatments (e.g., plant extracts, ultrasound). For instance, 14 studies reported a negative effect of physico-chemical characteristics on the efficacy of chlorine. Often, this negative effect on disinfectant efficacy was due to increasing levels of organic matter resulting from the processing of fresh produce. Other factors reported that may impact the disinfection efficacy are: turbidity, which is strongly related to the organic matter content as well as dissolved solids, the pH of the process water, and solids content.

In 28 of the studies, alternative disinfectants to the use of chlorine-based ones were tested, often comparing their efficacy with sodium hypochlorite. In most of these studies, this comparison with chlorine-based disinfectants was made to establish the efficacy of these alternative disinfectants, but no further analysis was performed to analyse the effect on the physico-chemical parameters and if these physico-chemical parameters can be used to monitor the changes in the microbiological quality of the process water. Only some studies did report the effect of disinfectants on the changes in the physico-chemical parameters. For instance, studies by de Oliviera et al. (2018) and Huang and Chen (2020) reported a negative effect when increasing COD levels on the efficacy of respectively, UV-A treatment in combination with curcumin and UV alone. In contrast, Cossu et al. (2016) and Li et al. (2012) did not observe an effect of increasing COD

levels on the disinfectant efficacy of UV-A in combination with gallic acid, and grape seed extract, respectively. In other studies, such as Abadias et al. (2021) turbidity was found to greatly affect disinfectant efficiency of UV-C treatment, whilst Huang et al. (2015), and Huang and Chen (2020) did not observe a negative effect of turbidity levels on the efficiency of pulsed light treatment, or UV treatment.

Furthermore, some studies focused on the clearing of spent process water and decreasing its turbidity, total solid content, as well as microbial load. For instance, Alharbi et al. (2017) reported on the successful use of electrocoagulation in combination with UV treatment for this purpose, and similarly Millan-Sango et al. (2017) utilised UV in combination with ultrasound to reduce microbial load, depletion of the COD, as well as a reduction of suspended particles.

3.6.2.1 Data on the use of physico-chemical parameters in establishing process water quality.

In several studies, physico-chemical parameters were measured as an indicator of water quality (Appendix E). For instance, in a study by Bertoldi et al. (2021), the physico-chemical properties of flume tank water samples from tomato processors showed strong correlations between turbidity and TDS (r = 0.81). Although correlations were found between ORP and FC and between turbidity and COD, these correlations were not strong (respectively r=0.66 and r=0.63). The authors found that COD, TDS, and turbidity were all suitable indicators of water quality during fluming operations, especially for organic matter, debris, and particles (Bertoldi et al., 2021). However, there are certain limitations when using turbidity and TDS as indicators, as these show a relatively low correlation with COD (correlation of 0.631 and 0.524, respectively. Thus, the use of these parameters as indicators may not be adequate to validate/verify and or monitor the microbiological quality of the process water and the disinfection capacity (Bertoldi et al., 2021). Abnavi et al. (2021a) selected COD as an indicator for the organic load of wash water, as well as the free chlorine on pathogens in the presence of organic content. These authors used these data to develop models to explain the free chlorine decay and the pathogen cross-contamination dynamics (Abnavi et al., 2021a). Taken together, from the tested parameters in these studies on chlorine demand or chlorine decay, namely COD, ORP, TDS, and turbidity, authors report that turbidity and TDS may provide a subjective indication of water quality and that ORP could not be used to accurately determine FC concentrations. Thus, according to the findings in these studies direct COD measurements may provide a better indication of water quality and chlorine demand.

Apart from the COD, a study by Chen and Hung (2016) explored the feasibility of using wash water quality parameters such as pH, ORP, COD, turbidity, UV 254nm, and other water properties to estimate the chlorine demand for different types of products (Appendix E). **Figure 93** illustrates the relationship between ORP and the residual disinfection concentration in the water. The figure agrees with the established ORP value that needs to be above 650 nm to maintain a residual concentration of chlorine in the water.

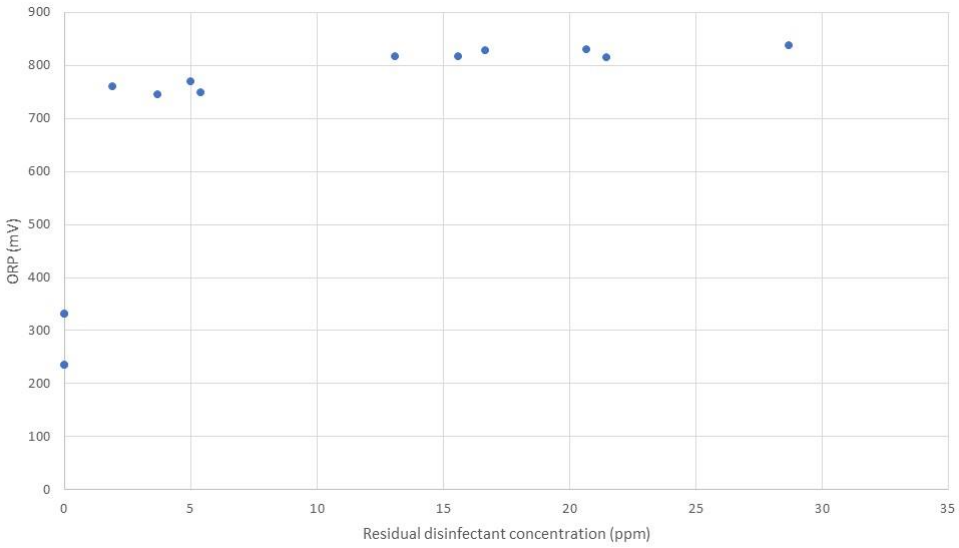


Figure 93. Relationship between ORP and residual disinfectant concentration in water used for processing and handling Romaine lettuce (data obtained from Fu et al. (2018))

Researchers developed a model to predict the chlorine demand based on the phenolic-to-protein ratio by measuring the UV 254nm because of its high correlation coefficient with chlorine demand in wash water among the tested parameters, including protein and phenolic content (Pearson correlation of 0.77). In a similar experiment, Chen and Hung (2017b) investigated the effect of several parameters (organic load, free chlorine, pH) on chlorine demand. In their earlier study, it was found that measuring UV 254nm absorbance has a strong relation with chlorine demand but this may be only valid at the specific conditions (non-buffered NaOCl at 100 mg/L free chlorine and pH 6). These authors investigated the effects of buffering and process water characteristics on chlorine demand, which are conditions not commonly used in process water. It was observed that organic load, pH, and initial chlorine concentration all significantly impacted chlorine demand ($P \leq 0.05$), but no effect was observed for the buffering capacity of NaOCl. In both studies, data were used to develop models to predict appropriate chlorine-based levels for the processing of produce. These researchers indicated that chlorine demand decreased from pH 2.5 to 6, slightly increased from pH 6 to 8 and decreased again from pH 8 to 9.5 (Chen and Hung, 2016, 2017b). The effect of pH on the efficacy of chlorine disinfection has been studied but none of the published papers evaluated log reduction at one set chlorine dose under varying pH values. There is a wide range of log reductions described that can be explained by the different experimental setups as well as the range of COD values in the water.

In two studies by Davidson et al. (2014 and 2017) on water used for processing iceberg lettuce, various physico-chemical parameters were assessed regarding the inactivation of *E. coli* O157:H7. Physico-chemical parameters analysed in these studies included pH, ORP, COD, as well as turbidity. In both studies, researchers demonstrated that physico-chemical parameters are suitable indicators of organic load in flume water. Organic load was negatively correlated with E.

coli inactivation by chlorine. Moreover, they also stated that at lower pH, the efficacy of chlorine is less affected by the increased organic load. To increase the efficacy of chlorine at higher organic loads, it is thus relevant to acidify the process water. According to the study by Davidson et al. (2014), total solids and COD in particular were the best indicator parameters that correlated with *E. coli* inactivation by chlorine. However, these researchers explained that total solids and COD measurement may not be practical for routine implementation as these measurements require a minimum of 2 h to perform.

A study on tomatoes investigated the use of chlorine dioxide in postharvest washing (Tomás-Callejas et al., 2012). Several physico-chemical parameters were investigated, such as ORP, pH, temperature, and turbidity to follow the changes in the water quality in commercial tomato packing houses. A negative correlation was reported between ORP and turbidity, between ORP and coliforms and between ORP and total bacterial counts, although correlations were small (r = -0.41, -0.31 and -0.34 respectively). Coliforms were also negatively correlated with conductivity and temperature (r = -0.22 and -0.45, respectively) (Tomás-Callejas et al., 2012). Researchers reported that the investigated process water in dump tanks contained high amounts of organic matter and high turbidity while in the flume tanks, ORP values were often higher, which contained higher quality process water. Due to the lower quality of dump tank process water, ORP values could not be properly maintained, compared to process water in flume tanks, and researchers argue that due to the poorer quality of the dump tank water, spoilage bacteria and foodborne pathogens may not be entirely inactivated by the chlorine dioxide. Abadias et al. (2021) also studied the log reductions in water used for processing tomatoes with several UV doses (0.7, 2.0, 3.4 and 4.7 kJ/m^2) at a lab scale. This study indicated that conductivity could be used as a parameter to assess water quality for treatments with UV as log reductions decrease when conductivity increases (**Figure 94**).

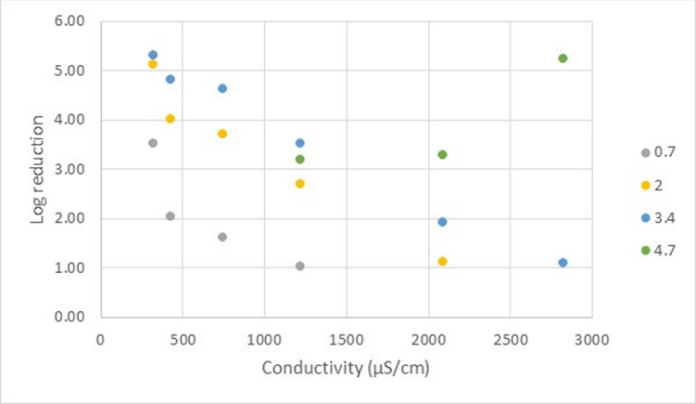


Figure 94. Log reductions in water used for processing tomatoes as a function of conductivity for various UV dose (0.7, 2.0, 3.4 and 4.7 kJ/m^2). Data obtained from Abadias et al. (2021)

In conclusion for RQ2a (i.e. which data on microbiological and physico-chemical parameters and methods are available to validate/verify and/or monitor the microbiological quality of the process water used for ffFVHs?), the literature review revealed that COD and to a lesser extent TDS and

turbidity are the most suitable parameters as indicators for water quality. In order to maintain disinfectant efficacy, parameters such as ORP, pH, or temperature can be used as indicators.

3.6.3 Results RQ2c

The literature review aimed to explore in-line and online monitoring methods tested at pilot and industrial scales to measure relevant physico-chemical and microbiological parameters for wash water quality monitoring at ffFVHs processing facilities to answer RQ2c. The identified literature showed various monitoring methods in terms of measurement types (i.e., online, in-line, offline), experiment scale (i.e., laboratory, pilot, industrial), and various physico-chemical and microbiological parameters. However, none of these studies discussed inline, online, or real-time monitoring methods for monitoring or validating the microbiological quality of ffFVHs process water applied in pilot or industrial settings. Therefore, the results will be discussed for lab scale settings only.

Methods found in the literature included those used to estimate dosing and residual disinfectant levels in the water (e.g., PAA, FC) (see section 3.6.3.1), UV/vis spectrometry to monitor wash water quality (see section 3.6.3.2) and methods to explore the microbial quality of the water (i.e. presence of pathogens and viable but non-culturable cells (VBNC)) (see section 3.3.6.3).

3.6.3.1 Methods estimating dosing of disinfectant and residual disinfectant levels

Maintaining residual disinfectant is a control approach to prevent cross-contamination due to underdosing of disinfectants or high costs related to overdosing (Van Haute et al., 2019; Albolafio et al., 2021). Van Haute et al. (2019) evaluated ORP to estimate residual free chlorine in fresh produce wash water. The study concluded that various factors, such as water source, organic matter, and the acidulant, complicate this relationship and consequently limit its usability to estimate free chlorine levels in wash water. Free chlorine levels in fresh-cut produce wash water can be better monitored if chlorine demand could be determined in real-time during processing (Van Haute et al., 2015). Chlorine dosing during fresh-cut produce washing typically depends on feedback control that automatically adds chlorine to the water when free chlorine levels drop below the set-point. Previous studies have investigated various parameters to predict chlorine demand including ORP, pH, UV absorbance at 254 nm (UVA_{254}), and COD. Among these tested parameters, UVA_{254} was highly correlated with chlorine demand and showed potential for predicting chlorine demand (Chen and Hung, 2016, 2017a). From a model using UVA to predict chlorine demand in lettuce wash water, Van Haute et al. (2018) also concluded that the use of UV absorbance to estimate chlorine demand is promising for online monitoring of fresh produce washing. These authors suggested that further studies should include possible variability in crops and potential interferences with the UVA signal. The authors also proposed to determine whether this UVA-based chlorine demand estimation contributes as a supporting measurement to the current methods for residual chlorine measurement, i.e., ORP, N,N-diethyl-p-phenylenediamine (DPD) and amperometric sensors. The amperometric technique is an electrochemical method measuring changes in electric current across two electrodes resulting from a redox reaction at the electrodes. In water treatment plants, the application of amperometric sensors for chlorine

monitoring is considered a common practice (Clark, ND). However, no scientific studies were obtained in our literature review that evaluated this monitoring system in ffFVHs processing.

In another study, an amperometric in-line probe and a chronoamperometric method with a disposable electrode sensor and other methods (i.e., redox titration kit, reflectometric method and high performance liquid chromatography (HPLC)) were tested at lab scale for PAA monitoring in various ffFVHs wash water types (Albolafio et al., 2021). In this study, HPLC was used as a reference technique in measuring PAA concentration to compare with the other methods. HPLC produced reliable results but was considered unsuitable for PAA monitoring due to the requirement of a highly-skilled technician and high installation and maintenance costs. The redox titration kit was less accurate as it overestimated PAA due to interference with other oxidants, such as hydrogen peroxide which is always present in PAA solutions. Similarly, the reflectometric method also overestimated PAA due to hydrogen peroxide interference, particularly when applied in process water with high organic matter that consequently needed high PAA, hence a high concentration of hydrogen peroxide. In-line amperometric sensors were accurate in estimating PAA in apple, tomato, and sweet pepper wash water, but they underestimated PAA in lemon wash water. In lemon wash water, PAA could not freely diffuse through the membrane and reach the active electrode for detection, probably due to lipophilic compounds from the damaged lemon peel. Finally, the chronoamperometric method showed reliable results across all wash water types and PAA concentrations. This method produced similar results as the HPLC method, indicating no interfering oxidants or other compounds (such as H_2O_2) that affected the measurement (Albolafio et al., 2021).

3.6.3.2 Using UV/vis spectrometry for wash water quality monitoring

UV/visual (UV/vis) spectrometry can be used to monitor water quality parameters as they absorb electromagnetic radiation at different wavelengths. Examples of parameters or compounds that can be measured using UV/vis spectrometry are total suspended solids, COD, biochemical oxygen demand, dissolved organic carbon, detergents and nitrates. Radzevičius et al. (2020) applied the UV/vis spectrophotometric technique at different wavelengths to test its suitability to monitor wash water and wastewater quality at on-farm root vegetable pack houses. Principal component analysis and partial least squares regression methods were applied to analyse the relationship between physico-chemical properties and UV/vis spectral data. Based on the results, these authors propose UV/vis measurements at 320 nm to monitor water quality monitoring, i.e., total suspended solids, total dissolved solids, and organic matter monitoring. The authors did not indicate whether the UV/vis absorbance method was an inline/online method or an offline system applied in a lab (Radzevičius et al., 2020).

3.6.3.3 Detection methods of pathogens and viable but non-culturable bacteria

Four studies explored different detection methods to test for the presence of pathogens in wash water in lab scale experiments. Microfluidic droplets (also known as ultra-miniaturized bioreactors) were used for the rapid contamination detection of *S. typhimurium* at a lab scale experiment. With this set-up *S. typhimurium* was detected in less than 5 hours in industrial shredded lettuce wash water (Harmon et al., 2020). Regarding detection methods, a dead-end ultrafiltration concentration (DEUF-C) combined with qPCR was a methodology described for the detection of

pathogens in process water at both lab scale (Magaña et al., 2014) and pilot scale (Kearns et al., 2019). However, filtration of wash water remains challenging because membrane fouling may decrease the filtration rate and filterable volume; therefore, further research is needed to evaluate the feasibility of using this technology for commercial use.

There are several commercially available devices/ sensors on the market for rapid or real-time monitoring of pathogens in water. Some examples are described below.

- Ecoli sense (https://ecoli-sense.com/) uses a nanomaterials-based detection sensor that acts like antibodies, which can detect the type and amount of *E. coli* strains in real-time monitoring.
- Bilanz Qualitat (https://tienda.bilanz.es/) uses a probe to estimate E. coli, total and fecal coliforms without reagents, which allows a result within one minute.
- Easychem® coli on-line from **Systea SpA** (Easychem COLI online | SYSTEA **) is a fully automated measurement for *E. coli*, total and fecal coliforms based on enzyme activity in which** colorimeters and fluorometers are integrated on thermostatic incubating positions. This allows a concurrent detection of both total coliforms and *E.coli* or fecal coliforms in water and wastewater.
- Colminder (https://www.colminder.com/) detects microbes in the water such as *E. coli, Enterococci* and total activity (bulk parameter of total microbiological activity) by measuring the specific metabolic (enzymatic) activity of the target organisms. Similar to Colminder, Colifast technology (https://www.colifast.no/) detects coliform bacteria based on a chemical reaction between a substrate in the growth medium and enzymes produced by the bacteria.
- Yarok Microbio technology (https://www.yaroktt.com/) detects bacterial presences (e.g. *E. coli, Salmonella, L. monocytogenes*) by identifying the intracellular metabolic activity of the live target bacteria.

In addition to the systematic literature review, a literature search was performed on Google Scholar to identify scientific papers evaluating or validating the application of these methods. However, no scientific papers were identified that validated or evaluated the devices/ sensors cited above. Some papers mentioned these devices to indicate that they are commercially available, but there was no further evaluation or discussion. Finally, a simple search was also performed to identify studies on molecular imprinting for pathogens detection in process wash water, but no references were obtained.

Foodborne bacterial pathogens are known to enter a viable but non-culturable (VBNC) state in response to environmental stress including the application of disinfectants (e.g., chlorine) during ffFVHs processing (Zhao et al., 2017; Highmore et al., 2018). However, the microbiological safety of fresh produce is mainly monitored by culture-based detection methods due to a lack of suitable methodologies that can distinguish between dead and VBNC cells. Truchado et al. (2020) examined three methods to quantify VBNC of *L. monocytogenes* including (i) flow cytometry, (ii) viability quantitative polymerase chain reaction (v-qPCR) assay using an improved version of propidium monoazide (PMAxx) dye as DNA amplification inhibitor, and (iii) v-qPCR combining both ethidium monoazide (EMA) and PMAxx. The last method was considered the best method although complete discrimination between dead and VBNC cells was not achieved, resulting in a slight

overestimation of VBNC cells. The method was tested in process water treated with chlorine only (Truchado et al., 2020).

For RQ2c (i.e. which inline/online monitoring systems are available to validate/verify and/or monitor relevant parameters related to the microbiological quality of the process water used for ffFVHs?), it can be concluded that although commercial online methods are available only limited information on inline or online methods was available in the scientific literature. The papers found showed that UV absorbance could be a promising online method to assess process water quality and to predict chlorine demand. Furthermore, chronoamperometric sensors could be used to detect residual disinfectant, such as for PAA.

3.7 Results of the literature review – Tier 2 modelling

This section describes the results of the literature review to obtain relevant dynamic models related to microbial water contamination used for processing ffFVHs (RQ1b) and models on microbial and physico-chemical parameters (RQ2b).

3.7.1 Analysis of records modelling microbiological and/or physico-chemical variables in process water used for ffFVHs

A total of 95 scientific papers were retrieved for RQ1b/RQ2b, as detailed in **Table 17**. After screening the title, abstract and methodology, 16 references were considered relevant and therefore fully read and analysed to build a general framework of the models considered in the literature.

The records were retrieved in **Step 1 (Systematic Review)** and **Step 2 (Selected Records after reading Title/Abstract/Methodology)** and an outline of the process is shown in **Table 18**.

Table 18. Outline of records obtained and analysed for finding a general modelling framework. Duplicates are records shared by both questions RQ1b/RQ2b.

	Number of retrieved works in Step 1 (Systematic Review)	Number of retrieved works in Step 2 (Selected Records after reading Title/Abstract/Methodology)
RQ1b	63	15
RQ2b	64	12
Duplicates	32	11
Total after deduplication	95	16

It should be noted that most of the analysed models, particularly those with relevant dynamics for microbial water contamination and/or disinfection, are extensions/simplifications/adaptations of the model by Munter et al. (2015), which was not retrieved in the systematic review. Nevertheless, excluding this model from the study would not leave out any relevant information, as it was built upon terms also present in the other examined models.

It should also be mentioned that, although the question uses the terms "microbiological and physico-chemical parameters", we are interested in the dynamics (changes with time) and

therefore instead of parameters (usually not changing with time) "state variable" or simply "variable" will be used in the following sections when referring to properties changing with time.

Table 19 shows the type of water, product and disinfectant used for the modelling in the selected 16 records. Note that some works only focus on modelling and do not include experimental data. The table also shows that some works consider either process water without product or product with water without organic matter or contamination. On the other hand, the most commonly used disinfectant is free chlorine (FC), sometimes without specifying the chlorine compound originally added.

Table 19. Specifications for the type of water, products and disinfectants used in the reviewed literature.

Nº	Reference	Type of water	Product	Disinfectant
1	(Abnavi et al., 2019)	Tap water	Imperator carrots and lettuce; romaine, iceberg, green leaf and red leaf	Non specified chlorine disinfectant (FC)
2	(Abnavi et al., 2021a)	Tap water	Chopped iceberg and red leaf lettuce	Sodium hypochlorite
3	(Abnavi et al., 2021b)	Wash water from iceberg lettuce, green cabbage, and carrots	Not used	Sodium hypochlorite
4	(Alborzi et al., 2018)	Deionised water	Fresh spinach leaves	Benzoic acid (BA), ethylenediaminetetraacetic acid (EDTA)
5	(Alenyorege et al., 2019)	Not specified	Chinese cabbage	Weeping frequency ultrasound
6	(Chen and Hung, 2016)	Wash water from iceberg lettuce, romaine lettuce, spinach, celery, mushroom, broccoli, strawberry, grape, cantaloupe and tomato, mixed with deionized water (dH2O)	Not used	Sodium hypochlorite
7	(Ding et al., 2018)	Deionised water with benzoic acid (BA)	Baby spinach	Benzoic acid (BA) and UVA light treatment
8	(Dunkin et al., 2017)	Wash water from FBO: shredded iceberg, chopped romaine and whole-leaf lettuce	Not used	Non specified chlorine disinfectant (FC)
9	(Li et al., 2019)	Wash water from romaine lettuce, iceberg lettuce, and carrot	Not used	Sodium hypochlorite
10	(Madamba et al., 2022a)	Clean water	Romaine lettuce	Non specified chlorine disinfectant (FC)

Nº	Reference	Type of water	Product	Disinfectant
11	(Madamba et al., 2022b)	Clean water	Romaine lettuce	Non specified chlorine disinfectant (FC)
12	(Mokhtari et al., 2018)	Fresh water	Fresh-cut romaine lettuce	Non specified chlorine disinfectant (FC)
13	(Srinivasan et al., 2020)	Tap water	Disk carrots, cabbage, and lettuce	Sodium hypochlorite
14	(Van Haute et al., 2018)	Wash water from FBO: sugarloaf, iceberg lettuce, endive and radicchio washed with tap water, and butterhead lettuce, iceberg lettuce, endive and radicchio washed with borehole water. Standardised process water made of: iceberg lettuce, and spinacia oleareacea	Not used	Peracetic acid (PAA) combined with lactic acid (LA)
15	(Wang and Ryser, 2020)	Wash water from roma tomatoes juice mixed with wash water	Roma tomatoes	Peroxyacetic acid, mixed peracid (peracetic acid), chlorine at pH 6.0 and chlorine in electrolyzed water at pH ~ 3.0
16	(Zhou et al., 2014)	Lettuce wash water	Not used	Sodium hypochlorite

3.7.2 Modelling variables considered in the different models in the literature.

Attending to modelling terms, the questions were interpreted in the following way:

- **Question RQ1b**: Let us denote by X_w the variable representing the microbial contamination of water (microbial concentration in water). The objective was to search for those works **including equations for $X_w(t)$, or ideally $\frac{dX_w}{dt}$**.
- **Question RQ2b:** The objective was to search those works modelling at least (1) microbiological contamination of water X_w and (2) other physico-chemical variables, as could be FC or COD. However, the search detected some relevant works modelling chlorine demand (CLD) that, although not including the modelling of X_w, could be used to understand and estimate the modelling of relevant physico-chemical parameters (see for example Zhou et al., 2014). Therefore, in modelling terms, the objective was to select those **works that either model X_w plus another physico-chemical variable or model CLD, even if X_w is not incorporated,** in the provided context. Note that if the

dynamic of microbial contamination of water X_w is modelled, the model is also included in RQ1b.

In summary, see **Table 20**, the type variables modelled in the literature are relevant to determine which model is useful to answer which question. At least the model needs to model the microbial contamination of water $\boldsymbol{X_w}$ or any other relevant physico-chemical variable, such as COD or some variable related to the disinfectant $\boldsymbol{C}$ (or related variables such as the chlorine demand, CLD, that is total chlorine minus free chlorine TC=FC+CLD). When useful for both questions (RQ1b&RQ2b) we use the term "Both".

Table 20. Modelled variables in RQ1b/RQ2b and type of model attending to its dynamic nature. The table also includes the classification of papers in each of the questions as detected during the literature screening and after full-text reading (Step 2).

Nº	References	Microbiological		Physico-chemical				Is a dynamic model (variables changing with time)?	Related Question	
		Water cont. X_w	Product cont. X_p	Disinfect. [Disinfect.] C	Disinfect demand CLD	Oxygen demand COD	Amino acids AA		Before Step 1	After Step 2
1	(Abnavi et al., 2019)	Yes	Yes	Yes [FC]	No	Yes	No	Yes	Both	Both
2	(Abnavi et al., 2021a)	Yes	Yes	Yes [FC]	No	Yes[(a)]	No	Yes	Both	Both
3	(Abnavi et al., 2021b)	No	No	Yes [FC]	No	No	Yes	Yes	RQ2b	RQ2b
4	(Alborzi et al., 2018)	Yes	No	No (BA, EDTA)	No	No	No	Yes	RQ1b	RQ1b
5	(Alenyorege et al., 2019)	Yes	No	No (ultrasound)	No	No	No	Yes	Both	RQ1b[(b)]
6	(Chen and Hung, 2016)	No	No	No	Yes	No	No	No	Both	RQ2b[(c)]
7	(Ding et al., 2018)	Yes	No	No (UV-A+BA)	No	No	No	Yes	RQ1b	RQ1b
8	(Dunkin et al., 2017)	Yes	No	Yes [FC]	No	No	No	Yes	RQ1b	Both[(d)]
9	(Li et al., 2019)	No	No	No	Yes	Yes	No	No	Both	RQ2b[(c)]
10	(Madamba et al., 2022a)	No	No	Yes [FC]	No	Yes	No	Yes	Both	RQ2b[(e)]
11	(Madamba et al., 2022b)	No	No	Yes[(f)] [FC]	No	Yes[(f)]	No	Yes[(f)]	Both	RQ2b[(e)]
12	(Mokhtari et al., 2018)	Yes	Yes	Yes [FC]	No	Yes	No	Yes	Both	Both
13	(Srinivasan et al., 2020)	No	No	Yes [FC]	No	Yes	No	Yes	Both	RQ2b[(c)]
14	(Van Haute et al., 2015)	Yes	No	Yes [PAA+LA]	Yes (PAA demand)	Yes	No	Yes	Both	Both
15	(Wang and Ryser, 2020)	yes	No	No	No	No	No	No	RQ1b	RQ1b
16	(Zhou et al., 2014)	No	No	Yes [FC]	Yes	Yes	No	No	Both	RQ2b[(c)]

(a): The model includes a mathematical expression for COD, but this is not modelled for the experimental data (see figure caption in the reference).

(b): Physicochemical parameters are mentioned in the text but are not modelled.

(c): Water contamination is mentioned in the text, but not modelled.

(d): Free chlorine is indirectly included in the X_w dynamics.

(e): Although microbial contamination is modelled, this microbial contamination is not in the context of washing, but after cross-contamination using Baranyi's model with secondary model for temperature.

(f): This second part of the model focuses on simulations, but the model is presented in Madamba et al. (2022a).

3.7.3 Modelling terms and assumptions found in the literature review

Two main groups of models were found:

- Group 1: Models that did not model dynamics of the microbiological or physico-chemical variables (Models 6,9,15,16) but were selected because they could provide helpful information to include inside the dynamic models. These models will be explained case-by-case as they are diverse and cannot be cast into a general form.
- Group 2: Dynamic models of water disinfection (Models 1-5,7,10-14). These models were further classified by attending to the different mechanisms considered.

3.7.3.1 Group 1: Non-dynamic models

- **Model with reference 6:** The model by Chen and Hung (2016) explains CLD as a function of the ultraviolet absorbance at 25nm (UV254) and the phenolics-to-protein/ΔE ratio (PPC), as follows:

$$CLD = \begin{cases} 295.23UV254 + 6.97, & if\ PPC < 0.6 \\ 119.77UV254 + 2.41, & if\ PPC \geq 0.6 \end{cases}$$

where ΔE denotes colour difference between deionized water and test samples.

- **Model with reference 9:** The model by Li et al. (2019) considers COD and CLD to be, respectively, linear and quadratic functions of physico-chemical parameters. The best correlations were found with Total Diluted Solids (TDS), where:

$$COD = b\,TDS - c$$

$$CLD = TC - FC = a\,TDS^2 + b\,TDS + c$$

and a, b and c are fitting parameters to be estimated. Good correlations were also found between COD/CLD and Total Organic Carbon (TOC).

- **Model with reference 15:** Wang and Ryser (2020) propose a model for microbial transferring during tomato dicing of the following form:

$$log_{10}\left(\frac{CFU}{g}\right) = Dexp\left(\frac{L}{B}\right)$$

where L denotes the mass of diced tomatoes, while D and B are fitting parameters.

- **Model with reference 16:** The model by Zhou et al. (2014) considers interesting linear correlations between lettuce extract concentration and the required amount of NaOCl to understand the chloramine hump and breakpoints. More importantly, the total amount of added NaOCl (TC) is modelled as the following function of CLD:

$$TC = FC + CLD_{COD} + CLD_{others}$$

where TC is the target level of FC after the chlorination breakpoint, CLD_{COD} and CLD_{others} denote, respectively, the amount of NaOCl required to react with the organic load up to the breakpoint and due to other losses (such as time and different pH). For the experimental data in the paper, they obtained the required stock of NaOCl needed to reach a certain FC level.

3.7.3.2 Group 2: Dynamic models, usually considering FC as disinfectant if not stated otherwise.

The following general model explains the mass balance between the different terms found in the selected papers of the systematic review.

$$\frac{dX_w}{dt} = (X_w\_\text{Inlet}) - \left(X_w\_\text{Transfer\_to\_}X_p\right) - (X_w\_\text{Inactivation\_by\_}C)$$

$$\frac{dC}{dt} = (C\_\text{Inlet}) - (C\_\text{Natural\_Decay}) - (C\_\text{Inactivation\_by\_}COD) - (C\_\text{Degradation\_by\_}AA)$$

$$\frac{dCOD}{dt} = (COD\_\text{Inlet}) - (COD\_\text{Degradation\_by\_}C)$$

$$\frac{dX_p}{dt} = (X_p\_\text{Transfer\_from\_}X_w) - (X_p\_\text{Inactivation\_by\_}C) - (X_p\_\text{Dilution\_by\_Product\_Removal})$$

$$\frac{dAA}{dt} = (AA\_\text{Inlet}) - (AA\_\text{Degradation\_by\_}C)$$

Model 1. Mass balance between the different terms contributing to the dynamics of the relevant variables identified as the most critical for simulating microbial, disinfectant and COD dynamics in the literature.

At least the model needs to include X_w or C as modelling variables. Other variables are COD, X_p and AA, which represent the chemical oxygen demand, the product microbial contamination and the amino acids concentration in water (as an indicator of chlorine demand).

The different terms of the mass balance in **Model 1** are illustrated in **Figure 95**, with the exception of amino acids dynamics that are described only in one work and not considered relevant for the purposes of this tender. The illustration shows a tank for washing lettuces and including the major mechanisms considered to find the dynamic changes of organic matter (COD in orange), disinfectant (C in turquoise) and pathogens (yellow), either in water (X_w) or in the product (X_p).

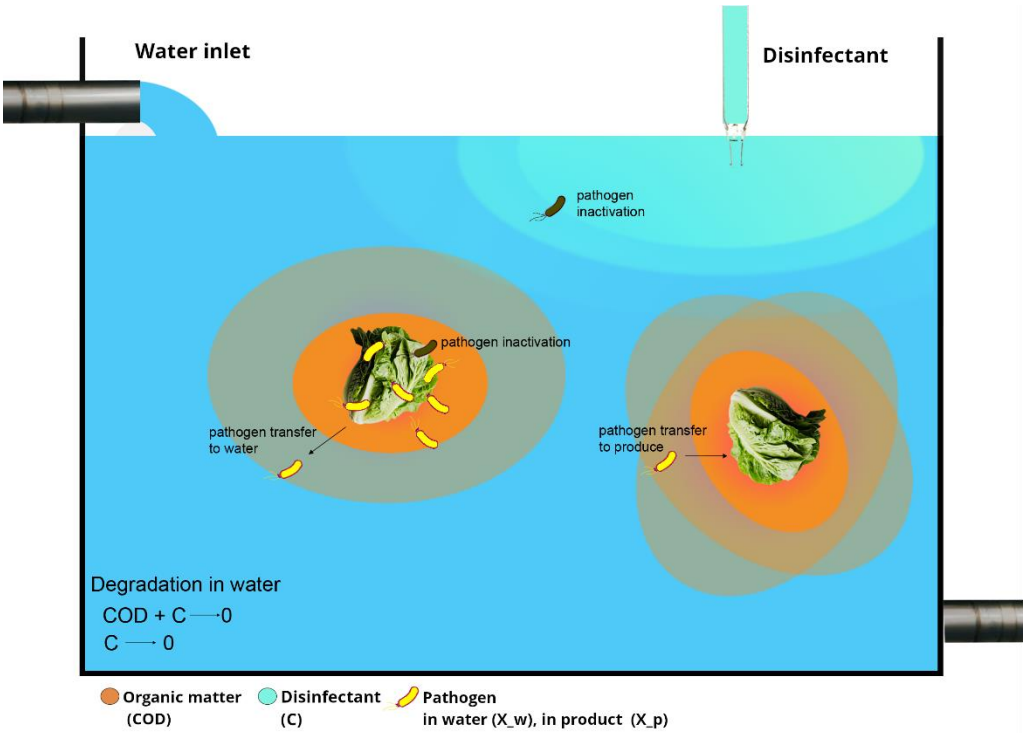


Figure 95. Illustration of the most common modelled mechanisms and variables ($\boldsymbol{COD}, \boldsymbol{C}, \boldsymbol{X_w}, \boldsymbol{X_p}$) in the literature.

Table 21 shows the mathematical models/formula for each contributing term to the mass balance found in the literature. For building the table, the different mathematical formulas were standardised both in notation and to obtain terms that can be plugged-in directly into the mass balance in **Model 1**. Note that some models in the literature were not derived directly from mass balances, or were expressed in their integral form. For example, inactivation dynamics (***X_w*_Inactivation_by_*C***) might be expressed in the literature using the derivative form $\frac{dX_w}{dt} = f(X_w, \dots)$, or its integral $X_w = g(X_w, \dots)$. When in its latter integral form, the expression $g(X_w, \dots)$ was differentiated with time to present always the same $f(X_w, \dots)$. Note, also that bacterial growth, so-common in predictive microbiology, was not considered in any of the studies, since the contact time is usually fast (around one minute) to be relevant compared with other terms.

Table 21. Mechanisms, assumptions and mathematical formulas for describing the different mechanisms in a mathematical formalism in the models found in the literature.

Dynamics for microbial contamination of water $\left(\frac{dX_w}{dt}\right)$			
Term	**Formula**	**Comments (type of model and assumptions)**	**References**
X_w***_Inlet***	β_{ws}	Addition of microbial contamination to water. In Abnavi et al. (2021a) modelled in function of inlet and outlet contamination fluxes.	Mokhtari et al. (2018), Abnavi et al. (2021a).
	$\begin{cases}\beta_{ws}, & \text{during contact time,}\\ 0, & \text{otherwise.}\end{cases}$	Same as previous, but explicitly including a step function (or if-condition) to simulate when the product is washing. Additionally, Abnavi et al. (2019) models this parameter as a function of the average pathogen level on prewashed lettuce (σ).	Abnavi et al. (2019).
X_w***_Transfer_to_***X_p	$\beta_{pw} X_w \frac{L}{V}$	Transport of pathogens from water to product (1st-order reaction).	Mokhtari et al. (2018), Abnavi et al. (2021a).
	$\begin{cases}\beta_{pw} X_w \frac{L}{V}, & \text{during contact time,}\\ 0, & \text{otherwise.}\end{cases}$	Same as previous, but explicitly including a step function to simulate when the product is washing.	Abnavi et al. (2019).
X_w***_Inactivation_by_C***	$\alpha X_w C$	Mass action law (2nd-order reaction) with two reactants.	Dunkin et al. (2017), Mokhtari et al. (2018), Abnavi et al. (2019), Abnavi et al. (2021a).
	$\frac{\alpha_M k_M}{k_M + COD} X_w C$	Mass action law combined with inactivation by COD following Michaelis-Menten kinetics.	Abnavi et al. (2021a).
	$\alpha X_w C^n$	Chick-Watson kinetics.	Dunkin et al. (2017).
	$\alpha X_w^x C^n$	Power law kinetics.	Dunkin et al. (2017).
	$\alpha m t^{m-1} X_w^x C^n$	Hom-power law kinetics.	Dunkin et al. (2017).
	$\alpha m t^{m-1} X_w C^n$	Hom model (note that Van Haute et al. (2015) uses an approximation of this expression as they need the integral form) kinetics.	Van Haute et al. (2015).
	$\frac{\alpha_M X_w}{1 + \exp(-\alpha_M t)\,(\exp(\alpha_M SL) - 1)}$	Geeraerd model where α_M and SL are functions of PAA+LA (used as disinfectant instead of FC) and UV254.	Van Haute et al. (2015).
X_w***_Inactivation*** *(C is not modelled)*[(a)]	$\alpha m t^{m-1} X_w$	Weibull inactivation model with benzoic acid (BA), EDTA (Ethylenediaminetetraacetic Acid) or combination (BA+EDTA).	Alborzi et al. (2018).

	$\frac{p}{\delta}\left(\frac{t}{\delta}\right)^{p-1} X_w$	Couvert et al. (2005) model. Instead of FC, the inactivation is due to UV-A light and benzoic acid (BA) in Ding et al. (2018) and ultrasounds in Alenyorege et al. (2019).	Ding et al. (2018), Alenyorege et al. (2018).
Dynamics for disinfectant $\left(\frac{dC}{dt}\right)$			
Term	**Formula**	**Comments (type of model and assumptions)**	**Reference**
C_Inlet	$\sum_{i=1}^{N} r_i \chi_i$	Addition of disinfectant to water in N doses. FC is added to water at rate r_i during the i-dose and χ_i are step functions that can also be expressed as an if-condition: $\chi_i = \begin{cases} 1 & \text{during } i \text{ dose,} \\ 0, & \text{otherwise.} \end{cases}$	Mokhtari et al. (2018), Madamba et al. (2022a,b).
	$\begin{cases} r_i & \text{during } i \text{ dose,} \\ 0, & \text{otherwise.} \end{cases}$	Same as previous, but using a step function instead of indicator functions χ_i to model the addition of disinfectant at the i-dose, and where r_i is the rate increase of FC for dosing.	Srinivasan et al. (2020).
C_Natural_Decay	λC	Mass Action Law (1st-order decay, one reactant).	Dunkin et al. (2017), Mokhtari et al. (2018), Abnavi et al. (2019), Srinivasan et al. (2020), Abnavi et al. (2021a), Madamba et al. (2022a,b).
C_Degradation_by_COD	$\beta CODC$	Mass Action Law (2nd-order reaction, 2 reactants).	Mokhtari et al. (2018), Abnavi et al. (2019), Srinivasan et al. (2020), Abnavi et al. (2021a), Madamba et al. (2022a,b).
C_Degradation_by_AA	$\beta_{AA}\gamma_{AA}AAC$	Mass Action Law (2nd-order reaction, 2 reactants), model was restructured, see comments below.	Abnavi et al. (2021b)
Dynamics for COD $\left(\frac{dCOD}{dt}\right)$			
Term	**Formula**	**Comments (type of model and assumptions)**	**Reference**
COD_Inlet	k_0	Constant addition of COD. This amount is proportional to the amount of product (see Li et al., 2019), although there is no explicit modelling of this proportionality in the literature.	Mokhtari et al. (2018), Madamba et al. (2022a,b).
	$\begin{cases} k_0 & \text{during contact time,} \\ 0, & \text{otherwise.} \end{cases}$	Same as previous, but explicitly including a step function to simulate when the product releases COD. The addition of COD is only active during contact time, i.e. when the product is in the water. In Abnavi et al. (2021a) this term is multiplied by a factor not included here (see comments below)	Abnavi et al. (2019, 2021a), Srinivasan et al. (2020).

COD_Degradation_by_C	$\beta\gamma CODC$	Mass Action Law (2nd-order reaction, two reactants). This reaction rate is assumed the same as the inactivation of FC, but we include the factor γ to assume different proportionality in the reaction between COD and C (see comments below).	Srinivasan et al. (2020), Abnavi et al. (2021a).
Dynamics for product contamination $\left(\frac{dX_p}{dt}\right)$			
Term	**Formula**	**Comments (type of model and assumptions)**	**Reference**
X_p***_Transfer_from_***X_w	$\beta_{pw}X_w$	This is equivalent to $\beta_{pw}X_w\frac{L}{V}$ (X_w**_Transfer_to_**X_p).	Mokhtari et al. (2018), Abnavi et al. (2021a).
	$\begin{cases}\beta_{pw}X_w & \text{during contact time,}\\ 0, & \text{otherwise.}\end{cases}$	Same as previous, but explicitly including a step function to simulate when the product is washing.	Abnavi et al. (2019).
X_p***_Inactivation_by_C***	$\alpha X_p C$	Assumed the same inactivation constant as in water.	Mokhtari et al. (2018), Abnavi et al. (2021a).
X_p***_Dilution***	c_1X_p	This is simulating the dilution of a system where there is no entering in the inlet flux, but the outlet flux is c_1.	Mokhtari et al. (2018), Abnavi et al, (2021a).
Dynamics for Amino acids $\left(\frac{dAA}{dt}\right)$			
Term	**Formula**	**Comments (type of model and assumptions)**	**Reference**
AA_Inlet	$\begin{cases}\gamma_{AA}k_0 & \text{during contact time,}\\ 0, & \text{otherwise.}\end{cases}$	Constant addition of amino acids to wash water.	Abnavi et al. (2021b).
AA_Degradation_by_C	$\beta_{AA}\gamma_{AA}AAC$	Mass action law (2nd -order reaction with two reactants).	Abnavi et al (2021b).

(a): Demand-free conditions, the term is not an explicit function of FC as concentration of FC is considered constant and therefore included in the α_M constant.

Other rearrangements were the following:

- Abnavi et al. (2021b) work points to the idea that the depletion of FC due to amino acids (AA) is at the same rate as AA due to FC (C). However, that is not necessarily true based on their results since AA is amplified by a factor γ_{AA} that is estimated. Note that if $R = \gamma_{AA} AA$, assuming this scaling constant for any concentration of AA, therefore $\frac{dR}{dt} = \gamma_{AA} \frac{dA}{dt}$ and, in terms of AA, the model in this work reads:

$$\frac{dAA}{dt} = -\beta_{AA} AAC$$
$$\frac{dC}{dt} = -\beta_{AA}\gamma_{AA} AA\, C - \lambda C$$

- This means that the depletion rate of amino acids due to disinfection is $-\beta_{AA} AAC$, but FC reacts with AA with a different rate equivalent to $-\beta_{AA}\gamma_{AA} AA\, C$. It should be stressed that different depletion rates and an amplifying factor for AA cannot be considered at the same time as the problem is structurally not identifiable (there are combinations of both that get the same result). The same rearrangement needed to be considered in Srinivasan et al. (2020) and Abnavi et al. (2021b), but here for the degradation rate of FC with COD.

3.8 Proposal of a general model describing different experimental conditions performed in laboratories and the FBOs

The aim of this section is to present a general modelling framework based on mass balance conservation law from which different models can be built when considering different types of experimental conditions, including from lab and the FBOs, that were not necessarily modelled in the literature. After proposing the general framework, different models are formulated and tested to describe lab and industrial experimental data.

3.8.1 Derivation of the general dynamic mass balance model.

The general model, in addition to the mechanisms already modelled in the literature (see **Model 1** with possible terms in **Table 21**), includes:

- Fluxes of water and product incoming and outgoing in and from the tank. In the literature, this is usually modelled in a discrete form using if-conditions, but for continuous water replenishment, the use of fluxes is a more adequate formalism.
- Dynamics of water volume (V) and product mass (M), since they change with time when incoming and outgoing fluxes are not equivalent, therefore being new states in the model.
- The possibility of having different, and more than one, product $X_{P,i}$, as in Abnavi et al., (2021a) work, where the subindex i is omitted when only one product is washed.
- Explicitly modelling that COD is transferred from the products.
- Focus on Free chlorine disinfectant (FC)

On the other hand, to avoid a very complex model, the focus is on processes using free chlorine as disinfectant and disregarding the dynamics for the amino acids and where sub-indexes w and p,i represent concentrations in water and product i, respectively. Therefore, the general modelling framework reads:

$$\frac{dCOD_w}{dt} = (COD_w\_\text{Inlet}) - (COD_w\_\text{Outlet}) + \left(COD_w\_\text{Transfer\_from}\_COD_{P,i}\right) - \left(COD_w\_\text{Transfer\_to}\_COD_{p,i}\right) - (COD_w\_\text{Degradation\_by}\_FC)$$

$$\frac{dCOD_{p,i}}{dt} = \left(COD_{p,i}\_\text{Inlet}\right) - \left(COD_{p,i}\_\text{Outlet}\right) - \left(COD_{p,i}\_\text{Transfer\_to}\_COD_w\right) + \left(COD_{p,i}\_\text{Transfer\_from}\_COD_w\right); \quad i = 1,2,\dots,n$$

$$\frac{dFC}{dt} = (FC\_\text{Inlet}) - (FC\_\text{Outlet}) + (FC\_\text{Injected}) - (FC\_\text{Natural\_Decay}) - (FC\_\text{Degradation\_by}\_COD_w)$$

$$\frac{dX_w}{dt} = (X_w\_\text{Inlet}) - (X_w\_\text{Outlet}) - (X_w\_\text{Inactivation\_by}\_FC_w) + (X_w\_\text{trans\_by}\_X_{p,i}) - (X_{p,i}\_\text{trans\_by}\_X_w)$$

$$\frac{dX_{p,i}}{dt} = \left(X_{P,i}\_\text{Inlet}\right) - \left(X_{P,i}\_\text{Outlet}\right) - \left(X_{p,i}\_\text{Transfer\_to}\_X_w\right) + \left(X_{p,i}\_\text{Transfer\_from}\_X_w\right) - \left(X_{p,i}\_\text{Inactivation\_by\_FC}\right); \quad i = 1,2,\dots,n$$

Model 2: Proposed general modelling framework based on mass balance conservation. There are different alternatives for modelling each of these terms, that will be studied next to find the best expressions that are presented in Model 3.

Each of the mechanisms described in this general modelling framework should be populated with specific mathematical formulations that will be presented at the end of this section (named **Model 3**) after studying different scenarios with experimental data in the literature to discern best formulas for each term.

It should be stressed that different simplifications of the model would be necessary depending on the experimental setup, as it is illustrated in the next sections. For example, if tank water volume and mass are constants, the last two ordinary differential equations are not needed, but can be included also making equivalent the influx and outflux. The maths inside each term can also depend on the type of experiment, as it is illustrated in the next section testing the model in very different conditions.

3.8.1.1 Modelling the inactivation dynamics (X_w_Inactivation_by_FC)

Inactivation dynamics are complex and require a special analysis where different alternatives must be tested using ad-hoc designed experiments in the literature. As shown in the modelling review, there are many and different models to describe these dynamics (see in **Table 21** the row referring to X_w_Inactivation_by_FC). In this section, different inactivation models were tested to determine which one better describes data specifically carried out to understand the inactivation dynamics and the protective effect of COD in this mechanism. The analysed alternatives include models not found in the literature review but common in other areas such as in water treatment.

The experimental data extracted from the literature were provided by the EFSA **ad hoc** Working Group established for the mandate on "***on microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and***

herbs (ffFVHs)" (EFSA-Q-2021-00374) and consist of two different sets of data (set 1 (Gómez-López et al., 2015) and set 2 (Gómez-López et al., 2014)) where there is only an incoming flux, equal to the outgoing flux, of contaminated water (with or without COD). On the other hand, when there is treatment, residual of FC is kept by different additions of sodium hypochlorite. Therefore, the model remains very simple as described here

$$\frac{dX_w}{dt} = \frac{F_w}{V}(X_w^{in} - X_w) - Inactivation\ \ model$$

where X_w and X_w^{in} are, respectively, the concentration of microbial contamination in the water tank (with volume V) and in the incoming and outgoing flux (F_w). X_w^{in}, F_w and V are known constants, however X_w^{in} is uncertain and estimated within the short uncertainty usually considered log10(CFU/ml) ±0.5 for each curve of experiments.

For the inactivation model, different alternatives were explored. On the one hand, the inactivation kinetics used for free chlorine found in the literature search were considered (other disinfectants or antimicrobial systems like UV were not considered). Note that in the literature review only Abnavi et al., (2021a) considers the protective effect of COD, using a model inspired by the Michaelis-Menten kinetics[5] (see equations in row 4 in **Table 22**). On the other hand, we included other possible inactivation rates that consider the effect of COD using Hill kinetics (row 5 in **Table 22**). The alternative inactivation models can be seen in **Table 22**, with the name of the model, the equations and the references.

Table 22 shows the two calculated indexes calculated to measure the ability of the inactivation model to describe the two sets of experimental data: the weighted least squares method (WLS, that is the log-likelihood estimator when variability in the data is considered constant, as in this case) and Akaike Information Criterion (AIC). Whereas WLS measures the error between the model and the data, the AIC measures the same but penalises the number of parameters to be estimated (and therefore penalises complex models).

Table 22. Performance of the alternative microbial inactivation models to reproduce data from Gómez-López et al. (2015) and Gómez-López et al. (2014). Two performance indexes are compared related with (a) the best description of the data (lower Weighted Least Squares Error) and the best compromise to represent the data with minimum complexity (lower Akaike Information Criterion).

Nº	Model name	Inactivation model	References	WLS	AIC
1	**Mass Action Law**	$\alpha \cdot X_W \cdot FC$	Dunkin et al. (2017), Mokhtari et al. (2018), Abnavi et al., (2019),	15.248	-64.206
2	**Chick-Watson kinetics**	$\alpha \cdot FC^m \cdot X_w$	Dunkin et al. (2017)	14.955	-63.096
3	**Rational kinetics**	$\alpha \cdot FC^m \cdot X_w^q$	Dunkin et al. (2017)	14.814	-61.363

[5] the term "inspired" is used as Michaelis-Menten kinetics are usually expressed for substrate inhibition, with substrate in the numerator and denominator. Nevertheless, similar arguments can be used for a variable inhibiting but not required for microbial growth resulting in Abnavi et al., (2021) equation.

Nº	Model name	Inactivation model	References	WLS	AIC
4	**Mass action Law with Michaelis-Menten kinetics for COD**	$\alpha \frac{K_m}{K_m + COD_w} X_W \cdot FC$	Abnavi et al., (2021a)	12.52	-72.695
5	**Mass action Law combined with Hill kinetics for COD**	$\boldsymbol{\alpha \left(\frac{{K_m}^n}{{K_m}^n + {COD_w}^n} \right) X_w \cdot FC}$	**----**	**9.5589**	**-85.021**

The Hill kinetics presented the best performance, an equation considering a very non-linear behaviour of the COD protective effect. The full model, therefore, considers the dynamics for the water contamination with a term describing the incoming and outgoing contamination (first term of the right-hand side of the next equation) and a term describing the inactivation using Hill kinetics of the form:

$$\frac{dX_w}{dt} = \frac{F_w}{V}(X_w^{in} - X_w) - \alpha \left(\frac{{K_m}^n}{{K_m}^n + COD^n} \right) X_w \cdot FC$$

where FC is the Free Chlorine concentration in water (ppm), COD the organic matter physico-chemical measure (ppm), α the inactivation rate constant (L/(mg-FC·min)), K_m a parameter representing the protective effect of the COD, which is relevant for lower values of this parameter, and the exponent n allows flexibility about the non-linear behaviour of the COD protective effect.

Figure 96 shows the results with this best-found model using Hill inactivation kinetics (equation 5 in **Table 22**). Control experiments (black, blue and red lines), $FC = 0$ ppm, are perfectly reproduced as inactivation kinetics are not needed. When inactivation kinetics are relevant (brown, turquoise, dark and light green lines), some minor differences between the experimental data and the results obtained using the Hill Model can be appreciated, especially during the first 10 minutes, when data is below the detection limit. Besides, we have only estimated one set of parameters for both sets of data that included different *Escherichia coli* cocktail of microorganisms and the washing water from different products (from Iceberg lettuce and Spinach). Therefore, the results are considered satisfactory.

It should be noted that Hill kinetics is a nested model, i.e., it includes other possible dynamics such as kinetics in row 4 of Table 22 (by using n =1) or in row 3 of Table 22 (by using n =1, m=1, q=1, and K_m orders of magnitude larger than COD). This is especially advantageous when experiments are not as informative about the inactivation kinetics as the ones tested here, as will be the case with industrial experiments where COD and FC are not perturbed significantly. For those cases n =1 will be assumed.

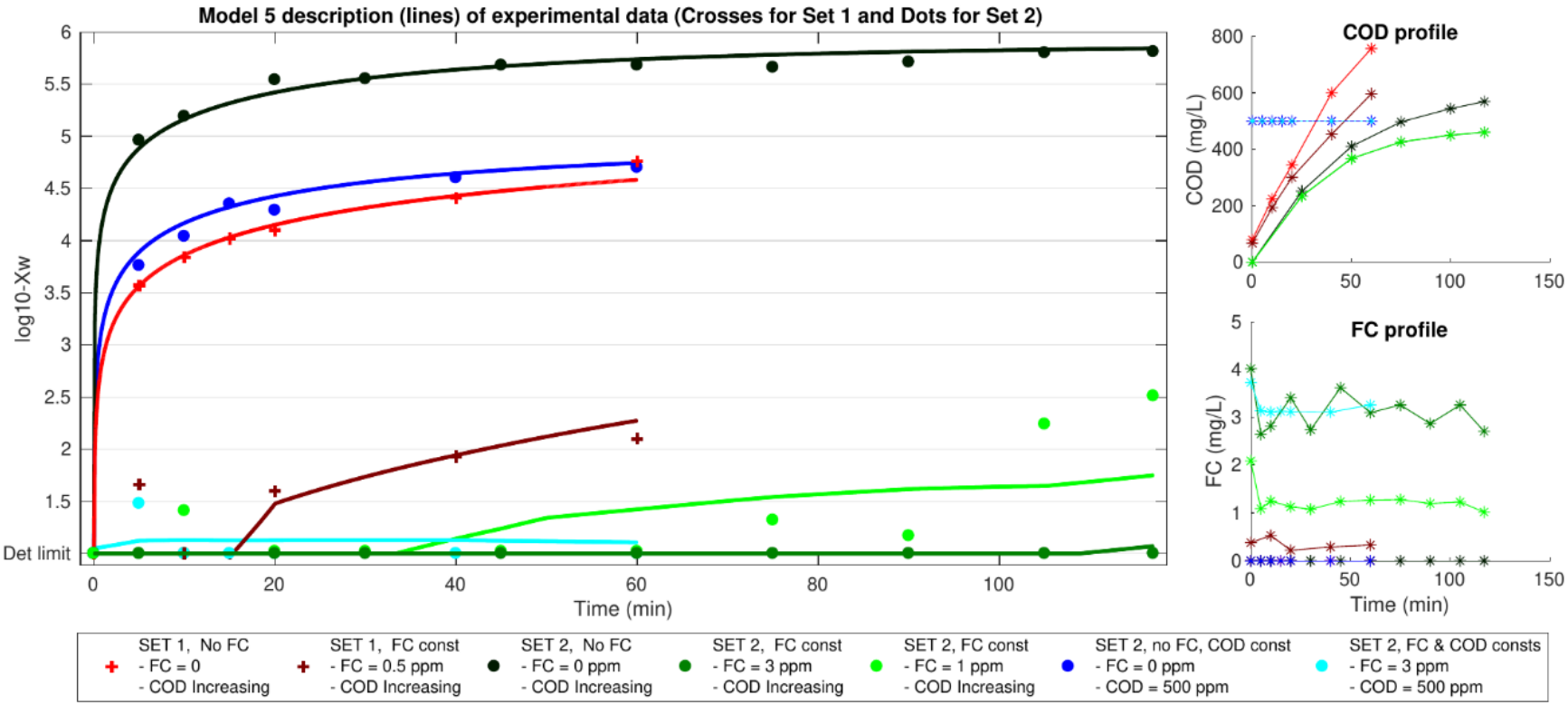


Figure 96: Results for the best-found model (using Hill kinetics inactivation in row 5 of table 22) describing inactivation data in Gómez-López et al. (2015) and Gómez-López et al. (2014). Note that COD and FC are not modelled but added as input to the different inactivation models considering a linear interpolation between the experimental measurements (plots in right column)

3.8.1.2 Modelling the other mechanisms in the general modelling framework

For modelling other mechanisms, the following assumptions were proposed:

- Inlet and outlet fluxes of water and product ($F_w^{in}, F_w^{out}, F_{p,i}^{in}, F_{p,i}^{out}$) were introduced in the model by following the standard theory in chemical reactors.
- Transfer and degradation dynamics are modelled following the mass action law. Note that in transfer dynamics, the change of compartment (tank water, food product) should be considered. To derive the final formulations, mass balances are derived and later transformed to concentrations resulting in transfer rate constants in units 1/time, as expected. However, if for example the concentration of COD in the product (mg-COD/g-produce) is unknown, this is included in the transfer constant to avoid structural identifiability problems, and therefore the units change to mg-COD/(g-produce· min). Along the document we represent by
  - $\widetilde{K}$ (with a tilde symbol) the transfer rate coefficient in units per time,
  - If the constant does not contain the tilde symbol (K), then it includes the contribution of the source (for instance, COD or X_w). The units of this constant are the concentration of the source divided by time. For example, if we consider the concentration of COD in the product i as the source ($COD_{p,i}$) then, the relation between both constants is:

$$K_{COD_{pi\to w}} = \widetilde{K}_{COD_{pi\to w}} COD_{p,i}$$

- Transfer of COD from the water to the product is assumed to be negligible.
- Inactivation dynamics are described, based on the previous results, as Hill kinetics: a nested model including other cases by fixing $n = 1$ (for Michaelis-Menten dynamics) and K_m to a large value if a protective effect of COD is not detected. Note that, even if it is not detected, it could still be a relevant effect (as proven in the last section). This would probably mean that the experiments, especially in industrial cases, are not sufficiently perturbed to alleviate the correlation issues between these parameters and other model parameters (practical identifiability problems).
- Inactivation of bacteria in the product is assumed to be a fraction of the inactivation in water: $\eta\left(\frac{\alpha {K_m}^n}{{K_m}^n+(COD_w)^n}\right) FC\ X_{p,i}$, with $\eta \in [0,1]$

The result is the next set of equations (Variables and parameters are described in **Table 23**:

$$\frac{dCOD_w}{dt} = \frac{F_w^{in}}{V}[COD_w^{in} - COD_w] + \sum_{i=1}^{n} \frac{M_i}{V} \widetilde{K}_{COD_{p,i\to w}} \cdot COD_{p,i} - \gamma \cdot \beta \cdot COD_w \cdot FC$$

$$\frac{dCOD_{p,i}}{dt} = \frac{F_{p,i}^{in}}{M_i}\left[COD_{p,i}^{in} - COD_{p,i}\right] - \widetilde{K}_{COD_{p,i\to w}} \cdot COD_{p,i} + \frac{V}{M_{p,i}} \widetilde{K}_{COD_{w\to p,i}} \cdot COD_w; \qquad i = 1,2,\dots,n$$

$$\frac{dFC}{dt} = \frac{F_w^{in}}{V}[FC^{in} - FC] + u(t) - \lambda \cdot FC - \beta \cdot COD_w \cdot FC$$

$$\frac{dX_w}{dt} = \frac{F_w^{in}}{V}[X_w^{in} - X_w] - \alpha\left(\frac{{K_m}^n}{{K_m}^n + {COD_w}^n}\right) X_w \cdot FC + \sum_{i=1}^{n} \frac{M_i}{V} \widetilde{K}_{X_{p,i\to w}} \cdot X_{p,i} - \sum_{i=1}^{n} \widetilde{K}_{X_{w\to p,i}} \cdot X_w$$

$$\frac{dX_{p,i}}{dt} = \frac{F_{p,i}^{in}}{M_i}\left[X_{p,i}^{in} - X_{p,i}\right] - \widetilde{K}_{X_{p,i\to w}} \cdot X_{p,i} + \frac{V}{M_i}\widetilde{K}_{X_{w\to p,i}} \cdot X_w - \eta\,\alpha\left(\frac{{K_m}^n}{{K_m}^n + {COD_w}^n}\right) \cdot FC \cdot X_{p,i}; \qquad i = 1,2,\ldots,n$$

Model 3. Mathematical model (including mathematical description for the different mechanisms) to test extracted data from the literature.

In this model, FC enters the washing tank in two different ways: (i) as a continuous addition of disinfectant $\left(\frac{F_w^{in}}{V}FC^{in}\right)$; and (ii) as discrete additions $(u(t))$, i.e. the plant operators add disinfectant at given times. This second way to add disinfectant by FBOs is quite common. The time (t) is in minutes and the main variables and parameters are described in **Table 23.**

Table 23. General model states, inputs and parameters. The specified units are those used internally within the model for computations. Nevertheless, they should agree with the data when fitting the model. Therefore, the internal units may be afterwards transformed to calculate the model outputs. For example, product mass is sometimes reported in kilograms and therefore $COD_{p,i}$ could be also reported in mg-COD/Kg-product, instead of mg-COD/g-product in the table. Another common example is the microbial load, that can be expressed in Log10(CFU(or MPN)/mL) or Log10(CFU(or MPN)/100mL) for bacteria in water.

	Definition	**Units**
States		
COD_w	COD in water	ppm-COD
$COD_{p,i}$	COD in product i	mg-COD/g-produce
FC	FC in water	ppm-FC
X_w	CFU in water	CFU/L
$X_{p,i}$	CFU in product i	CFU/g-produce
V	Volume tank	L
M_i	Mass of product i in the tank	g-product
Inputs		
F_w^{in}	Flux of water incoming in the tank	L/min
F_w^{out}	Flux of water out from the tank	L/min
$F_{p,i}^{in}$	Flux of product i incoming in tank	g-product/min
$F_{p,i}^{out}$	Flux of product i outgoing from tank	g-product/min
$u(t)$	Flux of injection of FC	ppm-FC/min
Parameters		
$\widetilde{K}_{COD_{p,i\to w}}$	Transfer rate of COD from product i	1/min
β	Degradation rate of COD by FC	1/(ppm-COD· min)
γ	Yield coefficient COD degradation with FC	mg-COD/mg-FC
λ	Natural inactivation rate of FC	1/min
$\widetilde{K}_{X_{p,i\to w}}$	Transfer rate constant of microorganisms from product i to water	1/min
$\widetilde{K}_{X_{w\to p,i}}$	Transfer rate constant of microorganism from water to product	1/min
α	Parameter in the Hill equation	1/(ppm-FC·min)
n	Exponent of the Hill equation	-
K_m	Protective effect of COD	ppm-COD
η	Relation between the inactivation rates in the product and in water	-

3.8.2 Model testing and calibration based on extracted data in Tier 2.

The aim in this section is twofold: to test the capability of the model to describe the data in the literature and, on the other hand, to provide some estimations of the unknown model parameters.

3.8.2.1 Selection of data for model testing

The steps for selecting the data for testing and calibration of the model were the following:

1. Free chlorine is the active disinfectant; therefore, data was filtered using column "Type of Water Disinfectant" and allowing only values "Calcium hypochlorite, Chlorine, hypochlorite, Other: Free chlorine concentration of AEW, and Sodium hypochlorite". Note that the model was derived for experiments using FC as disinfectant, although with a few modifications it can be adapted to other treatments, if necessary. Moreover, FC is the most studied and common disinfectant.
2. Only dynamic data were considered. For the structural identifiability of the parameters in the model, dynamic data are required, therefore, all manuscripts containing only static data were removed (10 records were retrieved after step 1 and 2: Abnavi et al. (2021a), Bertoldi et al. (2021), Afari et al. (2016), Davison et al. (2014), Dunkin et al. (2017), Borges et al. (2020), Chen et al. (2018), Mathew et al. (2018), Patange et al. (2019) and Shazer et al. (2017).
3. In addition to dynamic data describing the water microbial load (X_w), dynamic data of COD and FC in absolute values were needed (for example, Davison et al. (2014) includes percentage of COD reduced, making the model non-identifiable if inactivation is dependent on COD). Three records were retrieved after this step: Abnavi et al. (2021a), Bertoldi et al. (2021) and Afari et al. (2016).
4. Afari et al. (2016) performed the experiments without organic matter, therefore it is considered that this experiment does not simulate industrial conditions and it is also removed.
5. After this process two studies remained and were analysed further: Abnavi et al. (2021a) and Bertoldi et al. (2021)

3.8.2.2 Model development and calibration for experiments in Abnavi et al. (2021a)

Description of the process and model assumptions

In the paper by Abnavi et al. (2021a), the authors analyse the cross-contamination phenomena in a pilot study. They also provide the data taken during three batches or 'runs' of the washing process . In each of the three batches the following issues were considered:

- *E. coli* O157:H7 is considered as the strain to be inactivated
- Sodium hypochlorite is used as the disinfectant
- Two different products are washed: iceberg lettuce and red lettuce.
- The selected strain is inoculated only in red lettuce
- Five minutes after the disinfectant is added, iceberg and red lettuce are continuously introduced into and removed from the tank with a contact time of 30 s
- After 10 minutes of washing, the batch is considered finished and a new batch starts

For the particular process the following assumptions are considered:

- Input flows of water and product are equal to the respective output flows of water and product, i.e., $F_w^{in} = F_w^{out} = F_w$ and $F_{p,i}^{in} = F_{p,i}^{out} = F_{p,i}$.
- The rate of addition of FC is $u(t) = FC^{inj}$
- Two products are considered (iceberg lettuce and red lettuce).
- Rate of bacteria transfer from red lettuce to water is the same as from iceberg lettuce to water $\widetilde{K}_{X_{rl\to w}} = \widetilde{K}_{X_{il\to w}} = \widetilde{K}_{X_{p\to w}}$
- Transfer rate of COD from red lettuce to water is the same as transfer of COD from iceberg lettuce to water ($\widetilde{K}_{COD_{rl\to w}} = \widetilde{K}_{COD_{il\to w}} = \widetilde{K}_{COD_{p\to w}}$) and since no measurement of COD in the product is provided, we consider that the transfer of COD from each product to the water is constant. Therefore, $\widetilde{K}_{COD_{p\to w}} COD_{p,i} = K_{COD}$ where K_{COD} is the transfer rate of COD from product to water.

Under these conditions, the model remains as follows, where known parameters are in **Table 24**:

$$\frac{dCOD_w}{dt} = \frac{F_w}{V}\left[COD_w^{in} - COD_w\right] + \left(\frac{M_{il}}{V} + \frac{M_{rl}}{V}\right) K_{COD} - \gamma \quad \cdot \beta \quad \cdot COD_w \cdot FC$$

$$\frac{dFC}{dt} = \frac{F_w}{V}\left[FC^{in} - FC\right] + FC^{inject} - \lambda FC - \beta \quad \cdot FC \cdot COD_w$$

$$\frac{dX_w}{dt} = \frac{F_w}{V}\left[X_w^{in} - X_w\right] - \left(\frac{\alpha {K_m}^n}{{K_m}^n + (COD_w)^n}\right) FC\, X_w + \frac{M_{il}}{V}\widetilde{K}_{X_{p\to w}} X_{il} + \frac{M_{rl}}{V}\widetilde{K}_{X_{p\to w}} X_{rl} - \left(2\,\widetilde{K}_{X_{w\to p}}\right) X_w$$

$$\frac{dX_{il}}{dt} = \frac{F_{il}}{M_{il}}\left[X_{il}^{in} - X_{il}\right] - \widetilde{K}_{X_{p\to w}} X_{il} + \frac{V}{M_{il}}\widetilde{K}_{X_{w\to p}} X_w - \eta\left(\frac{\alpha {K_m}^n}{{K_m}^n + (COD_w)^n}\right) FC\, X_{il}$$

$$\frac{dX_{rl}}{dt} = \frac{F_{rl}}{M_{rl}}\left[X_{rl}^{in} - X_{rl}\right] - \widetilde{K}_{X_{p\to w}} X_{rl} + \frac{V}{M_{rl}}\widetilde{K}_{X_{w\to p}} X_w - \eta\left(\frac{\alpha {K_m}^n}{{K_m}^n + (COD_w)^n}\right) FC\, X_{rl}$$

Model 4. Mathematical model derived for describing Abnavi et al. (2021a) data. This model is obtained after simplifying the general Model 3 based on the described experimental design in the article

Table 24. Parameters provided (same units) in Abnavi et al (2021a). These parameters are not included in the estimation procedure.

Parameter	Value	Units	Description
V	20	L	Volume of the washing tank
λ	1.7×10^{-3}	1/min	Natural decay rate of FC in water
M_{il}	250	g-product	Mass of iceberg lettuce in the washing tank
M_{rl}	5	g-product	Mass of red lettuce in the washing tank
F_{il}	500	g-product /min	Input flow of iceberg lettuce in the washing tank
F_{rl}	10	g-product /min	Input flow of red lettuce in the washing tank
FC^{in}	0	ppm-FC	Concentration of FC in the replenishment water

Parameter	Value	Units	Description
V	20	L	Volume of the washing tank
λ	1.7×10^{-3}	1/min	Natural decay rate of FC in water
M_{il}	250	g-product	Mass of iceberg lettuce in the washing tank
M_{rl}	5	g-product	Mass of red lettuce in the washing tank
F_{il}	500	g-product /min	Input flow of iceberg lettuce in the washing tank
F_{rl}	10	g-product /min	Input flow of red lettuce in the washing tank
COD_w^{in}	0	ppm-COD	Concentration of COD in the replenishment water
X_w^{in}	0	MPN/L	Concentration of bacteria in the replenishment water
X_{il}^{in}	0	MPN/g	Concentration of bacteria in the input of iceberg lettuce
X_{rl}^{in}	10^5	MPN/g	Concentration of bacteria in the input of red lettuce

Model calibration and parameter estimation

The remaining parameters were estimated from the experimental data. Besides, input flows of water and injections of FC (F_w, FC^{inj}) are also estimated. The values of the parameters were obtained by fitting the model to the experimental data presented in **Figure 97**. The results are summarized in **Table 25**.

Table 25. Values of the model parameters estimated from the data provided in Abnavi et al (2021a). Flows of water replenishment and FC injection are also estimated.

Parameter	Value	Units	Description
α	6.74×10^3	1/(ppm-FC·min)	Parameter in the Hill equation
β	7.81×10^{-4}	1/(ppm-COD·min)	Rate constant of reaction FC and COD
γ	8.16	mg-COD/mg-FC	Yield coefficient COD of reaction FC and COD
$\widetilde{K}_{X_{p\to w}}$	4.55×10^2	1/min	Transfer rate coefficient of bacteria from product to water
$\widetilde{K}_{X_{w\to p}}$	8.44×10^2	1/min	Transfer rate coefficient of bacteria from product water to product
K_{COD}	4.94	mg-COD/(g-product·min)	Transfer rate at which COD is transferred from the product to the water
F_w	0.51	L/min	Input and output flow of water
FC^{inject}	430	ppm-FC/min	Flow of FC injected in the water during the first five seconds of the run
K_m	4.00×10^3	ppm-COD	Protective COD effect

Parameter	Value	Units	Description
n	2.91	-	Exponent in Hill equation
η	5.70×10^{-2}	-	Relation between inactivation in the product and in the water

Figure 97 shows the comparison between the simulation results (blue continuous lines), with optimal values for the parameters, and the experimental data (orange dots). The simulation begins five minutes after the addition of FC in the first run, i.e., when the first experimental measurement is taken. Initial conditions of the state variables were obtained from the first experimental measurement. The model simulation approximates well the experimental behaviour except for the COD where the simulation overestimates the increase but especially the decrease of COD caused by the addition of FC. It should be noted that the fitted model was not used to describe the evolution of COD, instead interpolation of the experimental data was used.

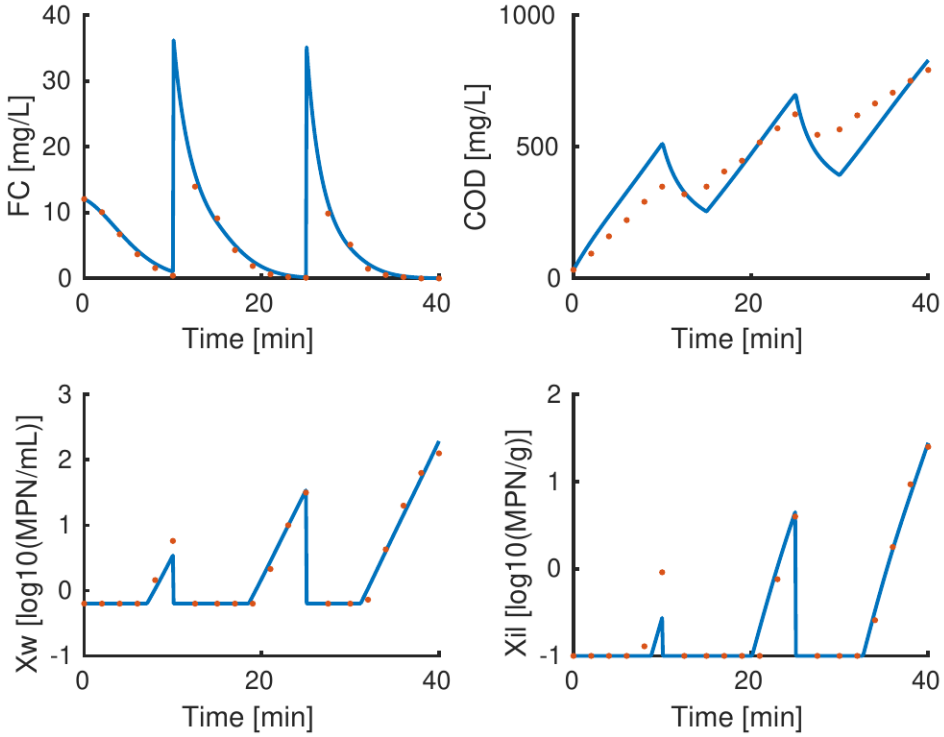


Figure 97. Comparison between the model simulation results (blue continuous lines) and experimental data (orange dots) provided in Abnavi et al (2021). Note that the authors in Abnavi et al (2021) used MPN/mL to report the data regarding bacterial concentration. The Log10 of the data was calculated to fit and plot the result in the graph.

3.8.2.3 Model development and calibration for experiments in Bertoldi et al. (2021)

Relevant disclaimer regarding this case study

In this case, the information on when the FC is added was missing. Therefore, a linear interpolation between the experimental measurements is considered. Unfortunately, we suspect that the obtained FC profile differ considerable from that which was used in the experiments. Therefore, **model results may not be reliable due to uncertainties related to data limitations in this experiment. Nevertheless, this example was kept to illustrate common problems that will be fixed when studying the industrial cases measured in this tender.**

Description of the process and model assumptions

In this research, data from three packinghouses (A, B, C) taken during two different visits to each of the packinghouses are provided.

- Total Aerobic Plate Count, equivalent to Total Bacterial Counts (TBC), and Total Coliforms (TC) are considered as possible surrogates of pathogens to be inactivated. The authors also mention that *Escherichia coli* is measured but dynamic data are only shown for APC and TC.
- Sodium hypochlorite is used as the disinfectant in packinghouses A and B, whereas Calcium hypochlorite is used in packinghouse C.
- The product considered in A, B and C is tomato

For the particular process described in Bertoldi et al. (2021), the assumptions are as in Abnavi et al. (2021a) regarding the fluxes, transfer coefficient of COD, and inactivation in the product, the only differences being:

- Only one product (tomatoes) is used, therefore, one of the equations of the product is removed and any reference to different products are removed, for example M_p if referred to only one product and M without subindex is used to simplify notation
- No information about the quantity and time of injection (or continuous injection) of disinfectant is provided. Different combinations of these variables would result into rather different parameter values. Therefore, instead of modelling FC as a state, a linear interpolation of the measured FC value is used as input to the model. This was also the needed simplification to be considered when modelling the industrial cases.
- The study provides dynamic data of TBC and TC. However, APC contain more values different from 0, so we will consider just APC in the study. Besides, negative values of COD in the measurements are fixed to 0.

Under these conditions, the model is reduced to:

$$\frac{dCOD_w}{dt} = \frac{F_w}{V}[COD_w^{in} - COD_w] + \frac{\mathrm{M}}{V}K_{COD} - \gamma \cdot \beta \cdot COD_w \cdot FC$$

$$\frac{dX_w}{dt} = \frac{F_w}{V}[X_w^{in} - X_w] - \alpha\left(\frac{{K_m}^n}{{K_m}^n + (COD_w)^n}\right)FC \cdot X_w + \frac{\mathrm{M}}{V}\widetilde{K}_{X_{p\to w}}X_p - \widetilde{K}_{X_{w\to p}}X_w$$

$$\frac{dX_p}{dt} = \frac{F_p}{\mathrm{M}}\left[X_p^{in} - X_p\right] - \widetilde{K}_{X_{p\to w}}X_p + \frac{V}{M}\widetilde{K}_{X_{w\to p}}X_w - \eta\alpha\left(\frac{{K_m}^n}{{K_m}^n + (COD_w)^n}\right)FC \cdot X_p$$

Model 5. Mathematical model derived for describing Bertoldi et al. (2021) data. This model is obtained from Model 3 (but resulting also in a simplified version of Model 4) based on the described experimental design in the article

Model calibration and parameter estimation

The volumes of the tanks are not indicated in the manuscript, the authors mention that they range between 38000 and 76000 L, so this will be a parameter to be estimated. This is also the case for the input flows of product. The information we have is that, during peak season 45000 to 57000 kg/h of tomato are processed. In this case, we have to estimate one flow per visit (1,2). This will be also the case for the contact time, that is not provided in the manuscript.

Table 26 shows the parameters provided in Bertoldi et al. (2021). The remaining parameters are separated in three sets: (i) those that are independent of the case study (ii) those that are different in each case study but coincide in each visit (tank volumes); (iii) those that differ from case study to case study and from visit to visit, this were transfer rate of COD from product to water ($\mathrm{K_{COD}}$), input flows of water and product (F_w, F_{to}), and contact time (τ).

Table 26. Parameters as provided (same units) in Bertoldi et al. (2021), and that are considered known and are not estimated.

Parameter	Value	Units	Description
COD_w^{in}	0.00	ppm-COD	Concentration of COD in the replenishment water
X_w^{in}	0.00	CFU/mL	Concentration of bacteria in the replenishment water
X_p^{in}	2.15×10^6	CFU/g-to	Concentration of bacteria in the product. Case B, Visit 1. Obtained from the manuscript data
X_p^{in}	7.36×10^6	CFU/g-to	Concentration of bacteria in the product. Case B, Visit 2. Obtained from the manuscript data

The parameter estimation procedure, using the data provided in manuscript Bertoldi et al. (2021), resulted optimal values that are presented in the following sections/tables. **Table 27** summarizes the values obtained for the parameters that are common to all cases and visits. The value obtained for β is close to 0, which indicates that FC has almost no effect on COD evolution. To simplify the reading of this report, only the most representative case (Packinghouse B) showing richer dynamics is included.

Table 27. Values of the unknown model parameters common to all cases that are estimated Bertoldi et al. (2021)

Parameter	Value	Units	Description
α	7.33×10^3	1/(ppm-FC·min)	Inactivation rate constant
$\gamma \cdot \beta$	4.21×10^{-11}	1/(ppm-FC·min)	Rate of COD degration by FC. Equivalent to rate of FC degradation by COD β multiplied by the mg consumed of COD per mg of FC (γ)
$\widetilde{K}_{X_{p\to w}}$	31.92	1/min	Transfer rate coefficient from tomato (product) to water
$\widetilde{K}_{X_{w\to p}}$	4.35×10^{-4}	1/min	Transfer rate coefficient to water to tomato (product)
K_m	17.11	ppm-COD	Parameter protective effect COD in inactivation

Parameter	Value	Units	Description
n	2.93	-	Exponent Hill inactivation
η	8.97×10^{-4}	-	Relation between inactivation in the product and in the water

Table 28 shows the estimated parameters for packinghouse B. As in the previous case, mass of product in the tank is computed from the flux of product (F_p) and the contact time (τ). Again, we obtain large differences between the same parameters in visits 1 and 2, except for F_p, which is in the same order of magnitude. Besides, contact time in visit 2 is 15 minutes, which is considered a very large value. The conclusions are similar in the analysis conducted for other packinghouses, either that the washing procedure was very different between the two visits or that the unknown parts of the procedure (in particular the times at which the disinfectant was added) have a large effect on the experimental data and, therefore, on the values obtained for the estimated parameters.

Table 28. Values of the unknown model parameters estimated for packinghouse B

Parameter	Value	Units	Description
$K_{COD,1}$	0.064	mg-COD/(g-to·min)	Rate at which COD is transferred the tomato the water in visit 1
$K_{COD,2}$	3.65	mg-COD/(g-to·min)	Rate at which COD is transferred from tomato to water in visit 2
$F_{w,1}$	3.27×10^{2}	L/min	Flow of water replenishment in visit 1
$F_{w,2}$	4.90×10^{-4}	L/min	Flow of water replenishment in visit 2
$F_{p,1}$	2.21×10^{5}	g-to/min	Mass flow of tomato in visit 1
$F_{p,2}$	1.04×10^{5}	g-to/min	Mass flow of tomato in visit 2
τ_1	0.13	min	Contact time in visit 1
τ_2	15.00	min	Contact time in visit 2
V	7.60×10^{4}	L	Tank volume in packinghouse A

Figure 98 shows the experimental data provided in Bertoldi et al. (2021) for packinghouse B and the corresponding model simulation**.** For the top and bottom-left figures, continuous blue lines represent model predictions, whereas dots represent experimental data. Bottom-right figure represents the FC evolution in the system assuming linear interpolation between two measurements. As shown in the Figure, the model is able to reproduce increase in COD observed in both visits in packinghouse B. It is also able to represent the mean evolution of the bacteria (in particular, in visit 1) but it is not able to reproduce the "noisy" behaviour, probably because the assumption of linear evolution of FC between two measurements is wrong. Again, the number of parameters to be estimated is too large given the quantity of experimental data. More experimental data are required to reduce the correlation between parameters.

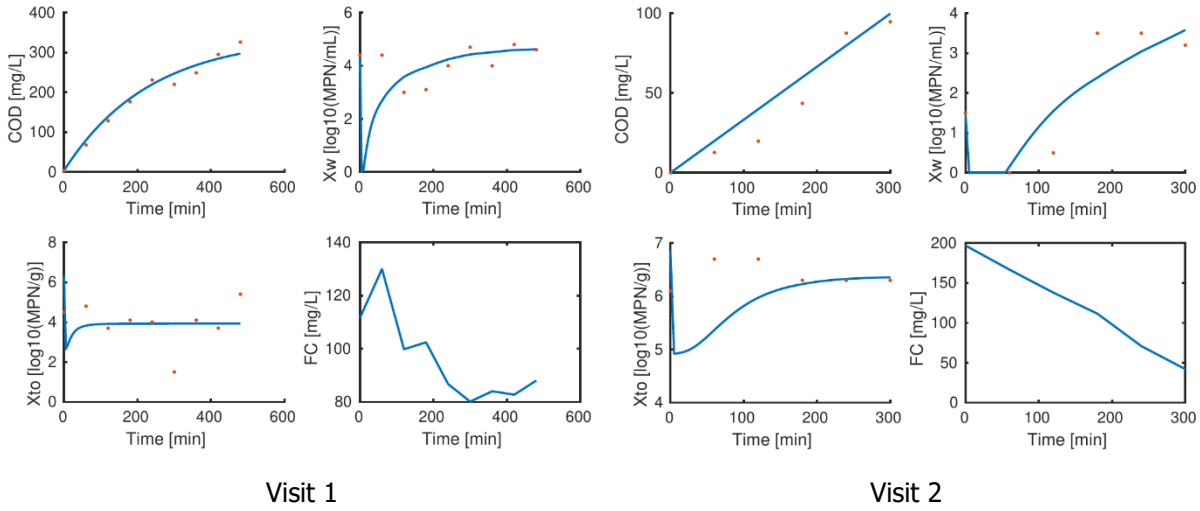


Figure 98. Comparison between the model simulation results (blue continuous lines) and experimental data (orange dots) provided by Bertoldi et al. (2021) for packinghouse B visit 1 (right) and visit 2 (left).

3.8.3 Conclusions of the study of the different data retrieved and model from the literature.

A general model (**Model 3** with definition of variables and parameters in **Table 23**) based on mass balance conservation was developed that can be used as starting point to understand the washing process of ffFVHs in the industry and in laboratory experiments. Depending on the modelled data, the general model can be simplified to obtain fit-for-purpose models, for different types of experiments.

The different fit-for-purpose models were able to reproduce the experimental data, but for some cases the experimental measurements were not sufficiently dynamic to infer relevant parameters with confidence. Note that in the literature, usually relevant information about the process was missing, such as the times at which the disinfectant is added. These issues led to a situation in which the influence of a given mechanism (protective effect of COD, natural decay of FC, transfer rates, etc.) cannot be discerned from the influence of the others. For example, an underestimation of the protective effect of COD may be compensated with an overestimation of the transfer rates without affecting the quality of the fitting.

Based on the experience modelling data in the literature, for the industrial scenarios in the next section the model will be reduced to the minimum to focus on the relevant dynamics.

3.9 Modelling the industrial case scenarios

In this section, a dynamic mass balance model is derived and used to analyse the microbiology and physico-chemical quality measured for different case scenarios described in section 2.1 (from now on named industrial cases or just cases followed by the corresponding identification code). First the general modelling framework is simplified and adapted to the available information from the FBOs and the type of measurements carried out. Later the model is analysed to understand microbial contamination and inactivation dynamics in the industry.

3.9.1 A model to analyse the industrial case scenarios

Model analyses in previous sections, using data retrieved from the literature, revealed that there are many different interesting and complex mechanisms playing a role when analysing different washing processes of ffFVHs. Nevertheless, to find useful modelling-based insight information, some of these mechanisms should be simplified or even disregarded to obtain a minimal model sufficiently informative to represent relevant mechanisms. After different preliminary tests (where parameter estimates and confidence intervals were calculated and analysed to understand which mechanisms were highly correlated), the following **Model 6** was selected, with mechanisms illustrated in **Figure 99.**

$$\frac{dX_w}{dt} = -D \cdot X_w + \frac{M}{V} K_X - \alpha \left(\frac{{K_m}^n}{{K_m}^n + COD_w^n} \right) X_w \cdot HOCl$$

$$\frac{dCOD_w}{dt} = -D \cdot COD_w + \frac{M}{V} K_{COD}$$

$$HOCl = \frac{FC}{1 + \frac{10^{-pK_a}}{10^{-pH}}} \quad \text{with} \quad pK_a = \frac{3000}{T} + 0.0253\,T - 10.06$$

$$M = \tau \frac{M_{batch}}{t_{batch}}$$

Model 6. Dynamic mass balance model selected among different alternatives to understand water contamination and inactivation dynamics in the studied industrial cases.

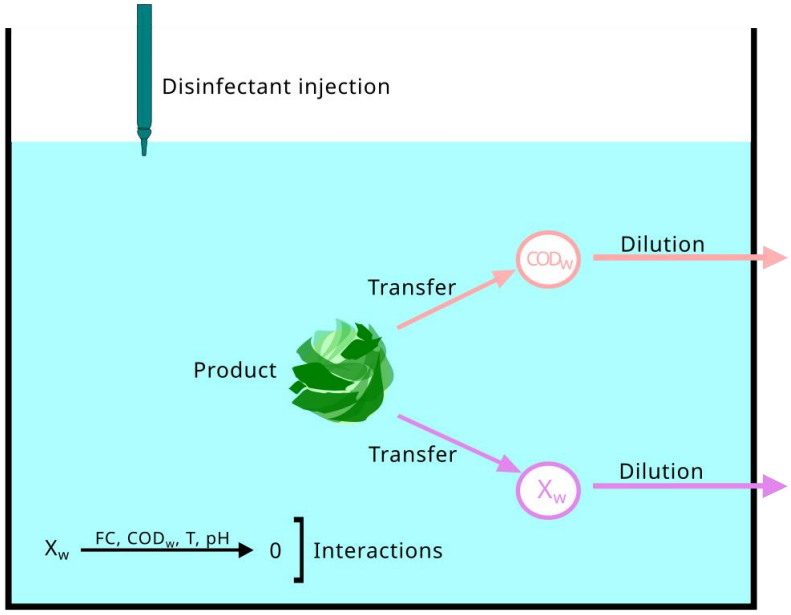


Figure 99. Illustration of mechanisms considered to model the industrial cases (using **Model 6**). Model state variables are represented within circles (X_w, COD_w), transfer mechanisms are with arrows and the interactions are

in the lower left part of the graph (inactivation due to FC). To simulate cases without disinfectants FC is zero and therefore the disinfectant inactivation has no effect

Model 6 focuses on the simulation of a batch (time while product is processed between full changes of the tank water, also named production time) and on the following two variables:

- $\boldsymbol{X_w}$ concentration of microbial contamination in the water, internally working with CFU/mL in the model but finally reported in outputs as Log10(CFU/100mL), for either Total Bacterial Counts (TBC) or *Listeria* spp. (Lis)
- $\boldsymbol{COD_w}$ concentration of organic content measured through chemical oxygen demand (ppm-COD)

It should be stressed that the initial conditions, unless being measured, are assumed zero for both variables since at the beginning of the batch process, the water was fully changed and considered clean (non-detectable microbial counts).

The inputs to the model are linear interpolations between measurements of the following time-changing variables:

- Free chlorine (FC) (expressed in ppm) was not included as a state variable in the model because it was unknown when and how much free chlorine was added to the process. Instead, FC was measured in the water at different times and for feeding the model a linear interpolation between measured points was considered (other types of interpolations were tested but did not improve the model results)
- Temperature (T) (expressed in Kelvin degrees) was needed because it affects the equilibrium of the hypochlorous acid ($HOCl$) that is considered in this model for the first time explicitly as the active compound in FC (see details in chapter 2 in the book "White's Handbook of Chlorination and Alternative Disinfectants", Black & Veatch Corporation (2010))
- The negative logarithm of the hydrogen ions (pH) was also needed because, similarly to T, affects the equilibrium of $HOCl$.

The parameters, which were retrieved from FBO cases or assumed, were

- Volume of the tank (V expressed in litres)
- Mass of product (M expressed in kg) calculated from information provided by the FBOs regarding the process contact time (τ), the duration of the batch (t_{batch}) and the total processed product in this batch (M_{batch})
- Hill exponent that was assumed to 1 (n =1), as data was not sufficiently rich to identify this parameter with confidence (obtained too large confidence intervals)

Finally, the unknown parameters to be estimated by model calibration with measured data of the variable estates ($\boldsymbol{X_w}$ and $\boldsymbol{COD_w}$) in the FBOs were:

- K_{COD} transfer rate of COD from product to water (expressed in ppm-COD/kg-product·min)
- K_X transfer rate of microbial contamination (either TC or *Listeria*) from product to water (expressed in CFU/kg-product·min)
- D dilution rate of the process, that is equivalent to the water flux divided by the tank volume in previous models $D = F_w/V$ (expressed in 1/min).
- α the inactivation rate in (expressed 1/(min·ppm-HOCl))

- K_m protective effect of COD in (1/ppm-COD)

The model is based on mass conservation principles and thus is valid for any zero or positive values of these parameters (except for tank volume that should be strictly positive). Moreover, the model is based on several assumptions considered here for the first-time to be able to deal with industrial data (representative of a normal process, but not necessarily sufficiently informative to infer certain type of information):

- Instead of working with inlet and outlet fluxes of water, that are unknown, the model assumes a constant dilution rate coefficient that is always the same for all cases. This is a major assumption as the product sweeps along some unknown quantity of water, and therefore the operator needs to refill the tank with new clean water at some times that are unknown. It was observed with previous analysis that this assumption was not impacting considerably the results but should be taken into account when interpreting the model outcome. Note that water inlet flux in the tank is assumed clean and therefore $COD_w^{in} = X_w^{in} = 0$
- As no contamination is measured in the product, the transfer rates are assumed constant parameters encoding inside the contribution of the contamination in the product and the net velocity of transferring from product to water along the batch. Note that the original general model did include the transfer from water to product and transfer and vice versa, that for example for microbial contamination was

$$\text{Net balance due to transfer rates in microbial water contamination} = \frac{M}{V}\widetilde{K}_{X_{p\to w}}X_p - \widetilde{K}_{X_{w\to p}}X_w$$

where subindex p is for product and w for water. However, in industrial cases (1) only a batch with similar product, or mixture of products, is analysed, (2) the contamination in the product is not measured and (3) the contact times are very short, usually of one minute. Therefore, the contribution from water to product is minimum (contrary to previous case where cross-contamination between batches was studied) and dynamics of contamination in the product are unknown. Therefore, the expression should be approximated by $\frac{M}{V}K_X$, where now the product contamination is included in the transfer rate coefficient, and therefore the units are not per time, but $\frac{\text{mg-COD}}{\text{kg-product·min}}$ for COD and $\frac{\text{CFU}}{\text{kg-product·min}}$ for water microbial contamination.

- The degradations due to the interaction between COD and disinfectant are considered zero ($\beta_X = \beta_{COD} = 0$). Note that all the modelling tests (with data from literature and measured in industrial cases) were based on measurements of free chlorine afterwards the interaction between COD and FC was produced. To estimate this relevant contribution, it is necessarily to measure, in addition to FC, when and how much total chlorine is added.
- The inactivation is modelled using the Hill kinetics with n=1, and α and K_m cannot be estimated if there is not disinfectant, similar to control experiments, because this term is zero.

3.9.2 Model-based understanding of the water contamination dynamics and variability depending on the type of operation, product and sector (data from cases without disinfectant in objective 1)

For model-based understanding of water contamination in the industry, all the cases without water treatment were selected except case 2 (only one visit) and 5 and 49 with hydro-cooling and water transport processes where the assumption of a tank holding water could not be considered. See **Table 29** for description of the considered cases (all from objective 1) for the understanding of contamination dynamics in the industry.

Table 29. Industrial cases analysed from objective 1 to understand microbial contamination in the industry

Case	Product	Sector	Process tank
1	Apples	Fresh-whole FVHs	Dumping
3	Peaches/Nectarines	Fresh-whole FVHs	Dumping
4	Peppers	Fresh-whole FVHs	Pre-washing
6	Carrots	Fresh-whole FVHs	Washing
7	Vegetable mix	Fresh-whole FVHs	Pre-washing
8	Celery	Fresh-whole FVHs	Washing
30	Shredded carrots	Fresh-cut FVHs	Washing
31	Curly endive/radicchio	Fresh-cut FVHs	Washing
32	Baby leaves	Fresh-cut FVHs	Washing
33	Parsley	Fresh-cut FVHs	Washing
34	Salad mix with carrots	Fresh-cut FVHs	Washing
50	Spinach	Frozen FVHs	Washing
51	Spinach	Frozen FVHs	Washing
52	Spinach	Frozen FVHs	Washing

For the analysis, **Model 6** was calibrated by maximising the log-likelihood assuming Gaussian error with uncertainty of 10ppm for COD and 0.5 logs for TC. That is equivalent of minimising the least squares error weighted with these quantities, therefore allowing a multi-experiment calibration (considering all experiments in the same optimisation) and making some parameters experiment-dependent (K_{COD} and K_{TC}) or global to all experiments (D). The reader is referred to the Methods section and references therein for details. Just to stress, that the estimated confidence intervals represent a lower bound of the parameter's uncertainty (not variability) very useful for detecting correlation problems. For example, in preliminary tests, estimating the dilution rate also for each experiment, resulted in a high correlation between the parameters, and even structural identifiability problems for experiments with poor dynamics, i.e. where only the final saturation state is measured. For this reason, it was assumed that dilution (the less expected varying parameter among experiments) was the same for all experiments.

The first conclusion is that the model, although simple, captures the main behaviour of all studied cases as can be seen in **Figure 100**. It should be stressed that y-axes are depicted using the same scale to allow comparisons among cases. This also hampers the clear visualisation of the results when contamination is

low. The reader is referred to the **Figure 1 in Appendix G** in the appendix to see experimental data and model simulations with y-axes scaled to the range in each experiment.

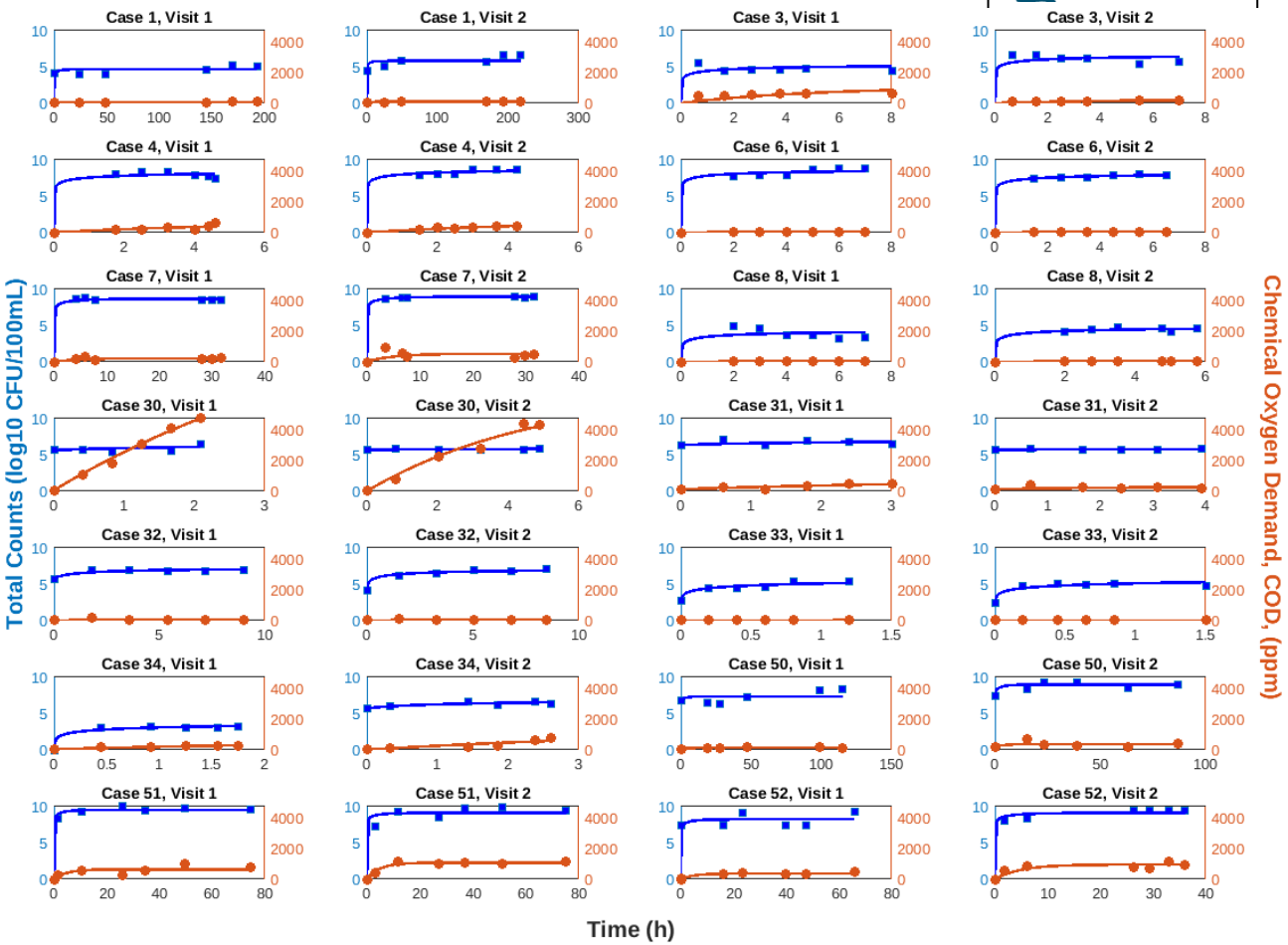


Figure 100. Dynamics of total counts (blue, left y-axes) and COD (orange, right y-axes) for the cases without disinfectant. Dots are experimental data and lines the model prediction for the estimated transfer rate parameters ($\boldsymbol{K_x}$, $\boldsymbol{K_{COD}}$) for each experiment (visit) assuming same dilution rate ($\boldsymbol{D}$) for all cases. The estimated parameters and confidence intervals are provided in an supplementary excel file named "Estimated_parameters_cases_WITHOUT_disinfectant.xlxs"

The estimated parameters can be analysed to find relevant patters in the industry since the model describes the experimental data and the estimated parameters have low uncertainty intervals. The calculated 95% confidence interval (CI), using the Cramér-Rao bound (see methods section for details), was 3.37e-3±1.45e-5 for the dilution rate constant. The maximum CI was detected for the transfer rate of TC in case 33 visit 2 (2.37e7±5.35e2) being the CI five orders of magnitude less than the estimated parameter. On the other hand, CIs are always orders of magnitude less than the estimate, being the worst estimation for COD transfer rate in case 50, visit 1 (4.51e-1±2.29e-3) with very low detected level of exudate from product to water. Even in this case, the CI is two orders of magnitude less than the estimated parameter. Therefore, the differences between estimates of transfer rates among cases can be assumed to be mostly due to variability and not uncertainty.

Figure 101, therefore, shows the variability between different visits and cases. The larger variability is observed for the microbial contamination transfer rates that may vary almost 7 orders of magnitude from the best (case 8 washing celery) and worst (case 51 washing spinach) cases. In addition, there is also a large variability between visits with a difference of almost two logs for case 50, although very small for case 30. The transfer rate of COD, however, is more homogeneous with a transfer rate in most cases within 1 and 2 orders of magnitude.

It should be stressed that those estimations assume same dilution rate, being the best estimated value for describing all cases 0.003 1/min. Therefore, estimations present a bias that should be considered, being the estimated transfer rates lower than expected if dilution was higher than 0.003 1/min and vice versa. Based on this bias, there are cases where both transfer rates either increase or decrease between visits (see for example case 1 where both transfer rates are larger in visit 2 than in visit 1) which could be due to variability of the transfer rates or due to different dilution between visits. Nevertheless, there are cases where that is not the pattern, as the case 3 washing Peaches/nectarines. Note that between visits, visit 1 presents a larger COD contamination and lower TC contamination rates than visit 2. This proves that there is in fact a very large variability in the transfer rates that could not be explained due to inaccurate estimations of the dilution rate.

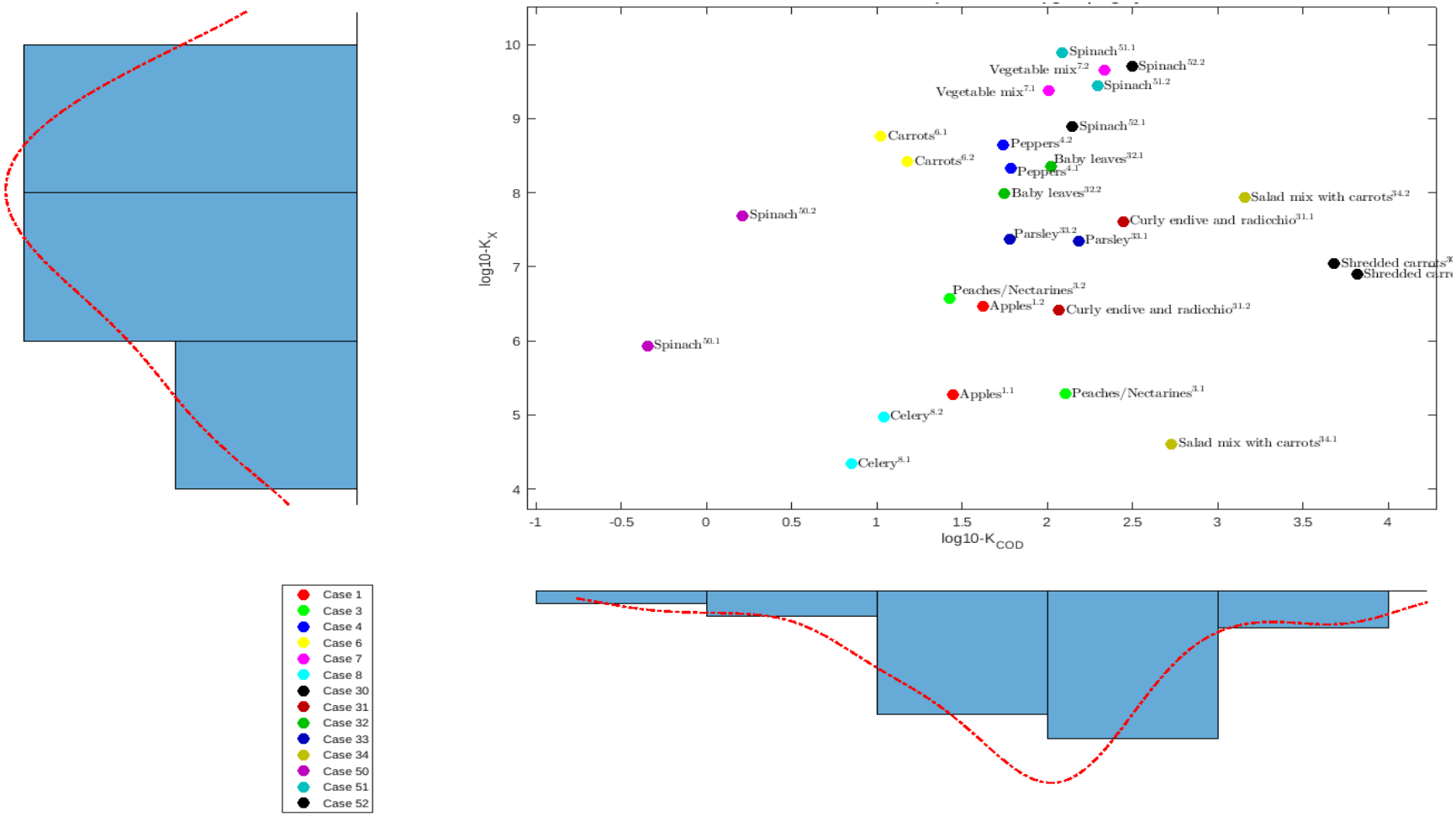


Figure 101. Estimated transfer coefficients, grouped by considered cases, for TC ($\boldsymbol{K_X}$ in $\frac{\text{CFU}}{\text{kg-product·min}}$) and for COD ($\boldsymbol{K_{COD}}$ in $\frac{\text{mg-COD}}{\text{kg-product·min}}$) using the log10 transformation (i.e. order of magnitude) for all visits with an estimated dilution of $\boldsymbol{D} = 0.003\ min^{-1}$. In the scatter plot colours represents the different cases, with two dots of the same colour for each visit and with extra-information added as text (product with superindex reporting the case and the visit). Distributions are estimated considering all visits for each of the transfer rates.

When grouping the transfer rates by type of operation, see **Figure 102**, it is observed that pre-washing operation presents the larger microbial contamination, probably for this reason this operation was included in the washing line. Remark that this model-based analysis also reveals that there are some cases with very contaminated products (see washing of spinach in cases 51 and 52), for which a pre-washing step could be a better option. Unfortunately, cases from pre-washing and washing did not correspond to consecutive operations in the same processing plant, which could have been a piece of critical information to understand the impact of pre-washing. Dumping tanks, on the other hand, usually wash fruits and therefore their lower microbial contamination could be due to the operation type or due to the product.

Same analysis was carried out by grouping the transfer rates by sector and by product, which can be seen in **Figures 2** and **3** in **Appendix G** respectively.

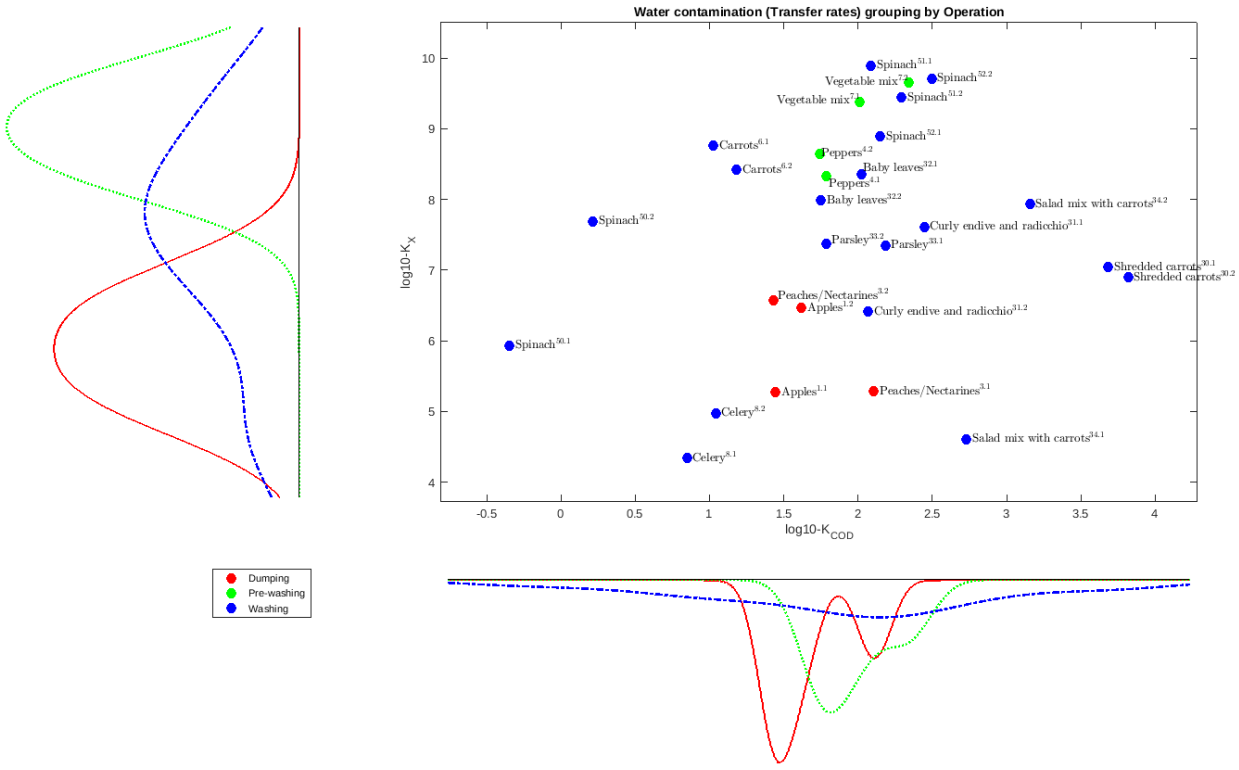


Figure 102. Estimated transfer coefficients, grouped by type of operation, for TC ($\boldsymbol{K_X}$ in $\frac{\text{CFU}}{\text{kg-product·min}}$) and for COD ($\boldsymbol{K_{COD}}$ in $\frac{\text{mg-COD}}{\text{kg-product·min}}$) using the log10 transformation (order of magnitude) for all visits with an estimated dilution of $\boldsymbol{D} = 0.003\ min^{-1}$. In the scatter plot colours represents the different operations. Distributions are estimated for the microbiological (y-axis) and COD (x-axis) transfer rates for each operation.

3.9.3 Model-based understanding of inactivation dynamics for total counts and *Listeria* spp. in the industry (data from cases with disinfectant in objective 2)

For model-based understanding of the effect of the disinfectant in the water microbiological contamination in the industry, all the cases with water treatment were selected except cases with only one visit (cases

10, 14, 17, 21, 27), using a disinfectant different from FC (cases 12, 13, 23, 53, 54, 56, 57), with an operation where a holding water tank cannot assumed (cases 24, 55, 58, 59) and the special case 29 where before starting the measurements a different product (with less COD contamination rate) was processed. **Table 30** shows an outline of the selected cases.

Table 30. Industrial cases analysed from objective 2 to understand microbial inactivation

Case	Product	Sector	Process tank
9	Apples	Fresh-whole FVHs	Dumping
11	Apples	Fresh-whole FVHs	Dumping
15	Pears	Fresh-whole FVHs	Pre-sorting
16	Peaches/Nectarines	Fresh-whole FVHs	Dumping
18	Peaches/Nectarines	Fresh-whole FVHs	Dumping
19	Peaches/Nectarines	Fresh-whole FVHs	Dumping
20	Peaches/Nectarines	Fresh-whole FVHs	Dumping
22	Peaches/Nectarines	Fresh-whole FVHs	Pre-sorting
25	Cherries	Fresh-whole FVHs	Dumping
26	Avocado	Fresh-whole FVHs	Pre-washing
28	Peppers	Fresh-whole FVHs	Washing
35	Tomatoes/Cucumbers	Fresh-cut FVHs	Pre-washing
36	Tomatoes/Cucumbers	Fresh-cut FVHs	Washing
37	Diced onion	Fresh-cut FVHs	Washing
38	Diced onion	Fresh-cut FVHs	Washing
39	Carrot sticks	Fresh-cut FVHs	Washing
40	Fresh-cut lettuce	Fresh-cut FVHs	Washing
41	Fresh-cut lettuce	Fresh-cut FVHs	Washing
42	Fresh-cut lettuce	Fresh-cut FVHs	Washing
43	Shredded lettuce	Fresh-cut FVHs	Pre-washing
44	Shredded lettuce	Fresh-cut FVHs	Washing
45	Baby leaves	Fresh-cut FVHs	Washing
46	Baby leaves	Fresh-cut FVHs	Washing
47	Baby leaves	Fresh-cut FVHs	Washing
48	Salad mix	Fresh-cut FVHs	Washing
60	Parsley	Frozen FVHs	Washing

Case	Product	Sector	Process tank
61	Chives	Frozen FVHs	Washing

The model for analysis is equivalent to the model presented in previous section, see Section 2.7.1 and **Model 6** for a detailed description, with the following adaptations

- Microbial contamination is studied not only for total Counts (TC) but also for *Listeria spp*.
- All the cases are with water treatment using chlorine disinfectants, therefore the inactivation term in the model is active and their parameters can be estimated from the data. Preliminary analyses reveal that there is a relevant correlation between this inactivation term and the contamination transfer, in addition, it is known that even if n=1 this type of Michaelis-Menten kinetics presents highly correlated parameters. Therefore, the inactivation rate constants ($\alpha_{TC}, \alpha_{Lis}$) are considered only bacteria-dependent, whereas the protective effect of COD (K_m) is considered experiment-dependent (but common to TC and Lis).
- The dilution rate constant was assumed with the value estimated for cases without disinfectant ($D = 0.0034\ 1/min$), since for high K_m and constant FC this parameter is structurally correlated with the inactivation rates.

Figure 103 shows that the model describes the dynamics of most of the cases under the considered assumptions. The only exceptions are cases like 38 visit 2 where the model dynamics are faster than the experimental data. This may be due to a non-realistic assumption of the dilution rate or because a product with a low contamination rate was processed previous to the first measurement (transfer rates changes along the batch). Note that measurements of *Listeria* spp. are usually low (or even below the detection limit for some cases), i.e. with poor informative dynamics, therefore dificulting the estimation of confidence parameters.

The estimated parameters, reported in "Estimated_parameters_cases_WITH_disinfectant.xlxs" were with low uncertainty for the inactivation rates with values $\alpha_{TC} = 7.66 \pm 0.02\ (\text{ppm-HOCl} \cdot \min)^{-1}$ for Total counts and $\alpha_{Lis} = 5.32 \pm 0.07 (\text{ppm-HOCl} \cdot \min)^{-1}$ for *Listeria* spp. However, these parameters were highly correlated with the protective effect of COD (K_m). When the protective effect is assumed not relevant for all cases (i.e, fixing K_m at large values of 1e4, data not shown), the model describes the data only slightly worse and with inactivation rates with lower values ($\alpha_{TC} = 5.48 \pm 0.02 (\text{ppm-HOCl} \cdot \min)^{-1}$ and $\alpha_{Lis} = 3.96 \pm 0.08 (\text{ppm-HOCl} \cdot \min)^{-1}$), but always being the inactivation rate of *Listeria* spp. lower than the inactivation rate of total counts, even for other tested alternatives.

Unfortunately, although expected, confidence intervals were more uncertain when modelling industrial cases with FC disinfection than when studying cases in the previous section without disinfectant. Therefore, it is not possible to distinguish if differences between Km estimations are due to uncertainty or variability and previous study of variability between estimations was not possible. Despite adding more information (*Listeria* dynamics), assuming dilution as a known parameter (estimated previously), and inactivation rates only dependent on the type of microorganisms (α_{TC} and α_{Lis}), there are still many unknown parameters. Four parameters are experiment-dependent (transfer rates of COD, TC and *Listeria* spp. and the protective effect of COD), that together with the inactivation rates results in a total of 218 estimates when analysing

the 27 cases with 2 visits each. In addition, the dynamics of *Listeria* spp. remains low in most of the experiments, not providing very informative information (the model cannot distinguish if *Listeria* spp. is low because of a lower transfer rate or a high inactivation rate). Therefore, the *Listeria* spp. transfer rate coefficient is the most uncertain parameter (worst case for case 45 visit 2 being 178.91±1.78e11).

Due to this uncertainty in the model estimations, only a case-by-case analysis for those experiments with low uncertainty can be considered. For example, case 37 (both visits) could be a good candidate as CI are less than the estimates for all parameters including the *Listeria* transfer rate. Estimated parameters from this case, are therefore used in the next section to illustrate how extending the model by including the FC dynamics could help to assess the impact of the FC additions into microbial and COD water contamination.

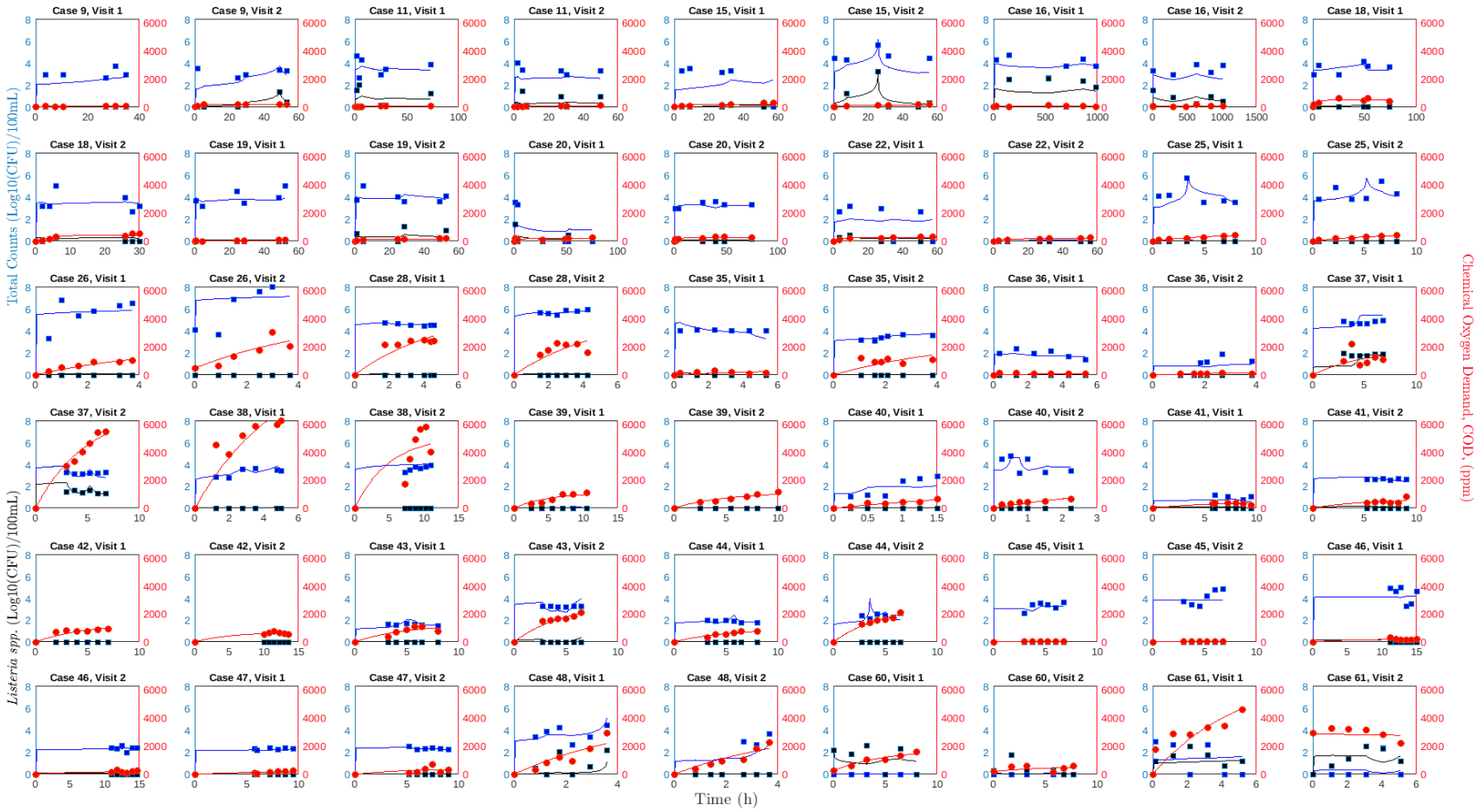


Figure 103. Dynamics of total counts (blue, left y-axes), *Listeria spp.* (black, left y-axes) and COD (orange, right y-axes) for the cases with disinfectant. Dots represent experimental data and lines the model prediction for the common-to-all-experiments estimated inactivation rates ($\boldsymbol{\alpha_{TC}, \alpha_{Lis}}$) and experiment-depending protective COD effect and transfer rates ($\boldsymbol{K_{TC}, K_{Lis}, K_{COD}, K_m}$) assuming same dilution rate ($\boldsymbol{D = 0.03 min^{-1}}$) for all cases. The estimated parameters are provided in an supplementary excel file named "Estimated_parameters_cases_WITH_disinfectant.xlxs" See same figure in Appendix G without y-axis normalization to observe the behaviour for dynamics with low values.

3.10 A model to simulate different what-if scenarios of interest for FBOs

In this section, a final model, **Model 7**, is presented with the objective to test different possible scenarios in industrial settings, or even run optimisation to determine the best operational conditions. The model is based on Model 6, used to describe the FBOs scenarios considered in this tender. The only difference is that FC is added to Model 6 as an input (interpolation from measured FC data) and in model 7 the dynamics of FC are modelled.

Based on standard mass balance, as illustrated in **Figure 104**, the FC dynamics is a function of (1) the dilution rate, (2) the added chlorine, (3) the FC natural decay and (4) the interaction of COD with chlorine. All terms can be modelled using parameters estimated in this tender for FBOs data (for example the dilution rate) or known from the literature (like the natural decay rate of FC in water from Abnavi et al (2021a)). The only exception is the COD and chlorine interaction. Although that is a critical mechanism when modeling how total added chlorine is transformed in combined and free chlorine, this is a mechanism that is highly uncertain as discussed at the end of the section.

Different types of intersections (or system controls) were allowed in the model to test different what-if scenarios

- changing the water that is continuously added ($\mathrm{F_w^{cont}}$)
- adding water at certain times ($\mathrm{F_w^{disc}}$)
- changing the FC that is continuously added ($\mathrm{FC_{in}^{cont}}$)
- adding FC at certain times ($\mathrm{FC_{in}^{disc}}$)

$$\frac{dX_w}{dt} = -D(t)\cdot \mathrm{X_w} + \frac{\mathrm{M}}{V}K_X - \alpha\left(\frac{{K_m}^n}{{K_m}^n + COD_w^n}\right)\mathrm{X_w}\cdot \mathrm{HOCl}$$

$$\frac{dCOD_w}{dt} = -D(t)\cdot COD_w + \frac{\mathrm{M}}{V}K_{COD} - \gamma\cdot\beta\cdot COD_w\cdot FC$$

$$\frac{dFC}{dt} = -D(t)\cdot FC + \mathrm{u(t)} - \beta\cdot COD_w\cdot FC - \lambda\cdot FC$$

$$HOCl = \frac{FC}{1+\frac{10^{-pK_a}}{10^{-pH}}} \quad with \quad pK_a = \frac{3000}{T} + 0.0253\,T - 10.06$$

$$M = \tau\frac{M_{batch}}{t_{batch}} \qquad D(t) = \frac{\mathrm{F_w^{cont}} + \mathrm{F_w^{disc}}}{\mathrm{V}} \qquad u(t) = \frac{\mathrm{FC_{in}^{cont}} + \mathrm{FC_{in}^{disc}}}{\mathrm{V}}$$

Model 7: Mathematical model to study what-if scenarios, such as changes in FC additions, processed products or contact time. The model is an extension of model 6 including the FC dynamics allowing discrete and continuous addition of water and FC.

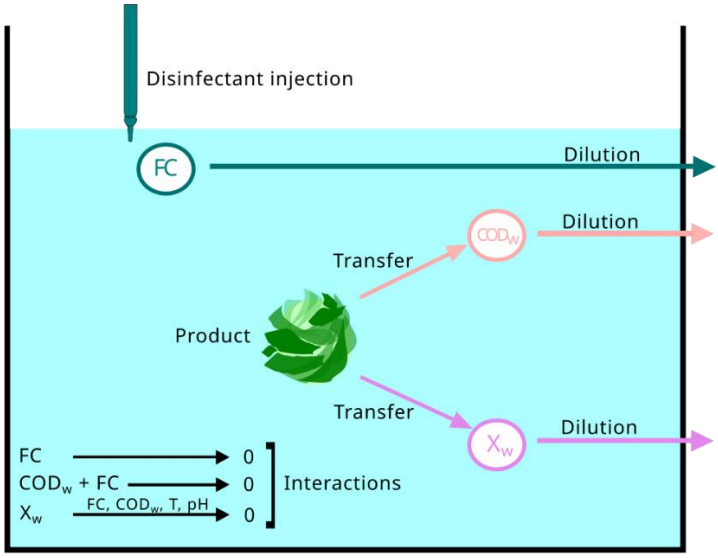


Figure 104. Illustration of mechanisms considered to model the what-if scenarios (model 7). The model is an extension of Model 6 to include free chlorine dynamics. Model state variables are represented within circles (X_w, COD_w, FC), transfer mechanisms with arrows and the interactions in the lower left part of the graph (inactivation due to FC, and FC/COD_w reaction)

As an illustrative example of the model potential, FC injections are designed to maintain the water contamination below 100CFUs/100 mL of TBC for a continuous water addition of 3.71 mg of FC per minute. Note that this continuous addition of water corresponds to the dilution rate constant. obtained for the industrial cases ($D = 0.0033\ (min)^{-1}$) for the size of the tank in case 37 ($V = 1100L$). Continuous addition of FC and discrete additions of water are considered zero ($\mathrm{F_w^{disc}} = 0, \mathrm{FC_{in}^{cont}} = 0$). Parameter values are retrieved from case 37 visit 2, considering no interaction between COD and FC (**Table 31**). Note that therefore the found FC injection is the minimum quantity to add in the tank to maintain contamination below the set limit, since any interaction between injected FC and COD would result in less concentration of FC in the water.

Table 31. Model parameters of case 37 visit 2 (washing of diced onions) used to simulate different FC injections.

Parameter	Value	Units	Description
$\boldsymbol{\alpha}$	7.66	1/(ppm-FC·min)	Inactivation rate coefficient
$\boldsymbol{K_m}$	5.54×10^3	ppm-COD	Protective COD effect
$\boldsymbol{n}$	1	-	Exponent in Hill equation
$\boldsymbol{K_X}$	7.46×10^6	CFU/(kg-product· min)	Transfer rate coefficient of bacteria from product to water
$\boldsymbol{K_{COD}}$	2.54×10^3	mg-COD/(kg-product· min)	Transfer rate coefficient of bacteria from product water to product

Parameter	Value	Units	Description
$\boldsymbol{\beta}$	0	1/(ppm-COD·min)	Rate constant of reaction FC and COD
$\boldsymbol{\gamma}$	0	mg-COD/mg-FC	Yield coefficient COD of reaction FC and COD
$\boldsymbol{\lambda}$	1.70×10^{-3}	1/min	Natural decay of FC
$\boldsymbol{V}$	1100	L	Tank water volume
$\boldsymbol{\tau}$	1	min	Contact time

Figure 105 shows the simulations for a designed FC injection profile to maintain microbial contamination in water around 2log10(CFU/100ml), starting with 15ppm of FC and injecting at 5 different equidistant times during the washing process. The figure shows the reported plots by the open-source code to allow to the FBOs simulate different scenarios. The code plots the model inputs (pH, Temperature and mass in the tank), the interventions or controls (for water and FC additions) and the model simulations, including the active compound in water (HOCl in red). This active compound shows a similar trend to FC till 3 hours where Temperature and pH drop, also affecting microbial water contamination.

It should be stressed that for more realistic simulations, the parameters determining the interaction between chlorine and COD need to be included. In the simulations total chlorine added is directly transformed in FC, without any COD effect (combined chlorine) by assuming rate constant $\beta = 0$. For realistic simulations, β and γ should be extracted from the literature or calculated in a lab. Alternatively, these values can be obtained from the estimations in this tender (Table 25), but knowing that these parameters were estimated with large uncertainty. In order to properly include the FC and COD interaction, more confidence estimations of both parameters are needed.

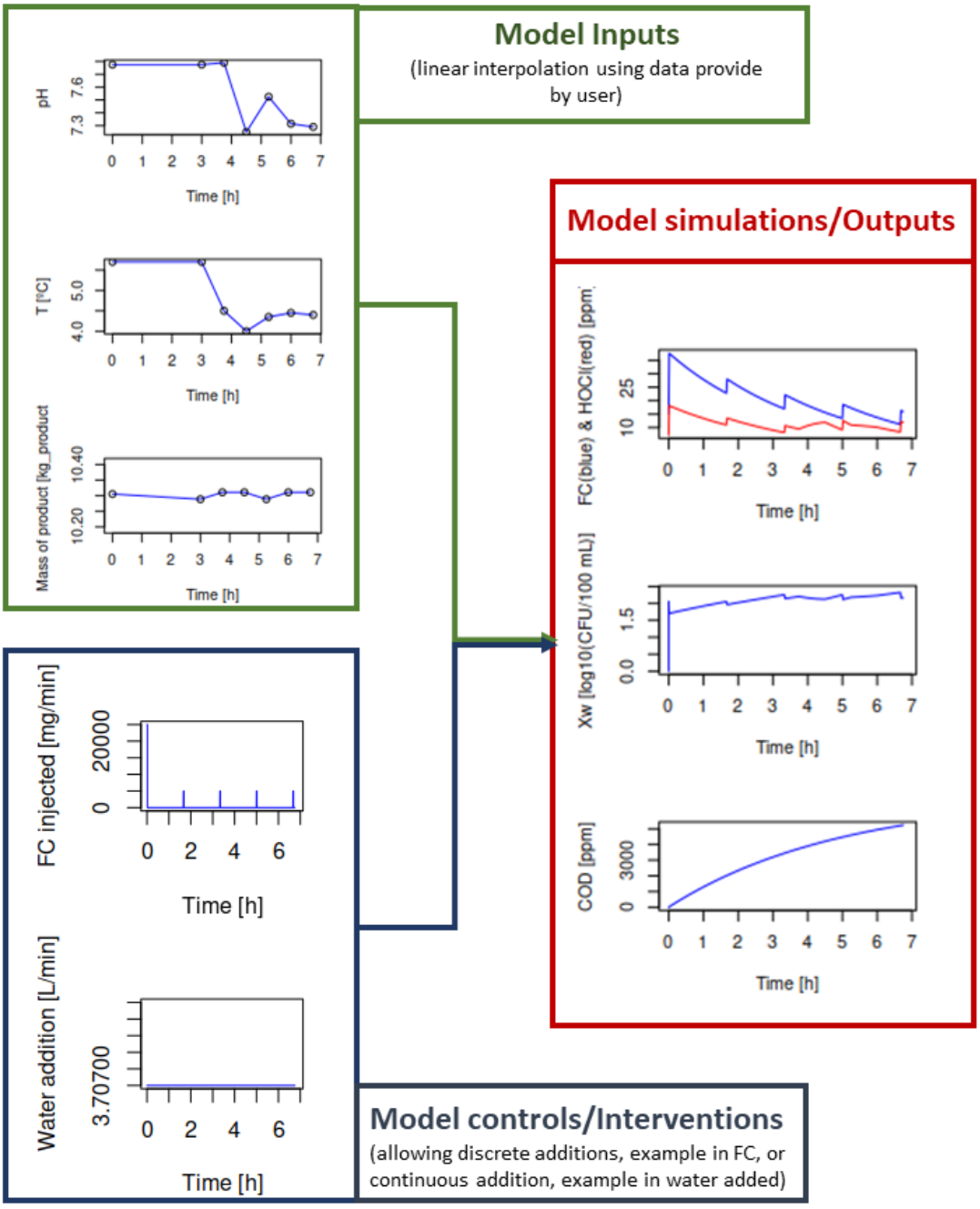


Figure 105. Illustration of plots using the release open-source code for simulating different what-if scenarios of interest for FBOs. The particular simulations represent a possible intervention (using FC discrete additions and some, but insignificant, water replenishment) to control the microbial contamination around 2 log (CFU/100 mL) in scenario 37 visit (parameters in Table 30).

Note that this example aims to illustrate the usefulness of Model 7 to assess what-if scenarios, and many different alternatives can be modelled including:

- Changing the interventions (what-if scenarios)
    - The chlorine concentration and dilution rate can be changed by adding FC and water at different times and with different amounts for each addition
    - the pH, temperature and processed mass of product can be changed dynamically along the duration of the production, including for example abrupt increases in the cumulative processed product or in pH for some periods of time. The code will interpolate these data to fed the model.
- Change some model parameters assuming non-changing values with time in the model like transfer rates. There are several alternatives:
    - Change the parameters for other values (what-if scenarios). For example, the model can be used to change the transfer rates from onions to carrots using data extracted from industrial scenarios
    - Perturb the parameter to understand its impact in the results (sensitivity analysis). For example, to change the dilution rate (that is flux of added water divided by tank volume) to assess its impact
    - Run Monte-Carlos to generate probabilities of water contamination by assigning probabilities to some of the critical model parameters (risk assessment)
- Finally, the model can be combined with appropriate algorithms to
    - Optimally design operational conditions of the process. Instead of exhaustive search by simulating many different alternatives, the model can be used used to find the best set of parameters to achieve a certain objective, such as: (i) minimum use of water, (ii) always guaranteeing safety (for example imposing as constraint that the maximum levels of TBC in process water are 2 logs).
    - Implement an online control of the process, if there is an online monitoring of some of the variables, to correct the optimal profile if there is some deviation of the model from the scenario where the optimal profile was calculated.

Given the potential of the model, attached to this tender is released an open-source code to allow FBOs and experts in food safety to exploit the model capabilities.

4 Discussion

In this section, the three main parts, data collection from FBOs, and analyses including the representation of data in box plots by sector, literature search, and modelling are discussed and interpreted. In the case of FBO data analyses, the physico-chemical parameters, the microbiological counts and the detection of pathogens are discussed by the food sector and type of disinfectant agent. The particular cases of some scenarios are presented.

4.1 Physico-chemical results

Residual concentration of the disinfectant in the process water is a critical step in ensuring the safety of ffFVHs. The presence of a residual disinfectant, such as chlorine, PAA, or H_2O_2, indicates that the water contains an active level of disinfectant sufficient to reduce or eliminate harmful microorganisms. This practice impacts the FBOs in several important ways such as ensuring microbial safety, compliance with regulations (Regulation (EC) No 852/2004). In the scenarios included in the

fresh-whole and fresh-cut sectors that used chlorinated water, there was very high variability in the residual concentration. The mean residual concentration in the fresh-whole and fresh-cut sectors ranged between 102 and 448 mg/L (IDs 09 and 22, in the lowest and the highest in the fresh-whole sector and IDs 37 and 47 the lowest and the highest in the fresh-cut sector). It is remarkably the high variability in the residual concentration of some scenarios e.g., ID 48 which showed that the residual concentration varied between 3.6 mg/L and 275 mg/L. This high variability in the residual level with very low residual in some cases and very high in other scenarios shows the poor water management practices that FBOs applied. In the fresh-whole sector, scenarios ID 01 and 05 with no water treatment also showed some chlorine residuals (lower than 5 mg/L) as companies used chlorine for reconditioning the water.

Regarding PAA, in theory, 80-200 mg/L is the concentration that falls within the effective range for many disinfection applications and presents some positive aspects compared to chlorine such as efficacy over a wide pH range, higher stability in the presence of organic matter, and fewer disinfection by-products than chlorine. However, other negative aspects include the effective dose and the sensors to monitor effectively the dose because of the interferences with the process water compounds and also with the H_2O_2 that is present as most commercial PAA formulations are an equilibrium mixture of PAA, H_2O_2, and acetic acid. Some methodologies such as titration kit overestimated PAA because of the interferences from other oxidizing agents such as H_2O_2. Inaccurate measurements of PAA result in underdosing and unsafe produce or overdosing with huge cost implications (Albolafio et al., 2021). Among the cases of studies selected, some scenarios from the three sectors using PAA showed residual concentrations ranging from very low PAA concentration to very high (2-2000 mg/L). In the fresh-cut sector, the washing system of diced onions (ID 38) was the only scenario that used PAA. Because of the problems with the high chlorine demand, the company changed chlorine for PAA as it has lower demand in situations with high COD. The target PAA concentration was 80 mg/L although the measurements varied from 18-88 mg/L with 18 samples out of the 24 with a concentration < 70 mg/L. In the frozen sector, five scenarios used PAA but the residual concentration varied from very low (5-70 mg/L in IDs 55, 58, and 59) to very high (200-2000 mg/L in IDs 60 and 61).

In the case of H_2O_2 as a disinfectant agent, only the fresh-whole and frozen sectors used it. In the fresh-whole sector, H_2O_2 was used in IDs 12, 13 and 23 in the dumping operation of apples and peaches/nectarines. The residual H_2O_2 concentration varied from 0-130 mg/L with high variability for IDs 13 and 23 (0-130 mg/L). In the frozen sector, scenarios IDs 53, 54, 56 and 57 used H_2O_2 in a range from 40-340 mg, particularly high residual concentrations in the scenario of the process operation of washing diced onions because of the high residual demand (ID 57).

pH: The pH level in chlorinated process water is crucial for several reasons, especially considering its impact on the effectiveness of chlorine as a disinfectant and its overall chemical stability. The importance of maintaining an optimal pH in chlorinated process water is crucial to ensure the efficacy of chlorine treatment of process water, as the antimicrobial effectiveness of chlorine in water depends significantly on the pH level. Chlorine exists in different forms in water, primarily as hypochlorous acid (HOCl) and hypochlorite ion (OCl-). Hypochlorous acid is a much more effective disinfectant than hypochlorite ion. The proportion of these two forms varies with pH: at lower pH levels, more hypochlorous acid is present, and as the pH increases, more hypochlorite ion is present. The optimal pH range for chlorine disinfection is typically between 6.0 and 7.0, where a higher percentage of chlorine is present as hypochlorous acid,

making the disinfection process more efficient. In the scenarios that used chlorine, the pH varied from 6.6 to 9.1 in the fresh-whole sector, and from 4.6-8.8 in the fresh-cut sector.

Results observed in the fresh-whole sector showed that when PAA was used, pH was low (3.15-5.26). For other scenarios with no water treatment or with H_2O_2, the pH varied from 6.42-8.28. The use of PAA as a disinfection treatment lowers the pH level of process water which influences the efficacy and stability of PAA. Unlike chlorine-based disinfectants, whose efficacy sharply decreases as pH increases, PAA remains a potent disinfectant over a wider pH range, though its optimal activity is within slightly acidic to neutral conditions. In some scenarios, the residual concentrations of PAA were very low (scenario IDs 26 and 27 for avocados and mangos, respectively) although the target concentration was very high (200 mg/L). The same was observed in the cooling operation of frozen spinach (5 mg/L of PAA and pH= 6.95-7.96).

ORP: Oxidation-Reduction Potential (ORP) is a critical parameter in process water whether chlorine is used as a disinfectant or not. It indicates the water ability to oxidize or reduce substances, which directly relates to the water disinfecting power. In chlorinated water, ORP is an excellent parameter for indicating the presence or absence of oxidizing species in the water. A higher ORP value of 650 mV means active disinfectant forms (e.g., oxidizing species such as hydroxyl radicals), suggesting that the water has a greater ability to oxidize and thus inactivate pathogens (Albolafio et al., 2021).

In scenarios where chlorine was not used, ORP was < 650 mV which is an indication of the low water oxidative capacity. For disinfectants like PAA and H_2O_2, low ORP indicated the low presence of oxidative conditions even though these systems might not add oxidants directly measurable by ORP in the same way chlorine does. In some scenarios (IDs 01, 49, 50, 51, 52 and 58), ORP was negative, indicating the presence of substances in the water that are acting as reducing agents. These could be organic matter from the spinach itself, such as soil or plant debris, or other contaminants that have entered the process water. Except for apples and onions, most of the negative ORP measurements corresponded to spinach process water with no water treatment or treated with PAA. A negative ORP reading in process water for washing spinach suggests a need for inmediate attention to ensure the water is effectively inactivating the microbial load entering in the tank. Adjusting the water treatment process to achieve a positive ORP value is crucial for food safety aspects.

Electrical conductivity (EC) in process water is a significant parameter that measures the ability of the water to conduct electric current which is primarily related to the presence of dissolved ions in the water, such as salts, minerals, and other inorganic substances. High conductivity values may suggest that the effectiveness of cleaning and sanitation processes is difficult to maintain and manage the process water quality from food processing plants. Thus, electrical conductivity is a vital parameter for the FBO, as it provides essential information about the chemical load and quality of process water. As expected, the highest EC (6310 µS/cm in ID 58) was shown in the frozen FVHs, because of the recirculation of process water at high temperature conditions. The scenarios of the fresh-cut sector showed also high EC because of the leaf exudates. In each sector, the variability in the EC depended on the product type and product-to-water ratio, changing from low EC (120 mS/cm in avocado) to extremely high (6310 mS/cm in frozen spinach).

Water temperature. The temperature of the process water is a critical factor related to the microbiological quality of ffFVHs. Effective temperature management can enhance the safety and quality of ffFVHs, but it requires careful consideration of the type of product, the specific microorganisms of

concern, and the balance between microbial reduction and quality preservation. Temperature plays a crucial role in controlling the survival and removal of microorganisms, including bacteria, viruses, and protozoa, which can contaminate ffFVHs. Understanding the effects of different temperatures during the washing process is essential for ensuring food safety and maintaining the quality of fresh FVHs. In the case of chlorinated water, the percentage of chlorine in the form of HOCl increases with decreasing temperature as the extent to which hypochlorous acid dissociates depends primarily on the pH and temperature of the water (Randtke, 2010).

Hot water washing or blanching (above 55°C) can significantly reduce the microbial load on FVHs as high temperatures can inactivate many pathogens and spoilage organisms. Among the scenarios studied, the lowest temperature was observed in the fresh-cut sector and the highest in the frozen sector. Temperature control should be part of a comprehensive food safety management system in the handling and processing operations that involve the use of water to minimize microbial contamination and ensure the safety of ffFVHs.

COD refers to the amount of oxygen required to chemically oxidize organic matter in the water. It is a measure of the organic compounds in the water. High COD levels indicate a higher disinfectant demand, which can lead to water quality issues and potentially affect the safety of washed ffFVHs. Monitoring and controlling COD levels are essential to maintain water quality and prevent microbial hazards in the washing process.

COD and residual concentration of the disinfectant levels are both important parameters to consider in the context of process water in the handling and processing of ffFVHs. This relation lies in the effectiveness of the disinfection process. High COD levels in process water can significantly impact the efficacy of disinfectants, particularly in the fresh-cut sector as it faces challenges with the accumulation of organic matter in washing water, which can promote microbial proliferation and cross-contamination if not managed properly. This necessitates the frequent renewal of water or the use of effective disinfection strategies to control microbial load and ensure the safety and quality of the final product (Gil et al., 2009; Manzocco et al., 2015). When COD levels are high, more disinfectant is consumed to oxidize these compounds, reducing the amount available to target and inactivate microorganisms. Therefore, controlling COD levels can indirectly influence the amount of residual concentration of the disinfectant required to maintain effective disinfection (Bornhorst et al., 2018). A study on the reuse of wash water in a fruit processing plant highlighted the necessity of evaluating the physico-chemical and microbiological properties of the water before and after treatment. Effective purification systems, including ultrafiltration and ozonation, can significantly reduce COD levels, thereby improving the efficiency of disinfectants used in subsequent washing steps (Breza-Boruta et al., 2024). Conversely, maintaining an appropriate residual concentration of the disinfectant levels can help control microbial contamination, but managing COD levels by reducing the introduction of organic matter is crucial as organic matter can react with disinfectants, reducing their effectiveness and potentially leading to the formation of harmful by-products (Gil et al., 2019). For instance, maintaining a certain level of free chlorine or other disinfectants in the wash water can significantly reduce microbial load and prevent the buildup of organic matter, thereby controlling COD levels (Banach et al., 2015). Thus, in process water management, there is an interplay between COD levels and residual concentration of the disinfectant to ensure the safety of the washing process. Among the three food sectors studied, COD varied between 0-3390 mg/L in fresh-whole FVHs, between 2-6280 mg/l in fresh-cut FVHs, and between 0-58250 mg/L

in frozen FVHs. The scenario that reached the highest COD level (58250 mg/L) was the process water for the transport of frozen onion with no water treatment (ID 49). Among the scenarios that used disinfectant, PAA and its interactions in process water could indirectly contribute to changes in COD levels over time. Proper management of PAA concentration and monitoring of COD are both important for maintaining water quality and ensuring the safety of ffFVHs during the handling and processing processes.

In the fresh-cut and frozen sectors, the scenarios of diced onions (IDs 38 and 55) that used PAA showed very high COD (1705-28800 mg/L). After cutting onions, the exudate from the cut onions rich in organic compounds such as sugars, amino acids, and sulfur-containing compounds are released into the water increasing the overall organic load and contributing to the increase in COD. Residual PAA in the process water, if present, may react with organic compounds released from onion exudate but to a lower extent than chlorine does. The effect of PAA on the disinfection process is similar to what happens when chlorine is used. Both PAA and chlorine are oxidizing agents that can oxidize organic compounds found on the surface of fruits and vegetables or in exudates from the produce, like onions in our case. The presence of organic matter can consume the disinfectant, reducing the ability to inactivate microorganisms effectively. PAA is often more stable in the presence of organic matter compared to chlorine and can be effective over a broader range of pH levels. While both chlorine and PAA might increase COD levels due to the oxidation of the organic matter, the type of organic matter and the extent of organic matter oxidation can affect their efficacy as disinfectants (Luongo et al., 2020). Proper management of PAA concentration and monitoring of COD are essential to ensure effective disinfection and water quality maintenance during the washing process of onions and other products.

Other physico-chemical parameters such as TDS, TSS and turbidity. In the process water used for washing FVHs, total dissolved solids (TDS), total suspended solids (TSS), and turbidity are all parameters that provide insights into the water quality and its suitability for the continuous use for the handing and processing operations. TDS refers to the total concentration of dissolved substances in water, including minerals, salts, and organic compounds. In the context of washing FVHs, TDS can come from various sources such as exudates, soil residues, pesticides, fertilizers, and organic matter. High TDS levels can affect the safety of the FVHs because of the difficulties in the disinfection process. Monitoring TDS levels is important to ensure that water quality meets acceptable standards for washing purposes. TSS refers to the total concentration of particles suspended in water from soil particles, plant residues, and other organic matter and debris. High TSS levels can lead to turbidity in the water and may affect the effectiveness of washing and disinfection processes. Controlling TSS levels is essential to maintain water clarity and prevent potential contamination of the FVHs. Turbidity is a measure of the cloudiness of water caused by suspended particles, such as TSS, that absorb light. In the context of washing FVHs, turbidity can indicate the presence of suspended solids and other contaminants in the water. High turbidity levels may impair visibility and hinder the inspection of FVHs during washing. Additionally, turbidity can interfere with the disinfection processes by shielding microorganisms from contact with disinfectants including UV-light (Domínguez Henao et al., 2018; Falcó et al., 2023). High turbidity, caused by organic matter like plant particles and juices, as well as inorganic materials such as soil, can significantly impair the efficacy of disinfectants. This is because the organic and inorganic matter can consume the disinfectants, reducing their availability for microbial inactivation. Therefore, monitoring and controlling turbidity levels are important for ensuring the effectiveness of washing and disinfection processes and maintaining water quality (Suslow, 2020). The relationship between TDS,

TSS, and turbidity in wash water for FVHs is that TDS contributes to the overall dissolved content in the water, while TSS and turbidity are indicators of the presence of suspended solids. High TDS levels can increase the potential for TSS and turbidity, as dissolved substances may contribute to the formation of suspended particles. Therefore, managing TDS levels is crucial to controlling TSS and turbidity and maintaining water quality for washing produce. Similarly, controlling TSS levels can help reduce turbidity and improve water clarity, which is essential for effective washing and disinfection processes.

The relationship between UV254 absorbance and COD lies in their correlation with the amount of organic matter present in the water. Higher UV254 absorbance values typically correspond to higher levels of COD, indicating a greater concentration of organic pollutants. Qi et al. (2020) indicated the effect of organic matter and concluded that UV254 is the primary indicator of the organic load effect. The results obtained in the case scenarios studied showed a high correlation (R>=0.6) between the COD and UV254 parameters. This relationship is often used in water quality analysis and monitoring to estimate organic pollution levels based on UV absorbance measurements, which are relatively quick and cost-effective compared to traditional COD testing methods that take 4 hours or more.

However, it's essential to note that the relationship between UV254 absorbance and COD can vary depending on factors such as the composition of organic matter, the presence of other contaminants, and the specific characteristics of the water sample. Chen et al. (2016) observed that both parameters, COD and ultraviolet absorbance at 254 nm (UV254) can be organic load indicators of fresh produce wash water. UV254 had the highest correlation coefficient (R = 0.77), followed by total phenolics (R = 0.65) and total protein content (R = 0.64) between wash water quality parameters (Chen and Hung, 2016). There is, however, considerable variability in the COD: UV254 ratio dependent on the type of produce that is washed, even among different leafy vegetables. For example, the process water derived from spinach had a much higher COD: UV254 ratio than that derived from iceberg lettuce (Van Haute et al., 2015). In the case scenarios studied, depending on the food sector this relationship changed according to the characteristics of the organic matter in the wash water. For example, in the fresh-cut sector, a correlation of 0.84 was obtained, while for fresh-whole and frozen it was 0.61 and 0.58, respectively. Therefore, while UV254 absorbance can provide a useful estimation of organic pollution levels, it may not always directly correlate with COD values, and additional testing may be necessary for accurate assessment and monitoring of water quality.

4.2 Microbiological results

In this section, the values for microbial counts are presented as mean values per scenario.

Moulds counts varied by FVHs sector with average counts between 3.0-6.0 Log CFU/100 mL for fresh-whole FVHs, 3.0-4.4 for fresh-cut FVHs, and 3.3-4.9 for frozen FVHs. Among disinfectant agents, those scenarios with no water treatment showed higher mould counts (3.16-5.97 Log CFU/100 mL), with chlorine decreasing the counts (below LoD-4.0 Log CFU/100 mL), particularly in those scenarios of fresh-cut FVHs in which the enumeration of mould counts was below LoD except for diced onions (ID 37). In the case of PAA, mold counts varied from below LoD to 4.24 Log CFU/100 mL in mango (ID 27). In frozen FVHs, only scenarios IDs 55 and 61 showed counts of 4.0 logs approx. Remarkably, the high mould counts observed in the scenarios that used H_2O_2 (IDs 12, 13, 23, 53, 54, 56, and 57) included the fresh-whole and frozen sectors. The presence of moulds on FVHs can lead to spoilage and reduce shelf life (Plaza et al. 2022), and could be a potential health risk for consumers due to mycotoxin

production (Morales et al. 2010). Soil residues, plant and fruit debris and FVH's containers, could be a potential source of mould contamination.

Yeast, the occurrence in the FVHs sector differed depending on the disinfection agent. For fresh-whole FVHs, high yeast counts were observed (3.43-7.87 Log CFU/100 mL) with no water treatment except for the dumping of apples (ID 08). When chlorine was used, yeast counts varied from below LoD- 4.0 Log CFU/100 mL with a low occurrence (< 8 except for ID 25 which was 15/24 samples). The three scenarios that used PAA (IDs 26, 27, and 28 of avocado, mango, and peppers) showed very different yeast counts (below LoD for peppers while for avocado and mangos was 7.5 and 4.3 Log CFU/100 mL, respectively). These differences in the yeast counts could be due to the differences in the residual concentration of PAA with peppers having residual PAA > 270 mg/L while the residual PAA concentration for avocado and mango was < 22 mg/L. In the case of H_2O_2 used in the fresh-whole FVHs sector, yeast counts were 4.4 Log CFU/100mL approximately in the process water of dumping apples and peaches/nectarines (IDs 12, 13, and 23). In the fresh-cut FVHs sector, those scenarios with no water treatment showed high yeast counts (4.7-6.2 Log CFU/100 mL) while in most of the scenarios that used chlorine levels decreased to below LoD except in diced onions, fresh-cut lettuce, and baby leaves (IDs 37, 40 and 46 respectively) with counts between 3.1-3.8 Log CFU/100 mL. The case studies selected in the frozen sector of FVHs showed very high yeast counts (4.4- 6.81 Log CFU/100 mL) particularly in scenarios with no water treatment and in those using H_2O_2. When PAA was used, yeast counts decreased (below LoD-3.9 Log CFU/100 mL) and also their occurrence (max 4/24).

Total bacterial counts: When total bacterial counts were examined, fresh-whole FVHs showed high counts, particularly in those scenarios with no water treatment where levels varied from 4.19 to 8.79 Log CFU/100 mL. When chlorine was used, the mean of total bacterial counts and the occurrence reduced (below LoD-4.48 Log CFU/100 mL) but PAA and H_2O_2 were not as effective as higher counts remained (3.59-6.05 Log CFU/100 mL for PAA and 4.98- 6.48 Log CFU/100 mL for H_2O_2). In the case of fresh-cut FVHs, a similar behaviour was observed as those scenarios with no water treatment accumulated high total bacterial loads (4.7-6.46 Log CFU/100 mL) and decreased with chlorine (below LoD- 4.0 Log CFU/100 mL). When the scenarios of frozen FVHs were examined, those scenarios with no water treatment and H_2O_2 showed high total bacterial counts such as the process water from washing spinach with total bacterial counts of 9.21 Log CFU/100 mL and occurrence of 24/24 (ID 51). In all scenarios that used PAA, total bacterial counts decreased from below LoD to 3.9 Log CFU/100 mL, except in scenario ID 55 for cooling diced onions which still showed a high count (6.88 Log CFU/100 mL). Monitoring total bacterial counts in process water from washing FVHs is an important aspect when considering the process intervention efficiency and quality assurance practices.

Coliforms are a group of bacteria that are commonly used as indicators of water quality and sanitation practices. Further, some of the coliform bacterial species, such as *E. coli* indicate potential fecal contamination. Monitoring coliform levels helps to verify the effectiveness of interventions such as disinfection practices used in the handling and processing operations of ffFVHs. Proper disinfection agents, such as chlorine, aim to reduce microbial contamination, including coliforms, in process water and minimize the risk of cross-contamination between batches of FVHs. Instead of testing for pathogens, which are rarely found on fresh FVHs, a FBO might analyse process water samples for viable gram-negative organisms, such as coliforms or Enterobacteriaceae. Although these organisms commonly occur on fresh FVHs, process water samples from validation trials should not show detectable levels of

these organisms (Gombas et al., 2017). The fresh-whole sector with no water treatment showed high variability in coliform counts from low counts of 2.94 Log CFU/100 mL (ID 03) in peaches/nectarines dumping to as high as 8.24 in vegetable mix pre-washing (ID 07). When chlorine was used, coliform counts were lower with counts below LoD-2.49 Log CFU/100 mL), decreasing also their occurrences. With PAA, as observed for total bacterial counts, different results were observed with below LoD for pepper washing (ID 28) or very high (5.06-6.12 Log CFU/100 mL for avocado (ID 26). When H_2O_2 was used, average coliform counts of 4 Log CFU/100 mL were detected in IDs 12, 13, and 23.

Escherichia coli (E. coli). Monitoring *E. coli* levels in process water is essential as the presence in process water indicates potential fecal contamination and harmful pathogens such as *Salmonella*, and other disease-causing bacteria. If contaminated water comes into contact with FVHs during handling or processing operations, transfer of pathogens to the produce can occur. High levels of *E. coli* in process water suggest poor water quality and inadequate disinfection practices, indicating a higher risk of contamination and foodborne illness. Monitoring *E. coli* levels helps verify the effectiveness of disinfection practices such as chlorination, that aim to reduce microbial contamination, including *E. coli*, in process water and minimize the risk of cross-contamination between batches of produce.

For fresh-whole FVHs, *E. coli* counts varied between 0.06-2.74 Log CFU/100 mL in those scenarios with no water treatment with a high occurrence of 12-24/24. Even with these high levels of *E. coli* counts, the probability of finding pathogens based on the data generated was very low and only norovirus was detected with *E. coli* counts of 1.34 Log CFU/100 mL in the pre-washing process of peppers (ID 04). When chlorine was used, *E. coli* counts decreased with mean counts < LoD to 1.39 Log CFU/100 mL and the occurrence decreased as well (max. 4/24) (17.7 vs 1.5% in non-treated process water versus process water treated with disinfectant. No *Salmonella* and pathogenic *E. coli* were detected independently of the *E. coli* counts when chlorine was added. Additionally, PAA was also very effective, as no *E. coli* counts were detected in the three fresh-whole FVHs scenarios and no *Salmonella* or pathogenic *E. coli* were detected. Unlike H_2O_2 showed a mean of 1.81 Log CFU/100 mL in scenarios IDs 12, 13, and 23 but in one of those (ID 13) *Salmonella* was detected.

In fresh-cut FVHs, low counts of *E. coli* < 0.94 Log CFU/100 mL were observed. It is notable that among the scenarios in which no water disinfectant agent was used (IDs 30, 31, 32, 33 and 34) even with low *E. coli* counts, *Salmonella* was detected and pathogenic *E. coli* as well in only two of them (IDs 31 and 32). When chlorine was added *E. coli* counts were below LoD except for ID 48 which showed an *E. coli* count of 0.4 Log CFU/100 mL but an occurrence of 1/24. The only case scenario that used PAA was ID 38 and the *E. coli* count was below LoD. In the frozen FVHs sector, high counts of *E. coli* (mean of 4.11 Log CFU/100 mL) were observed and a high occurence of *Salmonella* and pathogenic *E. coli* when no water disinfectant agent was used. When PAA was used, *E. coli* counts decreased to below LoD (IDs 60 and 61) to 1.96 Log CFU/100 mL (ID 55). A remarkable scenario of process water from cooling spinach (ID 58) showed high *E. coli* counts of 3.65 Log CFU/100 mL in 4 of the 24 samples analysed. It is also relevant to mention the high *E. coli* counts shown in scenarios of pre-washing and washing before cutting and freezing peppers (IDs 53 and 54).

***Listeria* spp**. The presence and levels of *Listeria* spp. in water used for washing FVHs may be used as an indicator for the occurrence of pathogenic *L. monocytogenes*. High numbers of *Listeria* spp. counts in process water from washing FVHs can indicate the presence of pathogenic bacteria that can be transferred from process water to FVHs and from one batch of FVH to another during washing. This can

lead to the widespread distribution of *Listeria* spp. on multiple batches of FVHs, and persist over time, posing a continuous risk of contamination to FVHs. Proper sanitation practices, water management, and monitoring of *Listeria* spp. levels are essential to prevent contamination and ensure food safety. In the scenarios studied of fresh-whole FVHs, *Listeria* spp. was detected in all the scenarios with no water treatment (IDs 01, 02, 03, 04, 05, 06, 07 and 08). From the data obtained in our case scenarios studied, no correlation has been found between *Listeria* spp. and the occurrence of *L. monocytogenes*. The average counts of *Listeria* spp. varied from 0.42 Log CFU/100 mL in water used in the washing of celery (ID 08) to 5.55 Log CFU/100 mL in the pre-washing water of the washing vegetable mix (ID 07). In the fresh-cut sector, the average count of *Listeria* spp. was 2.4 Log CFU/100 mL with a high occurrence (24/24) except for ID 34 where the occurrence of *Listeria* spp. was 22/24. Similarly, in frozen FVHs *Listeria* spp. average counts of 3.4 Log CFU/100mL were observed for IDs 49, 50, 51 and 52. When chlorine was used in fresh-whole FVHs scenarios, *Listeria* spp. counts decreased to below LoD in IDs 17, 25 and 29 to high counts as 1.6 Log CFU/100 mL in the dumping water of washing peaches/nectarines (ID 21). In the case of fresh-cut FVHs, *Listeria* spp. was always below LoD except in diced onions (ID 37) and salad mix (ID 48) even though the residual chlorine concentration was high (average of 119 mg/L and pH of 6.9). PAA was only used in the fresh-whole and frozen FVHs scenarios with *Listeria* spp. counts from below LoD in the fresh-whole FVHs sector to high counts (1.57-3.01 Log CFU/100 mL) and 5.7 Log CFU/100 mL in the frozen FVHs sector with high occurrence of *L. monocytogenes* (18.3% in the fresh-whole sector and 0.8% in the frozen sector). Relevant findings in the scenarios that used H_2O_2 were the high *Listeria* spp. counts and high occurrence (average mean of 4 Log CFU/100 mL and occurrence of 24/24 in scenarios IDs 12, 13 and 23 of the fresh-whole sector and in scenarios IDs 53, 54, 56, 57 of the frozen sector) whereas *L. monocytogenes* was only detected in scenario ID 12.

Norovirus: Currently, there is limited information available on the viral contamination of process water. A recent study, conducted with a limited number of samples, revealed the accumulation of *CrAssphage* and coliphages in process water, but human noroviruses were not detected (Cuevas-Ferrando et al., 2021). In the present survey, norovirus GI with counts > 4 Log GC/L were detected by RT-qPCR in the three sectors with no water treatments (IDs 04, 30, 31, 32, 33, 51 and 52). However, some scenarios using chlorine in the fresh-whole and fresh-cut FVHs sectors presented norovirus counts > 4 Log GC/L (IDs 15, 16, 22, 37, 41, 43, 44, and 47) with similar counts for sodium and calcium hypochlorite. For PAA-treated process water, the average mean for norovirus was 4.8 Log GC/L for scenarios of the fresh-whole and frozen FVHs sectors. In the case of H_2O_2-treated process water, the scenario of pre-washed peppers had counts of 4.93 Log GC/L (ID 53). The occurrence of human norovirus GI was higher than norovirus GII in process water. This may be explained by the higher resistance attributed to norovirus GI, which is more prevalent in treated and environmental waters (Cuevas-Ferrando et al., 2022). Norovirus GI strains are more often associated with waterborne transmission than norovirus GII, a trait that may relate to the proposal that norovirus GI have a higher stability in water than norovirus GII (de Graaf et al., 2016).When norovirus counts were compared with *E. coli* counts, it was noticed that *E. coli* was not detected in many scenarios from the three sectors such as IDs 22, 26, and 28 for the fresh-whole FVHs, IDs 37, 41, 43, 44, and 47 for the fresh-cut sector FVHs, and ID 61 for the frozen sector FVHs. Additionally, when positive samples for norovirus were compared with those positive for *CrAssphage* or coliphages, as faecal indicators, no clear correlation was observed.

Viability RT-qPCR was performed as a molecular detection method that combines viability marker pre-treatment (e.g., PMAxx) with RT-qPCR, which has been proposed to infer the capsid integrity of viruses. This is particularly relevant for non-culturable or tedious-to-culture viruses, such as human noroviruses. Out of the 30 samples initially identified as positive for norovirus GI by RT-qPCR alone, 13 remained positive after PMAxx pre-treatment. Despite PMAxx proving to be an improved method compared to direct RT-qPCR for estimating capsid integrity, the observed results following PMAxx pre-treatment do not provide full assurance that the samples indeed contained infectious viruses. Recently, viability RT-qPCR for norovirus was demonstrated as an improved method over direct RT-qPCR for estimating viral inactivation under certain conditions. However, the use of human enteroids proved to be a more robust model than viability RT-qPCR for assessing norovirus infectivity. Nevertheless, implementing human intestinal enteroids remains unfeasible for most water and food samples (Wales et al., 2024; Carmona-Vicente et al., 2024) including process water due to the extremely low levels observed.

Cryptosporidium (*C. hominis, C. parvum, C. meleagridis, C. tyzzeri, C. wrai, C. erinace, C. cuniculus, C. ferret* and *C. viatorum*) was not detected in any case scenario. The occurrence of *Cryptosporidium* spp. in water samples has been investigated; however, limited data is available on process water (Bourli et al., 2023).

Detection of pathogenic bacteria

Salmonella was present in the three sectors of FVHs. In the fresh-whole sector, in the process water disinfected with H_2O_2 used in apple dumping (ID 13) which also had high *E. coli* counts. In the fresh-cut sector, the presence of *Salmonella* was detected only in those scenarios with no water treatment (IDs 30, 31, 32, 33, and 34), most of them with high occurrence (13/24). Remarkably, the high occurrence in some of these fresh-cut scenarios such as parsley washing (ID 33 with a occurrence of 13/24) was also accompanied by high *E. coli* counts. *Salmonella* was also detected in scenarios of frozen FVHs with no water treatment (IDs 49, 50, 51, and 52) and those with PAA-treated water (IDs 55, 58, and 59).

L. monocytogenes was detected in 11 scenarios from the fresh-whole and frozen sectors of the total 61 scenarios studied (including the fresh-cut sector). Surprisingly, *L. monocytogenes* was not detected in any of the scenarios included in the fresh-cut sector. When the fresh-whole sector was examined, it was noticed that *L. monocytogenes* was detected in IDs 02, and 03 which corresponded to no water treatment. When disinfectants were used, *L. monocytogenes* was also detected in scenario ID 17 with chlorine in dumping of peaches and nectarines, in scenario ID 26 with PAA in avocado pre-washing, and IDs 12 and 23 with H_2O_2 (dumping of apples). It is remarkable to notice that *L. monocytogenes* was only detected in one scenario (ID 23) with high *Listeria* spp. counts (4.01 Log CFU/100 mL). In particular, *L. monocytogenes* occurrence was very high (11/24) in PAA-treated water for pre-washing of avocados (ID 26). Regarding ID 17, *L. monocytogenes* was present in samples with chorine residual <0.05 mg/L.

STEC was detected in two scenarios that belonged to the fresh-cut sector (ID 31, washing of curly endive and radicchio and ID 32, washing of baby leaves) and in one of the frozen sectors (ID 52, spinach leaves washing). In all these cases no water treatment was used.

VBNC. The induction of viable but non-culturable stages of bacteria was studied in 9 scenarios selected to compare in each sector the effectiveness of disinfectant agents (chlorine, PAA and H_2O_2) for the

inactivation of microbial cells in the process water used for the handling and processing operations of ffFVHs.

In the fresh-whole FVHs sector, scenario ID 05 for the hydro-cooling of carrots with no water treatment, scenario ID 26 for PAA-washed avocado, and ID 28 for PAA-washed peppers were examined. In all cases, the total VBNC bacteria remained as high as the total viable bacteria and showed the poor effectiveness of PAA in maintaining the microbiological quality of the water used in these handling operations.

In the fresh-cut sector, the four scenarios examined corresponded to process water treated with chlorine in: washing of onions (ID 37), pre-washing and washing of shredded lettuce (IDs 43 and 44) and washing of baby leaves (ID 47). Culturable bacterial counts were lower than VBNC bacteria due to the unsatisfactory inactivation. In these scenarios that used chlorine, the antimicrobial activity of chlorine maintaining the microbiological quality of the water was overestimated using the conventional plate count. In these scenarios, chlorine needed to be in higher concentrations (IDs 37 and 43 with mean residual disinfectant concentration values of 10 and 9 mg/L, and pH of 7.5 and 8.0, respectively) or although residual chlorine was higher, pH was too high for the maximum effectiveness of chlorine that is between 6.0-7.0) (IDs 44 and 47 with residual chlorine of 51 and 61 mg/L and pH of 8.3 and 7.8, respectively) (Marín et al., 2020). All these reasons could support the inadequate effectiveness of chlorine in reducing the likelihood of the induction of VBNC bacteria cells, representing a hazard that cannot prevent cross-contamination (Truchado et al., 2021b).

In the frozen sector, scenarios IDs 56 and 57 that used H_2O_2 for the pre-washing and washing processes of onions were selected to evaluate the effectiveness of this disinfectant agent inducing VBNC bacteria. The induction of VBNC was examined in total bacterial, coliforms, and *E. coli* counts as the three groups showed culturable counts which were compared with the viable bacteria and VBNC cells. Results showed the presence of VBNC cells with H_2O_2 treatment and the similarity with the culturable counts. These results proved the inefficacy of H_2O_2 maintaining the microbiological quality of the water use in the processing operations of frozen onions. These findings underscore the necessity for higher disinfectant concentrations or longer contact times to prevent the induction of VBNC bacterial cells.

Spores of *Clostridium perfringens* were examined in three case scenarios because of the higher risk of contamination such as in hydro-cooling of carrots (ID 05, non-treated process water) and washing of diced onions (ID 37, chlorine-treated process water) as underground vegetables or due to large surface-to-volume ratio such as washing of baby leaves (ID 47, chlorine-treated process water). In the two visits per scenario, the presence of spores was < LoD, and only 1 CFU/100 mL was detected in ID 05 visit 2 and ID 37 visit 1.

4.3 Discussion per sector

4.3.1 Fresh-whole FVHs sector

A total of 29 scenarios, spread over 18 FBOs, of fresh-whole fruits and vegetables process water were analyzed, of which 8 (27.6 %) did not use any disinfectant and 21 (72.4 %) used disinfectant. Chorine was the most frequently used disinfectant: sodium hypochlorite was used in 7 cases and calcium hypochlorite in 8. There were 3 scenarios using PAA and 3 scenarios that used H_2O_2. Regarding handling and/or processing operations, 15 scenarios corresponded to dumping, 4 to pre-sorting, 4 to pre-washing, 4 to washing and 2 to hydro-cooling. Production times ranged from 4 hours to 6 weeks and no automatic monitoring or dosage of disinfectant was present in any of the FBOs sampled.

In those FBOs that did not use any disinfectant, pH was in the range of 6.6-9.1 and ORP was <500 mV which is an indication of the low water oxidative capacity. ORP values varied greatly in ID 03, probably due to the addition of a fungicide in the processing water of the dumping tank. In this particular scenario, turbidity, TSS and filtered and non-filtered UV254 were high. In general, maximum moulds counts were observed when there was no water treatment, showing counts of 5.97 log CFU/100 mL in ID 07 (vegetable mix). However, in ID 06 and ID 08 (carrots and celery, respectively) mould counts were below LoD in all samples. In carrots, this could be due to the high total bacterial counts (8.06 log CFU/100 mL) that effectively competed with this group of microorganisms. Process water in pre-washing and washing operations of peppers, carrots and vegetable mix presented the highest coliform counts (>8 log CFU/100 mL). Moreover, *E. coli* was detected in all scenarios belonging to objective 1 and except in ID 05 and 06, more than 50% of the samples harboured these bacteria. No *Salmonella* spp. were found in fresh-whole FVHs sector even when no process water disinfection treatment was used. In those scenarios without disinfectant, *Listeria* spp. was present in more than 50% of the analysed samples. The highest *Listeria* spp. counts were observed in the pre-washing of bell peppers and vegetable mix, with counts ca. 5 log CFU/100 mL but none of these samples was positive for *L. monocytogenes*. *L. monocytogenes* was found in 2 scenarios belonging to the same FBO (ID 02 and 03).

In the scenarios where disinfectant was used, there was a very high variability in their residual disinfectant concentration in the process water, both among scenarios and within the same scenario; in some sampling points the residual disinfectant concentration was <0.02 mg/L, indicating the improper monitoring and poor management practices. Regarding residual chlorine, the concentration ranged between 0.02-5 mg/L while in other scenarios (e.g., ID 15, 17, 20, 22 and 29) the concentration reached levels >50 mg/L. Among the cases of studies selected, three scenarios used PAA. In fresh-whole avocado and mango (IDs 26 and 27, respectively) the target level was 200 mg/L but the values observed were between 2 and 20 mg/L. The FBO measured PAA following the recommendation of the PAA supplier using a method that could overestimate PAA because of the interferences from other oxidizing agents such as H_2O_2 (Albolafio et al., 2021). In fresh-whole peppers (ID 28), the residual concentration of PAA in the washing water was very high (270-380 mg/L), as the company uses an online amperometry probe to adjust PAA in the recirculation tank as needed. The online amperometry probe is a precise sensor essential for measuring PAA levels in fresh produce wash water, crucial for the accurate estimation of PAA concentration as part of a monitoring and control system aimed at ensuring an effective disinfection process. Monitoring and managing the residual concentration of the disinfectant within operational limits is crucial to mitigate microbial contamination associated with the washed water. Failure to adequately replenish the concentration could be problematic for the maintenance of the quality of the process water used in the dumping operation of apples and peaches/nectarines and its residual concentration varied from 0-130 mg/L. Low residual disinfectant concentrations were attributed to poor management of the dosage as no monitoring was done by the FBO.

The pH was not controlled in any of the fresh-whole FVHs scenarios studied and when chlorine was added, and high variability was observed (6.6 to 9.1), probably due to the differences in the residual concentration present in the process water. The lowest pH values were observed when PAA was used (3.2-5.3). The use of peracetic acid (PAA) as a disinfection treatment lowers the pH level of process water, so, the lowest pH (3.4-3.6) was observed in the scenario with highest PAA residual (ID 28, 477-556 mg/L). ORP values varied between 242 and 856 mV, and PAA and H_2O_2 scenarios showed lower values than those of chorine. EC increased during throughout the sampling points of each scenario,

regardless of the type of product, disinfectant used and operation. Regarding temperature, except for the hydro-cooling operation and ID 18 scenario, the water was not refrigerated for fresh-whole fruits and vegetables. It could be observed that when no refrigeration system is used, water temperature greatly depended on the ambient temperature (e.g., ID 12 and ID 14 sampled in winter versus ID 19 and ID 21 sampled in summer). Fruits entering the tank came from the field or the cold rooms, so this could cause the variation of water temperature during processing.

Concerning COD, the scenarios of the fresh-whole FVHs sector that reached the highest COD level (3390 mg O_2/L) was observed in the pre-washing process water of mango with PAA (ID 26). It is worth highlighting that the maximum COD value after 6 weeks and more than 2.500 tn of fruit processed was 276 mg O_2/L (ID 16), which indicates that the filtration system used was very efficient in terms of removing organic matter from the processing line (including dumping tank). In general, COD, TDS and TSS increased during sampling; some decreases could be attributed to a partial water replenishment. ID 22 and ID 15 (same FBO) showed the highest values of TDS, which may be attributed to the initial quality of processing water (surface water). These scenarios also presented high EC values.

Chlorine demonstrated high efficacy in reducing mould counts, in particular when the residual disinfectant concentration was > 5 mg/L. In those scenarios that used chlorine, total bacterial counts varied from below LoD to 4.68 log CFU/100 mL. However, many samples from scenarios using PAA- and H_2O_2-treated process water showed total bacterial counts above 4 Log CFU/100 mL which demonstrates the inefficacy of these disinfectants at the ranges used or the poor management practices applied. Similarly, Lopez-Galvez et al. (2020) found total bacterial counts of 8.7 and 4.3 log CFU/100 mL in the pre-washing (PAA < 3 mg/L) and washing step (417 mg/L) of peppers, respectively. Bertoldi et al. (2020) found an average range of 0.0 to 4.7 log CFU/mL in tomato flume tanks in three packinghouses in Florida, with chlorine levels that greatly differed among them (from 3 to 200 mg/L). In general, except for ID 26 (avocado, PAA), coliform counts were low in those industrial settings that used disinfectants. In chorine-based scenarios, coliform counts ranged from below LoD to 2.4 log CFU/100 mL, with the highest values related to low disinfectant residual in some sampling points. Similarly, it is worth mentioning that PAA in ID 26 failed to reduce coliforms probably due to the low residual disinfectant concentration found (ca. 2 mg/L). Lopez-Galvez et al. (2020) also found high coliform counts (7.4 log CFU/100 mL) in the pre-washing step of peppers, but it decreased in the washing step (1.8 log CFU/100 mL) where a higher concentration of PAA was found (417 mg/L). This fact underlines the importance of using the residual PAA concentration high enough to maintain the microbial quality of the process water. The occurrence of coliforms in H_2O_2-treated process water scenarios (ID 12, 13 and 23) was high, with average means >3.0 log CFU/mL. It should be highlighted that IDs 13 and 23 belonged to the same FBO that used untreated surface water, which could be the reason for the high counts observed. *E. coli* was found to be enumerated in 8 out of 15 scenarios using chlorine as a disinfectant, with an occurrence <33%. It has been reported that water samples from tomato flume tanks in three Florida packinghouses (using hypochlorite-based disinfectants) were sampled on an hourly basis in two different visits, with negative *E. coli* counts (Bertoldi et al., 2020). No *E. coli* was found in the three scenarios using PAA (ID 26, 27 and 28) unlike high occurrence was observed in those scenarios using H_2O_2 (70 samples out of 72, ID 12, 13 and 23). *Salmonella* was confirmed in 1 out of 624 samples analysed. The scenario in which it was found was ID 13 (apples, PAA), which was positive for *CrAssphage* and F-specific coliphages as well and had high total bacterial counts (4.98 log CFU/100 mL) and coliform counts (3.33 log CFU/100 mL). This FBO used untreated irrigation water as source and the sample showing the presence of *Salmonella* spp. had a residual

disinfectant concentration of 60 mg/L of PAA. However, the same FBO presented a lower residual disinfectant concentration in some sampling points with *Salmonella* absence. The same processing line was sampled later on (peach and nectarine scenario, ID 23) and no *Salmonella* was detected. It is important to remark that there were a higher number of isolates that were selected as presumptive *Salmonella* spp. from the selective medium but only one was confirmed using PCR analysis which counted for *Salmonella* occurence in fresh-whole of 0.16% (1 positive sample out of 624). Therefore, it is recommended to carry out a PCR confirmation in future *Salmonella* spp. analysis to avoid overestimation.

In chlorine scenarios, *Listeria* spp. occurrence was high in the dumping tank of the processing line that had continuous filtration (ID 16), in which 17 out of 24 samples were positive. In this scenario, water was reused for 6 weeks and more than 2500 tn of fruit were processed during this period. In the fresh-whole sector, despite the high occurrence of *Listeria* spp. in the samples (338 out of 624), only 22 (3.52 %) were positive for *L. monocytogenes*, 11 of them belonging to the same FBO. *Listeria* spp. and *L. monocytogenes* have been frequently isolated from irrigation and natural waters, due to their ubiquitous presence in natural environments and occurrence of *L. monocytogenes* in pond, river and irrigation water in different environments ranged from 0 to 98.5% in different studies reviewed by Bell et al. (2021) and Gartley et al. (2022).

It should be highlighted that in ID 17 (peaches&nectarines, dumping, chlorine) we had 4 missing data points for *Listeria* spp., due to the high levels after filtration of 100 mL, which made the plates uncountable. Two of these 4 samples were those with *L. monocytogenes* presence. In ID 26 (avocados, pre-washing, PAA) no *Listeria* spp. were found but *L. monocytogenes* was detected. It is difficult to explain the high occurrence of *L. monocytogenes* (11 out of 24 samples) when *Listeria* spp. was below the detection limit (< 1 CFU/100 mL). Some of the hypotheses could be related to the high numbers of the other microorganisms (> 3.22 log CFU/100 mL, moulds; >7.44 log CFU/mL yeasts; > 5.7 log CFU/mL total counts) and also high turbidity (from 24.4 to 480.2 NTU) and COD values (from 260 to 2998 mg O2/L) that could interfere with the growth of *Listeria* spp. in the selective media or may have outcompeted *Listeria* spp. On the contrary, *L. monocytogenes* was able to grow and develop in a selective medium after the enrichment procedure, as competing microbiota may have been inhibited while *L. monocytogenes* could be favoured.

In general, total and F-specific coliphages and norovirus presence were linked to the use of surface or well water in the processing lines. However, in ID 04, 07, 26, 27 and 28 municipal tap water was the source, and norovirus GI (ID 04, 26, 27, and 28) and total and F-specific viruses (ID07) were found. The occurrence of norovirus was higher when no disinfectant was used or in those scenarios with PAA addition. It should be highlighted that intact capsids of norovirus GI were detected in the process water of the pre-sorting of pears where chlorine residual was 13 and 43 mg/L (ID 15).

In summary, the occurrence of bacterial pathogenic microorganisms in the fresh-whole sector was 0.16% for *Salmonella* spp. (1 out of 624) and 3.52% for *L. monocytogenes* (22 out of 624) whereas no pathogenic *E. coli* were found. To highlight that 11 out of 22 *L. monocytogenes* found were from the same scenario (ID 26). Thus, from the 19 FBOs belonging to the fresh-whole sector, 5 (26.3 %) failed to control *L. monocytogenes*. In general, this failure in the control of pathogenic bacteria could be attributed to: (1) no use of disinfectant, (2) low residual concentration of disinfectant and (3) no appropriate management practices for disinfectant monitoring and/or dosage. The higher occurrence of *L. monocytogenes* than *Salmonella* spp. in dumping and pre-sorting operations could be explained by the fact that the surface of fruit containers that are used during harvest are in contact with potential

sources of this pathogen (e.g., soil, debris, weed) and afterward immersed in the dumping and pre-sorting tanks, being the most potential source of contamination. Regarding ID 26, PAA was used at a very low concentration. In general, chlorine and PAA disinfectants showed good efficacy when used at proper concentrations. The target of H_2O_2 used by the FBO was recommended by the supplier for drinking water (residual 10 to 20 mg/L), which could not be appropriate for recycled water with a high amount of organic matter. Moreover, the residual concentration of disinfectant was not controlled and was 0 mg/L in some samplings.

This study shows the complexity of water management in the fresh-whole FVHs sector, as there is not any guideline or common procedure for the FBO. Each FBO has its own procedures, mainly based on their own experience. Disinfectant monitoring is not done or sampling is done periodically or punctually using strips or colorimetric methods and subsequent dosage of disinfectant is done according to the residual obtained, which often demonstrated to fail in controlling microbial load and pathogenic microorganisms.

4.3.2 Fresh-cut FVHs sector

The fresh-cut FVHs industry was analyzed across 19 different scenarios, of which five involved scenarios without water treatment and 14 utilized chlorine-based water disinfection agents, including only one using PAA (ID 38). Among these scenarios, 17 were associated with washing operations and two with pre-washing processes (IDs 35 and 43).

Scenarios lacking disinfection typically employed municipal tap or well water for washing operations involving leafy greens (such as curly endive and radicchio, baby leaves, parsley, and a salad mix with carrots) as well as shredded carrots. These processes, characterized by brief production times, showed a relatively stable pH level around 8.0, although it fluctuated between 6.6 to 8.5. An exception was noted in the mixture of curly endive and radicchio, where the average pH was 6.2, influenced maybe by the well water used to fill the tanks.

Chemical Oxygen Demand (COD) levels saw dramatic rises from 2.3 mg/L to 4,795 mg/L, particularly notable in the shredded carrots (ID 30). This was accompanied by increased EC, turbidity, TDS, TSS, and UV absorption, indicating the release of organic matter such as carotenes and fibers. This release was observed even with brief production runs and despite complete water replenishment after 5.5 hours of production. Microbial load in the water samples increased, with high counts of yeasts, total bacterial counts, and coliforms (average values of 5.3-5.5 log CFU/100 mL). It's important to highlight the variability in *E. coli* presence, with an occurrence ranging from 6 to 15 out of 24 samples. The variability was particularly marked in the curly endive and radicchio mixture, where *E. coli* counts ranged from 0 to 2.2 log CFU/100 mL. The detection of pathogenic *E. coli* was noted in this scenario as well as in the washing of baby leaves (ID 32). *Salmonella* was detected in all five non-disinfected fresh-cut scenarios, of which in 48 of the 120 taken samples (= 40 %) *Salmonella* was found in one of the ten visits (= 10 %). The presumptive *Salmonella* positive samples were confirmed by the agglutination test, a serological test, as described in ISO 19250 (2013). When the presence of *Salmonella* O-, Vi, and H-antigens is confirmed by agglutination, the samples may be seen as *Salmonella* positive. Norovirus GI was identified in four out of these five scenarios, in one case reaching counts of 4.4 PFU/L, and two scenarios (IDs 30 and 33) testing positive for CrAssphage. Despite high occurrence and counts of *Listeria* spp. (22-24 out of 24 samples with an average of 2.4 log CFU/100 mL), *L. monocytogenes* was never detected.

Two scenarios involving the use of electrolyzed water (EW) for processing whole tomatoes and cucumbers before cutting were examined in detail. In the pre-washing stage (ID 35), controlling residual

chlorine levels proved challenging, with an average of 6.5 mg/L during the first visit and 28 mg/L in the second, coupled with a pH above 8.0. The COD, particularly in the second visit, increased due to the recycling of water. For the washing water (ID 36), although the residual chlorine was higher than in the pre-washing stage, the pH remained high (>8.5), but COD was better controlled with an average of 106 mg/L. The microbiological quality in both the pre-washing and washing operations with EW showed that all microbiological groups were below the limit of detection (LoD), except for the total bacterial counts, which were 3.75 and 1.69 Log CFU/100 mL, respectively.

Comparing these results to the pre-washing and washing of shredded lettuce treated with sodium hypochlorite (IDs 43 and 44), there was variability in physico-chemical properties, especially in residual chlorine. The pre-washing stage showed a decrease in residual chlorine from 20 to less than 1 mg/L, and the pH remained high (>8.0) with a significant COD (average of 1254 mg/L) due to the high demand because of the tissue exudates. The washing operation had a higher residual chlorine average (50 mg/100 mL) and a pH of 8.3. COD varied widely (600 mg/L during the first visit and 1600 mg/L during the second) due to the doubled product amount processed in the second visit. Microbial counts in both the pre-washing and washing operations were similar, with most below the LoD, except for coliforms during pre-washing (1.67 Log CFU/100 mL). Notably, norovirus counts were high (5.27 Log gc/100 mL) in both pre-washing and washing, while CrAssphages were only detected during pre-washing (3.45 Log GC/100 mL).

Faced with challenges in maintaining adequate residual free chlorine levels, FBOs explored alternative disinfection methods. For example, diced onions were treated either with chlorine (ID 37) or with PAA (ID 38). The chlorine treatment with municipal water resulted in residual chlorine levels of less than 2 mg/L, except at the start of the process, with a pH above 7.5. The PAA treatment with well water showed suboptimal residual PAA levels (average of 57 mg/L) with a pH of 5.3 and a very high COD (mean of 4760 mg/L). This rise in COD was attributed to the release of organic matter from the onions and the addition of PAA to the water. The microbiological quality of the chlorine-treated water was poor, with significant total bacterial counts of moulds, yeasts, total bacterial, coliforms, and *Listeria* spp. Remarkably, norovirus was present in high counts (5.03 Log GC/L), and *Listeria* spp. were detected in all samples, which is not unexpected for bulb products cultivated close to the ground and washed with poorly managed disinfectant water. The PAA-treated water also showed poor microbiological quality, with high counts of molds, total counts, and coliforms.

Different scenarios involving the washing of fresh-cut lettuce with various chlorine-based disinfectants also presented diverse outcomes in terms of residual chlorine, pH, and COD. Using calcium hypochlorite (ID 40), residual chlorine and pH levels were variable, with COD reaching up to 600 mg/L. A combination of calcium and sodium hypochlorite (IDs 41 and 47) resulted in a high residual chlorine range (33-198 mg/L) and variable COD levels (36-994 mg/L). When chlorine gas was used in conjunction with sodium hypochlorite, the residual chlorine levels were inconsistent, but the pH was stable at 6.5 (ID 42). Despite these variations, this scenario that used chlorine gas and sodium hypochlorite exhibited excellent microbiological quality, with total bacterial counts below the LoD.

For baby leaves washed with different disinfectants (IDs 45, 46, 47), the scenario using sodium hypochlorite (ID 45) had a higher mean residual chlorine, higher pH and very low COD, which could be due to fresh water refills. The scenario that added calcium hypochlorite (ID 46) showed better pH control. The combination of calcium and sodium hypochlorite (ID 47) resulted in a microbiological quality of the washing water generally good, except for certain bacterial counts.

A notable case was the washing of a salad mix with sodium hypochlorite (ID 48), where residual chlorine levels ranged widely, and the pH varied between 5.76 and 7.72. *Listeria* spp. counts during the first visit were notable with an occurrence of 6 out of 12 samples.

Overall, these scenarios highlight the complexities of managing water quality and disinfection by the fresh-cut FVHs FBOs, pointing to the need for continuous monitoring and adaptation of water treatment practices to ensure food safety.

4.3.3 Frozen FVHs sector

Within the frozen sector, 13 scenarios were investigated (spread over seven FBOs). No disinfection was used in four scenarios, while in the other nine scenarios, PAA or H_2O_2 was used as a disinfectant.

The scenarios where no disinfection was used all pertained to water used for washing or transport of the product (so at the beginning of the processing process), with production runs ranging from two to seven days. Levels of pH remained relatively constant, varying between 6.0 and 8.0. The chemical oxygen demand (COD) of the analysed water samples sometimes exhibited high increases (up to 58,250 mg/L as in ID 49) attributed to product-related factors, such as the release of organic matter (e.g. fibers and exudates from onions). The microbial load of the water samples was quite high (6 – 8 log CFU/100 mL), with no pronounced increase observed. This is possible because the water source was recycled process water, which was microbially loaded already at the start of production. Another contributing factor to this high microbial load is that in three of the four scenarios, spinach production (ID 50, 51, 52) occurred at the end of the harvest season as well as during the rainy season, so the raw material was already highly loaded. When it comes to pathogens, a high occurrence *of L. monocytogenes* was noted, however, this is possibly due to overestimation during analysis because only biochemical confirmation was performed and no PCR confirmation for these isolates by the consortium partner executing these analyses. *Salmonella* spp. was also frequently detected. In this case, the ISO method was modified slightly as the presumptive positive *Salmonella* spp. samples were confirmed *Salmonella* spp. positive by an agglutination test which is a serological confirmation test. The scenarios where disinfection was used, can be divided into two groups based on the type of disinfectant used. The first group (four scenarios, ID 53, 54, 56, 57) concerned water used for the pre-washing or washing step of the product or the cooling step after the blanching of the product. The pH and COD of these water samples remained relatively constant during production, with mean values of approximately 7.0 and 1500 mg/L; respectively. Hydrogen peroxide (H_2O_2) with a disinfection dose of 100 ppm was presumed to be used in these processing steps. However, the detected disinfectant concentrations varied between 40 and 240 ppm, which is lower or higher than the presumed dose. Nonetheless, its impact on the microbial load remained imperceptible, a phenomenon consistent with findings in the literature indicating that H_2O_2 necessitates a substantial residual concentration coupled with a heightened disinfectant demand owing to its significant interaction with organic matter in the washing solution, thereby resulting in rapid depletion and sluggish disinfection kinetics. Given this, current applications for H_2O_2 as process water disinfectants should not be recommended. This is also demonstrated in this study as the total bacterial counts remained constant through sampling (7 – 8 log CFU/100 mL), even in the one scenario where blanching occurred before the sampling. In this particular scenario (i.e. cooling water) also a high load (6 -7 log CFU/100 mL) of the fecal indicator *E. coli* was found. Similar findings may be seen for the other indicators with an occasional exception for *E. coli.* In contrast, bacterial pathogens were not detected in any of the scenarios.

The second group (five scenarios, ID 55, 58, 59, 60, 61) concerned water used for the washing step of the product or for the cooling step after blanching of the product, where PAA was used with a disinfection dose of either 20 to 50 mg/L or an unknown dose based on pH monitoring (keeping it below 4.5). When PAA was dosed based on pH, its value dropped remarkably (to 4.6) resulting in the detection of very high PAA concentrations (up to 1,795 mg/L). In contrast, when a dose of 20 to 50 mg/L was implemented, the pH remained relatively constant and no PAA was detected (< 5.0 mg/L). This indicates a lack of (proper) monitoring. The COD sometimes showed high increases (up to 28,800 mg/L), again due to product-related factors. The effect of PAA disinfection is evident through the microbiological properties as well. When high PAA concentrations were detected, microbial counts were very low (in most scenarios <LOD) and all analysed pathogens were undetected. When no PAA (< 5.0 mg/L) was found (ID 55, 58, 59), there was a considerable microbial load (total bacterial counts of 3 – 6 log CFU/100 mL). Similar findings may be seen for the other indicators for ID 55 with an exception for *E. coli,* but not for ID 58 and 59. Although the water source in this processing step was municipal tap water, the detected microbial load remained constant during sampling, which suggests this is product-related again and/or no proper cleaning and disinfection was performed before production. Nevertheless, the analysed pathogens were only sporadically detected in a few samples during one visit. The latter may be associated to the fact that a blanching step was applied preceeding this sampled processing step. However, it must be underlined that blanching cannot be considered a sufficient alternative to compensate for the lack of or improper disinfection monitoring.

4.4 Online monitoring case scenario

Objective 4 was focused on developing case studies within industrial settings to evaluate the effectiveness of measuring physico-chemical parameters that can be correlated with the maintenance of microbiological process water quality during the post-harvest handling and processing operations of ffFVHs. The monitoring and control systems should help maintain the optimal level of disinfection while avoiding the drawbacks of over or under-chlorination, such as harmful by-product formation or insufficient pathogen removal.

There were four scenarios initially selected for this objective but because of the problems that the different FBOs had with the calibration of sensors for the analyses of residual disinfectant, the data obtained did not allow for registering the monitoring and control of disinfectants. For this reason, a new scenario (ID CEBAS-OM) has been included with historical data that the CEBAS-CSIC group had. Managing the residual disinfectant and the regular concentration monitoring allows the fine-tuning of dosing rates, minimizing any time that the product is exposed to no water treatment without compromising food safety. Online monitoring using the "SmartWash™ system" can lead to cost savings and environmental benefits by reducing chemical consumption and minimizing the absorption of by-products by the washed product and the discharge into wastewater. Measuring residual disinfectant levels is a key component for validating the efficacy of the washing process and verifying that it consistently achieves the desired microbial reduction, avoiding accumulation. The historical results from ID CEBAS-OM using the "SmartWash™ System Solutions" showed how the control of the residual disinfectant to a desired operational limit of 15 mg/L approximately at the correct pH (6.0) can control the accumulation of total bacterial counts and coliforms, improving water management in the post-harvest handling and processing of ffFVHs.

4.5 Literature review

A systematic literature review was performed to obtain information on data and models that are available to quantify microbial contamination in process water as well as information on microbial and physico-chemical parameters that can be used to assess the microbial quality of the process water. In order to answer RQ1a (which data are available that can quantify the microbiological contamination of water used in post-harvest handling and processing operations of ffFVHs and between ffFVHs batches?), 69 scientific papers were evaluated. Overall, the data extraction from these papers appeared challenging since the papers not always clearly described the experimental setup or there were discrepancies between presented results in tables and described in the main text. Furthermore, the settings, water types, microorganisms, FVHs, and disinfection methods differed across studies, which made it hard to summarise the studies and draw general conclusions. For example, even though the majority of papers described leafy greens, the pathogens studied differed, the (laboratory) settings deviated, disinfection methods differed as well as how they were applied and at which concentration they were administered. These different experimental setups hampered the ability to draw conclusions on the efficacy of disinfection methods. Finally, most studies reported results from laboratory settings, which hampers drawing conclusions on microbial quality of process water as found in industrial settings.

The literature review on physico-chemical parameters showed that these parameters are used to characterize the quality of the process water and to identify the differences in the efficacy of disinfectants. Factors affecting disinfection and which were measured in the evaluated studies included: COD, conductivity, organic matter, ORP, pH, temperature, and turbidity. Several studies reported that in particular the organic load of process water measured as COD has a detrimental effect on the efficacy of chlorine-based disinfectants. The organic load can be reflected not only by COD, but also by turbidity and TDS. For other disinfection methods, these parameters were studied less frequently. Some papers report a negative effect of increasing COD or turbidity on UV efficacy. PAA (alone or in combination with UV), sodium acid sulfate, and pulsed light were reported to be less sensitive to the presence of organic matter compared to chlorine-based disinfectants. Combined treatments are sometimes used to first decrease the organic load and then disinfect the water such as a combination of electrocoagulation and UV treatment. Physico-chemical parameters were also investigated as indicators of the water quality for use in monitoring. Thus, parameters such as COD, TDS, and turbidity are suitable indicators of organic load in process water. Conductivity could be a suitable indicator for water quality when using UV as a disinfection method.

The systematic literature review was also performed to find information on inline/online methods to validate the microbial quality of process water. This showed that only a limited number of papers were available describing such methods at lab scale. No scientific publications on in-line and online monitoring methods were available at industrial scale regarding verification/validation or monitoring the microbiological quality of the process water. Since the methods described were all performed at lab scale and commercial methods available are not described in scientific literature, it is difficult to draw conclusions on the usability of such methods in practice. Therefore, more research is needed on inline/online methods, preferably at pilot or industrial scale.

4.6 Dynamic mass balance modelling

To understand industrial processes which involve many different mechanisms, with time-varying factors and highly interlined impacting parameters, it is necessary to use quantitative analysis and hypothesis testing that goes beyond common statistics. For these complex processes, mechanistic mathematical modelling provides a means to evaluate the validity of various hypotheses, infer relevant parameters and their variability depending on different changing factors and delivers simulations of what-if scenarios or even identify conditions for optimising certain performance objectives. However, the development of model structure, model fitting and validation requires many different steps where the available information, not only regarding experimental data but also mechanistic knowledge, is critical and most commonly not available. A model can only predict outside of the data range used for its development if there is a good description of the relevant mechanisms and it is fed with sufficiently informative data. This allows to select correct assumptions that simplify the model and make this tractable and useful.

Therefore, the model-based understanding of the dynamics of micro-organisms in process water applied in processing operations of ffFVHs required a long preparatory work with the steps described in the next subsections.

4.6.1 Review and analysis of the available models in the literature

The analysis of the available models in the literature allowed to find major relevant mechanisms, usual assumptions and critical affecting parameters. After a systematic review searching for a combination of common terms both in modelling and in the washing process, the title, abstract and methods of the 95 retrieved works were studied to identify 16 of major relevance (see **Table 18-20** and **Model 1**).

This selection was based on two criteria, the model should include either the dynamics of a microbiological factor or the interaction with a relevant physico-chemical factor. The latter were mostly non-dynamic models finding very useful relationships, but also commonly based on empirical formulas difficult to generalise outside of the range of experimental data used for their development. The group of dynamic models, however, resulted to be very useful. Most of them were inspired by the work by Munther et al. (2015) and each of the models was standardised to understand the different mathematical formula used to describe the different mechanisms. From these, the mechanisms of microbial inactivation due to water treatment with disinfectant was the most complex and presenting a large variety of possibilities. See **Table 21** for an outline of all the different alternatives.

4.6.2 Proposal of a general dynamic model based on mass balance conservation

The models and data retrieved from the literature were used to collect all the possible mechanisms affecting the process water quality. These mechanisms were interconnected based on mass balance conservation law in **Model 2**. This general framework allows to conceptually model very different experimental (lab and industrial) conditions by selecting the mechanisms of relevance of each of the specific experimental designs. Moreover, formulas for each mechanism can be derived based on standard theory of chemical reactors assuming mass action law for reaction terms.

The modelling of the disinfectant inactivation, however, required extra analysis to be able to propose a model describing the protective effect of COD. A battery of different modelling alternatives was built and considered for the analysis, including the only work in the literature modelling this effect (Abnavi et al.,

2021a). The confrontation of the models with the retrieved data from the literature, specifically designed to understand this effect, reveals that COD inhibits disinfectant inactivation in a highly non-linear form, best represented using Hill kinetics with an exponent larger than one. This means that COD might not affect significantly at low values, but the effect is higher than linear for large COD values. Although an interesting result, the Hill kinetics are functions of highly correlated parameters. Thus, the model had to be simplified to Michaelis-Menten kinetics (as in Abnavi et al. 2021a), by selecting the exponent equal to one, when COD dynamics were not very informative.

The general **Model 3** was obtained by collecting all the specific formulas for transport and reaction terms. The model describes very different conditions, like microbial contamination in the inlet flux or microbial contamination from product to water. Although the result is a large and complex model, it was designed to allow its simplification when needed for the different experiments found in the literature and scenarios in the industry. Figure **106** Ilustrats the models in this tender, all derived from **Model 3**, and being like nested models with different levels of simplifications.

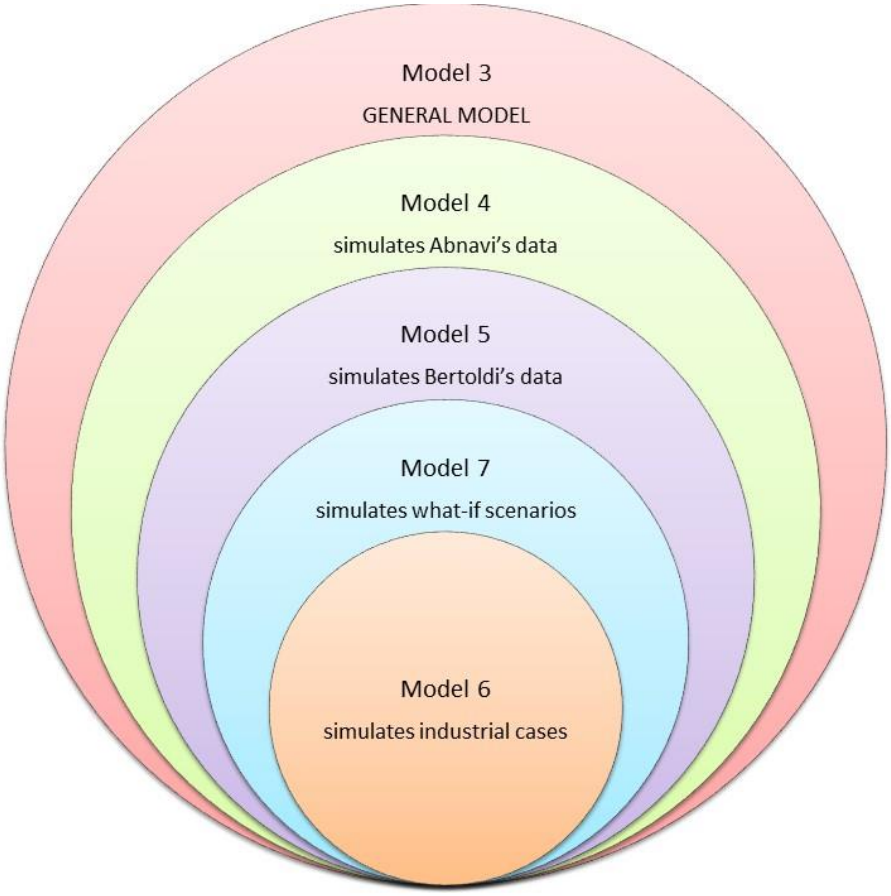


Figure 106. Illustration of the nested nature of the developed models in this tender. Models have different levels of complexity represented by the circle size. Nevertheless, the complexity of the model should be just the minimum to describe the data, and therefore simplifications are needed for different experimental designs. Thus, Model 3 is the most complex and large model, which can be simplified to

derive Model 4 (a subcircle of Model 3). Analogously Model 5 to 7 are simplifications of different level of Model 4. Models 1 and 2 are not included in the figure, as they are only related with the literature review.

4.6.3 Testing the model with different data retrieved from the literature

Two sets of data were modelled for being representative of the usual operations in industrial washing processes: the data by Abnavi et al. (2021a) with **Model 4** and by Bertoldi et al. (2021) with **Model 5**.

Model 4 describes the interplay between FC, COD and microbial contamination in two products and in the water, with cross-contamination (Abnavi et al., 2021a). The model reproduced the experimental data, but some of the parameters such as the inactivation rate presented large uncertainty (similar fits for different parameter values), probably due to a correlation between parameters within the Hill kinetics. Also, the estimated interplay between COD and FC was very low, and not relevant compared with other mechanisms. Similarly, the relevance of the inactivation in the product was minor comparing with the impact of the disinfectant in the water contamination. **Model 5**, on the other hand, shows the expressions for understanding the interplay between COD, product and the water microbiological contamination. The used data to fit the model proved to be a good representation of an industrial process but less informative that in Abnavi et al. (2021a). Therefore, different mechanisms included in model 4 had to be simplified or neglected (like inactivation of microbial contamination in the product). Finaly, data was reproduced without problems by the Model, but estimated parameters were very uncertain.

The modelling of these set of data from literature was critical to determine that an even simpler model was necessary to extract relevant information from the industrial scenarios studied in this tender.

4.6.4 A dynamic model to analyse industrial washing processing of ffFVHs

After different preliminary tests (data not shown), **Model 6** was selected for analysis of the industrial scenarios. It is a simplified model simulating the dynamics of COD and microbial contamination as a function of:

- time-varying measured inputs (pH, temperature and free chlorine concentration),
- constant known parameters such as the tank volume and the kilograms of processed product
- unknown parameters that should be estimated (for further analysis) from measured data. They can be split in three groups: (1) the dilution rate constant (related with tank volume and flux of water removed and added during a batch), (2) the transfer rates of COD and total bacterial counts and (3) the parameters of inactivation of the Hill kinetics.

Industrial scenarios without disinfectant, and therefore without inactivation, were of critical value to understand microbiological (in terms of total bacterial counts) and COD contamination of water, which were estimated for each of the visits for most of the scenarios without free chlorine. Note that these transfer rates have units of CFU per kilogram of product and minute and milligrams of COD per kilogram of product and minute, respectively. The results provide a picture where contamination rates are very variable, especially for bacterial contamination, but with interesting patterns regarding different visits, products, operations and sectors which helps us understand the industrial water contamination.

The transfer rates, estimated within this study, encode information that cannot be measured directly but is of major relevance to understanding water contamination. **Figure 101** shows the calculated transfer rates for several scenarios and visits. For example, in the Frozen sector, scenario 50 (spinach product, washing operation) the microbial transfer rates from product to water are higher for visit 2 than visit 1. Interestingly visit 1 sampling was at the beginning of the harvest season, whereas visit 2 was at the end when the product presented higher microbial loads. Other scenarios that were sampled at the end of the harvest season with the same operation and product (scenario 52, for example) show similar microbial contamination to scenario 50 visit 2, although slightly larger probably because of differences in dilution rate (same dilution rate was assumed for all scenarios). Regarding the Fresh-cut sector, as known from the literature, one of the products with more exudates is the carrots. Accordingly, the estimated transfer rates of COD for this product (scenarios 30, 34) were larger than for other products such as diced onions. Here differences between visits are also noted, in particular the differences between visit 2 and 1 in scenario 34, which can be easily explained, as previous to visit 1 an extensive cleaning of the tank with chlorine was carried out. Ardley et al (2019) reported that the most significant organic matter loads during carrot processing contributing to the high COD were the amounts of peel removed from the carrots. Tudela et al. (2019) compared different process wash water and maximum COD values of 266, 942, 2220, and 3150 mg/L were reported for baby leaves, shredded lettuce, shredded cabbage and diced onion, respectively. These conditions were established to mimic the real conditions of each type of process water in the fresh-cut industry. As expected, pre-washing operations (scenarios 04 and 07) also showed high COD and bacterial transfer rates, compared with washing operations (scenarios 06 and 08).

In a second step, industrial scenarios with chlorine-based disinfectants were modelled to understand the inactivation dynamics of total bacterial and *Listeria spp.* counts. In general, it can be said that the: (1) inactivation rate coefficients were in the same order of magnitude for both microbial groups, although lower for *Listeria* spp. which is a more resistant group when compared to total bacterial counts and (2) COD protective effect is relevant for some scenarios showing a Km constant similar to COD saturation levels. The parameter estimates, however, were more uncertain than when modelling scenarios without disinfectant.

Finally, the model is extended to include FC dynamics as a function of FC injections, dilution, natural decay and reaction with COD. This allows to simulate the impact of two different interventions: water disinfection and/or water replenishment. These interventions can be designed continuously, by assigning the constant addition of FC or constant change of water, and/or discretely by punctual additions of FC or water at certain times. Note that in steps 1 and 2, modelling industrial data, residual FC was measured and added as an input to the model. For illustration purposes, the tender shows the analysis for scenario 37, because parameters were estimated with confidence for this scenario (low CIs). For insignificant water additions (equivalent to the dilution rate estimated in industrial cases), the FC injections were calculated to maintain the microbial contamination in water around 2 log10(CFU/100mL) during the whole duration of the production. To keep microbial at this level, it was needed to start with a large FC injection, including other four injections afterwards with fewer milligrams of disinfectant to maintain the residual levels. As a final note, this profile of five equidistant-in-time FC injections was determined by testing different FC additions and comparing the resulting model simulations. However, other non-tested FC injections could provide similar or even better results (for example a higher initial

FC and avoiding therefore the first injection, or even using more frequent injections with less added FC at the start of the production). To guarantee that we operate at the optimum, the model can be used to solve an optimisation problem, which also allows including restrictions (for example maximum addition of FC) and minimise different or even multiple objectives, either attending to economic reasons (for example reducing contact times), to safety aims (for example reducing contamination in water) or to environmental purposes (such as reducing the amount of used water).

5 Conclusions

5.1 Conclusions on the data obtained in the 61 scenarios scenarios studied in the industrial sampling

5.1.1 Main conclusions from the physico-chemical results.

The presence of residual disinfectants like chlorine, PAA, or H_2O_2 in process water is crucial for microbial safety in the food processing industry. However, there were significant variations in residual disinfectant concentrations, indicating poor water management practices. A good process water management should include: (1) regular monitoring and adjustment of residual disinfectant concentration through continuous monitoring to ensure that the levels are within the optimal range; (2) in the case of chlorinated disinfectants maintaining the pH controlled (between 6.0 and 7.5); (3) filtration systems to remove organic matter that can reduce disinfectant efficacy and (4) regular water replacement to prevent organic load buildup, which can consume disinfectant and reduce its effectiveness. The literature review revealed that parameters such as ORP, pH, and temperature are important to maintain proper disinfection. The industrial scenarios indeed showed that pH levels influence the efficacy of disinfectants. Chlorine is most effective in slightly acidic to neutral conditions, while PAA remains potent over a wider pH range, although PAA itself may decrease the pH, as for example ID 60, 61. ORP values reflect the water ability to disinfect. Negative ORP readings indicate the presence of reducing agents, which may compromise disinfection. High EC levels suggest challenges in maintaining cleaning and sanitation processes, particularly in recirculating systems. Proper temperature management is essential to control microbial contamination during washing processes. Cold water may not effectively inactivate pathogens, but it is needed to maintain the quality of the product such as fresh-cut products, while hot water can improve microbial removal although the frozen industry cannot expect this to to maintain the microbial quality of the water, as blanching is only used to ensure enzyme inactivation. The literature review revealed that COD is a suitable parameter to monitor water quality.the industrial scenarios also showed that high COD levels increase disinfectant demand, affecting water quality and potentially compromising food safety. Effective COD control is crucial for maintaining water quality and disinfection efficacy. According to literature, TDS and turbidity can also be used for the same purpose, but are less effective. Indeed, the industrial scenarios showed that high levels can hinder disinfection processes. UV254 absorbance correlates with organic matter levels, providing a quick estimation of pollution levels. However, it may not always directly correspond to COD values and requires additional testing for accurate assessment in each case scenario.

In conclusion, proper monitoring and control of physico-chemical parameters and residual disinfectant levels are essential for ensuring effective disinfection, minimizing microbial risks, and maintaining process water quality in the handling and processing of ffFVHs. Additionally, addressing challenges such

as high COD levels and variability in disinfectant concentrations requires comprehensive water management strategies.

5.1.2 Main conclusions from the microbiological results

The industrial scenarios showed that mould, yeasts and total bacterial counts varied across different ffFVHs sectors and depending on the disinfection agent used. High mould, yeast and total bacterial counts were observed in scenarios with no water treatment, while chlorine and PAA generally reduced these counts. H_2O_2 showed mixed effectiveness, with some scenarios exhibiting high mould yeast and total bacterial counts. The literature review revealed that most studies reported on leafy greens and total bacterial counts for the process water in this type of crops ranged between LOD and 7 log cfu/100ml at pilot/industrial scale. In our industrial settings, PAA and H_2O_2 were not as effective in reducing total bacteria counts compared to chlorine. According to the literature review, disinfection methods most frequently studied at the lab scale were the use of chlorine and UV. These studies show that log reductions of up to 7 Log can be obtained depending on the dose applied and the COD levels in the water. On average, chlorine application results in a 4.7 log reduction (**Figure 79**). *E. coli* counts are considered as indicators of fecal contamination. Chlorine was effective in reducing coliforms and *E. coli* counts, while PAA showed variable effectiveness. The literature review revealed that pathogenic bacteria can be present in process water in a range between 1 log cfu/100ml and around 7.5 log cfu/100ml. The industrial scenarios showed high counts and occurrence of *E. coli* when no water treatment or H_2O_2 were used. *Listeria* spp. counts were detected in scenarios with no water treatment, which is logical as *Listeria* spp. may be found in the environment. y. The literature review indicated that chlorine, UV and PAA can all effectively reduce bacterial load in the water in lab experiments, although their efficacy depends both on the dose and on physico-chemical properties of the process water, such as pH and COD. The industrial scenarios showed that chlorine was generally effective in reducing *Listeria* spp. counts, but PAA and H_2O_2 showed mixed results. Norovirus were detected by means of RT-qPCR in scenarios with no water treatment and some scenarios using chlorine, PAA, or H_2O_2. Viability RT-qPCR indicated the presence of intact noroviral virions that could be potentially infectious, in some samples, highlighting the importance of thorough disinfection practices. *Cryptosporidium* was not detected in any scenario, while *CrAssphage* was detected in some scenarios across all sectors, indicating potential human fecal contamination.

Overall, the findings underscore the importance of effective water treatment and disinfection practices in ensuring the quality of the water used during the handling and processing operations of ffFVHs. Proper water management and monitoring are crucial to mitigate microbial risks and prevent contamination. As an example, scenario ID 42 of process water used in the washing operation of fresh-cut lettuce that used chlorine gas and because of the high demand (high COD) it was supplemented with sodium hypochlorite to maintain the residual chlorine needed.

The detection of pathogenic bacteria in the different sectors of fresh and frozen fruits, vegetables, and herbs (FVHs) reveals important insights into the microbiological hazards associated with handling and processing operations. *Salmonella* spp. was present in all three sectors of FVHs. In the fresh-whole sector, *Salmonella* was detected in the process water used for apple dumping, which was disinfected with H_2O_2. In the fresh-cut sector, *Salmonella* was detected in scenarios with no water treatment, indicating potential contamination during handling. *Salmonella* was also found in scenarios of frozen FVHs with no water treatment and those treated with PAA. *L. monocytogenes* was detected in scenarios

from the fresh-whole and frozen sectors but not in the fresh-cut sector. Detection occurred in scenarios with no water treatment and those treated with PAA or H_2O_2. Shiga toxin-producing *Escherichia coli* (STEC) was detected in scenarios from the fresh-cut and frozen sectors when no water treatment was used. Viable but non-culturable bacteria were induced in scenarios across all sectors, indicating the poor effectiveness of some disinfectant agents such as PAA and H_2O_2 and the overestimation of their effectiveness. In the fresh-whole sector, PAA treatment showed poor effectiveness in maintaining microbiological quality and the induction of VBNC stage. In the fresh-cut sector, chlorine treatment underestimated the presence of VBNC bacteria, posing a risk of cross-contamination. H_2O_2 treatment in the frozen sector also led to the induction of VBNC bacteria, highlighting its inefficacy in maintaining microbiological quality. Spores of *Clostridium perfringens*, a bacterium associated with foodborne illness, were examined in scenarios involving carrots, onions, and baby leaves. Detection was generally low, with only sporadic occurrences (1 CFU/100 mL detected in ID 05 visit 2 and ID 37 visit 1), suggesting a lower risk of contamination with these spores.

These findings emphasize the importance of effective disinfection strategies to minimize the risk of pathogenic bacterial contamination in ffFVHs throughout the handling and processing operation where water is used (e.g., IDs 39 and 42).

5.1.3 Main conclusion of the online monitoring case scenario

The control and optimization of residual disinfectant levels in process water are crucial for maintaining microbiological water quality and ensuring food safety during the handling and processing of ffFVHs. By implementing monitoring and control systems for residual disinfectants, such as chlorine, adjustments can be made in real-time to optimize dosing rates, minimize exposure to untreated water, and avoid over or under-chlorination. The literature review showed that UV absorbance is a promising online method to predict chlorine demand. Chronoamperometric sensor is another promising method for calibration of an online detector for monitoring the residual disinfectant, such as for PAA. SmartWash™ System Solutions is a unique monitoring, dosing, and control system, and an online cloud platform to enhance food safety and operational standards. This not only has the potential to enhance microbial reduction but also lead to cost savings and environmental benefits by reducing chemical consumption and minimizing the discharge of by-products into wastewater. Additionally, the comparison of monitoring methods, such as amperometric measurements of hypochlorous acid and measuring oxidation-reduction potential (ORP), can provide immediate feedback for chlorine dosing adjustments. These findings highlight the importance of integrating microbiological and non-microbiological parameters to validate and monitor water quality effectively in the handling and processing operations of ffFVHs.

5.2 Conclusions on the dynamic modelling

Different modelling alternatives were proposed along the WASHTOP tender to understand the interplay of microbiological and physico-chemical dynamics in process water. Some alternatives were very general, describing many different mechanisms, and others were more focused on industrial scenarios with only relevant impacting mechanisms. Simple models were useful for understanding the industrial washing processes and to estimate relevant unknown parameters, whereas more sophisticated and detailed dynamic models were needed to understand complex mechanisms such as the microbiological inactivation with disinfectants. In any case, all models were derived from a general large model (Model

3) based on mass balance conservation. That model includes many different possible mechanisms that can be easily adapted to represent different conditions.

Two open-source codes are released with this tender. The first code named "Industrial_Cases_SIMULATION" simulates all the modelled industrial scenarios considered within the tender, therefore including relevant realistic parameters, as well as the dynamics of COD and microbial contamination of water based on measured data and model simulation. The second code named What-if_scenarios_SIMULATION provides a refined model designed to assess the impact of the operation management of the washing process on the water contamination under different specific what-if scenarios. It allows to consider two types of intervention measures: water disinfection and water replenishment. When populated with realistic parameters, like those estimated in this tender and released with the first code, the scripts' results are useful to understand the impact of changing: (i) critical parameters (such as dilution rate, product to water transfer rate or contact time), or (ii) the dynamic changes of the interventions (including for example number and quantity of FC injections or water additions).This code, finally, can be combined with appropriate control and optimisation algorithms for in-line or on-line feedback control and process optimisation by FBOs in the set-up of their water management strategies.

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Abbreviations

Glossary of acronyms	
Acronym	**Meaning**
AESAN	Spanish Agency for Food Safety and Nutrition
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
BfR	German Federal Institute for Risk Assessment
CFU	Colony Forming Unit
FAO	Food and Agriculture Organization of the United Nations
FBO(s)	Food Business Operator(s)
ffFVHs	Fresh and Frozen Fruit, Vegetables and Herbs
FDA	U.S. Food and Drug Administration
VBNC	Viable but Non-Culturable
WHO	World Health Organisation

Most used modelling acronyms ordered alphabetically

C	Concentration of any disinfectant, when is free chlorine FC might be used instead
CLD	Disinfectant demand, usually chlorine demand.
$COD_{p,i}$	COD in produce i, without subindex when only one product
COD_w / COD	Chemical Oxygen Demand in water (for simplification of notation the w is sometimes ommited)
D	Dilution rate
F_w^{cont}	Continous change of water

F_w^{disc}	Discrete change of water
$F_{p,i}^{in}$	Flux of produce i incoming in tank, without subindex when only one product
$F_{p,i}^{out}$	Flux of produce i outgoing from tank, without subindex when only one product
F_w^{in}	Flux of water incoming in the tank
F_w^{out}	Flux of water out from the tank
$\mathrm{F_w^{cont}F_w^{disc}}$	
FC_w/FC	Free chlorine in water (for simplification of notation the w is sometimes ommited)
FC_{in}^{cont}	Continous addition of FC
FC_{in}^{disc}	Discrete addition of FC
K_m	Protective effect COD if low value
K_{COD}	Transfer rate constant of COD from product when concentration of COD product is not known and included in the constant, i.e, $K_{COD} = \widetilde{K}_{COD_{p,i\to w}} COD_{p,i}$
$\widetilde{K}_{COD_{p,i\to w}}/\widetilde{K}_{COD_{p\to w}}$	Transfer rate constant of COD from produce i, without subindex when only one product
K_X	Transfer rate constant of microbial load (X) from product when concentration of X in product is not known and included in the constant, i.e, $K_X = \widetilde{K}_{X_{p,i\to w}} X_{p,i}$
$\widetilde{K}_{X_{p,i\to w}}/\widetilde{K}_{X_{p\to w}}$	Transfer rate constant of microorganisms from product i to water, without subindex when only one product
$\widetilde{K}_{X_{w\to p,i}}/\widetilde{K}_{X_{w\to p}}$	Transfer rate constant of microorganism from water to produce i, without subindex when only one product
M_i/M	Mass of produce i in the tank, without subindex when only one product
M_{batch}	Total processed product in a batch
n	Hill exponent
t_{batch}	Total processed product in this batch
u	Additions of FC (either continuous or discrete additions) divided by tank volume

V	Volume tank
$X_{p,i}/X_p$	CFU in produce i, without subindex when only one product
X_w/X	Concentration of a relevant microorganism in water (for simplification of notation the w is sometimes ommited)
α	Inactivation rate coefficient
β	Degradation rate coefficient
γ	Yield coefficient COD degradation with FC
η	Relation between inactivation in the produce and in the water
λ	Natural inactivation rate of FC
τ	Residence time of product in the tank

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scatter plot colours represents the different operations. Distributions are estimated for the microbiological (y-axis) and COD (x-axis) transfer rates for each operation.

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List of Models

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Model 6. Dynamic mass balance model selected among different alternatives to understand water contamination and inactivation dynamics in the studied industrial cases. 384

Model 7. Mathematical model to study what-if scenarios, such as changes in FC additions, processed products or contact time. The model is an extension of model 6 including the FC dynamics allowing discrete and continuous addition of water and FC. 397

Appendix A **Benchmark papers**

The following papers were used as benchmark papers for the various RQs.

RQ1a: data

For viruses:

- Cuevas-Ferrando E, Allende A, Pérez-Cataluña A, Truchado P, Hernández N, Gil MI and Sánchez G, 2021. Occurrence and accumulation of human enteric viruses and phages in process water from the fresh produce industry. Foods, 10, 1853 2021. https://doi.org/10.3390/foods10081853

For bacteriology:

- Tudela JA, López-Gálvez F, Allende A, and Gil MI, 2019. Chlorination management in commercial fresh produce processing lines, Food Control, 106, https://doi.org/10.1016/j.foodcont.2019.106760.
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- Luo Y, Zhou B, Van Haute S, Nou X, Zhang B, Teng Z, Turner ER, Wang Q, and Millner PD, 2018. Association between bacterial survival and free chlorine concentration during commercial fresh-cut produce wash operation. Food Microbiology, 70, 120-12. https://DOI: 10.1016/j.fm.2017.09.013
- Luo Y, Nou X, Yang Y, Alegre I, Turner E, Feng H, Abadias M, and Conway W, 2011. Determination of free chlorine concentrations needed to prevent Escherichia coli O157:H7 cross-contamination during fresh-cut produce wash. Journal of Food Protection, 74 352-358. https:// DOI: 10.4315/0362-028X.JFP-10-429

RQ1b: Modelling

- Dunkin N, Weng SC, Jacangelo JG, and Schwab KJ, 2017. Inactivation of human norovirus genogroups I and II and surrogates by free chlorine in postharvest leafy green wash water. Applied and Environmental Microbiology 83, 22 e01457-17. https://doi.org/10.1128/AEM.01457-17.

RQ2a: data and methods on microbiological and physico-chemical parameters:

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- Van Haute S, Zhou B, Luo Y, Sampers I, Vanhaverbeke M, Millner P, 2019. The use of redox potential to estimate free chlorine in fresh produce washing operations: Possibilities and limitations, Postharvest Biology and Technology, 156, 110957, ISSN 0925-5214, https://doi.org/10.1016/j.postharvbio.2019.110957.
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- Li J, Teng Z, Weng S, Zhou B, Tumer ER, Vinyard BT, and Luo Y, 2019. Dynamic changes in the physico-chemical properties of fresh-cut produce wash water as impacted by commodity type and processing conditions, PLoS One 14(9):e0222174 https://dx.doi.org/10.1371%2Fjournal.pone.0222174
- Tudela JA, López-Gálvez F, Allende A, Hernandez N, Andujar S, Marin A, Garrido Y, and Gil MI, 2019. Operational limits of sodium hypochlorite for different fresh produce wash water based on microbial inactivation and disinfection by-products (DBPs). Food Control, 104 300-30. https://doi.org/10.1016/j.foodcont.2019.05.005
- Murray K, Aldossari H, Wu F, Warriner K, 2018. Dynamic changes in free-chlorine levels within a commercial post-harvest wash and prevention of cross-contamination between shredded lettuce batches. Food Control, 85, 127-134, ISSN 0956-7135, https://doi.org/10.1016/j.foodcont.2017.09.029.
- Tomás-Callejas A, López-Velasco G, Valadez A M, Sbodio A, Artés-Hernández F, Danyluk M D, Suslow TV, 2012. Evaluation of current operating standards for chlorine dioxide in disinfection of dump tank and flume for fresh tomatoes. Journal of Food Protection, 75, 304–313. https://doi: 10.4315/0362-028X.JFP-11-347
- Teng Z, Luo Y, Zhou B, Wang Q, and Hapeman C, 2021. Characterization and mitigation of chemical oxygen demand and chlorine demand from fresh produce wash water. Food Control, 127, 1008112

RQ2b: Modelling

- Chen X, Hung YC, 2016. Predicting chlorine demand of fresh and fresh-cut produce based on produce wash water properties. Postharvest Biology and Technology, 120, 10-15.
- Mundi GS, Zytner RG, Warriner K, Gharabaghi B,2017. Predicting fruit and vegetable processing wash-water quality. Water Science and Technology, 1), 256-269. https:// doi: 10.2166/wst.2018.109.
- Abnavi MD, Kothapalli CR, Munther D, Srinivasan P, 2021. Chlorine inactivation of *Escherichia coli* O157:H7 in fresh produce wash process: Effectiveness and modelling, International Journal of Food Microbiology, 356, ,109364, https://doi.org/10.1016/j.ijfoodmicro.2021.109364
- Srinivasan P, Abnavi MD, Sulak A, Kothapalli CRMunther D, 2020. Towards enhanced chlorine control: mathematical modelling for free chlorine kinetics during fresh-cut carrot, cabbage and

lettuce washing. Postharvest Biology and Technology 161, 111092. https://doi.org/10.1016/j.postharvbio.2019.111092.

RQ2c: inline/online methods

- Tudela JA, López-Gálvez F, Allende A, and Gil MI, 2019. Chlorination management in commercial fresh produce processing lines. Food Control 106: 106760. https://doi.org/10.1016/j.foodcont.2019.106760.

Appendix B **List of search terms used for the literature review**

When evaluating the results per RQ and including the number of benchmark papers found, the following final combinations of search strings were used per RQ as indicated in the table below.

Table B1. Details of search strings for literature searches performed on Scopus and Web of Science (2010 – present)

Set number	Search terms	No of records Scopus (a)	No of records WoS (a)
#1	**title-key-abstracts** (pathogen* OR "microbi* hazard*" OR bacteria* OR microbial* OR pathogen* OR total bacterial counts* or TC* or "viable but non-culturable*" or VBNC OR streptococcus OR "listeria monocytogenes" OR "l. monocytogenes" OR *virus* OR bacillus OR salmonella OR clostridium OR staphylococ* OR campylobacter OR "Escherichia coli" OR "E. coli" OR STEC OR yersinia OR shigella OR viral or surrogate* or NoV or HAV or HEV or MuNoV or MNV or Tulane* or MS2 or Mengo* OR FCV OR *calici* OR "microbial load" OR "microorganism count" OR *phage* OR O157 OR O104 OR "O:157" OR "O:104" OR "Shiga toxin*" OR Enterococ* OR VTEC OR EHEC OR Enterobacteriaceae OR coliform* OR EPEC OR parasite* or cryptosporidium or giardia or Cyclospora or C*rAssphage*)	1,263,357	2,625,839
#2	**title-key-abstracts** ("wash water" OR "wash-water" OR *washing OR "proces* water" OR "water quality" OR "wash* process" OR "tap water" OR "municipal water" OR "wash solution" OR "industrial water")	194,383	116,334
#2a	**title-key-abstract**s (post-harvest OR processing OR "wash* tank" OR cooling OR hydrocooling OR hydro-cooling OR blanching OR *sorting OR "dump* tank" OR "Water transport" OR drencher OR reused OR recirculated OR "flume tank" OR "produce wash*")	2,264,946	4,175,238

Set number	Search terms	No of records Scopus [a]	No of records WoS [a]
#3	**title-key-abstracts** ("mixed fruit*" OR "mixed vegetable*" OR "fresh produce" OR "fresh-cut produce" OR *fruit OR *berry OR *berries OR açai OR currant* OR grape OR citrus OR citron OR grapefruit OR lemon OR lime OR mandarin* OR orange OR tangerine OR *apple OR hawthorn OR loquat OR medlar OR pear OR quince OR apricot OR plum OR prune OR cherr* OR nectarine OR peach OR "Asian palmyra palm" OR avocado OR bael OR canistel OR coconut OR durian OR guava OR fig OR jujube OR kiwi OR langsat OR longan OR longkong OR lychee OR mafai OR mango* OR maprang OR papaya OR persimmon OR pitaya OR pomegranate OR rambutan OR roselle OR santol OR sapodilla OR soursop OR tamarind OR *melon OR cantaloupe OR honeydew OR galia OR "fruit* vegetable*" OR tomato* OR aubergine* OR eggplant* OR egg*plant OR pepper* OR courgette* OR zucchini* OR cucumber* OR cucurbit* OR gourd* OR pumpkin* OR squash* OR kabocha OR hokkaido OR tinda OR chilli* OR chili* OR okra OR *bean* OR *pea* OR "sweet corn" OR "leafy vegetable*" OR "green vegetable*" OR "mixed vegetable*" OR salad* OR arugula OR rucola OR "rocket lea*" OR "garden rocket" OR bitterleaf OR choy OR choi OR cabbage OR celery OR celtuce OR escarole* OR spinach OR chard OR chicory OR "mustard green*" OR "leafy green*" OR "collard green*" OR "beet green*" OR "microgreen*" OR "turnip green*" OR *cress OR endive OR epazote OR kale OR komatsuna OR lettuce OR mizuna OR mustard OR radicchio OR rapini OR tatsoi OR "turnip top*" OR "Chinese mallow" OR chickweed OR chaya OR "chrysanthemum green*" OR "fat hen" OR "fluted pumpkin" OR samphire OR "Greater plantain" OR "broadleaf plantain" OR "jute plant" OR karkalla OR "Lagos bologi" OR orache OR purslane OR sculpit OR stridolo OR soko OR "spleen amaranth" OR "brussel sprout*" OR carrot* OR arracacha OR "bamboo shoot*" OR beet* OR burdock OR chufa OR daikon OR *radish OR ginger OR turmeric OR gobo OR "hamburg parsley" OR horseradish OR *artichoke OR jicama OR mooli OR parsnip OR turnip OR salsify OR scorzonera OR skirret OR swede OR rutabaga OR "tiger nut*" OR tigernut OR ulluc* OR "water chesnut" OR wasabi OR yacón OR yacon OR asparagus OR cardoon OR celer* OR garlic OR kohlrabi OR kurrat OR keek OR "lotus root" OR nopal OR onion OR shallot OR *onion OR rhubarb OR "pie plant" OR samphire OR "bulb vegetable*" OR "stem vegetable*" OR "tuber vegetable*" OR "root vegetable*" OR "underground vegetable*" OR brocco* OR cauliflower* OR salad OR choi OR choy OR artichoke OR "courgette	4,787,168	3,473,755

Set number	Search terms	No of records Scopus [a]	No of records WoS [a]
	flower" OR "squash blossom" OR sprout* OR alfalfa OR "basil cress" OR "borage cress" OR mushroom* OR agaricus OR agrimonia OR agrocybe OR auricularia OR boletus OR clitocybe OR coprinus OR cortinarius OR craterellus OR flammulina OR ganoderma OR grifola OR gyromitra OR hericium OR hydnum OR hypsizygus OR lactarius OR lentinula OR lentinus OR lepista OR morchella OR pholiota OR pleurotus OR rhizopus OR sparassis OR stropharia OR terfezia OR tremella OR tricholoma OR tuber OR ustilago OR volvariella OR agaric OR agarikusutake OR "Callampa Agaricus" OR champignon* OR "Cogumelo do Sol" OR kawariharatake OR himematsutake OR cremini* OR portobello* OR matsutake OR "velvet pipoppini" OR "jew's ear*" OR "jelly ear*" OR porcini OR cèpe* OR "shaggy mane*" OR "lawyer's wig*" OR "cortinar webcap*" OR "trompette du mort" OR enoki OR lingzhi OR "hen-of-the-woods" OR maitake* OR "monkey's head*" OR "lion's mane*" OR "bear's head*" OR "hedgehog mushroom*" OR shimeji OR "indigo milk cap*" OR "candy cap*" OR "saffron milk cap" OR shiitake* OR "wood blewit*" OR morel* OR nameko OR "oyster mushroom*" OR "cauliflower mushroom*" OR roundhead* OR truffle* OR "paddy straw mushroom*" OR chanterelle* OR basil OR chervil OR chives OR cilantro OR coriander OR dill OR "lemon verbena" OR marjoram OR *mint OR oregano OR parsley OR rosemary OR sage OR savoury OR savory OR sorrel OR tarragon OR thyme OR "bay lea*" OR "Ocimum basilicum" OR "Anthriscus cerefolium" OR "Coriandrum sativum" OR "Anethum graveolens" OR "Aloysia citrodora" OR "Origanum majorana" OR "Mentha spicata" OR "Mentha piperita" OR "Origanum vulgare" OR "Petroselinum crispum" OR "Salvia rosmarinus" OR "Salvia officinalis" OR "Satureja hortensis" OR "Rumex acetosa" OR "Artemisia dracunculus" OR "Thymus vulgaris" OR "Laurus nobilis")		
#3a	**title-key-abstracts** (fresh OR frozen OR whole OR fresh-cut OR ready-to-eat OR cut OR diced OR sliced OR chopped OR shredded OR "minimally processed")	1,382,082	1,157,745
#4	**title-key-abstract** ("math* model" OR "mathematical description" OR dynamic* OR "kinetic model*"OR model* OR "model-based" OR "primary model" OR "secondary model" OR "equation*"OR "function*" OR "predictive microbiology" OR predict* OR regression OR correlation OR simulat* OR relationship OR distribution OR fitting OR calibration OR "Risk Assessment" OR "differential equation" OR EasyFit OR MicroHibro OR Combase OR Matlab OR Comsol	20,510,887	14,026,844

Set number	Search terms	No of records Scopus [a]	No of records WoS [a]
	OR Octave OR python OR Julia OR "R software" or "R package" or "Rstudio" or "package of R" or "R Core Team" OR NetLogo OR Bioinactivation OR "Microsoft excel" OR code OR "rate valu" OR "rate constant" OR "transfer constant" OR "inactivation*")		
#5	**title-key-abstract** ("physicochemical" OR acidity OR "chloride ion concentration" OR COD OR "chemical oxygen demand" OR "dissolved oxygen" OR "electrical conductivity" OR "five-day biochemical oxygen" OR "oxidation reduction potential" OR ORP OR "redox potential" OR pH OR salinity OR turbidity OR "total alkalinity" OR "total dissolved solid*" OR TDS OR "total suspended solid*" OR TSS OR "UV absorbance" OR "water temperature" OR disinfectant* OR sanitizer* OR residue* OR "peracetic acid*" OR "peroxyacetic acid*" OR PAA OR chlorin* OR "hydrogen peroxide" OR "sodium hypochlorite*" OR "calcium hypochlorite*")	1,701,044	1,276,061
#6	**title-key-abstract** ("in line" OR inline OR online OR "on line OR" automat* OR detection OR method* OR monitor* OR sensor* OR instrument* OR application* OR measurement OR "NIR spectroscopy" OR amperometr* OR "uv/vis spectro*" OR "Ultraviolet/visual spectro" OR "rapid monitoring" OR reflectometr* OR chronoamperometr* OR photometr* OR spectrophotometr* OR spectroscopy)	455,163	12,101,571
RQ1a[b]	**title-key-abstracts** (#1 AND #2) AND **title** (#3 AND #3a)	420	332
RQ1b[b]	**title-key-abstracts** (#1 AND #2) AND **title** (#3 AND #4)	175	170
RQ2a[b]	**title-key-abstracts** ((#1 OR #5) AND (#2 AND 2a AND #3a)) AND **title** #3	403	491
RQ2b[b]	**title-key-abstract** (#1 OR #5) AND #2 AND **title** (#3 AND #4)	328	306
RQ2c[b]	**title-key-abstract** (#1 OR #5) AND **title** (#2 AND #3 AND #6)	68	80

(a) Document type= all types; language = all language; time span = after 2009.

(b) For each research question, this search combination was considered the best in obtaining a feasible number of hits while retrieving the previously defined benchmark papers (see Appendix A). It was obtained from previous search trials and developed while improving the search terms.

Appendix C Data extraction from literature review

Papers that were evaluated as relevant based on title, keywords and abstracts were read in full and information extracted in two separate Excel sheets. The first sheet was for internal evaluation and included:

Table C1. Columns extracted for RQ1

Column	Column name	Description
A	**Ref**	Authors and publication year, e.g. Banach et al., 2017
B	**Title**	Title of the paper
C	**Type of paper**	Review, original article, book chapter
D	**IF**	The JCR impact factor of 2020 will be included, if available. Otherwise, SJR or CiteScore will be added
E	**Included/Excluded for further evaluation**	After reading the full text, the paper is either: 1. Included in the report (contains general relevant information) 2. Included for data extraction (Tier 2) 3. Excluded for further evaluation
F	**Rationale**	Rationale why to include or exclude the paper in the further evaluation (based on above mentioned inclusion or exclusion criteria or additional reasons)
G	**Included/Excluded for modelling**	After evaluating the full paper and discussion with the modelling group (CSIC-IMM), it was decided whether the paper contains relevant information for the modelling
H	**Rationale**	rationale why to include or exclude the paper in Tier 2 where all relevant data will be extracted
I	**Type of experiment**	Lab experiment, pilot experiment, industrial setting
J	**Nr of samples**	Number of samples
K	**Year of sampling**	Sampling period
L	**Region**	Continent: North America, South America, Europe, Asia, Australia, Africa
M	**country**	name of country in which the experiments were performed

Column	Column name	Description
N	**Food group**	Fruits, vegetables or herbs
O	**Food product**	Specific product(s) studied
P	**Type of format of FFVHs**	whole, fresh-cut, shredded, diced, frozen etc
Q	**Water source**	Source of water for filling the tank: **1) Surface water, 2) Well water, 3) Municipal tap water, 4) Recycled water, 5) Municipal + well water, 6) Municipal + recycled water**
R	**Water replenisment rate**	Time the water is used (% per product unit or time volume)
S	**Tank water volume (L)**	Amount of water in the tank used for the water treatment
T	**Product-water contact time range**	Time (min) the product is in contact with the water
U	**Handling or processing operation**	Cooling, dumping tank, hydrocooling, pre-sorting, pre-washing, washing
V	**Water agitation**	Air bubbling, water jet, none
W	**Microorganism group**	Pathogen, virus, parasite, hygiene indicator
X	**Microorganism**	Names of microorganisms tested
Y	**Microbial concentrations**	Range of concentrations during the experiment or number of table or graph containing the information
Z	**Type of water disinfectant**	None, sodium hypochlorite, calcium hypochlorite, peroxyacetic acid, hydrogen peroxide, ozone, others
AA	**Disinfectant time range**	Time the disinfectant was applied
AB	**Disinfectant set-point (dose)**	Dose of disinfectant used
AC	**Main message**	main conclusion of the article
AD	**Supporting information**	screenshots from tables or graphs

Table C2. Columns extracted additionally, for RQ2,

Column name	Description
Physico-chemical properties tested	Water T, pH, redox, type of UV (wavelength), Chemical oxygen demand (COD), electrical conductivity (EC), turbidity, total dissolved solds (TDS), total suspended solids (TSS), total hardness (TH), turbidity (TSS), residual concentration of disinfectant etc
Relationships found	Description of the relationship found between microbial and physico-chemical hazards (e.g. based on statistical analysis)
Methodology used	Method used to verify or validate microbial quality: off line, lab test, inline etc

Table C3. Columns extracted additionally for RQ2c

Column name	Description
Type of measurement	Inline or online
Method used	Sensor, spectrophotometer, UV/VIS spectrophotometer, amperometric probe, etc

Those papers that were evaluated as relevant for data extraction were compared by the EFSA WG to the papers selected by the EFSA WG members. The EFSA WG made a selection considering possible duplicates as well as the relevance for the modelling from which the following information was extracted:

Table C4. Data extraction columns aligned with the template file used by EFSA

Column	Column name	Description
A	Ref	Authors and publication year, e.g. Banach et al., 2017
B	Country	name of country in which the experiments were performed
C	Establishment category	size of the enterprise: small, medium-sized, large
D	Type of distribution	Business to Business (B2B) or Business to Consumer (B2C) or Both. This is not going to apply to many papers and probably very few papers may have this reported. When this information isn't reported in the papers or it does not apply to the relevant study you can indicate NA (= not available or not applicable)
E	Type of experiment (setting): review, lab, pilot plant, industrial	Lab experiment, pilot experiment, industrial setting
F	Type or source of water used	Source of water for filling the tank: municipal/tap water, rain water, process water, well water

Column	Column name	Description
G	Source of contamination/inactivation process studied	
H	Direct or indirect water use	product in contact with the water, e.g direct water use is the use in washing operations of the FVHs and indirect the use of water in the cleaning of boxes
I	Type FFV product (e.g. lettuce, spinach, etc.)	Specific product(s) studied
J	Type of production system	This refers to agricultural practices and the interest of extracting this information relates to possible emerging practices that may be associated also to emerging microbiological hazards when cultivating ffFVHs. Also in this case you may not need to fill in the information for this parameter if not available and can use NA as well.
K	Type of format of ffFVHs	whole, fresh-cut, shredded, diced, frozen etc
L	Handling and Processing operations	Cooling, dumping tank, hydro-cooling, pre-sorting, pre-washing, washing
M	Weight FFV (Kg)	weight of the produce washed in kg
N	Agitation during washing?	Air bubbling, water jet, none
O	Sampling point	point where the sample was taken
P	Volume water (L)	Amount of water in the tank used for the water treatment in L
Q	Ratio produce:water (calculated kg/l) (if available)	amount of produce washed divided by amount of water in the tank
R	Water replanieshment rate (% per product unit or time volume?)	Time the water is used (% per productunit or time volume)
S	Water Exchange	This refers to after how much time or days the water is fully changed e.g. in a washing tank.
T	Type of Water Disinfectant	None, sodium hypochlorite, calcium hypochlorite, peroxyacetic acid, hydrogen peroxide, ozone, others
U	Unit type	

Column	Column name	Description
V	Disinfectant set-point (Dose)	Dose of disinfectant used
W	Residual disinfectant concentration	
X	Sampling time (mins) (Contact time)	
Y	NTU (turbidity)	
Z	COD (mg/l)	
AA	Organic matter	
AB	T °C water	
AC	T °C FFV	
AD	pH	
AE	Conductivity (µS/cm)	
AF	Redox potential or oxidation reduction potential (ORP)	
AG	Microorganisms	Names of microorganisms tested
AH	One or multiple strains	
AI	Where the log CFU are inoculated	
AJ	Inoculation method in the ffFVH	
AK	log CFU initial (log CFU/mL or g) (MPN/ml or g)	
AL	On-line process monitorization? Which parameter?	AL -AQ columns refer to online monitorisation as well as methods followed by the industries for verification and validation of processes where water is used. The WG is doing a specific search to obtain this information, and we don't expect you to have a lot of info about these questions in the papers that you will retrieve with the search strategies that you will develop, so same reasoning applies here, in case no relevant information is available or reported in the publication please indicate NA.

Column	Column name	Description
AM	Process validation? Which parameter?	
AN	Process verification? Which parameter?	
AO	Method use for monitorization	
AP	Method use for validation	
AQ	Method use for verification	
AR	CULTURABLE: log CFU water/mL	culturable levels in the water should show in each of the multiple rows for each study the values of the data points obtained at different time and sampling points whereas AK should indicate the initial count/inoculum level placed in the water or in the product (when this available in the paper). AK may not be available depending on nature of the study.
AS	Log reduction CULTURABLE	
AT	CULTURABLE: log CFU product/g	
AU	Log reduction CULTURABLE	
AV	Method to enumerate CULTURABLE cells	
AW	Are VBNC cells enumerated?	
AX	VIABLE: log CFU water/mL	
AY	VBNC: log difference (VIABLE-CULTURABLE)/100 m<l	
AZ	DEAD = Log reduction (Initial - viable) water /100 mL	
BA	VIABLE: log CFU product/g	
BB	VBNC: log difference (VIABLE-CULTURABLE) product/g	

Column	Column name	Description
BC	DEAD = Log reduction (Initial - viable) product /sampling time	
BD	Is regrowth observed in the product? VBNC to culturable	
BE	Virulence_gene expression feature (both number and units, e.g., 2-fold, logs of invasion, etc.)	
BF	Disinfectant decay rate, disinfectant depletion rate	BF-BJ: answer with Yes or No. In case the specific rates are reported in the pare you could also indicate these under the column BZ 'Main conclusions and/or comments'.
BG	Is microbial transfer rate provided?	BF-BJ: answer with Yes or No. In case the specific rates are reported in the pare you could also indicate these under the column BZ 'Main conclusions and/or comments'.
BH	Is microbial inactivation rate provided?	BF-BJ: answer with Yes or No. In case the specific rates are reported in the pare you could also indicate these under the column BZ 'Main conclusions and/or comments'.
BI	Data inputs for the study (for models)	BF-BJ: answer with Yes or No. In case the specific rates are reported in the pare you could also indicate these under the column BZ 'Main conclusions and/or comments'.
BJ	Is modelling included in the paper?	BF-BJ: answer with Yes or No. In case the specific rates are reported in the pare you could also indicate these under the column BZ 'Main conclusions and/or comments'.
BZ	MAIN CONCLUSIONS AND/OR COMMENTS	main conclusion of the article

Appendix D **Results of Google Advanced Search**

Table D1. Results of the Google Advanced Search (Tier 1 based on Ti/Key/ABS) as performed on 22 September 2022 for RQ1 and rationale per evaluated report in case of exclusion in Tier 1

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
1) https://who.int		
https://apps.who.int/iris/rest/bitstreams/1401042/retrieve	Yes	
https://cdn.who.int/media/docs/default-source/food-safety/jemra/jemra-microbiological-hazards-in-fruits-vegetables-part1and2-summary-report.pdf?sfvrsn=152d08ba_15	No	No info on wash water
https://apps.who.int/iris/rest/bitstreams/1249847/retrieve	No	Irrigation water
https://apps.who.int/iris/bitstream/handle/10665/43193/9789241546690_eng.pdf;jsessionid	No	about ships
https://www.who.int/docs/default-source/food-safety/jemra/call-for-consultation/methodology-report-public-comments.pdf	No	No info on wash water
https://www.who.int/docs/default-source/wash-documents/wastewater-use/using-human-waste-safely---kit-1/recycling-realities---managing-health-risk-to-make-wastewater-an-asset.pdf	No	Irrigation water
https://apps.who.int/iris/bitstream/handle/10665/274939/9789241514705-eng.pdf	No	No info about microbiological contamination on fresh produce
https://apps.who.int/iris/bitstream/handle/10665/39313/9241544430_eng.pdf	No	about on-site sanitation of water
https://cdn.who.int/media/docs/default-source/food-safety/who-global-strategy-food-safety-2022-2030.pdf?sfvrsn=66cdef40_18&download=true	No	No info on wash water
https://apps.who.int/iris/bitstream/handle/10665/61297/WHO_HPP_FNU_93.1_eng.pdf?sequence=1	No	About food prepared by inhabitants along the peruvian amazon river
http://whqlibdoc.who.int/hq/1985-86/WHO_CDS_VPH_86.65.pdf	No	No info on wash water
http://apps.who.int/iris/bitstream/10665/193991/1/9789241565103_eng.pdf	No	Household washing
https://apps.who.int/iris/bitstream/handle/10665/40203/9241542063-eng.pdf?sequence=1	No	No info on wash water or fresh produce
https://apps.who.int/iris/rest/bitstreams/1031573/retrieve	No	Surface water
http://whqlibdoc.who.int/hq/pre-wholis/VPH_82.39.pdf	No	Noting about water
https://apps.who.int/iris/rest/bitstreams/1277753/retrieve	No	No info on wash water
http://whqlibdoc.who.int/publications/2011/9789241546690_eng.pdf	No	about ships
https://applications.emro.who.int/dsaf/dsa1203.pdf	No	About gray water from laundry

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
http://whqlibdoc.who.int/publications/2009/9789241598941_eng.pdf	Yes	
https://applications.emro.who.int/dsaf/dsa1203.pdf	No	About grey water from laundry
https://www.afro.who.int/sites/default/files/2017-10/Compendium_Report_Outbreaks_Fina%20_1.pdf	No	Nothing about our selected pathogens
https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/comments-on-iacg-discussion-papers-2nd-set.pdf?sfvrsn=137b60f_4	No	Comments of countries on discussion paper about AMR
http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf	Yes	
https://apps.who.int/iris/bitstream/handle/10665/255011/9789241565448-eng.pdf	No	About tropical diseases
https://applications.emro.who.int/imemrf/Egypt_J_Chem/Egypt_J_Chem_2018_61_5_883_896.pdf	No	Fresh produce' was mentioned in the reference list. Paper is about a membrane for wastewater reclamation
https://apps.who.int/iris/bitstream/handle/10665/36880/WHO_OFFSET_1_eng.pdf?sequence=17	No	About malaria
https://apps.who.int/iris/rest/bitstreams/1060492/retrieve	No	In beef and pork
https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/comments-on-iacg-discussion-papers-1st-set-270718.pdf?sfvrsn=e6099553_4	No	Comments of countries on discussion paper about AMR
http://apps.who.int › handle › 9789241565530-eng	No	Could not be evaluated, Google link not working
Code of Practice for Fish and Fishery Products	No	About fishery
https://apps.who.int/iris/bitstream/handle/10665/341822/WHO-PCS-2006.4.pdf?sequence=1	No	About pesticides
https://apps.who.int/iris/bitstream/handle/10665/207856/WPR_368_61_eng.pdf?sequence=1&isAllowed=y	No	Report on first zonal seminar on environmental sanitation
http://apps.who.int/iris/bitstream/handle/10665/136779/ccs_mmr_2014-18_9789290224495.pdf	No	Cooperation strategy Myanmar
http://apps.who.int/iris/bitstream/handle/10665/44635/9781843393108_eng.pdf?sequence=1	No	About drinking water in small communities
https://apps.who.int/iris/bitstream/handle/10665/44594/9789241548199_eng.pdf;sequence=1	No	About ship sanitation
https://apps.who.int/iris/bitstream/handle/10665/258760/seajph2017v6n2.pdf?sequence=1&isAllowed=y	No	About climate and environmental change in south east Asia
https://apps.who.int/iris/rest/bitstreams/1380862/retrieve	No	About infant and young child feeding
https://extranet.who.int/nutrition/gina/sites/default/filesstore/UGA%202010%20Agriculture%20Sector%20Development%20Strategy%20and%20Investment%20Plan.pdf	No	Development strategy agricultural sector Uganda
A key role for veterinary authorities and animal health ...	No	about parasitic zoonosis
Promoting Access to Medical Technologies and Innovation	No	Medical technologies, nothing about food

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
STATISTICS STATISTIQUES - World Health Organization (WHO)	No	World health statistics
MILK HYGIENE - Request Rejected	No	About milk
Microbiological Hazards in Fresh Leafy Vegetables and Herbs	Yes	
MANUAL ON INTEGRATED VECTOR MANAGEMENT	No	About insect vectors
Guidelines for personal protection when handling and ...	No	Guidelines personal protection
طسوتلما قشرل ةحصلا ةلحلما - Sign in	No	Could not be evaluated, Google link not working
Prévenir et combattre les maladies respiratoires aiguës à ...	No	Not in English
زا در سبزیجات برگی های بیماری های گیاهی بر میکروارگانیسم ...	No	Not in English
https://cdn.who.int/media/docs/default-source/food-safety/jemra/jemra-call-for-experts-and-data-water2021.pdf?sfvrsn=d20f66ce_5	No	Call for experts
14 June -2 July 2021 Experts participating in the meeting	No	List of experts
https://apps.who.int/iris/bitstream/handle/10665/249581/9789251090626-eng.pdf	No	Report about malnutrition
2) https://aesan.gob.es		
https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/evaluacion_riesgos/informes_cc_ingles/SODIUM_LAURIL_ETHER_SULPHATE.pdf	Yes	
https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/evaluacion_riesgos/informes_cc_ingles/CITROCIDE.pdf	Yes	
https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/evaluacion_riesgos/informes_cc_ingles/CITROCIDE_PLUS.pdf	Yes	
https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/control_oficial/2019_Informe_Zoonosis_One_Health_Union_Europea.pdf	No	Zoonosis report
https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/evaluacion_riesgos/informes_comite/COADYUVANTE_TECNOLOGICO_CITROCIDE_PLUS.pdf	No	Not in English
https://www.aesan.gob.es/AECOSAN/docs/documentos/publicaciones/revistas_comite_cientifico/comite_cientifico_18.pdf	No	Not in English
3) https://anses.fr		
https://www.anses.fr/en/system/files/EAUX2009sa0288EN.pdf	No	Report on effluents from animal by-product processing plants
4) https://food.gov.uk		
https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/acm891revised.pdf	No	Advice on re-washing salad at home

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.food.gov.uk/sites/default/files/media/document/irrigation-water-report-final.pdf	No	Irrigation water
https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/acm1116refs.pdf	No	List of papers
https://acmsf.food.gov.uk/sites/default/files/annex_b_campylobacter_report.pdf	No	Nothing about wash water
https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/acm479.pdf	No	about clostridium toxin production
https://acmsf.food.gov.uk/sites/default/files/2021-04/ACM-1359%20Items%20of%20interest%20from%20literature_0.pdf	No	List of papers
https://www.food.gov.uk/sites/default/files/media/document/Modelling%20framework%20to%20quantify%20the%20risk%20of%20AMR%20exposure%20via%20food%20products-%20example%20of%20chicken%20and%20lettuce.pdf	Yes	
https://acmsf.food.gov.uk/sites/default/files/campylobacter_consultation_letter_annex_a.pdf	No	Report of committee board
https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/acm613.pdf	No	Call for committee board
https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/acm484.pdf	No	Food-borne zoonosis
https://www.food.gov.uk/sites/default/files/media/document/FS101120%20NoV%20critical%20review%20report%20-%20FINAL%203%20June%202015.pdf	Yes	
https://www.food.gov.uk/sites/default/files/media/document/fs301020finreport.pdf	No	Not about wastewater from washing, but from other sources
https://www.food.gov.uk/sites/default/files/media/document/775-1-1323_FS425012.pdf	No	About fish
https://www.food.gov.uk/sites/default/files/media/document/reducing-campylobacter-cross-contamination-during-poultry-processing_0.pdf	No	About chicken
https://www.food.gov.uk/sites/default/files/media/document/assessment-comparing-meat-production-processes-in-selected-countries.pdf	No	About meat
https://www.food.gov.uk/sites/default/files/media/document/fs121014afinalreport.pdf	No	Campylobacter in slaughterhouses
https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/acm-1100-cerf1.pdf	No	Wash water from dairy parlour
https://cot.food.gov.uk/sites/default/files/cot/cotannualrep2006.pdf	No	About chemicals in food
5) https://fda.gov		
Guide to Minimize Microbial Food Safety Hazards for Fresh ...	Yes	
Fresh-Cut Produce Draft Guidance - FDA	Yes	
Commodity Specific Food Safety Guidelines for the Lettuce ...	Maybe	Maybe no quantitative data

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
Evaluation and Definition of Potentially Hazardous Foods | FDA	No	Nothing about wash water of fruits/veg/herb
Commodity Specific Food Safety Guidelines for the Fresh ...	No	No quantitative data
Commodity Specific Food Safety Guidelines for the Melon ...	No	No quantitative data
Commodity-Specific-Food-Safety-Guidelines-for-the ... - FDA	No	No quantitative data
Final Qualitative Assessment of Risk to Public Health from On ...	Maybe	Not sure if there is quantitative data
Fresh Culinary Herbs - FDA	No	Advice document and tells what to do, no quantitative data
Environmental Assessment for Food Contact Notification - FDA	No	Environmental assessment chemicals used in wash water
Hazard Analysis and Risk-Based Preventive Controls ... - FDA	No	Guidance document for industry, no quantitative data
Summary Report: Hot Peppers - FDA	Maybe	Sampled water, not sure what kind of water
commodity specific food safety guidelines for the production ...	No	Guidelines for industry
PART IV – ENVIRONMENTAL INFORMATION B - FDA	No	Environmental assessment chemicals used in wash water
Classification of Activities as Harvesting, Packing, Holding, or ...	No	Guidelines for industry
FDA Food Code 2017	No	General code for industry (e.g., restaurants)
Hazard Analysis and Risk-Based Preventive Controls ... - FDA	No	Draft guidelines for industry
Guidance for Industry: Sprouts - FDA	No	Guidelines for industry
ORA Outbreak Response Field Guide #1 - FDA	No	Outbreak response guide
Environmental Assessment for Food Contact Notification FCN ...	No	Environmental assessment chemicals used in wash water
Growers' Understanding and Implementation of FDA's GAPs ...	No	No info about pathogens
Report to Congress	Maybe	Not sure if there is quantitative data
FDA's CORE: A Food Safety Network 2011-2012	No	Outbreak reports
FDA Procedures for Standardization of Retail Food Safety ...	No	Manual for FDA food code
GRAS Notice 857, Phospholipase A1 produced by Aspergillus ...	No	Toxin from aspergillus in vegetable oil
K914145.pdf - Accessdata.fda.gov	No	Safety evaluation of a medical drug
GRAS Notification for Chlorine Dioxide and Withdrawal ... - FDA	No	about chlorine dioxide in wash water, nothing about pathogens

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
FDA Commissioner's Fellowship Program Class of 2010	No	Could not be evaluated, Google link not working
6) https://bfr.bund.de		
SPICES & HERBS – A Risk-Free Taste Experience? | BfR	Yes	
5th World Congress - Foodborne Infections and Intoxications	No	No data on water samples, only product
7) https://fao.org		
https://agris.fao.org/agris-search/search.do?recordID=US201800077106	No	infection risk of enteric pathogens from raw vegetable consumption washed with contaminated water; not focused on the contamination of wash water
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B53-2003%252FCXC_053e.pdf	No	Code of hygienic practice for fresh and vegetables
https://agris.fao.org/agris-search/search.do?recordID=US202000136935	maybe	susceptibility of foodborne pathogens to FC and PAA as simulated wash water (maybe relevant for 1b)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/ar/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FCRD%252Ffh51_crd23x.pdf	No	Codex committee on food hygiene, guideline for safe use and reuse of water in food production (2019)
https://agris.fao.org/agris-search/search.do?recordID=US201900342786	No	efficiency of wash waster additive/ decontamination
https://agris.fao.org/agris-search/search.do?recordID=US201900361060	No	efficiency of water-assisted decontamination system
https://agris.fao.org/agris-search/search.do?recordID=US201700154655	No	
https://www.fao.org/3/i0452e/i0452e00.pdf	maybe	review study on microbiological hazards in fresh leafy vegetables and herbs. See chapter 7. processing
https://www.fao.org/3/au623e/au623e.pdf	maybe	a review study on microbiological hazards in melon, see chapter 6.2- washing and sanitizing
https://agris.fao.org/agris-search/search.do?recordID=US201800013341	maybe	microbial population shifts on citrus carpoplane and the impact of irrigation and packinghouse processing water in four commercial farm
https://agris.fao.org/agris-search/search.do?recordID=US202100178280	maybe	testing efficacy of chemical disinfection against microorganism in wash water

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://agris.fao.org/agris-search/search.do?recordID=US201800305818	maybe	routes of contamination of ready-to-eat vegetables in the Middle east (out of Europe)
https://agris.fao.org/agris-search/search.do?recordID=PH2017000221	No	quality, safety, marketability of fresh-cut tropical fruits, full text not available
https://www.fao.org/3/i0096e/i0096e00.pdf	No	risk based food inspection manual; general document, not focused on wash water
https://www.fao.org/3/y5431e/y5431e.pdf	No	post-harvest management in assuring quality and safety of horticultural produce (2004); no contamination data
https://www.fao.org/3/i3901e/i3901e.pdf	No	food losses and waste in sustainable food system
https://www.fao.org/3/W7429E/w7429e0n.htm	No	working document FAO
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/it/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-52%252FLinks%252FINFORMATIONPACKAGESTECWORKINGGROUP.pdf	No	working document info package Codex committee on food hygiene on the draft" proposed guideline for the control of STEC in raw beef, fresh leafy vegetables, ..." (2022)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/ar/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FWD%252Ffh51_08_add1e.pdf	No	working document Codex committee on food hygiene on proposed guideline for the control of STEC in raw beef, fresh leafy vegetables, ..." (2019)
https://www.fao.org/fao-who-codexalimentarius/download/standards/13215/CXG_079e.pdf	No	broken link
https://www.fao.org/fileadmin/user_upload/IPM_Pesticide/JMPR/Evaluations/2017/ISOPYRAZAM__249_.pdf	No	Isopyrazam (fungicides), not about microbial contamination in wash water
http://extwprlegs1.fao.org/docs/pdf/phi209511.pdf	No	Food safety guidance for urban and peri-urban farms
https://www.fao.org/3/i5739e/i5739e.pdf	No	Training manual for GAP for fruits and vegetables
https://www.fao.org/3/cb4476en/cb4476en.pdf	No	not about contamination in wash water
http://extwprlegs1.fao.org/docs/pdf/kor190522.pdf	No	working document (guideline), too general
https://www.fao.org/home/en	No	Could not be evaluated, Google link not working
https://www.fao.org/3/ca9731en/ca9731en.pdf	No	about food security

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/3/i1357e/i1357e.pdf	maybe	Report of Joint FAO/WHO meeting on the use of chlorine-containing disinfectant in food production (2008)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FShared%2BDocuments%252FArchive%252FMeetings%252FCCFAC%252Fccfac37%252FFA37_16e.pdf	No	Codex committee on food additives
https://elearning.fao.org/blocks/mtfaocourse/redirect.php?tp=txtsolr&cid=393&cmid=704&src=aHR0cHM6Ly9lbGVhcm5pbmcuZmFvLm9yZy9wbHVnaW5maWxlLnBocC81MDEyOTMvYmxvY2tfbXRmYW9jb3Vyc2UvYXR0YWNobWVudHR4dHNvbHIvMzkzL05TQV9lbl9JbmRleEZpbGUuZG9jeA==	No	Working document (guideline)
https://www.fao.org/3/ca7430en/CA7430EN.pdf	No	Guideline for personal protection when applying pesticides
http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-14%252Fal78_13Ae.pdf	No	Codex committee on food hygiene (1977)
https://www.fao.org/3/i2382e/i2382e.pdf	No	Code of practice for fish and fishery
https://www.fao.org/3/contents/b31aee0d-5bee-517b-afbb-e40e51bf52fd/i2678e00.pdf	No	Could not be evaluated, Google link not working
https://www.fao.org/3/CA1201EN/ca1201en.pdf	No	transforming livestock for through SDGs
https://www.fao.org/fileadmin/user_upload/food-loss-reduction/Nairobi_congress/Peer-reviewed_conference_proceedings_-_All_Africa_PH_Congress_and_Exhibition__003_.pdf	No	conference proceeding
https://www.fao.org/3/i5317e/I5317E.pdf	No	intervention for control of non-typhoidal Salmonella in beef and pork
http://extwprlegs1.fao.org/docs/pdf/chn65190.pdf	No	Regulation in China for certification
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/fr/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B52-2003%252FCXC_052e.pdf	No	Code of practice for fish and fishery
https://www.fao.org/home/404	No	Could not be evaluated, Google link not working
https://www.fao.org/input/download/report/695/al31_18e.pdf	No	Code of practice for fish and fishery
https://www.fao.org/fileadmin/templates/lon/EGM_on_Nutrition/EGM_Background_Document.pdf	No	Expert Group Meeting on Nutrition and the SDGs under Review in Preparation for the High-Level Political Forum
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/de/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-722-18%252Fal89_18e.pdf	No	Code of practice for fish and fishery
https://www.fao.org/3/bl094e/bl094e.pdf	No	Environmental performance of pig supply chains

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FCircular%252520Letters%252FCL%2525202021-35-OCS%252Fcl21_35f.pdf	No	document in French
http://extwprlegs1.fao.org/docs/pdf/bhu159194.pdf	No	livestock regulation in Bhutan
https://www.fao.org/3/c1243e/c1243e.pdf	No	land degradation
http://faolex.fao.org/docs/texts/ind69299.doc	No	working document quality control for egg products
https://www.fao.org/input/download/report/930/REP16_FFPe.pdf	No	Code of practice for fish and fishery
https://www.fao.org/input/download/report/638/al28_13e.pdf	No	Codex report on food hygiene (2005) for egg products
https://www.fao.org/3/i0452e/i0452e.pdf	No	duplicate no. 8
http://extwprlegs1.fao.org/docs/pdf/USA181755.pdf	No	Eggs products inspection
https://www.fao.org/home/404	No	Could not be evaluated, Google link not working
http://extwprlegs1.fao.org/docs/pdf/ita192138.pdf	No	Italy's plan for sustainable use of plant protection product
https://www.fao.org/cofi/46220-0c01d74940144a468674c816958a1889f.pdf	No	Working document on fisheries
https://www.fao.org/3/cb7664en/cb7664en.pdf	maybe	Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables (November 2021)
https://www.fao.org/uploads/media/1008_IEA_Bioenergy_-_Current_status_and_potential_for_algal_biofuels_production.pdf	No	algal biofuels production
https://www.fao.org/3/at333e/at333e.pdf	No	Ethiopian soybeans and sunflowers value chains
http://www.fao.org/input/download/report/53/al91_13e.pdf	No	Codex committee on food hygiene (1989)
https://www.fao.org/3/ca6062en/CA6062EN.pdf	maybe	a review study on safety and quality of water used in food production and processing (maybe already identified from scopus and WoS?)
https://www.fao.org/home/en	No	Could not be evaluated, Google link not working
http://extwprlegs1.fao.org/docs/pdf/swa196350.pdf	No	dairy regulation

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-52%252Ffh52_08e.pdf	maybe	proposed draft guideline for the safe use and re-use of water in food production. This doc is quite recent, maybe interesting to check (2022)
https://www.fao.org/3/y5431e/y5431e05.htm	No	postharvest treatment to minimize contamination (2002), not about wash water
https://www.fao.org/3/x8735e/x8735e0n.htm	No	proposed draft for hygienic practice for primary production, harvesting, and packing of fresh fruits; not clear from which year
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/fr/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-48%252FReport%252FDraft%252FDraft%2BApp%2BIII.pdf	No	proposed draft for hygienic practice for fresh fruits and vegetables (2003)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-52%252Ffh52_08_add1e.pdf	No	comments to document no 60
https://www.fao.org/fsnforum/cfs-hlpe/sites/default/files/discussions/contributions/Thim-%20Low%20cost%20organic%20Agriculture%20manual.pdf	No	Could not be evaluated, Google link not working
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/fr/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-48%252FCRDs%252Ffh48_CRD08x.pdf	No	working document (draft) on hygienic practice for fresh fruits and vegetables (2016)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/pt/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-48%252FWorking%2BDocument%252Ffh48_06Add1e.pdf	No	comments for proposed draft revision for code of hygienic practice for fresh fruits and vegetables (2016)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/ru/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-48%252FWorking%2BDocument%252Ffh48_06e.pdf	No	proposed draft for code of hygienic practice for fresh fruits and vegetables (2016)
https://www.fao.org/3/i1909e/i1909e00.pdf	No	guideline for processing fresh-cut tropical fruits and vegetables (2011)
https://www.fao.org/home/404	No	Could not be evaluated, Google link not working
https://www.fao.org/3/y2515e/y2515e.pdf	No	principles and practices for SME fruit juice processing
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-52%252Ffh52_07e.pdf	maybe	proposed draft guideline for the control of STEC in raw beef, fresh leafy vegetables, ... (2022)
https://www.fao.org/3/s8620e/S8620E07.htm	No	factors to reduce losses in horticultural system

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/3/a1389e/a1389e00.pdf	maybe	Codex documents for fresh fruits and vegetables
https://www.fao.org/3/ak832e/ak832e.pdf	No	good practice post-harvest in Jamaica (2008)
https://www.fao.org/3/ae075e/ae075e11.htm	No	insect control during production
https://www.fao.org/3/i0782e/i0782e01.pdf	No	part of a guideline, not focus on washing/wash water
https://www.fao.org/3/x4296e/x4296e02.htm	No	Codex document on bottled/ packaged water
https://www.fao.org/3/i2448e/i2448e00.pdf	No	risk categorization of food and food establishments in Asian countries
https://www.fao.org/3/cb3839en/cb3839en.pdf	No	Call for experts on safety and quality of water in fishery and dairy
https://www.fao.org/3/x4296e/x4296e.pdf	No	Codex committee on food hygiene (2001)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FCircular%252520Letters%252FCL%2525202021-35-OCS%252Fcl21_35e.pdf	No	working document on guidelines on control of STEC
https://www.fao.org/3/i0782e/i0782e.pdf	No	horticultural chain management
https://www.fao.org/3/cb4955en/cb4955en.pdf	No	expert participation meeting "clean water"
https://www.fao.org/3/i8148en/I8148EN.pdf	No	guide to preventing post-harvest loss of apples in Lebanon
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FWD%252Ffh51_03e.pdf	No	Working document on food hygiene, not on wash water
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-48%252FReport%252FFinal%252FREP17_FHe.pdf	No	Codex report on food hygiene (2016)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/ar/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FCRD%252Ffh51_crd15x.pdf	No	discussion paper on principles for the safe use of water in food processing, only general info
https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation14/Dimethomorph.pdf	No	evaluation of dimethomorph (fungicides)
https://www.fao.org/3/i1357e/i1357e00.pdf	No	duplicate no. 28
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/ar/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FWD%252Ffh51_09e.pdf	No	discussion paper on principles for the safe use of water in food processing, only general info

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/3/bc273e/bc273e.pdf	No	grafting in-vitro of citrus plant
https://www.fao.org/3/i1357e/i1357e01.pdf	maybe	a review study, not clear from which year (> 2009), focus on use of chlorine as disinfectant
https://www.fao.org/3/a1505e/a1505e.pdf	No	implementing programmes to improve safety and quality of fruit and vegetable supply chains in Latin America (2007)
https://www.fao.org/3/i5347e/i5347e.pdf	No	microbial safety of lipid-based ready-to-use foods for malnutrition
https://www.fao.org/3/a1125e/a1125e05.pdf	No	overview of boscalid (fungicides)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-50%252FWD%252Ffh50_03e.pdf	maybe	work document FAO, microbiological risk assessment for water use in fresh produce (pre and post-harvest) and other products
https://www.fao.org/3/i1645e/i1645e00.pdf	No	GAP for horticultural production
https://www.fao.org/3/a1553e/a1553e00.pdf	No	code for fishery
http://www.fao.org/input/download/report/787/REP13_FHe.pdf	No	Codex draft report on food hygiene (2012)
http://www.fao.org/input/download/report/116/al03_13e.pdf	No	Codex draft report on food hygiene (2003)
https://www.fao.org/3/x8735e/x8735e.pdf	No	Codex draft report on food hygiene (2000)
https://www.fao.org/3/i4819e/i4819e.pdf	No	Nutritional and societal protection
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-701-43%252FWorking%2Bdocuments%252Fcac43_09e.pdf	No	Proposal for New York Codex program
http://www.fao.org/input/download/standards/13215/CXG_079e.pdf	No	guidelines on control of virus in food
http://www.fao.org/input/download/report/753/REP11_FHe.pdf	No	Report/ guideline on joint committee on food hygiene (2010)
https://www.fao.org/3/i3215e/i3215e.pdf	No	seafood safety and quality
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/de/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FReport%252FREP20_FHe.pdf	No	Report of Codex committee on food hygiene (2019)
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/de/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FReport%252FREP20_FHe.pdf	No	Report of Codex committee on food hygiene (2019)
https://www.fao.org/3/at509e/at509e.pdf	No	food safety manual for farmer field schools (Vietnam)

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/PT_Water/Docs/HLPE-Water-and-Food-Security_V0-Draft-1Oct2014.pdf	No	water and food security (draft)
https://www.fao.org/3/cb0658en/CB0658EN.pdf	No	Code of practice for fish and fishery
https://www.fao.org/3/w7429e/w7429e.pdf	No	Report of Codex committee on food hygiene (1999)
https://www.fao.org/3/cb0658en/CB0658EN.pdf	No	Code of practice for fish and fishery
https://www.fao.org/3/av045e/av045e.pdf	No	water for food security and nutrition
https://www.fao.org/3/I9610EN/i9610en.pdf	No	food loss analysis in tomato chain
https://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/HLPE_Reports/HLPE-Report-9_EN.pdf	No	water for food security and nutrition
https://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/PT_Food_Losses/Docs/HLPE_Food-Losses_Waste_Draft-V0_23-Dec-2013.pdf	No	food loss and waste
https://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/PT_Nutrition/Docs/HLPE-Nutrition-and-Food-Systems_Draft-V0-24_October_2016.pdf	No	nutrition and food system
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-47%252FSalmonella%2Bexpert%2Bmeeting%2Breport%2B-%2BOct%2B20%2B%25282%2529.pdf	No	Interventions on non-typhoidal Salmonella in beef and pork
https://www.fao.org/3/cb1306en/CB1306EN.pdf	No	smart irrigation - smart wash in Africa
http://www.fao.org/input/download/report/524/al79_13e.pdf	No	codex committee document from 1978
http://www.fao.org/input/download/report/734/al33_13e.pdf	No	draft guideline on food hygiene (2010)
https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation2017/FOSETYL-ALUMINIUM__302___PHOSPHONIC_ACID__301_.pdf	No	overview of fosetyl-aluminium
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-52%252Ffh52_07f.pdf	No	document in French
https://www.fao.org/3/cb1447en/online/cb1447en.html	No	water challenge in the future
https://www.fao.org/3/ne664en/NE664EN.pdf	No	Report on food security and nutrition
https://www.fao.org/3/cb6597en/cb6597en.pdf	No	freshwater macrophytes
https://www.fao.org/fileadmin/user_upload/IPM_Pesticide/JMPR/Evaluations/2014/dimethomorph.pdf	No	overview of dimethomorph
https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation11/Pyraclostrobin.pdf	No	overview pyralclostrobin

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/3/ah928e/ah928e.pdf	No	Report FAO consultant in Bhutan
http://www.fao.org/input/download/report/54/al93_13e.pdf	No	codex committee document from 1993
http://www.fao.org/input/download/report/517/al97_37e.pdf	No	codex committee document from 1997
https://www.fao.org/3/br269e/br269e.pdf	No	Global forum on food security and nutrition
https://www.fao.org/input/download/report/371/Al03_18e.pdf	No	Codex committee on fish and fishery products
https://www.fao.org/3/y9155b/y9155b0h.htm	No	the Effects of EU ban on Kenyan fisheries
https://www.fao.org/3/CA3182EN/ca3182en.pdf	No	wasabi cultivation in Japan
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-722-18%252Fal89_18e.pdf	No	Codex committee on fish and fishery products
https://www.fao.org/3/ca6798en/CA6798EN.pdf	No	FAO- China South-South Cooperation program
https://www.fao.org/3/i6494e/i6494e.pdf	No	environmental performance of large ruminants supply chains
https://www.fao.org/3/y5169e/y5169e.pdf	No	small-scale poultry production
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-52%252Ffh52_07s.pdf	No	document not in English
https://www.fao.org/3/w9253e/w9253e0o.htm	No	proposed draft for fish and fishery products
http://www.fao.org/input/download/report/523/al78_13Ae.pdf	No	codex committee document from 1977
http://www.fao.org/input/download/report/595/cx75_17e.pdf	No	code of principles concerning milk and milk products
https://www.fao.org/fsnforum/sites/default/files/files/132_decade_nutrition/PROCEEDING_EN_Decade_Nutrition.docx	No	Global forum on food security and nutrition
https://www.fao.org/docrep/018/ar122e/ar122e.pdf	No	organic recycling in Africa
https://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/PT_Water/Docs/HLPE_Water_food_Security_eConsultation_Proceedings-DRAFT-V0.pdf	No	water and food security (draft)
https://www.fao.org/3/ar122e/ar122e.pdf	No	organic recycling in Africa
https://www.fao.org/fileadmin/templates/tc/tce/pdf/Southern_African_Region_2008.pdf	No	preparedness response for floods
https://www.fao.org/3/av152e/av152e.pdf	No	environmental performance of large ruminants supply chains

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
https://www.fao.org/fileadmin/user_upload/aquatic-genetic-resources/FAMS_TBS_MG15.pdf	No	freshwater macrophytes
http://www.fao.org/input/download/report/597/cx78_19e.pdf	No	code of principles concerning milk and milk products
https://www.fao.org/fao-who-codexalimentarius/sh-proxy/de/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FCircular%252520Letters%252FCL%2525202021-35-OCS%252Fcl21_35s.pdf	No	document not in English
https://www.fao.org/fishery/docs/CDrom/bobp/cd1/Bobp/Publns/Reports/0084.pdf	No	expert consultation on cleaner fishery harbours
http://extwprlegs1.fao.org/docs/pdf/mdv172921.pdf	No	Maldives environment project
http://www.fao.org/input/download/report/369/al99_18e.pdf	No	Code of practice for fish and fishery
http://www.fao.org/input/download/report/364/al89_18e.pdf	No	Code of practice for fish and fishery
https://www.fao.org/fileadmin/user_upload/IPM_Pesticide/JMPR/Evaluations/2019/Valifenalate__318_.pdf	No	overview of valifenalate
http://extwprlegs1.fao.org/docs/pdf/uga152492.pdf	No	agriculture sector development strategy
http://www.fao.org/input/download/standards/10273/CXP_052e.pdf	No	Code of practice for fish and fishery
https://www.fao.org/fileadmin/templates/library/pdf/Soli_pollution__FAO_.pdf	No	soil pollution
https://www.fao.org/3/I3649ES/i3649es.pdf	No	document not in English
https://agris.fao.org/agris-search/search.do?recordID=CH2018113275	No	effects of disinfectant on preventing cross-contamination in fresh produce wash water
https://agris.fao.org/agris-search/search.do?recordID=US201800306065	maybe	efficacy of commercial washing treatments
https://www.fao.org/3/contents/b31aee0d-5bee-517b-afbb-e40e51bf52fd/i2678e00.pdf	No	not found
https://agris.fao.org/agris-search/search.do?recordID=DJ2022093044	maybe	Detection and Quantification Methods for Viable but Non-culturable (VBNC) Cells in Process Wash Water of Fresh-Cut Produce (maybe for 2a or 2c)
https://agris.fao.org/agris-search/search.do?recordID=NL2020060934	maybe	efficacy of chemical sanitizers to revent cross-contamination in the washing tank
https://agris.fao.org/agris-search/search.do?recordID=DJ20220138342	maybe	efficacy of chlorine dioxide to prevent microbial contamination in process wash water measured with several physico-chemical parameters (maybe for 2a)
https://www.fao.org/3/cc2007en/cc2007en.pdf	maybe	Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on the

Title (URL hyperlink)	Relevant (Yes/No)	Rationale when not relevant
		Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables (June 2022)

Table D2. Results of the Google Advanced Search as performed on 20 September 2022 for RQ2 the rationale per evaluated report in case of exclusion in Tier 1

Title (URL hyperlink)	Relevant (Yes/ No)	Rationale when not relevant
1) https://who.int		
Viruses in food: scientific advice to support risk management activities 13 MICROBIOLOGICAL RISK ASSESSMENT SERIES	Yes	
Safety and quality of water used with fresh fruits and vegetables 37 MICROBIOLOGICAL RISK ASSESSMENT SERIES	Yes	
Safety and Quality of Water Used in Food Production and Processing 33 MICROBIOLOGICAL RISK ASSESSMENT SERIES	Yes	
Draft Guidance of Microbiological Risk Assessment for Food, Public consultation, June 2020	No	Focus on food/drinking water, not on processing, washing etc.
Guide to ship sanitation, 3rd edition, 2011	No	Focus on ship sanitation
PROTECTING SURFACE WATER FOR HEALTH, IDENTIFYING, ASSESSING AND MANAGING DRINKING-WATER QUALITY RISKS IN SURFACE-WATER CATCHMENTS	No	Focus on surface water, not on food processing, washing water
Benefits and Risks of the Use of Chlorine-containing Disinfectants in Food Production and Food Processing, May 2008	Yes	
Safety evaluation of certain food additives. WHO food additives series 54	No	Focus on food additives
Interventions for the Control of Non-typhoidal Salmonella spp. in Beef and Pork 30 MICROBIOLOGICAL RISK ASSESSMENT SERIES	No	Focus on beef and pork, not on fruit, vegetables, herbs
CODE OF PRACTICE FOR FISH AND FISHERY PRODUCTS	No	Focus on fish and fishery products, not fruit, vegetables, herbs
2) https://aesan.gob.es		
Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the use of an antimicrobial aqueous solution containing hydrogen peroxide, acetic acid and peroxyacetic acid as a processing aid on citrus fruits and peppers, and their wash water, AESAN-2013-002	Yes	
Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) in relation to the use of an antimicrobial aqueous solution containing hydrogen peroxide, acetic acid and peroxyacetic acid (23/17/15) as a processing aid on citrus fruits and tomatoes, and their wash water, AECOSAN-2016-002	Yes	

Title (URL hyperlink)	Relevant (Yes/ No)	Rationale when not relevant
The European Union One Health 2019 Zoonosis Report European Food Safety Authority European Centre for Disease Prevention and Control, doi: 10.2903/j.efsa.2021.6406	No	Focus on zoonosis
3) https://anses.fr		
4) https://food.gov.uk		
ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD INFORMATION PAPER, Items of interest from the literature, ACM/1116	No	Only list of references
A review of the published literature and current production and processing practices in smoked fish processing plants with emphasis on contamination by Listeria monocytogenes, FS425012	No	Focus on smoked fish processing
Identification and prioritisation of risks to food safety and quality associated with the use of recycled waste-derived materials in agriculture and other aspects of food production, FS301020	No	Focus on reuse of waste streams
All references - Food Standards Agency -> Excel database with references for FSA project FS101120 about norovirus in food and on contact surfaces	No	Only list of references in excel file
5) https://fda.gov		
A Report of the Institute of Food Technologists for the Food and Drug Administration of the United States Department of Health and Human Services, IFT/FDA Report on Task Order 4	Yes	
FDA Commissioner's Fellowship Program Class of 2010	No	Only list of participants in a fellowship program
6) https://bfr.bund.de		
7) https://fao.org		
Safety and quality of water used with fresh fruits and vegetables 37 MICROBIOLOGICAL RISK ASSESSMENT SERIES https://www.fao.org/3/cb7678en/cb7678en.pdf (in WHO search)	*Yes*	
INFORMATION PACKAGE FOR THE Working group on the proposed draft "guidelines for the control of Shiga toxin producing Escherichia coli (STEC) in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses and sprouts: annexes", WG STEC Info	Yes	
Viruses in food: scientific advice to support risk management activities 13 MICROBIOLOGICAL RISK ASSESSMENT SERIES – broken link on FAO site - ***(in WHO search)***	*Yes*	
REPORT OF THE THIRTY-SEVENTH SESSION OF THE CODEX COMMITTEE ON FOOD HYGIENE, ALINORM 05/28/13	No	Meeting report, no particular focus on water use
REPORT OF THE THIRTY- FOURTH SESSION OF THE CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS, REP 16/FFP	No	Focus on fish and fishery products, not fruit, vegetables, herbs

Title (URL hyperlink)	Relevant (Yes/ No)	Rationale when not relevant
CODE OF PRACTICE FOR FISH AND FISHERY PRODUCTS https://www.fao.org/fao-who-codexalimentarius/sh-proxy/fr/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B52-2003%252FCXC_052e.pdf (in WHO search)	*No*	*Focus on fish and fishery products, not fruit, vegetables, herbs*
FAO Standards http://extwprlegs1.fao.org/docs/pdf/kor190522.pdf	No	General FAO standards
Interventions for the Control of Non-typhoidal Salmonella spp. in Beef and Pork 30 MICROBIOLOGICAL RISK ASSESSMENT SERIES https://www.fao.org/3/i5317e/I5317E.pdf (in WHO search)	*No*	*Focus on beef and pork, not on fruit, vegetables, herbs*
Land degradation Soils Bulletin 13 https://www.fao.org/3/c1243e/c1243e.pdf	No	Focus on soil, land degradation
Livestock rules and regulations of Bhutan http://extwprlegs1.fao.org/docs/pdf/bhu159194.pdf (not relevant, no pdf on W-drive)	No	Livestock regulations in Bhutan
Good practice in the design, management and operation of a ... error 404: broken link	-	-
Safety and Quality of Water Used in Food Production and Processing 33 MICROBIOLOGICAL RISK ASSESSMENT SERIES https://www.fao.org/3/ca6062en/CA6062EN.pdf (in WHO search)	*Yes*	
PROPOSED DRAFT GUIDELINES FOR THE SAFE USE AND RE-USE OF WATER IN FOOD PRODUCTION, CX/FH 22/52/8	Yes	
PROPOSED DRAFT GUIDELINES FOR THE CONTROL OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) IN RAW BEEF, FRESH LEAFY VEGETABLES, RAW MILK AND RAW-MILK CHEESES, AND SPROUTS, CX/FH 22/52/7	No	Focus on food products, not on water
PROPOSED DRAFT GUIDELINES FOR THE SAFE USE AND RE-USE OF WATER IN FOOD PRODUCTION Comments in reply to CL 2021/64-FH, CX/FH 22/52/8 Add.1	Yes	
Request for comments on the proposed draft Guidelines on the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts, CL 2021/35/OCS-FH	No	Focus on food products, not on water
REPORT OF THE THIRTY-SIXTH SESSION OF THE CODEX COMMITTEE ON FOOD HYGIENE, ALINORM 04/27/13	No	Meeting report, no particular focus on water use
Interventions for the Control of Non-typhoidal Salmonella spp. in Beef and Pork 30 MICROBIOLOGICAL RISK ASSESSMENT SERIES Preliminary report (in WHO search)	*No*	*Focus on beef and pork, not on fruit, vegetables, herbs*
Pesticide residues in food 2007, FAO Plant Production and Protection Paper 191	No	Focus on pesticides
USE OF CHLORINE-CONTAINING COMPOUNDS IN FOOD PROCESSING (264 pages pdf on chlorine use, not included on W-drive)	No	Focus on chlorine-containing compounds

Title (URL hyperlink)	Relevant (Yes/ No)	Rationale when not relevant
REPORT OF THE FORTY-SECOND SESSION OF THE CODEX COMMITTEE ON FOOD HYGIENE, REP 11/FH	No	Meeting report, no particular focus on water use
Assessment and management of seafood safety and quality, FAO Fisheries and Aquaculture Technical Paper 574	No	Focus on fish and fishery products, not fruit, vegetables, herbs
Good Agricultural Practices (GAP) on horticultural production for extension staff in Tanzania, FAO GAP Working Paper Series 13	No	Focus on agriculture, irrigation, etc. , not on processing/washing
Maldives Clean Environment Project Environmental and Social Assessment and Management Framework (ESAMF) & Resettlement Policy Framework (RPF), ESAMF-RPF-2016	No	Focus on environment
Soil Pollution – An Emerging Threat to Agriculture, Environmental Chemistry for a Sustainable World Volume 10	No	Focus on soil pollution
Total Yes	**13**	
Minus duplicates	**10**	

Appendix E **Summary of physico-chemical parameters studied in literature**

Table E1. Physico-chemical parameters analysed in selected literature, effect of these parameters on efficacy of chlorine-based disinfectants, and remarks on potential relationships between parameters

Study	NTU	COD	Organic matter	Temperature		Conductivity	ORP	Chlorine-based disinfectants tested - effect on efficacy?	Alternative disinfectant tested	Remarks from publication
(Abadias et al., 2021)	0.88 - 704 NTU	NA	0 - 15 % tomato pulp	7 °C		322 - 2155 µS/cm	215 - 277 mV	NA	UV	Turbidity and time greatly affected disinfectant efficiency of UV-C
(Abnavi et al., 2021)	NA	106 - 24743 mg/L	NA	4 °C		NA	NA	Chlorinated, negative effect of organic load	NA	Organic load has a negative effect on free chlorine disinfection efficacy, a model was developed for FC decay, as well as predicting *E. coli* inactivation, and free chlorine concentration using COD as an indicator for organic load
(Afari et al., 2015)	NA	NA	NA	4 °C		NA	760 mV	Chlorinated, no reported impact of physico-chemical properties	Electrolyzed water	Neutral electrolyzed (NEO) water containing 155 mg/L free chlorine efficiently inactivated *E. coli* and *Salmonella*
(Afari et al., 2016)	NA	NA	NA	4 °C		NA	815 - 950 mV	Chlorinated, no reported impact of physico-chemical properties	Electrolyzed water	NEO water with 155 mg/L free chlorine efficiently reduced pathogens, lowering the pH to 6.5 and ultrasound treatment increased disinfectant efficacy.
(Alharbi et al., 2017)	50 NTU	44 - 49 mg/L	NA	NA		500 µS/cm	NA	NA	Electrocoagulation, UV	Electrocoagulation can be used to reduce the turbidity and solids content of process water, a combination with UV treatment can be utilized for the reduction of microbial load.
(Anese et al., 2015)	NA	NA	NA	18 - 90 °C		NA	NA	NA	Ultrasound	Ultrasound and heat treatment can be used to decontaminate wastewater for recycling, however

Study	NTU	COD	Organic matter	Temperature		Conductivity	ORP	Chlorine-based disinfectants tested - effect on efficacy?	Alternative disinfectant tested	Remarks from publication
										disinfectants may be necessary for cost-effective decontamination
(Barrera et al., 2012)	0.036 - 0.123 A540 nm	NA	NA	1 - 18 °C	3 faciliti	83 - 240 µS/cm	650 - 850 m in 2 of facilities	Chlorinated, no reported effe of processing parameters on disinfectant efficacy	NA	Observations from industrial process water samplings revealed correlations between microbial inactivation and temperature, as well as conductivity, and pH
(Bertoldi et al., 2021)	2 - 155 NTU	1 - 326 mg/L	NA	NA	6.7 - 7	NA	632 - 886 m	Chlorinated, no reported effe of processing parameters on disinfectant efficacy	NA	Researchers conclude that ORP correlates poorly with free chlorine levels, turbidity may be used as an indicator for water quality and that disinfectant levels negatively correlate with micrbial counts
(Bornhorst et al., 2018)	13 - 44 NTU	597 - 2772 mg/L	382 - 1094 mg/L indicated by turbidity, TDS, and COD	4 °C	3.8 - 5	NA	NA	Chlorinated, no reported effe of processing parameters on disinfectant efficacy	NA	Of the measured paramaters only CO and turbidity inceased over time, indicating an increase in organic matter. Total chlorine levels increased but no trend in free chlorine levels wa observed, this may imply an accumulation of chlorine by-products.
(Chen and Hung, 2016)	0 - 187 NTU	52 - 764 mg/L	"Low - Medium - High"	4 °C	3.7 6	NA	314 - 586 m	Chlorinated, protein and phenolics react with chlorine resulting in chlorine loss	NA	Several parameters were tested as indicators for organic load of process water, UV absorbance at 254nm was shown to have the highest correlation with chlorine demand and may be used as an indicator for organic load
(Chen and Hung, 2017)	NA	NA	30 or 50 mg/L	NA	2. 6.0, 8	NA	NA	Chlorinated, organic load and pH were shown to affect disinfectant efficiency	NA	Organic load, pH and initial chlorine concentration were shown to affect th chlorine demand of process water. Maintaining near-neutral pH decrease chlorine demand and formation of disinfection by-products.

Study	NTU	COD	Organic matter	Temperature		Conductivity	ORP	Chlorine-based disinfectants tested - effect on efficacy?	Alternative disinfectant tested	Remarks from publication
(Chen et al., 2018)	NA	NA	NA	NA	3.3, 3.6, 3.9, or 7.0	NA	265 - 1020 mV	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	NA	Different acidic eletrolysed water conditions were tested, the condition resulting in 100 mg/L chlorine content used for 3 minutes was found most suitable to reduce contamination on red cabbage.
(Collazo et al., 2019b)	NA	NA	NA	5 °C	4.7, or 5.7	NA	478 - 526 mV	NA	UV	UV treatment at doses of 0.1 - 0.3 kJ/m2 were found to achieve effective inactivation of pathogenic bacteria (*Salmonella*, *Listeria*). A combination of low dose UV with PAA may be used as a preservation strategy to improve the safety of ready-to-eat leafy greens.
(Collazo et al., 2019a)	NA	NA	NA	5 °C	NA	NA	NA	Chlorinated control, no reported effect of processing parameters on disinfectant efficacy	Pulsed light, UV	Low-dose immersion-assisted UV-C (0.5 kJ/m2) was found to efficiently inactivate *L . Innocua*, however pulsed light treatment was ineffective.
(Cossu et al., 2016)	NA	0, 500, or 2000 mg/L	NA	NA	NA	NA	NA	NA	Gallic acid, UV	UV-A in combination with gallic acid achieved effective inactivation of *E. coli* O157:H7 in the presence of high and medium levels of organic content (up to 2000 mg/L O2 COD).
(Cuevas-Ferrando et al., 2021)	1 - 538 NTU	23 - 2118 mg/L	NA	6 - 22 °C	3.5 - 8.5	666 - 1397 µS/cm	147 - 759 mV	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	NA	The occurrence of viruses in process water is described, levels of bacteriophages were lower when residual chlorine was constantly maintained.
(Davidson et al., 2014)	0 - 0.127 A663 nm	23 - 4527 mg/L	0 - 10 % blended	13- 14 °C	6.5 - 8.5	NA	366 - 887 mV	Chlorinated, increasing organic load reduced chlorine efficacy in process water	NA	Total solids and COD were found to be the best indicators of organic load in process water. Organic load affected disinfectant efficacy

Study	NTU	COD	Organic matter	Temperature		Conductivity	ORP	Chlorine-based disinfectants tested - effect on efficacy?	Alternative disinfectant tested	Remarks from publication
			iceberg lettuce							against *E. coli* O157:H7 in flume water.
(Davidson et al., 2017)	0 - 0.155 A663 nm	255 - 5444 mg/L	0 - 10 % blended iceberg lettuce	12- 15 °C	5.3 - 7.0	NA	311 - 619 mV	NA	NA	Concentrations of 50 ppm PAA or mixed peracid resulted in minimal persistence of *E. coli* O157:H7 in process water, regardless of organic load. Increases in total solids, COD, turbidity, and ORP levels corresponded to increasing organic load in process water.
(de Oliveira et al., 2018)	NA	0, 500, or 1500 mg/L	NA	4 or 25 °C	3.0 in one experiment	NA	NA	NA	Curcumin, UV	UV-A in combination with 1- 10 mg/L curcumin was effective at inactivation of *E. coli* and *L. innocua* in simulated wash water. Lower pH increased the antimicrobial activity of curcumin, whilst COD reduced its activity.
(Dunkin et al., 2017)	14.9 - 61 NTU	140.5 - 1569 mg/L	NA	4 °C	5.4 - 6.7	220 - 938.7 µS/cm	NA	Chlorinated, the release of plant exudates due to vegetable cutting affects chlorine disinfection efficacy	NA	High turbidity, COD, and TOC reduced the inactivation of hNOV, indicating a protective matrix effect caused by these components in addition to residual free chlorine decay
(Fu et al., 2018)	0 - 210.8 NTU	NA	0 - 20 % lettuce juice	3 °C	6.3 or 7.2	NA	234.7 - 836.3 mV	Chlorinated, increasing organic load and solids content reduced chlorine efficacy in process water	NA	Organic load and the presence of solids reduced the inactivation of pathogens. Results indicated that ORP did not correlate linearly with free chlorine concentration.
(Gu et al., 2021)	NA	341.9 - 705 mg/L	NA	NA	4.0 -6.5	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	NA	*Salmonella* populations were readily inactivated on sponge/microfiber/papaya using process water containing free chlorine or PAA.

Study	NTU	COD	Organic matter	Temperature		Conductivity	ORP	Chlorine-based disinfectants tested - effect on efficacy?	Alternative disinfectant tested	Remarks from publication
(Guo et al., 2017)	61.9 - 232 NTU	1696 - 2100 mg/L	2 -4% fresh produce extract	4 or 30 °C	NA	NA	NA	Chlorinated, chlorine can react with organic matter and be decomposed by UV, impacting disinfection efficacy in process water. Therefore a higher initial chlorine concentration was used to maintain > 5 ppm free chlorine level	UV	A combined treatment of UV and chlorine efficiently reduced *Salmonella* in process water, UV treatment alone did not yield this same effect.
(Hagele et al., 2016)	NA	NA	NA	4 or 45 °C	NA	NA	NA	NA	UV	Bacterial viable counts could be reduced up to 2.1 log CFU/g using UV-C combined with warm water.
(Huang and Chen, 2019)	NA	NA	NA	NA	NA	NA	NA	NA	Pulsed light, UV	UV and Pulsed light treatment both were able to inactivate *Salmonella* in process water to approximately 1 - 2 log CFU/mL.
(Huang et al., 2020)	NA	0, 5000, or 50000 mg/L in one experiment	NA	4 °C	6.5	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	NA	Results show that an active free chlorine level of 5 ppm is needed to prevent survival of bacteria in process water during washing
(Huang and Chen, 2020)	0 - 100 NTU	0 - 2500 mg/L	2 - 6 % lettuce extract	4 or 30 °C	NA	NA	NA	Chlorinated control, no reported effect of processing parameters on disinfectant efficacy	UV	Increasing COD levels impacted the inactivation of *Salmonella* resulting in higher numbers of surviving bacteria, this effect was not observed with increasing levels of turbidity.

(Huang and Chen, 2018)	100 NTU	1800 mg/L	4%	4 °C	NA	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	Pulsed light, Ultrasound, UV	A combined treatment of pulsed light and chlorine was in general more effective in inactivation of *Salmonella* than chlorine alone.
(Huang et al., 2018)	64.2 - 258 NTU	1753.3 - 2276.7 mg/L	2 - 6 % lettuce extract	4 °C	NA	NA	NA	Chlorinated, increasing organic load reduced chlorine efficacy in process water	UV	UV treatment disinfection efficiency varied greatly between frsh produce types, it performed best on tomato but worst on spinach. UV in combination with chlorine or PAA consistently reached highest log reductions of *Salmonella*.
(Huang et al., 2015)	2.4 - 98.3 NTU	1043.3 - 2116.7 mg/L	0 - 2 % berry juice	NA	NA	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	Pulsed light	A combination of pulsed light with H2O2 enhanced disinfection efficacy, compared to pulsed light alone. Pulsed light treatment efficiency was not affected by organic load, or turbidity.
(Ignat et al., 2015)	NA	NA	NA	8 °C	NA	NA	NA	NA	UV	UV-C light at 0.4 kJ/m2 treatment resulted in 5-log reductions of pathogens in process water. Disinfection efficiency was not impacted by spectral properties.
(Jung et al., 2022)	NA	NA	NA	18 °C	2.7 - 7.4	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	Electrolyzed water	Electrolyzed water containing approximately 60 mg/L free chlorine was most effective at inactivating pathogens, compared to acid based disinfectants.
(Kramer et al., 2017)	NA	NA	NA	NA	NA	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	Pulsed light	Pulsed light treatment can reduce microbial loads in water to a low level (~2 log CFU/mL). However, treatments containing free chlorine were shown to reduce microbial load to below the LOD.
(Li et al., 2012)	NA	500, 800, or 1500 mg/L	NA	<10 °C	NA	NA	NA	NA	Grape seed extract	Grape seed extract at 2 mg/mL induced a > 2 log PFU/mL reduction of MNV-1 infectivity. This

										effect was not impacted by increasing COD levels.
(Lopez et al., 2016)	NA	NA	NA	20 °C	3.6 or 9.4	NA	NA	NA	NA	Commercial produce wash treatments were able to reduce pathogenic populations to below the detection limit in residual process water.
(López-Gálvez et al., 2020)	NA	137.9 - 350.4 mg/L	0.52 - 1.31 A254nm	20 °C	7.2 or 7.4	1573 - 1919 µS/cm	259 mV	Chlorinated, high organic load and presence of oxidizable substances in poor quality wash water as well as volatilization due to spraying may have impacted disinfectant efficacy	NA	Chlorine dioxide treatment reduced microbial populations in poor quality wash water, although high microbial loads were still detected.
(Maffei et al., 2016)	NA	NA	2.3 or 13.5 mg/L	25 °C	5.6 or 7.0	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	NA	Washing of five *Salmonella* inoculated lettuce portions with chlorinated water resulted in no measurable pathogen transfer to process water at concentrations ≥10 mg/L.
(Manzocco et al., 2015)	NA	NA	NA	8 °C	NA	NA	NA	NA	Pulsed light	Results show that independent of the number of washing cycles pulsed light treatment of process water can result in approximately 4-log reductions of total viable count and *Pseudomonas spp.*
(Mathew et al., 2018)	NA	0 - 315 mg/L	NA	21 - 46 °C	NA	NA	NA	Chlorinated, organic load, and residual disinfectant concentration impacted the disinfectant efficacy	NA	Treatment with 200 ppm chlorine or 80 ppm PAA efficiently inactivated *Salmonella* in process water, samples were still found to be positive for *Salmonella* after treatment with 5 ppm chlorine dioxide. Reduced disinfection efficiency was observed in the presence of organic matter.

(Millan-Sango et al., 2017)	NA	458 - 1115 mg/L	NA	NA	NA	NA	NA	NA	UV, ultrasound	UV-C light reduced the microbial load by 3.1 log CFU/mL, a combined UV and ultrasound treatment resulted in more efficient reduction of microbial load, depletion of COD, as well as decrease of suspended particles.
(Murray et al., 2018)	NA	861 - 5800 mg/L	NA	3 °C	6.3 or 7.1	550 - 1710 µS/cm	596 - 646 mV	Chlorinated, free chlorine depleted due to the formation of disinfection by-products, affecting disinfectant efficacy	NA	Free chlorine levels in commercial postharvest wash tanks vary. Process water samples often contain disinfection by-products htat contribute to antimicrobial capacity, resulting in 2.9 - 4.9 log reduction of *E. coli*, *Salmonella,* and *Listeria*
(Nicolau-Lapena et al., 2020)	0.9 NTU	NA	NA	8 °C	4.6 - 8.0	NA	256 - 891 mV	Chlorinated control, no reported effect of processing parameters on disinfectant efficacy	UV	UV-C treatment inactivated pathogenic bacteria, *Listeria* and *Salmonella*, and spoilage microorganisms to levels close to chemical disinfectants.
Ortiz-Sola et al 2021	NA	NA	NA	8 °C	5.3 - 7.7	NA	NA	Chlorinated control, no reported effect of processing parameters on disinfectant efficacy	UV	Water-assisted UV-C treatment in combination with PAA resulted in inactivation of *Listeria* and *Salmonella* equivalent to chlorine, UV-C alone did not reach a similar level of inactivation.
(Pablos et al., 2017)	NA	NA	NA	25 °C	NA	NA	NA	NA	allyl- and benzyl-isothiocyanates (AITC, BITC), chitosan	BITC may be used to disinfect fresh produce or water, but its antimicrobial effect is not as fast as chlorine-based disinfectants. The antimicrobial effect does, however, remain up to 48h in the water and BITC may be used at lower levels than chlorine. Chitosan did not exhibit significant antimicrobial effects.

(Pablos et al., 2022)	100 NTU	NA	150 or 500 mg/L total organic carbon	4 - 7 °C	6.2	1000 µS/cm	NA	Chlorinated, increasing levels of organic matter reduced chlorine efficacy in process water	quaternary ammonium compounds (QACs) isothiazolinones and carvacrol	QACs, carvacrol and chlorine exhibited the strongest antibacterial effect against *Salmonella* on lettuce, all tested disinfectants were effective against *Salmonella* in process water.
(Patange et al., 2019)	NA	NA	NA	<30 °C	3.2 - 7.3	NA	NA	Chlorinated control, no reported effect of processing parameters on disinfectant efficacy	cold plasma	Cold plasma treatment resulted in antimicrobial activity similar to that of 100 mg/L chlorine. Antibacterial activity was not or minimally impacted by pH.
(Sheng et al., 2020b)	NA	NA	NA	NA	6.7 - 6.9	NA	346.1 - 908 mV	Chlorinated control, no reported effect of processing parameters on disinfectant efficacy	Electrolyzed water, mineral oxychloride	Both electrolyzed water and mineral oxychloride were able to inactivate residual *Listeria* in process water.
(Sheng et al., 2020a)	NA	0 or 1000 mg/L	NA	NA	1.2 - 8.0	NA	433.7 - 738.7 mV	Chlorinated, increasing organic load reduced chlorine efficacy in process water	sodium acid sulfate (SAS)	SAS showed antimicrobial activity equivalent to 25 ppm chlorine in process water, but SAS exhibited greater efficacy than chlorine at low contamination levels, possibly due to its stability in the presence of organic matter.
(Tomás-Callejas et al., 2012b)	22 or 160 NTU	NA	NA	10 or 25 °C	NA	NA	240.9 - 760.1 mV	Chlorinated, organic load present in recirculated water reduces chlorine efficacy	NA	Chlorine levels tested in this study were unable to fully disinfect inoculated leaves, *Salmonella* was also not fully inactivated in process water. ORP values of process water were affected by turbidity.
(Tomás-Callejas et al., 2012a)	0 - 15.8 NTU	NA	NA	34 - 44 °C	7.1 - 8.2	>7.6*10^9	302 - 729 mV	Chlorinated, high turbidity and organic load in dump tanks reduced disinfectant efficacy	NA	Chlorine dioxide can be an effective santizer for flume and spray-wash systems, however it may not be suitable for use in dump tanks under typical commercial conditions due to the low water quality impacting disinfectant efficacy

(Wulfkuehler et al., 2013)	NA	NA	NA	4 or 45 °C	NA	NA	NA	Chlorinated, no reported effect of processing parameters on disinfectant efficacy	NA	Warm water was effective at retaining fresh produce quality but less effective in the disinfection of process water compared to chlorine.
(Wulfkuehler et al., 2015)	NA	7 - 156 mg/L	NA	4 °C	NA	NA	NA	NA	NA	Washing of radicchio prior to shredding resulted in a lower microbial load and organic contamination of process water.
(Zhang et al., 2022)	NA	1000 mg/L	NA	NA	5.0 - 5.3	NA	NA	NA	UV	Sequential application of PAA followed by UV/PAA resulted in strong inactivation efficacy of *E. coli* in process water. PAA levels can be more consistently controlled than chlorine in process water, as it reacts slower with organic exudates.

Appendix F **Supplementary figures supporting model-based analysis of industrial cases**

In this appendix extra information about the modelling results were included such as non-normalised y-axis plots or plotting of transfer rates by sector and product.

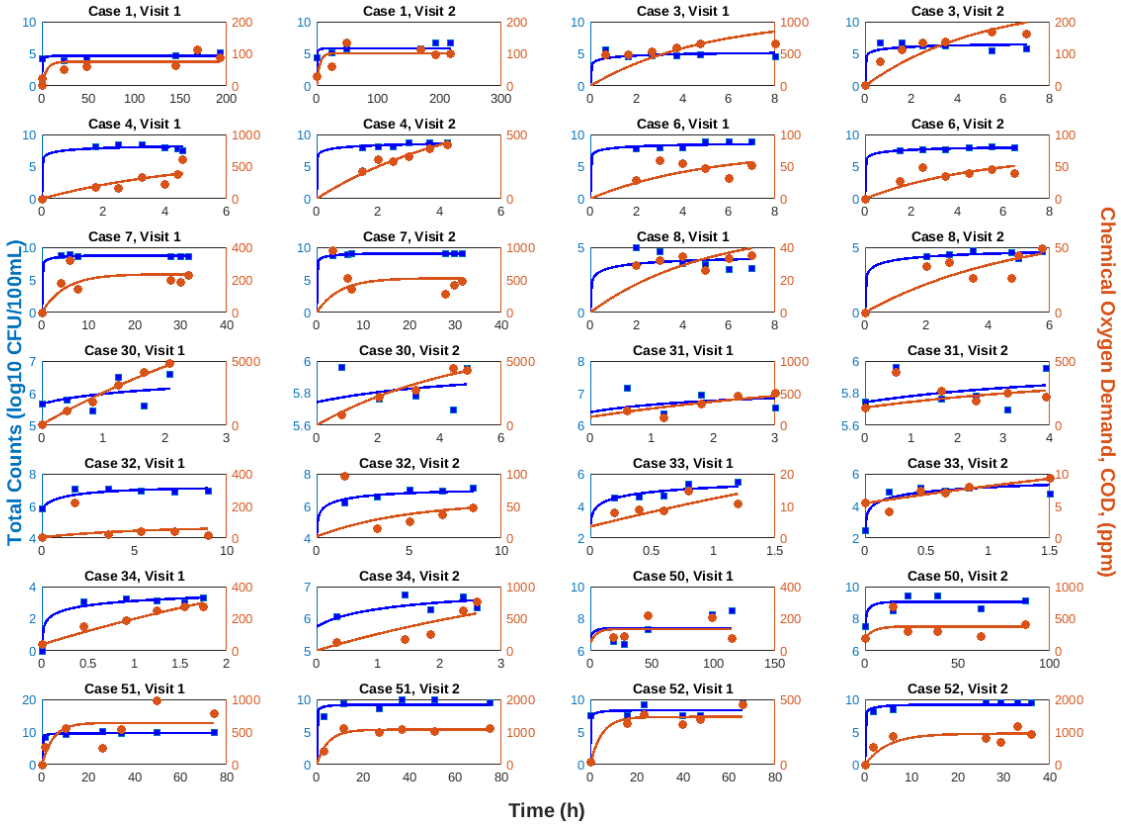

Figure F1. Dynamics of total bacterial counts (blue, left y-axes) and COD (orange, right y-axes) for the scenarios without disinfectant. Dots are experimental data and lines the model prediction after calculating the best transfer rate parameters ($\boldsymbol{K_x}$, $\boldsymbol{K_{COD}}$) for each experiment (visit) assuming same dilution rate ($\boldsymbol{D}$) for all cases.

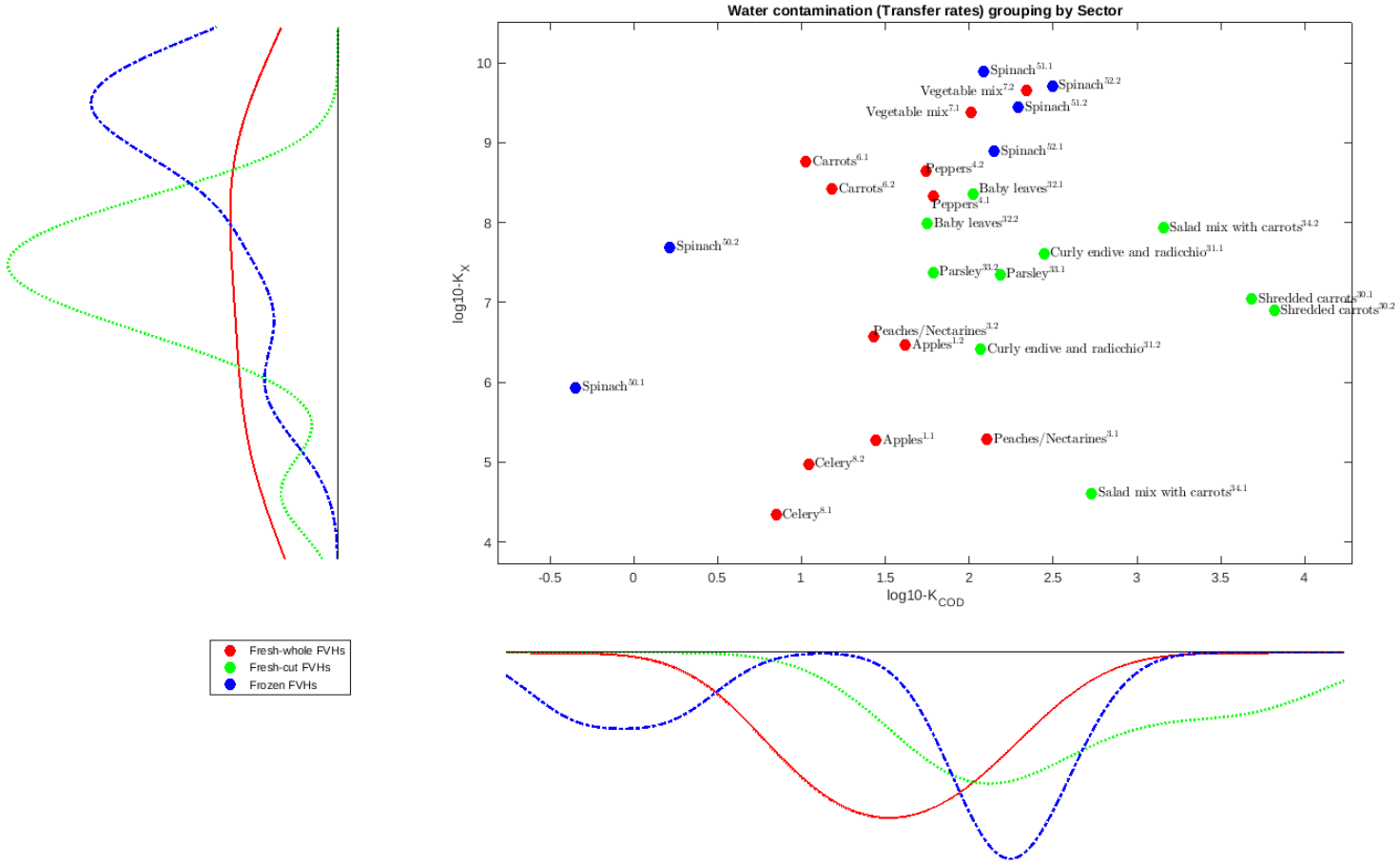

Figure F2. Estimated transfer coefficients, grouped by sector, for TC ($\boldsymbol{K_X}$ in $\frac{\text{CFU}}{\text{g-product·min}}$) and for COD ($\boldsymbol{K_{COD}}$ in $\frac{\text{mg-COD}}{\text{g-product·min}}$) using the log10 transformation (i.e. plotting the order of magnitude) for all visits with an estimated dilution of $\boldsymbol{D} = 0.003\ min^{-1}$. In the scatter plot colours represents the different sectors, same as in the estimated distributions in x-axis and y-axis.

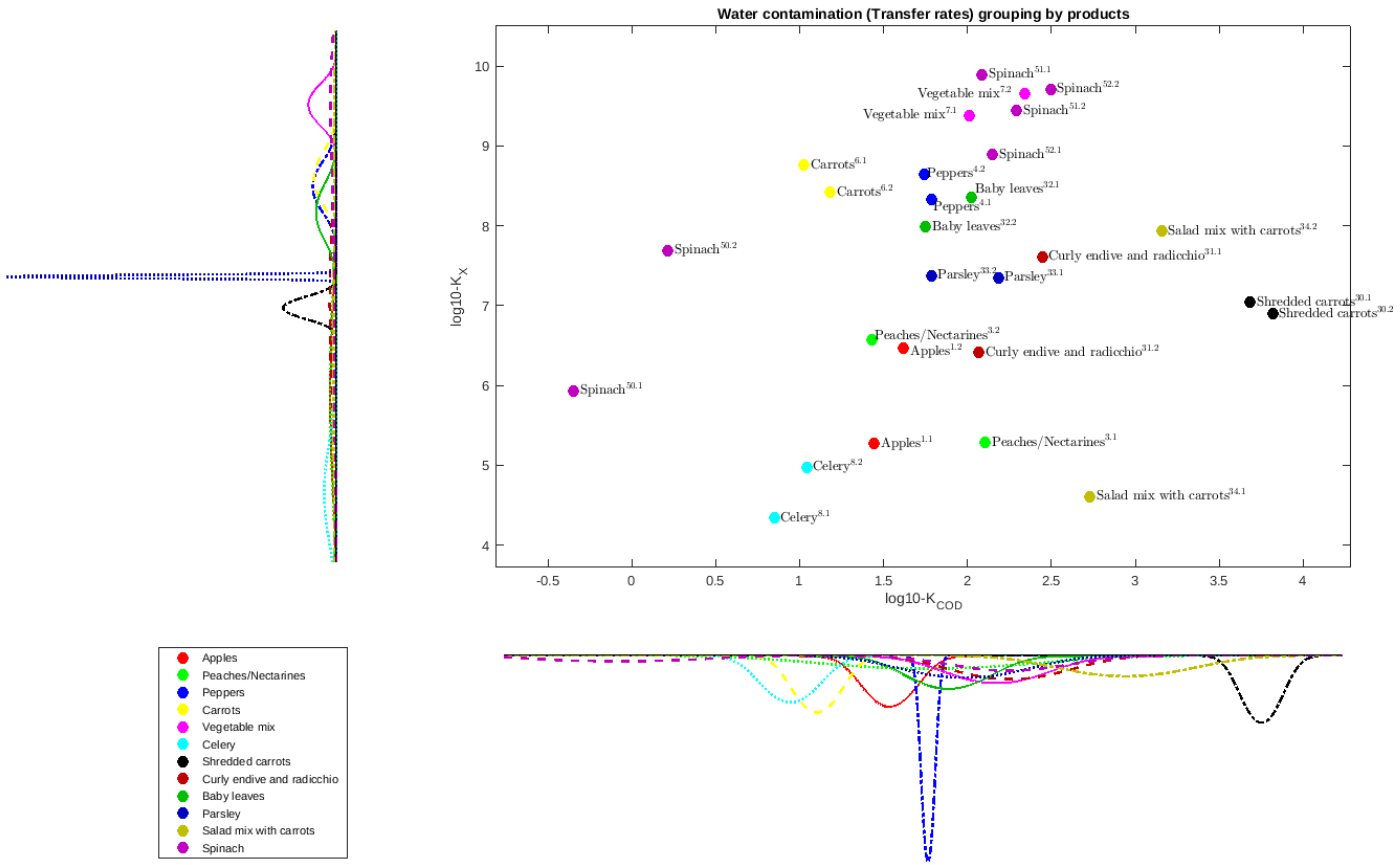

Figure F3. Estimated transfer coefficients, grouped by food product being washed, for TC ($\boldsymbol{K_X}$ in $\frac{\text{CFU}}{\text{g-product·min}}$) and for COD ($\boldsymbol{K_{COD}}$ in $\frac{\text{mg-COD}}{\text{g-product·min}}$) using the log10 transformation (i.e. plotting the order of magnitude) for all visits with an estimated dilution of $\boldsymbol{D} = 0.003\, min^{-1}$. In the scatter plot colours represents the different products, same as in the estimated distributions in x-axis and y-axis.

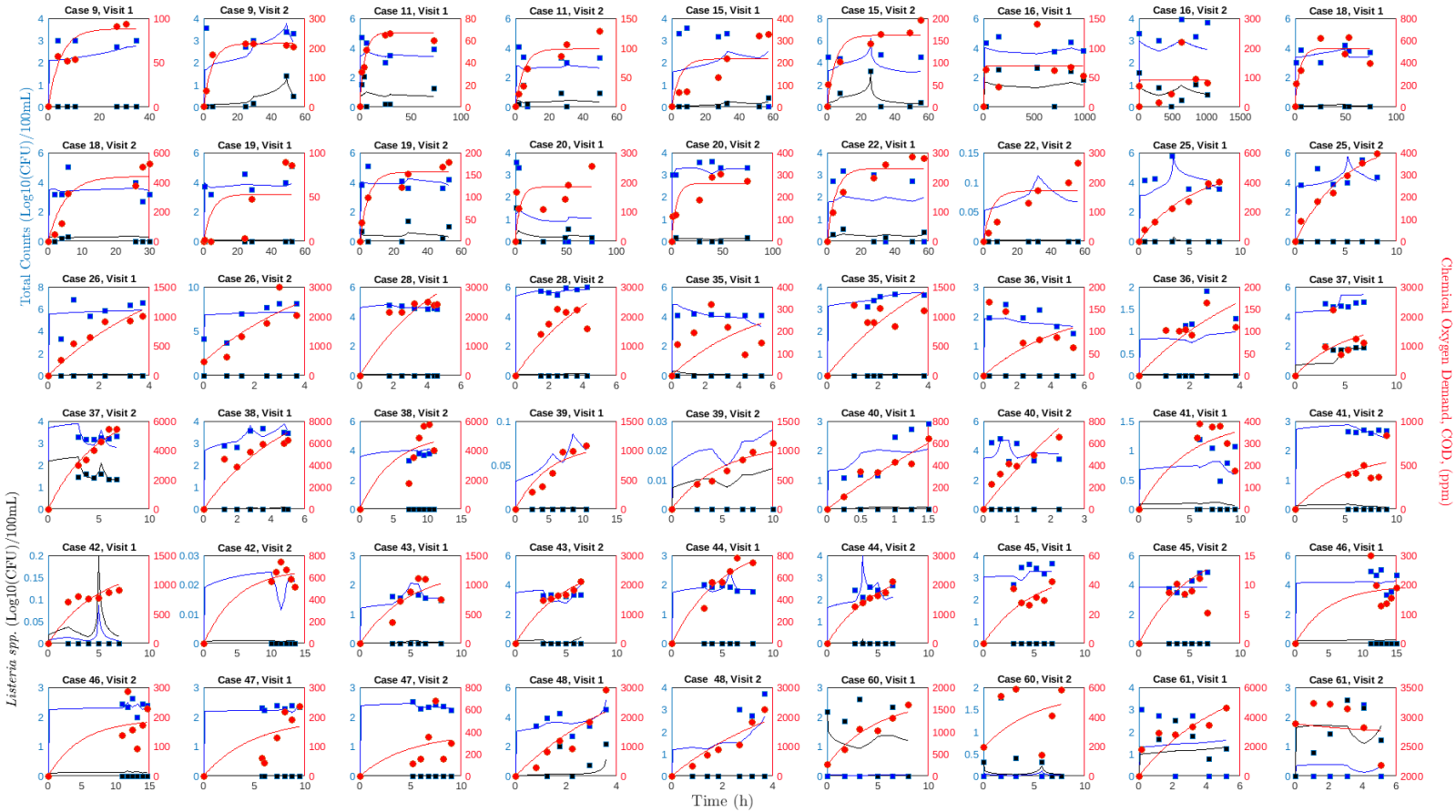

Figure F4. Dynamics without normalised scaling of total bacterial counts (blue, left y-axes), *Listeria spp.* (black, left y-axes) and COD (orange, right y-axes) for the scenarios with disinfectant. Dots are experimental data and lines the model prediction after calculating the inactivation rates ($\boldsymbol{\alpha_{TC}, \alpha_{Lis}}$) best transfer rate parameters and protective COD effect ($\boldsymbol{K_{TC}, K_{Lis}, K_{COD}, K_m}$) each experiment (visit) assuming same dilution rate ($\boldsymbol{D = 0.003 min^{-1}}$) for all scenarios. The estimated parameters are provided in an supplementary excel file named "Estimated_parameters_cases_WITH_disinfectant.xlxs"