The effectiveness of ozone, ultrafiltration, and low pH on *Escherichia coli* inactivation in fresh-cut endive process wash water at a pilot setting

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Summary

During the processing of fresh-cut produce, any pathogenic contamination that could occur must be prevented so that uncontaminated produce does not become contaminated via the washing water (cross-contamination). Thus, there is a need to disinfect water during processing. This study evaluated three disinfection technologies – ozone, ultrafiltration (UF), and lowering the pH to 4.0 using processing aid T128 – when used singly during fresh-cut endive washing. The goal was to investigate the effect of these technologies on the reduction of *Escherichia coli* in the process wash water (PWW) and identify potentials for saving water and energy on an industrial washing line. The study focused on disinfecting the PWW of fresh-cut endive, with the first two technologies applied in a bypass and the third directly in the wash tank. The study provided the first proof of application for three different water disinfection technologies during pilot-scale fresh-cut endive washing.

The main conclusions were that no *E.coli* reduction was found in the PWW during the experiments with ozone, UF or T128. Water and energy could be saved, although these savings were in the same order of magnitude as the technology costs. Furthermore, the pilot experiments allowed us to compare microbial and chemical aspects in the PWW and on the endive. This showed that although some chemical hazards like cadmium, lead, and nitrate accumulated over time, the final levels after two hours of recycling did not lead to an exceedance of legal limits in the PWW and endive. Future research should examine a modified bypass setup to allow treatment of higher water volumes. Especially ozone appeared interesting given that it is a more economical application than UF. The dosing needs to be adjusted, as the primary limitation was the insufficient dose applied. A possible combination of ozone and another technique, like UV, may be of interest to further research to achieve a higher log reduction.

1 Introduction

During the processing of fresh-cut produce, any pathogenic contamination that could occur must be prevented so that uncontaminated produce does not become contaminated via the washing water (cross-contamination). In the Netherlands, the available water disinfection technologies currently applied by fresh-cut processors are limited due to legislative barriers to applying chemical technologies. Nonetheless, several water disinfection technologies are available on the market. These can be applied post-harvest to disinfect the water used during (fresh-cut) produce washing (Gil et al., 2009; Banach et al., 2015; Meireles et al., 2016). Research has shown that disinfecting washing water is a relevant intervention strategy to tackle potentially high (10^6 CFU per batch of fresh-cut leafy greens; batch size non-specified) contamination events during processing (Chardon et al., 2016). Thus, the need to disinfect water during processing is evident.

In the public-private partnership (PPP) project *Safe & Save Water*, stakeholders associated with the Dutch fresh-cut produce sector have fervently discussed the advantages and disadvantages of fresh-cut produce washing with chemical and physical technologies. The discussions have shown that when considering technologies to disinfect water directly in contact with the produce in the wash tank (during washing), technologies like ozone, peracetic acid, or chlorine were preferable. This was because of their ability to inactivate microorganisms quickly in the water during processing. However, since the project aimed at a potential application of a disinfection technology within the next 3-5 years, the project's main emphasis was on alternative means to disinfect the washing water without decontaminating the product. Therefore, the study focused on lowering the pH of the wash water directly in the tank and disinfection technologies that could be applied via a bypass. A bypass was defined as where the water could be disinfected before it comes into contact with the produce. This could then be water collected after washing the produce, which can be treated and pumped back to wash the fresh produce. A treatment of the water in the by-pass will prevent a possible recontamination via the wash water. The discussions with the project stakeholders have shown that ozone and ultrafiltration were of interest to investigate. These technologies were preferable because of their feasibility in bacterial inactivation, legislation, and technology readiness level (TRL, i.e., the technology's maturity).

This study aimed to evaluate and develop the integration of three disinfection technologies – ozone, UF, and reducing the pH using processing aid T128 – when used singly during fresh-cut endive washing. The goal of using these technologies is to save water and energy on an industrial washing line without detrimentally affecting the chemical and microbiological water quality and safety. The study focused on disinfecting the process wash water (PWW) of fresh-cut endive, with the first two technologies applied in a bypass and the third directly in the wash tank. The microbial effectiveness of the technologies is defined by its ability to reduce *E. coli* by at least 5-log in the PWW.

2 Materials and methods

2.1 Experimental design

Pilot-scale experiments assessed the efficacy of (i) ozone and (ii) UF to disinfect the water during fresh-cut endive processing (Figure 1a). A bypass system was used connected to the washing line, which functions as a hydraulic transporter of the produce. In order to keep up with the flow rate of the washing line, only a part of the water could be treated. Therefore, the disinfection technologies were mounted on side stream of the by-pass. When disinfecting the water in a by-pass a possible recontamination of the wash water and subsequently the produce can be prevented. We also examined the efficacy of (iii) processing aid T128 at pH 4.0 to disinfect the PWW directly in the wash tank, i.e., in line, during fresh-cut endive processing (Figure 1b). Each experiment consisted of one run, in which about 400 kg of cut endive were washed in about 2 h. We examined each technology's effectivity when *E. coli* (10^6 CFU/mL) was added after 50 min and 110 min of processing to the PWW.

Figure 1 Pilot-scale experiments assessed the efficacy of (a) ozone and UF in a bypass (depicted in orange) and (b) T128 at pH 4.0 in the washing tank. Numbers show the sampling points: SP1 = before shaking table; SP2 = after cutting, also the inoculation point; SP3 = before treatment via the bypass; SP4 = after treatment via the bypass. Arrows indicate the flow of the water.

Before starting the experiments, the processing line was thoroughly disinfected with 80% ethanol. Swabs at four locations of the washing line were taken to verify hygiene. These were (i) the middle of the cutting board, (ii) the middle of the first transfer belt, (iii) the wash tank near the inoculation point

(i.e., SP2), and (iv) the middle of the output belt (Figure 2). Swab samples (10 x 10 cm²) were collected and analyzed with swab rinse kits (SRK; 922C, CR, SRK 10 mL TRIPLE PACKED, Copan Italia SpA, Brescia, Italy) as described by (Banach et al., 2018).

Figure 2 Swabbed surfaces before pilot experiments: (a) the middle of the cutting board, (b) the middle of the first transfer belt, (c) the wash tank near the inoculation point, and (d) the middle of the output belt. The red circle approximates the location of swabbing.

During the experiments, water samples were quantitatively examined for *E. coli*, total viable counts (TVC), and total coliforms. Also, water samples were analyzed for chemical oxygen demand (COD), cadmium, lead, nitrate, pesticide residues, and during the T128 pilot, for the ingredients of T128 (named chemical 1 and chemical 2 in this report). Depending on the experiment, water samples were analyzed for parameters relevant to the technology executed, such as the ozone concentrations. Collected endive samples were quantitatively examined for *E. coli*, TVC, *Enterobacteriaceae* (Enteros), lactic acid bacteria (LAB), cadmium, lead, nitrate, pesticide residues, and during the T128 pilot, for chemical 1 and chemical 2. A sampling plan/event schedule during pilot experiments with ozone and UF is shown in Table A1.1, and the pilot experiment with processing aid T128 is shown in Table A1.2 (Annex 1).

2.2 Processing line

A commercial small-scale processing line for leafy vegetables with a spiral washer (SW-50/350, Sormac B.V.) was used to process endive at a rate of about 200 kg endive per hour. The wash tank capacity was 440 L, and the pump tank water capacity was at least 400 L, meaning the total water capacity of these two tanks totaled at least 840 L. The pump flow rate was 50 m³/h. The pipework connection between the pump and the main pipe was as follows: pump: Vlakke lasflens DN65 (EN 1092-1), and main pipe: Triclamp DN50 (DIN 32676).

Figure 3 Schematic overview of the processing line with a cutting station, lettuce shredder, step conveyor belt, wash tank, and output belt with spray nozzles (Sormac, Venlo, the Netherlands).

From the spiral washer, a bypass system was constructed to connect the untreated PWW to either the ozone or UF technology to be then treated before pumping the water back to the washer. Figure 4 shows a schematic overview of the bypass setup.

Figure 4 Schematic overview of the bypass, including sampling points SP3 = before treatment via the bypass and SP4 = after treatment via the bypass.

2.3 Endive

Crated shipments of endive, which was grown in the Netherlands, were obtained from a supplier of Heemskerk BV. The same supplier was used for all three pilot experiments. One shipment of endive consisted of around 400 kg, which was used per pilot experiment. The shipment was delivered the morning of each pilot and was stored in a cold room (~ 15 °C) before experiments. The time between shipment arrival and experiments was about 2-3 h.

The endive was inspected before each experiment with some visual differences in the size of the endive heads and the amount of soil on the produce. For instance, the endive heads during the pilot with UF were smaller and had noticeably more soil.

Directly before the endive was placed on the transport belt, it was cored by hand with a knife, and any damaged outmost leaves were removed (pre-trimmed). The cut size of the lettuce shredder was 5 mm.

2.4 Bacterial strain and inoculum preparations

A commensal *E. coli* strain (12-123.2), which was initially isolated from surface water, was supplied by the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands. The strain was kept at -80 °C in Luria Broth (LB; L1704 LB Broth High Salt, Duchefa Biochemie B.V., Haarlem, the Netherlands) supplemented with 25% (v/v) glycerol. Bacteria were streaked on Brilliance *E. coli*/coliform selective agar (BECSA; Tritium Microbiologie B.V., Eindhoven, the Netherlands).

Cultures were prepared by transferring a single colony to 25 mL LB and incubated for 20 h at 37 °C in a 200-rpm shaking incubator to obtain stationary phase cells. Afterward, 0.5 mL of the prepared culture was added to 2 L Erlenmeyer flasks filled with 500 mL of freshly prepared LB. This culture was incubated for another 18 (± 1) h at 37 °C in a 200-rpm shaking incubator. After incubation for 18 (± 1) h, to obtain stationary phase cells, the liquid cultures (c.a. 10^9 CFU/mL) were collected in two 5 L plastic containers. All liquid (c.a. 4.5 L) was added directly to the wash tank.

2.5 Water treatment technologies

During each of the pilots, several parameters were measured; these included the temperature of the water, air, endive, main water flow, and, when applicable, the bypass flow and pH (Appendix B, Tables B1-B3).

2.5.1 Ozone

During the ozone experiment, the ozone equipment and generator were supplied and monitored by Nijhuis Industries, BV. The ozone generator continuously produced ozone with a gas concentration of 200 g /Nm³. With this setup, the amount of ozone produced during the experiments was 50 g O₃/h, which amounts to around 75 mg O₃/g COD in the by-pass at the start of the pilot ($t=47$ min using a flow rate of around 1 m³/h in the by-pass).

2.5.2 Ultrafiltration

During the UF experiment, the UF equipment and main filters were supplied by PB International. The washing line was fitted with an additional pre-filter of 280 μ m (supplied by Sormac B.V.). In a preliminary setup, the incoming water was then treated with 5 μ m with a surface area of 1288 cm². Then, we used a UF surface of 55 m² with a nominal diameter of 0.02 μ m. This was set to filter at 5.5 m³/h of the 37.5 m³/h, meaning 15% of the water in the bypass was set to be treated. This pre-setting was modified as the technology could not handle treating that percentage of water via the bypass. This was seen by the low pre-pressure of the UF after the first few minutes of treatment,

meaning the 5 µm and 280 µm filters were blocked. This was most likely a result of the sand and direct coming from the endive. Therefore, during the UF pilots, the amount of water that could be treated via the bypass had to be reduced, and the PWW was treated with UF at a rate of about 2 m³/h (0.055%).

2.5.3 Processing aid T128

During the T128 experiment, the processing aid materials and equipment were supplied and monitored by SmartWash Solutions BV. Prior to the pilot test, lab experiments with T128 at a pH of 3.5, 4.5, and 5.5 were examined and compared to a control treatment (2 min rinse with tap water, 11-12 °C). Results showed no adverse quality effects after storage at 7 °C for 24 h on the fresh-cut endive when there was a final rinse step. A target pH of 4.0 was selected for the pilot experiments in consultation with the supplier and processors. T128 is a blend made up of two chemicals. During the pilot experiment, these were added to the PWW directly during processing. The pH was targeted to 4.0 and monitored, along with the temperature of the water, throughout processing.

2.6 Microbiological analyses

Water samples collected were quantitatively examined for *E. coli* and TVC. Sodium thiosulfate (Sigma-Aldrich, the Netherlands) was used to neutralize the water samples from ozone and T128 experiments before microbial analyzes.

For on-site microbiological analyses (of *E. coli* and total viable numbers), 100 µL of the water sample was directly plated on BECSA or plate count agar (PCA; Tritium Microbiologie B.V., Eindhoven, the Netherlands), and 1 mL was serially diluted into peptone physiological salt solution (PPS; Tritium Microbiologie B.V., Eindhoven, the Netherlands), of which 100 µL of the appropriate dilutions were subsequently plated. All plating took place on-site. Plates were transported the same day to the laboratory. Plates were then incubated at 37 °C with daily inspection of colonies for up to two days.

Also, water samples were analyzed for *E. coli* and total coliforms and with the Colitag™ kit (Palintest, Gateshead, United Kingdom) to determine the most probable number (MPN) according to manufacturer's instructions.

Finally, collected endive samples were quantitatively examined for *E. coli* according to ISO 16649-2, Enteros according to ISO 21528-2, LAB according to ISO 15214, and TVC according to ISO 4833-1 (Mérieux NutriSciences, Ede, the Netherlands).

2.7 Chemical analyses

Collected water samples were periodically taken, as described in Table A1.1 and Table A1.2. Water was analyzed for COD in accordance with NEN 6633:2006/A1:2007 (Mérieux NutriSciences, Ede, the Netherlands). Water was also analyzed for cadmium and lead as well as nitrate (as NO₃) according to, respectively, Mérieux NutriSciences's in-house methods GK409 and GN063 (Mérieux NutriSciences, Ede, the Netherlands).

The Kemio™ heavy metal kit (KEMS10HM, Palintest, Gateshead, United Kingdom) was used to measure cadmium and lead on-site. Since the samples during the ozone and UF pilot experiments needed to be stored before analysis, they were acidified using nitric acid to stabilize the samples for measurement. In order to achieve stabilization, the pH of the samples was lowered to about 2.0. Before the acidified water samples could be measured with the kit, the pH was brought back to about 7.5 so that it fell within the kit's measuring capacity. Deacidification of the water samples was performed by adding sodium hydroxide. By adding nitric acid and sodium hydroxide, the volume of the samples increased. The measured concentrations Cd and Pb were multiplied by transversion factors to

estimate the concentrations of the initial water samples (transversion factor = total volume of measured sample/volume of the initial sample).

Furthermore, the water was analyzed for pesticide residues in accordance with in-house methods for LC-MSMS (A122) and GC-MSMS (A122) (Groen Agro Control, Delfgauw, the Netherlands). Finally, for the ozone pilot experiment, the concentration of ozone (mg/L) in the water was measured with the compact ozone meter (PTS043 Palintest, Gateshead, United Kingdom).

Collected endive samples were periodically taken, as described in Table A1.1 and Table A1.2. Cadmium and lead in these samples were analyzed in accordance with, respectively, NEN-EN 15763 and NEN-EN 15764 (Mérieux NutriSciences, Ede, the Netherlands). Pesticide residues were analyzed in accordance with in-house methods for LC-MSMS (A090) and GC-MSMS (A088) (Groen Agro Control, Delfgauw, the Netherlands).

2.8 Cost calculations for disinfection via the bypass

In order to estimate the economic feasibility, the costs of applying ozone and UF in a bypass operation, the operational expenditures (OPEX), including power consumption (kWh), maintenance (€), energy costs (€), as well as capital expenditures (CAPEX), were estimated. The total costs were subsequently calculated based on the treatment costs per m³ of water minus the savings on energy and water due to the treatment. Table 1 gives the input parameters used for the calculations. For ozone, a dosing of 4 mg/L was assumed.

Table 1 *Input values for cost calculations for disinfection via the bypass.*

General assumptions/characteristics of the washing lines (Sormac SW types 50 & 100)			
Effective freshwater intake (m³/h)	2	(intake equals purge)	
Amount of Treated Water, internal cycle (m³/h)	50	Sormac lines have 50 or 100m³/h recirculation	
Operation Time (days/year)	300	saved water (m3/h):	2
Depreciation Time (year)	5		
Operation Time (h/day)	14	h/year operation time	4200
Annual Amount of Treated Water (m³/year)	210000		
Annual Amount of used water (m³/year)	8400	ratio treated/saved	25
Energy price (€/kWh)	0.08	industrial price	
Water price (€/m³)	0.6	industrial price	
Total water costs/savings (no VAT, no BoL*) (€)	5040		
Savings on the reduction of energy losses by recirculation			
Tin (grdC)	15		
Tout (grdC)	2		
Heat saved (MJ/year)	456456	Cp (kJ/kgK)	4.18
Energy (kWh/year)	126793	3.6	MJ/kWh
Energy costs / saved (€/m3)	1.2		
Total energy costs/savings (€/year)	10143	(100% rendement e-> heat)	

* BoL, tax on drinking water (belastingdienst.nl).

3 Results and discussion

3.1 Results on the microbial effectiveness and the presence of chemical hazards in process wash water

3.1.1 *Escherichia coli*

The swabbed surfaces (see Figure 2, a-d) before each of the pilots showed that no *E. coli* was detected (results not shown).

When applied singly via a bypass system during fresh-cut endive processing, ozone and UF showed no clear reduction of *E. coli* in the PWW (Figure 5). These technologies' microbial effectiveness did not meet the aim of at least a 5-log reduction of *E. coli* in the PWW.

A possible explanation for the lack of reduction observed during the ozone pilot is attributed to the fact that ozone is largely used to oxidize the organic compounds present in the water, meaning there was an insufficient concentration of ozone available to reduce the *E. coli* present. This explanation is also supported by the observation of the return water from the skid, where the incoming color of the water was light green. In contrast, the water from the ozone skid returning to the washer was clear. Another explanation for the lack of reduction is that a limited amount of the water was treated with ozone via the bypass. Overall, given the high organic load, there was an insufficient concentration of ozone available to oxidize *E. coli* in the treated water. Therefore, with this bypass and processing setup, the 50 g O₃/h (a dosing of between 45 and 75 mg O₃/g COD) was insufficient to eliminate *E. coli* in the PWW.

The alternative setup with an additional pre-filter and treatment of the incoming water at 2 m³/h (0.055%) was insufficient during the UF experiments. The high organic load and larger particulate in the PWW from the fresh-cut endive and the soil on the produce also meant that the pre-filters had to be cleaned often. This need makes this technology less practical to apply on-site. Another explanation for the poor performance of both ozone and UF was due to the bypass design. Unfortunately, the sampling points were not placed directly after the technology, which allowed treated water to be mixed with untreated water. This will have influenced the bacterial loads detected. For UF, an additional water sample was taken at t = 116 min directly after the UF treatment before the treated water returned to the bypass. That sample showed a 2-log reduction of *E. coli* (Figure 5d). When the experiments were completed, a 'sucrose' test was performed to check leakage of the UF filters. It appeared that one filter was not functioning correctly anymore. Overall, with this bypass and processing setup, the UF (with pre-filters) was insufficient to eliminate *E. coli* in the PWW.

T128 was also examined during a pilot experiment, where the solution was applied directly to the PWW during fresh-cut endive processing. During this pilot, the average pH was 4.1, ranging from 3.8 to 4.6 during processing. This indication shows that the pH of the solution was kept stable. The concentration of chemical 1 and chemical 2 during the T128 pilot was not monitored on-line. T128 has been used as a processing aid and shown to effectively reduce *E. coli* when applied in combination with chlorine (Nou et al., 2011; Luo et al., 2012). However, when applied as a single component, the results were similar to the other two pilots, showing no clear reduction of *E. coli* in the PWW (Figure 6). The microbial effectiveness of this processing aid alone did not meet the aim of at least a 5-log reduction of *Escherichia coli* in the PWW.

Figure 5 Inactivation of *Escherichia coli* with ozone (a and b) and ultrafiltration (c and d) during pilot experiments. (a, c) First part of pilot experiments with inoculation at $t = 50$ min. (b, d) Second part of the pilot experiments with inoculation at $t = 110$ min. SP1 = before shaking table; SP3 = before treatment via the bypass; SP4 = after treatment via the bypass. ---, the limit of detection.

Figure 6 Inactivation of *Escherichia coli* with T128 during the pilot experiment. The first *E. coli* inoculation was at $t = 50$ min and the second inoculation was at $t = 110$ min. SP1 = before shaking table; SP2 = after cutting, also the point of inoculation.

3.1.2 Total Viable Count/Aerobic Plate Count

The microbial background levels in the PWW were about 5 log CFU/mL for the ozone pilot, about 4 log CFU/mL for the UF pilot, and about 3 log CFU/mL for the T128 pilot. Furthermore, the swabbed surfaces (see Figure 2, a-d) showed that TVC was maximally found at 6 CFU/cm² before the experiments.

Water collected during Ozone, UF, and T128 pilots showed that the TVC increased during processing. For instance, Figure 7 shows that during the T128 pilot, the background water contained about 3 log CFU/mL, water before the first inoculation about 4.5 log CFU/mL, and water after the first inoculation until the end of processing about 6.5-7 log CFU/mL.

Figure 7 Total viable count (TVC) during the T128 pilot experiment. The first *E. coli* inoculation was at $t = 50$ min and the second inoculation was at $t = 110$ min.

Additional water samples were collected (by Sormac) and analyzed for aerobic plate counts (APCs) during each of the pilots. Results showed an increase from about 2 log CFU/mL to 7 log CFU/mL for the ozone pilot; about 4.5 log CFU/mL to 7 log CFU/mL for the UF pilot; and about 4 log CFU/mL to 6.5 log CFU/mL for the T128 pilot. The increase over time is logical since *E. coli* was added at 6 log CFU/mL, so these levels are found back in the TVC.

3.1.3 Colitag™: *E. coli* and Coliforms

Results of the Colitag™ analyses of the PWW showed that coliforms were present in all the wash water samples collected. This result is plausible as coliforms are naturally present in the endive at high concentrations. Because of the natural, high presence of coliforms, the Colitag™ analyses of the PWW for *E. coli* were challenging.

3.1.4 Chemical Oxygen Demand and Ozone concentrations

The collected PWW (from SP1) shows that COD increases with time during processing (Figure 8). The results are similar for the pilot experiments, especially when ozone and UF were tested in the bypass. During the T128 pilot experiment, the COD values were higher than the ozone and UF pilots at 107 and 120 min. In previous laboratory experiments, which tested ozone disinfection of surface water, the COD was shown to decrease, most probably since the ozone concentration could oxidize the organic and inorganic compounds (Banach et al., 2021). In this pilot study, the COD during the ozone pilot increased over time, implying that the ozone concentration was insufficient to oxidize organic and inorganic compounds. This also concurs with our result that ozone, yet also UF and T128, did not meet the aim of at least a 5-log reduction of *E. coli* in the PWW. However, results from the compact ozone

meter of the PWW (SP4) showed that ozone was still present in the PWW returning to the washer, albeit at relatively low concentrations (0.19 – 0.33 mg/L) (Figure 9). It seems unlikely that ozone was present in these concentrations, seeing the fast reaction time of ozone, the high concentrations of COD present in the water and the lack of disinfection.

Figure 8 Average chemical oxygen demand (COD) of the process wash water during pilots with ozone, ultrafiltration (UF), and T128. Samples were collected from the wash tank at the end of the washing line (i.e., at sampling point 1) at time points 0, 47, 107, and 120 min after the technology started. The first *E. coli* inoculation was at $t = 50$ min and the second inoculation was at $t = 110$ min.

Figure 9 Average ozone concentrations ($n = 2$) of the process wash water after treatment via the bypass (i.e., at sampling point 4) during the ozone pilot. Samples were collected at time points 47, 51, 55, 107, 111, and 120 min after the technology started. The first *E. coli* inoculation was at $t = 50$ min and the second inoculation was at $t = 110$ min. ---, the limit of quantification.

3.1.5 Heavy metals: Cadmium and Lead

Cadmium and lead concentrations from PWW samples were collected from the wash tank at the end of the washing line before the shaking table (i.e., sampling point 1); they were measured with the Kemio™ heavy metal kit as well as with in-house methods of an accredited laboratory (Mérieux NutriSciences).

Results for cadmium show that concentrations increase during processing (Figure 10). This was observed for the ozone and UF pilots (via the bypass) at 107 and 120 minutes. During the T128 pilot (directly applied in the wash tank), cadmium was detected at 47 and 120 minutes. Although cadmium appears to accumulate in the wash water over time, the concentrations remain low and well below the legal limit of 5 µg/L (Directive 98/83/EC). Results for lead show that concentrations increased during processing for ozone and UF yet were not detected during the T128 pilot (Figure 11). The lead concentration at t=120 min for the UF experiment showed an exceedance of the legal limit of 10 µg/L as indicated in Directive 98/83/EC during the Kemio test. However, this level was not confirmed in the lab analysis.

Heavy metals, including cadmium and lead, can be present in leafy vegetables from the uptake during cultivation from the soil, air, or irrigation water. Many factors can influence the uptake capacity of heavy metals to the edible portion of leafy vegetables, including intrinsic factors like the plant species or extrinsic factors like previous water regimes (Banach et al., 2019). Given the results for cadmium and lead in the endive (see section 3.2.5), it may be possible that from cutting the endive, the heavy metals leached out in the water, and could thus explain the difference between background PWW samples before the technology as on (t = pre0) and results found during processing.

Figure 10 Average cadmium (Cd) concentrations of the process wash water during pilots with technologies ozone (O3), ultrafiltration (UF), and T128. (a) Results from the Kemio™ heavy metal kit and (b) results from Mérieux NutriSciences. Samples were collected from the wash tank at the end of the washing line before the shaking table (i.e., at sampling point 1) at time points 0, 47, 107, and/or 120 min after the technology started. T = pre0 reflects background samples before the technology was on. The first *E. coli* inoculation was at t = 50 min and the second inoculation was at t = 110 min. ---, the limit of quantification (LOQ) for (a) was 0.2 µg/L and for (b) was 0.3 µg/L.

Figure 11 Average lead (Pb) concentrations of the process wash water during pilots with technologies ozone (O3), ultrafiltration (UF), and T128. (a) Results from the Kemio™ heavy metal kit and (b) results from Mérieux NutriSciences. Samples were collected from the wash tank at the end of the washing line before the shaking table (i.e., at sampling point 1) at time points 0, 47, 107, and/or 120 min after the technology started. T = pre0 reflects background samples before the technology was on. The first *E. coli* inoculation was at t = 50 min and the second inoculation was at t = 110 min. ---, the limit of quantification (LOQ) for (a) was 2 µg/L and for (b) was 5 µg/L.

3.1.6 Nitrate

Nitrate concentrations from PWW samples from the wash tank at the end of the washing line before the shaking table (i.e., sampling point 1) were analyzed with in-house methods of an accredited laboratory (Mérieux NutriSciences). The nitrate results show that concentrations increase during processing for the ozone and T128 pilots, but nitrate was not quantified during the UF pilot (Figure 12). The highest concentration of nitrate was found during the ozone pilot at $t = 120$ min (42 mg/L). Previous research has shown that leafy vegetables can take up nitrate, with plant characteristics and the environment influencing the uptake into the edible portions of plants (Banach et al., 2019). Given the nitrate results in the endive (see section 3.2.6), it may be possible that nitrate leached out into the water from the cut endive. The levels found were, however, below the legal limit of 50 µg/L (Directive 98/83/EC).

Figure 12 Average nitrate concentrations of the process wash water during pilots with technologies ozone, ultrafiltration (UF), and T128. Samples were collected from the wash tank at the end of the washing line before the shaking table (i.e., at sampling point 1) at time points 0, 47, 107, and/or 120 min after the technology started. The first *E. coli* inoculation was at $t = 50$ min and the second inoculation was at $t = 110$ min. ---, the limit of quantification (LOQ).

3.1.7 Pesticide residues

During the ozone pilot, spirotetramat-keothydroxy and spirotetramat (sum) were detected, respectively, at 0.10 and 0.12 µg/L, in one of the wash water samples collected ($n=2$). During the UF pilot, fluopyram was detected in both wash water samples collected ($n = 2$, mean = 0.16 µg/L, standard deviation = 0). Finally, during the T128 pilot, spinosad was detected (0.038 µg/L) in one of the two wash water samples. Overall, the sum of the total pesticides in each water sample was less than 50 µg/L, which is the chemical parameter for water intended for human consumption as outlined in Directive 98/83/EC under Annex I, Part B.

3.1.8 Chemical 1 and Chemical 2 during T128 pilots

During the T128 pilot, the concentrations of the ingredients of T128 (i.e., chemical 1 and chemical 2) were measured in water samples collected from the wash tank at the end of the washing line (i.e., at sampling point 1). Results show that chemical 1 (Figure 13a) and chemical 2 (Figure 13b) are either

not quantified or very low in the water before the technology was turned on but are detected at the end of processing. This is expected as the chemicals were directly added to the wash water.

Figure 13 Average concentrations (a) chemical 1 and (b) chemical 2 of the process wash water during the T128 pilot. Samples were collected from the wash tank at the end of the washing line before the shaking table (i.e., at sampling point 1) at time point 0 and 130 min after the technology started. ---, the limit of quantification (LOQ).

3.2 Results on the microbial effectiveness and the presence of chemical hazards on endive

Before the experiments, the endive was visually inspected. This showed that the endive heads during the pilot with UF were smaller and had noticeably more soil than the other pilot experiments. According to the supplier and processor, it is usual to find such differences between the different batches; it can be attributed to weather conditions during growing or harvest. However, there was no major difference between the endive used for the pilot experiments.

3.2.1 *Escherichia coli*

Cut endive samples were sampled before and after washing, respectively, at the first transport belt and the final transport belt outlet and analyzed for *E. coli*. Results show that at $t=0$, all samples are below or around the limit of detection (LOD) (Figure 14). Washed endive samples from during the ozone and UF pilots had >4 log CFU/mL of *E. coli* detected. *E. coli* was not detected on the washed endive samples during the T128 pilot. This could partly be attributed to the final rinsing step during processing (i.e., the spray nozzles were on) from 118 to 125 min. It may be possible that chemicals 1 and 2 prevented *E. coli* attached to the produce; however, this hypothesis would need to be further validated with experiments.

Figure 14 Presence of *Escherichia coli* in unwashed and washed endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments. ---, the limit of detection (LOD).

3.2.2 Total Viable Count

Cut endive samples were sampled before and after washing, respectively, at the first transport belt and the final transport belt outlet and analyzed for TVC. Results show that the differences between the unwashed and washed endive are within about 0.5 log CFU/g of each other, levels are about 6 log CFU/g (Figure 15).

Figure 15 Total viable counts in unwashed and washed endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments.

3.2.3 Enterobacteriaceae

Cut endive samples were sampled before and after washing, respectively, at the first transport belt and the final transport belt outlet and analyzed for *Enterobacteriaceae*. Results show accumulation over time, with levels between about 4 and 6 log CFU/g (Figure 16).

Figure 16 Enterobacteriaceae in unwashed and washed endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments. ---, the limit of detection (LOD).

3.2.4 Lactic acid bacteria

Cut endive samples were sampled before and after washing, respectively, at the first transport belt and the final transport belt outlet and analyzed for LAB. Results show that several samples are below the LOD of 2 log CFU/g (Figure 17). During the T128 pilot, the endive was between 2 and 3 log CFU/g. This difference may be attributed to the batch of endive tested during that pilot.

Figure 17 LAB in unwashed and washed endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments. ---, the limit of detection (LOD).

3.2.5 Heavy metals: Cadmium and Lead

Cut endive samples were sampled after washing at the final transport belt outlet and analyzed for cadmium. Results show that concentrations range from 28.5 to 52.5 µg/kg during all three pilots (Figure 18). Compared to the EU legal limits for cadmium in leafy vegetables (Regulation (EC) No 1881/2006), these concentrations were well below the maximum limit of 200 µg/kg.

Similarly, cut endive samples were sampled after washing at the final transport belt outlet and analyzed for lead. Results show that concentrations range from 7.0 to 16.0 µg/kg during all three pilots (Figure 19). Compared to the EU legal limits for lead in leafy vegetables (Regulation (EC) No 1881/2006), these concentrations were well below the maximum limit of 300 µg/kg.

Figure 18 Average cadmium concentrations (µg/kg) in endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments.

Figure 19 Average lead concentrations (µg/kg) in washed endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments.

3.2.6 Nitrate

Cut endive samples were sampled before and after washing, respectively, at the first transport belt and the final transport belt outlet and analyzed for nitrate. Results show lower concentrations in washed endive samples, and concentrations during the T128 pilot are generally lower, ranging from 445-476 mg/kg (Figure 20). There are no legal limits for endive, but compared to the EU legal limits for nitrate in fresh spinach or lettuce (Regulation (EC) No 1881/2006), these concentrations were well below the maximum limit of 3,500 and 3,000 mg/kg, respectively.

Figure 20 Average nitrate concentrations (mg/kg) in unwashed and washed endive samples during ozone, ultrafiltration (UF), and T128 pilot experiments.

3.2.7 Pesticide residues

No pesticide residues were detected in the endive samples from any of the pilots.

3.2.8 Chemical 1 and Chemical 2 during T128 pilots

During the T128 pilot, the concentration of chemical 1 and chemical 2 was measured in endive samples collected from the final transport belt outlet. Results show that chemical 1 (Figure 21a) is present in the endive before and after processing with T128, with similar concentrations of around 500 mg/kg. The results for chemical 2 (Figure 21b) show that it was present before processing with T128 but is below the limit of quantification at the end of processing. The presence of chemical 2 in the endive before processing is not expected. Other experiments found that chemical 2 was not detected (detection limit 6 mg/kg) on spinach (n = 4) and lettuce (n = 4) when washed with and without chlorine and processing aid T-128 (at 0.1%) (Pascual et al., 2017). Research from Nou et al. (2011) suggested that T128 effects on produce may be reduced by including a rinse step following washing. During the T128 pilot, the endive sampled at t = 121 min was sprayed with tap water before being collected and analyzed. This rinsing step can explain the lower concentrations of chemical 1 and chemical 2 in endive at t = 121 min.

Figure 21 Average concentrations (a) chemical 1 and (b) chemical in endive during the T128 pilot. Samples were collected at the final transport belt outlet at time point 0 and 121 min after the technology started. ---, the limit of quantification (LOQ).

3.3 Cost calculations for disinfection via the bypass

Based on the characteristics of the washing line used and the assumptions made in section 2.8, the expected OPEX, CAPEX, and total costs were calculated for ozone and UF. Figure 22 shows the costs of the technologies attributed to the bypass as applied in the pilot experiment. Results align with expected costs for these processes and are based on the bypass flow of 50 m³/h to be treated.

If the bypass treatment is reliable and robust, the washing system is equipped to handle microbiological contaminations and a purge stream is then no longer needed. This leads to the elimination of the need for the intake of freshwater. If treating the total bypass flow enables one to minimize the purge, and, thereby, the intake of freshwater of 2 m³, then 25 times more water needs to be treated (volume of water of 210,000 m³/year) to save about 8,400 m³/year. When allocating these to the actual or potential savings on freshwater, these treatment costs will increase to about 2.10 €/m³ for ozone treatment and 2.70 €/m³ for UF treatment. Savings are then to be expected for reduced water intake costs of 5 k€/year and avoided energy costs for cooling this water to 2 °C of 127,000 kWh/year or about 10 k€/year. Including potential savings assuming a working zero purge system on both energy and water intake, then the total costs per m³ of treated water are 0.28€/m³ for Ozone treatment and 0.88€/m³ for UF treatment. This approach implies that the total amount of water to be treated is significantly more than the amount of water saved; in this pilot-setup, about 25 times. Consequently, treatment costs increase in the same order of magnitude. It should be noted that the costs in practice will be higher since the ozone dose and the UF as applied in the pilot were not sufficient to inactivate the *E.coli* present. Nevertheless, the calculations show that the ozone application, overall, is less expensive than UF.

Figure 22 Operational expenditures (OPEX), capital expenditures (CAPEX), and total costs for the application of ozone and ultrafiltration (UF) in a bypass to treat process wash water.

4 Conclusions and recommendations

4.1 Conclusions

This study evaluated, at the pilot scale, the effectiveness of single applications of ozone, UF, and T128 during fresh-cut endive processing on the reduction of inoculated *E. coli* to the PPW water. Using the water disinfection technologies in the bypass ensured that the product did not come into contact with the treatment technology, i.e. ozone or UV. During the pilot experiments, there were many challenges during processing, including the fast water recirculation (high water flow), high background contaminants (COD, microorganisms, etc.), and a vulnerable type of produce (curly leaves, cut). Overall, using this experimental design, the tested water disinfection technologies were insufficient in their microbial effectiveness. Contrary to expectations based on literature and comparable industrial applications, these technologies did not meet the aim of at least a 5-log reduction of *E. coli* in the PPW.

The main conclusions found are:

- There is no *E. coli* reduction detected in the PPW during the pilot experiments with ozone, UF, or T128.
- During the ozone and UF pilots, where the treatment occurred via a bypass, the amount of water treated was insufficient. For ozone, the dose applied was too low to have a significant effect, while for UF, there were problems with fouling of the membranes.
- Cadmium and lead were shown to accumulate in the PPW over time. The cadmium and lead concentrations overall did not exceed the legal limits in water and endive.
- Pesticide residues were not detected in the endive or were found in very low concentrations in the PPW.
- Application of the technologies in a bypass system resulted in water and energy savings. However, these savings were in the same order of magnitude as the technologies' costs. Overall, ozone applied in a bypass was cheaper than UF.

The implications of these findings are as follows:

- Monitoring and adjusting the dose of the technology – ozone – e.g., based on the water quality parameters, like the COD, is required given the bypass setup experimented within this study.
- Since the pilot experiment with UF showed clogging of the filters, a pre-treatment of the PPW might improve the performance of this disinfection technology.
- The bypass system's experimental design for the ozone and UF pilots did not result in a robust and reliable result for disinfection aims.

The study provides the first proof of application for three different water disinfection technologies during pilot-scale fresh-cut endive washing. The pilot experiments' main limitation was the design of the bypass system combined with the feasibility of applying (/dosing) ozone and UF at this scale in this setting. However, it allowed us to compare microbial and chemical aspects in the PPW and on the endive. It showed that recycling the wash water does not lead to an accumulation of chemical hazards studied (cadmium, lead, nitrate) in the PPW and endive above the EU legal limits.

4.2 Recommendations

Future research should examine a modified bypass setup for ozone, as the primary limitation was the insufficient dose applied. A possible combination of ozone and another technique, like UV, may be of interest to further research as a combined technology will improve the disinfection efficiency. Other bypass setups where the treatment time is longer and the organic load is less dynamic may be interesting for UF, provided the costs and application are feasible.

Furthermore, the bypass design should be adapted to allow a higher water volume to be treated. With this, the sampling points should be mounted directly after the technology to assess the technology's effectiveness more accurately.

Finally, this research focused on a model organism for Gram-negative bacteria. It would be worthwhile to explore the effectiveness of disinfection technologies on Gram-positive bacteria, such as *Listeria monocytogenes*. The type of pathogen and strain may respond differently to water disinfection technologies. Furthermore, *L. monocytogenes* has been implicated in outbreaks related to the consumption of contaminated fresh produce (Zhu et al., 2017).

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Annex 1 Experimental sampling plans

Table A1.1 Overview of the scheduled event or sampling plan during the (i) ozone and (ii) UF pilot experiments.

Time	Event and/or sample
7:00-7:30	Endive arrives at the location. It was stored in a cold room (~15 °C).
9:00	Arrive at Sormac location.
9:00 (on arrival)	Put the cutting equipment in the correct places.
9:00 (on arrival)	Visually clean and disinfect the line with 80% ethanol (as showed before experiment 1).
09:15-09:45	Briefing and explanation of time scheduling (when one must step back etc.)
09:15-13:30	Coordinate the experiment and track time schedule (in the processing facility, but not close to the washing line)
09:15-13:30	Take measurements of water and endive <ul style="list-style-type: none"> • Temperature • pH • Flow rate • Etc.
09:15-13:30	Technical help with line and equipment, fill in for cutters if needed
9:40	Take swabs before experiments (at the washing line) <ol style="list-style-type: none"> 1) Mid-cutting board 2) Middle 1st transfer belt 3) Wash machine near SP2 4) Middle of the last belt
09:45	Starting up the system (filling the washing line) (in front of the washing line)
09:45-10:20	Checking if the technology runs properly
10:00	Start production. Start cutting the endive.
10:09	Samples water for background at SP1 (3 L) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™ • Cd Kemio • Pb Kemio • COD Mérieux NutriSciences in duplicate • Cd Mérieux NutriSciences in duplicate • Pb Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate
10:10	Starting technology
10:15 (t=0)	Technology is set
10:50 (t=35 min)	Sample unwashed endive (before inoculation) after the cutter (2 x 200 g) <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate (2 x 300 g)
10:55 (t=40 min)	Sample washed endive (before inoculation) at the outlet (2 x 200 g) <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate (2 x 300 g) • Pb Mérieux NutriSciences in duplicate • Cd Mérieux NutriSciences in duplicate • Pesticides Groenlab in duplicate (2 x 1 kg)

Time	Event and/or sample
11:02 (t=47 min)	Sample water at SP1 (3 L in total) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™ • Cd Kemio • Pb Kemio • COD Mérieux NutriSciences in duplicate (2 acidified bottles) • Nitrate Mérieux NutriSciences in duplicate (2 normal bottles)
11:02 (t=47 min)	Sample water at SP4 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
11:05 (t=50 min)	First inoculation (SP2)
11:06 (t=51 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i>
11:06 (t=51 min)	Sample water at SP 3 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> Sample water at SP 4 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
11:07 (t=52 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i>
11:07 (t=52 min)	Sample water at SP 4 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
11:10 (t=55 min)	Sample water at SP 1 (121 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • TVC • Colitag™
11:10 (t=55 min)	Sample water at SP 2 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
11:10 (t=55 min)	Sample water at SP4 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
~11:15 (t=60 min)	Sample of the inoculum for <i>E. coli</i>
12:02 (t=107 min)	Sample water at SP1 (3 L in total) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™ • Cd Kemio • Pb Kemio • COD Mérieux NutriSciences in duplicate (2 acidified bottles) • Nitrate Mérieux NutriSciences in duplicate (2 normal bottles)
12:02 (t=107 min)	Sample water at SP4 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
12:05 (t=110 min)	Second inoculation
12:06 (t=111 min)	Sample water at SP 1 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • Colitag™
12:06 (t=111 min)	Sample water at SP 3 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> Sample water at SP 4 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
12:07 (t=112 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
12:07 (t=112 min)	Sample water at SP 4 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • Colitag™

Time	Event and/or sample
12:10 (t=115 min)	Sample water at SP 1 (121 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™
12:10 (t=115 min)	Sample water at SP 2 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • Colitag™
12:10 (t=115 min)	Sample unwashed endive after cutter (2 x 200g) <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate (2 x 300g)
12:10 (t=115 min)	Sample water at SP 4 (121 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™
12:10 (t=115 min)	Sample washed endive at the outlet (2 x 200g) <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate
12:15 (t=120 min)	Stop cutting endive
12:15 (t=120 min)	Sample water at SP 1 (4.4 L in total) <ul style="list-style-type: none"> • Cd Kemio in duplicate • Pb Kemio in duplicate • COD Mérieux NutriSciences in duplicate (2 acidified bottles) • Cd Mérieux NutriSciences in duplicate (2 normal bottles) • Pb Mérieux NutriSciences in duplicate (2 normal bottles) • Nitrate Mérieux NutriSciences in duplicate (2 normal bottles) • Pesticides Groenlab in duplicate (2 x 1 L)
12:15 (t=120 min)	Sample washed endive at the outlet (3 kg in total) (2 x 300g): <ul style="list-style-type: none"> • Cd Mérieux NutriSciences in duplicate • Pb Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate • Pesticides Groenlab in duplicate (2 x 1 kg)
12:15	Stop production
12:20	Remove cut and washed endive
12:20	All samples to cooled storage or to lab
12:20	Water samples for Cd and Pb to lab and perform Kemio test with nitric acid
12:45	Mount UV unit on the washer
13:00	Lunch and debriefing
13:00	Treat rest water in washing line before draining with UV Adjust pH between 6.5-10.
13:15	Discard water
13:30	Fill with clean water and start cleaning with cleaning agents
14:20	Disassembling the bypass/the treatment
Next morning	Transport waste endive

Table A1.2 Overview of the scheduled event or sampling plan during the (iii) T128 at pH 4 pilot experiment.

Time	Event and/or sample
7:00-7:30	Endive arrives at the location. It was stored in a cold room (~15 °C).
9:00	Arrive at Sormac location.
9:00 (on arrival)	Put the cutting equipment in the correct places.
9:00 (on arrival)	Visually clean and disinfect the line with 80% ethanol (as showed before experiment 1)
09:15-09:45	Briefing and explanation of time scheduling (when one must step back etc.)
09:15-13:30	Coordinate the experiment and track time schedule (in the processing facility, but not close to the washing line)
09:15-13:30	Take measurements of water and endive <ul style="list-style-type: none"> • Temperature • pH • Flow rate • Etc.
09:15-13:30	Technical help with line and equipment, fill in for cutters if needed
9:40	Take swabs before experiments (at the washing line) <ol style="list-style-type: none"> 1) Mid-cutting board 2) Middle of the first transfer belt 3) Wash machine near SP2 (point of inoculation) 4) Middle of the last belt
09:45	Starting up the system (filling the washing line) (in front of the washing line)
10:00	Start production. Start cutting the endive.
10:00	Turn on spray nozzles
10:02	Sample of washed endive at the outlet as background sample (2 x 500 g) for chemical 1 and chemical 2
10:05	Turn off spray nozzles
10:09	Samples water for background at SP1 (3.3 L) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™ • Cd Kemio • Pb Kemio • COD Mérieux NutriSciences in duplicate • Cd Mérieux NutriSciences in duplicate • Pb Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate • Chemical 1 and chemical 2 in duplicate (2 x 250 mL)
10:10	Start technology
10:15 (t=0)	Technology is set
10:50 (t=35 min)	Sample unwashed endive (before inoculation) after the cutter (2 x 200 g): <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate (2 x 300 g)
10:53 (t=38 min)	Turn on spray nozzles
10:55 (t=40 min)	Sample washed endive (before inoculation) at the outlet (2 x 200g): <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate
10:57 (t=42 min)	Turn off spray nozzles
11:00 (t=45min)	Sample washed endive (before inoculation) at the outlet (2 x 300g): <ul style="list-style-type: none"> • Nitrate Mérieux NutriSciences in duplicate • Pb Mérieux NutriSciences in duplicate • Cd Mérieux NutriSciences in duplicate • Pesticides Groenlab in duplicate (2x1kg)

Time	Event and/or sample
11:02 (t=47 min)	Sample water at SP1 (3 L in total) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™ • Cd Kemio • Pb Kemio (2 acidified bottles) • COD Mérieux NutriSciences in duplicate (2 normal bottles) • Nitrate Mérieux NutriSciences in duplicate
11:05 (t=50 min)	First inoculation at SP2
11:05:30 (t=50.5 min)	Sample water at SP 2 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i>
11:06 (t=51 min)	Sample water at SP 2 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i>
11:07 (t=52 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i>
11:10 (t=55 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • TVC
11:10 (t=55 min)	Sample water at SP 2 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i>
11:15 (t=60 min)	Sample water at SP 1 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
11:15 (t=60 min)	Sample water at SP 2 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> • Colitag™
~11:20 (t=65 min)	Sample of the inoculum for <i>E. coli</i>
12:02 (t=107 min)	Sample water at SP1 (3 L in total) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate • Colitag™ • Cd Kemio • Pb Kemio (2 acidified bottles) • COD Mérieux NutriSciences in duplicate (2 normal bottles) • Nitrate Mérieux NutriSciences in duplicate
12:05 (t=110 min)	Second inoculation at SP2
12:05:30 (t=110.5 min)	Sample water at SP 2 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
12:06 (t=111 min)	Sample water at SP 2 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
12:07 (t=112 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
12:10 (t=115 min)	Sample water at SP 1 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • TVC in duplicate
12:10 (t=115 min)	Sample water at SP 2 (6 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate
12:13 (t=118 min)	Start the spray nozzles
12:15 (t=120 min)	Sample washed endive at the outlet (2 x 200 g) <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate
12:16 (t=121 min)	Sample of washed endive at the outlet (2 x 500 g) for chemical 1 and chemical 2
12:20 (t=125 min)	Turn off spray nozzles
12:15 (t=120 min)	Sample water at SP 1 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • Colitag™

Time	Event and/or sample
12:15 (t=120 min)	Sample water at SP 2 (106 mL) <ul style="list-style-type: none"> • <i>E. coli</i> in duplicate • Colitag™
12:15 (t=120 min)	Sample unwashed endive after cutter (2 x 200 g) <ul style="list-style-type: none"> • TVC Mérieux NutriSciences in duplicate • <i>E. coli</i> Mérieux NutriSciences in duplicate • Enteros Mérieux NutriSciences in duplicate • LAB Mérieux NutriSciences in duplicate (2 x 300 g) • Nitrate Mérieux NutriSciences in duplicate
12:21 (t=126 min)	Sample washed endive at the outlet (3 kg in total) (2 x 300 g) <ul style="list-style-type: none"> • Cd Mérieux NutriSciences in duplicate • Pb Mérieux NutriSciences in duplicate • Nitrate Mérieux NutriSciences in duplicate (2 x 1 kg) • Pesticides Groenlab in duplicate
12:25 (t=130 min)	Sample water at SP 1 (4.4L in total) <ul style="list-style-type: none"> • Cd Kemio in duplicate • Pb Kemio in duplicate (2 acidified bottles) • COD Mérieux NutriSciences in duplicate (2 normal bottles) • Cd Mérieux NutriSciences in duplicate • Pb Mérieux NutriSciences in duplicate (2 normal bottles) • Nitrate Mérieux NutriSciences in duplicate (2 x 1 L) • Pesticides Groenlab in duplicate • Chemical 1 and chemical 2 in duplicate (2 x 250 mL)
12:20 (t=130 min)	Stop cutting endive
12:20 (t=130 min)	Stop technology
12:30 (t=130 min)	Stop production
12:30	Remove cut and washed endive
12:30	All samples to cooled storage or to lab
12:30	Water samples for Cd and Pb to lab and perform Kemio test with nitric acid
12:55	Mount UV unit on the washer
13:10	Lunch and debriefing
13:10	Treat rest water in washing line before draining with UV Adjust pH between 6.5-10.
13:25	Discard water
13:40	Fill with clean water and start cleaning with cleaning agents
14:30	Disassembling the bypass/the treatment
Next morning	Transport waste endive

Annex 2 Additional parameters monitored during the pilots

Table A2.1 Overview of the additional parameters measured during the ozone pilot.

Test 18 June 2020 Ozone					
Time [min]	Temp. water [°C]	Temp. air [°C]	Temp. Product [°C]	Main flow [m ³ /h]	Bypass flow [m ³ /h]
0	9.7	17.8	11.7	37.5	1 – 1.5
10	9.5	17.8	10.4	37.5	1 – 1.5
20	9.5	17.8	11.7	37.5	1 – 1.5
30	9.7	17.8	11.5	37.5	1 – 1.5
40	11.4	18.0	11.5	37.5	1 – 1.5
50	12.1	18.0	11.7	37.5	1 – 1.5
60	12.4	18.0	11.5	37.5	1 – 1.5
70	14	18.0	11.9	37.5	1 – 1.5
80	15	18.1	12.0	37.5	1 – 1.5
90	13.3	18.2	12.2	37.5	1 – 1.5
100	12.9	18.2	11.8	37.5	1 – 1.5
110	12.1	18.2	10.9	37.5	1 – 1.5
120	12.5	18.2	11.8	37.5	1 – 1.5

Table A2.2 Overview of the additional parameters measured during the ultrafiltration (UF) pilot.

Test 25 June 2020 UF						
Time [min]	Temp. water [°C]	Temp. air [°C]	Temp. Product [°C]	Main flow [m ³ /h]	Bypass flow [m ³ /h]	pH washer
0	8.5	18.7	11	37.5	4.8	7.94
10	8.7	18.6	12.3	36	3.3	8.00
20	10.5	18.6	12.3	34	n/a	7.96
30	10	18.6	12.7	30	n/a	7.92
40	9.9	18.6	12.7	27	n/a	7.92
50	9.6	18.7	12.8	32	n/a	7.90
60	10.5	18.8	13.0	27	n/a	7.91
70	10.3	18.8	12.8	37	n/a	7.89
80	9.7	18.8	12.9	22	n/a	7.85
90	11.3	18.8	12.5	32	n/a	7.85
100	10.4	18.8	12.6	20	n/a	7.81
110	10.1	18.9	13.2	27	n/a	7.82
120	9.7	18.8	13.4	20	n/a	7.85

Table A2.3 Overview of the additional parameters measured during the T128 pilot.

Test 2 July 2020 T128							
Time [min]	Temp. water [°C]	Temp. air [°C]	Temp. Product [°C]	Main flow [m³/h]	Bypass flow [m³/h]	pH washer	pH T128
0	9.9	17.9	8.5	37	n/a	4.06	
10	9.5	17.9	8.7	37	n/a	4.26	
20	9.4	17.9	8.5	37	n/a	4.28	4.08
30	11.5	17.8	8.8	37	n/a	4.12	3.88
40	12	17.8	8.9	37	n/a	4.54	4.38
50	11.9	17.9	8.8	37	n/a	4.44	4.17
60	11.3	18.0	10.7	37	n/a	4.22	3.95
70	11.8	18.0	10.8	37	n/a	4.27	4.07
80	11.7	18.0	12.4	37	n/a	4.42	4.12
90	12.6	18.0	9.0	37	n/a	4.24	4.02
100	12.8	18.0	11.1	37	n/a	4.36	4.06
110	14.3	18.0	12.3	37	n/a	4.62	4.51
120	13.8	18.0	13.5	37	n/a	4.35	4.15
130	13.3	17.9	13.5	37	n/a	4.29	3.93

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