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Beetroot ketchup as a stable carrier of potential probiotic *Lacticaseibacillus rhamnosus* K3 and *Lactobacillus johnsonii* K4: A study on sensory attributes, storage viability, and *in vitro* gastrointestinal survival

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ABSTRACT

This research aimed to assess the viability of *Lacticaseibacillus rhamnosus* K3 and *Lactobacillus johnsonii* K4 in beetroot ketchup during storage and simulated digestion to examine fermentation effects on sensory quality. The findings revealed that both strains maintained viability above 8 $\log_{10}$ CFU/ml during storage, confirming their potential as probiotics. pH levels changed significantly over three-week storage period indicating fermentation's impact on shelf stability. The control sample maintained consistent pH level of 4.6, while pH of ketchup fermented with *L. rhamnosus* K3 decreased from 3.84 to 3.79, and ketchup fermented with *L. johnsonii* K4 decreased from 3.96 to 3.69. Sensory evaluations showed statistically significant differences in odor, texture, flavor, and overall quality between samples. Fermentation with *L. johnsonii* K4 improved sensory attributes, achieving the highest overall quality score with mean value of 7.31 out of 10, compared to 6.28 for the control and 6.23 for the *L. rhamnosus* K3 fermented ketchup. Survival rate of *L. johnsonii* K4 was 27 %, compared to 2.8 % for *L. rhamnosus* K3 in dynamically simulated gastrointestinal system TIM-1. Both fermented ketchups contained over 10^9 CFU of viable cells. These results demonstrate that plant-based food products can effectively serve as carriers for potential probiotic strains, preserving their viability during storage and digestion, while enhancing sensory quality of food products.

1. Introduction

Probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," have gained importance in the development of functional foods and health promotion (Cordaillat-Simmons et al., 2020). They are primarily found in fermented foods and dietary supplements with strains of the *Bifidobacterium* genus and lactic acid bacteria such as *Enterococcus, Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus, Pediococcus*, and *Streptococcus* prevailing (Soemarie et al., 2021). Food products containing probiotics are generally derived from dairy sources, which means that not everyone can benefit from them. It is especially important for people who suffer from allergies, or lactose intolerance, have vegan eating habits, or are concerned about their health and sustainability (Küçükgöz and Trząskowska, 2022). As a result of these concerns, the market has seen an increase in the number of non-dairy probiotic-containing foods. The food matrix specialties of vegetables, including their low pH, sugar and fiber content, along with their overall nutritional values, make them ideal candidates for carrying probiotic strains (Maia et al., 2023). However, bioactive compounds in plant-based food matrices, and the amount of vegetables included in the product, can affect the survival of probiotic strains. In this context, interesting and little researched are beetroot and tomato, which are rich in bioactive compounds that can affect probiotic viability. Beetroot contains betalains like betacyanins and betaxanthins, phenolic compounds such as flavonoids and phenolic acids, and nitrates, all of which have antioxidant properties that can create a favorable environment for probiotics (Czyżowska et al., 2020; Sentkowska and Pyrzyńska, 2023). Tomatoes are packed with carotenoids, including lycopene and beta-carotene, which protect probiotics from oxidative damage (Boulaajine and Hajjaj, 2024). They also contain phenolic compounds such as caffeic acid, p-coumaric acid, and ferulic acid and glycoalkaloids

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and can enhance the growth environment for probiotics by reducing oxidative stress and inflammation. Vitamins and essential minerals in tomatoes further stabilize and support probiotics in tomato-based food matrices (Coelho et al., 2023). These bioactive compounds collectively can create a conducive environment for probiotics growth, enhancing their viability and efficacy in non-dairy, vegetable-based fermented products.

Therefore, food matrix selection will have a direct impact on probiotic strains (Dinkçi et al., 2019). Probiotic strains have already been successfully tested in different kinds of vegetables and have shown good sensory properties with ability to carrying these strains (Lillo-Pérez et al., 2021). As part of the potential probiotic selection for this study, 38 strains from traditional fermented foods were screened and strains were isolated from pickled cucumbers and sauerkraut found in homes in central Poland and was characterised by its ability to survive at low pH levels, tolerance of bile salts, excess of phenols. It is also important to draw attention for the remarkable abilities of *L. johnsonii* K4 growth during the exposure of phenols and hydrophobicity by over 60 %. This makes *L. johnsonii* K4 a promising probiotic species that could benefit from further study and potential integration into functional foods. (Zielińska et al., 2015). Beetroot and tomato, both rich sources of essential nutrients, antioxidants, and bioactive compounds, offer a unique opportunity for the development of fermented products that combine taste and nutrition (Ali et al., 2020; Punia Bangar et al., 2022). Nevertheless, these food matrices should also ensure that the probiotic strains remain viable during storage and be resistant to digestion. The recommended minimum viability level of 10^6 colony-forming units (CFU) per millilitre or gram of the consumed product is crucial for potential health effect (Shori, 2016). pH is one of the most important criteria to determine the potential activity of microorganisms and food quality, as the low pH levels are one of the main restrictive parameters for storage viability of selected strains, while at the same time necessary for preventing growth of other, food-spoilage, microorganisms (Nematollahi et al., 2016; Tripathi & Giri, 2014). It is also possible for acidity and pH to have opposite effects on the sensory characteristics and overall quality of a product.

Survival of probiotics during food processing and digestion is a critical factor in determining the efficacy of probiotic-containing products. Several challenges, including acidic conditions, enzymes, and bile salts in the gastrointestinal tract, can impact the viability and functionality of probiotics. Digestion is a complex process of breaking down food into its nutrients for absorption by the body and plays an important role in determining the bioavailability of bioactive compounds present in foods (Naissinger da Silva et al., 2021). To gain a comprehensive understanding of the survival of probiotics during gastrointestinal passage, it is a reliable choice to use simulated digestion models, because *in vitro* digestion models allow us to examine the digestion process in every step. The TNO Intestinal Model (TIM) system is a computer controlled dynamic *in vitro* model that closely simulates the conditions of the human gastrointestinal tract, including the stomach, small intestine, and colon. This model allows for the precise control and monitoring of digestion parameters, the survival of probiotics, and the overall nutritional quality of food products (Barroso et al., 2015). The use of the TIM-1 system to simulate gastrointestinal conditions for evaluating the survival of probiotic strains in these novel food matrices is another innovative aspect of this study.

This study aims to evaluate beetroot ketchup as a novel non-dairy carrier for probiotics, focusing on sensory attributes, storage viability, and gastrointestinal survival, which distinguishes it from previous research on dairy-based matrices.

2. Materials and methods

2.1. Selection of potential probiotic strains

The strains of *L. rhamnosus* K3(ID: KM186164) and *L. johnsonii* K4 (ID: KM186165) were collected from the laboratory of the "Department of Food Gastronomy and Food Hygiene, Warsaw University of Life Sciences in Poland" (Zielińska et al., 2015, 2019). Bacteria were activated from a frozen culture stored at −80°C and incubated at 37°C in 10 ml of MRS broth (pH 6.8 ± 7.2, Merck, Darmstadt, Germany) for 24 hours, with non-inoculated media as controls to ensure no contamination. After completing the incubation period, the tubes were centrifuged at 10, 000 rpm for 5 minutes (MPW-251; MPW MED Instruments, Warsaw, Poland) to separate bacterial cells from the medium. The supernatant was replaced with 8.5 g/kg of saline and the centrifugation procedure was performed three times to remove residual growth medium.

2.2. Preparation of beetroot ketchup

The raw materials are selected from common ketchup recipes and for improving the food matrices and decrease acidy of tomatoes and vinegar, beetroots were added. During product development, various proportions of ingredients were tested to obtain optimal textural and sensory parameters, which allowed the establishment of target amounts (Table 1). Tomato concentrate, beetroots, garlic powder, black pepper, white sugar and apple vinegar were purchased from local market. Beetroots were washed, boiled in a pressure cooker for 30 minutes. After cooking, the softened beetroots were mashed using a high-powered mixer (Bosch ErgoMixxThe mixture was pasteurized at 72°C for 15 minutes, followed by cooling to room temperature for inoculation. For each sample, a 1 ml bacterial solution (9 $\log_{10}$ CFU/ml) of *L. rhamnosus* K3 or *L. johnsonii* K4 in 0.85 % saline solution was added to 100 g of the ketchup mixture and fermented for 5 hours at 37°C. Next, the samples were immediately cooled to 4°C and stored in the refrigerator for 24 hours before further analysis.

2.3. pH analyses

The pH value of the samples was determined by a calibrated pH meter (ORION STAR A211, Thermo SCIENTIFIC). Measurements were made the day after fermentation and weekly for 3 weeks of storage. During the measurement, the product temperature was equal to the ambient temperature of 21 ± 1°C.

2.4. The viability of probiotics during storage

The viability of *L. rhamnosus* K3 and *L. johnsonii* K4 in ketchup samples was checked every week during the storage of 3 weeks at 4°C. A serial dilution method was conducted to check bacterial viability with MRS agar (pH 6.8 ± 7.2, Merck-Darmstadt, Germany) used for the plating at 37°C for 48 hours. The counting results are shown as log CFU/ml.

2.5. In vitro digestion

The TIM-1 *in vitro* gastrointestinal model (TNO Nutrition and Food Research Institute, Zeist, the Netherlands) has been extensively explained (Venema et al., 2019) and has been used on numerous occasions to study probiotic survival (Minekus, 2015; Venema et al., 2020). Before starting the experiments, a cleaning process involved immersing glass and plastic components in a solution containing 40 g of sodium hydroxide (NaOH) and 200 g of a phosphate-free cleansing agent (RBS® by Carl Roth) per liter of distilled water. These parts were then manually brushed to enhance the removal of contaminants and subsequently rinsed with tap water. Following this, the entire system was sequentially submerged in a solution of sodium hypochlorite (2.5 % w/v) for approximately 30 minutes and afterward rinsed with tap water, a 0.5 M hydrochloric acid (HCl) solution, distilled water, and finally cleaned with a 70 % ethanol solution. This model includes different compartments to simulate the stomach, duodenum, jejunum, and ileum, with a connection with peristaltic pumps. The digestion process is

Table 1
Proportion of the ingredients in the developed ketchup.

Sample	Ingredients						
	Tomato concentrate	**Beetroots**	**Garlic powder**	**Black Pepper**	**Sugar**	**Apple Vinegar**	**Probiotic**
KTC	100 g	30 g	1 g	0.3 g	5 g	1 g	-
KT3	100 g	30 g	1 g	0,3 g	5 g	1 g	*L. rhamnosus* K3
KT4	100 g	30 g	1 g	0,3 g	5 g	1 g	*L. johnsonii* K4

Explanatory notes: KTC—control sample, KT3 ketchup with *L. rhamnosus* K3 fermentation, KT4 ketchup with *L. johnsonii* K4 fermentation.

computer-controlled by different sensors and stays at 37°C during the entire process. Digestion lasted for 5 hours, during which approximately 90 % of the intake passed through the model. At the end of the experiment, the residue in the model was collected as well, to measure remaining viable counts in the system for details see (Venema et al., 2019). The process of mixing via peristalsis was controlled using computer-regulated peristaltic valve pumps, which simultaneously managed the movement of meals through distinct compartments. The pH levels within these compartments were under computer surveillance, with adjustments made by introducing HCl (1 M) for the stomach compartment and $NaHCO_3$ (1 M) for the small intestinal compartments. Electrolytes, bile, enzymes and pancreatic juice were adjusted according to a healthy adult. Pancreatic solution was simulated by secreting 10 % pancreatin ((Pancrex V) in a small intestinal electrolyte solution (containing NaCl 5 g l−1, KCl 0·6 g l−1, $CaCl_2$ 0·22 g l−1) at 0.25 ml per minute. We simulated biliary output by secreting 4 % bile solution (porcine bile extract, Sigma) at 0.5 ml per minute. The compartments were filled with start residues as described before (Minekus et al., 1995), except for the gastric residue, which was mixed with the 'meal'. Digested and dissolved low-molecular weight compounds were continuously dialyzed from the jejunum and ileum compartments through hollow fiber membrane systems, mimicking nutrient absorption in the body, and maintaining physiological bile and electrolyte concentrations. The dialysis fluid in the jejunum comprised 5.43 g per liter of NaCl, 0.65 g per liter of KCl, 0.37 g per liter of $CaCl_2$, and included 1.55 % bile extract. Similarly, the ileum dialysis fluid had the same composition, with the exception of bile salts, which were absent. The flow rates through the hollow fibers were set at 10 ml/min. In each TIM-1 run, 30 grams of citrate buffer ($C_6H_5Na_3$-O_7·2 H_2O, pH 4.5, Sigma) and approximately 5 g of a starting residue solution with 30 g of samples (non-fermented or fermented ketchup) were introduced. The starting residue solution consisted of 5 g from a prepared gastric electrolyte solution containing the enzymes Lipase (37.5 mg; Rhizopus F-AP15 from Amano Pharmaceuticals), Pepsin (42.0 mg, Sigma P7012), and acetate buffer pH 5.0 (prepared by adding 21.6 g of acetic acid to 87.1 g/liter sodium salt trihydrate, Sigma). For each experimental condition, two replicates were included in the experiments.

2.6. Determination of bacterial survival

The viable counts of *L. rhamnosus* K3 and *L. johnsonii* K4 were assessed at the start of the experiment (designated as t=0) and in the ileum efflux of TIM-1 over a five-hour period, with hourly sampling (at t=1, 2, 3, 4 and 5 h). To determine the viable counts, all collected samples were subjected to serial ten-fold dilution in sterile PBS. Subsequently, 10 µl from each dilution (ranging from 10^0 to 10^7-fold) were evenly spread onto the surface of Rogosa agar plates, which were then placed in anaerobic conditions (Anaerobic System Anaerogen from Oxoid, Basingstoke, UK), and incubated for 48 hours at 37°C. Viable counts were determined by examining agar plates that resulted in colony counts ranging from 3 to 30 Colony Forming Units (CFU). The cumulative survival percentage was calculated by dividing the viable bacteria in every-hour efflux samples by the number of bacteria in the intake.

2.7. Sensory analysis

The Quantitative Descriptive Profile (QDP) method, following ISO Standard 13299:2016 (ISO 13299:2016*(En), Sensory Analysis — Methodology — General Guidance for Establishing a Sensory Profile*, n.d.), was utilized to objectively evaluate the sensory quality of the produced ketchup samples. In the preliminary session, descriptors were selected and defined during the panel discussion. Sensory descriptors, including Tomato Odour, Beetroot Odour, The Odour of Spices, Fermented Odour, Sweet Odour, Other Odour, Density, Viscosity, Fermented Flavour, The Flavour of Salt, Beetroot Flavour, Acid Flavour, Sweet Flavour, Flavour of Spices, Flavour of Bitter, Other Flavour, and Overall Quality, were employed for this purpose. Table S1 in the supplementary materials contains the definitions of these descriptors. The trained panel included eight assessors, each with two to ten years of experience in sensory evaluation. They very command understanding of the sensory methodology and were familiar with the sensory quality being evaluated. To maintain scientific accuracy and standardization, 50 ml transparent containers with lids were used for sample preparation, each assigned a unique 3-digit code, and served randomly to experts at room temperature. Neutralization between samples was ensured by providing still water. Experts evaluated the sensory quality of samples using a 100 mm scale, converted to numerical values from 0 to 10 and named as conventional units [c.u.]. Attributes were rated from "none" to "very strong". Evaluation was conducted twice with 16 individual results calculated to determine the average result. Samples were coded with three-digit codes and served randomly to avoid bias. These measures collectively established a systematic and rigorous approach to assess the sensory attributes of the produced ketchup, aiming for precision and objectivity in the evaluation process.

2.8. Statistical analysis

The statistical analyses for this study involved a combination of R software package (version 3.6.2) and Statistica 13.3 (StatSoft, Kraków, Poland). The data were analyzed by multivariate analysis of variance (ANOVA) and Tukey HSD post hoc test and for sensory analysis results, a principal component analysis (PCA) was conducted in R, utilizing a correlation matrix to explore patterns and relationships within the sensory data. The difference was considered statistically significant when $p < 0.05$ in relation to bacterial counts, pH, and the outcomes of sensory evaluation.

3. Results and discussion

3.1. Viability of L. rhamnosus K3 and L. johnsonii K4 during storage

Studies show that the viability of probiotic strains is highly dependent on the relationship of the selected raw materials with probiotic strains. In general, the final product should contain 10^6 to 10^7 CFU/ml counts of strains to have a probiotic effect. Additionally, it is crucial that the selected strains remain viable throughout the storage process until consumption and have a minimum of 10^6 CFU/ml (Maia et al., 2023; Marinova et al., 2019). For the samples, KT3 and KT4 the microbial counts varied over the three-week period. The count of selected probiotic strains dropped significantly ($p<0.05$) after one week of storage

but there was no significant change during the following 2 weeks of storage ($p>0.05$). Specifically, directly after fermentation, KT3 exhibited a log CFU/ml of 9.12, which slightly decreased to 8.73 after one week of storage and further to 8.49 after three weeks. Similarly, KT4 showed values of 9.16 log CFU/ml after fermentation and 8.55 log CFU/ml, 8.56 log CFU/ml, 8.47 log CFU/ml at one, two, and three weeks, respectively (Table 2). Thus, both KT3 and KT4 showed a decreasing trend in microbial counts over the storage period, suggesting potential changes in the microbial population or metabolic activity. However, all samples stayed at more than 8 log CFU/ml throughout the storage process. The control sample, KTC, consistently showed non-detectable levels of microbial growth throughout the entire fermentation period, indicating the absence of viable bacteria and showed that the pasteurization process was successful, which suggests that in the fermented ketchup, there is no growth other than the added strains. Other research on the storage stability of a mixture of *L. johnsonii* and *L. plantarum* strains in fermented vegetables showed that the storage period did not affect the viability of strains for one month at 4°C and the counts of bacteria stayed above 8.5 log CFU/g (Manowan et al., 2020)[OBJ]. Other research on fermented beetroots and beetroot juices showed that lactic acid bacteria stayed viable after 6 months of storage (Klewicka & Czyzowska, 2011). Table 3 also illustrates the storage viability of similar food matrices with probiotic strains. Hence, our developed ketchup with beetroots can be considered suitable food carriers for *L. johnsonii* K4 or *L. rhamnosus* K3 and have potential probiotic effects due to the amounts of viable cells of the selected strains.

3.2. pH Changes After Fermentation and During Storage

The pH changes in ketchup samples were monitored over the three-week storage period. The control sample, KTC, maintained a consistent pH level of 4.6 at the start and throughout the storage weeks. Conversely, the pH of KT3, ketchup fermented with *L. rhamnosus,* exhibited a decrease, starting at 3.84 after initial fermentation to 3.79 by the end of the third week. Similarly, KT4, fermented with *L. johnsonii,* decreased in pH from 3.96 to 3.69 over the same storage period. There were significant changes found for the pH levels for all the samples during storage ($p<0.05$) (Table 4), but not between the two strains.

Similar to our results, another study working on the fermentation of vegetable matrices including red cabbage, carrot, and radish also found that all fermented vegetable pH levels decreased significantly, not only during the fermentation process, but also during storage at room temperature, with final pH levels ranging from 3.70 to 3.99 (Vatansever et al., 2017). That is why controlling pH in a different period of storage for the probiotic food products helps to understand potential effects of lactic acid fermentation. Regarding research on tomato juices with *Lactobacillus plantarum* and *Leuconostoc mesenteroides* addition showed that pH levels of all samples decreased after the bacteria addition and during storage at 4°C, while a non-fermented control sample stayed stable throughout the storage period (Bah et al., 2019). It is possible that the enzymes from microorganisms can hydrolyse the substrates and produce metabolites inside of the product and this can explain the pH drop over the storage period (Nematollahi et al., 2016). The pH drop during storage is likely due to lactic acid production from carbohydrate fermentation by the probiotics. Key enzymes involved include lactate dehydrogenase, which converts pyruvate to lactic acid (Wang et al., 2021). On the other hand, the viability of probiotic strains is highly influenced by changes in pH. Most probiotics, especially those in the *Lactobacillus* and *Bifidobacterium* genera, thrive in mildly acidic environments with a pH range of 4.5–6.5. As the pH drops below this optimal range, particularly below pH 3.5, the survival of these strains decreases significantly due to disruptions in cell membrane integrity and metabolic functions (Bustos et al., 2024). It is also possible to see in our research at the end of the storage period, pH reached the lowest level as well as it also affected the viability of the strains.

These findings highlight the impact of storage on the acidity of ketchup formulations, particularly those subject to fermentation with specific lactic acid bacteria, suggesting potential implications for shelf stability.

Table 2
The viable count of both strains in the fermented ketchup fermentation and during storage.

Sample	Total count (log CFU/g)			
	After fermentation	**1 week**	**2 weeks**	**3 weeks**
KTC	nd	nd	nd	nd
KT3	9.12^{a}	8.73^{b}	8.69^{b}	8.49^{b}
KT4	9.16^{a}	8.55^{b}	8.56^{b}	8.47^{b}

Explanatory notes: KTC—control sample, KT3 ketchup mixtures with *L. rhamnosus* K3 fermentation, KT4 ketchup mixtures with *L. johnsonii* K4 fermentation, nd-not determined. Tukey HSD test shows that statistical differences in lowercase are applicable to all samples in the same row.

Table 3
Comparative analysis of probiotic viability during storage in similar matrices (log CFU/ml or g).

Study/ Product Variant	Probiotic Strains/ Matrix Type	Initial Count	Storage Conditions	Final Count	References
Beetroot Ketchup	*L. rhamnosus* K3	9.12	4°C, 3 weeks	8.49	Current Research
Beetroot ketchup	*L. johnsonii* K4	9.16	4°C, 3 weeks	8.47	Current Research
Fermented Vegetables	*L. johnsonii, L. plantarum*	9.50	4°C, 1 month	8.50	(Manowan et al., 2020)
Beetroot Juice	Lactic Acid Bacteria	9.11	4°C, 6 months	6.8	(Klewicka and Czyzowska, 2011)
Fresh Beetroot Cubes	*Lactobacillus plantarum* BL3	7.71	4 °C,3 weeks	9.16	(Barbu et al., 2020)
Dried Beetroot Chips	*Lactobacillus plantarum* BL3	7.85	4 °C,3 weeks	~7	(Barbu et al., 2020)
Freeze-Dried Beetroot	*Lactobacillus plantarum* BL3	~8	4 °C,3 weeks	~7	(Barbu et al., 2020)

Table 4
The pH values after fermentation and during storage.

Sample	pH Measurement			
	After fermentation	**1 week**	**2 weeks**	**3 weeks**
KTC	4.68 ± 0.01^{a}	4.66 ± 0.02^{a}	4.63 ± 0.03^{a}	4.65 ± 0.02^{a}
KT3	3.84 ± 0.02^{a}	3.80 ± 0.01^{b}	3.81 ± 0.05^{b}	3.79 ± 0.02^{b}
KT4	3.96 ± 0.04^{a}	3.89 ± 0.09^{b}	3.77 ± 0.03^{c}	3.69 ± 0.02^{d}

Explanatory notes: KTC—control sample, KT3 ketchup mixtures with *L. rhamnosus K3* fermentation, KT4 ketchup mixtures with *L. johnsonii K4* fermentation, Tukey HSD test shows that statistical differences different lowercase letters (a, b, c, d, e) within the same column indicate significant differences ($p < 0.05$) between samples at a specific time point, Different lowercase letters (a, b, c, d, e) within the same row indicate significant differences ($p < 0.05$) between weeks for the same sample, ± indicates SD.

3.3. Survival of L. rhamnosus K3 and L. johnsonii K4 During Simulated Digestion

It is necessary to study the survival of probiotic microorganisms in industrial processing and storage conditions, but also during gastrointestinal transit to their site of action when incorporating them into a food matrix. It is therefore imperative to conduct *in vitro* studies simulating digestion to ensure that these microorganisms survive (Bernat

et al., 2015). The validated TIM-1 Digestion System (Marteau et al., 1997) served as a crucial platform for elucidating the intricate dynamics of survival of the strains in ketchup fermented with *L. johnsonii* K4 or *L. rhamnosus* K3 over a 5-hour period. The cumulative survival expressed as percentages of bacterial intake for *L. johnsonii K4* displayed a steady increase, with a final cumulative delivery of 27 % at the end of the experiment. This progressive trend indicates a robust survival and persistence of *L. johnsonii* throughout the simulated gastrointestinal tract, suggesting its potential resilience in the human digestive system. Conversely, *L. rhamnosus* exhibited a distinctive survival pattern, with a lower cumulative percentage of intake, reaching 2.8 % after 5 hours (Fig. 2). *L. johnsonii* K4's higher survival rate in gastrointestinal conditions may be due to its robust cell wall structure and higher tolerance to acidic pH, as reported by (Zielińska et al., 2015). A study on the survival of different formulations of *Lactobacillus* probiotic strains, after a complete run on the TIM-1 system, showed up to 12 % cumulative survival (%-age of intake) (Venema et al., 2019). Similarly, another survival experiment on TIM-1 systems for probiotics showed that non capsulated cells of *Lactiplantibacillus plantarum* isolated from fermented buffalo milk survived 18.5 % cumulative survival after a complete run of upper gastrointestinal systems, including residue, while in the same conditions, *Enterococcus faecium* strains cumulative survival percentage was 15 % (Surono et al., 2018). These results showed that the survival of strains was related to the selection of specific strains, even under the same conditions.

In the investigation of beetroot ketchup samples KT3 and KT4, the cell counts (CFU) were assessed at 5-hour time complete digestion process, between 0 and 60 minutes, 60–120 minutes, 120–180 minutes, 180–240 minutes, 240–300 minutes, and in the residue. For KT3, the cell counts ranged from 6.78×10^{7} CFU at 0–60 minutes to 1.68×10^{9} CFU in the residue. In the case of KT4, higher cell counts were observed, starting from 3.78×10^{9} CFU/ml at 0–60 minutes and reaching 2.41×10^{10} CFU in the residue (Table 5). (de Oliveira et al., 2023) conducted study to investigate the effect of red beetroot on the viability of probiotic lactobacilli and showed that all strains had viable cell counts of more than 3.5×10^{10} CFU, at the end of the digestion process in static simulated digestion models. In different research on survival of *Lactobacilli* on TIM-1 upper gastrointestinal systems for 6 hours showed that probiotic as a form of powder showed 2.10×10^{8} while probiotic powder enriched with Ahiflower oil 4.14×10^{8} (Venema et al., 2020).According to (Valero-Cases et al., 2017), research on *Lactobacillus plantarum* strain in tomato juice survived during the digestion process and even improved the intestinal barrier function in *in vitro* cell cultures.

It is recommended that for foods with probiotics to have a therapeutic effect, between 10^{6} and 10^{8} CFU of viable probiotic cells should remain after the intestinal digestion stage (Wendel, 2022). Therefore, a 30 g serving of ketchup fermented with *L. johnsonii* K4 or *L. rhamnosus* K3 provides above 10^{9} CFU viable probiotic cells. This is a sufficient amount to promote consumer benefits, making the ketchup potentially probiotic. The study shows that fermentation in a non-dairy food matrix rich in nutrients and, enabled the growth of bacterial strains in the matrix.

3.4. Sensory Analysis

In this study, fermented ketchup with beetroots was prepared as an alternative functional food. That's why it is important to develop products with acceptable sensory properties. Table 4 shows the sensory characteristics of non-fermented ketchup (KTC), ketchup fermented with *L. rhamnosus* (KT3) and ketchup fermented with *L. johnsonii* K4 (KT4) that were evaluated for odour, texture and flavour attributes. The fermentation odour showed significant differences between all samples. However, *L. johnsonii* fermentation has significantly increased the intensity of beetroot and spices odour; *L. rhamnosus* fermentation affected tomato odour, fermentation odour and sweet odour, while the non-fermented sample highest intensity of another other described by panellists, "earth odour" and "bitter odour". This variation highlights the influence of fermentation on the development of distinctive profiles, which can be attributed to the metabolic activities of the bacteria strain (Zhang et al., 2023). Fermentation-induced changes in ketchup samples also influenced the product density. The density of the KT3 and KT4 changed significantly compared to KTC ($p<0.05$). This can be the reason for substrate degradation during the fermentation process and lead to changes in texture and mouthfeel, thereby affecting sensory differences (Senanayake et al., 2023). Flavour profiles also differed between selected strains. Fermented and sour flavour has the highest score in KT3, with a significant difference in other fermented samples, KT4, while KTC has the lowest scores for these attributes ($p<0.05$). These results showed that fermentation with different bacterial strains can result in different biochemical pathways leading to the generation of unique flavour compounds with the production of various metabolites, including organic acids and alcohol (Zhang et al., 2023). Overall quality scores showed significant differences, highlighting the impact of *L. johnsoni* K4 fermentation on the sensory attributes and overall acceptability of ketchup ($p<0.05$). Fermentation can improve overall quality by enhancing flavour complexity and improving textural attributes. In conclusion, the fermentation process, particularly with *L. johnsonii* K4, significantly influences the sensory characteristics and overall quality of ketchup, contributing to distinct flavour profiles, textural properties, and consumer acceptability. Similarly, in beetroot beverages *Lactobacillus casei* fermentation for 2 hours at 37°C resulted in the highest sensory acceptability, with viable lactic acid bacteria counts maintained during storage (Gamage et al., 2016). Furthermore, the optimization of fermented tomato production highlighted the importance of selecting specific lactic acid bacteria strains, such as *L. fermentum, L. plantarum, P. pentosaceus*, and *L. paracasei*, with scores to enhance sensory scores (Zhao et al., 2024). These studies collectively demonstrate the impact of lactic acid bacteria on sensory attributes in tomato and beetroot products.

PCA revealed interesting findings about the fermented samples KT3, KT4, and KTC. The KT4 sample showed an improvement in overall quality. The fermented flavour and sour flavour were dominated by KT3 and KT4. On the other hand, KTC sample had a more diverse range of flavours, including sweet and beetroot flavours (Fig. 1).KT4 has higher intensity of beetroot odour and lower intensity of fermentation odour, beetroot flavour and fermented flavour compared to other fermented sample KT3 and these attributes can be related that KT4s has highest overall quality compared to both other fermented sample and non-fermented sample.

Further investigation revealed that the sweet flavour range was higher in KTC, which could be attributed to the absence of fermentation. During the fermentation process, *L. johnsonii* and *L. rhamnosus* consumed the sugars, resulting in the production of fermented and sour and decreased sweet flavour. The PCA analysis showed that the fermented

Table 5
Cumulative CFUs as determined by microbiological cell count in the ketchup samples KT3 and KT4, and average cumulative survival during complete TIM-1 runs.

Sample	Time of Interval [minutes]					
	0–60	**60–120**	**120–180**	**180–240**	**240–300**	**Residue**
KT3	6.78×10^{7}	1.03×10^{9}	1.33×10^{9}	1.57×10^{9}	1.60×10^{9}	1.68×10^{9}
KT4	3.78×10^{9}	1.13×10^{10}	1.31×10^{10}	1.46×10^{10}	1.69×10^{10}	2.41×10^{10}

Explanatory notes: KT3 ketchup mixtures with *L. rhamnosus* K3 fermentation, KT4 ketchup mixtures with *L. johnsonii* K4 fermentation.

Table 6
Intensity of defined attributes for ketchup samples (0–10 c.u).

Attribute	Sample		
	KTC	KT3	KT4
Tomato Odour	6.06 ± 1.91[a]	6.16 ± 1.59[a]	5.85 ± 2.32[b]
Beetroot Odour	4.46 ± 1.76[a]	3.54 ± 1.79[b]	4.56 ± 1.52[a]
Fermentation Odour	2.30 ± 1.76[a]	4.29 ± 1.87[b]	3.63 ± 1.53[c]
Sweet Odour	3.44 ± 1.19[a]	3.72 ± 2.08[b]	2.72 ± 1.54[c]
Spices Odour	2.59 ± 1.80[a]	2.76 ± 1.91[a]	2.90 ± 1.78[a]
Another Odour	1.56 ± 2.09[c]	1.23 ± 1.70[b]	1.25 ± 1.50[b]
Density	4.24 ± 1.67[a]	3.40 ± 2.06[b]	3.75 ± 1.27[c]
Viscosity	3.20 ± 1.73[b]	3.13 ± 2.19[b]	3.19 ± 1.37[b]
Tomato Flavour	5.74 ± 1.96[a]	5.89 ± 1.69[a]	5.72 ± 2.26[a]
Beetroot Flavour	5.48 ± 1.64[a]	4.65 ± 1.63[a]	3.94 ± 2.03[b]
Sour Flavour	3.24 ± 0.97[a]	6.21 ± 1.55[b]	5.71 ± 1.61[b]
Fermented Flavour	2.44 ± 1.48[a]	5.14 ± 1.56[b]	4.78 ± 1.77[c]
Sweet Flavour	4.48 ± 1.83[b]	2.34 ± 0.99[c]	2.83 ± 1.59[c]
Salty Flavour	3.35 ± 1.09[a]	2.84 ± 1.16[a]	2.44 ± 1.57[a]
Spice Flavour	3.66 ± 1.82[a]	3.71 ± 2.01[b]	3.65 ± 2.12[a]
Another Flavour	1.48 ± 1.93[a]	1.63 ± 1.32[b]	1.64 ± 0.88[b]
Overall quality	6.28 ± 1.93[a]	6.23 ± 1.32[a]	7.31 ± 0.88[b]

Explanatory notes: KTC—control sample, KT3 ketchup mixtures with *L. rhamnosus* K3 fermentation, KT4 ketchup mixtures with *L. johnsonii* K4 fermentation; c.u. – conventional units; Tukey HSD test shows that statistical differences between samples are represented by means in the same row followed by different lowercase letters of. The alphabet, they differ significantly ($p<0.05$).

samples were distinct from the non-fermented samples, indicating that the fermentation process had a significant impact on the resulting sensory characteristics.

4. Conclusion

This study demonstrates that beetroot ketchup can effectively serve as a non-dairy carrier for probiotics, with high sensory acceptability and significant viability over a three-week storage period. These findings suggest potential for broader applications in functional foods. Over the storage duration, microbial counts in the fermented samples gradually decreased, yet consistently remained above 8 $\log_{10}$ CFU/ml, indicating the probiotic potential of both strains. The overall quality of developed ketchup also improved with the addition of a potential probiotic strain. The pH changes observed in ketchup underscored the impact of fermentation, raising considerations for shelf-life stability. Notably, both *L. rhamnosus* K3 and *L. johnsonii* K4 were able to survive the digestive process to some degree, although there were some differences between the strains. In particular, *L. johnsonii* K4 was able to survive up to 30 % through the gastrointestinal system. This strain also had a positive impact on the overall sensory quality of ketchup, making it better than the other samples. The ability of these probiotics to withstand the simulated gastrointestinal conditions in TIM-1 suggests their potential to reach the colon in a viable state, a critical factor for realizing their health benefits in that part of the gastrointestinal tract. The findings highlight the importance of *in vitro* digestion models like TIM-1 in assessing the behaviour of probiotics under conditions that closely simulate the complexities of the human digestive system. As consumers increasingly seek food options with potential health-promoting benefits, fermented ketchup stands as a noteworthy candidate that combines taste preferences with potential nutritional advantages.

In conclusion, the most important achievements of our research include the investigation of a non-dairy matrix as a carrier of potentially probiotic bacteria to increase the availability and offer of these functional foods. Moreover, we have demonstrated the possibility of designing a product with very good sensory properties and health potential resulting from the viability of beneficial microorganisms both in the product and under digestion conditions in the gastrointestinal tract. Incorporating fermented beetroot ketchup into regular diets could provide a non-dairy source of probiotics, offering potential health benefits such as improved gut health and enhanced immune function. Limitations of this study include the storage period and limited strain diversity. Future research should explore different storage conditions, alternative probiotic strains, and varying fermentation substrates to expand the applicability of these findings.

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CRediT authorship contribution statement

Monika Trząskowska: Writing – review & editing, Supervision, Methodology. **Kübra Küçükgöz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Koen Venema:** Writing – review & editing, Supervision,

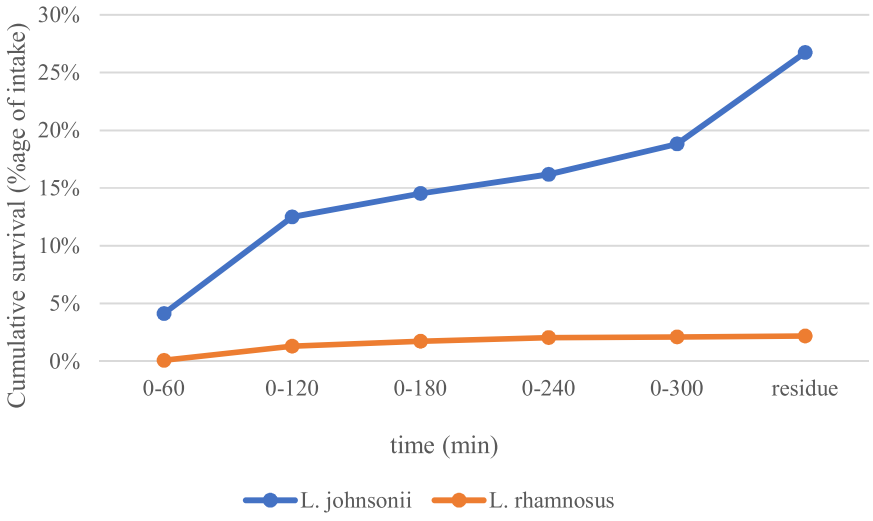

Fig. 1. Survival of *L. johnsonii* K4 *and L. rhamnosus* K3 (expressed as cumulative delivery from the ileal compartment) in the samples collected from the *in vitro* model at different time points (min).

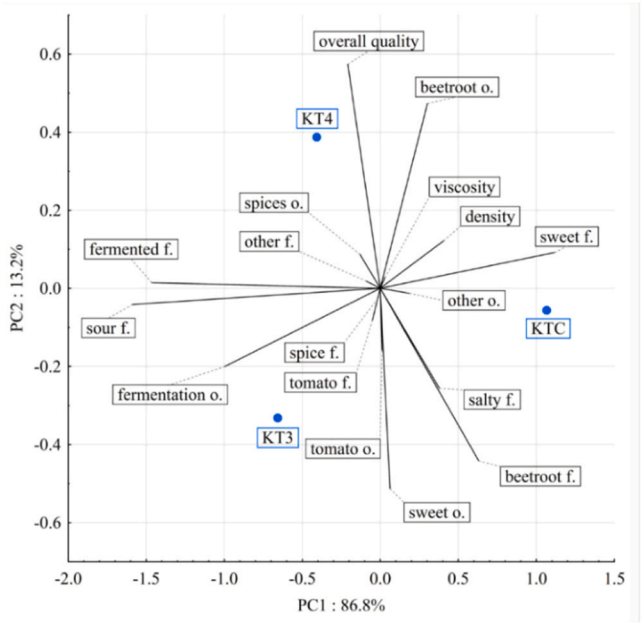

Fig. 2. The principal component analysis (PCA) analysis of sensory attributes the ketchup samples. Explanatory notes: KTC—control sample, KT3 ketchup mixtures with *L. rhamnosus K3* fermentation, KT4 ketchup mixtures with *L. johnsonii K4* fermentation.

Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fbp.2024.10.004.

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