LWT - Food Science and Technology 182 (2023) 114795

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Development of a fermented plant-based beverage from discarded bread flour

Teresa Sigüenza-Andrés [a], Manuel Gómez [a], José M. Rodríguez-Nogales [a], Irma Caro [b,*]

[a] Food Technology Area, College of Agricultural Engineering, University of Valladolid, 34004, Palencia, Spain
[b] Department of Nutrition and Food Science, Faculty of Medicine, University of Valladolid, 47005, Valladolid, Spain

ARTICLE INFO

Keywords:
Discarded bread flour
Glucose
Probiotics
Lactic acid bacteria
Functional beverage

ABSTRACT

This work aimed to reuse bread flour from discarded bread as a substrate for the growth of probiotic lactic acid bacteria (LAB) and *Bifidobacterium*. Commercial starters were used to ferment beverages based on bread waste flour and water, with and without enzymes (α-amylase and β-glucoamylase) and with and without a desalting treatment. All the fermentations were carried out at 38 °C during 24 h. Microbial counts, pH and titratable acidity (TA) were determined at 0, 3, 6, 9 and 24 h and carbohydrates content at 0, 9 and 24 h. The pH, TA and water holding capacity (WHC) were also analysed at 15 and 21 days during the storage of the beverages. Both starters showed good growth during the first 9 h although Nu-trish® BY showed a slight decrease until the end of the fermentation. All the beverages reached pH levels lower than 4.1 after 24 h. Samples treated with enzymes achieved faster a higher acidity and a lower WHC. In general, salted samples showed higher maximum rates of growth (μ_{max}) and maximum rates of pH reduction (V_{max} pH red) and acidification (V_{max} acid), as well as lower pH and WHC and higher TA, in all the studied analyses.

1. Introduction

Food waste and reuse are included in 12.3 of the United Nations Sustainable Development Goals (United Nations, 2015). According to Ishangulyyev et al. (2019), approximately one-third of all food produced for human consumption in the world is lost or wasted causing one of the most serious environmental, social and economic issues. Bread and bakery products are among the most discarded food in the world (FAO, 2013).

The European Commission (2008) recommends using all waste suitable for human consumption and reintroducing it into the food chain. However, few alternatives have been developed to retain or recycle bread waste for use in human food. Currently, the most frequent solution is starch hydrolysis and fermentation due to the high content of this carbohydrate in breads (Melikoglu & Webb, 2013).

Fermentation is one of the oldest, simplest and most economical methods for producing and preserving food and beverages, as well as for improving the nutritional, sensory and shelf-life properties of the products (Petrova & Petrov, 2020). In addition, fermentation provides a natural way to reduce the volume of the material to be transported, eliminate undesirable components and reduce the energy required for cooking to make a safer product with minimal quality loss (Blandino et al., 2003).

Cereals have been used for fermented beverages production due to their composition since they are essential sources of carbohydrates and their widespread cultivation worldwide (Tsafrakidou et al., 2020). Recent studies have shown that cereals are good substrates for the development of functional beverages with enhanced nutritional properties, based on their fermentation by LAB (Angelov et al., 2018; Chavan et al., 2018). Only some LAB can ferment complex carbohydrates, such as starch, the main component in bread (Adessi et al., 2018). Nevertheless, the addition of amylolytic enzymes, such as α-amylase and glucoamylase, can help the fermentation process, because they break the starch into dextrins, oligosaccharides and glucose, increasing the availability of fermentable carbohydrates (Luana et al., 2014). Besides, cereal-based beverages are an excellent alternative to dairy-based drinks because these contain cholesterol as well as lactose and milk proteins that can cause intolerance and allergy, respectively (Chavan et al., 2018; Granato et al., 2010). The increase in vegetarianism around the world is also an opportunity for cereal-based drinks.

Probiotic bacteria like *Lacticaseibacillus* and *Bifidobacterium* have been used to produce functional foods. Probiotics are microorganisms

* Corresponding author.
E-mail address: irma.caro@uva.es (I. Caro).

https://doi.org/10.1016/j.lwt.2023.114795
Received 16 January 2023; Received in revised form 20 April 2023; Accepted 24 April 2023
Available online 25 April 2023

that, administered in adequate amounts, confer a health benefit to the host.

A wide variety of plant-based functional drinks has been developed using LAB and probiotics. Various researchers have proposed the use of starchy matrices such as cereals, pseudocereals and legumes (Angelov et al., 2018; Aparicio-García et al., 2021; Chavan et al., 2018; Luana et al., 2014) Only a few have considered cereal industry byproducts to develop new probiotic drinks (Verni et al., 2019). Recently, a review of the use of surplus bread as food ingredient was published (Gómez & Martinez, 2023). Moreover, Nguyen et al. (2022) used surplus bread to develop a probiotic beverage using *Lacticaseibacillus rhamnosus GG (LGG)* and *Saccharomyces cerevisiae* and Massa et al. (2022) discarded buckwheat bread to create sustainable non-alcoholic drinks.

However, to date, these authors have not examined the use of other probiotic bacteria or amylolytic enzymes or the effect of desalting in the development of fermented beverages from bread waste.

This work aimed to develop a new probiotic cereal-based beverage by reusing bread flour from discarded bread as a substrate for the growth of probiotic LAB and *Bifidobacterium*, with or without an enzymatic addition, and with and without a previous desalting treatment. The pH, titratable acidity (TA), water holding capacity (WHC) and carbohydrates were analysed in each beverage. Moreover, microbial growth was also studied to see the effect of each treatment on the growth and acidification kinetics of the bacteria.

2. Materials and methods

2.1. Materials

2.1.1. Bread flour

Discarded wheat bread loaves were purchased from the local bakery La Tahona de Sahagún (Sahagún, Spain) to produce bread flour. The bread was dried at room temperature for three days and ground in an LM 3100 hammer mill (Perten Instruments, Huddinge, Sweden) with a sieve of 1 mm (see Table S1). Each bread weighted ~250 g and its formulation (per 100 g of wheat flour) was 1.8% salt, 4% fresh yeast and 55% water.

2.1.2. Microorganisms

The commercial probiotic cultures Nu-trish® LGG® (**starter LGG**) and Nu-trish® BY-01 DA (**starter BY**) were kindly donated by CHR Hansen (Hørsholm, Denmark). LGG (*L. rhamnosus*) was kept at −20 °C and BY (*Bifidobacterium, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) was stored at −80 °C until use, according to the manufacturer's instructions. Dilutions of each starter were made in peptone water 0.01% (p/v) (WWR BDH Chemicals, Wayne, USA) with 0.08% of NaCl (Panreac ITW Companies, Barcelona, Spain) to reach a

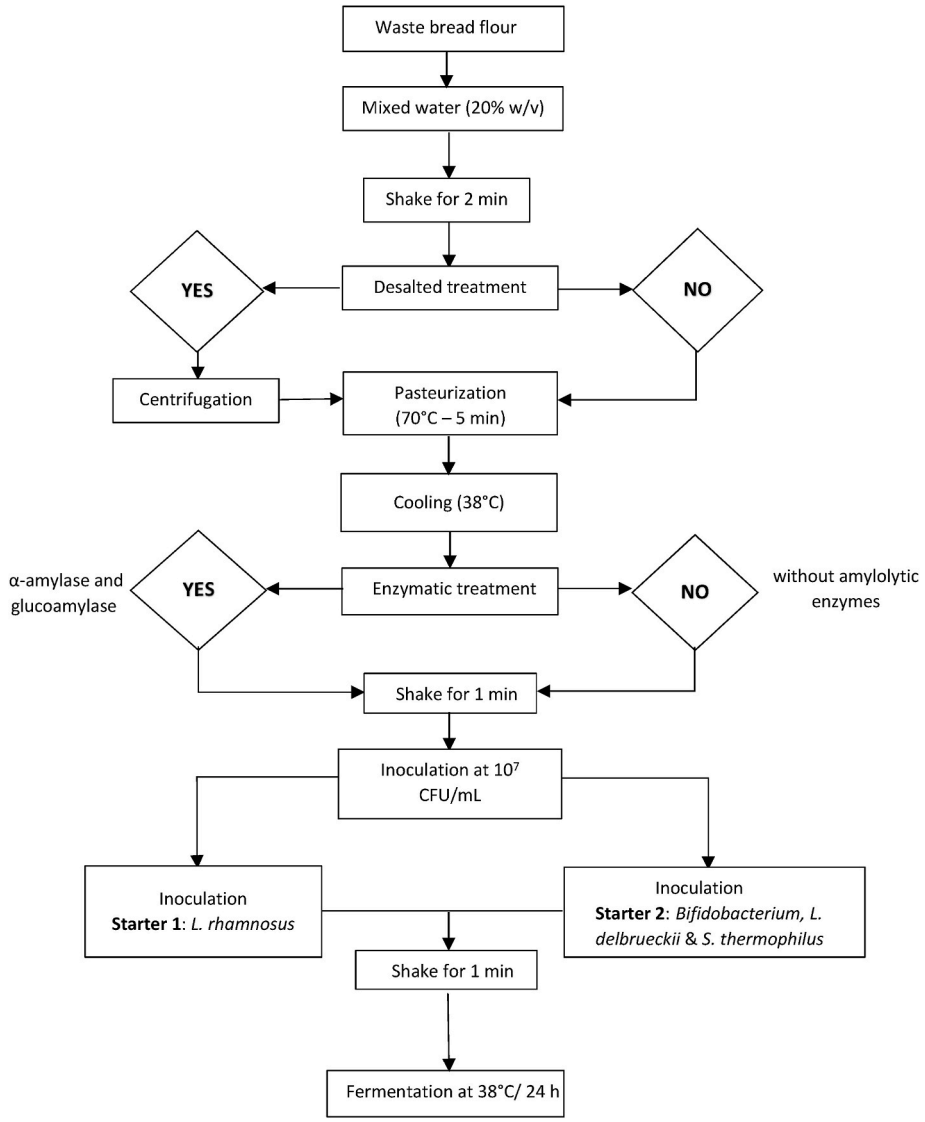

Fig. 1. Process for making fermented beverages from bread waste flour.

10^7 CFU/g cell density in the product before fermentation.

2.1.3. Enzymes

The starch hydrolysis was conducted by adding α-amylase (Liquoflow® Yield, Novozyme JSC, Bagsværd, Denmark) and β-glucoamylase (Saczyme® Go, Novozyme JSC) at the same time. The amount of enzyme added was the maximum recommended by the manufacturer.

2.1.4. Fermentation procedure and storage

Fig. 1 shows the whole process. Bread flour was mixed with a final volume of 900 mL sterile water (20% w/v) for 5 min and then pasteurised in a water bath at 70 °C for 5 min. Then, the mixture was cooled in a cold-water bath until 37 °C was reached (approximately 10 min). Two samples were processed without desalting and in the other two the salt was removed.

For the desalting process, after shaking it for 2 min, the mixture (flour bread-water) was centrifuged for 5 min at 5000 rpm in a centrifuge (Beckman Coulter J2-HS, Pasadena, California). The supernatant was discarded, and the pellet was rehydrated again with sterile water until the initial volume was reached.

Once the desalted and non-desalted blends were prepared, 0.0179 mL/100 g flour of α-amylase (0.021%) and 0.029 mL/100 g flour of β-glucoamylase (0.033%) were added to half of them immediately before incubation and shaken for 1 min; control samples were kept without enzymes. Then, the mixture was inoculated with approximately 10^7 CFU/g of each starter per essay, shaken for 1 min and incubated at 38 ± 2 °C for 24 h in a water bath. The fermentation was carried out in 1L glass bottles (180 g of flour + 720 mL of sterile water).

Samples for microbial counts, pH and TA determination were taken at times 0, 3, 6, 9 and 24 h, while carbohydrates analysis samples were taken at times 0, 9 and 24 h. Finally, WHC was done just at the end of the fermentation (24 h). The pH, acidity and WHC were also measured after 15 and 21 days of storage at 4 °C.

2.2. Microbiological determination

Ten g-samples were taken according to above mentioned times with a sterile pipette, mixed with 90 mL of peptone water (peptone 0.01%, NaCl 0.85%) (WWR BDH Chemicals, Wayne, USA) in a 100-mL flask and shaken for 2 min. After that, decimal serial dilutions were made in sterile peptone water.

Starter LGG was grown in MRS Agar (Agar Man, Rogosa and Sharpe, VWR BDH, Chemicals, Radnor, USA) using the drop method (Cal--Sabater et al., 2019) in triplicate in drops of 10 μL. The plates were incubated at 37 °C for 48 h in a bacteriological oven (Giralt S.A, Barcelona, Spain).

Each bacterium of **starter BY** was grown in different conditions. *Bifidobacterium* was grown in MRS Agar containing 25 ppm of mupirocin lithium (European Pharmacopeia Reference Standard) and 500 ppm of L-cysteine hydrochloride (Merck, Darmstadt, Germany). Plates with 1 mL of the appropriated dilutions were incubated under anaerobic conditions using an Oxoid AnaeroGen 3.5 L sachet (Thermo Scientific, Massachusetts, USA) and an anaerobic jar (Oxoid) for 72 h at 37 °C. *S. thermophilus* were counted onto M17 agar plates (Oxoid CM0785) supplemented with 10% lactose monohydrate (Merck, Darmstadt, Germany). Three drops of 10 μL from appropriate serial dilution were placed in the plates following the drop method and incubated for 24 h at 37 °C. Finally, *L. delbrueckii* subsp. b*ulgaricus* were counted in MRS Agar covered with a double layer after the inoculation of 1 mL of the appropriate dilution and incubated at 42 °C for 48 h.

2.3. pH and acidity determination

pH was measured by using a BASIC 20 pH-meter (Crison, Barcelona, Spain). TA was determined by titrating 10 g of the sample previously homogenised with 10 mL of distilled water with 0.1 M NaOH (WWR BDH Chemicals, Wayne, USA) using phenolphthalein (Alfa Aesar, Massachusetts, USA) as an indicator. Both parameters were measured in duplicate. The acidity or TA was expressed as % (g/100 mL) acidity using this formula:

$$\%\ \text{Acidity} = \frac{\text{NaOH (mL)x N de NaOH (0.1M) x Meq lactid acid (0.090)}}{\text{Sample weight (g)}} \text{x}100$$

2.4. WHC

Fifteen g of each sample (W_0) was weighed in a 50 mL-falcon tube and was centrifuged in a Sorvall ST 16R centrifuge (Thermo Scientific, Massachusetts, USA) for 15 min at 5706 rpm. The residue (W_1) was taken into account to calculate the water retention of each beverage after fermentation. The test was calculated as follows:

$$\text{WHC}\ (\%) = \left(\frac{W_1}{W_0}\right) \text{X}\ 100$$

2.5. Analysis of carbohydrates by HPAEC-PAD method

Glucose, fructose, isomaltose, maltose and maltotriose were analysed in duplicate and were quantified based on areas to assess the changes in carbohydrate composition during fermentation. These carbohydrates were extracted according to the Pico et al. (2015) method with slight modifications. Briefly, 2.5 g of each sample was weighed in a 50 mL-falcon tube, and 15 ml of distilled water and 2 mL of Carrez II (potassium hexacyanoferrate II, Sigma Aldrich, Missouri, USA) were added. The falcon tube was shaken for 1 min at 1000 rpm using Mini-shaker (IKA Model-S2 Staufen, Germany) and centrifuged (Beckman Model J2-21) at 12,000 rpm for 20 min at 20 °C. Then, the supernatant was transferred to a 50 mL flask. Afterwards, 15 mL of water was added to the residue obtained in the previous centrifugation and the sample was shaken and centrifuged as explained above. The supernatant of the second extraction was mixed with the first one and the flask was filled to 50 mL with distilled water, and frozen until analysed. A suitable aliquot was filtered with a 0.45 μm pore nylon membrane and used for the chromatography analysis.

HPAEC-PAD analyses were carried out on a Metrohm system (Metrohm, Herisau, Switzerland). All the instruments used in this study have similar characteristics to those used by Pico et al. (2015).

Before the HPAEC-PAD analysis of glucose, it was necessary to perform a dilution 1:20 of the samples obtained after 9 h of fermentation. The eluent A gradient program was also modified: time 0 at 97% (50 mM NaOH) and then a linear decrease to 85% in 15 min; these characteristics were maintained for 10 min, followed by an increase to the initial conditions (97%) with a linear increase from 25 min to 30 min to finish the run time. The detection limits were: glucose, 13.939; fructose, 0.686; isomaltose, 0.422; maltose, 1.447 and maltotriose, 0.136 (mg/L). These analyses were carried out at the Laboratory of instrumental techniques (LTI) of the University of Valladolid.

2.6. Statistical analysis

Counts were expressed as the logarithm of unit-formed colonies (Log-CFU). Total BY Log CFU counts was the sum of three microorganisms counts. The DMFit web edition (Institute of Food Research, Norwich, UK; Baranyi & Roberts, 1994) was used to model the curves and calculate the growth parameters from the microbial kinetics. *L. rhamnosus* growth kinetics were modelled from the beginning to the end of the fermentation (24 h). Starter BY growth kinetics curves were done after 9 h of fermentation. The data obtained at 24 h did not allow modelling to grow since there was a growth decrease in all samples. All data presented are mean values of replicates obtained from two independent fermentations.

The maximum rates of pH reduction (V_{max} pH red) and acidification

(V_{max} acid), as well as the remaining acidification kinetics parameters were calculated according to Caro et al. (1999). Analysis of variance (ANOVA) for the other experiments was done using the STATGRAPHICS Centurion XV (StatPoint Technologies, Inc., Warrenton, VA). Values of $p < 0.05$ were considered statistically significant.

3. Results and discussions

3.1. Microbial growth

Growth kinetics parameters obtained from two starters with and without enzymes and salt or without salt are shown in Table 1. The maximum rate of growth (μ_{max}) was significantly higher for starter BY with enzymes than for starter LGG with enzymes. Focusing on BY, significant differences were only observed between BY NE S and BY E DS. Moreover, the starter BY had a rapid growth up to 9 h and then it slowed down its growth, reaching the stationary and death phase at 24 h specially in non-enzymes and desalted samples (see Table 1).

The lowest rate was reached by LGG, and no significant effect of any treatment on the growth rate of LGG was observed. However, an increasing trend was observed in the enzymatic treatment.

According to the maximum population (growth T_9-T_0), none of the treatments significantly affected the starter LGG, whereas, in the starter BY, a positive effect of salt was evidenced, reaching a maximum population of 3 log CFU/g. The highest net increase (growth T_{24}-T_0) was reached by LGG E DS, LGG NE S and BY NE S samples with no significant differences. The growth of BY samples at 24 h was lower than the growth at 9 h, particularly in desalted samples whose growth was negative (Tables S2–S3). This fact indicates that this starter reached the stationary phase before 9 h and the death phase between 9 and 24 h. On the contrary, starter LGG continued growing until the end of the fermentation. Interestingly, its growth was higher in LGG NE S and LGG E DS samples, than in LGG E S and LGG NE DS samples, respectively. The possible explanation for this observation could be that *L. rhamnosus* contains several genes that allow it to have great flexibility in the utilization of sugars (Toh et al., 2013). In this work, it has been observed that LGG might use starch and/or starch hydrolysis-derived intermediates because it grew properly with and without enzymes.

Regarding microbial counts at 9 h (Log CFU/g t_{9h}) of starter BY, there were no differences between samples without enzymes; the best growth was achieved using the treatment with enzymes and salt. No significant effect of enzymatic or desalting treatments was observed in the LGG samples, nor in the growth increase at 9 h. However, the type of starter had a significant influence on the microbial counts at 9 and 24 h (data not shown see, Table S5). Moreover, an increase in LGG growth was observed from 9 to 24 h while BY counts decreased, especially in the BY NE DS sample, since the microorganisms have reached the death phase, as mentioned before.

The growth curves of the two starters used for fermentation, with or without enzymes, salted or desalted are shown in Fig. 2. It has been possible to estimate microbial growth from the Baranyi and Roberts (1994) model as it fits the function.

Comparing the growth of both starters, we can see that the LGG E S sample (Fig. 2a); reached its maximum population before 8 h while the desalted beverage took longer to reach it (see Fig. 2b). The LGG cell counts were similar to those observed in other studies based on maize porridge (Helland et al., 2004) and bread waste (Nguyen et al., 2022) at 24 h (8.2 and 7.7 Log CFU/g, respectively), although their initial values were slightly different. BY E sample did not reach the stabilization phase until the first 9 h of fermentation (Fig. 2c), while the BY DS reached it before, around 4–5 h (Fig. 2d). Microorganism counts of all drinks at 24 h exceeded the minimum amount necessary (7 log CFU/g) for a food item to be considered probiotic (Bernat et al., 2014).

Regarding pH, it decreased faster with the addition of enzymes in all cases (Fig. 2). Nevertheless, if we compared the reduction of pH between starters, pH curves from starter LGG started slowly down until it suffered a sharp fall. In contrast, the decrease of pH from starter BY was more progressive. Microbial growth and pH reduction are inversely related. LGG reached a lower pH than BY, probably because *L. rhamnosus* required more time for matrix adaptation than the starter BY, but then it produced more lactic acid and the end of fermentation. It is known that *L. rhamnosus* GG is a facultative heterolactic acid bacterium and it produces other compounds (Jyoti et al., 2004). Besides, in this experiment, with higher quantities of glucose, LGG could also produce larger amounts of lactic acid, although the time to reach the V_{max} pH was longer. This is probably because bacteria cannot do the glycolysis and the oxidation balance in parallel and they need more time to do the last reaction (Jyoti et al., 2004). Likewise, the differences observed between the two starters could be attributed to the optimum pH for *Bifidobacterium* growth (6–7) (Gomes & Malcata, 1999).

Table 1
Growth kinetics parameters obtained from LGG and BY, using a bread waste beverage, with E and NE and S and DS[a].

Starter	μ_{max} (log CFU/g/h)	Growth T_0-T_{24} (log CFU/24h)	Growth T_9-T_0 (log CFU/g)	R^2	Log CFU/g at t_{9h}	Log CFU/g at t_{24h}
LGG E S	0.263 ± 0.035 ab	1.54 ± 0.22cd	1.51 ± 0.04a	0.990 ± 0.013a	8.07 ± 0.16a	8.11 ± 0.02 ab
LGG NE S	0.193 ± 0.025a	2.17 ± 0.03d	1.69 ± 0.17a	0.955 ± 0.049a	7.97 ± 0.08a	8.45 ± 0.22bc
LGG E DS	0.223 ± 0.007a	2.09 ± 0.07d	1.44 ± 0.27a	0.994 ± 0.005a	7.98 ± 0.27a	8.63 ± 0.07c
LGG NE DS	0.162 ± 0.009a	1.31 ± 0.06c	1.25 ± 0.10a	0.842 ± 0.198a	7.89 ± 0.17a	7.95 ± 0.01a
BY E S	0.525 ± 0.145cd	1.59 ± 0.61cd	3.03 ± 0.43b	0.776 ± 0.304a	21.34 ± 0.21d	19.64 ± 0.37f
BY NE S	0.536 ± 0.041d	2.13 ± 0.42d	2.66 ± 0.69b	0.998 ± 0.002a	20.47 ± 0.02c	19.94 ± 0.25f
BY E DS	0.390 ± 0.003bc	−0.32 ± 0.13b	1.56 ± 0.08a	0.967 ± 0.047a	19.79 ± 0.11b	17.91 ± 0.15e
BY NE DS	0.447 ± 0.058cd	−2.38 ± 0.18a	1.42 ± 0.19a	0.752 ± 0.114a	20.33 ± 0.16c	16.54 ± 0.15d

[a] LGG, *L. rhamnosus GG*; BY, *Bifidobacterium, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus;* E, with enzymes; NE, without enzymes; S, salt; DS, desalted

Data are the means of two independent experiments ± standard deviations (n = 2). BY Log CFU/g include the sum of the three microorganisms counts.

Growth data were modelled according to Baranyi and Roberts (1994). Values in the same column with different superscript letters differ significantly ($P < 0.05$). μ_{max} is the maximum rate of bacterial growth expressed in log CFU/g/h; growth T_9-T_0 is the maximum population expressed in log CFU/g; growth T_{24}-T_0 is the net increase expressed in log CFU/g; R^2, fit to the Barany and Roberts model and t_{9h} and t_{24h} are the microorganisms counts at 9 and 24 h, respectively (log CFU/g).

[a] Check Table 1 for LGG, BY, E, NE, S and DS meaning.

3.2. Carbohydrates analysis

Table 2 shows the evolution of the concentration of different sugars in the beverages studied, during fermentation. The glucose content only increased over time in the treatments with enzymes. In samples without enzymes, glucose values did not exceed 0.02 g/100 g in any case. While glucose increase was high in LGG E samples between 0 and 9 h, in BY E samples this rise was between 9 and 24 h of fermentation. As expected, the use of amylolytic enzymes increased glucose availability (Sigüenza-Andrés et al., 2022) and this approach has been successfully

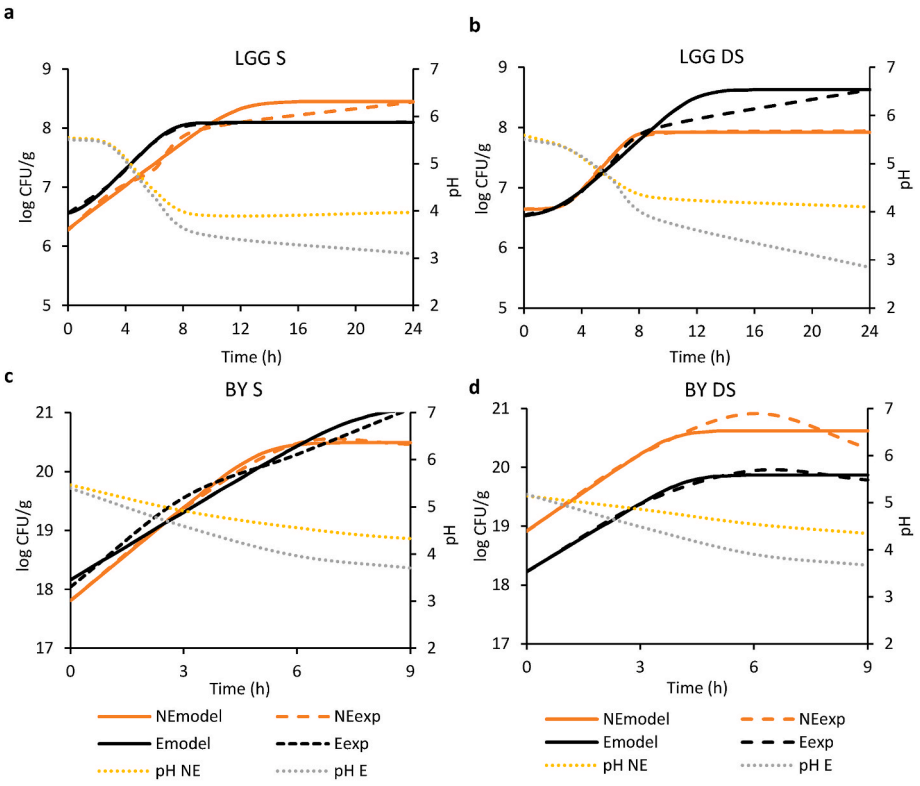

Fig. 2. Growth were modelled according to Baranyi and Roberts (1994). Modelled (model) and experimental (exp) growth kinetics curves of LGG and BY with E and NE and S and DS against pH reduction curves along the fermentation. BY curves include the sum of the three microorganisms counts.

Table 2
Evolution of glucose, fructose, isomaltose, maltose, maltotriose concentration in the fermented drinks analysed. Factors: Nu-trish® LGG® (LGG), Nu-trish® BY-01 DA (BY), addition of enzymes (E), without addition (NE), with (S) and without salt (DS). Data are expressed as mean ± standard deviation (n = 8).

Sample	Fermentation time (h)	Glucose (g per 100 g)	Fructose (g per 100 g)	Isomaltose (g per 100 g)	Maltose (g per 100 g)	Maltotriose (g per 100 g)
LGG E S	0	5.159 ± 0.9533^{cd}	0.0536 ± 0.0067^{de}	0.0147 ± 0.0006^{g}	1.4878 ± 0.1515^{g}	0.2763 ± 0.2877^{c}
LGG E S	9	9.8858 ± 2.4522^{e}	0.0494 ± 0.0062^{de}	0.0208 ± 0.0047^{fg}	0.7515 ± 0.5767^{e}	0.0132 ± 0.0100^{a}
LGG E S	24	10.9632 ± 0.9499^{e}	0.0679 ± 0.0085^{ef}	0.0341 ± 0.0092^{i}	0.0605 ± 0.0352^{abc}	0.0144 ± 0.0216^{a}
LGG NE S	0	0.0131 ± 0.0114^{ab}	0.0189 ± 0.0181^{ab}	0.0145 ± 0.0005^{ef}	0.6394 ± 0.0034^{de}	0.0252 ± 0.0021^{a}
LGG NE S	9	0.0144 ± 0.0130^{abc}	0.0206 ± 0.0195^{abc}	0.0147 ± 0.0005^{ef}	0.6328 ± 0.0072^{de}	0.0287 ± 0.0057^{a}
LGG NE S	24	0.0024 ± 0.0011^{a}	0.0034 ± 0.0001^{a}	0.0145 ± 0.0004^{ef}	0.6304 ± 0.0120^{de}	0.0267 ± 0.0013^{a}
LGG E DS	0	6.1629 ± 0.7989^{d}	0.0338 ± 0.0059^{bcd}	0.0069 ± 0.0008^{abcd}	1.1975 ± 0.2403^{f}	0.0033 ± 0.0012^{a}
LGG E DS	9	9.8234 ± 0.9688^{e}	0.0417 ± 0.0057^{cd}	0.0102 ± 0.0003^{bcde}	0.2551 ± 0.01596^{c}	0.0836 ± 0.0061^{ab}
LGG E DS	24	10.7377 ± 1.5658^{e}	0.0503 ± 0.0074^{de}	0.0244 ± 0.0005^{gh}	0.1035 ± 0.0084^{abc}	0.0239 ± 0.0031^{a}
LGG NE DS	0	0.0196 ± 0.0149^{ab}	0.0129 ± 0.0016^{ab}	0.0054 ± 0.0003^{abc}	0.2348 ± 0.0077^{bc}	0.0096 ± 0.0097^{a}
LGG NE DS	9	0.0101 ± 0.0141^{a}	0.0012 ± 0.0001^{a}	0.0059 ± 0.0007^{abc}	0.2478 ± 0.0098^{c}	0.0124 ± 0.0026^{a}
LGG NE DS	24	0.0015 ± 0.0005^{a}	0.0014 ± 0.0004^{a}	0.0059 ± 0.0005^{abc}	0.2557 ± 0.0028^{c}	0.0114 ± 0.0009^{a}
BY E S	0	3.8857 ± 0.4324^{bc}	0.0415 ± 0.0052^{cd}	0.0116 ± 0.0014^{cde}	1.1132 ± 0.0668^{f}	0.1495 ± 0.0280^{b}
BY E S	9	6.1934 ± 0.9401^{c}	0.0439 ± 0.0046^{de}	0.0135 ± 0.0025^{de}	0.2012 ± 0.1087^{abc}	0.0026 ± 0.0004^{a}
BY E S	24	9.6691 ± 2.8424^{e}	0.0482 ± 0.0019^{de}	0.0295 ± 0.0030^{hi}	0.0169 ± 0.0051^{ab}	0.0119 ± 0.0122^{a}
BY NE S	0	0.0027 ± 0.0034^{a}	0.0114 ± 0.0132^{a}	0.0044 ± 0.0051^{abc}	0.1965 ± 0.2232^{abc}	0.0111 ± 0.0129^{a}
BY NE S	9	0.0093 ± 0.0127^{a}	0.0074 ± 0.0086^{a}	0.0029 ± 0.0034^{a}	0.1330 ± 0.1525^{abc}	0.0022 ± 0.0025^{a}
BY NE S	24	0.0056 ± 0.0078^{a}	0.0025 ± 0.0042^{a}	0.0010 ± 0.0018^{a}	0.0474 ± 0.0814^{abc}	0.0002 ± 0.0002^{a}
BY E DS	0	2.9603 ± 1.1328^{b}	0.0446 ± 0.0338^{d}	0.0043 ± 0.0018^{ab}	0.4997 ± 0.2909^{d}	0.0288 ± 0.0130^{a}
BY E DS	9	3.4261 ± 1.0890^{b}	0.0557 ± 0.0306^{de}	0.0221 ± 0.0077^{g}	0.0718 ± 0.0515^{abc}	0.0050 ± 0.0016^{a}
BY E DS	24	5.6437 ± 2.0498^{d}	0.0843 ± 0.0510^{f}	0.0483 ± 0.0202^{j}	0.0606 ± 0.0173^{abc}	0.0034 ± 0.0032^{a}
BY NE DS	0	0.0048 ± 0.0062^{a}	0.0004 ± 0.0005^{a}	<LQ	0.0097 ± 0.0149^{a}	0.0001 ± 0.0002^{a}
BY NE DS	9	0.0015 ± 0.0030^{a}	<LQ	<LQ	0.0025 ± 0.0030^{a}	<LQ
BY NE DS	24	0.0014 ± 0.0028^{a}	0.0002 ± 0.0004^{a}	<LQ	0.0051 ± 0.0073^{a}	<LQ

LQ Limit of quantification. Below detention limit. See Materials and Methods.
Values with the same superscript letter were not statistically significantly different at $p < 0.05$.

used as a mechanism to produce substrates for LAB growth (Khalid, 2011). Helland et al. (2004) showed that LGG was an active starter that reduced glucose levels by 90% in maize porridge with barley malt. In our study, glucose content appeared to increase due to the enzymes effect on starch hydrolysis, even though LGG consumed glucose, as its growth showed. According to Kankainen et al. (2009) LGG was capable to use

glucose. However, in this study it was not possible to observe the glucose used because amylolytic enzymes could still release it from starch. Concerning BY E, the microorganisms could also metabolise glucose although the evolution was different from LGG.

It is known that the microorganisms present in the BY starter ferment lactose, glucose, mannose, and fructose and preferably use lactose (O'Leary & Woychik, 1976; Van de Guchte et al., 2006; Zheng et al., 2020). According to Chervaux et al. (2000), glucose import is inefficient in *Lactobacillus delbrueckii* subsp. *bulgaricus*. Nevertheless, O'Leary and Woychik (1976), after culturing this microorganism in different media, found that these bacteria utilized glucose preferentially. In the BY samples of this study, higher concentrations of glucose were found at 24 h (approx. 10 g/100g) and lower values of pH were observed but the amount of lactic acid was lower (0.39%). The *Streptococcus* and *Lactobacillus* in this matrix may have used glucose partially as an energy source in the first times of fermentation.

Desalting did not change the final 24 h glucose concentration in LGG samples. However, in the case of BY, a higher glucose concentration at 24 h was observed in the salted samples. This result in BY cannot be explained by a reduction in glucose transformation to lactic acid during fermentation caused by the presence of salt in the bread flour, as a higher TA, and a higher LAB counts were observed in the BY E samples without desalting. This treatment may remove some growth factors like vitamins, polyamines or free amino acids that could be necessary to grow *L. delbrueckii* subsp. *bulgaricus* or *Streptococcus thermophilus* (Muller et al., 2009). According to the multifactorial ANOVA (Table S4), at 24 h of fermentation, the presence or absence of salt had no effect on glucose concentration, whereas both, the starter and the enzymes, and their interaction had a significant effect on glucose concentration.

Regarding fructose, the quantities of this sugar were lower in all the beverages and the highest content was reached in BY E DS with 0.084 g/100 g at 24 h of fermentation. This sugar levels did not change through the whole fermentation, so no fructose consumption or formation were observed in the beverages. LAB are able to metabolise fructose although they show preference for glucose as a main source of energy (Bernat et al., 2015). Fructose content is low because starch is composed of glucose chains and enzymatic hydrolysis produce glucose instead of fructose (Martinez & Gómez, 2020).

Isomaltose was also found in the beverages in low concentrations. Its content in treatments without enzymes remained stable along with fermentation. In contrast, significant differences were found between times 0 and 24 h in samples with enzymes. The microorganisms present in the starters were not able to metabolise this sugar so its slight increase results from the action of the amylolytic enzymes used (Robyt, 2009).

In the case of maltose, its content showed a contrary trend to glucose. The amount of maltose decreased as fermentation progressed and this was observed in the samples with enzymes. The cause for this decline can be attributed to β-glucoamylase which breaks down maltose into glucose, reducing its concentration in the beverages (Warren et al., 2015). Significant differences were noticed between 0 and 9 h in both starters with enzymes. In the samples without enzymes, no evolution was observed, and the content was lower. It is known that *Bifidobacterium* spp. Can use maltose as a fermentable carbohydrate (Mårtensson et al., 2001; Muller et al., 2009). However, there were no significant differences between the maltose content at 0 and 24 h in both starters without enzymes. Therefore, *Bifidobacterium* preferentially utilized glucose to maltose. Its reduction in BY samples could be due to the enzymatic hydrolysis of maltose, not to the ability of the *Bifidobacterium* strains present in this starter to ferment this carbohydrate. These results contradict those obtained by Vaz Rezende et al. (2022) who observed that *B. animalis* subsp. *lactis* used maltose in commercial dietary fibres. The difference between our results and Vaz Rezende et al.'s (2022) is that the latter, small quantities of glucose and free amino acids were present during their fermentation. Bacteria present in BY may need the presence of these compounds to use maltose as an energy source or, as mentioned before, as a preferred source. The desalting treatment did not seem to affect the reduction of maltose content during the 24 h of fermentation.

For maltotriose, its content was much lower and did not exceed 0.3 g/100 g in any case. A significant decrease of the content of this trisaccharide was observed between 0 and 24 h in the LGG E S and BY E S samples which had higher starting concentrations. This reduction was produced by enzymatic hydrolysis which breaks the bonds between the three glucose units (Beschkov et al., 1984). In the rest of the samples, the amount of maltotriose remained stable.

3.3. Acidification kinetics during fermentation

The results of V_{max} pH red, V_{max} acid, pH (pH V_{max}) and lactic acid (LA V_{max}) achieved at the maximum rate and the times at which the maximum rate of pH reduction (t V_{max}) and pH 4.5 (t $_{pH4.5}$) were reached, are shown in Table 3. The V_{max} pH red were higher in the experiments with salt and enzymes of each starter, reaching values of 0.37 dpH/h for LGG and 0.28 dpH/h for BY. The rates were lower in comparison with the rates reached by Coda et al. (2011) for *L. plantarum* in fermented emmer beverages (0.40–0.76 dpH/h) in 4 h of fermentation at 30 °C. However, similar results (0.24 dpH/h) were obtained by Magala et al. (2015) in the rice beverage fermented with a mixed starter (*Bifidobacterium* and L. *plantarum)*. The desalted treatment influenced V_{max} pH red since all the samples that underwent desalting had lower values of this parameter (0.11–0.29 dpH/h). Chikthimmah et al. (2001) reported that small amounts (0–2.5%) of NaCl stimulated the growth of

Table 3
Acidification kinetics parameters obtained from two starter cultures: LGG and BY, using a bread waste beverage, with E and NE and S and DS[a].

Sample	V_{max} pH red (dpH/h)	t V_{max} (h)	pH V_{max}	t $_{pH4.5}$ (h)	V_{max} acid (dacidity/h)	LA V_{max} (%)
LGG E S	0.37 ± 0.02^{e}	6.00 ± 0.00^{b}	4.30 ± 0.11^{ab}	5.45 ± 0.35^{abc}	0.04 ± 0.01^{d}	0.25 ± 0.03^{ab}
LGG NE S	0.32 ± 0.01^{de}	6.00 ± 0.00^{b}	4.45 ± 0.18^{b}	5.80 ± 0.57^{bc}	0.02 ± 0.00^{bc}	0.13 ± 0.00^{ab}
LGG E DS	0.29 ± 0.01^{cd}	8.25 ± 1.06^{c}	3.99 ± 0.26^{a}	6.45 ± 1.34^{c}	0.02 ± 0.00^{abc}	0.26 ± 0.18^{b}
LGG NE DS	0.21 ± 0.06^{b}	6.60 ± 0.85^{b}	4.56 ± 0.2^{bc}	7.25 ± 2.47^{c}	0.01 ± 0.00^{a}	0.09 ± 0.01^{a}
BY E S	0.28 ± 0.04^{cd}	3.60 ± 0.85^{a}	4.43 ± 0.04^{b}	3.50 ± 0.71^{ab}	0.03 ± 0.00^{c}	0.22 ± 0.07^{ab}
BY NE S	0.18 ± 0.03^{b}	3.00 ± 0.00^{a}	4.91 ± 0.21^{c}	6.50 ± 1.41^{c}	0.01 ± 0.00^{ab}	0.15 ± 0.03^{ab}
BY E DS	0.23 ± 0.00^{bc}	3.00 ± 0.00^{a}	4.49 ± 0.07^{b}	3.00 ± 0.28^{a}	0.02 ± 0.00^{abc}	0.16 ± 0.03^{ab}
BY NE DS	0.11 ± 0.01^{a}	5.25 ± 1.06^{b}	4.61 ± 0.13^{bc}	6.50 ± 0.71^{c}	0.01 ± 0.00^{a}	0.11 ± 0.01^{ab}

[a] LGG, *L. rhamnosus* GG; BY, *Bifidobacterium, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus;* E, with enzymes; NE, without enzymes; S, salt; DS, desalted

Data are the means of two independent experiments ± standard deviations (n = 2).

Values in the same column with different superscript letters differ significantly ($P < 0.05$). **V_{max} pH red** is the maximum rate of pH reduction expressed in (dpH/h). **T V_{max}** is the time at the maximum rate of pH reduction (h); pH **V_{max}** is the pH reached at the maximum rate of pH reduction; **t $_{pH4.5}$** is the time at which pH 4.5 is reached (h); **V_{max} acid** is the maximum acidification rate (dacidity/h) and **LA V_{max}** is the percentage of lactic acid reached at the maximum rate of acidification (g per 100 mL of lactic acid).

[a] Check Table 1 for LGG, BY, E, NE, S and DS meaning.

LAB, whereas higher concentration (5%) inhibited them. Results show that the presence of salt in the initial matrix was beneficial for the metabolism of the starters used, hence the production of lactic acid and pH reduction. The enzymatic treatment also affected this parameter and significant differences were observed between samples with and without enzymes except for sample LGG with salt.

Low times to obtain t V_{max} were observed by starter BY, approximately 3 h except for BY NE DS (5.25 h) which was significantly different. The t V_{max} in LGG was approximately twice higher than BY until a maximum time of 8.25 h. It seems that the three microorganisms of the starter BY created a synergy combination, and they acidified the medium earlier. Although they reached similar or higher pHs than LGG in the final beverages. These high pH values could result from a lower growth of the starter acidification strains *(Streptococcus thermophilus)* (Muller et al., 2009) throughout fermentation. Moreover, the starter BY had a rapid growth up to 9 h, reaching the stationary at this time and death phase at 24 h specifically, for samples without amylolytic enzymes and desalted samples. The cooperation between *Lactobacillus* and *Streptococcus* has been studied in milk since they stimulate each other during milk process fermentation. Some of them are proteolytic and others hydrolyse polysaccharides and produce monosaccharides or carbonyl compounds using as a source of energy (Li et al., 2021; Van de Guchte et al., 2006). However, protocooperation of these bacteria in vegetal matrix has not been investigated according to the bibliography reviewed.

The pH V_{max} oscillated between a range of 3.99 and 4.91 in all beverages. Starter LGG allowed to reach a low pH, but it took more time to do it. The minimum pH was reached by culture LGG E DS although the time to get this pH was the highest of all of them.

BY E samples reached pH 4.5 earlier than the rest of the treatments. The pH 4.5 is suitable because it prevents the growth of pathogens (Motarjemi & Nout, 1996). A good acidified starter is known to reduce the pH by 1.3 units in 6 h at 30 °C (Beresford et al., 2001). All treatments with enzymes and LGG NE S were successful in reducing by 1.3 units the pH during the fermentation (see Fig. 2). This fact can indicate that the matrix, bread flour and water, is a good substrate for the growth of both starters. No significant differences were found, regardless of whether it had been desalted or not, while the enzymatic treatment only affected this parameter in the case of BY S and BY DS (see Table 3).

The desalting treatment only reduced V_{max} acid in the starter LGG but not in the starter BY. Besides, the enzymatic treatment increased this parameter but only in salted samples. These results matched with V_{max} pH red values because the pH reduction and the TA increase are linked.

The percentage of lactic acid reached at LA V_{max} ranged between 0.11 and 0.25. The highest content was found in treatments with enzymes and significant differences were only observed in LGG DS samples (Table 3). The enzymatic treatment was the only factor that significantly affected this parameter according to multifactorial ANOVA (Table S5).

3.4. Evolution of pH, TA and WHC during storage

The evolution of pH, TA and WHC values were statically treated to analyse the lifespan of each final product (Table 4).

Regarding pH, the lack of enzymes greatly affected the pH reached at the end of the fermentation in the LGG samples and, to a lesser extent, in the BY samples. In general, low values of pH were detected in samples treated with enzymes in both starters. A negative correlation between pH_{1d} and V_{max} pH red ($r = -0.50$; $p < 0.05$) and a positive correlation between pH_{1d} and pH V_{max} ($r = 0.70$; $p < 0.05$) were observed. These correlations make sense since higher rates of pH reduction allow lower pH to be reached in less time. Therefore, those samples with higher V_{max} pH red and those which reached the maximum rate with lower pH values attained the most acidic pH. At 15 days, we noted again significant differences between enzymes and non-enzymes samples fermented by both starters. No significant differences were found in the evolution of pH in any beverage during its storage. Nevertheless, at 21 days, samples with enzymes and salt showed a slight decrease in pH compared to that obtained after 15 days; while desalted samples with and without enzymes showed an increase on pH. In contrast, Aparicio-García et al. (2021) noticed a strong pH decrease in a sprouted oat beverage, during the first 5 days of storage until 4.0. According to them, this sharp decline in pH matches with the fermentative capacity of *L. plantarum* during storage at 4 °C. Salmerón et al. (2015) observed similar pH values (3.27–3.72) in the nine cereal drinks fermented by different strains of *Lactobacillus*.

High TA was observed in the samples with enzymes on all days studied, except for BY E DS samples on day 21. The enzymatic treatment had a clear positive effect on TA on all three days studied (see Table S6). Curiously, in BY E sample, as mentioned before, higher glucose concentration at 24 h was observed in comparison with the desalting sample (see Table 2). It is possible, that the presence of salt was also an important factor for this parameter. The TA in the final products

Table 4
PH, titratable acidity and WHC measures obtained from different fermented beverages after their fermentation (1 day) and during their storage at 15 and 21 days. Factors: LGG and BY; E and NE; S and DS. Data are expressed as mean ± standard deviation (n = 8). [a]

Sample/ Time	pH			Titratable acidity (g per 100 mL of lactic acid)			WHC (%)		
	1d	15d	21d	1d	15d	21d	1d	15d	21d
LGG E S	3.10 ± 0.26^{ab1}	2.84 ± 0.01^{a1}	2.82 ± 0.09^{d1}	0.48 ± 0.01^{f1}	0.55 ± 0.01^{e2}	0.59 ± 0.03^{c2}	38.73 ± 3.11^{a1}	39.28 ± 1.77^{a1}	39.07 ± 1.08^{a1}
LGG NE S	3.97 ± 0.18^{d1}	3.84 ± 0.03^{d1}	3.84 ± 0.08^{e1}	0.21 ± 0.05^{bc1}	0.17 ± 0.01^{a1}	0.17 ± 0.00^{a1}	74.92 ± 4.03^{c1}	76.20 ± 2.22^{c1}	71.72 ± 9.36^{c1}
LGG E DS	2.85 ± 0.01^{a1}	2.90 ± 0.06^{a1}	3.13 ± 0.42^{b1}	0.40 ± 0.01^{e1}	0.43 ± 0.03^{d1}	0.47 ± 0.18^{bc1}	35.85 ± 0.88^{a1}	33.67 ± 0.42^{a1}	34.87 ± 1.32^{a1}
LGG NE DS	4.10 ± 0.00^{d1}	4.19 ± 0.04^{e1}	3.97 ± 0.23^{a1}	0.11 ± 0.02^{a1}	0.14 ± 0.03^{a1}	0.19 ± 0.09^{a1}	68.70 ± 0.00^{bc1}	62.04 ± 4.43^{b1}	65.77 ± 1.79^{bc1}
BY E S	3.38 ± 0.19^{b1}	3.26 ± 0.11^{b1}	3.03 ± 0.25^{d1}	0.39 ± 0.03^{e1}	0.36 ± 0.00^{c1}	0.40 ± 0.05^{b1}	36.88 ± 0.21^{a1}	35.90 ± 0.28^{a1}	36.47 ± 3.06^{b1}
BY NE S	3.77 ± 0.14^{cd1}	3.67 ± 0.08^{cd1}	3.89 ± 0.36^{c1}	0.30 ± 0.03^{d1}	0.22 ± 0.02^{b1}	0.17 ± 0.08^{a1}	72.85 ± 0.40^{c1}	67.10 ± 3.54^{b1}	67.47 ± 6.60^{bc1}
BY E DS	3.46 ± 0.21^{bc1}	3.47 ± 0.23^{bc1}	3.40 ± 0.00^{b1}	0.27 ± 0.04^{cd1}	0.22 ± 0.01^{b1}	0.20 ± 0.01^{a1}	34.17 ± 0.33^{a1}	33.47 ± 1.98^{a1}	32.99 ± 1.68^{a1}
BY NE DS	3.90 ± 0.09^{d1}	3.95 ± 0.21^{de1}	3.89 ± 0.09^{b1}	0.18 ± 0.01^{ab1}	0.15 ± 0.02^{a1}	0.13 ± 0.09^{a1}	66.26 ± 6.10^{b1}	60.72 ± 5.64^{b1}	59.05 ± 5.59^{b1}

[a] LGG, *L. rhamnosus* GG; BY, *Bifidobacterium, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus;* E, with enzymes; NE, without enzymes; S, salt; DS, desalted

Significant differences between columns are indicated by letters and those between rows by numbers.

[a] Check Table 1 for LGG, BY, E, NE, S and DS meaning.

(0.11–0.48%) was, in general terms, higher than the values registered by Salmerón et al. (2015). This difference could be due to the short fermentation time of 10 h. However, our results were lower than those reported for dairy probiotic products with growth supplements added (0.44–0.63%) (Gomes et al., 1998). No changes in TA were observed during storage, with the exception of LGG E S sample after 15 days, where it increased slightly without affecting the final pH value. Nguyen et al. (2022) also reported an increase of lactic acid content at 5 °C in a bread beverage fermented with *L. rhamnosus* after 6 weeks of storage.

There are significant differences in the percentage of WHC between samples with and without enzymes. Enzymes addition facilitated starch hydrolysis in the fermented beverages. Due to the starch hydrolysis, the fermented beverages have a lower WHC capacity (Luana et al., 2014). Along the storage time, Aparicio-García et al. (2021) reached similar WHC (33.7–35.3%) to our samples with enzymes, although they used sprouted oat and just 4 h of fermentation. No differences in the WHC evolution were noticed during storage. The absence of changes makes the product more stable during storage.

4. Conclusion

Bread waste flour is a good substrate for the growth of lactic acid bacteria and *Bifidobacterium* present in the two starters. The combination of starter BY and enzymatic and non-enzymatic treatments with salt proved to be the best option for the fermentation of a bread waste-based beverage according to the growth kinetics parameter. The enzymatic treatment resulted in a pH reduction, an increased total acidity, and a desirable decrease in the WHC of the fermented beverages. Moreover, the enzymes addition caused a higher production of glucose and isomaltose, whereas maltose decreased due to its hydrolysis. The desalting process contributed to produce worse results, but the sample LGG E DS sample reached the maximum log CFU/g at 24h and the lowest pH. In general, the enzymatic treatment would be the most suitable option if short fermentation times are sought. However, starter BY could be used without adding enzymes, in longer fermentations reaching a good microbial growth.

More studies are needed to evaluate the nutritional, sensorial and organoleptic beverage properties, as well as the microbial evolution during their storage.

CRediT authorship contribution statement

Teresa Sigüenza-Andrés: Data curation, Investigation, Formal analysis, Methodology, Resources, Writing – original draft, Writing – review & editing. **Manuel Gómez:** Conceptualization, Validation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **José M. Rodríguez-Nogales:** Conceptualization, Validation, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Irma Caro:** Conceptualization, Validation, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

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.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was supported financially by Junta de Castilla y León (VA177P20), Spain, and the TRANSCOLAB FEDER-Interreg Spain-Portugal project (0612_TRANS_CO_LAB_2_P). The authors are also grateful to the Laboratory of instrumental techniques of the University of Valladolid for the analysis of samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114795.

References

Adessi, A., Venturi, M., Candeliere, F., Galli, V., Granchi, L., & De Philippis, R. (2018). Bread wastes to energy: Sequential lactic and photo-fermentation for hydrogen production. *International Journal of Hydrogen Energy, 43*(20), 9569–9576. https://doi.org/10.1016/j.ijhydene.2018.04.053

Angelov, A., Yaneva-Marinova, T., & Gotcheva, V. (2018). Oats as a matrix of choice for developing fermented functional beverages. *Journal of Food Science and Technology, 55*(7), 2351–2360. https://doi.org/10.1007/S13197-018-3186-Y

Aparicio-García, N., Martínez-Villaluenga, C., Frias, J., & Peñas, E. (2021). Production and characterization of a novel gluten-free fermented beverage based on sprouted oat flour. *Foods, 10*(1). https://doi.org/10.3390/foods10010139

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology, 23*(3–4), 277–294. https://doi.org/10.1016/0168-1605(94)90157-0

Beresford, T. P., Fitzsimons, N. A., Brennan, N. L., & Cogan, T. M. (2001). Recent advances in cheese microbiology. *International Dairy Journal, 11*, 259–274.

Bernat, N., Cháfer, M., Chiralt, A., & González-Martínez, C. (2014). Hazelnut milk fermentation using probiotic *Lactobacillus rhamnosus* GG and inulin. *International Journal of Food Science and Technology, 49*(12), 2553–2562. https://doi.org/10.1111/ijfs.12585

Bernat, N., Cháfer, M., Chiralt, A., & González-Martínez, C. (2015). Development of a non-dairy probiotic fermented product based on almond milk and inulin. *Food Science and Technology International, 21*(6), 440–453. https://doi.org/10.1177/10820132145437

Beschkov, V., Marc, A., & Engasser, J. M. (1984). A kinetic model for the hydrolysis and synthesis of maltose, isomaltose, and maltotriose by glucoamylase. *Biotechnology and Bioengineering, 26*(1), 22–26. https://doi.org/10.1002/bit.260260106

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., & Webb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International, 36*(6), 527–543. https://doi.org/10.1016/S0963-9969(03)00009-7

Cal-Sabater, P., Caro, I., Castro, M. J., Cao, M. J., Mateo, J., & Quinto, E. J. (2019). Flow cytometry to assess the counts and physiological state of *Cronobacter sakazakii* cells after heat exposure. *Foods, 8*(12). https://doi.org/10.3390/foods8120688

Caro, I., Mateo, J., & Franco Fernandez, M. J. (1999). Métodos de determinación de la actividad de acidificacion de las bacterias acidolácticas utilizadas en la industria láctea. *ILE: Industrias Lácteas Españolas, 245*, 36–42.

Chavan, M., Gat, Y., Harmalkar, M., & Waghmare, R. (2018). Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume. *LWT - Food Science and Technology, 91*, 339–344. https://doi.org/10.1016/j.lwt.2018.01.070 (October 2017).

Chervaux, C., Ehrlich, S. D., & Maguin, E. (2000). Physiological study of *Lactobacillus delbrueckii* subsp. *bulgaricus*. Strains in a novel chemically defined medium. *Applied and Environmental Microbiology, 66*(12), 5306–5311.

Chikthimmah, N., Anantheswaran, R. C., Roberts, R. F., Mills, E. W., & Knabel, S. J. (2001). Influence of sodium chloride on growth of lactic acid bacteria and subsequent destruction of *Escherichia coli* O157:H7 during processing of Lebanon bologna. *Journal of Food Protection, 64*(8), 1145–1150. https://doi.org/10.4315/0362-028X-64.8.1145

Coda, R., Rizzello, C. G., Trani, A., & Gobbetti, M. (2011). Manufacture and characterization of functional emmer beverages fermented by selected lactic acid bacteria. *Food Microbiology, 28*(3), 526–536. https://doi.org/10.1016/j.fm.2010.11.001

European Commission. (2008). Directive 2008/98/EC of the European Parliament and of the council of 19 November 2008 on waste and repealing certain Directives (Text with EEA relevance). http://data.europa.eu/eli/dir/2008/98/oj.

FAO. (2013). Food wastage footprint: Impacts on natural resources - summary report. http://www.fao.org/3/a-i3347e.pdf.

Gomes, A. M. P., & Malcata, F. X. (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science & Technology, 10*(4–5), 139–157. https://doi.org/10.1016/S0924-2244(99)00033-3

Gomes, A. M. P., Malcata, F. X., & Klaver, F. A. M. (1998). Growth enhancement of *Bifidobacterium lactis* Bo and *Lactobacillus acidophilus* Ki by milk hydrolyzates. *Journal of Dairy Science, 81*, 2817–2825. https://doi.org/10.3168/jds.S0022-0302(98)75840-0

Gómez, M., & Martinez, M. M. (2023). Redistribution of surplus bread particles into the food supply chain. *Lwt, 173*. https://doi.org/10.1016/j.lwt.2022.114281. November 2022.

Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., & Faria, J. A. F. (2010). Functional foods and nondairy probiotic food development: Trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety, 9*(3), 292–302. https://doi.org/10.1111/j.1541-4337.2010.00110.x

Helland, M. H., Wicklund, T., & Narvhus, J. A. (2004). Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. *International Journal of Food Microbiology, 91*(3), 305–313. https://doi.org/10.1016/j.ijfoodmicro.2003.07.007

Ishangulyyev, R., Kim, S., & Lee, S. H. (2019). Understanding food loss and waste - why are we losing and wasting food? *Foods, 8*(8). https://doi.org/10.3390/foods8080297

Jyoti, B. D., Suresh, A. K., & Venkatesh, K. V. (2004). Effect of preculturing conditions on growth of *Lactobacillus rhamnosus* on medium containing glucose and citrate. *Microbiological Research, 159*(1), 35–42. https://doi.org/10.1016/j.micres.2004.01.008

Kankainen, M., Paulin, L., Tynkkynen, S., von Ossowski, I., Reunanen, J., Partanen, P., Satokari, R., Vesterlund, S., Hendrickx, A. P. A., Lebeer, S., De Keersmaecker, S. C. J., Vanderleyden, J., Hämäläinen, T., Laukkanen, S., Salovuori, N., Ritari, J., Alatalo, E., Korpela, R., Mattila-Sandholm, T., … de Vos, W. M. (2009). Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human- mucus binding protein. *Proceedings of the National Academy of Sciences, 106*(40), 17193–17198. https://doi.org/10.1073/pnas.0908876106

Khalid, K. (2011). An overview of lactic acid bacteria. *International Journal of Biosciences, 1*(3), 1–13.

Li, S. N., Tang, S. H., Ren, R., Gong, J. X., & Chen, Y. M. (2021). Metabolomic profile of milk fermented with *Streptococcus thermophilus* cocultured with *Bifidobacterium animalis* ssp. *lactis, Lactiplantibacillus plantarum*, or both during storage. *Journal of Dairy Science, 104*(8), 8493–8505. https://doi.org/10.3168/jds.2021-20270

Luana, N., Rossana, C., Curiel, J. A., Kaisa, P., Marco, G., & Rizzello, C. G. (2014). Manufacture and characterization of a yogurt-like beverage made with oat flakes fermented by selected lactic acid bacteria. *International Journal of Food Microbiology, 185*, 17–26. https://doi.org/10.1016/j.ijfoodmicro.2014.05.004

Magala, M., Kohajdová, Z., Karovičová, J., Greifová, M., & Hojerová, J. (2015). Application of lactic acid bacteria for production of fermented beverages based on rice flour. *Czech Journal of Food Sciences, 33*(No. 5), 458–463. https://doi.org/10.17221/74/2015-CJFS

Mårtensson, O., Andersson, C., Andersson, K., Öste, R., & Holst, O. (2001). Formulation of an oat-based fermented product and its comparison with yoghurt. *Journal of the Science of Food and Agriculture, 81*(14), 1314–1321. https://doi.org/10.1002/jsfa.947

Martinez, M. M., & Gómez, M. (2020). Insight of the α-amylase family of enzymes. *Microbial Enzyme Technology in Food Applications*, 21–45. https://doi.org/10.1201/9781315368405-4

Massa, A., Axpe, E., Atxa, E., & Hernández, I. (2022). Sustainable, carbonated, non-alcoholic beverages using leftover bread. *International Journal of Gastronomy and Food Science, 30*(October). https://doi.org/10.1016/j.ijgfs.2022.100607

Melikoglu, M., & Webb, C. (2013). Use of waste bread to produce fermentation products. In C. W. Maria Kosseva (Ed.), *Food industry wastes* (pp. 63–76). Elsevier. https://doi.org/10.1016/B978-0-12-391921-2.00004-4.

Motarjemi, Y., & Nout, M. J. (1996). Food fermentation : A safety and nutritional. *FAO/WHO Workshop on Assessment of Fermentation as a Household Technology for Improving, 74*(6), 553–559.

Muller, J. A., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2009). Manufacture of probiotic bacteria. *Prebiotics and Probiotics Science and Technology*, 725–759. https://doi.org/10.1007/978-0-387-79058-9_18

Nguyen, T. L., Toh, M., Lu, Y., Ku, S., & Liu, S. Q. (2022). Biovalorization of market surplus bread for development of probiotic-fermented potential functional beverages. *Foods, 11*(3), 1–17. https://doi.org/10.3390/foods11030250

O'Leary, V. S., & Woychik, J. H. (1976). Utilization of lactose, glucose, and galactose by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk treated with lactase enzyme. *Applied and Environmental Microbiology, 32*(1), 89–94. https://doi.org/10.1128/aem.32.1.89-94.1976

Petrova, P., & Petrov, K. (2020). Lactic acid fermentation of cereals and pseudocereals: Ancient nutritional biotechnologies with modern applications. *Nutrients, 12*(4), 1–26. https://doi.org/10.3390/nu12041118

Pico, J., Martínez, M. M., Martín, M. T., & Gómez, M. (2015). Quantification of sugars in wheat flours with an HPAEC-PAD method. *Food Chemistry, 173*, 674–681. https://doi.org/10.1016/j.foodchem.2014.10.103

Rezende, E. S., Lima, G. C., Lima, M., Coelho, A. S., & Naves, M. M. (2022). Prebiotic potential of isolated commercial dietary fibres compared to orange albedo in *Lactobacillus* and *Bifidobacterium* species. *Bioactive Carbohydrates and Dietary Fibre, 28* (March), Article 100316. https://doi.org/10.1016/j.bcdf.2022.100316

Robyt, J. F. (2009). Enzymes and their action on starch. In J. BeMiller, & R. Whistler (Eds.), *Starch* (pp. 237–292). Elsevier. https://doi.org/10.1016/B978-0-12-746275-2.00007-0.

Salmerón, I., Thomas, K., & Pandiella, S. S. (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. *Journal of Functional Foods, 15*, 106–115. https://doi.org/10.1016/j.jff.2015.03.012

Sigüenza-Andrés, T., Pando, V., Gómez, M., & Rodríguez-Nogales, J. M. (2022). Optimization of a simultaneous enzymatic hydrolysis to obtain a high-glucose slurry from bread waste. *Foods, 11*(12), 1793. https://doi.org/10.3390/foods11121793

Toh, H., Oshima, K., Nakano, A., Takahata, M., Murakami, M., Takaki, T., Nishiyama, H., Igimi, S., Hattori, M., & Morita, H. (2013). Genomic adaptation of the *Lactobacillus casei* group. *PLoS One, 8*(10). https://doi.org/10.1371/journal.pone.0075073

Tsafrakidou, P., Michaelidou, A.-M., & Biliaderis C, G. (2020). Fermented cereal-based products: Nutritional aspects, possible impact on gut microbiota and health implications. *Foods, 9*(6), 734. https://doi.org/10.3390/foods9060734

United Nations. (2015). Transforming our world: The 2030 agenda for sustainable development. https://sdgs.un.org/publications/transforming-our-world-2030-agenda-sustainable-development-17981.

Van de Guchte, M., Penaud, S., Grimaldi, C., Barbe, V., Bryson, K., Nicolas, P., Robert, C., Oztas, S., Mangenot, S., Couloux, A., Loux, V., Dervyn, R., Bossy, R., Bolotin, A., Batto, J.-M., Walunas, T., Gibrat, J.-F., Bessières, P., Weissenbach, J., … Maguin, E. (2006). The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. *Proceedings of the National Academy of Sciences, 103* (24), 9274–9279. https://doi.org/10.1073/pnas.0603024103

Verni, M., Rizzello, C. G., & Coda, R. (2019). Fermentation biotechnology applied to cereal industry by-products: Nutritional and functional insights. *Frontiers in Nutrition, 6*(April), 1–13. https://doi.org/10.3389/fnut.2019.00042

Warren, F. J., Zhang, B., Waltzer, G., Gidley, M. J., & Dhital, S. (2015). The interplay of α-amylase and amyloglucosidase activities on the digestion of starch in in vitro enzymic systems. *Carbohydrate Polymers, 117*, 185–191. https://doi.org/10.1016/j.carbpol.2014.09.043

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology, 70*(4), 2782–2858. https://doi.org/10.1099/ijsem.0.004107