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Full Length Research Paper

Functional Characterization of the Weissella Cibaria BAL3C-5 C120T Strain, which Produces Dextran and Riboflavin in Excess, for the Creation of Biofortified Plant-based Drinks

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Humans require riboflavin (vitamin B2), which can only be obtained through a balanced diet. An excellent method for creating functional drinks through in situ biofortification is fermentation using riboflavin-producing lactic acid bacteria (LAB). These drinks have the potential to improve consumer health and fill in dietary gaps. Weissella cibaria BAL3C-5 C120T's functional skills for riboflavin overproduction and dextran production during the fermentation of drinks made from oats, rice, soybeans, and almonds have been assessed in this work. The strain's ability to produce dextran and riboflavin in the examined beverages was verified. The strain's ability to raise riboflavin and dextran levels to 3.4 mg/L and 3.2 g/L, respectively, after 24 hours of fermentation was particularly noticeable in the oat-based beverage. Furthermore, the strain was able to add up to 6.6 g/L of the prebiotic oligosaccharide panose to fermented oat-based beverages under ideal circumstances. Furthermore, after a month of storage, BAL3C-5 C120T shown a high 80% cell viability and a good capacity to reduce pH (from 7.0 to 3.8) in the oat-based drinks. The fermented oat-based beverages that were produced had a thixotropic structure and gel-like behavior, which was not seen in the non-fermented control drinks, according to rheological research. In conclusion, these findings validated the special qualities of the W. cibaria BAL3C-5 C120T strain for the production of functional and bio-fortified plant-based drinks with enhanced rheological and nutritional qualities. Analysis of the BAL3C-5 C120T strain's autoaggregation characteristics and survival in gastrointestinal settings suggested that it might be used as a probiotic in a fermented beverage made from oats. In this regard, the study also encourages the use of W. cibaria species in food and health industries where they have not yet been employed as an auxiliary or starter culture.

Key words: Lactic acid bacteria, Vitamin B2, Dextran, Functional drinks, Biofortification, Rheology.

INTRODUCTION

According to Nazir et al. (2019), functional foods are foods or beverages that contain one or more components that influence several biological processes, enhancing overall health and lowering the risk of contracting illnesses. They also serve as a means of addressing nutritional deficiencies, halting the emergence of illnesses linked to nutrition, and enhancing both mental and physical health. Accordingly, in situ biofortification of potentially functional foods with a variety of vital metabolites, including vitamins, minerals, and others, is the direction of current advances in food science and microbiology (Nazir et al., 2019; Ofori et al., 2022).

Additionally, consumers are becoming more conscious of the development of foods known as "clean-label" products, which have less artificial additives and healthier substitutes, such as low-sugar, low-fat, or gluten-free items (Wakeel et al., 2018). Using lactic acid bacteria (LAB) that produce useful metabolites is a very promising way to address these issues. Among these, the generation of vitamin B2 (riboflavin, RF) and dextran-type exopolysaccharides is notable (Díaz-Montes, 2021; Levit et al., 2021). According to Zannini et al. (2016), dextrans are α-glucans made up of a main chain of D-glucopyranosyl residues with α-(1,6) links and different proportions of branches with α-(1,4), α-(1,3), or α-(1,2) bonds. They are produced by hydrolyzing the sucrose disaccharide to produce fructose and glucose, then transferring this latter monosaccharide to the expanding chain of the polymer. The synthesis is catalyzed by dextransucrases (Dsr, glycoside hydrolase GH 70 family). Depending on their molecular weight and branching, dextrans can function as novel hydrocolloids with a variety of rheological characteristics, enhancing the structure and texture of a variety of foods (e.g. in the formulation of low-fat dairy products or gluten-free baked goods) (Lynch et al., 2018; Werning et al., 2022). Liquorilactobacillus hordei TMW 1.1907 has been used to create cloudy dextran at high levels up to 8.5 g/L in apple or grape juices, demonstrating that in situ production of dextran has also been studied in non-dairy beverages (Eckel et al., 2019). The postbiotic high molecular weight dextrans that LAB produces also has antiviral (Nacher-Vazquez et al., 2015), anti-inflammatory (Zhou et al., 2022), immunomodulatory (Zarour et al., 2017), antioxidant, and hypocholesterolemic (Mohd Nadzir et al., 2021) qualities, as well as prebiotic (Kim et al., 2022).

The World Health Organization (WHO) designated RF as one of the key micronutrients to evaluate growth, development, and nutritional status (WHO, 1967). It is the precursor of the cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are vital for cellular energy metabolism and a number of oxidation-reduction processes (Levit et al., 2021). RF is mostly found in foods derived from animals, with smaller levels found in vegetables and goods made from cereals or plants. The EFSA recommends a daily intake of 0.4 mg for breastfed newborns and 1.6 mg for healthy adults. Additional limits are required for pregnant and breastfeeding women, with values of 1.9 and 2.0 mg/day taken into consideration, respectively (EFSA, 2017). Numerous illnesses, including anemia, migraines, cancer, oxidative stress, hyperglycemia, and hypertension, have been associated to the prevention of RF (Said and Ross, 2012; Udhayabanu et al., 2018). Therefore, iron absorption, tryptophan metabolism, mitochondrial dysfunction, brain dysfunction, skin disorders, migraines, circulatory issues, and digestive disorders are all impacted by RF deficiency, also known as ariboflavinosis, whose symptoms may manifest with an intake of less than 0.2 to 0.3 mg/day (Suwannasom et al., 2020; Thakur et al., 2017). RF is intriguing as an antiviral and antibacterial drug due to its photo-sensitizing qualities (Farah et al., 2022).

Despite the fact that RF is found in many different foods, significant deficiencies have been reported globally as a result of insufficient diets in both industrialized and developing nations (Plantone et al., 2021). Since it is a more sustainable, natural, and consumer-friendly option than chemically synthesized RF, vitamin B2 biofortified foods produced by RF-overproducing LAB have garnered a lot of attention in this regard (Revuelta et al., 2017). Furthermore, the sector continues to face significant challenges due to RF-overproducing genetically modified microbes, mostly because to consumer or governmental concerns around genetic engineering (Zhang et al., 2022). In this context, utilizing strains of Lactiplantibacillus plantarum (Ye´pez et al., 2019), Limosilactobacillus fermentum (Russo et al., 2014), Limosilactoba-cillus reuteri (Spacova et al., 2022), or Weissella cibaria (Hernandez-Alca´ntara et al., 2022), the use of selected LAB for biofortification of various foods has been successfully confirmed. The food and health sectors are actually interested in the ability to raise the RF concentration in food matrices that either lack or have very low concentrations of this vitamin. Therefore, a highly intriguing strategy for the creation of possible functional beverages is the biofortification of plant-based goods. This is due to the fact that they are a significant component of some populations' diets and have been gaining popularity recently as a nutritious substitute for dairy products (Drewnowski et al., 2021).

A variety of spontaneous RF-overproducing W. cibaria strains with mutations in the regulatory area of the rib operon, which encodes the enzymes involved in the RF biosynthetic pathway, were previously chosen by treatment with the lethal RF analogue roseoflavin (Diez- Ozaeta et al., 2023). The target strain, W. cibaria BAL3C-5 C120T, demonstrated constitutive RF production with an unregulated expression of the rib operon and was able to boost production levels by 70 times compared to its parent strain, BAL3C-5. The C120T mutation at the regulatory region of the rib operon was the only variation found in the whole genome sequencing of the parental and mutant strains (Diez-Ozaeta et al., 2023). This was the first time, as far as we know, that a single mutation was identified as the cause of the overproducing phenotype in a strain of LAB. Furthermore, we have confirmed a safety evaluation of the strain and validated its ability to enrich via fermentation with dextran (≈1 g/100 g) and RF (350–150 μg/100 g) gluten-free doughs (Russo et al., 2024). Therefore, we have assessed the behavior of this strain and that of its parental BAL3C-5 during fermentation of various commercial vegetable drinks, taking into account the low concentration of RF that they present, in order to develop new plant-based functional drinks and expand the potential utility of W. cibaria BAL3C-5 C120T. The detection of their dextran manufacturing capability and the associated rheological features of the resultant beverages have been analyzed in addition to their metabolic performance and RF overproducing ability. Lastly, the BAL3C-5 C120T strain's resilience to simulated gastrointestinal conditions and autoagglutination ability were examined to verify its appropriateness as a possible starter and probiotic culture.

2. Material and methods

2.1. Bacterial strains and growth conditions

The parental wild-type and mutant strains of W. cibaria, BAL3C-5 and BAL3C-5 C120T, respectively, were employed in this investigation. Its derivation, the spontaneous mutant RF-overproducing BAL3C-5 C120T strain, was earlier selected after BAL3C-5 strain treatment with roseoflavin (Diez-Ozaeta et al., 2023). The BAL3C-5 strain was previously isolated from rye sourdough (Llamas-Arriba et al., 2021). The liquid RF assay medium, which is a semidefined broth without RF (RAM, Difco, Le Pont de Claix, France), and the de Man, Rogosa, and Sharpe rich medium without dextrose (Condalab, Torrejo´n de Ardoz, Spain) supplemented with either 2% sucrose (MRSS) or 2% glucose (MRSG), were used to cultivate the bacteria. The culture was conducted at 30 ◦C.

2.2. Analysis of stability of the BAL3C-5 C120T RF-overproducing phenotype

For more than 100 generations, W. cibaria BAL3C-5 C120T was cultivated in RAM medium at 30 °C. To verify the stability of the RF-overproducing phenotype, cell survival was examined by plate counting every 14 generations, and levels of RF production expressed as fluorescence were calculated as described in Section 2.

2.3. Plant-based beverage fermentations

Commercial plant-based beverages were used to inoculate both strains. These matrices included rice-based drinks (Yosoy), almond-based drinks (Ecomil), soy-based drinks (Carrefour Soy beverage), and oat-based drinks (Alitey oat beverage). Table 1 displays the nutritional profiles of the various plant-based substitutes. The W. cibaria strains cultivated in MRSS were centrifuged at 9300 ×g for 10 minutes after overnight cultures, and the cells were then resuspended in new medium to produce an optical density of 0.1 at 600 nm (OD600 nm). The cultures were then centrifuged as previously described and resuspended in the appropriate plant-based beverage to yield an initial cell density of roughly 5 × 107 colony-forming units (CFU)/mL after being grown to an OD600 nm of 1 (≈5 × 108 CFU)/mL. 5% sucrose was added as a supplement to all drinks. Fermentations were conducted at 30 °C for 24 hours. Samples were taken for pH and cell viability analysis, as well as for the quantification of RF and dextran, at the time points of 0 and 24 hours. The Crison pH/mV-meter 501 was used to measure the pH, and successive dilutions of cultures in phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO, and 1.47 mM KH2PO) pH 7.0 were used to plate the cultures on MRS agar and quantify the CFU/mL.

The following oat-based beverage fermentations were carried out as previously described, but with the addition of various sugars. The following three conditions were used for the fermentations: no sugar addition, 2.5% maltose + 2.5% sucrose supplementation, and 5% sucrose supplementation. Following a 24-hour fermentation period, the beverages were kept for four weeks at 4 °C. Samples were collected every seven days during the storage period, as well as at time points 0 and 24 after fermentation. Every assay was run in triplicate. Samples were analyzed to determine the pH and cell viability, quantify RF and dextran, and characterize the rheological characteristics of fermented beverages.

2.4. Analysis of fermentation products (RF, dextran, sugars and metabolites)

High-performance liquid chromatography (HPLC) and fluorescence detection using a microplate reader were used to quantify RF. Samples were somewhat pre-treated before to RF analysis. Coagulation was chemically generated for non-fermented control beverages by adding 1 M HCl until pH 4.0 was achieved. Following that, samples that were fermented and those that weren't were centrifuged at 12,000 ×g for Ten minutes. Following the previously described procedure, supernatants were extracted, and the RF contained in them was identified and measured by a fluorescence detection using a Varioskan Flask System (Thermo Fisher Scientific, Waltham, MA, USA) microplate reader, following HPLC fractionation (Mohedano et al., 2019). In short, a sterile 96-well polystyrene flat bottom plate (Corning, Maine, USA) was used to analyze 200 µL aliquots in triplicate. A wavelength of 440 nm for excitation and 530 nm for emission detection were used to measure fluorescence. A calibration curve was used to determine the RF concentration.

The Shimadzu-Prominence chromatograph (Shimadzu, Duisburg, Germany) with a Shimadzu fluorescence detector RF 10A XL was used for HPLC analysis. A TRC-160K1 precolumn (Teknokroma, Barcelona, Spain) and an EC 250/4.6 Nucleosil 120-5 C18 column (Macherey-Nagel, Düren, Germany) that are both thermostated at 40

C, were employed. The measurements were conducted in an isocratic conditions with a mobile phase made up of 0.083 M sodium acetate and methanol (60:40, v/v) at pH 5 at a flow rate of 0.8 mL/min. The elution time was 12 minutes, and the injection volume was 20 µL. Comparing the retention period of RF to that of a pure standard (98 percent, Sigma-Aldrich, Gillingham, England) allowed for its identification, and an external calibration was used for quantification.

As previously mentioned, gas chromatography–mass spectrometry (GC–MS) analysis of 100 µL of beverage supernatant samples was used to assess the concentration of the sugars that were initially present in the beverages as well as the metabolites produced during fermentation (Besrour-Aouam et al., 2021). As an internal standard, myo-inositol was employed, and the compound's concentration was quantified based on peak area, adjusted with response factors determined using the internal standard and Agilent's

GC-ChemStation Rev. E.02.00 (2008) software (Palo Alto, CA, USA).

The amount of soluble dextran in the beverage supernatants was measured using the isomaltose produced by the hydrolysis of polymers, with the following adjustments, as previously reported for bread samples (Hernandez-Alca´ntara et al., 2022). In short, 150 µL of dextranase solution containing 0.18 g of Chaetomium erraticum dextranase (Sigma-Aldrich, Darmstadt, Germany) was added to 750 µL of each sample supernatant in order to convert dextran into isomaltose. After 18 hours of incubation at 30 °C, the samples were centrifuged for 10 minutes at 12000 ×g, and the supernatants were filtered through a 0.22 µm filter. Finally, as previously mentioned, aliquots were kept at -20 ◦C until they could be further examined by GC–MS. For every tested condition, three replicates were examined.

2.5. Rheological characteristics of fermented drinks made from oats

A stress-controlled rotating rheometer, the Physica MCR 101 (Anton Paar, Graz, Austria), was used to characterize the rheological properties of fermented and non-fermented control oat beverages in both continuous and oscillatory modes. Weekly assessments were carried out, and rheological analyses were carried out at the first time point, 24 hours into the fermentation process, and during a month of chilled storage.

At 5 °C, continuous flow analysis was performed with a concentric Cone-plate system CP25 (25 mm diameter, 1◦ cone angle, and 48 µm gap) for fermented beverages and cylinder geometry, CC17/T200 (Rotor Diameter = 16.7 mm, Length = 25 mm, and Cup Diameter = 18 mm) for control beverages. Shear stress and apparent viscosity were determined as a function of shear rate in three steps: 1) range from 0.1 s—1 to 100 s—1, 2) maintaining 100 s—1 for 20 s, and 3) decreasing finally the shear rate from 100 s—1 to 0.1 s—1.

Flow curves, data of shear stress (τ)-shear rate (γ˙), were fitted by the power law-Ostwald de Waele model (Eq. (1)), and the Herschel–Bulkley model (Eq. (2)), respectively,

$$\tau = K p \dot{\gamma}^{np} \quad (1)$$

where Kp is the consistency coefficient (Pa·sn), np is the pseudoplasticity index (dimensionless), that reflect the closeness to Newtonian flow. When the magnitude of n < 1, the fluid is shear-thinning, and when n >1, the fluid is shear thickening in nature.

$$\tau = \tau y + KH—B\dot{\gamma}^{nH—B} \quad (2)$$

where KH-B is the consistency coefficient (Pa·sn), nH-B is the pseudo- plasticity index (dimensionless). τy is the yield stress (Pa).

Loop test analysis (Barnes, 1997) was used to examine the thixotropy property. The program was set up as previously mentioned, using three shear phases. This test made it possible to see how the structure broke down at rising shear rates and then recovered during the down-ramp. The recovery of the structure is slowed down and the initial viscosity is not anticipated to be attained in the case of thixotropic fluids. The energy involved in the breakdown of thixotropic structure is thus indicated by the area, A, (in Pa s—1), within the hysteresis loop, which represents dimension-ally the "energy" in relation to the volume of the sheared sample.

Using the same geometry as previously mentioned, the oscillatory shear mode was used to examine the viscoelastic characteristics. Five degrees Celsius was the constant temperature. In the linear viscoelastic region LVR, which was previously assessed by strain sweep testing at a fixed angular frequency (1 rad/s), the analysis was conducted under linear viscoelastic conditions. Since a linear range between 0.1% and 10% strain has been established, the LVR strain chosen was roughly 1%. In the range of 0.1–10 rad/s, the storage modulus (G′) and loss modulus (G″) were examined as functions of angular frequency.

2.6. Survival under the gastrointestinal circumstances that were simulated

The ability of W. cibaria strains to withstand gastrointestinal tract conditions was examined using simulated gastric and intestinal fluids. Late exponential growth cultures were centrifuged for 10 minutes at 8000 ×g, rinsed twice with PBS, and then resuspended at 1 × 108 CFU/mL in 35 mL of the appropriate test medium, which may be either an oat-based beverage or saline solution (0.9 % NaCl). Additionally, without additional bacterial inoculation, the LAB survival was assessed in the 24-hour fermented oat-based beverage. Evaluating the matrix's protective effect on bacterial viability, whether or not fermentation was present, was the goal of the testing in oat-based drinks. For the gastric simulation assay, a saline solution, the oat-based beverage, and the 24-hour fermented oat-based beverage, the three different matrices were assessed. After using 1 M HCl to bring each matrix's pH down to 2.5, 875 µL of a pepsin solution (at 100,000 U/mL) was added to the 35 mL treated samples, resulting in a final concentration of 2500 U/mL of the enzyme. To mimic peristalsis, suspensions were incubated for 90 minutes at 37 °C while being stirred. Samples were collected at the 0 and 90 minute time periods. The pH was then raised with 2.5 M NaOH to 4.0 in order to simulate the in-testine phase. The gastric solution was then supplemented with 15 mL of a solution that contained 3% bile salts (Sigma-Aldrich) and 1% pancreatin (Sigma-Aldrich) dissolved in 0.1 M NaHCO3 (pH 7.0). Samples were obtained at 0, 90, and 180 minutes after the suspensions had been incubated for 180 minutes as described above. Lastly, 100 µL of the samples were plated on MRS agar in the proper dilutions and incubated for 24 hours at 30 °C. CFU/mL was used to represent cell viability.

2.7. Evaluation of autoaggregation

After being cultivated in MRS broth for the entire night, the bacterial suspensions were collected by centrifugation at 8000 ×g for 8 minutes at room temperature. Following two PBS (pH 6.6) washes, the cells were adjusted using the same buffer to achieve an OD 600 nm of 0.5 (A0). Auto-aggregation values were assessed at 3, 6, and 24 hours (At) after each bacterial suspension was incubated at 30 ◦C.

Auto-aggregation values were calcu- lated using the following formula:

$A = (1–At/A0) \times 100\%$

2.8. Statistical analysis

One-way ANOVA was used to analyze every measurement. A p-value of less than 0.05 was deemed significant. Tukey's test ($\alpha = 0.05$) was used to calculate mean pairwise comparisons when ANOVA tests were significant. R software version 4.3.1 was used for all studies (R Core Team, 2023).

3. Results and discussion

3.1. Stability of the RF-overproducing phenotype

Food biofortification is the process of adding certain nutrients to food through exogenous addition or fermentation of food matrices in order to boost nutritional content and support human development and health. In lieu of chemical and transgenic methods, several strains of LAB are thought to be suitable candidates for creating RF-rich meals due to their high adaptability for commercial food fermentation and their capacity to synthesize RF (RF-producing LAB). However, fermentations using wild-type strains typically provide low RF productivity.

In this regard, we chose the W. cibaria BAL3C-5 C120T strain for testing in commercial drinks because it can constitutively synthesize the vitamin regardless of the presence of RF or FMN in the growth medium and can produce about 7 mg/L of RF in a medium devoid of this compound (Diez-Ozaeta et al., 2023). If the only genetic difference between this strain and its parental wild type strain is a punctual mutation at the regulatory region of the rib operon, then there was a chance that a simple spontaneous mutational event (a change of T by C) could return BAL3C-5 C120T to the RF wild type phenotype. Therefore, it was vital to ascertain whether the ability to produce high amounts of RF was stable before testing the BAL3C-5 C120T in drinks. In order to evaluate the amount of free RF in the culture supernatants and the development of the bacteria, the mutant strain was cultivated for more than 100 generations in RF-free RAM medium. The RF-overproducing phenotype was confirmed to have been stable for at least 100 generations by the data displayed in Fig. 1, which revealed no discernible variations in the amounts of RF produced by the bacteria during the continuous growth.

3.2. RF-overproducing phenotype confirmed in plant-based beverages

There is either no RF present in plant-based beverages or very little of it. Therefore, the performance of the RF-overproducing BAL3C-5 C120T in commercial plant-based drinks was compared to that of its parental RF-producing BAL3C-5 (wild-type) strain in order to generate new fermented foodstuff. Drinks before the addition of LAB were used as controls. Additionally, the LAB's capacity to generate dextran was examined. Since sucrose is the substrate used by LAB to make dextran, 5% sucrose was added to the drinks. The concentration of RF and dextran in the inoculated samples of commercial beverages made from oats, rice, soy, and almonds, as well as the LAB strains' capacity to reduce pH and cell viability, were assessed following a 24-hour fermentation period at 30 °C (Fig. 2). Fig. 2A shows the experimental design.

Regarding the RF levels (Fig. 2B), the rice-based drink showed a 2.8-fold rise in RF levels compared to the non-fermented control, whereas the wild-type strain showed no statistically significant RF increase following fermentation. In contrast to the vitamin concentrations found in the control non-fermented beverages and those fermented with BAL·C-5, BAL3C-5 C120T markedly raised the RF concentration in all studied beverages (Fig. 2B). Therefore, in comparison to their respective beverage baseline, we saw a rise of 24.7 times for oat-based beverages, 8.7 times for rice and soy-based beverages, and 8.1 times for almond-based beverages (Fig. 2B).

As previously mentioned, the oat-based beverage had the highest RF value, with BAL3C-5 C120T increasing the RF content from 0.11 mg/L to over 3 mg/L. Similar to this, several studies have effectively examined the use of various LAB strains for the biofortification of beverages. Accordingly, strains of L. plantarum have been utilized to raise the amount of RF in a soy-based beverage to 0.7–1.86 mg/L (Juarez del Valle et al., 2016) or in a beverage that resembles kefir to 0.5–1.5 mg/L (Ye´pez et al., 2019). The oat-based beverage was the best matrix for RF biofortification by the W. cibaria strain BAL3C-5 C120T among the plant-based drinks tested here, according to the results presented here and the prior knowledge. The levels of RF (2.73 mg/L) attained in the oat-based drink fermented with this strain were higher than those previously reported for other drinks fermented by LAB.

Additionally, both strains examined in this study produced 600–800 mg/L of dextran in an oat-based beverage, and 85–100 mg/L and 50–70 mg/L in drinks made with rice and soy, respectively (Fig. 2C). Neither the fermented almond-based beverage nor any of the non-fermented plant-based

control drinks contained dextran. Therefore, the drink made from oats was the most intriguing matrix for both LAB's dextran biofortification. In the past, LAB has been seen as a potential method for producing exopolysaccharide (EPS) in a variety of meals in situ. Some strains of Leuconostoc, Weissella, and Pediococcus, along with some lactobacilli, have been identified as promising vehicles for the fortification of fermented milks, plant- and fruit-based juices, or grain-based foodstuffs with homopolysaccharides (dextran, levan, and/or β-glucan) (Hamet et al., 2015; Han et al., 2014; Herna- dez-Alca´ntara et al., 2022; Juvonen et al., 2015; Pérez-Ramos et al., 2017; Song, 2013). The EPS generated by LAB has been linked to various biological activities, including antioxidant, prebiotic, anti-inflammatory, cholesterol-lowering, and health benefits, in addition to enhancing technological and organoleptic qualities (Bhat and Bajaj, 2018; Dini´c et al., 2018; Farinazzo et al., 2020; Pan et al., 2020; Zannini et al., 2016). Furthermore, the current work has proven that BAL3C-5 C120T can raise the concentration of dextran in plant-based beverages in addition to its potential to overproduce RF. The amounts of sugars and a few of their metabolites in the beverages were measured in addition to the assessment of dextran synthesis (Table 2).

Along with the added sucrose (about 200 mM), glucose (up to 259 mM and 347 mM in the oat- and rice-based beverages, respectively) and fructose (up to 4.9 mM in the rice-based drink) were found in all control drinks. Furthermore, the oat-based beverage had the largest carbohydrate content, along with the rice-based beverage. Maltose (94 mM and 83 mM, respectively) and maltotriose (9228 mM and 272 mM, respectively) were only found in these two beverage types. Both LAB displayed a comparable fermentation pattern, as was to be predicted.

Accompanying the formation of dextran was the detection of lactic acid generation (up to ≈415 mM in soy-based drinks) and a rise in fructose concentration, likely due to the hydrolytic activity of sucrose by W. cibaria Dsr. In the fermented oat-based drinks, the two LABs caused the highest increase in fructose (from 1.9 mM to ≈30 mM), the highest decrease in sucrose concentration (from 192 mM to ≈78 mM; Table 2), and the highest yield in dextran production (12 mM, Table 2 and Fig. 2C). Furthermore, maltotriose (273 mM) was reportedly eaten during fermentation, while glucose was not metabolized in the oat-based beverage. This information may help to explain the superior behavior of the BAL3C-5 C120T strain in the oat drink. It is also important to note that panose generation was seen following fermentation in the oat drink. A α-1,6 glycosidic link connects a glucose and maltose unit to form the trisaccharide known as panes. By verifying its bifidogenic action in vitro, this trisaccharide has been characterized as a prebiotic candidate (Ma¨kel¨ainen et al., 2009).

After 24 hours of fermentation, the BAL3C-5 C120T performed well, matching the performance of its parent BAL3C-5 strain when the pH (Fig. 2D) and cell viability (Fig. 2E) were assessed. These findings confirmed that the strain's capacity for fermentation was unaffected by the RF-overproducing phenotype. Since the drink showed the greatest pH drop and the highest count increase after fermentation in comparison to the other matrices, the suitability of oat-based beverages was once again proven (Fig. 2D). Following fermentation by BAL3C-5 C120T, the pH of beverages made from oats, soy, almonds, and rice significantly decreased (down to 3.72, 5.05, 4.14, and 3.74, respectively), whereas the parental strain had nearly identical results. It is important to note that pH-lowering qualities are crucial for the finished product's safety and antibacterial qualities. From an initial population of 3–4 × 107 CFU/mL to 1–3 × 108 CFU/mL, all beverages displayed an increase in viable cells of about 10 times, albeit the oat-based drink showed a somewhat larger increase (Fig. 2E). Overall, it was determined that strain BAL3C-5 C120T could produce substantial amounts of RF and dextran in the plant-based beverages that were tested, with the oat beverage demonstrating the most notable performance. The population consumes a lot of plant-based goods these days, and they have a lot of potential to replace dairy products in the years to come. Additionally, they have a very low RF concentration, thus the food business and the general public are particularly interested in the creation of these kinds of beverages that are biofortified with this vitamin in situ.

3.3. Characterizing the rheological characteristics of oat-based beverages and biofortifying them in situ

In order to create a new biofortified beverage with novel rheological characteristics, it was determined to further explore the fermentation of the oat-based beverage since it produced the most promising matrix. Maltose can be used by the LAB Dsr as a donor substrate in addition to sucrose, but not as an acceptor for further polymer elongation. Accordingly, as previously mentioned (Fernandes and Rodrigues, 2007), a larger creation of the panose found in this experiment (Table 2) can also be induced by an increase in the drink's maltose concentration. Furthermore, a larger yield of dextran may be obtained by increasing the concentration of sucrose before fermentation. Thus, using the procedure shown in Fig. 3, the impact of adding sucrose or sucrose plus maltose was assessed by contrasting the behavior of the two LAB strains in oat-based beverages: (i) without added sugars, (ii) supplemented with 2.5% maltose plus 2.5% sucrose, and (iii) supplemented with 5% sucrose. The beverages were kept for 28 days at 4 °C after the fermentations were completed for 24 hours at 30 °C. The beverages were assessed for viable levels, RF, and dextran production. Following fermentation and at the conclusion of the refrigerated storage period, the levels of different chemicals, pH, cell viability, and rheological parameters were examined (Fig. 4).

3.3.1. A notable rise in the RF content of beverages made with oats

The BAL3C-5 C120T's RF-overproducing capacity was once again validated by the RF analysis (Fig. 4A) in comparison to BAL3C-5. The levels of RF during a 24-hour fermentation with BAL3C-5 C120T were comparable in the three circumstances examined: 3.40 mg/L, 3.23 mg/L, and 3.16 mg/L were found for the sugar-free drinks that were supplemented with either 2.5% of each sugar or 5% sucrose, respectively (Fig. 4A). Furthermore, a 30-fold increase from the baseline (from 0.11 mg/L to 3.40 mg/L) is possible in the ideal scenario (without the addition of sugar). Therefore, these findings demonstrated that sugar supplementation was not required to enhance RF behavior and production and that it did not significantly impair vitamin production.

The resulting beverage demonstrated a potential shelf-life stability of 28 days after 24 hours in all three fermentation conditions, and RF levels remained constant after 4 weeks of refrigeration. This is a crucial factor in terms of the beverage's functional potential. When it came to the parental strain, the values were consistent with the baseline. The spontaneous L. reuteri mutant's great ability for RF overproduction was recently described (Spacova et al., 2022). RF overproduction in food matrices is typically expressed as the total fluorescence intensity of the beverages without identifying the precise concentration of RF accessible, despite the fact that it was reported to be able to create around 18 mg/L in culture medium. More than those previously reported in food bio-fortification utilizing LAB (Juarez del Valle et al., 2016; Russo et al., 2014; Ye´pez et al., 2019), a 30-fold increase over the baseline was noted in the current study. Therefore, the fluorescence of the samples was evaluated both before and after HPLC fractionation in order to verify the appropriateness of direct measurement of RF fluorescence in an oat-based beverage. The two approaches yielded nearly identical findings for every drink that was evaluated (Supplementary Fig. S1). As a result, the straightforward technique of direct fluorescence measurement in a Varioskan microplate type reader may be used to accurately determine the RF content in oat-based beverages.

About 0.7 mg would be consumed daily from one 200 mL glass of this beverage. This indicates that a full and healthful diet would provide a significant portion of the recommended daily quantities of RF (0.4–2 mg/day, depending on the demographic group and condition). It is important to note that high calorific dietary patterns in the USA, Canada, and Europe have been linked to a nutritional deficit in vital micronutrients including vitamins and minerals in recent years (Cashman, 2018; Mantadakis, 2020; Ofori et al., 2022). Furthermore, hidden hunger, sometimes referred to as micronutrient deficiencies, is a prevalent phenomenon in developing nations as a result of excessive intake of basic foods brought on by limited access to sufficient food due to low economic and political standing (Go¨decke et al., 2018).

In both developed and developing nations, the creation of novel functional foods and appropriate dietary patterns may be taken into consideration to enhance overall health. Since plant-based products make up a large portion of the diet in many people, they provide a possible solution to this issue. As dairy substitutes, plant-based drinks are becoming more and more well-liked (McClements et al., 2019; Montemurro et al., 2021). But it's important to make sure the new substitutes have a sufficient nutritional profile and aren't nutritionally worse than dairy products. Only 16% of plant-based beverages out of 641 products had RF, according to a recent study, and none of them met the 15% daily value that should be designated as the "Source of," or 0.09 mg/100 mL (Drewnowski et al., 2021). The RF rise in this study reached 3.4 mg/L, nearly tripling the value of 1.2 mg/L typically seen in milk, which is one of the primary dietary sources of RF (Drewnowski et al., 2021).

Therefore, it is accurate to say that the resulting oat-based beverages are a better source of RF than the dairy and non-dairy products that are already on the market.

Regarding the ability to decrease pH, the BAL3C-5 C120T strain's significant fermentative capacity was demonstrated by the fact that the pH was consistently lowered to about 3.8 (Fig. 4B). Throughout the storage period, this feature stayed constant. This criterion is crucial for ensuring the drink's safety and microbiological stability as well as for extending its shelf life. Regarding cell viability, the population grew by more than ten times, reaching 108–109 CFU/mL, following 24 hours of fermentation. The population was 100% of the original inoculum after 7 days in refrigeration, and after 4 weeks in storage, about 80% of the population was preserved in every instance (Fig. 4B). Another aspect to emphasize is population stability, which guarantees high counts over the storage period and may improve the strains' potential probiotic nature (Montemurro et al., 2021).

Due to their low levels of cholesterol and saturated fats, plant-based beverages are regarded as a healthy alternative to dairy. The primary factors now propelling their rising consumption include a greater understanding of their health benefits, a rise in the number of individuals who are allergic to or intolerant to dairy products, or a growing preference for veganism. In this regard, the rising demand for plant-based drinks presents an intriguing approach to creating novel functional foods. The BAL3C-5 C120T strain's capacity for RF biofortification of oat-based (and other) beverages was validated in the current investigation. This aspect is particularly important because RF is typically added chemically to this kind of beverage, which does not naturally contain it or only in very little amounts. Furthermore, because of its anti-aging, anti-cancer, anti-inflammatory, and antioxidant qualities, RF is important. The framework of RF and the prevention of many health conditions have, in fact, been the subject of numerous research studies recently (Farah et al., 2022; Revuelta et al., 2017; Suwannasom et al., 2020; Thakur et al., 2017).

3.3.2. Dextran and prebiotic oligosaccharide production in oat-based drinks

In order to ascertain the production of dextran by the two LAB following the 24-hour fermentation period and at the conclusion of the fermented drinks storage at 4 ◦ C (28

days), we analyzed three fermentation conditions (Fig. 5A). Without adding LAB, no production was seen in the control drinks (Fig. 5A).

Both LAB produced large levels of dextran during the fermentation phase for drinks laced with 5% sucrose. The values obtained with BAL3C-5 (4.5 g/L) and BAL3C-5 C120T (3.3 g/L) were also ten and thirteen times higher, respectively, than those found in the similar fermented beverages that did not include any added sugar. Additionally, although somewhat lower amounts of dextran were noted, the outcomes of the supplementation with 2.5% sucrose plus 2.5% maltose were comparable to those of the 5% sucrose supplement. It must be mentioned that upon fermentation, the BAL3C-5 strain's CFU/L was consistently greater than that of the BAL3C-5 C120T strain (Fig. 4B). With levels of 977 mg vs 257 mg and 663 mg vs 519 mg under supplementation with 2.5% sugars and 5% sucrose, respectively, it was evident that BAL3C-5 C120T was a superior producer of dextran when the amount produced by 1011 CFU was determined. (Figure 5B). As a consequence, the results showed that BAL3C-5 C120T should be optimized as a dextran producer by enhancing fermentation conditions to promote better growth.

Furthermore, a statistically significant drop in dextran levels was not observed when fermented drinks were refrigerated for 28 days (Fig. 5A). Thus, without additional hydrolysis, it appears to remain stable at a high molecular weight. Since only high molecular weight dextran produced by LAB has this impact, this is significant for the postbiotic action of the dextran as an immunomodulator (Zarour et al., 2017). Furthermore, dextrans from LAB has antiviral action because it stimulates the innate immune system by inducing the production of interferons (IFN-1 and IFN-γ), according to in vitro and in vivo experiments conducted with fish models (Na´cher-Va´zquez et al., 2015). Innate immune responses are crucial for primary defense against viruses; for instance, SARS-CoV-2 uses these as a springboard for immune evasion (Silva et al., 2022). Additionally, older adults are more susceptible to SARS-CoV-2 infections because they are experiencing immunosenescence. As a result, dextran-enriched beverages may assist the elderly by strengthening their resistance to viral infections.

As anticipated, the fermentation conditions had an impact on both the generation of panose and dextran, according to an analysis of the metabolism of sugars (Table 3). The optimal conditions for increasing the two LAB's production of panose were the addition of 2.5% maltose and 2.5% sucrose. The wild-type strain produced the maximum level (20 g/L, 42.2 mM), whereas BAL3C-5 C120T also produced significant quantities (6.6 g/L, 25.8 mM). Furthermore, it was shown that the amounts of the prebiotic trisaccharide produced in drinks fermented by either LAB in the aforementioned supplementation increased by almost 15 times when compared to the levels produced without the addition of sugar. Lastly, in the

A 6–9-fold rise in panose concentration over the baseline values was found in the presence of 5% sucrose. Thus, BAL3C-5 C120T and its parent strain can be utilized to produce new oat-based functional beverages enhanced with dextran and panose, according to the results above.

With a compound yearly growth rate of 6.7% from 2020 to 2027, the market for plant-based beverages is expected to increase from USD 14.46 billion in 2019 to USD 24.30 billion by 2027 (Fior Markets, 2020). In this regard, it appears that there is industry interest in using BAL3C-5 C120T to create a novel kind of fermented beverage based on oats that is biofortified with RF, prebiotic panose, and postbiotic dextran. Additionally, this novel functional beverage should succeed in this significant market because it benefits geriatric, vegetarian, vegan, and milk-intolerant populations.

3.3.3. Rheological characteristics of beverages made from fermented oats

As a result of acidification during fermentation, the oat-based beverages underwent a discernible change in texture and structure (see Supplementary Fig. S2). As with milk, the acidification of the medium caused the beverages to coagulate after they reached the isoelectric point of the primary oat proteins (oat globulins). Additionally, the microbes themselves and their byproducts (such dextran) can affect how proteins aggregate. They may potentially interact with other matrix elements to produce structural alterations. The rheological properties of these samples are depicted in Fig. 6 with respect to the flow behavior and the viscoelastic response, which will be discussed later.

The viscosity of the non-fermented control beverage was unaffected by the applied shear rate, demonstrating Newtonian flow behavior. When the control drink was acidified and chemically coagulated, the behavior changed from Newtonian to non-Newtonian, indicating a change in fluid structure. As shown in Fig. 6A and B, these alterations were considerably more noticeable in the beverages fermented with the parental and mutant strains.

Fitting the shear stress-shear rate flow curves with the Herschel-Bulkley (H-B) model (Eq. (2)) and the power law model (Eq. (1)) allowed for the quantification of the non-Newtonian behavior; the related values are listed in Table 4. The lower shear rate regime, where plastic behavior was found, was well-fitted by the H-B model to non-Newtonian behavior. The stress that needs to be exceeded for flow to occur, known as the yield stress, τ_y, was computed. The stress below which the material acts as a solid and absorbs the strain energy without flowing is known as the critical value.

As previously stated, the remaining samples were yield stress fluids, in contrast to the Newtonian control beverage with $\tau_y = 0$ and $n = 1$. All samples showed a general shear thinning behavior (index $n < 1$) after flow was commenced above the yielding regime, with the viscosity decreasing as the shear rate increased.

Fig. 6. Rheological behaviour of oat-based drinks fermented with W. cibaria strains. Non-fermented beverages (Δ and ⋄) and beverages fermented with BAL3C-5 C120T (○) and BAL3C-5 (□) strains under the different conditions are displayed: control beverage (symbols in red), the pH-controlled beverage (symbols in grey), and the fermented beverages: (w/o) sugars (symbols in green), 2.5 % sugars (symbols in yellow), and 5 % sucrose (symbols in blue). (A) and (B) The pseudoplastic behaviour or shear thinning flow is characterized by a viscosity which decreases as the shear rate increases. (C) and (D) The thixotropy is identified by the presence of a hysteresis loop in the down and up ramp flow curves. (E) and (F) The viscoelastic behaviour is studied by the dependence with frequency of the storage modulus, G′, (filled symbols) and the loss modulus, G″, (empty symbols). “Gel” behaviour (G′ > G″) characterizes all the samples.

A thorough examination of the structure's development as a function of shear and time was of interest in addition to the yield stress characterisation. In a lot of fluids, viscosity just depends on temperature and shear rate and is not affected by time. However, the viscosity does not reach a steady state during the testing period for certain extremely concentrated dispersions. Rather, the stability of the internal network structure—which is damaged by shear pressures and requires time to repair—determines its value. This results in thixotropic behavior, which is a time-dependent shear thinning behavior. Because the up ramp flow curve and the down ramp flow curve had distinct trajectories and created a hysteresis loop, it is easy to see the thixotropy property in Figs. 6C and D. The magnitude of the thixotropy is defined by the computed area within the hysteresis loop, A, (in Pa s—1), which indicates the energy used in the structure's breakdown (values of A are given in Table 4).

The degree of complexity and robustness of the fluid structure is indicated by the rheological parameters that characterize the flow curve, yield stress, τy, consistency index, K, and pseudoplasticity index, n, as well as the thixotropy property. Therefore, it is commonly believed that these values will rise as molecular structures get more complicated. Given the two strains of W. cibaria that produce dextran, other studies have reported on the potential network formed by the EPS and other beverage components that is disrupted when applying a high shear rate (Juvonen et al., 2015; Zannini et al., 2016). This network may account for the high values for the complex behavior of yielding, shear thinning, and thixotropic nature of these fluids. Because of the low amount of interactions between the ingredients, the control beverages displayed low values for these flow parameters. In contrast, more structured fermented beverages shown a significant increase, which was even larger for the beverage without additional sugars. Notably, fermented structures with added sugars exhibit a better ability to recover viscosity and have lower yield stress values than beverages without added sugars. On the other hand, hysteresis loops and thixotropic indices were larger in sugar-free beverages. This suggests that a longer recovery time would be required to rebuild the produced structures, which have a high viscosity at rest. In fact, viscosity increased significantly after fermentation, rising from 3 mPa·s (control beverage) to >30 mPa·s for the various fermentation settings when assessed at a calculated shear rate of 100 s—1 (Table 5). Although it was far less than the viscosity seen during fermentation, the chemically acidified control beverage also showed an increase in viscosity when compared to the non-fermented control. Additionally, it should be mentioned that the resulting beverages' behavior and viscosity did not change over the course of storage, indicating that the drink's structure was unaffected (data not shown).

Figs. 6E and F show the samples' linear viscoelastic moduli, storage modulus G′, and loss modulus G″. Because of the microstructure's reaction to flow, visco-elastic fluids in the linear zone are usually time or frequency dependent. The structures' inability to react rapidly at short times (high frequencies) causes an elastic response (G′ > G″). On the other hand, the material can continually adapt at significantly longer experimental times (low frequencies), enabling flow and the discovery of a viscous effect (G″ > G′). Consequently, the behavior is viscoelastic at various frequency scales. The fundamental distinction between the time-dependent flow behavior responding to the thixotropic event and the time-frequency dependence seen in linear viscoelastic experiments is that, while the structure responds in the linear region but stays constant, it degrades through deformation during continuous flow. In the frequency range of the experimental data, the viscoelastic behavior of the fermented beverages was found to be substantially elastic (G′ > G″), as shown in Figs. 6E and F. The physical cross-linking or aggregation of protein molecules into a three-dimensional solid-like network would cause this reaction, which is typical of "gel behavior." Variations in the network's size and/or component interactions may be the cause of the different elastic modulus values seen in the fermented beverages. The capacity of the network to stop the flow, even at low frequencies, leads to the conclusion that the fermented beverages exhibit significant elastic behavior (modulus independent of frequency). Conversely, a lower elastic nature is suggested by the observed behavior of chemically acidified beverages, which have a similar contribution from both moduli (G′ ≈ G). The non-fermented beverage, on the other hand, showed newtonian behavior, a common viscous reaction, for which elasticity cannot be quantified.

In conclusion, using BAL3C-5 and BAL3C-5 C120T strains to ferment oat-based beverages produced well-structured fluids that were distinguished by a strong elastic "gel behavior" (G′ > G). Complex interactions between the EPS, protein network, and other beverage ingredients may be the cause of the observed response, which is most likely the result of the construction of a solid-like three-dimensional network.

Additionally, these fermented structures showed severe

shear thinning flow with a yield stress and strong sensitivity to shear. Furthermore, the fermented beverages without additional sugars showed a time-dependent flow behavior, where the thixotropy index was obviously important. These complicated behaviors contrast with the viscous newtonian behavior of the chemically acidified beverage and the elastic (G′ ≈ G″) but weak shear thinning and non-thixotropic behavior of the non-fermented beverage.

Due to its capacity to bind water, dextran is known to improve the rheological characteristics of fermented dairy products, such as acidified milk gels, by decreasing syneresis and increasing viscosity and creaminess (Mende et al., 2013). Furthermore, it has been demonstrated that dextran improves the texture of dairy products by stabilizing milk proteins, presumably via a sort of depletion flocculation mechanism (Pachekrepapol et al., 2014). Thus, it is possible that the amounts of dextran (3.5–4.5 g/L) that the Weissella strains produce are mostly responsible for the rheological characteristics shown in the oat-based beverages that were fermented with them. The oat-based beverage produced by BAL3C-5 C120T fermentation appears to have high rheological qualities in addition to its useful qualities, even if other elements also play a role in the rheological alterations in the fermented drinks.

3.3.4. BAL3C-5 C120T survival in gastrointestinal simulations

As a preliminary test for the BAL3C-5 C120T strain's potential as a probiotic bacterium, it was necessary to examine its potential for in vivo applications targeting the gastrointestinal system after its ability for RF and dextran biofortification in plant-based beverages was confirmed. Three matrices were employed to simulate the gastrointestinal conditions: a saline solution and the oat beverage, both of which were inoculated with 5–8 × 108 CFU/mL. The oat beverage fermented for 24 hours, and upon fermentation, it contained a LAB population of 5 × 108 CFU/mL (Fig. 7). It was clear that the food matrix had a protective impact. The population in the saline solution dropped significantly to 2 × 104 CFU/mL after 90 minutes in simulated gastric conditions, but it stayed roughly in the same range in the inoculated and fermented oat beverages (2–4 × 108 CFU/mL) and 2-log lower (5–6 × 106 CFU/mL), respectively. The same pattern was reproduced 180 minutes later under intestinal simulation circumstances. The saline solution showed a final drop of up to 4–3 × 103 CFU/mL, whereas the oat-based drink showed a drop of up to 5–3 × 105 CFU/mL for the two circumstances (Fig. 7). After the experiment, a high population was still detected despite a notable decline in viability. The BAL3C-5 C120T strain's resistance to gastrointestinal disorders is significant in this regard since it may promote the in situ synthesis of RF in the digestive tract, increasing its bioavailability, promoting absorption, and enhancing the intestinal microbiota. Nonetheless, it is imperative to examine the intestinal cells' ability to adhere and their potential to generate RF in these circumstances. Actually, strains can only be suggested as probiotics if they exhibit verified gastrointestinal stability and biological safety (de Melo Pereira et al., 2018; George Kerry et al., 2018).

The capacity to self-aggregate, which is a means of colonizing various settings as well as a survival, communication, and recognition technique that enables them to withstand and persist in the gastrointestinal tract, is another crucial characteristic of potential probiotics. Additionally, this capacity may prevent prospective infections from colonizing by acting as a barrier (Del Re et al., 2000; Krausova et al., 2019; Saito et al., 2019). For both LAB strains, the autoaggregation capability rose over the course of the incubation period. After three hours, the autoaggregation values were 19%, and after six hours, they were 26%. Additionally, following a 24-hour incubation period, both strains showed a significant level of autoaggregation, with values of about 80% (Fig. 8). These findings are consistent with those of earlier studies that assessed the autoaggregation capabilities of probiotic strains of Lactobacillus and Bifidobacterium (Abouloifa et al., 2020; Cizeikiene and Jagela-viciute, 2021). In this regard, the ability to produce dextran is another property that might affect gut colonization and, consequently, the probiotic qualities of the strains that are now available. Several studies have confirmed that the dextran-producing capacity promotes gut colonization, stabilizes bacterial biofilm, improves microbial adhesion and autoaggregation capacities, and increases tolerance to environmental stresses (Deng et al., 2020; Tuo et al., 2013). However, the relationship between the strains' adhesion and dextran-producing capacities has not been assessed in this study.

4. Conclusions

Because of its RF-overproducing phenotype, W. cibaria BAL3C-5 C120T has been functionally characterized in this work, confirming its utility as a food biofortifier. By creating new functional foods with increased RF content, it appears that fermenting oat-based beverages is an excellent way to meet the daily recommended RF values. Furthermore, W. cibaria BAL3C-5 C120T's ability to produce dextran can give the oat-based beverage unique rheological characteristics. Additionally, BAL3C-5 C120T's biofortification of oat-based beverages with prebiotic panose and postbiotic dextran will improve the beverage's functionality. In addition to having a high capacity for autoaggregation, BAL3C-5 C120T demonstrated good survivability in the oat-based beverage both after storage and under in vitro simulated gastrointestinal circumstances. Therefore, if BAL3C-5 C120T's RF-overproducing and dextran-producing properties are exerted in the digestive system, they could be used as probiotic bacteria supplied in the functional oat drink. Nevertheless, despite encouraging outcomes, in vivo research is still required to validate its probiotic potential.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

References

Abouloifa, H., Rokni, Y., Bellaouchi, R., Ghabbour, N., Karboune, S., Brasca, M., Ben Salah, R., Chihib, N.E., Saalaoui, E., Asehraou, A., 2020. Characterization of probiotic properties of antifungal Lactobacillus strains isolated from traditional fermenting green olives. Probiotics Antimicrob. Proteins 12 (2), 683–696. https://doi.org/10.1007/s12602-019-09543-8.

Barnes, H.A., 1997. Thixotropy—a review. J. Nonnewton. Fluid Mech. 70 (1–2), 1–33. https://doi.org/10.1016/S0377-0257(97)00004-9.

Besrour-Aouam, N., Fhoula, I., Herna´ndez-Alca´ntara, A.M., Mohedano, M.L., Najjari, A., Prieto, A., Ruas-Madiedo, P., Lo´pez, P., Ouzari, H.-I., 2021. The role of dextran production in the metabolic context of Leuconostoc and Weissella Tunisian strains. Carbohydr. Polym. 253, 117254 https://doi.org/10.1016/j.carbpol.2020.117254.

Bhat, B., Bajaj, B.K., 2018. Hypocholesterolemic and bioactive potential of exopolysaccharide from a probiotic Enterococcus faecium K1 isolated from kalarei. Bioresour 254, 264–267. https://doi.org/10.1016/j.biortech.2018.01.078.

Cashman, K.D., 2018. Vitamin D deficiency: A Public Health Issue in High- and Low- income Countries or Just Hype?, pp. 206–214. https://doi.org/10.1159/000484391.

Cizeikiene, D., Jagelaviciute, J., 2021. Investigation of antibacterial activity and probiotic properties of strains belonging to Lactobacillus and Bifidobacterium genera for their potential application in functional food and feed products. Probiotics Antimicrob. Proteins 13 (5), 1387–1403. https://doi.org/10.1007/s12602-021-09777-5.

de Melo Pereira, G.V., de Oliveira Coelho, B., Magalha˜es Júnior, A.I., Thomaz-Soccol, V., Soccol, C.R., 2018. How to select a probiotic? A review and update of methods and criteria. Biotechnol. Adv. 36 (8), 2060–2076. https://doi.org/10.1016/j.biotechadv.2018.09.003.

Del Re, B., Sgorbati, B., Miglioli, M., Palenzona, D., 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of Bifidobacterium longum. Lett. Appl. Microbiol. 31 (6), 438–442. https://doi.org/10.1046/j.1365-2672.2000.00845.x.

Deng, Z., Luo, X.M., Liu, J., Wang, H., 2020. Quorum sensing, biofilm, and intestinal mucosal barrier: involvement the role of probiotic. Front. Cell. Infect. Microbiol. 10 https://doi.org/10.3389/fcimb.2020.538077.

Díaz-Montes, E., 2021. Dextran: sources, structures, and properties. Polysaccharides 2 (3), 554–565. https://doi.org/10.3390/polysaccharides2030033.

Diez-Ozaeta, I., Martín-Loarte, L., Mohedano, M.L., Tamame, M., Ruiz-Maso´, J.A´., delSolar, G., Duen˜as, M.T., Lo´pez, P., 2023. A methodology for the selection and characterization of riboflavin-overproducing Weissella cibaria strains after treatment with roseoflavin. Front. Microbiol. 14 https://doi.org/10.3389/ fmicb.2023.1154130.

Dini´c, M., Pecikoza, U., Djoki´c, J., Stepanovi´c-Petrovi´c, R., Milenkovi´c, M., Stevanovi´c, M., Filipovi´c, N., Begovi´c, J., Goli´c, N., Luki´c, J., 2018. Exopolysaccharide produced by probiotic strain Lactobacillus paraplantarum BGCG11 reduces inflammatory hyperalgesia in rats. Front. Pharmacol. 9 https://doi.org/ 10.3389/fphar.2018.00001.

Drewnowski, A., Henry, C.J., Dwyer, J.T., 2021. Proposed nutrient standards for plant- based beverages intended as milk alternatives. Front. Nutr. 8 https://doi.org/10.3389/fnut.2021.761442.

Eckel, V.P.L., Vogel, R.F., Jakob, F., 2019. In situ production and characterization of cloud forming dextrans in fruit-juices. Int. J. Food Microbiol. 306, 108261 https://doi.org/10.1016/j.ijfoodmicro.2019.108261.

EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel), Turck, D., Bresson, J.L., Burlingame, B., Dean, T., Fairweather-Tait, S., Heinonen, M., et al., 2017. Dietary reference values for riboflavin. EFSA J. 15, e04919. https://doi.org/ 10.2903/j.efsa.2017.4919.

Farah, N., Chin, V.K., Chong, P.P., Lim, W.F., Lim, C.W., Basir, R., Chang, S.K., Lee, T.Y., 2022. Riboflavin as a promising antimicrobial agent? A multi-perspective review. Curr. Res. Microb. Sci. 3, 100111 https://doi.org/10.1016/j.crmicr.2022.100111.

Farinazzo, F.S., Valente, L.J., Almeida, M.B., Simionato, A.S., Carlos Fernandes, M.T., Ishii Mauro, C.S., Bosso Tomal, A.A., Garcia, S., 2020. Characterization and antioxidant activity of an exopolysaccharide produced by Leuconostoc pseudomesenteroides JF17 from juçara fruits (Euterpe edulis Martius). Process Biochem. 91, 141–148. https://doi.org/10.1016/j.procbio.2019.12.005.

Fernandes, F.A.N., Rodrigues, S., 2007. Evaluation of enzymatic reactors for large-scale panose production. Appl. Biochem. Biotechnol. 142 (1), 95–104. https://doi.org/10.1007/s12010-007-0046-z.

Fior Markets, 2020. Plant-based Beverage Market by Type (Milk, Other Drinks), Distribution Channel (Convenience Stores, Hypermarkets and Supermarkets, Specialty Stores, and Others), Source (Coconut, Rice, Fruits, Soy, Nuts, Almond), Region, Global Industry Analysis, Market Size, Share, Growth, Trends, and Forecast 2020 to 2027 (n.d.).

George Kerry, R., Patra, J.K., Gouda, S., Park, Y., Shin, H.-S., Das, G., 2018. Benefaction of probiotics for human health: a review. J. Food Drug Anal. 26 (3), 927–939. https://doi.org/10.1016/j.jfda.2018.01.002.

Go¨decke, T., Stein, A.J., Qaim, M., 2018. The global

burden of chronic and hidden hunger: trends and determinants. Glob. Food Sec. 17, 21–29. https://doi.org/10.1016/j.gfs.2018.03.004.

Hamet, M.F., Piermaria, J.A., Abraham, A.G., 2015. Selection of EPS-producing Lactobacillus strains isolated from kefir grains and rheological characterization of the fermented milks. LWT Food Sci. Technol. 63 (1), 129–135. https://doi.org/10.1016/ j.lwt.2015.03.097.

Han, J., Hang, F., Guo, B., Liu, Z., You, C., Wu, Z., 2014. Dextran synthesized by Leuconostoc mesenteroides BD1710 in tomato juice supplemented with sucrose. Carbohydr. Polym. 112, 556–562. https://doi.org/10.1016/j.carbpol.2014.06.035.

Hern´andez-Alc´antara, A.M., Chiva, R., Mohedano, M.L., Russo, P., Ruiz-Maso´, J.A´., del Solar, G., Spano, G., Tamame, M., Lo´pez, P., 2022. Weissella cibaria riboflavin- overproducing and dextran-producing strains useful for the development of functional bread. Front. Nutr. 9 https://doi.org/10.3389/fnut.2022.978831.

Joint F, 1967. WHO Expert Committee on Nutrition, World Health Organization. Joint FAO/WHO Expert Committee on Nutrition: Seventh Report, Rome, Italy, 12–20 December 1966. World Health Organization (n.d.). Juarez del Valle, M., Lain˜o, J.E., de Moreno de LeBlanc, A., Savoy de Giori, G., LeBlanc, J.G., 2016. Soyamilk fermented with riboflavin-producing Lactobacillus plantarum CRL 2130 reverts and prevents ariboflavinosis in murine models. Br. J. Nutr. 116 (7), 1229–1235. https://doi.org/10.1017/S0007114516003378.

Juvonen, R., Honkap¨aa¨, K., Maina, N.H., Shi, Q., Viljanen, K., Maaheimo, H., Virkki, L., Tenkanen, M., Lantto, R., 2015. The impact of fermentation with exopolysaccharide producing lactic acid bacteria on rheological, chemical and sensory properties of pureed carrots (Daucus carota L.). Int. J. Food Microbiol. 207, 109–118. https://doi.org/10.1016/j.ijfoodmicro.2015.04.031.

Kim, G., Bae, J.-H., Cheon, S., Lee, D.H., Kim, D.H., Lee, D., Park, S.-H., Shim, S., Seo, J.-H., Han, N.S., 2022. Prebiotic activities of dextran from Leuconostoc mesenteroides SPCL742 analyzed in the aspect of the human gut microbial ecosystem. Food Funct. 13 (3), 1256–1267. https://doi.org/10.1039/D1FO03287A.

Krausova, G., Hyrslova, I., Hynstova, I., 2019. In vitro evaluation of adhesion capacity, hydrophobicity, and auto-aggregation of newly isolated potential probiotic strains. Fermentation 5 (4), 100. https://doi.org/10.3390/fermentation5040100.

Levit, R., Savoy de Giori, G., Moreno de LeBlanc, A., LeBlanc, J.G., 2021. Recent update on lactic acid bacteria producing riboflavin and folates: application for food fortification and treatment of intestinal inflammation. J. Appl. Microbiol. 130 (5), 1412–1424. https://doi.org/10.1111/jam.14854.

Llamas-Arriba, M.G., Herna´ndez-Alca´ntara, A.M., Mohedano, M.L., Chiva, R., Celador-Lera, L., Vela´zquez, E., Prieto, A., Duen˜as, M.T., Tamame, M., Lo´pez, P., 2021. Lactic acid bacteria isolated from fermented doughs in Spain produce dextrans and riboflavin. Foods 10 (9), 2004. https://doi.org/10.3390/foods10092004.

Lynch, K.M., Coffey, A., Arendt, E.K., 2018. Exopolysaccharide producing lactic acid bacteria: their techno-functional role and potential application in gluten-free bread products. Food Res. Int. 110, 52–61. https://doi.org/10.1016/j. foodres.2017.03.012.

M¨akela¨inen, H., Hasselwander, O., Rautonen, N., Ouwehand, A.C., 2009. Panose, a new prebiotic candidate. Lett. Appl. Microbiol. 49 (6), 666–672. https://doi.org/10.1111/j.1472-765X.2009.02698.x.

Mantadakis, E., 2020. Iron deficiency anemia in children residing in high and low- income countries: risk factors, prevention, diagnosis and therapy. Mediterr. J. Hematol. Infect. Dis. 12 (1), e2020041 https://doi.org/10.4084/mjhid.2020.041.

McClements, D.J., Newman, E., McClements, I.F., 2019. Plant-based milks: a review of the science underpinning their design, fabrication, and performance. Compr. Rev. Food Sci. Food Saf. 18 (6), 2047–2067. https://doi.org/10.1111/1541-4337.12505.

Mende, S., Peter, M., Bartels, K., Dong, T., Rohm, H., Jaros, D., 2013. Concentration dependent effects of dextran on the physical properties of acid milk gels. Carbohydr. Polym. 98, 1389–1396. https://doi.org/10.1016/j.carbpol.2013.07.072.

Mohd Nadzir, M., Nurhayati, R.W., Idris, F.N., Nguyen, M.H., 2021. Biomedical applications of bacterial exopolysaccharides: a review. Polymers 13 (4), 530. https://doi.org/10.3390/polym13040530.

Mohedano, M.L., Hern´andez-Recio, S., Ye´pez, A., Requena, T., Martínez-Cuesta, M.C., Pel´aez, C., Russo, P., LeBlanc, J.G., Spano, G., Aznar, R., Lo´pez, P., 2019. Real-time detection of riboflavin production by Lactobacillus plantarum strains and tracking of their gastrointestinal survival and functionality in vitro and in vivo using mCherry labeling. Front. Microbiol. 10 https://doi.org/10.3389/fmicb.2019.01748.

Montemurro, M., Pontonio, E., Coda, R., Rizzello, C.G., 2021. Plant-based alternatives to yogurt: state-of-the-art and perspectives of new biotechnological challenges. Foods 10 (2), 316. https://doi.org/10.3390/foods10020316.

N´acher-V´azquez, M., Ballesteros, N., Canales, A´., Rodríguez Saint-Jean, S., Pe´rez-

Prieto, S.I., Prieto, A., Aznar, R., Lo´pez, P., 2015. Dextrans produced by lactic acid bacteria exhibit antiviral and immunomodulatory activity against salmonid viruses. Carbohydr. Polym. 124, 292–301. https://doi.org/10.1016/j.carbpol.2015.02.020.

Nazir, M., Arif, S., Khan, R.S., Nazir, W., Khalid, N., Maqsood, S., 2019. Opportunities and challenges for functional and medicinal beverages: current and future trends. Trends Food Sci. Technol. 88, 513–526. https://doi.org/10.1016/j.tifs.2019.04.011.

Ofori, K.F., Antoniello, S., English, M.M., Aryee, A.N.A., 2022. Improving nutrition through biofortification–a systematic review. Front. Nutr. 9 https://doi.org/

10.3389/fnut.2022.1043655.

Pachekrepapol, U., Horne, D.S., Lucey, J.A., 2014. Interactions between acidified dispersions of milk proteins and dextran or dextran sulfate. J. Dairy Sci. 97, 5371–5382. https://doi.org/10.3168/jds.2014-8144.

Pan, L., Han, Y., Zhou, Z., 2020. In vitro prebiotic activities of exopolysaccharide from Leuconostoc pseudomesenteroides XG5 and its effect on the gut microbiota of mice. J. Funct. Foods 67, 103853. https://doi.org/10.1016/j.jff.2020.103853.

P´erez-Ramos, A., Mohedano, M.L., Lo´pez, P., Spano, G., Fiocco, D., Russo, P., Capozzi, V., 2017. In situ β-glucan fortification of cereal-based matrices by Pediococcus parvulus 2.6: technological aspects and prebiotic potential. Int. J. Mol. Sci. 18 (7), 1588. https://doi.org/10.3390/ijms18071588.

Plantone, D., Pardini, M., Rinaldi, G., 2021. Riboflavin in neurological diseases: a narrative review. Clin. Drug Investig. 41 (6), 513–527. https://doi.org/10.1007/s40261-021-01038-1.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Revuelta, J.L., Ledesma-Amaro, R., Lozano-Martinez, P., Díaz-Fern´andez, D., Buey, R.M., Jim´enez, A., 2017. Bioproduction of riboflavin: a bright yellow history. J. Ind. Microbiol. Biotechnol. 44 (4–5), 659–665. https://doi.org/10.1007/s10295-016-1842-7.

Russo, P., Capozzi, V., Arena, M.P., Spadaccino, G., Duen˜as, M.T., Lo´pez, P., Fiocco, D., Spano, G., 2014. Riboflavin-overproducing strains of Lactobacillus fermentum for riboflavin-enriched bread. Appl. Microbiol. Biotechnol. 98 (8), 3691–3700. https://doi.org/10.1007/s00253-013-5484-7.

Russo, P., Diez-Ozaeta, I., Mangieri, N., Tamame, M., Spano, G., Duen˜as, M.T., Lo´pez, P., Mohedano, M.L., 2024. Biotechnological potential and safety evaluation of dextran- and riboflavin-producing Weisella cibaria strains for gluten-free baking. Foods 13 (1), 69. https://doi.org/10.3390/foods13010069.

Said, H.M., Ross, A.C., 2012. Riboflavin. In: Modern Nutrition in Health and Disease: Eleventh Edition. Saito, K., Tomita, S., Nakamura, T., 2019. Aggregation of Lactobacillus brevis associated with decrease in pH by glucose fermentation. Biosci. Biotechnol. Biochem. 83 (8), 1523–1529. https://doi.org/10.1080/09168451.2019.1584522.

Silva, M.J.A., Rodrigues, Y.C., Lima, K.V.B., Lima, L.N.G.C., 2022. Innate immunity to SARS-CoV-2 infection: a review. Epidemiol. Infect. 150 (e142), 1–16.

Song, Y.-R., 2013. Exopolysaccharide produced by Pediococcus acidilactici M76 isolated from the korean traditional rice wine, Makgeolli. J. Microbiol. Biotechnol. 23 (5), 681–688. https://doi.org/10.4014/jmb.1301.01032.

Spacova, I., Ahannach, S., Breynaert, A., Erreygers, I., Wittouck, S., Bron, P.A., Van Beeck, W., Eilers, T., Alloul, A., Blansaer, N., Vlaeminck, S.E., Hermans, N., Lebeer, S., 2022. Spontaneous riboflavin-overproducing Limosilactobacillus reuteri for biofortification of fermented foods. Front. Nutr. 9 https://doi.org/10.3389/fnut.2022.916607.

Suwannasom, N., Kao, I., Pruß, A., Georgieva, R., B¨aumler, H., 2020. Riboflavin: the health benefits of a forgotten natural vitamin. Int. J. Mol. Sci. 21 (3), 950. https://doi.org/10.3390/ijms21030950.

Thakur, K., Tomar, S.K., Singh, A.K., Mandal, S., Arora, S., 2017. Riboflavin and health: a review of recent human research. Crit. Rev. Food Sci. Nutr. 57 (17), 3650–3660. https://doi.org/10.1080/10408398.2016.1145104.

Tuo, Y., Yu, H., Ai, L., Wu, Z., Guo, B., Chen, W., 2013. Aggregation and adhesion properties of 22 Lactobacillus strains. J. Dairy Sci. 96 (7), 4252–4257. https://doi.org/10.3168/jds.2013-6547.

Udhayabanu, T., Karthi, S., Mahesh, A., Varalakshmi, P., Manole, A., Houlden, H., Ashokkumar, B., 2018. Adaptive regulation of riboflavin transport in heart: effect of dietary riboflavin deficiency in cardiovascular pathogenesis. Mol. Cell. Biochem. 440 (1–2), 147–156. https://doi.org/10.1007/s11010-017-3163-1.

Wakeel, A., Farooq, M., Bashir, K., Ozturk, L., 2018. Micronutrient malnutrition and biofortification: recent advances and future perspectives. In: Plant Micronutrient Use Efficiency. Elsevier, pp. 225–243.https://doi.org/10.1016/B978-0-12-812104-7.00017-4.

Werning, M.L., Herna´ndez-Alca´ntara, A.M., Ruiz, M.J., Soto, L.P., Duen˜as, M.T., Lo´pez, P., Frizzo, L.S., 2022. Biological functions of exopolysaccharides from lactic acid bacteria and their potential benefits for humans and farmed animals. Foods 11 (9), 1284. https://doi.org/10.3390/foods11091284.

Y´epez, A., Russo, P., Spano, G., Khomenko, I., Biasioli, F., Capozzi, V., Aznar, R., 2019. In situ riboflavin fortification of different kefir-like cereal-based beverages using selected Andean LAB strains. Food Microbiol. 77, 61–68. https://doi.org/10.1016/j. fm.2018.08.008.

Zannini, E., Waters, D.M., Coffey, A., Arendt, E.K., 2016. Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. Appl. Microbiol. Biotechnol. 100 (3), 1121–1135. https://doi.org/10.1007/s00253-015-

7172-2.

Zarour, K., Llamas, M.G., Prieto, A., Rúas-Madiedo, P., Duen˜as, M.T., de Palencia, P.F., Aznar, R., Kihal, M., Lo´pez, P., 2017. Rheology and bioactivity of high molecular weight dextrans synthesised by lactic acid bacteria. Carbohydr. Polym. 174, 646–657. https://doi.org/10.1016/j.carbpol.2017.06.113.

Zhang, J.-R., Ge, Y.-Y., Liu, P.-H., Wu, D.-T., Liu, H.-Y., Li, H.-B., Corke, H., Gan, R.-Y., 2022. Biotechnological strategies of riboflavin biosynthesis in microbes. Engineering 12, 115–127. https://doi.org/10.1016/j.eng.2021.03.018.

Zhou, L., Zhou, L., Wei, C., Guo, R., 2022. A bioactive

dextran-based hydrogel promote the healing of infected wounds via antibacterial and immunomodulatory. Carbohydr. Polym. 291, 119558 https://doi.org/10.1016/j.carbpol.2022.119558.

Table 1
Nutritional profile of plant-based beverages used in this study (per 100 mL).

	Oat beverage	Soy beverage	Almond beverage	Rice beverage
Energy (kJ)	194	138	145	221
Energy (kCal)	46	33	35	52
Fat (g)	0.8	1.8	1.9	1
Saturated fatty acids (FA) (g)	0.2	0.3	0.3	0.1
Monounsaturated FA (g)	0.3	not listed	not listed	0.3
Polyunsaturated FA (g)	0.3	not listed	not listed	0.6
Carbohydrates (g)	8.1	1	3.2	10
Sugars (g)	5.4	0.7	0.3	4
Proteins (g)	1.3	3.2	1	0.3
Salt (g)	0.07	0.06	0.14	0.07

Table 2
Metabolic analysis of each plant based-beverage before and after fermentation with the corresponding *W. cibaria* strain.

	Sucrose (mM)	Fructose (mM)	Dextran (mM)	Glucose (mM)	Lactic acid (mM)	Maltose (mM)	Maltotriose (mM)	Panose (mM)
Oat								
Control	192.43 ± 10.41^{a}	1.95 ± 0.03^{b}	–	258.57 ± 12.80^{a}	–	82.68 ± 6.14^{a}	271.97 ± 56.16^{a}	–
BAL3C-5	72.80 ± 8.53^{b}	27.43 ± 4.66^{a}	10.93 ± 2.85^{a}	266.86 ± 19.24^{a}	132.33 ± 17.99^{a}	74.42 ± 9.86^{a}	–	0.33 ± 0.08^{a}
BAL3C-5 C120T	83.55 ± 5.53^{b}	34.72 ± 8.05^{a}	12.44 ± 1.87^{a}	263.08 ± 1.97^{a}	80.78 ± 12.85^{a}	42.39 ± 1.29^{b}	–	0.53 ± 0.25^{a}
Soy								
Control	205.85 ± 0.63^{a}	0.74 ± 0.01^{b}	–	3.30 ± 0.08^{a}	0.50 ± 0.02^{b}	–	–	–
BAL3C-5	206.00 ± 17.91^{a}	13.01 ± 0.46^{a}	1.36 ± 0.13^{a}	1.80 ± 0.35^{b}	425.18 ± 28.91^{a}	–	–	–
BAL3C-5 C120T	193.55 ± 0.54^{b}	12.97 ± 0.54^{a}	0.92 ± 0.06^{a}	1.62 ± 0.08^{b}	405.12 ± 23.06^{a}	–	–	–
Rice								
Control	228.61 ± 59.74^{a}	4.88 ± 0.02^{b}	–	347.41 ± 81.47^{a}	–	94.20 ± 16.02^{a}	9228.24 ± 439.37^{a}	–
BAL3C-5	174.88 ± 5.17^{a}	7.73 ± 0.48^{a}	1.36 ± 0.13^{a}	295.81 ± 9.92^{a}	78.38 ± 7.03^{a}	90.69 ± 1.12^{a}	4899.34 ± 639.07^{b}	–
BAL3C-5 C120T	182.90 ± 3.00^{a}	7.88 ± 1.19^{a}	1.12 ± 0.22^{a}	313.86 ± 4.59^{a}	63.57 ± 15.28^{a}	88.12 ± 0.8^{a}	4230.02 ± 387.62^{b}	–
Almond								
Control	224.98 ± 26.64^{a}	0.81 ± 0.03^{b}	–	1.88 ± 1.08^{a}	0.43 ± 0.61^{b}	–	–	–
BAL3C-5	242.38 ± 44.35^{a}	1.61 ± 0.47^{b}	–	0.42 ± 0.17^{b}	77.51 ± 15.08^{a}	–	–	–
BAL3C-5 C120T	236.23 ± 20.67^{a}	2.87 ± 0.13^{a}	–	0.36 ± 0.01^{b}	59.73 ± 7.71^{a}	–	–	–

Fermentations were performed during 24 h with either BAL3C-5 C120T or BAL3C-5. Then, samples were used to quantitatively identify sugars and metabolites by GC–MS analysis. Samples of the unfermented drinks were used as control. Data are presented as mean ± standard deviation. The different letters among the same matrix indicate statistical significant differences with one-way ANOVA analysis.