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Vila-Real, Catarina

2022-12

Vila-Real , C , Pimenta-Martins , A , Mbugua , S , Hagretou , S-L , Katina , K , Maina , N H , Pinto , E & Gomes , A M P 2022 , ' Novel synbiotic fermented finger millet-based yoghurt-like beverage : Nutritional, physicochemical, and sensory characterization ' , Journal of functional foods , vol. 99 . https://doi.org/10.1016/j.jff.2022.105324

http://hdl.handle.net/10138/352856
https://doi.org/10.1016/j.jff.2022.105324

Journal of Functional Foods 99 (2022) 105324

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Novel synbiotic fermented finger millet-based yoghurt-like beverage: Nutritional, physicochemical, and sensory characterization

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ARTICLE INFO

Keywords:
Fermentation
Whole grains
Lactic Acid Bacteria
Nutritional value
Synbiotic
Functional cereal-based product

ABSTRACT

This study presents the nutritional, physicochemical, and sensory characterization of a functional fermented African finger millet-based beverage, using a co-culture containing an exopolysaccharide-producer strain and a probiotic strain. First, the fermentation factors affecting the beverage were studied to ascertain required starter culture and cereal matrix which would support its production. Co-culturing *Weissella confusa* 2LABPT05 and *Lactiplantibacillus plantarum* 299v in finger millet showed the best fermentative performance. The fermented yoghurt-like beverage contained both strains above 10^8 CFU/mL and showed improved nutritional and physicochemical profiles, compared to the unfermented control: higher content in threonine, arginine, GABA and glutamine, increased protein digestibility, 25 % *vs* 64 %, a significant production of dextran, 0 % *vs* 16 %, and increased apparent viscosity, 12 mPa.s *vs* 35 mPa.s. The developed functional prototype is innovative, organoleptically acceptable, with high nutritional quality, and promising potential for targeting international markets and different population groups from children to the elderly.

1. Introduction

The development of functional probiotic foods and beverages has shown a marked increase over the last years, and the consumer and market demands for these products are increasing all over the world (Grand View Research, 2019). In particular, non-dairy functional probiotic beverages have been gaining an interesting market positioning. Besides some nutritional drawbacks of dairy products, such as cholesterol or lactose contents, trends such as plant-based diets linking human health to environmental sustainability, the high prevalence of lactose intolerance/malabsorption and gluten intolerance around the world have opened an opportunity for these products (Min et al., 2018). In consequence, fruit-, legume-, cereal-based products have been developed (Min et al., 2018).

Among cereals, traditional minor grain crops native to Africa such as sorghum and millets, not only reveal advantages in terms of production and stress adaptation, but also have an interesting nutritional profile, and, consequently, a potential positive impact on human health (Vila-Real et al., 2017). In Africa, the use of spontaneous fermentation for the development of cereal-based products is common (Aka et al., 2014). However, naturally fermented products contain both functional and non-functional microorganisms, liable to affect both positively and negatively product attributes, eventually posing a hazard to human health.

In response, lactic acid bacteria (LAB) have been used as starter cultures in controlled fermentations, successfully contributing to product quality and safety, increased shelf-life, improved texture, and sensory properties, adding value to the product (Waters et al., 2015). *Lactiplantibacillus* (former *Lactobacillus)* is one of the most common LAB genera used in fermented probiotic foods, given its ability to ferment many carbohydrates, tolerate low pH and high oxygen concentrations, possess enzyme activity against polyphenol compounds and, in case of *L. plantarum* 299v, the ability to adhere to human mucosa cells, and demonstrate antimicrobial and immunomodulatory activities (Mack

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https://doi.org/10.1016/j.jff.2022.105324
Received 20 July 2022; Received in revised form 16 September 2022; Accepted 5 November 2022
Available online 28 November 2022

et al., 2003). On the other hand, the *Weissella* genus has been gaining interest in the cereal industry given its ability to produce exopolysaccharides (EPS) (Fusco et al., 2015). EPS play a major technological role not only on the increasing of specific volume, lowering of crumb firmness and reducing of staling in baking technology, but also in the texture creation of the final product, being suitable natural alternatives to texture improving chemical food additives in many food applications (Lynch et al., 2018). Some studies have focused on EPS' applications in sourdough and baking products as revised by Torino et al. (2015), yet there is limited information on their application for the development of cereal-based beverages (Lorusso et al., 2018). Moreover, the development of synbiotic novel beverages using traditional crops is scarce (Fasreen et al., 2017; Sanni et al., 2013).

Based on these rationale, the authors hypothesised that the combination of an EPS-producing strain with a probiotic strain would perform better than either single strain alone on the fermentation of indigenous cereal matrices and, consequently, improve the yoghurt-like beverage's technological and functional properties. Furthermore, the authors truly believe that the selection of the most suitable cereal matrix and fermentation conditions, will drive the enhancement of nutrients production/accessibility, texture, and organoleptic profiles contributing to a product with additional health benefits, without impairing physical and sensory quality.

Hence, the main objectives of this study were to select the best bacterial strain consortium (combination of EPS-producing strain co-cultured with a probiotic strain) and the African native whole grain matrix that would fit the design of a fermented yoghurt-like beverage followed by its nutritional, physicochemical, and sensory characterization. To the best of our knowledge this is the first time that research looks at the potential of benefiting from the double advantage of nutrient richness and potential prebiotic activity of African whole grain finger millet as a fermentation medium for carefully selected probiotic and EPS-producing strains to generate a synbiotic yoghurt-like beverage with improved nutritional properties and enhanced rheological profile; such development creates novelty in the sector of functional foods, provides an added value health-promoting solution for local populations and the envisaged valorisation of underutilised cereals contributes to regional socio-economic sustainability.

2. Methodology

The research work presented is divided into two sequential phases according to its main goals:

Phase 1: Evaluation of the fermentation capacity of single LAB (two indigenous *Weissella* strains) or combined with probiotic strain cultures (probiotic strain *Lactiplantibacillus plantarum* 299v co-cultured with each *Weissella* strain) of three African whole grain flours in order to select the most favourable starter culture:cereal combination to enhance technological and nutritional traits of cereal matrix;

Phase 2: Development of a novel synbiotic fermented African cereal-yoghurt like beverage, aligned with the previously selected conditions and its nutritional, physicochemical, and sensory characterization.

2.1. Phase 1

2.1.1. Sample preparation

Cleaned whole grains red sorghum (*Sorghum bicolor* (L.) Moench) and finger millet (*Eleusine coracana* (L.) Gaertn) were obtained from a local producer in Nairobi, Kenya. Grains were individually milled, firstly using an industrial milling equipment (CD1 mill, Chopin Technologies, France), then, using a lab-scale Thermomix (Type TM5-2, Vorwerk Elektrowerke, Germany), and, finally, a mortar and pestle were used to reduce the remaining particles larger than 500 μm to the final granulometry < 500 μm. Previously cleaned and milled (<500 μm) pearl millet (*Pennisetum glaucum* (L.)) whole grain flour was obtained from a local producer in Ouagadougou, Burkina Faso. The three cereal flours were separately suspended in a sucrose water-based solution (10 % (w/v)) (cereal:solution, 1:9 (w/w)), suspensions which will be referred to hereafter as slurries.

2.1.2. Microorganisms, growth conditions and inoculation

Two strains of *Weissella confusa/cibaria* previously isolated from traditionally fermented cereal-based products from Kenya (strain *W. confusa/cibaria C2*, isolated from a traditionally fermented multi-cereal beverage, named *Uji*) and Burkina Faso (strain *W. confusa* 2LABPT05, from a traditionally fermented pearl millet pancake, named *Massa*) and the commercial *Lactiplantibacillus plantarum* 299v (LP299V®), obtained from Probi AB, Lund, Sweden, were used. The microorganisms, previously kept at −80 °C, were individually activated at 2 % (v/v) in Man-Rogosa-Sharpe (MRS) broth (Biokar Diagnostics, Beauvais, France), at 37 °C, overnight, and the inoculum was propagated (at least twice) at 1 % in MRS-Broth, every 24 h until the day of inoculation. For each experiment, single (only *Weissella* strains) and combined (*L. plantarum* 299v and each *Weissella* strain) cultures were used. In co-cultures both inocula were standardised to the same initial viable cell numbers (10^6 CFU/mL or 10^8 CFU/mL, which value was indigenous strain-dependent) and, immediately mixed in a 1:1 ratio and inoculated at 1 % (v/v) in each slurry (Table 1). The final number of inoculated slurries were 12, corresponding to all possible combinations of two *Weissella* strains × two types of culture (single or combined) × three cereals (sorghum, finger millet and pearl millet). Each slurry was prepared in duplicate.

2.1.3. Fermentation process and enumeration of microorganisms

Each slurry fermentation occurred in an orbital incubator (Wiggen Hauser, Germany) at 30 °C, 200 rpm, until reaching pH around 4–5. Samples were collected at different time points (before inoculation and at 0, 2, 4, 8 h after fermentation) for chemical analysis (pH, organic acids and sugars concentrations) and microbial growth (lactobacilli, *Weissella* and contaminant bacteria).

A differential medium was used for individual counts of each bacterium, which preparation was based on Lee and Lee (2008). Briefly, to the MRS agar prepared medium, bromophenol blue was added at 0.002 % (final concentration), followed by autoclaving, after which L-cysteine/HCl (final concentration of 0.05 %, Sigma Aldrich, St Louis, MO, USA) was filtered (0.22 μm Minisart® High Flow syringe filters, Sartorius AG, Germany) and added. Enumeration of the two types of bacteria was based on specific colony morphology. Other media were prepared,

Table 1
Description of the experimental conditions for each cereal slurry inoculated with single cultures or combined cultures with *Lactiplantibacillus plantarum* 299v and indigenous *Weissella* strains - *W. confusa/cibaria C2* (from Kenya) or *W. confusa* 2LABPT05 (from Burkina Faso).

Run	Cereal	Type of culture	Probiotic strain	Indigenous *Weissella* strain
1	Sorghum	Single	–	*W. confusa/cibaria C2*
		Combined	*L. plantarum* 299v	*W. confusa/cibaria C2*
2	Finger millet	Single	–	*W. confusa/cibaria C2*
		Combined	*L. plantarum* 299v	*W. confusa/cibaria C2*
3	Pearl millet	Single	–	*W. confusa/cibaria C2*
		Combined	*L. plantarum* 299v	*W. confusa/cibaria C2*
4	Sorghum	Single	–	*W. confusa* 2LABPT05
		Combined	*L. plantarum* 299v	*W. confusa* 2LABPT05
5	Finger millet	Single	–	*W. confusa* 2LABPT05
		Combined	*L. plantarum* 299v	*W. confusa* 2LABPT05
6	Pearl millet	Single	–	*W. confusa* 2LABPT05
		Combined	*L. plantarum* 299v	*W. confusa* 2LABPT05

namely Potato Dextrose Agar (PDA) (Biokar Diagnostics, Beauvais, France), for the enumeration of yeasts and moulds, Plate Count Agar (PCA) (Biokar Diagnostics, Beauvais, France), for the total bacterial count, and Violet Red Bile Glucose Agar (VRBGA), for enumeration of Enterobacteriaceae (BioMérieux France). MRS Agar and PCA were used in all sampling time points, whereas PDA and VRBGA were only used before inoculation of the strains to confirm the microbiological quality of the cereal slurries (absence of contaminants). Microorganisms were incubated at 37 °C for 72 h in the case of PCA and for 48 h for MRS and VRBGA, and at 30 °C for 48 h for PDA.

2.1.4. Fermentative capacity - acidification, sugars' consumption, and organic acids' production

The pH was measured, at every time point, at room temperature using a pH meter, equipped with a pH electrode (Crison micro pH 2002, Spain). Concentrations of sugars and organic acids were measured simultaneously with refractive index and ultra-violet detection, respectively, using high-performance liquid chromatography (HPLC). HPLC analysis was performed according to Sousa et al. (2015), with some modifications: approximately 2 g of each sample were diluted in 5 mL of sulphuric acid 13 mmol/dm^3 (95–97 %, Merck), homogenized with an Ultra-Turrax (T18 Basic; IKA Works, Inc., Wilmington, NC, USA) at 18.000 rpm for 3 min, then filtered with no. 1 filter paper (V. Reis, Lisbon, Portugal) and, immediately prior to injection, with 0.45 μm pore size filters (Chromafil® PET − 45/25, Macherey-Nagel, Germany). Each sample was injected once, data were collected and analysed by Clarity System Software (version 5.0.5.98) and quantified using the appropriate chromatographic standards' calibration curve.

2.2. Phase 2

After the selection of the best combination match between bacterial consortium and cereal matrix, a new fermentation run, under the same operational conditions, was carried out in triplicate, aiming the development of the fermented yoghurt-like beverage (YLB). In this fermentation process, strains were inoculated at the same percentage (1 % (v/v)) but not standardised to the same initial viable cells numbers (in a ratio of 1:100, *Weissella*:*Lactiplantibacillus*). Preliminary trials (not published) showed that such protocol step allowed the probiotic strain to achieve higher viable cell numbers than when inoculated on a similar viable cell number basis. Samples were collected at different time points of fermentation process (before inoculation and at 0, 4, 6, 8 h of fermentation).

2.2.1. Free amino acids content

The content of free amino acids was determined by using the pre-column derivatization with orthophthalaldehyde methodology, according to the procedure of Pripi-Nicolau et al. (2000). Briefly, the derivatization reaction was performed by the auto-sampler: to 100 μL of sample, three reagents were sequentially added, mixed, and injected (reagent A: composed of homoserine (Merck) and norvaline (Merck) internal standards, mercaptoethanol (Sigma-Aldrich), sodium tetraphenylborate (Sigma-Aldrich) and borate (Boric Acid, Merck) buffer; reagent B: composed of iodoacetic acid (Sigma-Aldrich) and borate buffer, pH 9.5;

and reagent C: composed of OPA (Merck), methanol, borate buffer and mercaptoethanol). Separation and quantification were performed by injecting 10 μL of the derivate, with a linear multistep solvent gradient, and were detected by a fluorometric detector ($\lambda_{excitation}$ = 356 nm, $\lambda_{emission}$ = 445 nm). Samples were analysed in duplicate, by 32 Karat Software (version 8.0, Beckman Coulter) and quantified using the amino acids pure standards' calibration curve.

2.2.2. Protein digestibility

The *in vitro* protein digestibility (IVPD) of the samples was determined by a combination of the methods of Arte et al. (2015) and Elmaki et al. (1999) with some modifications. Freeze-dried samples (500 mg) were incubated with 1.5 mg of pepsin in 15 mL of 0.1 mol/dm^3 HCl at 37 °C, at 150 rpm, for 3 h. After neutralization with 2 mol/dm^3 NaOH, 4 mg of pancreatin in 7.5 mL of phosphate buffer (pH 8.0) were added, followed by 1 mL of toluene to prevent microbial growth, and the solution was incubated for 24 h at 37 °C, at 150 rpm. After 24 h, the enzyme was inactivated by the addition of 10 mL of trichloroacetic acid (10 %, w/v), and the undigested protein was precipitated. Samples were centrifuged at 5000 *g* for 20 min, at room temperature. Nitrogen (N) in the supernatant was determined by the Kjeldahl method. A negative control (following the same protocol, but without sample) was included in the run together with the samples. Digestibility was calculated as follows:

$$IVPD(\%) = [(N_{supernatant} - N_{control})/N_{originalsample}]100 \quad (1)$$

2.2.3. Mineral content

Prior to the analysis of mineral qualitative and quantitative profiles, samples were digested in Speedwave MWS-3 (Berghof, Germany) microwave system, in a specific digestion programme based on the manufacturer's instructions (Berghof Products + Instruments GmbH). A certified reference material (rice flour standard – IRMM 804) was used as a positive control for digestion. Briefly, 400 mg of previously freeze-dried sample were mixed with 5 mL of nitric acid (65 %, Merck, Germany) and 2 mL of hydrogen peroxide (30 %, Merck, Germany) in a Teflon vessel and heated in the microwave system. The digestion was divided into five steps (temperature, °C/time, min): 130/5, 170/5, 190/15, 100/2, 100/2. The resulting solutions were diluted with deionised water and then, mineral concentration was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) model Optima™ 7000 DualView (PerkinElmer, USA) with a radial plasma configuration, according to Rodrigues et al. (2015). A calibration curve was prepared with multi analyte custom grade solution of HNO_3 5 %, which was used to express the results.

2.2.4. Proximate composition

The fermented YLB was nutritionally characterized. Protein (Kjeldahl method in which assessed nitrogen content was multiplied by 6.25 to estimate protein content), total fat (Soxhlet method), total sugars (Munson and Walker technique), fibre (AOAC 991.43, and AOAC 985.29), sodium (Flame Atomic absorption spectroscopy), and moisture (drying at atmospheric pressure at 102 °C) were analysed.

2.2.5. Apparent viscosity analysis

The apparent viscosity of the samples was determined using the rotating springless viscometry B-ONE PLUS (Lamy Rheology, Champagne au Mont d'Or, France), with the MS ASTM measuring system, and a R-2 mobile disc. Apparent viscosity was measured at 20 ± 2 °C and 8 ± 2 °C, at constant shear rate (60 s^{-1}), after 10 s, in triplicate. This combination of parameters intended to simulate the texture of the YLB perceived by the consumer at the moment of tasting. Moreover, viscosity [η (γ)] was also measured at different shear rates in order to study the type of fluid in question. Viscosity values were given by the viscometer, which calculation is based on the following equation:

$$\eta = \tau\gamma = K_{TAU} \times M \times K_D \times \omega \quad (2)$$

where η is the apparent viscosity (Pa.s), τ is the shear stress (Pa), γ is the shear rate (s^{-1}), K_{TAU} and K_D are constants related to the measuring system (K_{TAU} = 55.65 and K_D = 1), M is the torque (mN.m) and ω is the rotational speed (rpm).

2.2.6. Exopolysaccharides' characterization and quantification

Prior to structural analysis, EPS were extracted from the cell mass of *W. confusa* 2LABPT05, grown on MRS agar, according to Maina et al. (2008). EPS structural analysis was carried out using nuclear magnetic resonance (NMR) spectroscopy, according to Maina et al. (2008).

EPS (dextran) quantification was accomplished using an enzyme-assisted method, using the unfermented and fermented freeze-dried samples according to the methodology of Katina et al. (2009).

2.2.7. Sensory analysis

The fermented YLB was subjected to sensory analysis by a non-trained consumer tasting panel (n = 30), using a questionnaire with open and closed questions, developed by the authors together with an expert in sensory evaluation. Five different cereal YLB samples were tasted in two phases: the objective of the first phase was to evaluate the original YLB (with no added aroma) in terms of appearance, colour, consistency, and texture using a 9-point hedonic scale varying from dislike extremely to like extremely. The second phase (first step) intended to evaluate several parameters, namely, global acceptability, sweetness, acidity, quality of the aroma and flavour of four samples flavoured with different aromas: coffee, chocolate, hazelnut and blueberry. All the aromas were kindly provided by FRULACT S.A (Maia, Portugal). Understanding that the fermented cereal beverage may stand on its own but may also be used as an ingredient to be incorporated in a dairy or non-dairy food matrix, this second phase included a second step in which participants were also asked to taste the flavoured cereal beverage together with a non-sweetened natural yoghurt and to evaluate it in terms of global acceptability using the same 9-point hedonic scale. Final questions about the hypothetical purchase of the product, regardless of the aroma, were also asked.

2.2.8. Statistical analysis

For phase 1, one-way analyses of variance (ANOVA) followed by the Tuckey HSD post hoc test were used aiming to study the effect of cereal (differences between the cereal matrices) towards fermentative capacity. Moreover, the independent *T*-student test was used to study differences between bacteria and, also, between type of cultures. The independent variables used were the absolute difference (△) between measurements registered at 0 and after 8 h, for each analysed parameter: $\triangle t_8 - t_0$. For phase 2, in order to study variables before and after fermentation, pairwise comparisons were carried out, using paired-samples T-tests. Normality and homoscedasticity of data were checked by Shapiro-Wilk and Levene tests, respectively. In those cases in which normality and/or homoscedasticity of data were not verified, Kruskal-Wallis, Mann-Whitney and Wilcoxon non-parametric tests were used.

Sensory data were descriptively expressed by the median, minimum, and maximum. The Wilcoxon test was applied to study differences between the global appreciation of the YLBs with and without yoghurt. The chi-square (χ^2) test of independence or Fisher test was used to study associations between texture and global appreciation/certainty of purchase. The Kruskal-Wallis test was used to study differences in classifications between the four aromas. The significance level was set at 5 % (p-values ≤ 0.05) for all tests performed. Statistical analyses were conducted using IBM® SPSS® Statistics, version 24 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Phase 1

3.1.1. Acidification and microbial growth

The evolution of pH values of dry-milled sorghum, pearl and finger millets slurries, inoculated with the single or combined cultures, throughout 8-h fermentation is presented in Fig. 1. Reduction in pH values was shown to be more effective for pearl and finger millets (ca. 2 pH units) than for sorghum (ca. 0.5 pH units in case of *W. confusa* 2LABPT05, and a reduction of 1 pH unit observed for *W. confusa/cibaria C2*). Besides a higher acidification capacity, pearl and finger millets also enabled bacterial strains to grow effectively (at least two log cycles) achieving final viable cell numbers around 10^8-10^9 CFU/mL, for both indigenous strains (Fig. 2), revealing their prebiotic potential. In contrast, in sorghum's fermentations only *W. confusa/cibaria C2* was able to trigger acidification, yet no growth was observed (Fig. 1 and Fig. 2). Sorghum is a cereal matrix rich in tannins that might inhibit the growth and limit the metabolism of the selected strains. It has been reported that *L. plantarum* 299v possesses tannase (esterase) activity (Osawa et al., 2000), demonstrated by its capacity to breakdown these phenolic compounds. This characteristic gives this microorganism the ability to mildly ferment sorghum matrices, as observed in previous experiments with single probiotic cultures (not published). On the contrary, there is no evidence that *Weissella* strains have this capacity, and thus, they may be more sensitive to the presence of tannins. Nevertheless, according to the present results, no differences were observed even when co-cultured with the probiotic strain in the fermentation of sorghum.

Generally, the final pH at 8 h of fermentation was lower in single cultures than in co-cultures, more evidently in pearl millet fermentation by both strains and in finger millet by *W. confusa* 2LABPT05, showing that *Weissella* strains produced a more acidic environment when fermenting the cereal slurries alone, without any eventual competition from other bacteria. Despite the final pH reached during finger millet's fermentation with *C2* co-culture was slightly lower when compared to 2LABPT05 co-culture in the same cereal, the observed delta ($\triangle t_8 - t_{0,\,pH}$) was similar ($p > 0.05$). The establishment of a final pH around 4–5 was the target defined to ensure the inhibition of pathogenic and spoilage organisms, contributing to the safety and stability of the final fermented product.

The observed delta growth ($\triangle t_8 - t_{0,\,Log\ (CFU/mL)}$) for the indigenous *Weissella* strains was considered similar between single and combined

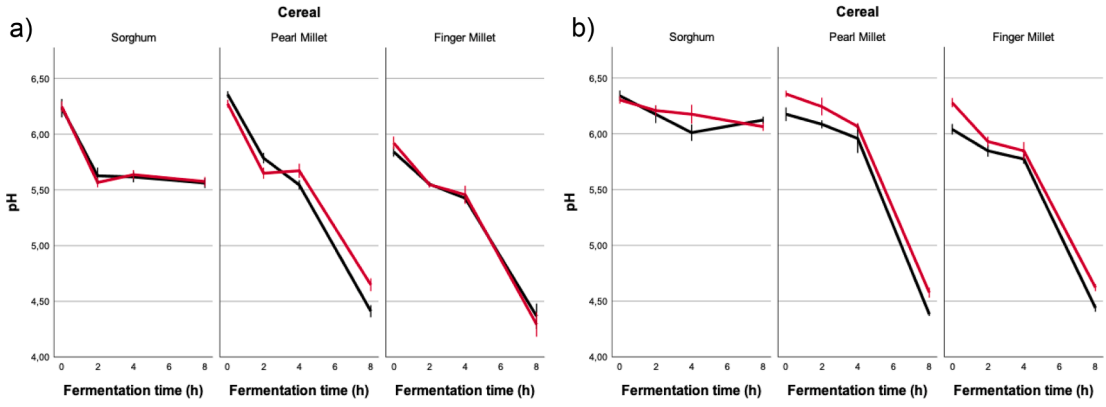

Fig. 1. Evolution of pH values of sorghum, pearl millet, and finger millet slurries, inoculated with *W. confusa/cibaria C2* (a) and *W. confusa* 2LABPT05 (b) strains in single cultures (black lines) or combined cultures (red lines) with *L. plantarum* 299v, over 8 h of fermentation at 30 °C and 200 rpm in an orbital incubator. Error bars represent the 95 % confidence intervals of independent replicate experiments.

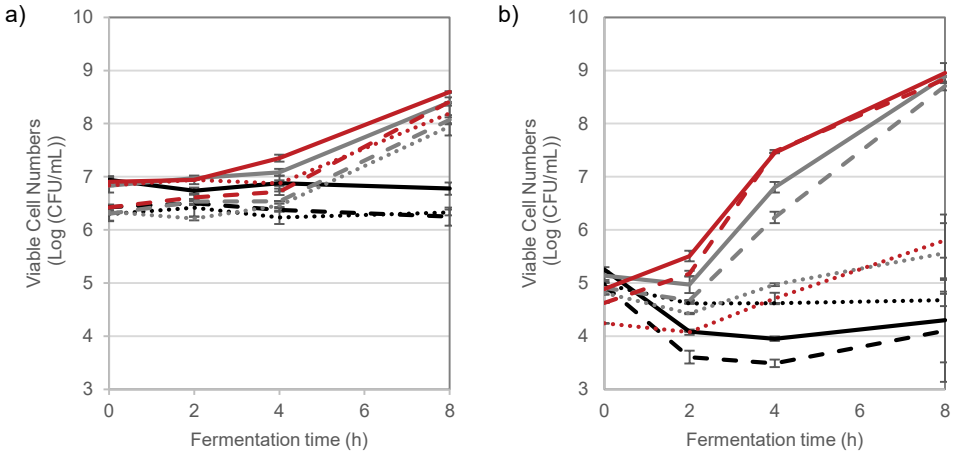

Fig. 2. Evolution of the viable cell numbers (Log (CFU/mL)) of *W. confusa/cibaria C2* (a) and *W. confusa* 2LABPT05 (b) inoculated in single cultures (full lines) or combined cultures (the indigenous *Weissella* strains are represented in dashed lines and the *L. plantarum* 299v strain in dotted lines) in dry-milled sorghum (black lines), pearl millet (grey lines) and finger millet (red lines), over 8 h of fermentation at 30 °C and 200 rpm in an orbital incubator. Error bars represent the standard deviation of independent replicate experiments.

cultures, for each cereal and bacterial combination, showing that the presence of the probiotic strain did not influence much their performance. When comparing both bacterial combinations, 2LABPT05 strain reported a significantly higher growth than *C2* in both millets ($p < 0.05$) regardless the type of culture (Table 2). On average, *L. plantarum* 299v reported a growth increase between one and two log cycles, during the fermentation process. The fact that both strains were able to grow in co-culture indicates the potential for their use as a mixed starter culture in co-fermentation. In terms of co-cultures, there were no differences, between both millets tested, in the growth of the strain 2LABPT05, while the strain *C2* grew better in finger millet ($p \leq 0.05$). *Lactiplantibacillus plantarum* 299v registered a similar growth in both millets, regardless of the bacterial combination. Nevertheless, when looking for differences between strains' combination, in finger millet, the ones that resulted in higher $\triangle t_8 - t_{0,\ Log\ (CFU/mL)}$, for both bacteria, was 2LABPT05 ($p \leq 0.05$).

3.1.2. Sugars' consumption and organic acids' production

Metabolic activity was followed by measuring sugar consumption and subsequent organic acid production profiles. Results are presented in Table 3 only for pearl and finger millets, which were the cereals where metabolic activity was significant; given its lack of impact on growth and acidification capacity, sorghum was not pursued any further. Lactic acid was the only organic acid produced upon 8-h fermentation in considerable amounts. It would be expectable that the production of acetic acid was also observed, given its natural production by both bacteria, however, it was not detected (limit of detection = 0.05 g/L). Wang et al. (2019), who used a pearl millet fermentable matrix with *Weissella confusa* as starter culture, reported lower quantities of lactic acid over 24-h fermentation, but the production of acetic acid was observed, even in low amounts. Despite the absence of statistically significant differences either between strain combinations for the same cereal or between cereals for the same strain combination, clear variances in metabolic performance were observed. When comparing single with combined cultures, it is possible to observe that the final lactic acid concentrations were higher in the former, which is in agreement with the higher acidification observed in these single cultures. Differences in pearl and finger millets were observed; fermentation of finger millet generated a more acidic environment, when compared with that of pearl millet, for both culture types.

As expected, the levels of sucrose decreased during fermentation, due to its utilization by the microorganisms. Specifically, *W. confusa* is known for producing acids, and also EPS, from sucrose (Fusco et al., 2015). Sucrose was both metabolized as a carbon source for microbial growth, and more evidently after 6 h of fermentation (not shown), it was used for EPS production. This decrease of sucrose, due to the activity of the glycansucrases (glucansucrases or fructansucrases) characteristic of *Weissella* strains, leads to the formation of glucans, releasing fructose into the medium (Amari et al., 2013); such trend is patent in the results achieved given the existence of fructose at the end of fermentation. Similar results were also observed by Wang et al. (2019).

Summing up the previous results, an interesting combination between the probiotic strain *L. plantarum* 299v and Burkinabé *Weissella* strain 2LABPT05 was found. In comparison with the combination including the *Weissella* strain from Kenya, *C2*, the growth of 2LABPT05 strain was significantly higher considering both types of cultures, and in what concerns to the *Lactiplantibacillus* strain, it was similar. Furthermore, the combination with *W. confusa* 2LABPT05 clearly produced a fermented product with higher viscosity (not shown). Concerning the cereal matrix, the millets revealed a higher prebiotic potential as previously discussed. Moreover, in terms of nutritional properties, finger millet has richer-fibre and lower-fat profiles, more phenolic compounds and micronutrients such as calcium, potassium, and thiamine than pearl millet (Vila-Real et al., 2017). Based on these rationale, the starter culture selected for the next phase was composed by *W. confusa* 2LABPT05 and *L. plantarum* 299v, and the selected fermenting cereal matrix was finger millet.

Table 2
Absolute differences of the number of viable cells (Log (CFU/mL)) between the onset and the end of fermentation (over 8 h, at 30 °C and 200 rpm in an orbital incubator) of sorghum, pearl millet, and finger millet slurries, inoculated with *W. confusa/cibaria C2* and *W. confusa* 2LABPT05 strains in single cultures or combined with the probiotic bacterium *L. plantarum* 299v.

Single cultures				Combined cultures		
Strain	**Sorghum**	**Pearl Millet**	**Finger Millet**	**Sorghum**	**Pearl Millet**	**Finger Millet**
C2	$0.2 \pm 0.1^{a,x}$	$1.57 \pm 0.02^{a,y}$	$1.69 \pm 0.04^{a,y}$	$0.18 \pm 0.01^{a,x}$	$1.6 \pm 0.1^{a,y}$	$2.0 \pm 0.1^{a,z}$
299v[1]		n.a.[2]		$0.022 \pm 0.006^{A,x}$	$1.6 \pm 0.1^{A,y}$	$1.4 \pm 0.2^{A,y}$
2LABPT05	$1.0 \pm 0.6^{b,x}$	$3.74 \pm 0.06^{b,y}$	$4.1 \pm 0.1^{b,y}$	$0.9 \pm 0.8^{a,x}$	$3.80 \pm 0.08^{b,y}$	$4.2 \pm 0.2^{b,y}$
299v[3]		n.a.		$0.33 \pm 0.08^{B,x}$	$1.3 \pm 0.1^{A,y}$	$1.6 \pm 0.3^{A,y}$

Values are expressed as mean ± standard deviation of independent replicate experiments. For a given type of culture and cereal, different letters (a and b) indicate significant differences between *Weissella* strains ($p \leq 0.05$), and different letters (A and B) indicate significant differences between *Lactobacillus* strains' combination ($p \leq 0.05$), using independent *T*-student test; For a given type of culture strain strain, different letters (x, y, and z) indicate significant differences between cereals ($p \leq 0.05$), using one-way ANOVA. [1] In combined culture with *W. confusa/cibaria C2*. [2]n.a. – Not applicable. [3] In combined culture with *W. confusa* 2LABPT05.

3.2. Phase 2

3.2.1. Fermentation process

Fermentation of finger millet with the selected co-culture shortened the fermentation period similarly to other reported studies in literature for finger millet (6–10 h) (Fasreen et al., 2017; Mugocha et al., 2000). Furthermore, it enabled *L. plantarum* 299v to increase one log cycle and reach final viable cell numbers around 10^8 CFU/mL (above the minimum required threshold of 10^7 CFU/g). On the other hand, *W. confusa* 2LABPT05 achieved a much more impactful increment of about four log cycles, proving that it is not negatively affected by the probiotic strain's presence; on the contrary, its growth and metabolic activities are enhanced and it can still perform its crucial technological role (Fig. 3).

Bacterial metabolic activity followed the growth behaviour trends and the production of lactic and acetic acids was detected in the fermented product (Table 3). It is well-known that these fermentation end-products have antimicrobial effects, although, such activity is not equal among all organic acids; specifically, acetic acid has a higher potential to inhibit yeasts, moulds, and bacteria than lactic acid providing good product preservative properties (Liptáková et al., 2017). The production of acetic acid reported in this final fermented product, compared to the previous experiment during the selection of the best strain combination, may be justified by the presence of the initial higher number (~10^7 CFU/mL *vs* ~ 10^4 CFU/mL, respectively) of *L. plantarum* 299v viable cells. In the work of Lorusso et al. (2018), in which quinoa flour was fermented by *L. plantarum* T6B10 (ca.10^7 CFU/mL) for 20 h, acetic acid concentration was slightly higher (1.5-fold) than the present results; such differences may be related with the different fermentation times (8 h *vs* 20 h) or with the cereal matrix itself. In fact, in the study of Coda et al. (2011), in which emmer flour was fermented for 4 h by *L. plantarum* A6, very low concentrations of acetic acid (around 0.7 mM) were found. The higher values of acetic acid obtained in the present study may be related to the presence of *W. confusa*, which is also known for the production of this metabolite. In fact, in the abovementioned study of Lorusso et al. (2018), when the fermentation was performed singly by *W. confusa* higher values of acetic acid were obtained, when compared with the single cultures of *L. plantarum* and *L. rhamnosus*. As observed previously, sucrose was utilized by the microorganisms resulting in the release of fructose (Table 3). In the work of Zannini et al. (2013) higher levels of fructose were also observed at the end of fermentation, and the authors mentioned that fructose formation by *W. cibaria* MG1 was positively correlated with the initial concentration of sucrose. Also, Katina et al. (2009) observed significant amounts of fructose in the final sourdough fermented by *W. confusa*, which microorganism has proven to not use fructose for growth, EPS or mannitol production during the fermentations.

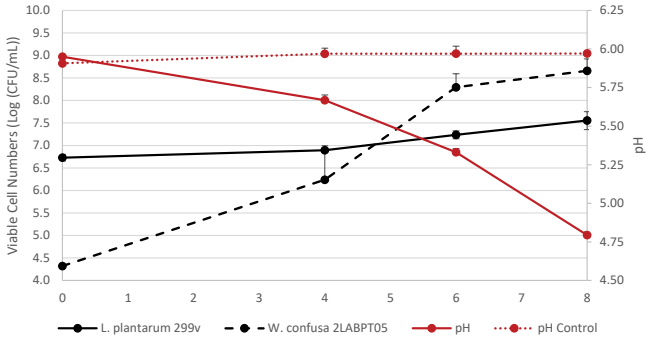

Fig. 3.. Evolution of acidification (red lines) and bacterial growth (black lines) of finger millet slurry, inoculated with *W. confusa* 2LABPT05 (dashed black line) co-cultured with *L. plantarum* 299v (full black line), over an 8-h fermentation period, at 30 °C and 200 rpm in an orbital incubator. A pH control is represented by the dotted red line. Error bars represent the 95 % confidence intervals of independent replicate experiments.

Table 3
Physicochemical characterization of the non-fermented slurry and the fermented finger millet yoghurt-like beverage (YLB), by *W. confusa* 2LABPT05 co-culture with *L. plantarum* 299v, over 8 h, at 30 °C and 200 rpm in an orbital incubator.

	Unfermented slurry		Fermented YLB	
Lactic Acid (g/100 g YLB)	< LOD[1]		0.268 ±	0.009
Acetic Acid (g/100 g YLB)	< LOD		0.070 ±	0.008
Sucrose (g/100 g YLB)	6.3 ±	0.8 [a]	3.8 ±	0.6 [a]
Glucose (g/100 g YLB)	1.0 ±	0.2 [a]	0.7 ±	0.08 [a]
Fructose (g/100 g YLB)	0.49 ±	0.08 [a]	2.1 ±	0.3[b]
Protein digestibility (%)	25 ±	2 [a]	64 ±	4[b]
Fe (mg/100 g YLB)	0.474 ±	0.009 [a]	0.46 ±	0.03 [a]
Mg (mg/100 g YLB)	13.4 ±	0.1 [a]	12.6 ±	0.1[b]
Mn (mg/100 g YLB)	1.83 ±	0.07 [a]	1.71 ±	0.01 [a]
K (mg/100 g YLB)	30.3 ±	0.1 [a]	31.2 ±	0.8 [a]
Na (mg/100 g YLB)	0.34 ±	0.01 [a]	3.2 ±	0.2[b]
P (mg/100 g YLB)	24.4 ±	0.3 [a]	23.8 ±	0.2[b]
Ca (mg/100 g YLB)	36.5 ±	0.9 [a]	31.9 ±	0.2[b]
Zn (mg/100 g YLB)	0.113 ±	0.004 [a]	0.095 ±	0.001[b]
Apparent viscosity (mPa.s), 20 °C	11.9 ±	0.4 [a]	35 ±	2[b]
Apparent viscosity (mPa.s), 8 °C	13.2 ±	0.4 [a]	102 ±	35[b]
Average Dextran (%), dry weight	0.30 ±	0.09 [a]	16.1 ±	0.9[b]

For a given parameter, different letters (a and b) indicate significant different ($p \leq 0.05$) between samples, using the Paired sample T-test or Wilcoxon test. [1]LOD: Limit of Detection (Lactic and acetic acids: 0.05 g/L).

3.2.2. Free amino acids content

The amino acids present at the highest concentrations in finger millet were aspartic acid, glutamic acid, and asparagine, and their contents decreased over the 8 h-fermentation process ($p \leq 0.05$); tyrosine content also decreased during fermentation (Fig. 4). The essential amino acids, methionine, phenylalanine, isoleucine, and leucine revealed a slightly different trend: their contents increased between two- to elevenfold upon 6-h fermentation but were then depleted over the next 2 h from fermented slurries. In the pioneering work of Garvie (1967) dextran-forming strain groups require few amino acids, among which the more relevant are glutamic acid, isoleucine, and leucine, a trend that may justify the observed decrease of those amino acids after the 6 h (EPS were produced during this period as discussed in subchapter 3.2.7.). Nonetheless, other amino acids contents were also reduced and, apparently, are not specially required by these EPS-producing strains. On the other hand, threonine, arginine, GABA, and glutamine increased one to threefold upon 8-h fermentation (Fig. 4). These amino acids have shown a positive impact on the maintenance of intestinal mucosal integrity and barrier function (threonine), modulation of GI motility (GABA), athletic improvement (arginine and glutamine) and other important body functions (arginine) (Auteri et al., 2015; Mahan & Escott-Stump, 2008; Mao et al., 2011; Popolo et al., 2014). The increased availability of free amino acids plays a beneficial role on the host nutritional status, particularly if a deficiency in endogenous protease production is present (Houngbédji et al., 2021), upholding the important contribution of this fermented yoghurt-like beverage to human health promotion.

3.2.3. Protein digestibility

The IVPD of the fermented product (64 %) was highly enhanced when compared to the unfermented flour counterpart (25 %) (Table 3). Fermentation of sorghum, pearl and finger millets and legume flours impacts positively protein digestibility, as reviewed by Nkhata et al. (2018). Explanations for such potentially improving effect include the fact that pH reduction triggers protein breakdown into smaller bio-peptides, with potential added biological value, a consequence of augmented proteolytic enzymes activity of fermenting bacteria (Sripriya et al., 1997). Furthermore, the reduction in levels of anti-nutrients, such as tannins, which are negatively involved in protein metabolism, allow digestive enzymes to gain easier access to proteins, resulting in increased protein bioavailability and digestibility (Joye, 2019). The

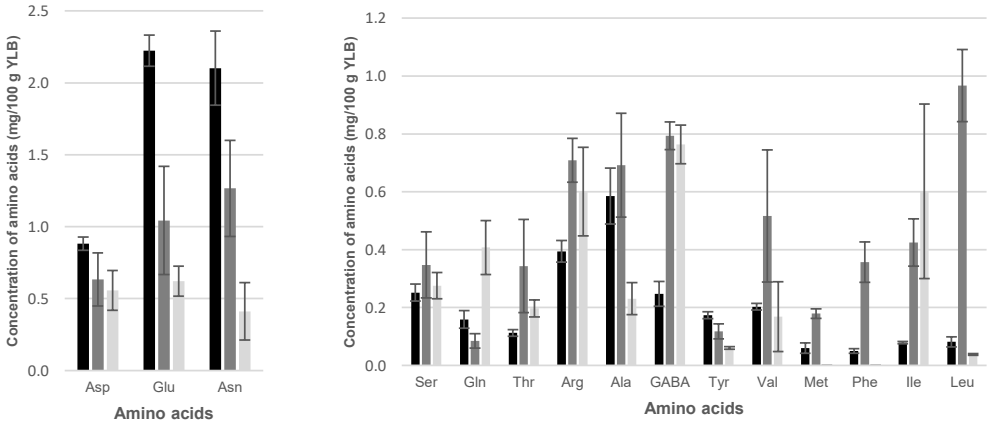

Fig. 4. Free amino acids content (mg/100 g YLB) of finger millet's slurry, fermented by *W. confusa* 2LABPT05 co-cultured with *L. plantarum* 299v, before fermentation (black columns) and 6 h (dark grey columns) and 8 h (light grey columns) after fermentation, at 30 °C and 200 rpm in an orbital incubator. Error bars represent the 95 % confidence intervals of independent replicate experiments. (Asp: Aspartic acid; Glu: Glutamic acid; Asn: Asparagine; Ser: Serine; Gln: Glutamine; Thr: Threonine; Arg:Arginine; Ala: Alanine; Tyr:Tyrosine; Val:Valine; Met: Methionine; Phe: Phenylalanine; Ile: Isoleucine; Leu: Leucine). For a given amino acid, different letters indicate significant differences ($p \leq 0.05$) between times, using the Paired sample T-test or Wilcoxon test.

higher IVPD complemented with the increase of some limiting amino acids, two parameters used to assess a product's nutritive value (Lorusso et al., 2018), makes the fermented yoghurt-like beverage more interesting in terms of nutritional profile and more beneficial to consumers relative to the starting flour.

3.2.4. Mineral content

In general, the 8-h fermentation period slightly decreased the mineral content, although without significant impact on concentrations, except for potassium and sodium (Table 3). Given the alleged reduction of anti-nutrients mediated by fermentation, the bioavailability of divalent or trivalent minerals would be expectably enhanced (Nkhata et al., 2018), however, such effect was not observed. Phytate reduction is driven by phytase activity, which might be cereal endogenous or produced by microorganisms. In the present study, the non-increase in the mineral content might be justified by the non-degradation of phytate. This could have happened mainly for two reasons, either because the intrinsic cereal phytases were inactivated (by autoclaving) (Reale et al., 2007), or because the bacterial strains employed do not produce intra- or extracellular phytases. Reale et al. (2007), who screened 50 lactic acid bacteria, namely *L. plantarum*, *L. amylovorus*, *L. acidophilus*, *L. sanfranciscensis*, *Leuconostoc mesenteroides* strains for intra- and extracellular phytate-degrading activity, concluded that all strains lacked extracellular phytase activity and intracellular phytase activity was not significant. Nevertheless, the work of Sripriya et al. (1997) showed that even in single cultures or sequential inoculation, probiotic fermentation with *Lactiplantibacillus* strains resulted in a reduction of phytic acid levels. Another issue that may interfere in the action of the phytase enzyme is the process temperature. It is well-studied that phytase activity is optimum at temperatures around 35–45 °C, a range which does not include the fermentation temperature used in the present study. Other works (Rasane et al., 2015; Sindhu & Khetarpaul, 2001), revealed that when the food matrices were heat-treated, low levels of phytic acid were obtained with LAB fermentation, at 37 °C. It must be highlighted, however, that these are all possibilities that cannot be confirmed since phytase activity was not quantified in the present work.

3.2.5. Proximate composition

The resultant fermented fresh YLB offers an improved nutritional profile, namely per 100 g: 57 kcal (238.65 kJ) of energy, including 15.2 g of carbohydrates (of which 6.2 g are sugars), 4.0 g of fibre, 0.7 g of protein and 0.1 g of total fat. The high-fibre (7 g/100 kcal), fat-free, and low-salt (<0.1 g/100 g) nutritional claims characteristic of the product enable an improved food market availability in African and European countries, characterized in many cases by products rich in fat, sugars, and salt, the major contributors to non-communicable chronic diseases. Moreover, the lactose- and gluten-free matrix makes this product an excellent option for lactose or gluten intolerants.

3.2.6. Apparent viscosity

The 8-h fermentation had a considerable impact on the apparent viscosity of the product, which increased from 12 to 35 mPa.s (192 %), measured at 20 °C (Table 3). This texture modification is a consequence of EPS production, mainly generated by the *W. confusa* 2LABPT05 strain. After refrigeration at 4–6 °C for approximately 12 h, viscosity underwent a tenfold increase, showing the impact of temperature on viscosity (Table 3); indeed during cooling, starch retrogradation (realignment of amylose and amylopectin hydrogen bonds and recovery of crystalline structure (Kubo et al., 2010)) occurs leading to increased viscosity. This YLB revealed to be a dilatant or shear-thickening fluid, as apparent viscosity increases with increasing shear rate (Fig. 5) (Rapp, 2017).

3.2.7. Exopolysaccharides

The analysis of the 1D ^{1}H spectrum of the EPS produced by *W. confusa* 2LABPT05 reveals two different distribution peaks' zones (Fig. 6). Downfield of 4.9–5.3 ppm, with a typical dextran with main chain α- (1 → 6)-linked-D-glucopyranosyl residues, with the anomeric proton signal at 4.98 ppm, and a low-intensity anomeric signal is observed at 5.32 ppm, which corresponds to α- (1 → 3)-linked-D-glucopyranosyl residues (Maina et al., 2008). The intensity (%) of both peaks, obtained from the integration of relative intensity of the signals, is 97 % of α- (1 → 6), which constitute the main chain, and 3 % of α- (1 → 3), which are the glycosidic branched linkages. These results are aligned with those obtained by Galli et al. (2020) and Wang et al. (2019), in which EPS produced by *W. confusa* Ck15 and *W. confusa* A16, respectively, were analysed. The other peaks' zone, upfield of 3.5–4.0 ppm, is characterized by bulk region protons, corresponding to ^{6b}H, ^{5}H, ^{6a}H, ^{3}H, ^{2}H, and ^{4}H of the α- (1 → 6) -linked glucosyl main chain, respectively.

The fermented beverage showed high levels of dextran (16.1 ± 0.9 %, dry-weight, or 3 g/100 g fresh fermented YLB), in comparison to the

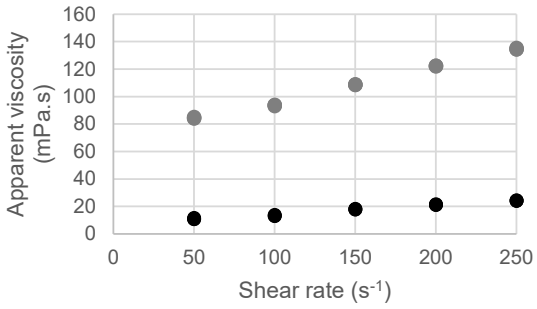

Fig. 5. Apparent viscosity as a function of shear rate of the unfermented slurry (black colour) and the fermented finger-millet yoghurt-like beverage (grey colour), at 8 °C, for 10 s.

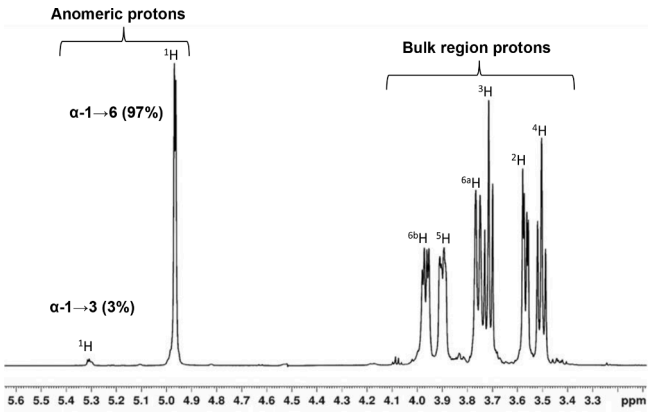

Fig. 6. The 1D ^{1}H nuclear magnetic resonance spectrum of EPS produced by *W. confusa* 2LABPT05 recorded at 600 MHz in D_2O at 50 °C. The peaks are referenced to internal acetone (^{1}H = 2.225 ppm).

non-fermented slurry (0.30 ± 0.09 %, dry-weight), with a considerable *in situ* production of this compound by the *Weissella* strain (Table 3). According to the growth kinetics of *W. confusa* 2LABPT05 and the evolution of sucrose concentration over fermentation (not shown), it seems that sucrose utilization for EPS production might have happened mostly at the late exponential phase. Galli et al. (2020) also observed that the production of EPS started during the exponential phase, and thereby, they concluded that LAB might use sucrose simultaneously for both growth and EPS production. Nevertheless, according to other authors (Han et al., 2014), the viscosity increase was higher during the stationary phase, regardless of having previously increased during the late exponential phase. Nevertheless, this is not possible to confirm by the present work, since fermentation ends after 8 h, *i. e.* before entering the stationary phase.

EPS yield is influenced by several factors including, pH, the food matrix and the presence of oxygen (Katina et al., 2009). In addition, Pintado et al. (2006) concluded that different media influence both the quantity and the composition of the produced EPS. It has been shown that pH around 4.7–4.8 (coinciding with the pH value of the final hour of the present fermentation) leads to a higher production of EPS, even when the involved enzymes have shown optimal activity at different pHs (Kaditzky & Vogel, 2008). Furthermore, the influence of the cereal matrix on EPS yield may be related to the buffering capacity of the substrate (Kaditzky & Vogel, 2008). The study of Mennah-Govela et al. (2020) concluded that the protein content and the initial pH value of a food product are the major influencers on the buffering capacity. In what concerns the product developed in this study, the protein content seems to have no major influence, given its low value; however, the levels of aspartic and glutamic acids, in particular, may have affected buffering capacity in an acidic environment (Mennah-Govela et al., 2020). Although their levels were reduced upon fermentation, they remained the majority among the amino acids detected. Upon fermentation, a pH below 5, and the increased organic acids levels may have contributed to the finger millet YLB buffering capacity (Mennah-Govela et al., 2020). Lastly, the aeration, improved by the slurry agitation over fermentation, leads to higher growth rates of the involved microorganisms (Katina et al., 2009), and thereby, may also have influenced positively the EPS yield.

3.2.8. Sensory analysis

Globally, the unflavoured and flavoured YLBs were well appreciated ('like slightly'), with texture being the less rated parameter (Fig. 7). This attribute was explored in terms of its relationship with global appreciation and certainty of purchase, and for that, based on the median texture's classification it was divided into two classes, namely the respondents who classified texture with<4 points (53.5 %), including 4 ('dislike slightly') and those who classified texture with more than 4 points. The latter group tended to classify product global appreciation better and classify more equally the product with or without yoghurt, but no statistical differences were observed. Consequently, those who liked more the texture would buy the product with more certainty. Half of the panel would buy the product if it was offered with yoghurt, and when no yoghurt was added, the certainty of the purchase decreased. Concerning the quality of the aromas, there was a difference in the classification by the panel ($p \leq 0.05$). In terms of mean rank, the hazelnuts aroma was the highest-rated aroma registering the highest value (70.2), blueberry was the second most favourite (62.1), coffee followed (56.1) and the lowest value was registered for chocolate (53.6). For some respondents adding the yoghurt improved the product in terms of texture, however, the aroma was not so well perceived. The mixing of the cereal YLB with another food product, namely yoghurt, was planned in order to broaden market positionings and enable reaching a wider number of population groups including children and the elderly. The combination of both products could be more easily accepted and successful in terms of consumer appreciation.

4. Conclusions

The present work was able to identify a successful bacterial consortium composed of the Burkina Faso indigenous EPS-producing strain *W. confusa* 2LABPT05 and the probiotic strain *L. plantarum* 299v to be used as a starter culture for the effective fermentation of an African whole cereal matrix, enabling the development of a novel functional probiotic cereal-based product with improved sensory and nutritional properties.

The selected co-culture demonstrated a high fermentative performance on the nutritionally rich traditional minor finger millet cereal.

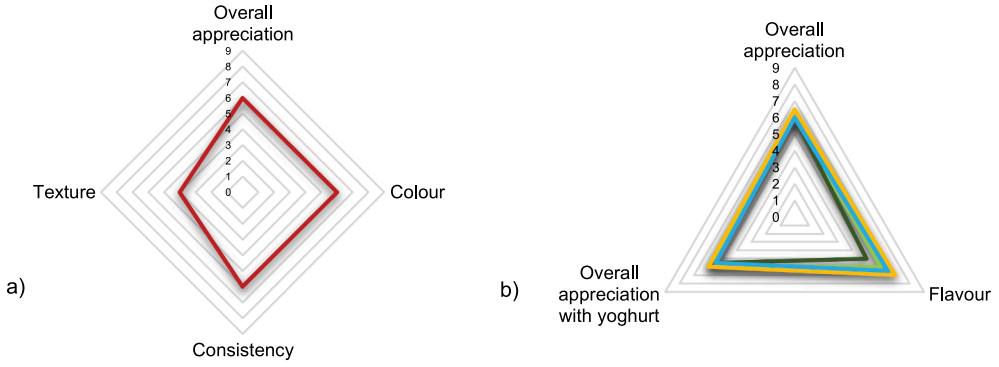

Fig. 7. Spider diagram of the sensory analysis for the non-flavoured finger millet fermented yoghut-like beverage (a) and the aroma-flavoured (dark green line: chocolate; light green line: coffee; blue line: blueberry; yellow line: hazelnut) finger millet fermented, incorporated or not in natural yoghurt (b). Hedonic scale from 1 (to dislike extremely) to 9 (to like extremely). The results are expressed as medians.

This symbiotic consortium allowed the development of a novel functional finger millet-based yoghurt-like beverage, characterized by high viable cell numbers of the probiotic microorganisms, above the minimum required threshold of 10^7 CFU/g, and an interesting slimy texture, naturally improved by the *in loco* production of EPS. The fermented YLB revealed a high-fibre, low-sugar, low-fat nutritional profile, with a higher quantity of dextran, an increased protein digestibility, and a higher content of limiting amino acids, in comparison to the native raw flour. Furthermore, the YLB was organoleptically acceptable, either *per si* or when incorporated in a dairy matrix, enabling more versatility in terms of consumption and new product development.

Hence, the resulting prototype showed to be an added-value product with adequate potential for industrial application: innovative, interesting from the organoleptic, nutritional, and healthy points of view, targeting different population groups from children to the elderly and lactose or gluten intolerants.

CRediT authorship contribution statement

Catarina Vila-Real: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Ana Pimenta-Martins:** Conceptualization, Methodology. **Samuel Mbugua:** Resources. **Sawadogo-Lingani Hagrétou:** Resources. **Kati Katina:** Project administration. **Ndegwa H. Maina:** Conceptualization, Methodology, Funding acquisition, Project administration. **Elisabete Pinto:** Conceptualization, Methodology, Funding acquisition, Supervision, Project administration. **Ana M.P. Gomes:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

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

Data will be made available on request.

Acknowledgements

We would like to thank the African local producers who provided the cereal grains and flour, to *FRULACT S.A.*, for providing us the aromas, through Mentoring Program Comendador Arménio Miranda By FRULACT Academy, to the consumer tasting panel and, also, to *Germen - Moagem de Cereais S.A.*, a Portuguese cereal-milling company. We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020.

Source of funding

This work was supported by National Funds from FCT – *Fundação para a Ciência e a Tecnologia* through project ERA-AFR/0002/2013 - grant ERA-AFR/0002/2013 BI_I and doctoral grant SFRH/BD/133084/2017].

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