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Changes in phenolic composition, antioxidant, sensory and microbiological properties during fermentation and storage of maize products

Anna Mikulajová^{1*}, Zuzana Matejčeková¹, Zlatica Kohajdová², Silvia Mošovská¹, Eva Hybenová¹ and Ľubomír Valík¹

Abstract

In this study, we assessed the potential of maize (*Zea mays*) flour to serve as a substrate for the growth, metabolism, and survival of the Fresco culture cocci (*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris* and *Streptococcus thermophilus*) and *Bifidobacterium* spp. (*B. choerinum* K1/1, *B. pseudolongum* K4/4, *B. animalis* subsp. *animalis* J3II, *B. thermophilum* DSM 20212), which could result in improved nutritional and sensory properties. Maize dairy-free mashes (flavoured with saccharose or caramel) were effectively fermented with Fresco culture for 8 h at 37 °C (3 log increase of counts on average). The functionality of products was proven within the cold storage period (21 days at 6 °C) when viable cell densities of potentially probiotic bacteria were sufficient to demonstrate health-promoting effects (> 5 log CFU/mL). Fermentation process positively changed the contents and compositions of phenolic compounds. Total phenolic content was higher by about 11.5–94.68% in comparison to initial values (0 h). Caffeic acid recorded the highest increase, by about 21.7–151.7%. The antioxidant activity of fermented mashes was also improved. Overall sensory acceptance was enhanced from 2.1 (8 h) to 3.1–3.6 from 4.0 (21 d), which revealed pleasant acceptance of the final caramel products.

Keywords Fermentation, Maize mashes, Bifidobacteria, Phenolics, Antioxidant

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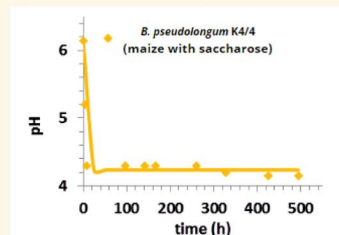
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Graphical Abstract

Laboratory experiments in real growth media
maize flour, caramel or saccharose
with *Bifidobacterium* spp.



+ Application of predictive microbiology



+ Analytical measurements



+ Sensory evaluation

Optimisation of fermentation processes

Functional foods with bioactive
properties and probiotic potential

Introduction

World's population may suffer from adverse food reactions due to various forms of food allergy and food intolerance. It seems that its prevalence is increasing (Aaron et al. 2018; De Petrillo et al. 2021; Lopes & Sicherer 2020). One of the most frequent food intolerances is lactose intolerance, with a diagnosed prevalence of about 57% of the world's population. There are variable percentages of prevalence across the world and ethnic groups as well. Lactose intolerance is widespread, especially in African and Asian countries, with a prevalence ranging from 70 to almost 100%. In the USA, the prevalence is about 36% on average, and in Europe, ranges from 5% in the north-western countries to 70% in the southern countries (e.g., Italy) (Dewiasty et al. 2021; Storhaug et al. 2017). Lactose intolerance may develop in people with celiac. Chronic small intestine inflammation associated with celiac disease may lead to a reduction of lactase activity in the enterocyte brush border (Ojetti et al. 2005). On the other hand, lactose and additional intolerances may play role in non-responsive celiac disease, defined as persistent symptoms in patients on a gluten-free diet (Ojetti et al. 2005; Usai-Satta et al. 2022). The symptoms of these diseases often overlap and should be thoroughly diagnosed.

Overall prevalence of celiac disease ranges from 0.5% to 2%. It is less common in countries of East Asia (Japan, Vietnam) or sub-Saharan Africa, where gluten-containing cereals are not the dominant part of diet. Celiac disease is an autoimmune disorder, and although it is a T-cell-mediated immune response to gluten, it is not classified as a food allergy (Catassi et al. 2022). A lactose- and gluten-restricted diet is the common therapeutic approach in lactose intolerance, celiac disease, and non-celiac gluten sensitivity although this strategy may have nutritional disadvantages with reduced calcium, phosphorus, iron, and vitamin intake (Alkalay 2022; Catassi et al. 2022; Montalto et al. 2006). In addition, intestine inflammation, and reduced absorption of nutrients from food may lead to associated health problems (Alkalay 2022; Dewiasty et al. 2021). Proper management of food intake to provide a nutritionally balanced diet is an essential part of patients' lives.

Maize (*Zea mays*) is one of the most common cereals used to produce foodstuffs for celiac patients. Major maize grain constituents are starch (70–75%), proteins (8–10%) and lipids (4–5%) (Arendt & Zannini 2013). Zeins and glutelins are the most abundant protein fractions. Amino acid composition is deficient in lysine and tryptophan (Wang et al. 2008). Maize dietary

fibre content is 2%, mineral contents range from 1.0 to 1.3%, and levels of vitamins (E and B group), phytosterols and phenolics are also significant. β -carotene and β -cryptoxanthin are the predominant provitamins A. On the other hand, maize is deficient in vitamin B₁₂ (Arendt & Zannini 2013). The main phenolics in maize grain are phenolic acids, among which the predominant is ferulic acid and *p*-coumaric acid. The presence of caffeic acid, gallic acid, *p*-coumaric acid, sinapic acid, and syringic, protocatechuic acid, and vanillic acid was also reported (Salinas-Moreno et al. 2017). Phenolic acids have strong pharmacological activities, and many of them are associated with their antioxidant properties. These compounds are metabolized in humans after absorption by the intestine/colon mucosa through the portal vein to the liver. Their transformation results in the change of structure and biological effects (Kumar & Goel 2019).

A potential alternative to improve the nutritive quality of maize, and overall, to patient diet enrichment can be microbial fermentation treatment. The previous studies indicate that fermentation improves the quality of proteins, digestibility of protein, and starch, bioavailability of minerals (iron, zinc, calcium, magnesium), production or increasing of phenolic compounds, vitamins K, B group, and folate (Liptáková et al. 2017; Petrova & Petrov 2020). Food products fermented with probiotic microorganisms, involving mainly genera *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Saccharomyces*, have both nutritional and health benefits (Liptáková et al. 2017). The cereal grains contain also native prebiotic substances that improve the survival of probiotic microorganisms in the gastrointestinal tract (Voss et al. 2021). Additionally, volatile compounds produced contribute to the product's sensory qualities.

Among the available fermented products, cereal-based ones regained popularity in line with the ever-increasing health consciousness of consumers. Despite common features, many differences exist concerning substrates and the types of microbes involved in the manufacture of fermented foods and beverages. To our knowledge, no studies involving bifidobacteria in fermented maize products are available. Based on the above, the objective of our study was to prepare fermented gluten- and dairy-free products with probiotic, health and preventive characteristics based on maize flour. Thus, we have evaluated chemical, microbiological, and sensory properties to develop a new functional gluten-free alternative not only for metabolically handicapped people.

Methodology

Microorganism

The following microbial strains were utilized: Fresco DVS 1010 starter culture consisting of *Lactococcus lactis* ssp.

lactis, *Lactococcus lactis* ssp. *cremoris*, and *Streptococcus thermophilus* (commercial culture from Christian and Hansen, Hørsholm, Denmark), *Bifidobacterium thermophilum* DSM 20212 (collection strain Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany), *Bifidobacterium choerinum* K1/1 and *Bifidobacterium pseudolongum* K4/4 (isolated from faeces of goat), *Bifidobacterium animalis* subsp. *animalis* J3II (isolated from faeces of lamb). Fresco DVS 1010 starter culture was provided by Rajo a. s., Bratislava, Slovakia and bifidobacterial strains were provided by Prof. Vlková (Czech University of Life Sciences, Prague, Czech Republic). The starter cultures of studied bacteria were prepared according to the procedure of Matejčková et al. (2019).

Enumeration of bacteria

Selective transgalactosylated oligosaccharide mixture propionate agar supplemented with mupirocin (TOS-MUP) (Merck, Darmstadt, Germany) for cultivation and enumeration of bifidobacteria was used. Petri dishes were cultivated at anaerobic conditions using anaerobic jars and Anaerocult A system (Merck, Darmstadt, Germany) for 72 h (37 ± 0.5 °C).

The presumptive numbers of the cocci were enumerated on M17 agar plates (Biokar Diagnostics, Beauvais, France) according to the EN ISO 15214 (2002). Petri dishes were incubated at aerobic conditions for 24 h (30 ± 0.5 °C).

Assessment of growth and metabolic parameters

The model proposed by Baranyi and Roberts (1995) was fitted to the data of studied bacteria to estimate metabolic and kinetic parameters of growth (lag phase duration, specific growth rate, rate constant for decrease of counts, rate constant for a decrease of pH). The Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA) add-in DMFit (Version 3.5, ComBase, University of Tasmania, Australia and the USDA Agricultural Research Service, Washington, DC, USA) was used. The growth rates (G_R) were recalculated to the specific growth rates according to the equation $\mu = \ln 10 \times G_R$. The changes in pH values were measured using a pH meter with a penetration electrode (Knick Portamess, Berlin, Germany).

Sample preparation

Natural maize mash was prepared by mixing maize flour (8%, w/v; nutritional value in 100 g: energy 1401 kJ; carbohydrates 69.5 g; sugar 1.3 g; protein 8.6 g; fat 2.0 g; saturated fat 0.3 g; Solčanka, Solčany, Slovak Republic) with water (90%), and saccharose (2%, w/v). Caramel maize mash was prepared from maize flour (8% w/v), water (72%), and caramel component (20%, w/v; composed of 35% caramel syrup, 10% glucose-fructose syrup, 28.5%

saccharose, modified maize starch, caramelized sugar, water; nutritional value in 100 g: energy 1201 kJ; carbohydrates 63.9 g; sugar 58.2 g; protein 1.4 g; fat 2.3 g; saturated fat 1.4 g; Agrana, Vienna, Austria). The intention was to design a product with spoon-eating consistency. Mashers were heated with stirring at 100 °C for 20 min, and subsequently sterilized at 121 °C for 20 min and cooled down to 37 °C. Caramel component was added after sterilization. The sterility of prepared samples was regularly confirmed by the plating method prior to the inoculation.

Sample fermentation, and storage

Prepared samples were inoculated with Fresco DVS 1010 culture (5%; v/v) to achieve inoculation levels of approximately 10^6 CFU/mL. The fermentation process was carried out at 37 ± 0.5 °C for 8 h (5% CO₂). After this period, studied bifidobacteria were singly inoculated (cell counts of approximately 10^8 – 10^9 CFU/mL) into fermented mashers and stored at 6 ± 0.5 °C for 21 days. Periodical evaluation of pH values and viability of the studied microorganisms was performed. The experiments were carried out in duplicate. Unfermented mashers, mashers after fermentation (8 h) and after storage (14 and 21 days) were freeze-dried and stored at -40 °C until extraction procedures. The sensory analysis was conducted in the freshly fermented mashers (37 ± 0.5 °C/8 h) and stored products (6 ± 0.5 °C for 14 and 21 days).

Phenolic compound extraction

Freeze-dried samples were extracted as previously reported by Mikulajová et al. (2007a) with 65% ethanol (1:20, w/v, 80 °C, 1 h, three times). The combined extracts were re-extracted with ethyl acetate (1:20, v/v, 25 °C, 1 h, three times; Centralchem, Bratislava, Slovak Republic) to recover the phenolic compounds. The solvents were then dried under a vacuum, and the remainders were reconstituted in 96% ethanol. The ethanolic extracts were used for the analysis of total phenolic content, phenolic compounds profile and antioxidant activity.

Analysis of total phenolic compounds

The total phenolic content was estimated using the Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) method according to Mikulajová et al. (2007b). Absorbance was determined at 765 nm. The calibration curve was prepared by using gallic acid (Sigma-Aldrich, Steinheim, Germany) as the calibration standard. The total phenolic content was expressed in terms of gallic acid equivalent per gram of sample (mg GAE/g).

Analysis of phenolic compounds profile

The phenolic composition was determined using HPLC/DAD (Agilent 1200 Series, Agilent Technologies, Santa Clara, California, USA) method based on the modified study of Seal 2016. The separation was conducted using a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm, Agilent Technologies, Santa Clara, California, USA). A gradient system of elution, a constant flow rate of 1 mL/min, an injection volume of 10 μl and a column temperature of 25 °C were used. The mobile phase included water/acetic acid adjusted to pH 2.8 (solvent A) and acetonitrile (solvent B). The solvent A/solvent B ratio was changed from 95/5 to 70/30. Detection was carried out at 272 nm and 350 nm, according to the absorption maxima of evaluated compounds. Quantification of individual components in samples was performed by an external standard method with gallic, protocatechuic, vanillic acid, syringic acid, caffeic, *p*-coumaric acid, and *trans*-ferulic acid used as standards. ChemStation software 12.2 (Agilent Technologies, Santa Clara, California, USA) for the collection and processing of chromatographic data was used. Values were expressed as μg per gram of sample (μg/g).

Analysis of antioxidant activity

Free radical scavenging by DPPH test

The method of Mikulajová et al. (2007b) was adopted for 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) (Sigma-Aldrich, Steinheim, Germany) assay. Absorbance of the samples was measured at 517 nm at 10 min of reaction. Different concentrations of DPPH solution in 96% ethanol were used to prepare the calibration curve, and results were expressed as the amount of scavenged DPPH radicals per gram of sample (mg DPPH/g).

Free radical scavenging by ABTS test

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test was measured according to Mikulajová et al. (2007b). Absorbance of the samples was measured at 730 nm at 10 min of reaction. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox; Sigma-Aldrich, Steinheim, Germany) was used as standard. The antioxidant activities were displayed as mg of Trolox equivalent antioxidant capacity per gram of sample (mg TEAC/g).

Ferric reducing antioxidant potential test (FRAP)

FRAP test was performed based on the method of Pohanka et al. (2009). Absorbance of the samples was measured at 595 nm after 30 min of reaction. The reducing power was determined from the calibration curve prepared with Trolox and results were expressed as mg of

Trolox equivalent antioxidant capacity per gram of sample (mg TEAC/g).

Sensory analysis

The sensory evaluations were conducted with 10 trained assessors (9 women and 1 man) between the ages of 25 and 43 (mean 32.6 years) from Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava. The panellists were trained regarding basic sensory evaluation method (STN EN ISO 8586–2, 2014). The sensory quality of maize caramel products was assessed as fresh fermented products (37 ± 0.5 °C/8 h) and after 14 and 21 days of storage (6 ± 0.5 °C) with implementation of Quantitative Descriptive Analysis (QDA), International Standard ISO 13299 (2010) and the procedure of Stone et al. (2012). Finally, following attributes were evaluated: colour, consistency, texture, overall acceptability as well as individual descriptors of aroma and taste. The 4-point hedonic scale used for the evaluation ranged from dislike extremely (point 0) to like extremely (point 4).

Statistical analysis

Fermentation experiments and extractions were carried out in duplicates and analyses were performed in triplicates. The results were reported as means \pm standard deviation (SD). The results were analysed by Student's t-test, one-way analysis of variance, Fisher's LSD procedure at $p=0.05$ significance level, and by Pearson correlation analysis. Data were also analysed by principal component analysis (PCA). Statistical analyses were conducted using Statgraphic Plus, Version 3.1 (Statsoft; Tulsa, Oklahoma, USA) software.

Results and discussion

Fermentation process and microbiological analyses

In our study, eight plant-based, dairy-free fermented products with probiotic potential were studied. In water-based mashes, cocci of the Fresco culture (5%

(v/v)) entered immediately exponential phase of growth. Within 8 h of the fermentation process the levels reached populations of 9.16 ± 0.26 log CFU/mL representing 3 log units increase compared to the initial state. In our previous study, we observed similar good growth of Fresco culture in non-dairy gluten-free amaranth and buckwheat products, where Fresco culture reached counts 8–9 log CFU/mL after 8 h of fermentation, representing 2 to 3 log CFU/mL increase compared with the initial state (Matejčeková et al. 2015 2018). In maize mash added with saccharose, the specific growth rate of Fresco was about 22% higher ($\mu_{\text{saccharose}}=0.99$ 1/h) compared to caramel one ($\mu_{\text{caramel}}=0.78$ 1/h). Despite the lower calculated specific growth rate in caramel product, both specific growth rates indicated optimal fermentation conditions for the growth of the cocci. Thus, maize products provided nutrients required for the growth of the Fresco culture. After 8 h of the fermentation process as the bacterial growth and metabolic activity occurred, pH reached values of 4.18 and 4.55 in saccharose and caramel maize mash, respectively (decrease of about 2.0 and 1.4 units). The calculated rate constants for pH decrease within 8 h were -0.76 and -0.27 1/h in saccharose and caramel maize mash, respectively. In our study, pH values reached after fermentation remained almost constant within cold storage period, and after 21 days ranged from 4.10 to 4.54 (Table 1). pH levels below 4.5 are necessary from the point of microbiological safety of final products, suppressing and minimizing the outgrowth of undesirable microbiota (Schoustra et al. 2022). In our study, pH values were 4.5 or below, demonstrating that 6 log CFU/mL of inoculum was sufficient to decrease the pH during the fermentation period.

Different levels of probiotic bacteria in yoghurts have been recommended and specified, to exert the claimed health effects of probiotic products. The National Yogurt Association (NYA) of the United States specifies that 8 log CFU/mL of probiotic lactic acid bacteria at the time of manufacture are required to use the NYA 'Live and

Table 1 Parameters evaluating the behaviour of potentially probiotic bifidobacteria after they had been added to fermented maize mashes with saccharose and caramel, stored for 21 days at 6 °C

| | maize/caramel | | | | maize/saccharose | | | |
|---|---------------|------------------|-----------------|--------------------------|------------------|------------------|-----------------|--------------------------|
| | k_d | N_{end} | k_{pH} | pH_{end} | k_d | N_{end} | k_{pH} | pH_{end} |
| Fresco + <i>B. animalis</i> subsp. <i>animalis</i> J311 | -0.007 | 5.7 ^c | -0.263 | 4.50 | -0.006 | 5.8 ^b | -0.766 | 4.18 |
| Fresco + <i>B. choerinum</i> K 1/1 | -0.006 | 5.6 ^d | -0.520 | 4.10 | -0.006 | 5.1 ^c | -0.521 | 4.10 |
| Fresco + <i>B. pseudolongum</i> K 4/4 | -0.004 | 6.2 ^a | -0.284 | 4.45 | -0.004 | 6.2 ^a | -0.250 | 4.15 |
| Fresco + <i>B. thermophilum</i> DSM 20212 | -0.236 | 6.1 ^b | -0.595 | 4.32 | -0.008 | 5.1 ^c | -0.572 | 4.54 |

k_d rate constant for a decrease of counts (log CFU/mL h), N_{end} counts after storage period (log CFU/mL), k_{pH} rate constant for a decrease of pH (1/h), pH_{end} pH value after storage period

^{a–d}Means within a column with different superscript letters differ significantly ($p < 0.05$)

Active Culture' logo on the products containers. In Japan, the Fermented Milks and Lactic Acid Bacteria Beverages Association has specified a minimum of 7 log CFU/mL of bifidobacteria to be present in fresh dairy products as a standard (Lani et al. 2022). To achieve health benefits, probiotic products are expected to support the growth and survival of probiotic strains with a minimum level of 6 log CFU/mL at the expiration date of the products (Liptáková et al. 2017). Thus, maintaining the probiotic bacteria viability and survivability during product manufacturing and storage is an important criterion to achieve health benefits. Species from the genus *Bifidobacterium* are considered more susceptible to oxygen and acidic conditions, thus food applications of bifidobacteria could be much limited compared to lactobacilli. However, the level of sensitivity to oxygen or pH may be strain- and substrate- dependent. Even though bifidobacteria are considered to be strictly anaerobic, some species can survive in the presence of oxygen (Kawasaki et al. 2006). In our study, significant differences in viable cells of bifidobacteria were observed after 21 days of storage. On average, counts of bifidobacteria were variable ranging from 5.1 to 6.2 log CFU/mL, with the highest levels being for *B. pseudolongum* K 4/4. In general, decrease in counts of about 2.9 log order on average was recorded. The inhibitory effect of lactic and acetic acid and the decrease in

pH during fermentation were likely manifested. Despite the decline in bacterial levels, the counts remained above the level of 5 log CFU/mL, the minimum level of probiotics suggested by some authors (Gueimonde et al. 2004). In our previous study, species of bifidobacteria survived adequately (> 5 log CFU/mL) in fermented maize milk products (Matejčková et al. 2019).

Phenolic compounds

Total phenolic contents of maize/caramel fermented mashes in particular stages of fermentation were examined. Results are presented in Table 2. Phenolic content of unfermented sample (0 h) was 0.274 mg GAE/g. After 8 h of fermentation with Fresco culture, the amount of phenolics was 0.338 mg GAE/g, representing increase of 23.4% in comparison to the initial content (0 h) of unfermented sample. After 14 days of storage, the phenolic content of mashes with *B. animalis* J3II and *B. thermophilum* DSM 20212 increased by about 7.5 and 16.1% in comparison to the content after 8 h of fermentation, respectively. In contrast, mashes with *B. choerinum* K1/1 and *B. pseudolongum* K4/4 were shown a decrease in phenolics. This result indicates that phenolic compound metabolism is strain- or species- dependent. In the next storage period, phenolic content did not change significantly ($p > 0.05$) in all mashes. However, at the end of the

Table 2 Total phenolic content and antioxidant activity of maize mashes determined by DPPH test, ABTS test, FRAP test

| Mashes | time | TPC (mg GAE/g) | | DPPH test (mg DPPH/g) | | ABTS test (mg TEAC/g) | | FRAP test (mg TEAC/g) | |
|------------------------------------|------|------------------------------|-----------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|-----------------------------|
| | | maize/caramel | maize/saccharose | maize/caramel | maize/saccharose | maize/caramel | maize/saccharose | maize/caramel | maize/saccharose |
| Unfermented | 0 h | 0.274 ± 0.003 ^a | 0.117 ± 0.003 ^a | 0.240 ± 0.003 ^a | 0.221 ± 0.002 ^a | 0.525 ± 0.001 ^b | 0.336 ± 0.002 ^a | 0.322 ± 0.002 ^a | 0.186 ± 0.000 ^a |
| fermentation with Fresco | 8 h | 0.338 ± 0.001 ^b | 0.154 ± 0.000 ^b | 0.360 ± 0.003 ^b | 0.328 ± 0.002 ^b | 0.555 ± 0.003 ^c | 0.390 ± 0.001 ^b | 0.334 ± 0.001 ^b | 0.251 ± 0.002 ^b |
| + <i>B. animalis</i> J3II | 14 d | 0.359 ± 0.002 ^c | 0.215 ± 0.002 ^{ce} | 0.335 ± 0.003 ^c | 0.345 ± 0.002 ^{cd} | 0.531 ± 0.004 ^{bd} | 0.393 ± 0.001 ^{bc} | 0.485 ± 0.000 ^c | 0.303 ± 0.002 ^c |
| + <i>B. choerinum</i> K1/1 | 14 d | 0.310 ± 0.003 ^d | 0.176 ± 0.001 ^d | 0.336 ± 0.003 ^c | 0.334 ± 0.004 ^{bde} | 0.477 ± 0.006 ^{ae} | 0.389 ± 0.003 ^{bc} | 0.376 ± 0.001 ^d | 0.249 ± 0.002 ^b |
| + <i>B. pseudolongum</i> K4/4 | 14d | 0.304 ± 0.006 ^d | 0.219 ± 0.001 ^c | 0.292 ± 0.006 ^d | 0.316 ± 0.002 ^{be} | 0.480 ± 0.003 ^a | 0.416 ± 0.004 ^d | 0.411 ± 0.002 ^{ef} | 0.283 ± 0.002 ^d |
| + <i>B. thermophilum</i> DMS 20212 | 14d | 0.382 ± 0.004 ^e | 0.169 ± 0.001 ^f | 0.287 ± 0.006 ^d | 0.321 ± 0.002 ^b | 0.504 ± 0.001 ^{ef} | 0.396 ± 0.001 ^{ce} | 0.448 ± 0.003 ^a | 0.258 ± 0.000 ^{be} |
| + <i>B. animalis</i> J3II | 21 d | 0.325 ± 0.008 ^{bdf} | 0.183 ± 0.003 ^d | 0.323 ± 0.006 ^{cd} | 0.356 ± 0.002 ^c | 0.539 ± 0.003 ^{cd} | 0.394 ± 0.002 ^{be} | 0.412 ± 0.000 ^f | 0.262 ± 0.002 ^{eg} |
| + <i>B. choerinum</i> K1/1 | 21 d | 0.306 ± 0.000 ^d | 0.227 ± 0.003 ^c | 0.288 ± 0.006 ^d | 0.322 ± 0.002 ^{be} | 0.499 ± 0.004 ^{ae} | 0.355 ± 0.002 ^f | 0.387 ± 0.004 ^{dh} | 0.362 ± 0.002 ^f |
| + <i>B. pseudolongum</i> K4/4 | 21 d | 0.339 ± 0.003 ^{bcd} | 0.208 ± 0.001 ^e | 0.308 ± 0.003 ^d | 0.317 ± 0.000 ^e | 0.510 ± 0.003 ^g | 0.338 ± 0.000 ^a | 0.394 ± 0.003 ^{ghi} | 0.293 ± 0.001 ^c |
| + <i>B. thermophilum</i> DMS 20212 | 21 d | 0.331 ± 0.003 ^{bf} | 0.171 ± 0.001 ^{df} | 0.343 ± 0.003 ^{bc} | 0.315 ± 0.002 ^{be} | 0.498 ± 0.004 ^{ae} | 0.406 ± 0.004 ^{bde} | 0.381 ± 0.001 ^{di} | 0.261 ± 0.000 ^g |

TPC total phenolic content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), FRAP ferric reducing antioxidant potential, TEAC Trolox equivalent antioxidant capacity

^{a-e} Means within a column with different superscript letters differ significantly ($p < 0.05$)

storage period, phenolic amounts were higher by about 11.5–23.7% compared to the initial state (0 h).

For comparison, in natural maize/saccharose mashes total phenolics increased from 0.117 mg GAE/g in the unfermented mash to 0.154 mg GAE/g in mash fermented (8 h) with Fresco culture, which represents increase about 31.6%. Following inoculation with microorganisms from genus *Bifidobacterium* and storage for 14 days led to the continued increase in phenolic compounds by about 12.8–56.0%. This trend was maintained for up to 21 days of storage in mashes with K1/1 isolate only, while a decrease or no changes ($p > 0.05$) were observed in the remaining mashes. The final phenolic values (after 21 days) were higher by about 46.1–94.6% compared to the initial value (0 h) and were lower compared to caramel mashes. Our results indicate that cocci of the Fresco culture positively affected the content of phenolic compounds within the fermentation process. The positive effect on phenolics content had also subsequent addition of bacteria from genus *Bifidobacterium*. Probiotics have proven to have an anti-inflammatory effect on

small intestine through their gluten detoxification ability (Petrova & Petrov 2020). Moreover, phenolic compounds are involved in the modulation of gut microbiota population, composition, and activity (Loo et al. 2020). This can be beneficial for celiac patients, too, because a gluten-free diet was found to negatively influence the presence of healthy and potentially unhealthy gut bacteria and related immune responses (Loo et al. 2020).

The profile of phenolic compounds

The results of analysis of individual phenolic acid changes are illustrated in Fig. 1. In the unfermented maize/caramel sample, two benzoic acid derivatives – vanillic acid (4.97 µg/g), syringic acid (4.65 µg/g) and three cinnamic acid derivatives – caffeic acid (13.90 µg/g), *p*-coumaric acid (57.60 µg/g), ferulic acid (11.74 µg/g) were found. The benzoic acid derivatives increased by about 22.5% after 8 h of fermentation, and by about 93.5–102.8% (depending on the bifidobacteria used) after 14 days of storage. Even though it dropped in the following phase, the amount of these compounds was still about

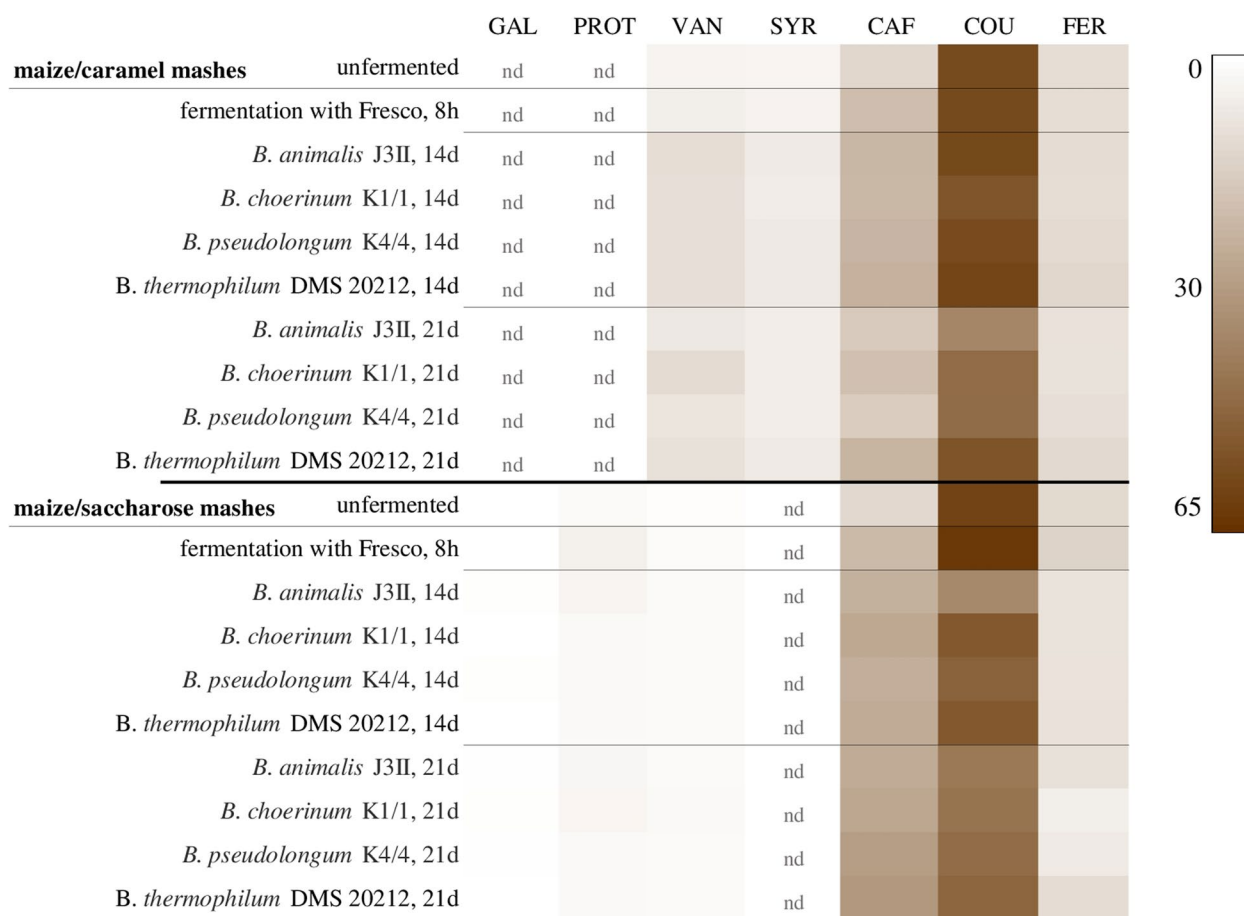


Fig. 1 Heatmap of phenolic acids profile in maize mashes obtained by HPLC analysis (µg/g). GAL: gallic acid; PROT: protocatechuic acid; VAN: vanillic acid; SYR: syringic acid; CAF: caffeic acid; COU: *p*-coumaric acid; FER: ferulic acid; nd: not detected

56.7–100.8% more compared to the unfermented sample. A similar trend was observed for caffeic acid (an increase about of 21.7–76.5%, after 21 days), while *p*-coumaric and ferulic acids gradually decreased during storage to values lower than the initial ones (0 h), apart from ferulic acid content in mash with *B. thermophilum* DMS 20212.

In the natural maize/saccharose sample, three benzoic acid derivatives – gallic acid (1.05 µg/g), protocatechuic (2.86 µg/g), vanillic acid (1.86 µg/g) and three cinnamic acid derivatives – caffeic acid (13.31 µg/g), *p*-coumaric acid (59.76 µg/g), ferulic acid (13.20 µg/g) were detected. After 8 h of fermentation, the benzoic acid derivatives increased by about 69.2% and cinnamic acid derivatives by about 17.7%. In subsequent phases, the changes of individual phenolic acids were different in the different fermentation systems. Gallic, vanillic and caffeic acid levels continued to increase, except for mash with *B. thermophilum* DMS 20212, where gallic acid level did not show a significant difference ($p > 0.05$). Protocatechuic acid content was decreased but was still about 3.4–59.6% higher than the initial content (0 h). Overall, the content of benzoic acid derivatives increased by about 22.7–55.6% in comparison to initial level (0 h). The highest increase (about 106.6–151.7%) was observed for caffeic acid content at the end of storage. Contrariwise, the content of ferulic and *p*-coumaric acids was lower than the initial one.

p-Coumaric acid is the most abundant phenolic acid in both type of mashes, followed by caffeic, and ferulic acids. *p*-Coumaric acid represents on average 63.5%, and 49.2% of total phenolic acids in initial (0 h), and final (21 d) mashes, respectively. The proportion of caffeic acid to total phenolic acids increased from 14.7% (on average) to 20.9% (maize/caramel mashes) and 32.5% (maize/saccharose mashes). Ferulic and *p*-coumaric acids are the predominant insoluble-bound phenolic acids in maize, where they are linked to cell wall polymers through covalent linkages. The presence of diferulates in maize have been also reported (Salinas-Moreno et al. 2017). Microbial enzymes like esterase and xylanase can release phenolics from their insoluble-bound forms with the support of proteases, cellulases, and amylase, which can hydrolyse the structural components of maize flour. Furthermore, the lower pH can contribute to the disintegration of complex forms through acid hydrolysis and support the activity of hydrolytic enzymes (Khan et al. 2020). Only soluble phenolics are considered bioavailable (Ayyash et al. 2018). *Lactococcus* ssp. and *Bifidobacterium* ssp. have the potential to secrete carbohydrate-degrading enzymes (Grujović et al. 2020). The production of esterases, β -glucosidases, and β -galactosidases by *Streptococcus* and *Bifidobacterium* strains was reported (Ayyash et al. 2018; Wu et al. 2021). In our study, the initial

increase in *p*-coumaric and ferulic acids content (after 8 h and 14 d, resp.) was followed by a decrease in the next days. This course of changes suggests that the releasing of these phenolic acids from their complex bond forms, as a consequence of enzymatic and reduced pH effects, prevails in the initial stage. The next period is probably more characterized by the metabolization, degradation and/or transformation of phenolic acids, resulting in a reduction in their content. The phenolic acids can be degraded by microbial decarboxylases and reductases. Ferulic and *p*-coumaric acids can be decarboxylated into corresponding *p*-vinyl derivatives that affect the sensory characteristics of final fermented product. The activity of phenolic acid reductase results in the production of hydroxyphenylpropionic acid, e.g., dihydroferulic acid, from ferulic acid (Miyagusuku-Cruzado et al. 2020; Szwajgier & Jakubczyk 2010; Wu et al. 2021). Moreover, our results indicate that microorganisms used have variable capacities for phenolic acids liberation and metabolism.

Antioxidant activity

Antioxidant efficacy was studied by three different methods. The results are presented in Table 2. Both the reducing power and the radical scavenging activities of DPPH and ABTS⁺ were significantly ($p < 0.5$) increased after fermentation (8 h) by about 3.8% (FRAP), 49.9% (DPPH) and 5.8% (ABTS) in maize/caramel fermented mashes. The increasing trend of reducing power was maintained until the 14 days of storage in each mash to which bifidobacteria were added (increase by about 16.7–50.5%). In contrast, the radical scavenging activities decreased after 14 days, and the activities against ABTS⁺ radical declined below the initial value. After 21 days of storage, antioxidant activities in evaluated mashes did not change significantly ($p > 0.05$), and showed higher activity (FRAP test), or did not exceed activities (ABTS and DPPH test) after fermentation (8 h). However, final reducing power and antiradical activity against DPPH radicals were higher by about 18.2–27.8% and 19.6–42.6%, respectively in prepared mashes than in unfermented mashes. The final antiradical activity against ABTS⁺ radicals was only higher ($p < 0.05$) in the mash with *B. animalis* J3II (about 2.7%).

The course of changes in antioxidant activity in maize/saccharose fermented mashes was slightly different from caramel mashes. Particularly, the decrease in antiradical activity after 14 days of storage was not observed. Antiradical activity against ABTS⁺ and DPPH radicals was higher by about 15.6–23.7% and 43.3–56.2%, respectively. The DPPH radical scavenging activity remained constant in all mashes during the subsequent storage days. A similar trend was observed for ABTS⁺ radical scavenging activity, except for mashes with *B. choerinum*

K1/1 and *B. pseudolongum* K4/4, where the activity dropped. Reducing power increased throughout the experimental period, except for mash with *B. animalis* J3II, which reached the maximum after 14 days. Finally, the resulting reduction power values in prepared mashes were higher by about 40.9–94.9% than in unfermented mashes. The results of correlation analysis showed that antioxidant activity determined by ABTS test correlates with the activity determined by FRAP test ($r=0.749$), but not by DPPH test ($r=0.060$). Moreover, a high positive correlation was found between TPC and ABTS test ($r=0.884$) and between TPC and FRAP tests ($r=0.923$). Conversely, no correlation was observed between TPC and DPPH test ($r=0.127$). Different antioxidant method mechanisms and different contributions of phenolics, phenolic metabolites, and other bioactive compounds to overall antioxidant activity may be responsible for these differences. For example, caffeic acid has higher antioxidant activity than ferulic acid, while dihydroferulic acid, the fermentation metabolite of ferulic acid, was noticed to have a stronger antioxidant property than ferulic acid (Boudaoud et al. 2021).

The relationship between samples and attributes that contributed to the antioxidant potential of samples was visualized PCA. The PCA indicated that the first 3 principal components (PC), accounted together for 88.9% of the total variation, with PC1 explaining 59.6%. The plot of PC1 vs. PC2 loadings (Fig. 2A) shows along the PC1 axis, a close relationship between, ABTS and FRAP tests and the content of TPC, vanillic and syringic acids, while are inversely correlated with gallic and protocatechuic acids. The PC2 and PC3 explained 19.3% and 9.9% of the total variation and reflected mainly the contribution of the attributes as DPPH test, coumaric and ferulic acids. Scatter plot diagram of samples resulting from PCA in coordinates of PC1 and PC2 is presented in the Fig. 2B. PCA divided samples into 5 groups according to the applied flavouring agent and fermentation process: samples prepared from maize and saccharose fermented by Fresco culture and with added bacteria of genus *Bifidobacterium* characterised by the highest content of caffeic acid; sample prepared from maize and saccharose fermented only by Fresco culture for 8 h characterised by the highest content of *p*-coumaric and ferulic acids; unfermented sample prepared from maize and saccharose characterised by the lowest content of vanillic acid; samples prepared from maize and caramel fermented by Fresco culture and *Bifidobacterium* spp. strains characterised by the highest content of TPC, vanillic and syringic acids and non-fermented samples prepared from maize and caramel characterised by low concentration of syringic acid. On the other side PCA non-separated samples based on the applied strains of bifidobacteria.

Sensory analysis

Caramel component was added to the products to make them more attractive for consumers. The sensory characteristics and consumer acceptance of fermented caramel mashes were conducted based on indicators such as colour, consistency, texture, taste, aroma, and overall acceptability of products (Table 3).

Fresh fermented caramel products (8 h) and products stored for 11 and 21 days were evaluated, to determine the differences within the storage period. Scores given for colour to the water-based maize mashes ranged from 2.8 to 3.4, indicating very good colour of prepared products. In milk-based caramel maize mashes, the evaluation of colour ranged from 2.9 to 3.7, indicating very good, the almost excellent colour of the prepared products (Matejčková et al. 2019). The final consistency of the products varied from 2.1 to 3.0, representing lumpy to an almost reasonably smooth consistency. The attributes of taste and aroma were divided by the descriptors that could positively or negatively affect the overall sensory character of the final products. The results are presented in spider web graphics (Fig. 3). In prepared products, the intensity of total aroma ranged from 2.2 to 3.3, representing moderate to strong intensity. In the stored products, caramel and cereal aroma were evaluated as the strongest. Several assessors identified fruity, vanilla, and nutty aroma, but they were scored with low intensity. An unacceptable rancid and grassy aroma was not recorded. As bifidobacteria can produce acetic and lactic acid in a proportion of 3:2, the evaluation of unacceptable taste was an important step in the assessment. In the product with *B. choerinum* K1/1 after 11 days, the foreign taste was noted (0.38), but after 21 days of storage, the unnoticeable intensity was recorded (0.00). In all prepared mashes, a positive effect on the overall acceptability of the storage period was noted and an increase in final scores was recorded. After 21 days of storage, levels of overall acceptance ranged from 3.1 to 3.6 that revealed pleasant acceptance of the final caramel products. The highest score was found in the product with *B. thermophilum* DSM 20212 (3.6) and was significantly different compared ($p<0.05$) to the other products. A positive effect of storage period was noted also in our previous study Matejčková et al. (2019) in caramel milk maize products.

Conclusion

Our results show that fermentation of maize with Fresco culture and bifidobacteria is an appropriate approach combining the benefits of maize prebiotics, starter culture and probiotics. At the end of the storage period, statistically significant changes in counts of bifidobacteria were noted ($p<0.05$). Nonetheless,

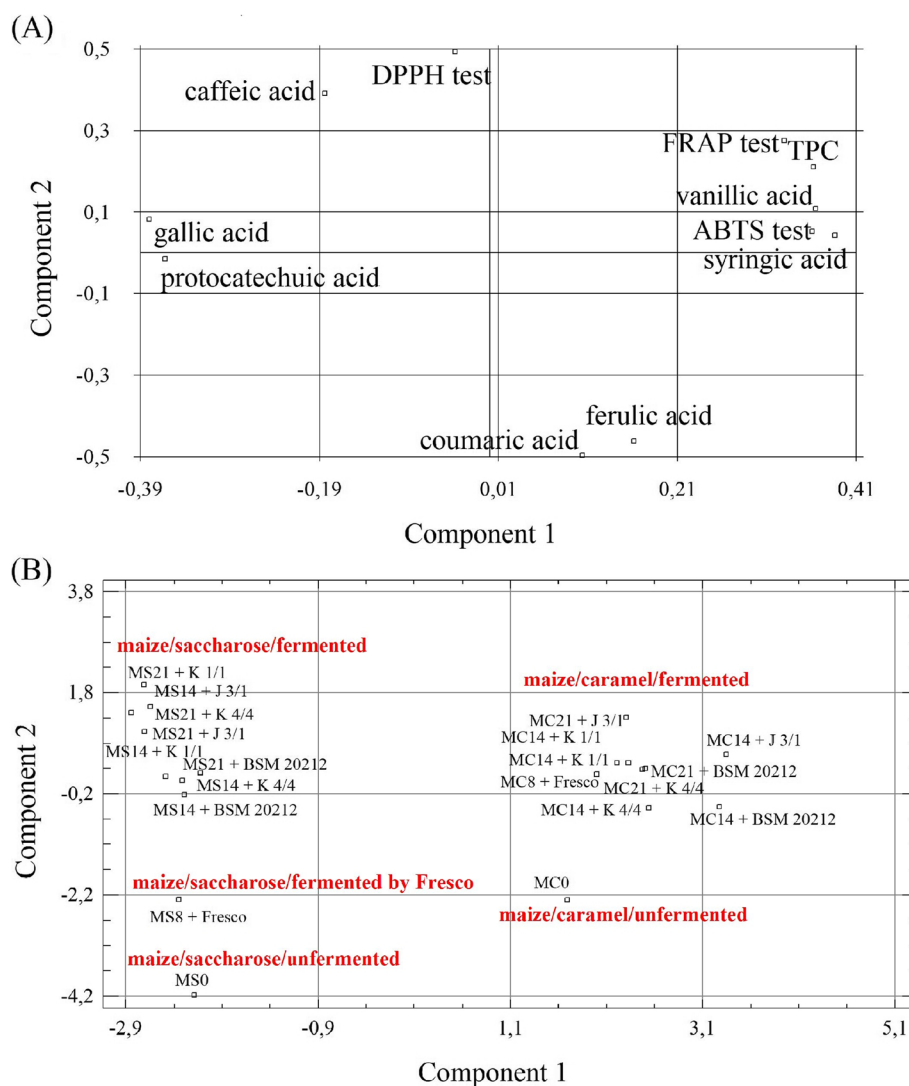


Fig. 2 **A** The plot of PC1 vs. PC2 loadings of analysed attributes. TPC: total phenolic content; FRAP: ferric reducing antioxidant potential; ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; **(B)** Scatter plot diagram of samples resulting from PCA in coordinates of PC1 and PC2. MS0: maize/saccharose unfermented 0 h; MS8: maize/saccharose fermented 8 h; MS14: maize/saccharose stored 14 days; MS21: maize/saccharose stored 21 days; MC0: maize/caramel unfermented 0 h; MC8: maize/caramel fermented 8 h; MC14: maize/caramel stored 14 days; MC21: maize/caramel stored 21 days

the population of potentially probiotic bifidobacteria did not drop below 5 log CFU/mL. Fermentation induces the liberation of phenolic acids, leading to their enhanced levels as well as the formation of phenolic metabolites that may promote their bioavailability and subsequent health effect. However, the health-promoting effects need additional investigation, particularly through in vivo studies. Generally, the highest increase was observed for caffeic acid, followed by syringic, vanillic, and protocatechuic acids, while ferulic and

p-coumaric acids were more extensively metabolised, so their amounts were gradually decreased. Fermentation also improved the antioxidant activity, mainly reducing potential. The results of the sensorial analysis suggest that products also within the storage period may be accepted by consumers. The designed products meet the requirements to be gluten-free and lactose-free, and can be served as a snack or for breakfast. Maize fermented products represent a promising alternative to dairy products at a time of change in

Table 3 Sensory evaluation of maize/caramel products

| | Time | Colour | Consistency | Texture | Total aroma | Overall acceptability |
|--|------|----------------------|----------------------|----------------------|----------------------|-----------------------|
| Fermentation with Fresco | 8 h | 3.4±0.5 ^d | 2.9±0.5 ^c | 2.9±0.7 ^b | 2.8±0.7 ^c | 2.1±0.6 ^a |
| + <i>B. animalis</i> subsp. <i>animalis</i> J311 | 14 d | 3.2±0.5 ^c | 2.5±0.5 ^b | 2.9±0.5 ^b | 2.6±0.7 ^b | 3.1±0.8 ^d |
| | 21 d | 3.3±0.5 ^c | 2.1±0.3 ^a | 2.5±0.5 ^a | 2.9±0.8 ^c | 3.6±0.5 ^e |
| + <i>B. choerinum</i> K 1/1 | 14 d | 2.8±0.9 ^a | 2.9±0.7 ^c | 3.0±0.7 ^b | 2.5±0.9 ^b | 2.4±0.5 ^b |
| | 21 d | 3.0±0.8 ^b | 2.6±0.5 ^b | 3.4±0.7 ^c | 2.6±0.5 ^b | 3.5±0.7 ^e |
| + <i>B. pseudolongum</i> K 4/4 | 14 d | 3.2±0.5 ^c | 3.0±0.8 ^d | 2.9±0.5 ^b | 2.5±0.5 ^b | 2.8±0.7 ^c |
| | 21 d | 3.3±0.5 ^c | 2.6±0.5 ^b | 3.1±0.8 ^b | 2.4±0.7 ^b | 3.1±0.3 ^d |
| + <i>B. thermophilum</i> DSM 20212 | 14 d | 3.2±0.4 ^c | 2.8±0.6 ^c | 2.8±0.4 ^b | 2.2±0.4 ^a | 2.8±0.4 ^c |
| | 21 d | 3.0±0.5 ^b | 2.8±0.6 ^c | 3.3±0.6 ^c | 3.3±0.6 ^d | 3.2±0.6 ^d |

^{a–e}Means within a column with different superscript letters are significantly different ($p < 0.05$); 0: worst possible value, 4: best possible value

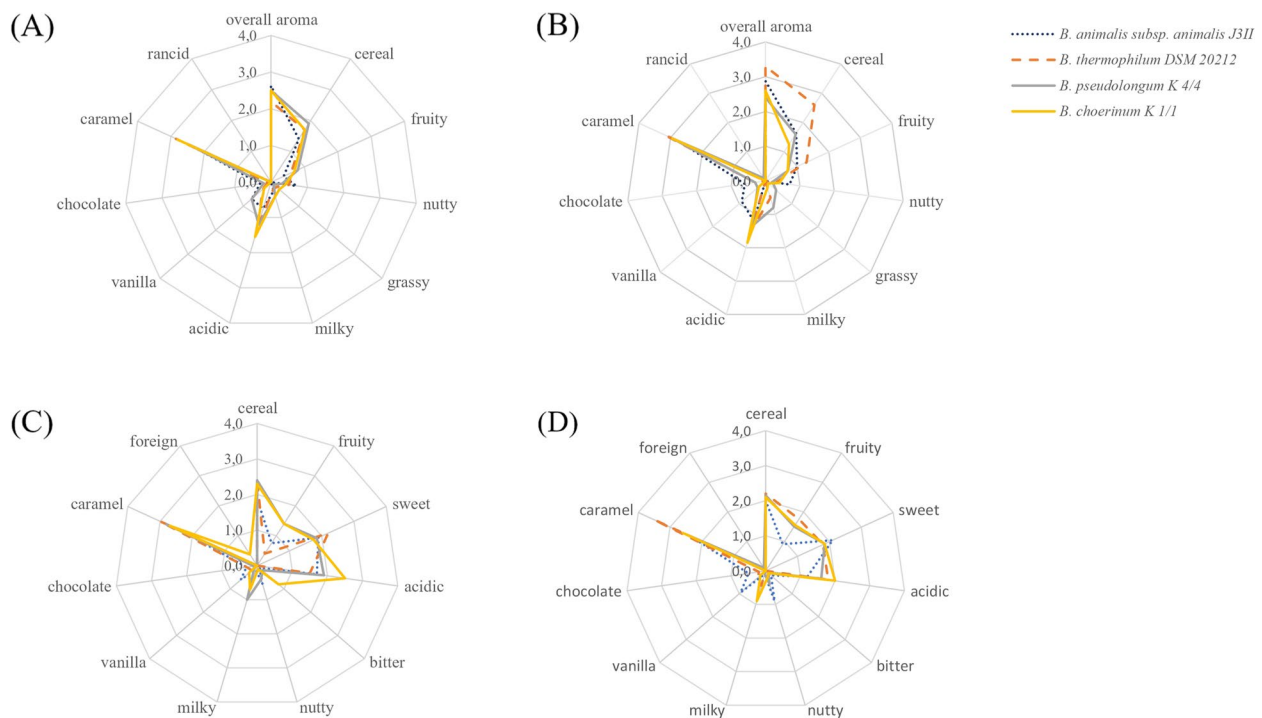


Fig. 3 The qualitative sensory evaluation of maize/caramel mashes within cold storage with bifidobacteria. **A** aroma after 14 days of storage; **B** aroma after 21 days of storage; **C** taste after 14 days of storage; **D** taste after 21 days of storage

consumer habits and a move towards increasing consumption of non-dairy products. Our results provide a theoretical reference for the industrial production of fermented maize caramel mashes. Accordingly, further optimization of the large-scale production technology and formula is an important step to meet the needs of industrialized production.

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Authors' contributions

Anna Mikulajová: conceptualization, methodology, writing—original draft, review, experiments, data analysis. Zuzana Matejčková: conceptualization, methodology, writing—original draft, review, experiments, data analysis. Zlatica Kohajdová: data analysis, writing—original draft, review. Silvia Mošovská: methodology, data analysis, review. Eva Hybenová: experiments, review. Ľubomír Valík: review, supervision. All authors read and approved the final manuscript.

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Availability of data and materials

All data supporting this study are included in this manuscript. Further details are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The research was carried out in accordance with the Declaration of Helsinki and the Code of Ethics of the Slovak University of Technology in Bratislava. According to national legislation, the sensory analysis does not need approval by the ethics committee, and it is not mandatory to get the written consent of participants. Participation was voluntary. Before the sensory evaluation was conducted, each participant was informed of the conditions for participating, the purpose of research and sensory evaluation, the process of evaluation, the evaluated products, the possible risks, and the possible benefits of the study. All participants agreed to participate in the study. The procedure of sensory evaluation was performed in compliance with International Standard ISO 13299.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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