

## Design of Bacterial Cultures in Fermented Functional Maize Product Formulation

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In this work, the effect of single fermentation of maize mashes with Fresco DVS 1010 culture (*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, and *Streptococcus thermophilus*) was studied for 8 h (37°C) followed by a storage period of 21 days (6°C). Although milk is a typical growth medium for lactic acid cocci of the Fresco culture, fermentation increased its counts by about 3 log CFU/mL after 8 h (37°C), whereas pH of 4.54 and 4.69 was achieved after the fermentation in products with sucrose and caramel, respectively. Fermentation process was observed to significantly increase ( $p < 0.05$ ) the total phenolics content (143.9–206.1%) and the antioxidant activities (53.7–107.4%) of the samples. Potentially probiotic *Lactobacillus plantarum* HM1, *Bifidobacterium choerinum* K1/1, *Bifidobacterium pseudolongum* K4/4, *Bifidobacterium animalis* ssp. *animalis* J3II, and *Bifidobacterium thermophilum* DSM 20212 inoculated after the fermentation process were well maintained ( $> 5$  log CFU/mL) in combination with the mixed Fresco culture in the prepared maize products within 21 days of storage. Based on the overall sensory acceptance, caramel mashes after 12 days of storage period were evaluated as satisfactory (2.4 to 3.1 from 4.0), while 21-day stored products achieved good acceptability scores (3.0 to 3.6 from 4.0), hence the tendency of the positive effect of prolonged shelf-life was noted.

### INTRODUCTION

Increased awareness of consumers over health and a steady increase in life expectancy have resulted in the demand for food products that can improve consumer well-being [Ogunremi *et al.*, 2015]. Therefore, the industry is directing development of new products towards the area of functional foods. Research in this area has moved progressively towards introducing the concept of synbiotics, a combination of pro- and prebiotics in a single product that may affect gut microbial composition. Cereals have a significant role in human nutrition in most parts of the world and are grown at over 73% of the total world harvest area [Charalampopoulos *et al.*, 2002]. Cereal grains represent an important nutritive component both in developed and in developing countries. They are considered as one of the most important sources of carbohydrates (starch and fiber), vitamins (group B), minerals, and phenolic compounds with many proven health effects. They also have the potential to offer consumer prebiotic and whole grain benefits [Lamsal & Faubion, 2009]. While lysine represents a limiting amino acid in cereals, a combina-

tion of such substrates with milk or dairy products constitutes one of the most appropriate ways of improving the nutritional value of final products. Although a variety of technologies (*e.g.*, cooking, sprouting, and milling) are used for cereal processing, fermentation still remains the most appropriate choice to improve the nutritional, sensorial, and shelf-life properties [Coda *et al.*, 2011]. This is the main reason why a large proportion of cereals is processed into foods and beverages by fermentation prior to consumption [Nout, 2009]. Indeed, lactic acid bacteria (LAB) (*Lactobacillus*, *Pediococcus* spp.), yeasts (*Candida*, *Debaryomyces*, *Hansenula*, *Pichia*, and *Saccharomyces* spp.), and filamentous fungi (*Amylomyces*, *Aspergillus*, *Mucor*, and *Rhizopus* spp.) are mainly involved in the manufacture of cereal-based beverages [Blandino *et al.*, 2003]. There are also few reports on the suitability of cereals as a carrier of probiotic LAB. An oat-based synbiotic drink made by fermenting an oat substrate with *Lactobacillus plantarum* B28 was developed in a study of Prado *et al.* [2008]. Pelikánová *et al.* [2015] proved the potential of maize flour for the production of fermented products containing lactobacilli that were well maintained above the suggested minimum level (6 log CFU/mL) during 21 days of storage at 6°C. While selecting a preferable probiotic strain, several aspects of functionality have to be considered. Bifidobacteria have long been recognized as bacteria possessing probiotic,

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nutritive, and therapeutic properties [Picard *et al.*, 2005]. Various health-promoting functions of bifidobacteria have poised them to be an ideal dietary supplement. There have been some studies describing incorporation of bifidobacteria in several kinds of dairy products, *e.g.*, fermented dairy milks [Kongo *et al.*, 2006] or cheese [Dinakar & Mistry, 1994]. Traditional fermented foods prepared from common types of cereals (rice, wheat, maize) are well known mainly in Asia and Africa. As the European market for probiotic products is expected to grow continuously, research on the development of food products containing probiotics and the isolation of new strains with potential probiotic properties is important. A considerable potential at manufacturing new products towards the area of functional foods led us to prepare functional food products with the probiotic potential for celiac patients based on gluten-free cereal. Thus, this study aimed at manufacturing and characterizing the chemical and microbiological properties of prepared fermented maize products as well as at determining the survival of selected bacteria of *Bifidobacterium* and *Lactobacillus* spp. genera. The next part of our study involved detailed sensory evaluation of final products, where sucrose was adjusted by caramel flavor to make products more attractive to consumers.

## MATERIALS AND METHODS

### Microorganism

A Fresco DVS 1010 starter culture consisting of *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, and *Streptococcus thermophilus* is a commercial culture from Christian and Hansen (Hørsholm, Denmark) and was kindly provided by Rajo a. s. (Bratislava, Slovakia). The Fresco culture was kept in a deep freezer. The starter culture was obtained aerobically overnight at  $30 \pm 0.5^\circ\text{C}$  in M17 broth (Biokar Diagnostics, Beauvais, France). A potentially probiotic isolate *L. plantarum* HM1 was isolated from breast milk and identified by Liptáková *et al.* [2016]. The isolate was first sub-cultured three times for 24 h at  $37 \pm 0.5^\circ\text{C}$  (5%  $\text{CO}_2$ ) in de Man, Rogosa and Sharpe (MRS) broth (Biokar Diagnostics, Beauvais, France) from the frozen stock containing MRS broth and 25% of glycerol before using it as an inoculum (stored at  $-30^\circ\text{C}$ ). The following potentially probiotic bifidobacterial strains were provided by prof. Vlková (Czech University of Life Sciences, Prague, Czech Republic): *Bifidobacterium choerinum* K1/1 and *Bifidobacterium pseudolongum* K4/4 were isolated from goat feces, *Bifidobacterium animalis* ssp. *animalis* J3II was isolated from lamb faeces, and *Bifidobacterium thermophilum* DSM 20212 is a collection strain (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Göttingen, Germany (DSMZ)). The sub-cultivation of bifidobacteria was performed in serum bottles in Wilkins-Chalgren (WCH) broth (Oxoid, Brno, Czech Republic) supplemented with 0.5% (w/v) of soya peptone (Oxoid, Brno, Czech Republic), Tween (0.1% (v/v); Biolife, Milan, Italy) and cysteine-hydrochloride (0.05% (w/v) Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was added to act as an oxygen scavenger to provide a low redox potential. Bifidobacterial strains were stored in WCH broth added with glycerol (20% v/v) before using it as an inoculum (stored at  $-20^\circ\text{C}$ ). Pure cultures of the studied bacteria were centrifuged ( $3461 \times g$  for

5 min, Centrifuge EBA 20, Hettichlab, Tuttlingen, Germany); the cell pellet was washed in 10 mL of sterile distilled water and centrifuged again under the same conditions. After centrifugation, supernatant was removed and pellets were re-suspended in distilled water to its original volume in compliance with the procedure of Matejčková *et al.* [2018].

### Enumeration of bacteria

The presumptive numbers of the Fresco culture were enumerated on M17 agar plates (Biokar Diagnostics, Beauvais, France) according to the STN ISO 15214. Inoculated Petri dishes were cultivated at aerobic conditions for 24 h ( $30 \pm 0.5^\circ\text{C}$ ). Presumptive numbers of *L. plantarum* were estimated using Vegiton MRS agar (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Inoculated Petri dishes were cultivated at anaerobic conditions at  $37 \pm 0.5^\circ\text{C}$  (5%  $\text{CO}_2$ ). Bifidobacteria were cultivated and enumerated on selective transgalactosylated oligosaccharide mixture propionate agar with added mupirocin (TOS-MUP) (Merck, Darmstadt, Germany). Petri dishes were cultivated at  $37 \pm 0.5^\circ\text{C}$  for 72 h under anaerobic conditions using anaerobic jars and Anaerocult A system (Merck, Darmstadt, Germany).

### Evaluation of growth and metabolic parameters

Growth and metabolic parameters (specific growth rate, rate constant for decrease of counts, rate constant for decrease of pH) of the studied LAB in maize mashes were fitted and calculated using the mechanistic model DMFit by Baranyi & Roberts [1995]. Growth and metabolic parameters were calculated from each growth curve. Specific growth rates  $\mu$  (1/h) were recalculated from the  $\log_{10}$  based growth rates ( $G_R$ ) according to the equation  $\mu = \ln 10 \times G_R$ .

The pH values were measured during fermentation and storage using a pH meter with a penetration electrode (Knick Portamess, Berlin, Germany).

### Preparation of maize substrate

The maize mash used as a substrate was prepared from maize flour (8% (w/v)) (Solčanka, Solčany, Slovak Republic), 2% (w/v) of sucrose, and ultra-pasteurized (UHT) milk (fat content 1.5%). Mashes were heated at  $100^\circ\text{C}$  for 20 min while stirred, and then sterilized for 20 min at  $121^\circ\text{C}$ . The mashes were subsequently cooled. In part of the samples, sucrose was replaced with a commercial caramel component (35% caramel syrup, 10% glucose fructose syrup, 28.5% sucrose, modified maize starch, caramelized sugar, water) (Agrana, Vienna, Austria), to achieve better sensory characteristics. In the flavored mashes, the caramel component was added after sterilization.

### Fermentation process

For static fermentation, maize mashes were inoculated with 5% (v/v) of Fresco DVS 1010 culture (Danisco, Copenhagen, Denmark) to achieve inoculation levels of approximately 6 log CFU/mL. Static fermentation was performed for 8 h at  $37 \pm 0.5^\circ\text{C}$  (5%  $\text{CO}_2$ ). The samples for microbiological analyses of counts and pH values were taken every 2 h. Potential probiotic strains of LAB ( $n=5$ ) were inoculated into the substrates after the fermentation process in concen-

trations of approximately 8–9 log CFU/mL. Subsequently, the mashers remained for another 21 days at  $6 \pm 0.5^\circ\text{C}$  with periodical determination of pH values and viability of the studied LAB. The experiments were carried out in duplicate.

### Sensory evaluation

The procedure of sensory evaluation was conducted in accordance with ISO 13299 in the Laboratory of Sensory Analysis of the Slovak University of Technology in Bratislava, Slovak Republic. The detailed sensory assessment of the samples was performed with implementation of the Quantitative Descriptive Analysis (QDA) according to the procedure described by Stone *et al.* [2012]. The individual samples of each maize mash with caramel products (weighing approximately 15 g) were placed in transparent, odorless, plastic boxes (125 mL) covered with lids. The samples were evaluated at room temperature ( $20\text{--}22^\circ\text{C}$ ). Still mineral water was used for rinsing palates between samples as a neutralizer. The sample sets for each evaluator were coded individually with three-digit numbers to avoid the carry over effect. The samples were presented randomly. Finally, 10 trained assessors (9 women and 1 men) between the ages of 25 and 43 (mean = 32.6) were chosen from sensory experts trained in accordance with STN EN ISO standard 8586–2, having broad experience in sensory evaluation of different food products.

Sensory analysis included characteristics of color, consistence, texture, overall acceptability as well as individual descriptors of aroma and taste. Overall sensory quality was evaluated using a hedonic scale for each attribute (0 representing “dislike extremely” and 4 representing “like extremely”). Fresh fermented mashers ( $37 \pm 0.5^\circ\text{C}/8$  h) and stored products ( $6 \pm 0.5^\circ\text{C}$  for 12 and 21 days) were evaluated at the same time.

### Sample extract preparation

Samples were freeze-dried and extracted using 65% (v/v) ethanol for 1 h at  $80^\circ\text{C}$ , three times [Mikulajová *et al.*, 2007a]. Phenolic compounds were subsequently reextracted with ethyl acetate (Centralchem, Bratislava, Slovak Republic), concentrated to dryness, and dissolved in 96% (v/v) ethanol. The ethanolic extracts obtained were used for determination of total phenolics content, total flavonoids content, and antioxidant activity that was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay and by ferric reducing antioxidant power (FRAP) assay.

### Analysis of total phenolics content (TPC)

The total phenolics content was determined using the Folin–Ciocalteu’s reagent (Sigma-Aldrich, Steinheim, Germany) according to Yu *et al.* [2002]. Gallic acid (Sigma-Aldrich, Steinheim, Germany) was used as a standard. Gallic acid base solution in 96% (v/v) ethanol (1.0 mg/mL) was diluted to obtain 0.01–0.80 mg/mL concentrations. Results were expressed in mg of gallic acid equivalent per gram of sample (mg GAE/g).

### Analysis of total flavonoids content (TFC)

The total flavonoids content was determined using the AlCl<sub>3</sub> method described by Kumar *et al.* [2011]. Quercetin

(Sigma-Aldrich, Steinheim, Germany) was used as a standard. Quercetin base solution in 96% (v/v) ethanol (1.0 mg/mL) was diluted to obtain 0.01–0.40 mg/mL concentrations. Results were expressed in mg of quercetin equivalent per gram of sample (mg QE/g).

### Determination of antiradical activity

The antiradical activity was determined by using stable free radicals (DPPH<sup>•</sup>) (Sigma-Aldrich, Steinheim, Germany) as described by Yen & Chen [1995]. Absorbance of the samples was measured at 517 nm after 10 min of reaction. Results were expressed as the amount of scavenged DPPH radicals per gram of sample (mg DPPH/g).

### Analysis of ferric reducing antioxidant power (FRAP)

FRAP was measured according to Pohanka *et al.* [2009]. Absorbance of the samples was measured at 730 nm after 30 min of reaction. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox; Sigma-Aldrich, Steinheim, Germany) was used as a standard and results were expressed in mg of Trolox equivalent per gram of sample (mg Trolox/g).

### Statistical analysis

All the results are expressed as means  $\pm$  standard deviation (SD). Each fermentation experiment was carried out in duplicate and each analysis was performed in three separate trials. Statistical analyses were carried out using Microsoft Excel 2013 (Microsoft, Redmond, U.S.A.). Data were analyzed by Student’s *t*-test with a least significant difference of 95% and by correlation analysis. Correlation coefficients were determined according to Asuero *et al.* [2006].

## RESULTS AND DISCUSSION

In this study, maize flour was selected as a substrate for lactic acid fermentation and vehicles for probiotics. To protect probiotic bacteria, especially bifidobacteria from oxygen in yoghurt, the incorporation of high oxygen consuming strains of *S. thermophilus* has been proposed. *S. thermophilus* alone or as a part of mesophilic cultures has been suggested as a solution for reduction of fermentation time with increasing growth rates; absence of certain sensory and textural defects and further improvement of nutritional value of ‘bifidus’ products [Gomes & Malcata, 1999]. Thus, based on our previous research [Matejčková *et al.*, 2018], and the above facts, a mixed Fresco DVS 1010 culture was selected for lactic acid fermentation of the prepared maize mashers.

### Microbial counts and stability during cold storage period

Within 5% (v/v) concentration of the mixed Fresco culture at 8 h fermentation the levels reached populations of  $9.15 \pm 0.24$  log CFU/mL representing 3 log units increase compared to the initial state. Short fermentation time (8 h) was preferable in order to minimize the risk of contamination. Pelikánová *et al.* [2015] also proved the potential of maize substrates for lactic acid fermentation within 10 h when growth rates of lactobacilli ranged from 0.155 to 0.811 log CFU/(mL $\times$ h). In our study, Fresco culture entered immediately the exponential phase of growth with almost the same

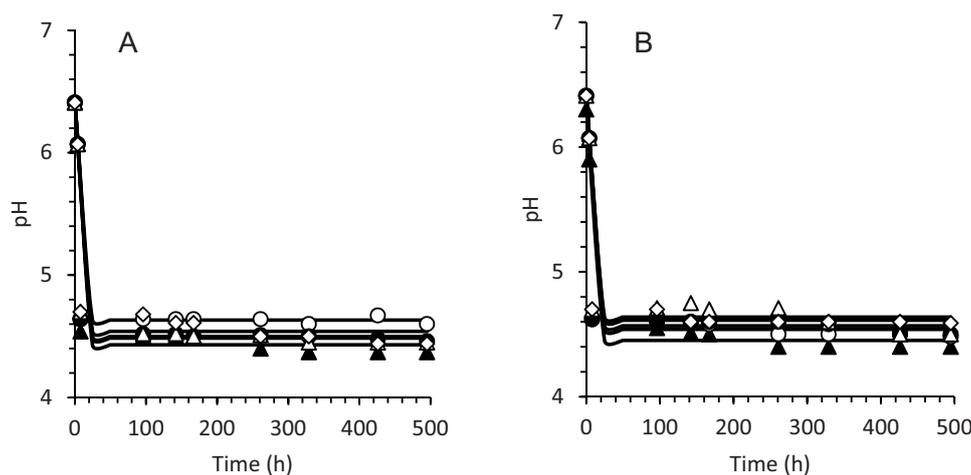


FIGURE 1. Changes in pH as a function of incubation time of milk-based maize mashes with sucrose (A) and caramel (B) inoculated with Fresco DVS 1010 and next (after 8 h) with *L. plantarum* HM1 (▲), *B. thermophilum* DSM 20212 (○), *B. animalis* ssp. *animalis* J3II (●), *B. pseudolongum* K4/4 (Δ), and *B. choerinum* K1/1 (◇).

specific growth rates in milk-based maize mash with sucrose ( $\mu_{\text{suc}} = 1.34$  1/h) and caramel ( $\mu_{\text{car}} = 1.33$  1/h) (equivalent to a generation time of 31 min; data not shown). In our previous study, addition of chocolate flavour in buckwheat substrates increased the specific growth rates of the cocci of the Fresco culture by about 1.4–30.3% compared to those with sucrose only [Matejčková *et al.*, 2017]. Due to the accumulation of lactic acid produced as a result of metabolic activity, a rapid drop in pH was observed in single fermentation process (Figure 1). The pH values measured after 8 h of fermentation were 4.54 and 4.69 in maize products with sucrose and caramel, respectively. In our previous study, efficient acidification profiles (pH below 4.5) were generally achieved during co-culture fermentation of Fresco culture with *L. plantarum* [Matejčková *et al.*, 2018]. Salmerón *et al.* [2014] noted a decrease of pH below 3.7 after 10 h in cereal beverages (oat, barley, and malt substrates), whereas Rathore *et al.* [2012] recorded pH below 3.5 for all cereal fermentations both with *L. acidophilus* and *L. plantarum* strains.

Good viability of the microbial starter culture is the first and the most important criterion to ensure good quality and health-promoting properties of the product. To achieve health benefits, the minimum level of 6 log CFU/mL, concerning the population of probiotic microorganism at the end of the product shelf life, is usually considered as acceptable [Georgieva *et al.*, 2009]. Thus, the changes in cell counts of potentially probiotic bacteria were evaluated. In general, the bacterial counts in samples decreased by about 0.3–1.7 log units at 6°C during the period of 21 days and were well maintained above the limit of 6 log CFU/mL except *B. choerinum* K1/1 (Figure 2). Despite the decline in levels of *B. choerinum* K1/1 strain in the product with sucrose by about 3.8 log units, the counts remained above 5 log CFU/mL, *i.e.* at the minimum level of probiotics suggested by some authors [Gueimonde *et al.*, 2004]. Hence, a beneficial relationship occurred between cocci of the Fresco culture and *Bifidobacterium* spp. (*L. plantarum* HM1) in the prepared products. Concentrations of Fresco culture were not significantly affected ( $p > 0.05$ ) by the potentially probiotic strains tested, whereas

average counts varied within  $9.08 \pm 0.36$  log CFU/mL after 21 days (data not shown). Gueimonde *et al.* [2004] evaluated counts of LAB in commercial fermented milks, whereas counts of streptococci were present at levels ranging from 7 to 9 log CFU/mL during cold storage (4°C), while bifidobacteria showed a decrease between 0.17 and 1.10 log units. Co-culture fermentation of milk by *B. lactis* and *L. acidophilus* (1:1 inoculum ratio) led to the enhanced growth rates and acidification profiles when compared with single strain, suggesting some degree of symbiosis [Gomes & Malcata, 1999].

Over 21 days, *L. plantarum* and *Bifidobacterium* spp. strains continued metabolism of saccharides and accumulation of organic acids resulting in pH decrease to 4.37–4.60 (Table 1), representing a decline of about 1.8–2.0 units. Survival of bifidobacterial strains is dependent on pH of the environment. While low pH decreases their survival, tolerance of *Bifidobacterium* spp. to acidic conditions has been reported to be strain specific [Kailaspathy & Chin, 2000]. Despite this fact, in our study, all tested bifidobacterial strains of different origin were well maintained in the final products as reported above. Nowadays, the origin of probiotics from the human gastrointestinal tract intended for human consumption is not an essential criterion. Zielińska & Kolożyn-Krajewska [2018] showed that several microorganisms found in consumed food products did not originate from human hosts, including *e.g.* *B. animalis* ssp. *lactis* and *Saccharomyces cerevisiae* var. *boulardii*.

#### Determination of contents of total phenolics and total flavonoids

An increase in TPC (Table 2) after the fermentation process (8 h) from 0.093 to 0.226 mg GAE/g and from 0.130 to 0.398 mg GAE/g was observed in maize mashes with sucrose and caramel, respectively. Inoculation with LAB strains and storage for 21 days (6°C) resulted in an increase of TPC by about 10.1–33.7% in the samples with sucrose, and in a decrease by about 11.7–17.9% in caramel mashes except the product with *B. pseudolongum* K4/4 strain, where TPC did not change significantly ( $p > 0.05$ ). Lactic acid fermentation of the prepared samples resulted in significantly higher

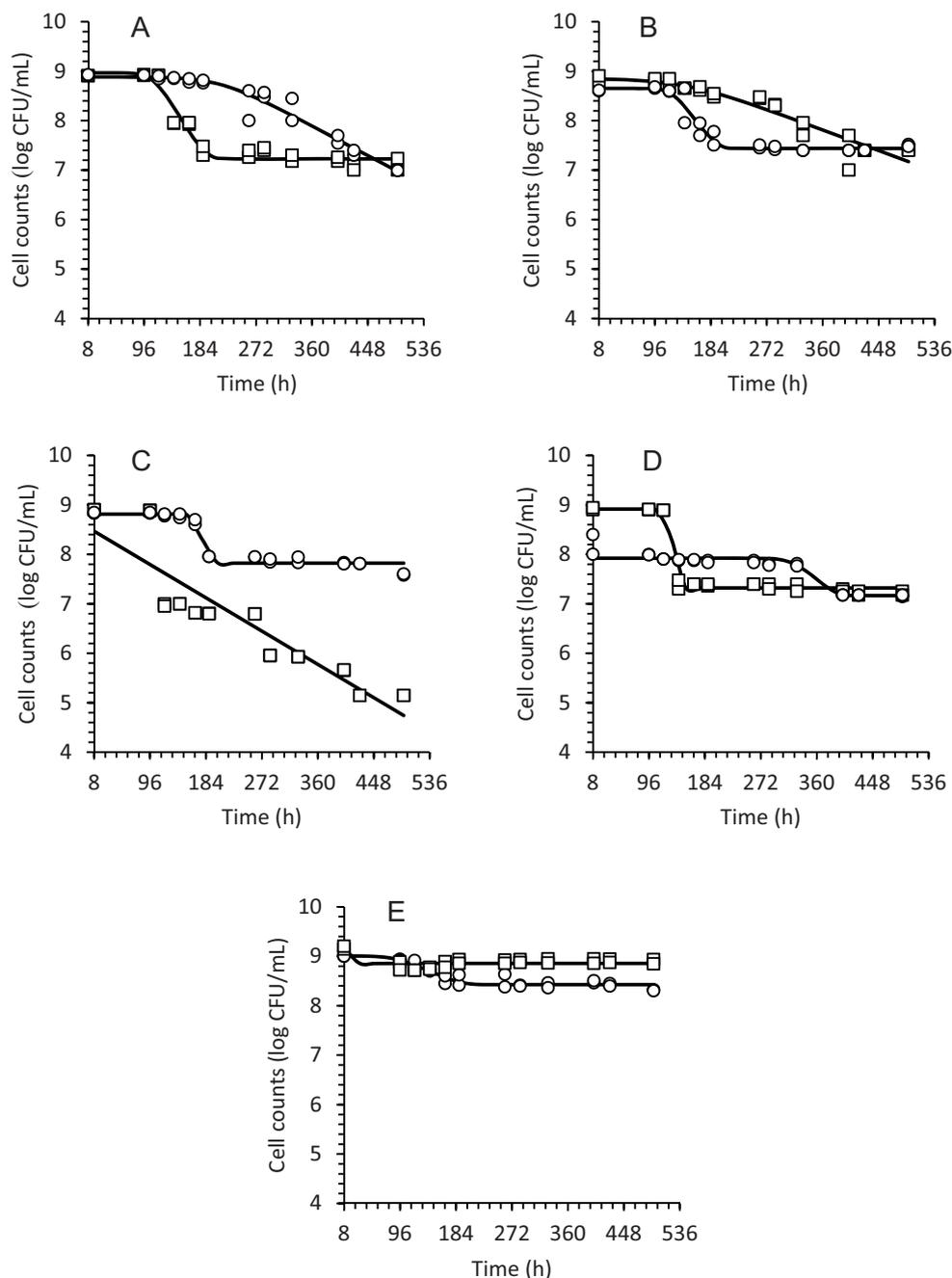


FIGURE 2. Survival of *B. thermophilum* DSM 20212 (A), *B. animalis* ssp. *animalis* J3II (B), *B. choerinum* K 1/1 (C), *B. pseudolongum* K 4/4 (D), and *L. plantarum* HM1 (E) strains during cold storage of fermented (8 h, Fresco DVS 1010) milk-based maize mashes with sucrose ( $\square$ ) and caramel ( $\circ$ ).

levels of phenolic compounds (151.4–226.1%) in comparison to the non-fermented ones. Katina *et al.* [2007] observed an increase in TPC by about 17% in rye fermented by *L. plantarum* at 30°C (20 h) compared to the non-fermented sample.

Fermentation process also significantly ( $p < 0.05$ ) increased TFC by about 50.8 and 92.1% in the product with sucrose and caramel, respectively. In fermented and 21-day stored with LAB strain products, TFC was significantly higher compared to the non-fermented samples (17.6–136.7%).

Our results showed that even short fermentation time (8 h) was able to increase phenolic compounds levels. Several mechanisms of increasing phenolics content are reported. Microbial enzymes together with those derived from cereals (amylase,

xylanase, and protease) may disrupt cell walls of grains resulting in the release of components bound in the cell structures. By this means, an increase is observed in the amount of phenolic compounds occurring in the bounded insoluble form of covalent bonds linked to the cell wall components [Katina *et al.*, 2007; Hur *et al.*, 2014]. Furthermore, *Bacillus subtilis* and *L. plantarum* strains showed a glucosidase activity [Wang *et al.*, 2014], leading to the release of phenolic compounds from their soluble conjugated forms (bond to the carbohydrate components). Moreover, lactic acid produced by microbiota can help release bounded phenolics to their free forms. Increasing substrate acidity may have various effects on the stability of releasing phenolic compounds, and it can result in their dif-

TABLE 1. Parameters evaluating behaviour of lactic acid bacteria after they had been added to fermented (8 h, Fresco DVS 1010) milk-based maize mashes with sucrose and caramel and stored for 21 days.

Maize mash product	$k_{d1}$ (log CFU/(mL×h))	$N_{end1}$ (log CFU/mL)	$k_{pH1}$ (1/h)	$pH_{end1}$	$k_{d2}$ (log CFU/(mL×h))	$N_{end2}$ (log CFU/mL)	$k_{pH2}$ (1/h)	$pH_{end2}$
	Sucrose				Caramel			
Fresco + <i>L. plantarum</i> HM1	-0.183	8.9 <sup>a</sup>	-0.440	4.37	-0.005	8.3 <sup>a</sup>	-0.326	4.40
Fresco + <i>B. pseudolongum</i> K 4/4	-0.088	7.2 <sup>c</sup>	-0.374	4.45	-0.011	7.2 <sup>d</sup>	-0.409	4.50
Fresco + <i>B. animalis</i> subsp. <i>animalis</i> J3II	-0.004	7.4 <sup>b</sup>	-0.401	4.46	-0.016	7.5 <sup>c</sup>	-0.439	4.50
Fresco + <i>B. thermophilum</i> DSM 20212	-0.021	7.2 <sup>c</sup>	-0.511	4.60	-0.007	6.9 <sup>c</sup>	-0.424	4.48
Fresco + <i>B. choerinum</i> K 1/1	-0.008	5.1 <sup>d</sup>	-0.381	4.44	-0.035	7.6 <sup>b</sup>	-0.396	4.59

$k_d$  – rate constant for decrease of counts,  $N_{end}$  – counts after storage period,  $k_{pH}$  – rate constant for decrease of pH,  $pH_{end}$  – pH value after storage period, <sup>a-c</sup> Means within a column with different superscript letters differ significantly ( $p < 0.05$ ).

TABLE 2. Content of total phenolics and total flavonoids in fermented products of milk-based maize mashes with sucrose and caramel.

Maize mash product	Storage	Total phenolics content (mg GAE/g)		Total flavonoids content (mg QE/g)	
		Sucrose	Caramel	Sucrose	Caramel
After sterilization (non-fermented)		0.093±0.001 <sup>e,y</sup>	0.130±0.002 <sup>d,x</sup>	0.396±0.012 <sup>f,x</sup>	0.326±0.015 <sup>e,y</sup>
8 h after fermentation (Fresco DVS 1010)	F	0.223±0.005 <sup>d,y</sup>	0.398±0.008 <sup>a,x</sup>	0.597±0.012 <sup>d,y</sup>	0.627±0.006 <sup>d,x</sup>
Fresco + <i>L. plantarum</i> HM1	21 D	0.303±0.008 <sup>a,y</sup>	0.327±0.002 <sup>c,x</sup>	0.697±0.003 <sup>b,y</sup>	0.748±0.030 <sup>ab,x</sup>
Fresco + <i>B. pseudolongum</i> K 4/4	21 D	0.249±0.006 <sup>c,y</sup>	0.410±0.003 <sup>a,x</sup>	0.577±0.013 <sup>d,y</sup>	0.650±0.009 <sup>c,x</sup>
Fresco + <i>B. animalis</i> ssp. <i>animalis</i> J3II	21 D	0.253±0.003 <sup>c,y</sup>	0.345±0.011 <sup>c,b,x</sup>	0.466±0.005 <sup>e,y</sup>	0.723±0.017 <sup>b,x</sup>
Fresco + <i>B. thermophilum</i> DSM 20212	21 D	0.295±0.004 <sup>a,y</sup>	0.351±0.010 <sup>b,x</sup>	0.632±0.008 <sup>c,y</sup>	0.773±0.019 <sup>a,x</sup>
Fresco + <i>B. choerinum</i> K 1/1	21 D	0.265±0.003 <sup>b,y</sup>	0.339±0.001 <sup>b,x</sup>	0.818±0.018 <sup>a,x</sup>	0.664±0.008 <sup>c,y</sup>

F – fermented product; 21 D – fermented stored product after 21 days of storage; <sup>a-c</sup> Means within a column with different superscript letters differ significantly ( $p < 0.05$ ), <sup>x,y</sup> Means within a line with different superscript letters differ significantly ( $p < 0.05$ ).

ferent extractability. According to Schmidt *et al.* [2014], ferulic and gallic acid showed the most substantial increase during fermentation of rice bran with *Rhizopus oryzae*.

#### Determination of antioxidant activity

The antioxidant activity of maize mash products was evaluated as antiradical activity against DPPH radicals and as FRAP. These methods are widely used to evaluate the antioxidant capacity [Rufino *et al.*, 2010]. The antioxidant activity of maize mashes determined by both methods increased within 8 h of fermentation (like TPC and TFC). Compared to the non-fermented product, the antiradical activity was higher about 80.5 and 53.7% for the samples with sucrose and caramel, respectively, FRAP increased about 80.2 and 107.4%, respectively (Table 3). After 21 days of storage, an increase was observed in DPPH radical scavenging activity and FRAP of maize mash with sucrose and *L. plantarum* HM1 (Table 3). Products containing sucrose and *Bifidobacterium* strains showed a lower antioxidant activity at the end of storage compared to the fermented product (8 h). DPPH<sup>•</sup> scavenging activity of all products with caramel decreased during 21 days of storage. FRAP decreased only in products with

*B. animalis* J3II and *B. pseudolongum* K 4/4. Different studies demonstrated that phenolics content and antioxidant activity firstly increased significantly to the certain time, and then increased slightly [Schmidt *et al.*, 2014] or decreased [Salar *et al.*, 2012] during fermentation. Fermentation of maize by *Thamnidium elegans* CCF-1456 at 25°C in a study of Salar *et al.* [2012] proved the maximum TPC content and the highest antiradical activity on the 5<sup>th</sup> day of incubation, with similar change tendency noted in the activity of carbohydrate hydrolysing enzymes ( $\alpha$ -amylase,  $\beta$ -glucosidase, and xylanase) during fermentation. In cereal matrixes, antioxidant activity was highly correlated to the phenolics content [Mikulajová *et al.*, 2007b, 2015]. Results of the present study also supported this finding, where moderate, high and/or very high correlations were found between TPC and results of DPPH assay (correlation coefficient ( $r$ )=0.628 and  $r$ =0.795 for products with sucrose and caramel, respectively), between TPC and FRAP ( $r$ =0.877 and  $r$ =0.918, respectively), as well as between TFC and FRAP ( $r$ =0.770 and  $r$ =0.889, respectively). This fact indicates that phenolic compounds are involved in the antioxidant properties of final products. These results are in agreement with our previous findings [Mikula-

TABLE 3. Antioxidant activity of fermented products of milk-based maize mashes with sucrose and caramel determined by DPPH and FRAP assays.

Maize mash product	Storage	DPPH scavenging activity (mg DPPH/g)		FRAP (mg Trolox/g)	
		Sucrose	Caramel	Sucrose	Caramel
After sterilization (non-fermented)		0.525±0.010 <sup>e,y</sup>	0.569±0.003 <sup>d,x</sup>	0.172±0.002 <sup>e,y</sup>	0.215±0.004 <sup>c,x</sup>
8 h after fermentation (Fresco DVS 1010)	F	0.948±0.011 <sup>b,x</sup>	0.874±0.008 <sup>a,y</sup>	0.309±0.001 <sup>b,y</sup>	0.446±0.012 <sup>a,x</sup>
Fresco + <i>L. plantarum</i> HM1	21 D	0.983±0.012 <sup>a,x</sup>	0.670±0.021 <sup>c,y</sup>	0.344±0.007 <sup>a,y</sup>	0.424±0.017 <sup>a,b,x</sup>
Fresco + <i>B. pseudolongum</i> K 4/4	21 D	0.778±0.021 <sup>c,x</sup>	0.764±0.024 <sup>b,y</sup>	0.297±0.007 <sup>b,c,y</sup>	0.406±0.004 <sup>b,x</sup>
Fresco + <i>B. animalis</i> ssp. <i>animalis</i> J3II	21 D	0.775±0.010 <sup>c,x</sup>	0.664±0.018 <sup>c,y</sup>	0.260±0.009 <sup>d,y</sup>	0.407±0.006 <sup>b,x</sup>
Fresco + <i>B. thermophilum</i> DSM 20212	21 D	0.790±0.022 <sup>c,x</sup>	0.706±0.020 <sup>b,c,y</sup>	0.284±0.003 <sup>c,y</sup>	0.423±0.008 <sup>a,b,x</sup>
Fresco + <i>B. choerinum</i> K 1/1	21 D	0.602±0.015 <sup>d,y</sup>	0.664±0.012 <sup>c,x</sup>	0.301±0.005 <sup>b,y</sup>	0.446±0.002 <sup>a,x</sup>

F – fermented product; 21 D – fermented stored product after 21 days of storage; <sup>a-f</sup> Means within a column with different superscript letters differ significantly ( $p < 0.05$ ), <sup>x,y</sup> Means within a line with different superscript letters differ significantly ( $p < 0.05$ ).

TABLE 4. Evaluation of sensory attributes of fermented products of milk-based maize mashes with caramel.

Maize mash product	Storage	Colour	Consistency	Texture	Overall acceptability
8 h of fermentation (Fresco DVS 1010)	F	3.7±0.5 <sup>a</sup>	1.5±0.5 <sup>c</sup>	1.3±0.5 <sup>c</sup>	1.7±0.5 <sup>f</sup>
Fresco + <i>L. plantarum</i> HM1	12 D	2.9±0.5 <sup>d</sup>	2.0±0.5 <sup>b</sup>	2.5±0.5 <sup>b</sup>	2.9±0.6 <sup>b,c</sup>
	21 D	3.3±0.4 <sup>b,c</sup>	2.3±0.4 <sup>b</sup>	2.8±0.4 <sup>b</sup>	3.3±0.4 <sup>b</sup>
Fresco + <i>B. pseudolongum</i> K 4/4	12 D	3.3±0.6 <sup>a,b,d</sup>	2.4±0.5 <sup>a,b</sup>	2.8±0.7 <sup>a,b</sup>	3.0±0.6 <sup>b</sup>
	21 D	3.4±0.5 <sup>a,b</sup>	2.3±0.4 <sup>a,b</sup>	2.8±0.7 <sup>a,b</sup>	3.2±0.4 <sup>b</sup>
Fresco + <i>B. animalis</i> ssp. <i>animalis</i> J3II	12 D	3.1±0.5 <sup>b,d</sup>	2.5±0.5 <sup>a</sup>	2.9±0.5 <sup>a,b</sup>	3.1±0.8 <sup>a,b,d</sup>
	21 D	3.3±0.5 <sup>a,b,d</sup>	2.1±0.3 <sup>b</sup>	2.5±0.5 <sup>b</sup>	3.6±0.5 <sup>a</sup>
Fresco + <i>B. thermophilum</i> DSM 20212	12 D	3.5±0.5 <sup>a,b</sup>	2.4±0.5 <sup>a,b</sup>	2.8±0.4 <sup>b</sup>	2.8±0.4 <sup>e,d</sup>
	21 D	3.1±0.4 <sup>c,d</sup>	2.3±0.7 <sup>a,b</sup>	2.8±0.4 <sup>b</sup>	3.1±0.5 <sup>b,d</sup>
Fresco + <i>B. choerinum</i> K 1/1	12 D	3.3±0.8 <sup>a,b,d</sup>	2.4±0.5 <sup>a,b</sup>	2.6±0.5 <sup>b</sup>	2.4±0.5 <sup>c</sup>
	21 D	3.4±0.6 <sup>a,b</sup>	2.4±0.5 <sup>a,b</sup>	3.3±0.6 <sup>a</sup>	3.3±0.7 <sup>a,b</sup>

F – fermented product; 12 D – fermented stored product after 12 days; 21 D – fermented stored product after 21 days; 0 – worst possible value, 4 – best possible value; the results are means±standard deviation of ten evaluations, <sup>a-f</sup> Means within a column with different superscript letters differ significantly ( $p < 0.05$ ).

joyá et al., 2007b, 2015], where correlations with  $r$  ranging from 0.794 to 0.984 were noted depending on types of cereals. In baked round rolls fermented with *Saccharomyces cerevisiae*, a very high correlation ( $r=0.987$ ) was recorded compared to fermented milk maize products. This fact could be attributed to the interactions of raw material used, e.g. formation of complexes of phenolic compounds with milk components [Muniandy et al., 2016]. Within fermentation process, changes in component structure are observed with synthesis of new components, which differ in their features and availability for extraction and analytical determinations.

### Sensory analysis

Detailed sensory evaluation of final products with caramel flavour was assessed in this section. Caramel flavour was added to make products more attractive and sensory accept-

able to consumers, thus only caramel mashes were sensory analysed. Fresh fermented products and products stored for 12 and 21 days were evaluated, to determine the differences within the storage period.

According to the employed method of profile assessment, the following significant quality characteristics were determined for maize mash products with caramel: evaluation of colour, consistency, texture and overall acceptability (Table 4), as well as taste and aroma as individual descriptors (Figure 3). Scores given for colour to the milk-based maize mashes ranged from 2.9 to 3.7, indicating very good, almost excellent colour of the prepared products. In our previous study [Matejčková et al., 2018], storage period did not significantly ( $p > 0.05$ ) affect the evaluation of colour, which was almost the same compared to the products right after single fermentation process. The attributes of taste and aroma were

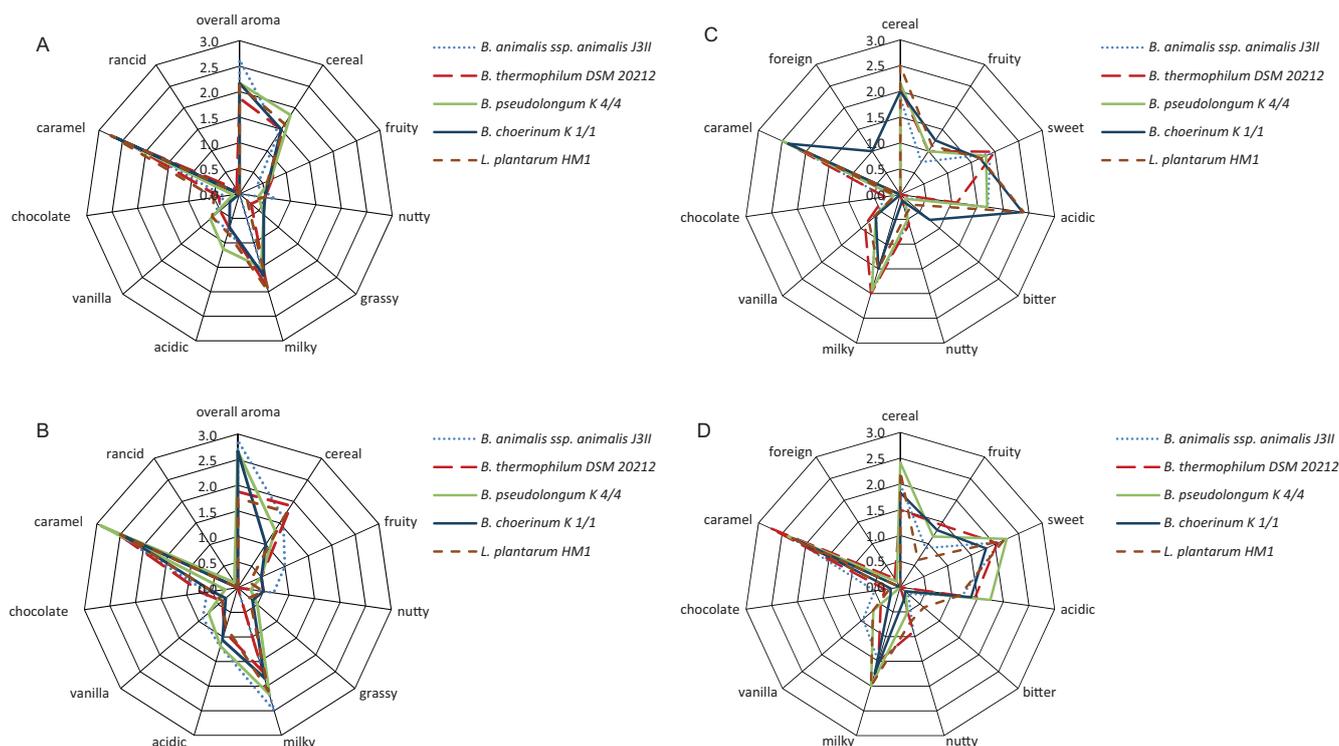


FIGURE 3. The qualitative sensory evaluation of milk-based maize mashes with caramel after fermentation (8 h, Fresco DVS 1010) and cold storage with lactic acid bacteria strains. A – aroma after 12 days of storage; B – aroma after 21 days of storage; C – taste after 12 days of storage; D – taste after 21 days of storage.

divided by the descriptors that could have positive and negative effects on the overall sensory value of final products. For better interpretation, the results are presented in spider web graphics (Figure 3). In the final flavoured products, the main purpose was to preserve the primary descriptors – caramel, cereal, and milky aroma/taste. In addition, the evaluation of unacceptable aroma/taste (e.g., rancid, foreign), was an important step in the assessment. Several species or subspecies of LAB may provide different sensory and physicochemical characteristics to the products. The final metabolic compounds of some cultures, either pure or mixed, may provide unpleasant sensory characteristics. Moreover, bifidobacteria are able to produce acetic and lactic acids in a proportion of 3:2 during fermentation, hence, the growth and survival of these bacteria may enhance the acetic flavour of final products, which undermines their sensory acceptance [Cruz *et al.*, 2010]. In the prepared mashes, intensity of the overall aroma ranged from 1.8 to 2.9 (Figure 3), representing moderate to strong intensity. In the stored products, cereal aroma was evaluated as moderate, representing an average value of 2.3, while in the product right after the fermentation (8 h) it was evaluated as the strongest (2.9). The same results were reported in our previous research [Matejčková *et al.*, 2018], when cereal aroma was evaluated as the strongest (2.3) in buckwheat lactose-free products after the fermentation (8 h). In our study, several assessors identified nutty, fruity, vanilla or chocolate aroma, but they were rated as with low intensity. Unacceptable foreign aroma (0.02) was also noted in the samples but with almost unnoticeable intensity. As for the evaluation of taste (Figure 3), the descriptor denoting cereal taste prevailed in the mashes with *L. planta-*

*rum* HM1 and *B. pseudolongum* K4/4 strains (average value of 2.5). In other products, cereal taste was scored from 1.5–2.2, indicating moderately strong cereal taste. Unacceptable bitter and foreign taste were evaluated as slight (0.2).

Final evaluation of the overall acceptability (Table 4) of fermented milk-based mashes with caramel was one of the most important steps pointing to the total sensory acceptability of the final products. Caramel mashes after 21 days of storage received higher scores (3.0–3.6), which indicated good acceptability when compared to the fermented product (1.7), thus the positive effect of storage period was noted. In our previous study, we had reported an increase of the overall acceptability over a storage period in buckwheat chocolate and caramel products [Matejčková *et al.*, 2017]. On the other hand, Kocková & Valík [2014] noted a negative effect of 21-day storage period on the overall acceptability of buckwheat product with salt fermented by *L. rhamnosus* GG which decreased from 3.31 to 2.44 points.

## CONCLUSION

Ensuring high quality and safety of fermented products requires a deep understanding of the fermentation process, as well as types and roles of microbes in final products characteristics. In the present study, we assessed the use of potentially probiotic microorganisms with emphasis on *L. plantarum* and *Bifidobacterium* spp. as live supplements in the prepared fermented (8h) milk-based maize mashes. *Bifidobacterium* spp. strains and *L. plantarum* survived adequately (>5 log CFU/mL) to confer potentially probiotic properties of the fermented and stored mashes. The high levels of LAB

and the production of organic acids did not cause undesirable changes in the overall acceptability of final caramel products. Our results showed that even short fermentation time (8 h) was able to increase levels of phenolic compounds and anti-radical activity of fermented samples compared to the non-fermented product. Thus, if the right amount of cereal substrate and promoting synergy between selected LAB within storage is observed, good bioactive properties and probiotic potential are formulated. Therefore, inclusion of probiotic bacteria in fermented products enhances their value as therapeutic functional foods.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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