Suitability of lactic acid bacteria for fermentation of maize and amaranth

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Summary
Fermentation of cereals by lactic acid bacteria may represent a cheap way of production of nutritionally rich foods. It can also bring a new trend in the development of new functional foods suitable for celiac patients or for people suffering from lactose intolerance. In our work, we focused on the growth and metabolic activity of selected lactobacilli in cereal mashes prepared from amaranth or maize flour, saccharose and water or milk. All strains showed sufficient growth and survival in mashes with the growth rates ranging from 0.155 log CFU·ml^{-1}h^{-1} to 0.819 log CFU·ml^{-1}h^{-1}. At the end of cold storage, densities of lactobacilli were maintained above the limit 10^6 CFU·ml^{-1} (10^6 CFU·g^{-1}) that is essential to maintain a potential probiotic effect in the gastrointestinal tract. After storage, 264–8 100 mg·kg^{-1} lactic acid was found to be contained, with significantly higher contents in milk-based mashes. The pH levels in the products were reduced to 3.6–5.5. From 2 g of saccharose added to 100 g of the mashes, only small amounts of 0.11–0.95 mg were utilized by lactobacilli during fermentation. This means that they used other easily accessible carbohydrate sources from the cereal components.

Keywords
Lactobacillus; celiac disease; cereal; pseudocereal; fermentation

Cereals have a significant role in human nutrition. They are grown on over 73% of the total world harvested area and contribute by more than 60% to the world food production. Cereals are a source of specific carbohydrates, proteins, lipids, dietary fibre and of a wide spectrum of vitamins and minerals [1–3]. In recent years, cereals have been investigated regarding their potential use in developing functional foods. Although cereals are deficient in some basic components, e.g. essential aminoacids, fermentation of these substrates by lactic acid bacteria may represent the simplest and economical way in improving their nutritional value, sensory properties and functional qualities [2]. Cereal-based fermented foods could be potential vehicles for many functional compounds, such as antioxidants, dietary fibre, minerals, probiotics or vitamins [4].

Lactic acid fermentation of cereals is a long-established processing method, which is being used in Asia and Africa for the production of foods in various forms such as bread, beverages, gruels and porridge prepared from most common types of cereals (such as rice, wheat, corn or sorghum) [5]. In most of these products, the fermentation is natural and involves mixed cultures of bacteria, yeasts and fungi. The type of bacterial flora developed in each fermented food depends on the water activity, pH, salt concentration, temperature and the composition of the food matrix. Most fermented foods, including the major products that are common in the western world, are dependent on lactic acid bacteria to mediate the fermentation process. Lactic acid fermentation contributes to the safety, nutritional value, shelf life and acceptability of a wide range of cereal-based foods [2]. Several studies confirmed that lactobacilli are able to grow and metabolism in the cereal substrates.

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CHARALAMPOPOULOS et al. [6] showed that the potentially probiotic lactic acid bacteria are able to grow in malt, barley and wheat media. The studied strains utilized the available saccharides during the exponential phase and produced organic acids (lactic and acetic acid) and ethanol. HELLAND et al. [5] studied the growth and metabolism of four selected probiotic strains in cereal-based puddings prepared from rice and maize flour with milk or water. All four strains tested showed good growth and survival in cereal-based puddings. In these substrates, the production of organic acids was observed. The same research team investigated the growth and metabolism of probiotics in maize porridge. All examined strains showed good growth and lactic acid production [7].

The most known diseases related to wheat gluten and similar proteins of barley and rye are wheat allergy and celiac disease. They are mediated by adaptive immune systems. In both conditions, the reaction to gluten is mediated by T-cell activation in the gastrointestinal mucosa [8]. Celiac disease is an immune-mediated enteropathy triggered by the ingestion of glutenin by susceptible individuals. The disease affects about 1% of the world population. Ingestion of gluten causes self-perpetuating mucosal inflammation and subsequent loss of absorptive villi and hyperplasia of the crypts leading to malabsorption of several important nutrients including iron, folic acid, calcium and fat-soluble vitamins [9–12]. Other reactions to gluten are commonly described as gluten sensitivity. These do not involve allergic or autoimmune mechanisms [13].

As a result of mucosal damage of the small intestine, celiac patients may suffer from lactose or histamine intolerance. Celiac disease and antitissue transglutaminase antibodies occur more frequently in patients with diabetes type 1 than in the general population, depending on the age of patient; at most, 10% of children and 2% of adults with type 1 diabetes have positive tests for such antibodies. Both diseases (celiac and diabetes type 1) are associated with the HLA class II genes on chromosome 6p21 [14].

The only acceptable treatment to date for celiac disease is the strict lifelong elimination of gluten from the diet [9–12]. The grains allowed in a gluten-free diet are rice, maize, sorghum, millet, soybean and teff [15]. Oat, rice, soya, maize, sorghum and millet flours may be contained in fermented beverages [16, 17]. Several alternatives to common gluten-containing grains exist, such as the pseudocereals amaranth, quinoa and buckwheat [18, 19]. Over the last 20 years, cereal technologists have tried to produce gluten-free bread that could meet the expectations of celiac consumers. The incorporation of nutrient-dense whole grains, such as amaranth, buckwheat, millet, quinoa, brown rice, sorghum and teff, in gluten-free bread formulations recently showed the potential of increasing the nutritional value of these products, especially in terms of fibre, protein and mineral contents [20]. Iron-fortified amaranth-based bread with satisfactory sensory characteristics was also recently produced by KISKINI et al. [21]. Mawé is the uncooked fermented maize dough. It is an important ingredient for the preparation of cooked beverages, stiff gels and steamed cooked bread in Benin. Amahewu or mahewu is a sour maize-based fermented gruel or beverage consumed mainly by the indigenous people of South Africa [22].

Probiotics are defined as „viable microorganisms that exhibit a beneficial effect on the health of the host upon ingestion by improving the properties of its indigenous microflora“ [23, 24]. Common microorganisms used in probiotic preparations are *Lactobacillus* species, such as *Lactobacillus acidophilus*, *Lb. casei*, *Lb. reuteri*, *Lb. rhamnosus*, *Lb. johnsonii*, *Lb. plantarum*, and *Bifidobacterium* species, such as *Bifidobacterium longum*, *B. breve*, *B. lactis* [25, 26]. Probiotics, particularly *Lactobacillus* spp., are used to improve the microecology of the gut and the production of antibacterial substances (lactic acid, hydrogen peroxide, diacetyl and bacteriocins). In order to exert positive health effects, the microorganisms need to be viable, active and abundant at more than 10^6 CFU·g^{-1} in the product throughout the specified shelf life [27–29]. TRIPATHY and GIRI [24] noted that probiotic products should be consumed with an approximate amount of 100 g per day in order to deliver about 10^9 viable cells into the intestine.

The aim of this work was to study the growth of selected strains of lactobacilli in cereal substrates such as the amaranth and maize mash, during 8 h fermentation at 37 °C, and to study the survival of lactobacilli during storage at 6 °C for 3 weeks. We also monitored the ability of saccharose utilization and lactic acid production by selected lactobacilli using reflectometry.

MATERIALS AND METHODS

Microorganisms

The following lactobacilli were studied: *Lb. acidophilus* 145 (commercial probiotic strain from Christian and Hansen, Hørsholm, Danmark), *Lb. rhamnosus* GG (probiotic strain from the col-

lection of Finnish microbiologists Ouwehand and Salminen, University of Turku, Turku, Finland), *Lb. rhamnosus* VT1 (isolated from tartar sauce, a potentially probiotic strain from the microbial collection of Institute of Chemical Technology, Prague, Czech Republic [30]), *Lb. paracasei* subsp. *paracasei* 1753 (strain from the collection of Masaryk University, Brno, Czech Republic), *Lb. helveticus* X A/2, *Lb. paracasei* VII B/10, *Lb. casei* VII B/6 and *Lb. plantarum* III A/5 (strains from the collection of National Agricultural and Food Centre – Food Research Institute, Bratislava, Slovakia).

These strains were maintained in de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 4–6 °C. Starter cultures were obtained by 24 h incubation (CO_2 incubator ATP Line CB, Binder, Tuttlingen, Germany) at 37 °C in MRS broth. They were centrifuged (centrifuge EBA 20, Andreas Hettich, Tuttlingen, Germany) at 6000 ×*g* for 5 min, washed in distilled water and re-suspended in distilled water to its original volume in compliance with the procedure of Angelov et al. [31].

Substrate preparation and microbiology

The cereal mashes were prepared from amaranth flour (18 g protein, 63 g carbohydrates, 8 g fat) or maize flour (8 g protein, 76 g carbohydrates, 3 g fat; Kroner, Bratislava, Slovakia), saccharose (2%) and water or milk (1.5% fat content). The consistency of the mash in this study was adjusted to make it suitable for eating with a spoon, as a dessert product. These selected flours are suitable for celiac diet due to its high nutritional value and they represent the replacement of the cereal flours strictly prohibited for gluten-free diet [32]. The water-based mashes were chosen as an alternative for people suffering from lactose intolerance or allergy to milk proteins. The content of the amaranth flour was 20% in the water-based mash and 14% in the milk-based mash. The content of the maize flour was 10% in both mashes. The mashes were heated at 100 °C for 15 min and then sterilized (Timo 88944, PBI International, Milano, Italy) at 121 °C for 15 min. After cooling, the mashes were inoculated and fermented.

The substrate was inoculated with 5% starter culture in accordance with the study of Helland et al. [5], in order to achieve approximately 10^6 CFU·ml^{-1} in the mash. The static anaerobic fermentation was carried out in duplicate experiments at 37 °C for 8 h. The mashes were subsequently kept at 6 °C for 3 weeks. The experiments were carried out in duplicate. During the fermentation process (37 °C for 8 to 10 h) and cold storage (at 6 °C for 21 days), the growth of lactic acid bacteria was determined in accordance with Slovak Technical Standard STN ISO 15214 [33] as viable cell counts on MRS agar (Merck) at 37 °C for 48 h.

Measurements, analyses and mathematical modelling

The pH levels were measured using a pH meter with a penetration electrode (Knick Portamess, Berlin, Germany). Total titratable acidity was determined in 10 g of mash homogenized with 90 ml of distilled water and expressed as the amount (in millilitres) of 0.1 mol·l^{-1} NaOH to get pH 8.3 [8]. Saccharose and lactic acid contents were determined by reflectometry (Reflectometer RQflex 10, Merck).

In addition to growth parameters, such as the growth rate during fermentation, the lag-phase and the growth rate during storage, we also examined the rate of decline in pH, the rate of saccharose utilization and the rate of lactic acid production using Baranyi DMFit-model version 2.0 [34]. Standard deviation (Eq. 1) was calculated from the sum-of-squares (*SS*) and degrees of freedom (*df*):

$$s_{yx} = \sqrt{\frac{SS}{df}} = \sqrt{\frac{\sum(\log N_{obs} - \log N_{pred})^2}{n-1}} \qquad (1)$$

where N_{obs}, N_{pred} are concentrations of lactobacilli (in colony forming units per millilitre) determined in the experiments and predicted by the model, respectively. To indicate how well were the data fitted, coefficient of determination was calculated by the DMFit model at each of growth curve (Tab. 1, Tab. 2).

Statistical analysis

Growth parameters of individual strains, including rate constants of lactic acid production, saccharose consumption and pH decrease, were statistically evaluated using Analyse-it Method Validation package version 3.50 (Analyse-it Software, Leeds, United Kingdom). In order to better characterize the effects of mash type (water or milk basis, cereal type – amaranth or maize), the differences between the growth and fermentation parameters were calculated. Significant differences between given pairs of mash types and cereal types were identified by the Tukey method using Wilcoxon test with Hodges-Lehmann location shift ($\alpha = 0.05$). To determine if there were significant differences between the growth and fermentation parameters in two mash types made from two cereal types, Student's t-test was used.

Tab. 1. Growth parameters of lactic acid bacteria during fermentation and storage of amaranth mashes.

Strain	Substrate	*Gr* [log CFU·ml^{-1}h^{-1}]	Lag-phase [h]	R^2
Lb. rhamnosus GG	amaranth + water	0.708	0.68	1.00
Lb. rhamnosus VT1	amaranth + water	0.513	–	1.00
Lb. acidophilus 145	amaranth + water	0.280	1.76	0.94
Lb. paracasei 1753	amaranth + water	0.313	1.29	0.99
Lb. paracasei VII B/10	amaranth + water	0.218	0.41	0.96
Lb. casei VII B/6	amaranth + water	0.248	1.13	0.99
Lb. plantarum III A/5	amaranth + water	0.333	0.30	0.98
Lb. helveticus X A/2	amaranth + water	0.496	1.74	1.00
Lb. rhamnosus GG	amaranth + milk	0.690	1.00	1.00
Lb. rhamnosus VT1	amaranth + milk	0.690	0.55	1.00
Lb. acidophilus 145	amaranth + milk	0.543	4.30	0.96
Lb. paracasei 1753	amaranth + milk	0.430	0.76	0.99
Lb. paracasei VII B/10	amaranth + milk	0.252	0.66	1.00
Lb. casei VII B/6	amaranth + milk	0.227	2.04	0.99
Lb. plantarum III A/5	amaranth + milk	0.391	1.42	0.99
Lb. helveticus X A/2	amaranth + milk	0.273	1.64	0.99

Gr – growth rate during fermentation, R^2 – coefficient of determination.

Tab. 2. Growth parameters of lactic acid bacteria during fermentation and storage of maize mashes.

Strain	Substrate	*Gr* [log CFU·ml^{-1}h^{-1}]	Lag-phase [h]	R^2
Lb. rhamnosus GG	maize + water	0.517	–	0.98
Lb. rhamnosus VT1	maize + water	0.439	–	0.97
Lb. acidophilus 145	maize + water	0.155	2.62	0.96
Lb. paracasei 1753	maize + water	0.297	2.83	0.98
Lb. paracasei VII B/10	maize + water	0.158	–	0.95
Lb. casei VII B/6	maize + water	0.212	0.84	0.92
Lb. plantarum III A/5	maize + water	0.338	0.78	0.99
Lb. helveticus X A/2	maize + water	0.214	1.23	0.93
Lb. rhamnosus GG	maize + milk	0.585	–	0.99
Lb. rhamnosus VT1	maize + milk	0.811	–	1.00
Lb. acidophilus 145	maize + milk	0.380	1.37	0.98
Lb. paracasei 1753	maize + milk	0.259	0.70	0.93
Lb. paracasei VII B/10	maize + milk	0.273	1.22	0.99
Lb. casei VII B/6	maize + milk	0.197	–	0.99
Lb. plantarum III A/5	maize + milk	0.320	0.53	0.98
Lb. helveticus X A/2	maize + milk	0.254	0.60	0.98

Gr – growth rate during fermentation, R^2 – coefficient of determination.

RESULTS AND DISCUSSION

Growth and survival of lactobacilli

An overview of growth parameters of studied lactobacilli in water- and milk-based mashes prepared from amaranth or maize flours is presented in Tab. 1 and Tab. 2. In general, most of the studied strains of lactobacilli attained a higher cell population in milk-based mashes during anaerobic fermentation at 37 °C, reaching a maximum of 10^8 CFU·ml^{-1} within 8 h to 10 h. Significantly lower concentrations of lactobacilli were obtained during fermentation and storage of water-based mashes. This indicates a lower growth and stability of starter cultures in these products without the milk component. According to Charalampopoulos et al. [3] and Curry and Crow [35], lactobacilli require fermentable saccharides, amino acids,

peptides, fatty esters, salts, derivatives of nucleic acids and vitamins, especially of the B group, and minerals for their growth, and these requirements are strain-depending. At the end of fermentation process, higher cell populations of lactobacilli were observed in amaranth mash in comparison to the maize mash (Fig. 1).

HELLAND et al. [5] evaluated growth and metabolism of probiotic strains in milk- and water-based cereal (maize and rice) puddings. It was found that *Lb. rhamnosus* GG showed the highest viable cell counts in both puddings, reaching 9.1 log CFU·g^{-1} and 8 log CFU·g^{-1}, respectively, in 12 h, with significantly higher viable counts in milk-based puddings. This research team studied also growth and metabolism of probiotic strains in water-based maize porridge, in which all strains reached maximum cell densities of 7.2–8.2 log CFU·g^{-1} in 12 h [7]. In our study, *Lb. rhamnosus* GG reached 8.5 log CFU·ml^{-1} and 8.1 log CFU·ml^{-1} in milk- and water-based maize mash, respectively. For this strain, the greatest difference in cell populations before and after fermentation (3.05 log CFU·ml^{-1}) in amaranth milk mash was observed. The lowest counts at the end of storage from all studied lactobacilli (7.5 log CFU·ml^{-1}) was reached by

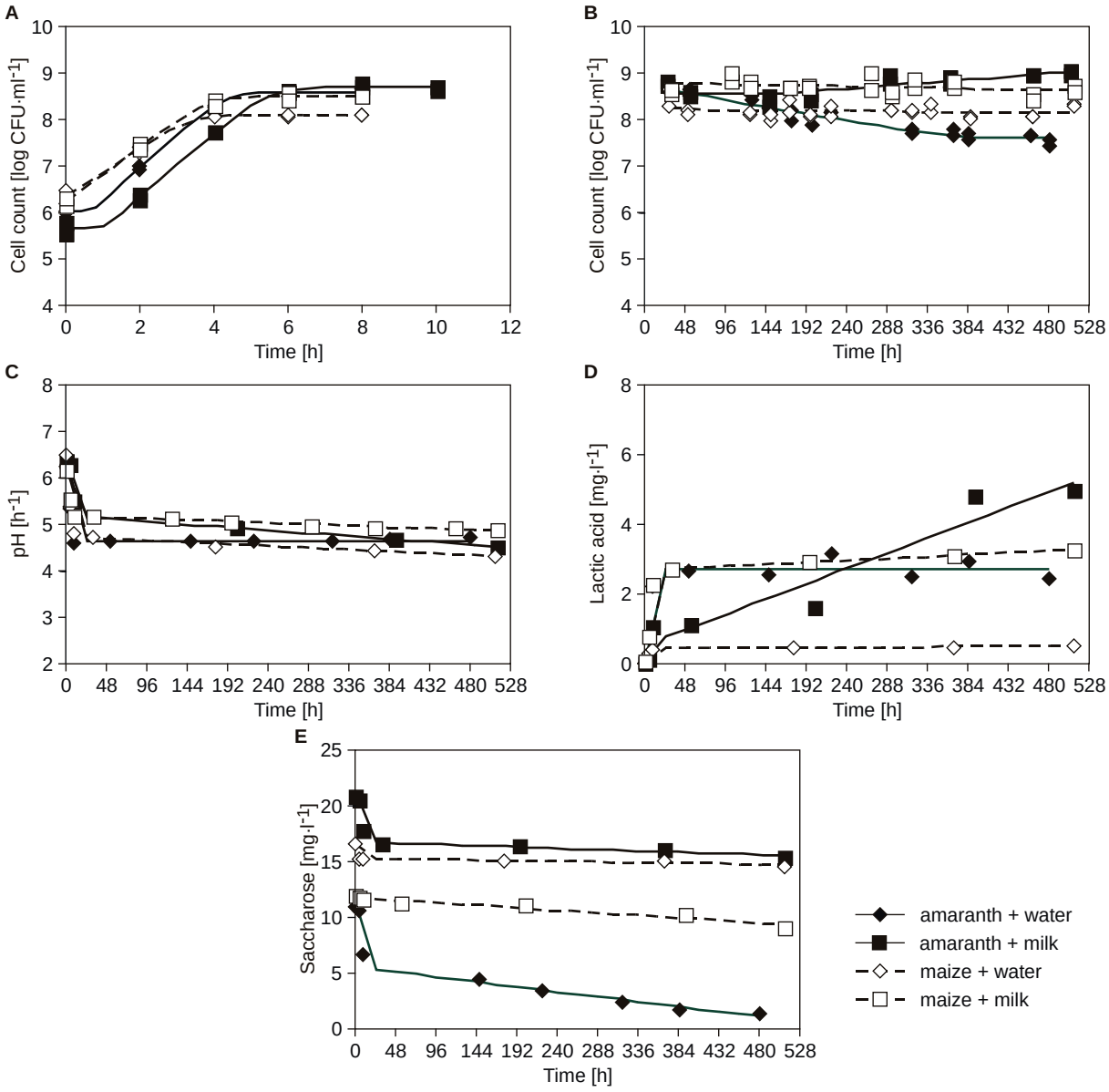

Fig. 1. Fermentation of amaranth and maize mashes by *Lb. rhamnosus* GG.

A – cell counts during fermentation at 37 °C; B – cell counts during storage at 6 °C; C – change in pH; D – formation of lactic acid; E – utilization of saccharose.

Lb. rhamnosus GG in the water-based amaranth mash (Fig. 1). A significant decrease in counts during storage (1 log order) was determined, with the rate of decline of –0.0025 log CFU·ml^{-1}h^{-1} (0.06 log CFU·ml^{-1}d^{-1}). This strain grew at about the same rate in milk-based amaranth mash (growth rate *Gr* = 0.690 log CFU·ml^{-1}h^{-1}) and in water-based amaranth mash (*Gr* = 0.708 log CFU·ml^{-1}h^{-1}). In the milk-based maize mash, the strain grew by about 15% slower than in the milk-based amaranth mash. The growth rate in the water-based maize mash was by about 27% lower than in the water-based amaranth mash. No important difference between the growth rate of *Lb. rhamnosus* GG in the maize mash with milk and water (0.585 log CFU·ml^{-1}h^{-1} versus 0.517 log CFU·ml^{-1}h^{-1}) was determined.

In contrast with *Lb. rhamnosus* GG, *Lb. acidophilus* 145 reached the highest growth rate during storage (*Gr* = 0.003 log CFU·ml^{-1}h^{-1}) with the increase of 1 log order in the milk-based amaranth mash (Fig. 2). HELLAND et al. [5] investigated the growth of *Lb. acidophilus* La5 in maize-rice pudding. After 21 d storage, viable counts of this strain were reduced almost to zero in water-based pudding. In the same

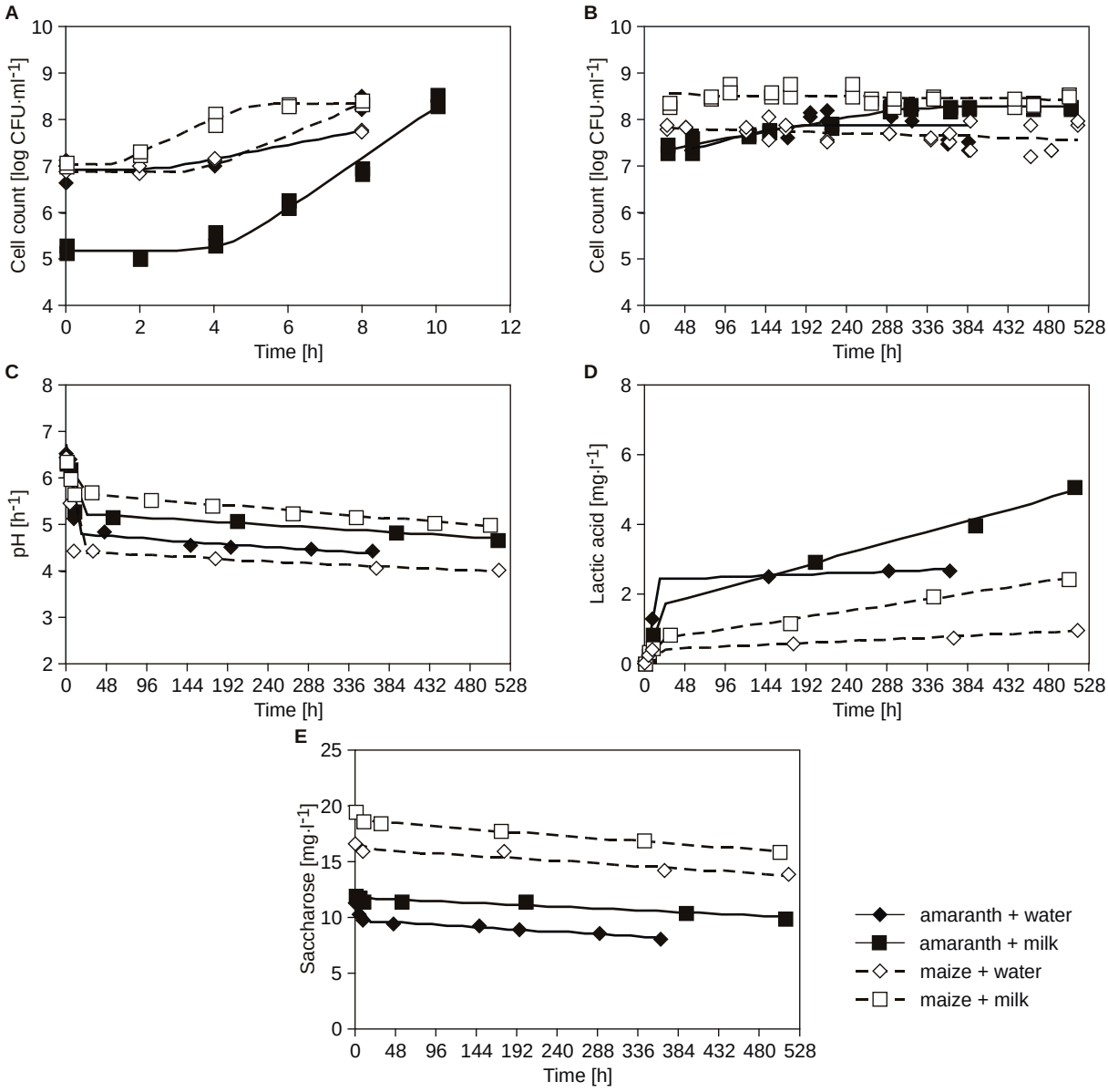

Fig. 2. Fermentation of amaranth and maize mashes by *Lb. acidophilus* 145.

A – cell counts during fermentation at 37 °C; B – cell counts during storage at 6 °C; C – change in pH; D – formation of lactic acid; E – utilization of saccharose.

Tab. 3. Statistical analysis.

	Amaranth mash		Maize mash	
	Water-based	Milk-based	Water-based	Milk-based
Gr [log CFU·ml^{-1}h^{-1}]	0.398 ± 0.166^{ac}	0.414 ± 0.184^{ac}	0.294 ± 0.130^{ad}	0.387 ± 0.210^{ac}
N_{end_37} [CFU·ml^{-1}]	8.38 ± 0.14^{ac}	8.44 ± 0.64^{ac}	8.02 ± 0.12^{ad}	8.49 ± 0.10^{bc}
k_{pH} [h^{-1}]	–0.166 ± 0.056^{ac}	–0.096 ± 0.045^{bc}	–0.237 ± 0.027^{ad}	–0.102 ± 0.034^{bc}
k_{LA} [g·l^{-1}h^{-1}]	0.204 ± 0.101^{ac}	0.080 ± 0.055^{bc}	0.041 ± 0.011^{ad}	0.118 ± 0.078^{bc}
k_{sac} [g·l^{-1}h^{-1}]	–0.382 ± 0.152^{ac}	–0.313 ± 0.421^{ac}	–0.216 ± 0.128^{ad}	–0.271 ± 0.158^{ac}
k_{N_stac} [log CFU·ml^{-1}h^{-1}]	0.0002 ± 0.0013^{ac}	0.0009 ± 0.0014^{ac}	0.0001 ± 0.0003^{ac}	0.0003 ± 0.0006^{ac}

The values represent mean ± standard deviation calculated from the parameters of *Lactobacillus* strains used in the substrate fermentation. The same letters in the superscript indicate that differences were not significant (a, b – amaranth, maize; c, d – water-based, milk-based).
Gr – growth rate, N_{end_37} – counts at the end of fermentation, k_{pH} – rate of decline in pH, k_{LA} – rate of production of lactic acid, k_{sac} – rate of utilization of saccharose, k_{N_stac} – growth rate during storage.

pudding, *Lb. acidophilus* 1748 declined from 7 log CFU·g^{-1} at the end of 12 h fermentation, to 3.7–4.3 log CFU·g^{-1} after 21 d storage. Charalampopoulos et al. [3] observed poor growth of *Lb. acidophilus* NCIMB 8821 in cereal-based substrates (malt, wheat and barley media). In our study, *Lb. acidophilus* 145 reached the final counts of 7.6–8.7 log CFU·ml^{-1} after 21 d storage depending on the type and main component of the substrate. In our earlier study, however, viable counts of this strain in water-based buckwheat mash after 21 d storage were significantly reduced to 2.6 log CFU·ml^{-1} with the rate of decline of –0.025 log CFU·ml^{-1}h^{-1} [36].

Salmerón et al. [37] evaluated the growth of *Lb. acidophilus* NCIMB 8821, *Lb. plantarum* NCIMB 8826 and *Lb. reuteri* NCIMB 11951 in oat, barley and malt beverages during fermentation at 37 °C for 10 h. They observed the highest *Lb. plantarum* density in the oat and barley media (N_O = 8.2 log CFU·ml^{-1} and N_B = 7.9 log CFU·ml^{-1}, respectively). In our study, similar results for the cell counts of *Lb. plantarum* III A/5 were observed. At the end of fermentation, a density of about 8.1 log CFU·ml^{-1} and 8.5 log CFU·ml^{-1} was reached in maize and amaranth mash, respectively.

The highest growth rate (*Gr* = 0.819 log CFU·ml^{-1}h^{-1}) was observed for *Lb. rhamnosus* VT1 in milk-based maize mash, which was by about 87% faster in comparison with the water-based mash. The difference between the growth rates of *Lb. rhamnosus* VT1 in milk- and water-based amaranth mash was 23%, where milk was a more suitable substrate, and the densities of *Lb. rhamnosus* VT1 at the end of fermentation reached 8.8 log CFU·g^{-1}. The water or milk content had no statistically significant effect on the growth rate (Tab. 3).

According to our previous study, *Lb. paracasei* 1753 grew in milk (0.201 log CFU·ml^{-1}h^{-1}) by about 53% and 22% slower in comparison to the amaranth and maize mash with milk, respectively, and even by about 36% and 32% slower in comparison to the water-based amaranth and maize mash, respectively. This fact indicates that the composition of amaranth and maize flour (content of vitamins, minerals, proteins, polysaccharides, aminoacids and other components) promotes the growth of lactobacilli [36, 38].

Decline in pH, saccharose utilization and production of lactic acid

In addition to the growth parameters, the rate of pH decline, the rate of saccharose utilization and lactic acid production depending on lactobacilli in the cereal-based mashes were also examined (Tab. 4, Tab. 5). After 21 days of storage of milk-based mashes, pH values ranged between 4.3 and 5.5, whereas in water-based mashes, pH values were between 3.6 and 4.7. In a previous study of maize-rice puddings fermented by *Lb. rhamnosus* GG, *Lb. acidophilus* 1748 or *Lb. acidophilus* La5, the pH values were 4.0–4.8 and 3.4–4.4 after 21 days of storage in milk- and water-based puddings, respectively [5]. Reduction in pH was also faster in water-based mashes in comparison with milk-based mashes due to lower buffering capacity of the water-based mashes.

The largest decrease in pH, up to 3 units, was observed in water-based maize mash fermented by *Lb. plantarum* III A/5. At the end of cold storage, the pH was 3.6 and it dropped with the rate of –0.264 h^{-1}. In the rate of pH decline, statistically

significant differences in the impact of water or milk content were observed (Tab. 3). SALMERÓN et al. [37] evaluated the growth and metabolism of potentially probiotic lactobacilli in cereal beverages (oat, barley and malt substrates). It was determined that *Lb. plantarum* decreased pH values below 3.7 after 10 h of fermentation. In water-based maize porridge fermented by *Lb. rhamnosus* GG, *Lb. reuteri* SD 2112 or *Lb. acidophilus* LA5, the pH value dropped from 5.8 to 3.1–3.7 during the fermentation period [7].

The acids production during fermentation of saccharose is the primary functional requirement for lactic acid starter bacteria. The strains under study produced significantly higher content of lactic acid in milk-based mashes in comparison to the water-based mashes. At the same time, the amaranth mashes were better substrates for lactic acid production in comparison with maize. In milk-based mashes, 2759–8100 mg·kg^{-1} of lactic acid was determined in comparison with water-based mashes, in which the same strains or iso-

Tab. 4. Rate of decline in pH, saccharose utilization and production of lactic acid in amaranth mashes.

Strain	Substrate	k_{pH} [h^{-1}]	k_{sac} [g·l^{-1}h^{-1}]	k_{LA} [g·l^{-1}h^{-1}]
Lb. rhamnosus GG	amaranth + water	–0.230	–0.525	0.274
Lb. rhamnosus VT1	amaranth + water	–0.186	–0.562	0.281
Lb. acidophilus 145	amaranth + water	–0.173	–0.275	0.146
Lb. paracasei 1753	amaranth + water	–0.222	–0.425	0.280
Lb. paracasei VII B/10	amaranth + water	–0.105	–0.113	0.077
Lb. casei VII B/6	amaranth + water	–0.126	–0.473	0.130
Lb. plantarum III A/5	amaranth + water	–0.204	–0.416	0.342
Lb. helveticus X A/2	amaranth + water	–0.080	–0.265	0.099
Lb. rhamnosus GG	amaranth + milk	–0.104	–0.075	0.064
*Lb. rhamnosus*VT1	amaranth + milk	–0.180	–1.251	0.198
Lb. acidophilus 145	amaranth + milk	–0.130	–0.108	0.101
Lb. paracasei 1753	amaranth + milk	–0.036	–0.608	0.024
Lb. paracasei VII B/10	amaranth + milk	–0.068	–0.179	0.033
Lb. casei VII B/6	amaranth + milk	–0.066	–0.032	0.043
Lb. plantarum III A/5	amaranth + milk	–0.111	–0.056	0.090
Lb. helveticus X A/2	amaranth + milk	–0.073	–0.197	0.080

k_{pH} – rate of decline in pH, k_{sac} – rate of utilization of saccharose, k_{LA}– rate of production of lactic acid.

Tab. 5. Rate of decline in pH, saccharose utilization and production of lactic acid in maize mashes.

Strain	Substrate	k_{pH} [h^{-1}]	k_{sac} [g·l^{-1}h^{-1}]	k_{LA} [g·l^{-1}h^{-1}]
Lb. rhamnosus GG	maize + water	–0.208	–0.342	0.048
Lb. rhamnosus VT1	maize + water	–0.283	–0.360	0.061
Lb. acidophilus 145	maize + water	–0.249	–0.064	0.047
Lb. paracasei 1753	maize + water	–0.216	–0.160	0.026
Lb. paracasei VII B/10	maize + water	–0.209	–0.006	0.034
Lb. casei VII B/6	maize + water	–0.235	–0.258	0.030
Lb. plantarum III A/5	maize + water	–0.264	–0.270	0.043
Lb. helveticus X A/2	maize + water	–0.229	–0.270	0.041
Lb. rhamnosus GG	maize + milk	–0.153	–0.405	0.276
Lb. rhamnosus VT1	maize + milk	–0.150	–0.214	0.159
Lb. acidophilus 145	maize + milk	–0.088	–0.113	0.052
Lb. paracasei 1753	maize + milk	–0.105	–0.270	0.143
Lb. paracasei VII B/10	maize + milk	–0.060	–0.360	0.136
Lb. casei VII B/6	maize + milk	–0.079	–0.009	0.068
Lb. plantarum III A/5	maize + milk	–0.074	–0.299	0.046
Lb. helveticus X A/2	maize + milk	–0.109	–0.495	0.062

k_{pH} – rate of decline in pH, k_{sac} – rate of utilization of saccharose, k_{LA}– rate of production of lactic acid.

lates of lactobacilli produced between 264 mg·kg^{-1} and 2691 mg·kg^{-1} lactic acid. The highest production was observed as a result of *Lb. plantarum* III A/5 growth in milk-based amaranth mash (8100 mg·kg^{-1}), while *Lb. paracasei* VII B/10, grown in water-based maize mash, produced only small amounts of lactic acid (264 mg·kg^{-1}). In barley and oats, *Lb. plantarum* was able to produce about 850 mg·kg^{-1} and 770 mg·kg^{-1} lactic acid, respectively [38]. Similar to our study, Helland et al. [5] demonstrated that substitution of water for milk in cereal puddings led to an increase in lactic acid content from 560–2600 mg·kg^{-1} to 4300–9800 mg·kg^{-1}. The same research team observed the largest production of 3600–4000 mg·kg^{-1} of lactic acid after 24 h fermentation in maize-barley porridge fermented by *Lb. rhamnosus* GG [7]. In our study, the production of lactic acid by *Lb. rhamnousus* GG in maize mash after 21 days of storage was 3206 mg·kg^{-1} and 500 mg·kg^{-1} in milk- and water-based mash, respectively. Kocková et al. [39] observed the highest lactic acid production in the case of amaranth fermentation by *Lb. rhamnosus* GG. The lactic acid content at the end of fermentation ranged from 93.82 mg·kg^{-1} to 1043.46 mg·kg^{-1} in samples inoculated with approximately 10^5 CFU·g^{-1} and from 104.07 mg·kg^{-1} to 5572.72 mg·kg^{-1} in samples inoculated with approximately 10^6 CFU·g^{-1}.

The initial contents of saccharose were in maize mash between 21420 mg·kg^{-1} and 15500 mg·kg^{-1}, and in amaranth mash between 15030 mg·kg^{-1} and 9600 mg·kg^{-1}. The water or milk content had no statistically significant effect on utilization of saccharose. On the other hand, statistically significant differences were observed using amaranth or maize flour in water-based mashes (Tab. 3). The strain *Lb. rhamnosus* VT1 showed the highest utilization rate of saccharose in all types of mashes, with the exception of milk-based maize mash. Overall, the fastest utilization of saccharose (rate of utilization of saccharose k_{sac} = –1251 mg·kg^{-1}h^{-1}) was found in milk-based amaranth mash fermented by this strain. The greatest change in the content of saccharose was observed in water-based amaranth mash (9500 mg.kg^{-1}) fermented by *Lb. rhamnosus* GG. On the other hand, a minimal change in the content of saccharose (1080 mg·kg^{-1}) was seen as a result of *Lb. casei* VII B/6 growth in milk-based maize mash. For *Lb. acidophilus* La5, a reduction of approx. 10000 mg·kg^{-1} lactose and 2200 mg·kg^{-1} fructose resulted in lactic acid production of approx. 6000–7000 mg·kg^{-1} [5]. In our study, the content of lactic acid for *Lb. acidophilus* 145 was 2444 mg·kg^{-1} and 5060 mg·kg^{-1} as a result of utilization of 3690 mg·kg^{-1} and 2060 mg·kg^{-1} saccharose in maize and amaranth milk-based mash, respectively.

CONCLUSIONS

The studied lactobacilli were able to grow and metabolize in cereal substrates. The viable lactobacilli counts were well maintained above the suggested minimum limit of 10^6 CFU·ml^{-1} during 21 days of storage at 6 °C. This or higher content is required for efficacy of a probiotic product. The milk base of mashes proved to be a better substrate for bacterial growth in comparison to water, and amaranth in comparison to maize, too. Overall, strains *Lb. rhamnosus* GG and *Lb. rhamnosus* VT1 showed the highest growth rates in all types of substrates. Moreover, *Lb. rhamnosus* VT1 showed the fastest decrease in pH, the greatest rate of utilization of saccharose and the highest rate of production of lactic acid. The lowest growth rate was observed for the strain *Lb. acidophilus* 145 in water-based maize mash during fermentation. On the other hand, this strain reached the highest growth rate with an increase of 1 log order in the amaranth mash based on milk during storage. In fermentation of cereal substrates by 8 strains of lactic acid bacteria, water or milk had no statistically significant effect on their numbers, on changes in the stationary phase or on the utilization of saccharose. In two different substrates (amaranth and maize), statistically significant differences in the impact of water or milk environment were observed only in the decrease of pH, which was connected with the rate of formation of lactic acid. The type of fermented substrate (amaranth or maize) caused statistically significant differences in all parameters. Milk as a good source of nutrients, compensated for differences in all constants in both matrices.

Currently, the interest of consumers in fermented cereal-based products is growing. Since the offer of probiotic products on the market is limited to dairy products, this study was devoted to development of non-dairy functional foods containing probiotics. Advancing the development of fermented products based on cereals or pseudocereals, including probiotics, can lead not only to enrichment of the the diet in patients suffering from celiac disease, individuals with food allergies, or otherwise metabolically handicapped people, but also to a balanced diet in healthy subjetcs. The cereal mash represents a cheap and nutritionally rich substrate for the growth of lactic acid bacteria. Although milk is a typical growth medium for lactic

acid bacteria, according to our results also cereal mashes appeared to be suitable for fermentation by these microorganisms. Our findings follow the new trend in the field of probiotic functional foods, which would be suitable for celiac patients (water- and milk-based fermented products) and for people suffering with lactose intolerance (water-based fermented cereals).

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