Development of New Cereal-, Pseudocereal-, and Cereal-Leguminous-Based Probiotic Foods

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Abstract

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The suitability of the selected cereals, pseudocereals, and legumes for new probiotic foods development was tested. Probiotic products were produced by inoculating buckwheat, dark buckwheat, barley, oat, soya, and chickpea in combination with oat with *Lactobacillus rhamnosus* GG and subsequent moulding to eliminate water from the cooked grains. The cell growth, pH and organic acid profiles were monitored during fermentation process at 37°C for 10 h followed by the storage period at 5°C for 21 days. The growth and metabolic parameters were calculated using principles of the predictive microbiology. *Lb. rhamnosus* GG was able to grow in all substrates during fermentation and reached the cell density of 6.68–7.58 log CFU/g, the highest growth rate having been calculated in the oat product (0.341 log CFU/g/h). After the fermentation, the lowest pH value was observed in the barley product (4.52), while after the storage in the oat-soya product (4.32). The greatest amount of lactic acid after the storage period was measured in the oat-soya product (1977.8 mg/kg). Sensory characteristics of the fermented and stored products were also monitored.

Keywords: lactose intolerance; milk allergy; predictive microbiology; *Lactobacillus rhamnosus* GG; fermentation

Probiotics are defined as live microorganisms which, upon ingestion in certain numbers, exert health benefits beyond the inherent general nutrition (EFSA 2011). Traditionally, probiotics are added to yoghurt and other fermented dairy products. Nowadays, there is an increasing consumers' demand for non-dairy probiotic foods, which can overcome some of the disadvantages associated with the fermented dairy products like lactose intolerance or allergy to milk proteins (Prado *et al*. 2008; Rivera-Espinoza & Gallardo-Navarro 2010).

Cereals, pseudocereals, and legumes are considered as the most important sources of proteins, carbohydrates, lipids, vitamins, and minerals in human diet (Fletcher 2004). Furthermore, they are a good source of non-digestible carbohydrates (fibre, oligosaccharides) that can stimulate the growth of probiotic strains (Kedia *et al*. 2007).

Lactobacillus rhamnosus GG is one of the most monitored probiotic strains. It belongs to Gram-positive, non-spore-forming, non-motile, catalase-negative, facultatively anaerobic or microaerophilic and mesophilic bacteria. The metabolism of *Lb. rhamnosus* GG is facultatively heterofermentative (Jyoti *et al*. 2003). It enhances human natural resistance and healthy digestive system and inhibits the adhesion of some pathogenic bacteria. It relieves the syndromes of gastro-intestinal tract irritation tract, atopic dermatitis, and cow milk allergy (FAO/WHO 2001; Collado *et al*. 2007; EFSA 2011).

The aim of this study was to estimate the suitability of buckwheat, dark buckwheat, barley, oat, soya, and chickpea for the development of new probiotic products containing the probiotic strain *Lactobacillus rhamnosus* GG. The calculation of the growth and metabolic parameters and sensory evaluation of products were also done, using the tools of predictive microbiology.

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MATERIAL AND METHODS

Starter culture. Probiotic strain *Lb. rhamnosus* GG (ATCC 53103; Dicofarm, Roma, Italy) was used in the presented work.

Samples. Six kinds of grains were used – buckwheat, dark buckwheat, barley, oat, soya, and chickpea (Kroner, Bratislava, Slovak Republic).

Preparation of substrates for fermentation. The substrates were prepared according to the procedure described in Table 1, being boiled at 100°C for 15 min, cooled down and inoculated by *Lb. rhamnosus* GG to give approximately 5 log CFU/g, and moulded in laboratory conditions (by means of one kg weight on 28 cm^2 area until the drain of water was observed).

Fermentation and storage process. The moulded substrates were fermented at 37°C for 10 h and then stored at 5°C for 21 days.

Biological and chemical analyses. The enumeration of viable cells was performed by the estimation of the colony forming units number on de Man-Rogosa-Sharpe agar (Merck, Darmstand, Germany) plates according to the STN ISO 15214. The pH was measured by pH-meter CG 843 (Schott, Mainz, Germany). The identification and quantity of the organic acids were determined by isotachophoretic analysis using the Isotachophoretic Analyser ZKI 01 (Villa Labeco, Spišská Nová Ves, Slovak Republic). The electrolytic system according to Kocková *et al*. (2013a) was used. Quantitative analysis was performed by calibration using standard solutions of acids (Lachema, Brno, Czech Republic).

Table 1. Composition and treatment of substrates prior to fermentation

Substrate	Total amount (g)	Composition (w/w)
Buckwheat	321.3	24.9 buckwheat 74.7 water 0.4 salt
Dark buckwheat	401.2	19.9 dark buckwheat 79.8 water 0.3 salt
Barley	361.8	33.2 barley 66.3 water 0.5 salt
Oat	302.3	49.6 oat 49.6 water 0.8 salt
Oat + soya	301.5	33.2 oat 16.6 soya* 49.8 water 0.4 g salt
Oat + chickpea	301.5	33.2 oat 16.6 chickpea* 49.8 water 0.4 salt

*soya and chickpea were precooked 30 min at 100°C

Estimation of growth and metabolic parameters. The growth and metabolic curves were modelled by means of the mechanistic model of Baranyi and Roberts (1994). The growth and metabolic parameters were calculated from each curve.

Sensory analyses. The products were evaluated by means of 5-point hedonic scale for the colour, cohesiveness, mastic ability, aroma, and taste. A panel of 10 trained members was chosen. Fresh fermented matrices and matrices after the storage period were evaluated at the same time.

Statistical analyses. Each experiment was performed in thriolicate. The results represented means with standard deviations. Statistical analyses were carried out using Microsoft Excel 2007 (Microsoft, Redmond, USA). The data were treated by Student *t*-test with the least significant difference of 95%.

RESULTS AND DISCUSSION

The presented results show the changes in the viable cell counts, pH, and organic acids during the fermentation period at 37°C for 10 h and the following storage at 5°C for 21 days.

Viable counts. The growth of *Lb. rhamnosus* GG is presented in Figure 1 and the growth parameters in Table 2. The substrates were inoculated with the probiotic strain at the initial cell density of 5.00 to 5.64 log CFU/g. The cell concentration at the end of fermentation ranged from 6.68 to 7.58 log CFU/g, which was lower than the density of *Lb. rhamnosus* GG in cereal and pseudocereal water-based porridges (Kocková *et al*. 2013b) and the density of *Lb. plantarum*, *Lb. acidophilus*, and *Lb. reuteri* in oat, barley and malt beverages (Salmerón *et al*. 2013), probably due to lower water content. The lag phase ranged from 2.31 to 4.69 h, in the case of dark buckwheat and oat-soya products the lag phases were not observed. The growth rate varied from 0.225 to 0.341 log CFU/g/h, being lower than in cereal and pseudocereal porridges (Kocková *et al*. 2013b) or in milk during fermentation at 35 and 41°C (Valík *et al*. 2008). During the storage period, *Lb. rhamnosus* GG was able to survive in the products. The viable cell counts at the end

Table 2. Growth parameters of *Lb. rhamnosus* GG during fermentation of cereal, pseudocereal, and cereal-leguminous products and storage period

	Fermentation					Storage		
	Gr	t_d	λ	N_0	N_{max}	Gr	N_0	N_{max}
Buckwheat	0.322^{b}	0.934^{a}	3.56^{b}	5.28^{c}	7.32^{b}	0.00060^{c}	7.58^{c}	7.99^{b}
Dark buckwheat	0.225^{a}	1.335^{b}	–	4.87^{a}	7.32^{b}	0.00006^{a}	7.26^{b}	7.32^{a}
Barley	0.252^{a}	1.194^{b}	3.21^{b}	5.02^{b}	6.68^{a}	0.00122^{d}	7.00^{a}	7.54^{a}
Oat	0.341^{b}	0.882^{a}	4.69^{c}	5.39^{d}	7.23^{b}	0.00033^{b}	7.06^{a}	7.34^{a}
Oat + soya	0.254^{a}	1.214^{b}	–	4.88^{a}	7.38^{b}	0.00587^{e}	7.54^{c}	7.95^{b}
Oat + chickpea	0.254^{a}	1.497^{c}	2.13^{a}	5.65^{e}	7.58^{c}	0.00135^{d}	7.64^{c}	7.85^{b}

Gr – growth rate (log CFU /g/h); t_d – time to double (h); λ – lag phase (h); N_0 – initial density of *Lb. rhamnosus* GG (log CFU/g); N_{max} – final density of *Lb. rhamnosus* GG (log CFU/g); $^{a–e}$means within a column with different superscript letters are significantly different ($P < 0.05$); $n = 3$

of the storage ranged from 7.32 to 7.99 log CFU/g. *Lb. rhamnosus* GG was also able to survive in cereal and pseudocereal porridges (Kocková *et al.* 2013b) and in amaranth and buckwheat water- and milk-based puddings (Pelikánová *et al.* 2011).

pH and organic acids. The changes of pH value and the parameters of these changes are presented in Figure 2 and in Table 3. During fermentation, pH values decreased from the initial 5.16–6.13 to the final 4.52–5.79, with the rate ranging from –0.009 to

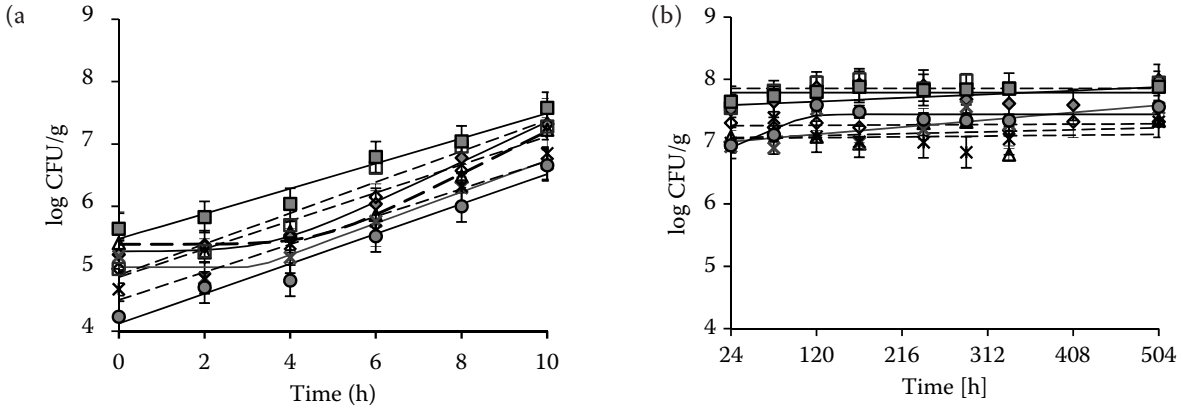

Figure 1. Evaluation of growth of *Lb. rhamnosus* GG in cereal, pseudocereal and cereal-leguminous products during (a) fermentation and (b) storage period

buckwheat; dark buckwheat; barley; oat; oat + soya; oat + chickpea; $n = 3$

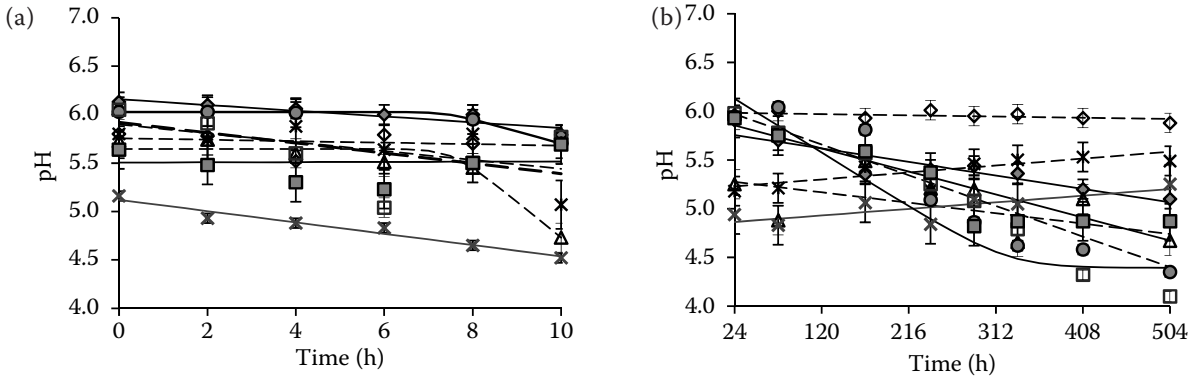

Figure 2. Evaluation of changes of pH value in cereal, pseudocereal and cereal-leguminous products during (a) fermentation and (b) storage period

buckwheat; dark buckwheat; barley; oat; oat + soya; oat + chickpea; $n = 3$

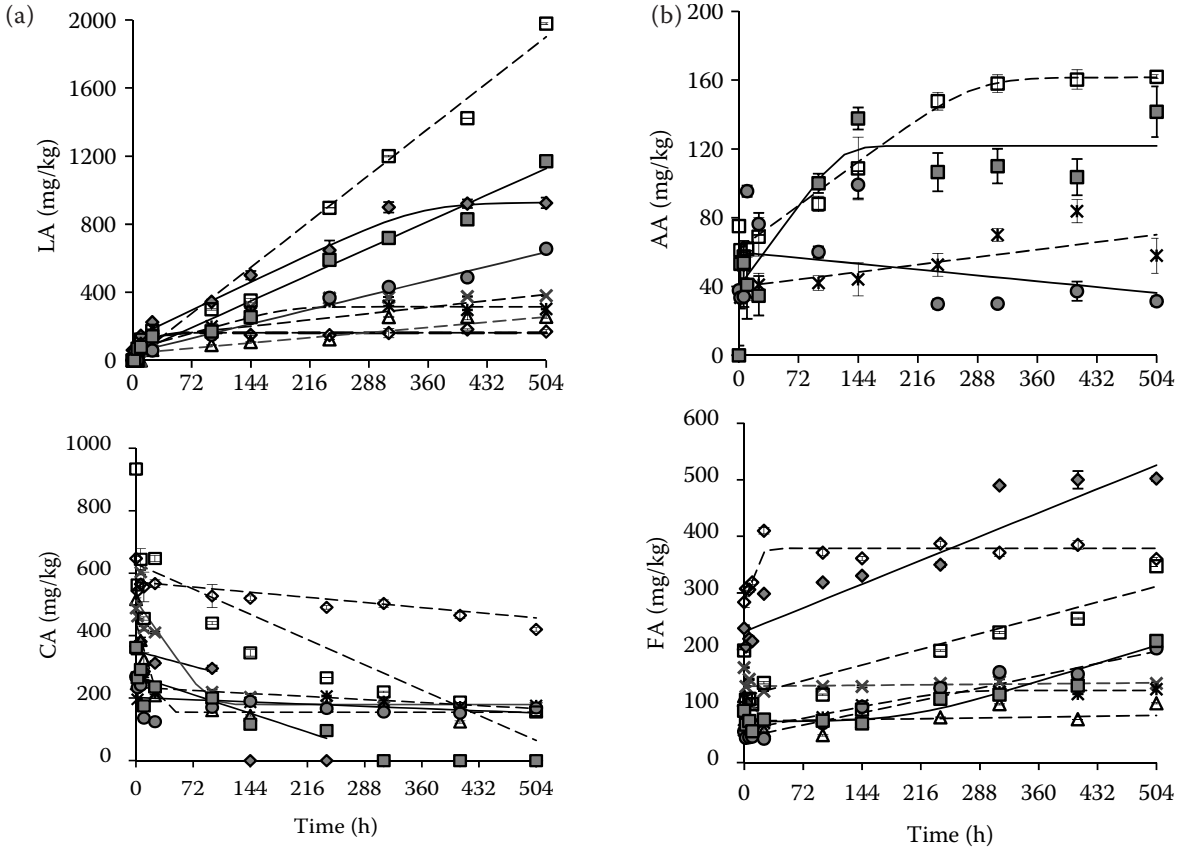

Figure 3. Evaluation of changes in concentration organic acids in cereal, pseudocereal and cereal-leguminous pressed matrix during (a) fermentation and (b) storage period

buckwheat; dark buckwheat; barley; oat; oat + soya; oat + chickpea; $n = 3$; LA – lactic acid; AA – acetic acid; CA – citric acid; FA – formic acid, $n = 3$

–0.370 per hour. Generally, *Lb. rhamnosus* GG is not able to acidify the environment very much. In MRS broth, it decreased the pH value to 4.00 (Zalán *et al*. 2010), in milk to 6.00 (Valík *et al*. 2008) in cereal and pseudocereal porridges to 4.31–5.99 (Kocková *et al*. 2013b), and in milk-based cereal puddings and maize porridges with barley below 4.00 (Helland *et al*. 2004a,b). The lag phase of the pH changes was observed only in the oat product, 7.53 h. During the storage period, the decrease of pH values continued, except for the barley product. pH values at the end of the storage period ranged from 4.32 to 5.88. Water-based cereal and pseudocereal substrates were stable during storage in view if the pH values, excepting

Table 3. Parameters of changes pH value during fermentation of cereal, pseudocereal, and cereal-leguminous products and storage period

	Fermentation				Storage		
	k_{pH}	λ_{pH}	pH_0	pH_{end}	k_{pH}	pH_0	pH_{end}
Buckwheat	-0.030^{b}	–	6.16^{d}	5.79^{d}	-0.00144^{b}	5.76^{c}	5.10^{d}
Dark buckwheat	-0.009^{a}	–	5.76^{c}	5.70^{c}	-0.00013^{c}	5.98^{c}	5.88^{f}
Barley	-0.058^{c}	–	5.12^{a}	4.52^{a}	0.00070^{d}	4.86^{a}	5.25^{e}
Oat	-0.370^{d}	7.53	5.65^{b}	4.73^{b}	-0.00111^{b}	5.27^{b}	4.67^{b}
Oat + soya	-0.046^{c}	–	5.90^{c}	5.77^{d}	-0.00327^{a}	5.97^{c}	4.32^{a}
Oat + chickpea	0.001^{e}	–	5.51^{b}	5.69^{c}	-0.00246^{a}	5.85^{c}	4.87^{c}

k_{pH} – rate of changes in pH value (h^{-1}); λ_{pH} – lag phase of pH changes (h); pH_0 – initial pH; pH_{end} – final pH; $^{a–f}$means within a column with different superscript letters are significantly different ($P < 0.05$); $n = 3$

Table 4. Parameters of changes in concentration organic acids in cereal, pseudocereal, and cereal-leguminous products during fermentation and storage period

	Lactic acid			Acetic acid			Citric acid			Formic acid		
	k_{acid}	c_0	c_{end}	k_{acid}	c_0	c_{end}	k_{acid}	c_0	c_{end}	k_{acid}	c_0	c_{end}
Buckwheat	2.674^{d}	126.3^{e}	927.7^{d}	–	–	–	–0.677^{b}	350.4^{b}	–	0.681^{e}	231.5^{d}	500.0^{f}
Dark buckwheat	4.555^{f}	50.6^{c}	161.6^{a}	–	–	–	–0.267^{a}	571.7^{e}	464.8^{c}	4.482^{f}	287.5^{e}	378.9^{e}
Barley	1.185^{b}	102.5^{d}	365.2^{c}	–	–	–	–4.457^{e}	512.5^{d}	179.4^{b}	0.014^{a}	135.0^{c}	141.2^{b}
Oat	0.547^{a}	31.7^{b}	255.5^{b}	–	–	–	–10.821^{f}	441.6^{c}	154.6^{a}	0.023^{b}	73.5^{a}	76.5^{a}
Oat + soya	3.764^{e}	4.3^{a}	1977.8^{f}	0.353^{a}	61.6^{b}	161.6^{b}	–1.121^{d}	629.7^{f}	155.4^{a}	0.386^{c}	117.1^{b}	347.4^{d}
Oat + chickpea	2.148^{c}	44.7^{c}	1170.2^{e}	0.654^{b}	39.7^{a}	121.8^{a}	–0.833^{c}	271.2^{a}	–	0.441^{d}	72.7^{a}	215.5^{c}

k_{acid} – rate of acid concentration changes (mg/kg/h); c_0 – initial concentration of acid (mg/kg); c_{end} – final concentration of acid (mg/kg); $^{a–f}$means within a column with different superscript letters are significantly different ($P < 0.05$); $n = 3$

those prepared from whole buckwheat and amaranth flours (Kocková *et al*. 2013b).

The changes in the levels acids organic are presented in Figure 3 and Table 4. The parameters of organic acids changes were calculated for both periods together, because of the continuing metabolic activity during storage. The rate of lactic acid production ranged from 0.547–4.555 mg/kg/h, which is similar to the rate during the storage period of cereal and pseudocereal porridges (Kocková *et al*. 2013b). The concentration of lactic acid at the end of the storage was 161.6–1977.8 mg/kg/h. The level of lactic acid in cereal and pseudocereal porridges at the end of the storage period ranged from 236.4–1122.9 mg/kg (Kocková *et al*. 2013b). The amount of lactic acid in oat, barley and malt beverages fermented at 37°C for 10 h with *Lb. plantarum* NCIMB 8826, *Lb. acidophilus* NCIMB 8821 and *Lb. reuteri* NCIMB 11951 ranged from 180 to 2670 mg/l (Salmerón *et al*. 2013). According to Helland *et al*. (2004a), lactic acid concentration in maize-rice water- and milk-based pudding fermented with *Lb. rhamnosus* GG was 2600 and 9800 mg/kg, respectively. The production of lactic acid in maize-barley porridge fermented with *Lb. rhamnosus* GG reached 4000 mg/kg (Helland *et al*. 2004b).

Acetic acid was produced only in the products prepared from oats in combination with soya and chickpea. The rate of production and final concentration of acetic acid in these substrates were lower in comparison with those of lactic acid, which is similar to fermented cereal and pseudocereal water-based porridges (Kocková *et al*. 2013b).

During the fermentation and storage periods utilisation of citric acid was observed. The rate of utilisation ranged from 0.267 to 10.821 mg/kg/h. The final citric acid concentration as calculated varied from

Table 5. Sensory evaluation of fermented and stored probiotic cereal, pseudocereal, and cereal-leguminous products

		Colour	Cohesiveness	Mastic ability	Aroma	Taste
Buckwheat	F	1.57 ± 0.53^{a}	2.72 ± 0.57^{a}	3.63 ± 0.52^{b}	2.75 ± 0.46^{b}	3.31 ± 0.46^{c}
	S	1.67 ± 0.52^{a}	2.45 ± 0.60^{a}	3.00 ± 0.82^{b}	2.13 ± 0.64^{a}	2.44 ± 0.62^{b}
Dark buckwheat	F	2.67 ± 0.82^{c}	3.17 ± 0.41^{b}	3.70 ± 0.48^{b}	2.63 ± 0.74^{b}	2.38 ± 0.52^{b}
	S	2.33 ± 0.52^{b}	2.75 ± 0.46^{a}	3.50 ± 0.53^{b}	2.88 ± 0.83^{b}	2.19 ± 0.53^{b}
Barley	F	3.00 ± 0.50^{c}	2.89 ± 0.93^{a}	2.25 ± 0.46^{a}	2.63 ± 0.52^{b}	3.38 ± 0.52^{c}
	S	3.00 ± 0.71^{c}	2.57 ± 0.53^{a}	2.33 ± 0.50^{a}	1.94 ± 0.81^{a}	2.38 ± 0.52^{b}
Oat	F	3.33 ± 0.50^{c}	2.86 ± 0.38^{a}	2.44 ± 0.73^{a}	2.57 ± 0.53^{b}	3.38 ± 0.52^{c}
	S	3.25 ± 0.46^{c}	2.56 ± 1.13^{a}	2.33 ± 0.87^{a}	1.89 ± 0.78^{a}	3.00 ± 0.76^{c}
Oat + soya	F	2.83 ± 0.41^{c}	2.83 ± 0.75^{a}	3.00 ± 0.63^{b}	2.67 ± 0.52^{b}	3.92 ± 0.20^{d}
	S	2.83 ± 0.41^{c}	2.33 ± 1.03^{a}	2.67 ± 0.52^{a}	2.50 ± 0.55^{b}	1.25 ± 0.50^{a}
Oat + chickpea	F	2.83 ± 0.41^{c}	3.17 ± 0.75^{b}	2.83 ± 0.75^{b}	2.83 ± 0.41^{b}	3.67 ± 0.52^{d}
	S	2.83 ± 0.41^{c}	2.83 ± 0.98^{a}	2.50 ± 0.55^{a}	2.33 ± 0.82^{b}	1.25 ± 0.50^{a}

F – fermented products; S – fermented products after storage period. 0 – worst possible value, 4 – best possible value; $^{a–d}$means within a column with different superscript letters are significantly different ($P < 0.05$); $n = 3$

154.6 to 464.6 mg/kg, while citric acid amounts in buckwheat and oat-chickpea products were under the detection limit. The level of citric acid in fermented cereal and pseudocereal porridges after the storage ranged from 136.9 to 823.0 mg/kg (Kocková *et al*. 2013b). In all products, the production of formic acid was observed, with the rate ranging from 0.014 to 4.482 mg/kg/h. The concentration of formic acid at the end of the process was higher in comparison with the cereal and pseudocereal substrate (Kocková *et al*. 2013a).

According to Kocková *et al*. (2013b), in cereal and pseudocereal water-based porridges no metabolic activity of *Lb. rhamnosus* GG was observed during storage, excepting the porridges prepared from amaranth and whole buckwheat flours.

Sensory analyses. The sensory parameters of the followed cereal, pseudocereal, and cereal-leguminous products after fermentation and storage period were determined. The storage influenced the sensory evaluation, especially in the case of the aroma of the buckwheat, barley, and oat products and the taste of the buckwheat, barley, and oat-leguminous products, with which significant differences ($P > 0.05$) were observed.

CONCLUSION

The probiotic strain *Lb. rhamnosus* GG was able to grow and metabolise in cereal, pseudocereal, and leguminous substrates with only a small content of residual water, unlike in porridges or beverages. It was also able to survive in fermented products during storage at refrigerating temperature, with its metabolic activity continuing. The fermented products were acceptable for consumers, but the sensory values of the stored products were lower compared to those of fresh fermented products, due to the metabolic activity of the probiotic strain during storage.

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