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LEGUMES AS POTENTIAL PLANTS FOR PROBIOTIC STRAIN *LACTOBACILLUS RHAMNOSUS* GG

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Abstract

PETRULÁKOVÁ MONIKA, VALÍK ĽUBOMÍR. 2015. Legumes as Potential Plants for Probiotic Strain *Lactobacillus rhamnosus* GG. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 63(5): 1505–1511.

The aim of the study was evaluation of growth and metabolic activity of *Lactobacillus rhamnosus* GG during fermentation of leguminous porridges (soybean flour, soybean, chickpea flour, chickpea, white bean, red bean, speckled bean, green lentil, husked lentil, yellow pea), and the evaluation of their stability during storage. A mixture of leguminous sample with water was inoculated after sterilization with equal number of *L. rhamnosus* GG, to obtain 5 log cfu/g in the porridge. Fermentation was led at 37 °C during 10 hours and storage at 5 °C for 21 days. Monitoring of the lactobacilli counts, pH value, and concentration of organic acids during fermentation and storage was done. Calculation of growth and metabolic parameters during fermentation and storage period was performed by the mechanistic model of Baranyi and Roberts. *L. rhamnosus* GG was able to grow up to 6.8–7.9 log cfu/g during fermentation, cell density during storage period was stable, except whole soybean, yellow pea and red bean. Metabolic activity of *L. rhamnosus* GG during fermentation caused decrease of pH value to the final 5.6–6.0, increase of lactic and acetic acid concentration to 89.3–341.7 mg/kg and 129.2–525.2 mg/kg, respectively. During storage period, metabolic activity of *L. rhamnosus* GG continued.

Keywords: legumes, fermentation, *Lactobacillus rhamnosus* GG, probiotic, growth parameters, metabolic parameters

INTRODUCTION

Legumes (*Leguminosae*) are plants producing seeds for human nutrition. They are subdivided into two groups: pulses and leguminous oilseeds. Legumes with higher content of carbohydrates and low amount of lipids, like lentils or beans, belong to the first group. Typical representative of the second group, legumes with higher content of lipids and lower content of carbohydrates, is soybean. As the legumes are good and cheap source of proteins, carbohydrates, vitamins and minerals they are important component of nutrition mainly in developing countries. Legumes in daily diet have physiological effects in controlling or preventing some metabolic diseases thanks to high content of dietary fibre (Michaels, 2004; Boschin and Arnoldi, 2011; Sreerama *et al*., 2012; Tharanathan and Mahadevamma, 2003).

Fermentation is probably one of the oldest methods of processing legumes, besides germination, soaking or boiling. Fermented leguminous products are widespread mainly in countries of Asia, Africa and South America (Frias *et al*., 2005). During fermentation, reduction of anti-nutritional factors (oligosaccharides, allergenic proteins, proteinase inhibitors, lectins and cyanogenic glycosides) and immunoreactivity can occur (Egounleta and Aworh, 2003; Song *et al*., 2008). In additional, fermented leguminous foods could be one of the carriers of probiotic bacteria and variegate market with probiotic foods, which are usually milk based and thus unsuitable for people who have to restrict the dairy products consumption (due to lactose intolerance, cow's milk allergy, low-protein diet, phenylketonuria) or who dislike dairy products (Němečková *et al*., 2011).

Lactobacillus rhamnosus GG belongs to the group of facultatively heterofermentative lactic acid bacteria. Evidence of the ability of *L. rhamnosus* GG to tolerate presence of bile salts and ability to survive during gastric and duodenal digestion exist. *L. rhamnosus* GG effectively colonizes gastrointestinal tract after three days of use (Görner and Valík, 2004; Lam *et al*., 2007; Pitino *et al*., 2010; Succi *et al*., 2005). According to Valík *et al*. (2008) and Kocková et *al*. (2013a; 2013b), *L. rhamnosus* GG showed good growth in milk, cereal and pseudocereal porridges. Based on these results and increasing interest in plant probiotic foods we decided to evaluate growth and metabolic activity of *L. rhamnosus* GG also in the leguminous porridges during fermentation and storage period.

MATERIALS AND METHODS

Materials

Soybean flour, whole soybean and chickpea flour obtained from mill house (Mill Zrno, Šíšov, Slovakia), and green lentil, white bean, speckled bean (Svitko, Banská Bystrica, Slovakia), red bean, chickpea (Lagris, Dolní Lhota u Luhačovic, Czech Republic), husked lentil (Marianna, Ivánka pri Dunaji, Slovakia) and yellow pea (Kroner, Bratislava, Slovakia) obtained from stores were used in this work.

Microorganism, Inoculation and Cultivation Conditions

The probiotic strain *L. rhamnosus* GG ATCC 53103 was used in this work. It was kept in de Man – Rogosa – Sharpe broth (MRS; Merck, Darmstadt, Germany) at 5 ± 1 °C. The standard suspension of the microorganism was prepared from an 18 h culture grown in MRS broth at 37 °C.

Preparation of Media and Fermentation Process

Sixty grams of flours or milled and sieved (0.5 mm sieve size) grains were mixed with 540 mL of deionised water and autoclaved (121 °C, 15 min). After cooling, inoculation with overnight culture of *L. rhamnosus* GG at initial density of cells at approximately 5 log colony form units per gram (cfu/g) was done. Static fermentation was performed at 37 °C for 10 hours. Storage observations were carried out at 5 °C for 21 days. Samples for analyses were taken every 2 hours and every 2–3 days during the fermentation and storage tests, respectively.

Microbial Analysis

The numbers of *L. rhamnosus* GG were determined on MRS-agar (Merck, Darmstadt, Germany), according to the Slovak Technical Standard STN ISO 15214 (2002).

Chemical Analysis

The pH of samples was measured by pH-meter CG 843 (Schott, Mainz, Germany). The quality and quantity of the produced organic acids were measured by isotachophoretic analysis by using the Isotachophoretic Analyser ZKI 01 (Villa Labeco, Spišská Nová Ves, Slovak Republic). Electrolytic system according to Kocková *et al*. (2013b) was used. Quantitative analysis was performed by calibration of standard solution of lactic, acetic and citric acids (Lachema, Brno, Czech Republic).

Estimation of Growth and Metabolic Parameters of *L. rhamnosus* GG

Growth and metabolic curves and the corresponding parameters of *L. rhamnosus* GG behaviour in each substrate were fitted with DMFit tool, a mechanistic model of Baranyi and Roberts (1994).

Statistical Analysis

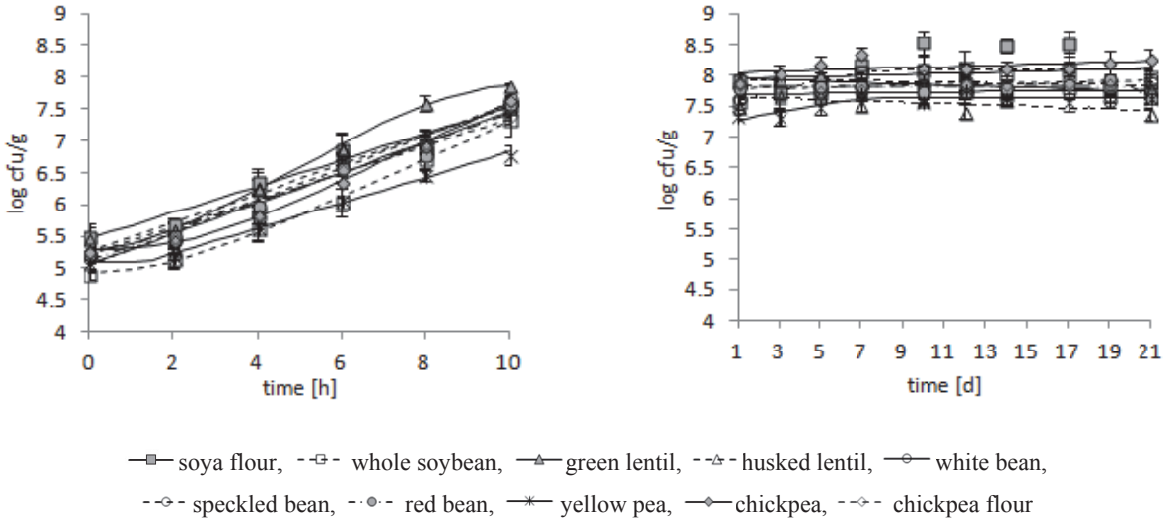
Each experiment was performed in three separate trials. Results represented means with standard deviations. Statistical analysis were carried out using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA). Data were treated by independent two-sample Student t-test with a least significant difference of 95%.

RESULTS AND DISCUSSION

Growth of *L. rhamnosus* GG

According to the results shown in Fig. 1, *Lactobacillus rhamnosus* GG was able to grow in all leguminous porridges during ten-hour fermentation process to final counts 6.8–7.9 log cfu/g. Higher cell counts of *L. rhamnosus* GG after fermentation period were observed in cereal and pseudocereal porridges (Kocková *et al*., 2013a), rice-maize puddings prepared with water or milk (Helland *et al*., 2004a) and in maize porridge (Helland *et al*., 2004b). Growth rates (Tab. I) of probiotic strain in leguminous porridges ranged from 0.20 to 0.35 log cfu/g/h, while in cereal and pseudocereal porridges ranged from 0.25 to 0.59 log cfu/g/h (Kocková *et al*., 2013a). Higher growth rate of *L. rhamnosus* GG was observed in milk at 35 °C (Valík *et al*., 2008) and in buckwheat and amaranth milk or water based puddings fermented at 37 °C (Pelikánová *et al*., 2008). Lag phase, a period for adapting microorganism to new environment, was observed only in case of whole soybean, green lentil, yellow pea and chickpea and ranged from 1.3 h to 2.5 h (Tab. I).

During storage period, an increase in the cell counts was observed in case of whole soybean and yellow pea at the beginning of period. The cell counts decreased only in red bean porridge at the end of storage period (after 467.8 h). *L. rhamnosus* GG was also able to survive in cereal and pseudocereal porridges during storage period



1: *Evaluation of cell counts of Lb. rhamnosus GG during fermentation and storage*

I: *Growth parameters of Lb. rhamnosus GG during fermentation and storage*

	Fermentation			Storage
	Gr [log CFU/g/h]	**t_d [h]**	**λ [h]**	**Gr [log CFU/g/d]**
Soya flour	0.21^{a}	1.5^{e}	-	0.01^{e}
Whole soybean	0.29^{c}	1.0^{b}	1.9^{b}	0.09^{g}
Green lentil	0.35^{d}	0.9^{a}	1.3^{a}	–0.01^{b}
Husked lentil	0.23^{b}	1.3^{d}	-	–0.01^{b}
White bean	0.24^{b}	1.3^{d}	-	0.00^{d}
Speckled bean	0.25^{b}	1.2^{d}	-	–0.01^{c}
Red bean	0.23^{b}	1.3^{d}	-	–0.38^{a}
Yellow pea	0.20^{a}	1.5^{e}	1.4^{a}	0.06^{f}
Chickpea	0.31^{c}	1.0^{b}	2.5^{c}	0.01^{e}
Chickpea flour	0.21^{a}	1.4^{e}	-	0.01^{e}

Gr – growth rate, t_d – time to double, λ – lag phase
$^{a–h}$ – means within a column with different superscript letters are significantly different ($P < 0.05$)

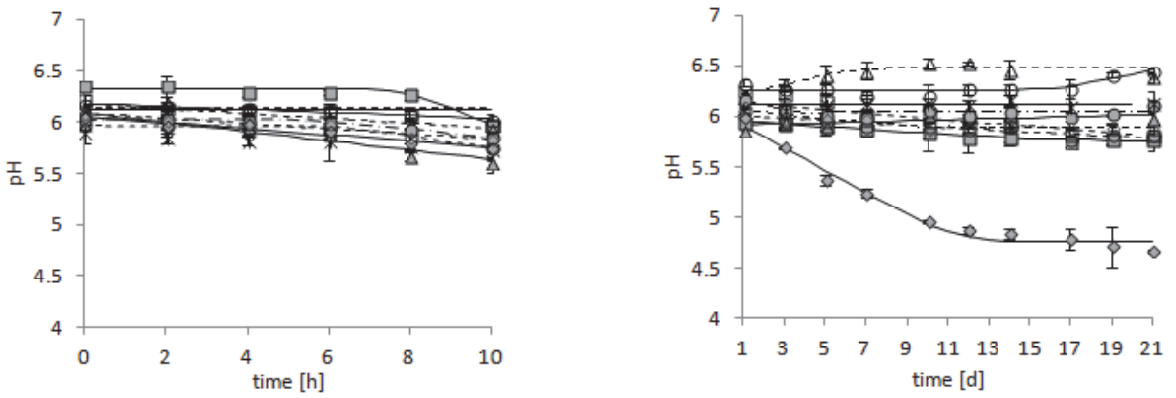
(Kocková *et al*., 2013a) and additionally, *L. rhamnosus* GG was the only strain able to survive in water based rice – maize puddings among several probiotic strains used by Helland *et al*. (2004a).

Metabolic Activity of *L. rhamnosus* GG

Metabolic activity of *L. rhamnosus* GG in leguminous porridges led to decrease of pH values very slightly (from initial 5.9–6.4 to final 5.6–6.0; Fig. 2) with the rates ranged from –0.01 to –0.15 1/h (Tab. II). Long lag phases were observed in the porridges prepared from soya flour, speckled beans and red beans (Tab. II). Faster decrease in pH values was observed in case of cereal and pseudocereal porridges (Kocková *et al*., 2013a) and in milk and water based buckwheat and amaranth puddings (Pelikánová *et al*., 2011). *L. rhamnosus* GG also caused slight acidification in milk, only to 6.5 (Valík *et al*., 2008) and in MRS broth, to 4.0 (Zalán *et al*., 2010). In comparison, pH values in malt, barley and malt-barley substrates fermented by *L. acidophilus* and *L. plantarum* were below 3.5 (Rathore *et al*., 2012) and pH values of vegetable substrates fermented with lactic acid bacteria ranked from 3.7 to 4.5 (Němečková *et al*., 2011).

The pH values in the most of porridges were unsettled during storage period. In porridges prepared from green lentil, husked lentil, white beans and yellow pea, pH values increased with the rates ranged from 0.01/h to 0.08/h, was observed. In other porridges, decrease in pH values occurred, significantly in case of chickpea porridge with rate –0.11/h. Cereal and pseudocereal substrates were metabolically stable during storage period, except amaranth porridge and porridge from whole buckwheat flour (Kocková *et al*., 2013a).

Production of lactic acid by *L. rhamnosus* GG was observed in all porridges during fermentation period, except those prepared from white beans and speckled beans, from initial 43.6–227.3 mg/kg to final 89.3– 341.7 mg/kg (Fig. 3). The highest rate of lactic acid production was calculated for soya flour



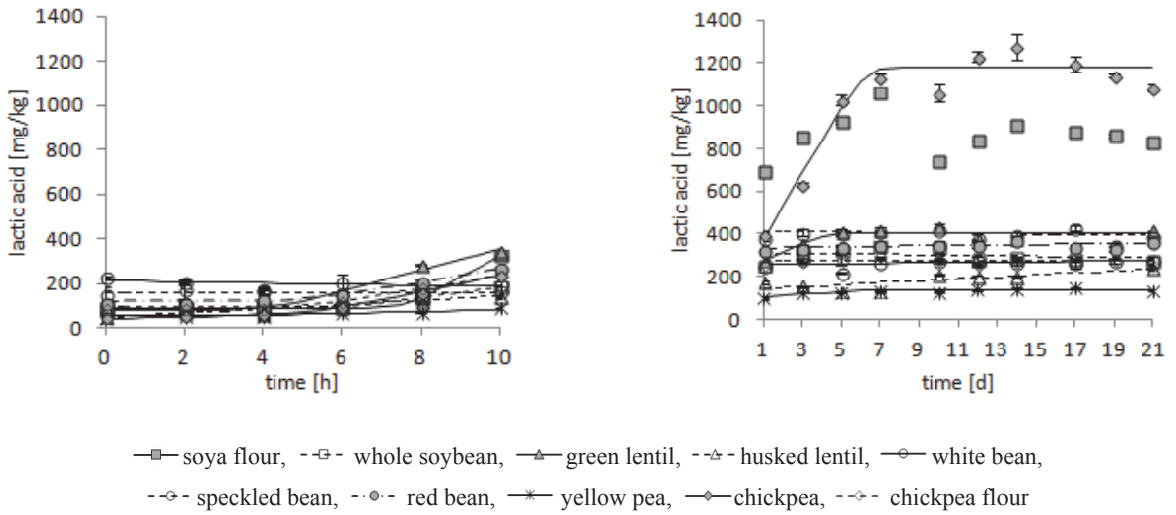
2: *Evaluation of pH values during fermentation and storage*

II: *Parameters of pH value changes and changes of lactic acid concentration during fermentation and storage*

	pH			Lactic acid		
	Fermentation		Storage	Fermentation		Storage
	k_{pH} [1/h]	λ_{pH} [h]	k_{pH} [1/d]	k_{acid} [mg/kg/h]	λ_{acid} [h]	k_{acid} [mg/kg/h]
Soya flour	–0.15^{g}	7.8^{a}	–0.01^{c}	97.5^{i}	7.6^{d}	0.06^{d}
Whole soybean	0.00^{b}		–0.02^{c}	24.0^{c}	6.1^{c}	–0.04^{a}
Green lentil	–0.04^{e}		0.01^{c}	48.1^{h}	4.2^{a}	1.68^{g}
Husked lentil	–0.02^{d}		0.05^{f}	9.3^{d}	-	0.19^{e}
White bean	–0.01^{c}		0.04^{d}	–2.6^{a}	-	0.04^{c}
Speckled bean	–0.06^{f}	6.2^{a}	–0.01^{c}	0.3^{b}	-	–0.04^{a}
Red bean	–0.04^{e}	5.7^{a}	–0.05^{b}	28.9^{f}	5.0^{b}	0.05^{c}
Yellow pea	0.08^{a}	20.4^{b}	0.08^{f}	3.9^{c}	-	0.30^{f}
Chickpea	–0.03^{d}		–0.11^{a}	36.0^{g}	5.1^{b}	6.54^{h}
Chickpea flour	–0.03^{d}		–0.02^{c}	29.5^{f}	5.0^{b}	0.00^{b}

k_{pH} – rate of changes in pH values, λ_{pH} – lag phase of pH changes, k_{acid} – rate of acid concentration changes, λ_{acid} – lag phase of lactic acid concentration changes

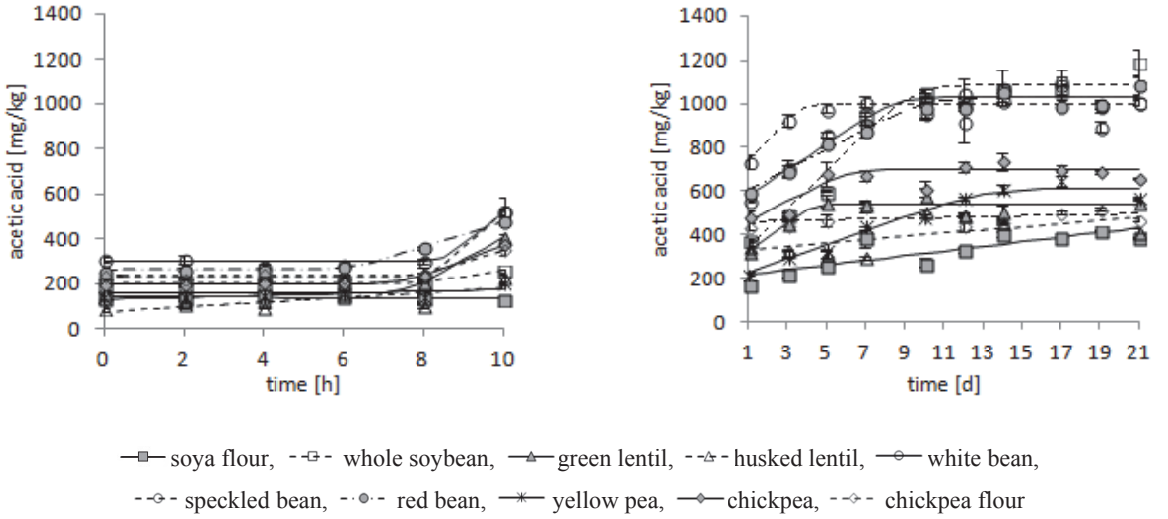
$^{a–h}$ – means within a column with different superscript letters are significantly different ($P < 0.05$)



3: *Evaluation of concentration of lactic acid during fermentation and storage*

porridge, 97.5 mg/kg/h. Lag phase was observed in most of porridges ranged from 4.2 h to 7.6 h (Tab. II). Lactic acid levels at the end of fermentation of cereal and pseudocereal porridges ranged from 174.7 mg/kg to 644.2 mg/kg (Kocková *et al.*, 2013a). Higher lactic acid levels after fermentation by *L. rhamnosus* GG was observed in MRS broth (Zalán *et al.*, 2010), in milk (Valík *et al.*, 2008), in maize porridges (Helland *et al.*, 2004b) and in rice-maize puddings (Helland *et al.*, 2004a). During storage period, continuation of lactic acid production was observed in all porridges except those prepared from whole soybean and speckled bean. The highest rate of lactic acid production during storage was calculated for chickpea porridge, 6.54 mg/kg/h. In cereal and pseudocereal porridges fermented with *L. rhamnosus* GG, no significant changes in lactic acid level during storage was observed, except amaranth flour and whole buckwheat flour, in which the production of lactic acid was observed (Kocková *et al.*, 2013a).

The concentrations of acetic acid increased in all porridges, except soya flour porridge, from initial 86.41–308.96 mg/kg to final 129.2–525.2 mg/kg (Fig. 4). The rate of acetic acid production was higher in compare to rate of lactic acid production in most of porridges, what effected final sensory quality of porridges in negative way. In all porridges, except those prepared from soya flour, husked lentil and yellow pea, the lag phases of acetic acid production were also observed. They ranged from 6.3 h to 8.5 h (Tab. III). Production of acetic acid continued, however, with the rates ranged from 0.08 mg/kg/h to 3.92 mg/kg/h during storage period. Final acetic acid levels after storage period ranged from 389.3 mg/kg to 1187.5 mg/kg (Tab. III). Comparing to our results, Kocková *et al.* (2013a) found slower production of acetic acid than production of lactic acid in the the cereal and pseudocereal porridges. While acetic acid was also produced with the similar or higher rates of 0.01–6.79 mg/kg/h in case of

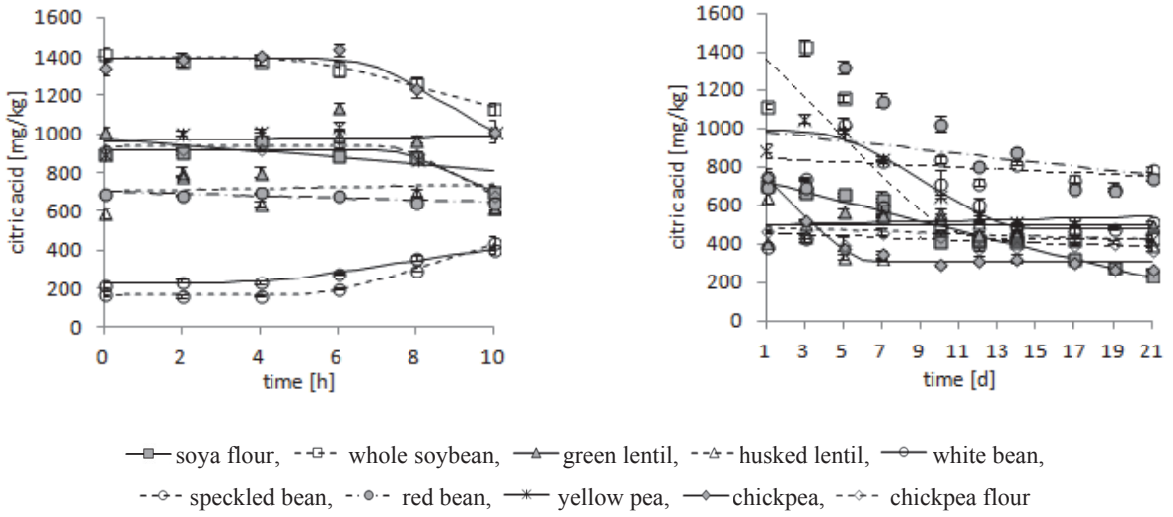


4: *Evaluation of concentration of acetic acid during fermentation and storage*

III: *Parameters of acetic acid and citric acid concentration changes during fermentation and storage*

	Acetic acid			Citric acid		
	Fermentation		Storage	Fermentation		Storage
	k_{acid} [mg/kg/h]	λ_{acid} [h]	k_{acid} [mg/kg/h]	k_{acid} [mg/kg/h]	λ_{acid} [h]	k_{acid} [mg/kg/h]
Soya flour	–0.57^{a}	-	0.44^{c}	–94.55^{h}	7.7^{e}	–1.04^{g}
Whole soybean	26.95^{d}	7.9^{b}	3.76^{h}	–55.22^{g}	5.4^{b}	–4.26^{i}
Green lentil	102.58^{g}	7.6^{b}	2.75^{g}	–16.68^{f}	-	0.00^{b}
Husked lentil	10.36^{c}	-	0.31^{b}	3.47^{c}	-	–0.13^{c}
White bean	142.94^{h}	8.5^{b}	2.47^{f}	30.92^{b}	4.1^{a}	0.09^{a}
Speckled bean	167.08^{h}	8.2^{b}	3.92^{i}	64.94^{a}	5.9^{c}	–0.20^{c}
Red bean	57.84^{e}	6.3^{a}	1.93^{e}	–5.47^{e}	-	–0.46^{f}
Yellow pea	4.40^{b}	-	1.34^{d}	2.42^{d}	-	–2.68^{h}
Chickpea	69.79^{f}	7.5^{b}	1.98^{e}	–117.38^{i}	6.8^{d}	–4.02^{i}
Chickpea flour	56.31^{e}	8.0^{b}	0.08^{a}	–112.46^{i}	7.7^{e}	–0.14^{d}

k_{acid} – rate of acid concentration changes, λ_{acid} – lag phase of lactic acid concentration changes
$^{a–h}$ – means within a column with different superscript letters are significantly different ($P < 0.05$)



5: *Evaluation of concentration of citric acid during fermentation and storage*

amaranth grain during storage, in the buckwheat flour porridges, an reduction of acetic acid level was observed.

Utilization of citric acid during fermentation period was observed in porridges prepared from soya flour, whole soybean, green lentil, red bean, chickpea and chickpea flour, from initial 692.7–1413.6 mg/kg to final 642.2–1131.8 mg/kg (Fig. 5), with the rates of utilization ranged from –5.5 mg/kg/h to –117.4 mg/kg/h (Tab. III). In porridges prepared from husked lentil, white bean, speckled bean and yellow pea, the production of citric acid was observed, with the rates ranged from 2.42 mg/kg/h to 64.94 mg/kg/h (Tab. III). Utilization of citric acid by *L. rhamnosus* GG was also observed in cereal and pseudocereal porridges (Kocková *et al.*, 2013a), maize porridge (Helland *et al.*, 2004b) and rice-maize puddings (Helland *et al.*, 2004a). During storage period in all porridges, except white bean, the utilization of citric acid was observed, with the rates of utilization ranged from –0.13 mg/kg/h to –4.26 mg/kg/h. Its concentration after storage period ranged from 230.1 mg/kg to 787.0 mg/kg. Citric acid level in cereal and pseudocereal porridges during storage was stable, except those prepared from amaranth flour and whole buckwheat flour, in which continuing of citric acid utilization was observed (Kocková *et al.*, 2013a).

CONCLUSION

According to our results, probiotic strain *L. rhamnosus* GG is able to grow and metabolize during fermentation of leguminous porridges. Cell counts of *L. rhamnosus* GG during storage were stable, except red bean porridge, in which decrease in cell counts after 19 days was observed. Cell counts were over 6 log cfu g^{-1}, what is a legislative limit for labelling food as probiotic, in all substrates at the end of storage period. On the other hand, metabolic activity of *L. rhamnosus* GG continued during storage, what influenced sensory quality of porridges in negative way. Nevertheless, we believe that the use of legumes as carriers of probiotic bacteria has a future. It will, however, necessary to resolve the question of shelf-life of fermented products prepared in this way.

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REFERENCES

BARANYI, J. and ROBERTS, T. A. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.*, 23(3–4): 277–294.

BOSCHIN, G. and ARNOLDI, A. 2011. Legumes are valuable sources of tocopherols. *Food Chem.*, 127(3): 1199–1203.

EGOUNLETY, M. and AWORH, O. C. 2003. Effect of soaking, dehulling, cooking and fermentation with

Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. Walp) and groundbean (Macrotyloma geocarpa Harms). *J. Food Eng.*, 56(2–3): 249–254.

FRIAS, J., MIRANDA, M. L., DOBLADO, R. and VIDAL-VALVERDE, C. 2005. Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of Lupinus albus L. var. Multolupa. *Food Chem.*, 92(2): 211–220.

GÖRNER, F. and VALÍK, Ľ. 2004. *Aplikovaná mikrobiológia požívatín*. Bratislava: Malé Centrum.

HELLAND, M. H., WICKLUND, T. and NARVHUS, J. A. 2004a. Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. *Int. Dairy J.*, 14(11): 957–965.

HELLAND, M. H., WICKLUND, T. and NARVHUS, J. A. 2004b. Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. *Int. J. Food Microbiol.*, 91(3): 305–313.

KOCKOVÁ, M., DILONGOVÁ, M., HYBENOVÁ, E. and VALÍK, Ľ. 2013a. Evaluation of cereal and pseudocereal suitability for the development of new probiotic foods. *J. Chem.*, 2013: 8.

KOCKOVÁ, M., MENDEL, J., MEDVEĎOVÁ, A., ŠTURDÍK, E. and VALÍK, Ľ. 2013b. Cereals and pseudocereals as substrates for growth and metabolism of a probiotic strain *Lactobacillus rhamnosus* GG. *J. Food Nutr. Res.*, 52(1): 25–36.

LAM, E. K. Y., TAI, E. K. K., KOO, M. W. L., WONG, H. P. S., WU, W. K. K., YU, L. et al. 2007. Enhancement of gastric mucosal integrity by *Lactobacillus rhamnosus* GG. *Life Sci.*, 80(23): 2128–2136.

MICHAELS, T. E. 2004. Pulses, Overview. Wrigley, C. (ed), *Encyclopedia of grain science.* Oxford: Elsevier Academic Press.

NĚMEČKOVÁ, I., DRAGOUNOVÁ, H., PECHAČOVÁ, M., RYSOVÁ, J. and ROUBAL, P. 2011. Fermentation of vegetable subs*trate* by lactic acid bacteria as a basis of functional foods. *Czech J. Food Sci.*, 29(2011): S42–S48.

PELIKÁNOVÁ, J., LIPTÁKOVÁ, D., VALÍK, Ľ. and STANČEKOVÁ, K. 2011. Evaluation of the growth of selected lactobacilli in pseudocereal substrate. *Potravinarstvo,* 5(4): 53–57.

PITINO, I., RANDAZZO, C. L., MANDALARI, G., LO CURTO, A., FAULKS, R. M., LE MARC, Y. et al. 2010. Survival of *Lactobacillus rhamnosus* strains in the upper gastrointestinal tract. *Food Microbiol*, 27(8): 1121–1127.

RATHORE, S., SALMERÓN, I. and PANDIELLA, S. S. 2012. Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. *Food Microbiol.*, 30(1): 239–244.

SONG, Y. S., FRIAS, J., MARTINEZ-VILLALUENGA, C., VIDAL-VALDEVERDE, C. and GONZALEZ DE MEJIA, E. 2008. Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chem.*, 108(2008): 571–581.

SREERAMA, Y. N., SASHIKALA, V. B., PRATAPE, V. M. and SINGH, V. 2012. Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chem.*, 131(2012): 462–468.

STN ISO 15214. 2002. *Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30 °C* [in Czech: *Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu mezofilných kyslomliečnych baktérií. Metóda počítania kolónií kultivovaných pri 30 °C*]. Bratislava, Slovak Republic: Slovak standards institute.

SUCCI, M., TREMONTE, P., REALE, A., SORRENTINO, E., GRAZIA, L., PACIFICO, S. et al. 2005. Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *FEMS Microbiol Lett*, 244(1): 129–137.

THARANATHAN, R. N. and MAHADEVAMMA, S. 2003. Grain legumes – a boon to human nutrition. *Trends Food Sci. Tech.*, 14(12): 507–518.

VALÍK, Ľ., MEDVEĎOVÁ, A. and LIPTÁKOVÁ, D. 2008. Characterization of the growth of *Lactobacillus rhamnosus* GG in milk at suboptimal temperatures. *J. Food Nutr. Res.*, 47(2): 60–67.

YADAV, S. and KHETARPAUL, N. 1994. Indigenous legume fermentation: Effect on some antinutrients and in-vitro digestibility of starch and protein. *Food Chem.*, 50(4): 403–406.

ZALÁN, Z., HUDÁČEK, J., ŠTĚTINA, J., CHUMCHALOVÁ, J. and HALÁSZ, A. 2010. Production of organic acids by *Lactobacillus* strains in three different media. *Eur. Food Res. Technol.*, 230(3): 395–404.

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