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Application of Lactic Acid Bacteria for Production of Fermented Beverages Based on Rice Flour

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Abstract

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We investigated the suitability of rice flour for fermented beverage production using various strains of lactic acid bacteria. Fermentation led to a decrease in pH from 5.04–5.17 to 3.74–4.35. At the same time, total acidity increased (1.28–2.59 g/l) due to lactic acid (0.59–2.76 g/l) and acetic acid (0.11–0.30 g/l) production. Fermentation of rice beverages also caused a gradual decrease in glucose and fructose concentration. Lactic acid bacteria proliferated in the first phases of fermentation, and cell counts reached a maximum after 12 h. The highest growth rate (v_{LAB} = 0.44 Log_{10} CFU/ml/h) was observed in a sample with the culture of *Lactobacillus brevis* CCM 1815. Viscosity of beverages decreased significantly after 24 h of fermentation. The highest values of sensory parameters were observed in a monoculture of *Lactobacillus plantarum* CCM 7039 and in a sample with a mixed culture of *Lactobacillus plantarum* CCM 7039 and *Bifidobacterium longum* CCM 4990.

Keywords: cITP; HPLC; fermentation; rice; sensory analysis; viscosity

A large portion of cereals is traditionally processed into foods and beverages through fermentation (Nout 2009). Foods that are fermented have been subjected to the action of microorganisms or enzymes, leading to desirable biochemical changes. Fermentation is a relatively cost-effective, low-energy preservation process, which is essential in ensuring the shelf-life and microbiological safety of the product (Liu *et al.* 2011).

Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein and fibre digestibility, by enhancing micronutrient bioavailability, and by degrading anti-nutritional factors. Fermentation processes also enhance food safety by reducing toxic compounds, such as aflatoxins, and by producing antimicrobial factors, such as lactic acid, bacteriocins, CO_2, H_2O_2, and ethanol, which facilitate inhibition or elimination of food-borne pathogens. In addition to its nutritive, safety and preservative effects, fermentation enriches the diet through the production of a diversity of flavours, textures, and aromas (Giraffa 2004).

The demand of consumers for non-dairy milk substitutes with high acceptance and functionality is increasing. Cereal-based beverages have a huge potential to fulfil this expectation and to act as potential vehicles for functional compounds such as antioxidants, dietary fibre, minerals, prebiotics, and vitamins (Nionelli *et al.* 2014). Nowadays, many studies examine traditional and novel cereal-based beverages fermented with various microorganisms and with potentially probiotic and functional properties (Gupta *et al.* 2010; Coda *et al.* 2012; Rathore *et al.* 2012; Nionelli *et al.* 2014; Ghosh *et al.* 2015).

The aim of this study was to prepare fermented rice beverages by application of different strains of lactic acid bacteria and to observe the fermentation process by chemical, physical, and microbiological analysis, and to characterise the sensory properties of final products.

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MATERIAL AND METHODS

Bacterial strains and culture media. *Lactobacillus brevis* CCM 1815, *Lactobacillus fermentum* CCM 7192, *Lactobacillus plantarum* CCM 7039, and *Bifidobacterium longum* CCM 4990 were purchased from the Czech Collection of Microorganisms (Masaryk University, Brno, Czech Republic) as lyophilisate. Strains of lactobacilli were reconstituted in De Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37°C for 24 hours. For *Bifidobacterium longum* CCM 4990, MRS medium with L-cysteine was used and cultivation was carried out under strictly anaerobic conditions at 37°C. Cells were centrifuged at 5000 rpm for 20 min after incubation. Next, cells were washed twice in sterile saline solution and resuspended in sterile saline solution before being added to the beverages.

Preparation of rice beverages. *S*amples of fermented rice beverages (RB): RB *Lbp* – rice beverage inoculated with *Lactobacillus plantarum* CCM 7039; RB *Lbb* – rice beverage inoculated with *Lactobacillus brevis* CCM 1815; RB *Lbf* – rice beverage inoculated with *Lactobacillus fermentum* CCM 7192; and RB *Lbp* + *B. long* – rice beverage fermented with a mixed culture (1 : 1 v/v) of *Lactobacillus plantarum* CCM 7039 and *Bifidobacterium longum* CCM 4990 were prepared according to Coda *et al.* (2012). Briefly, rice flour 12% (w/w) was mixed with tap water and the mixture was heated at 95°C for 10 minutea. Afterwards, red grape must (10% v/w) was added. After homogenisation and cooling at ca. 40°C the inoculum of starter culture was applied. Fermentation was carried out at 30°C for 24 h (Coda *et al.* 2012).

pH, total titratable acidity and kinetics of acidification. The pH was determined using a digital pH meter (inoLab pH Level 2; WTW, Weilheim, Germany). Total titratable acidity was determined by visual titration with a standard solution of NaOH (0.1 mol/l) and using phenolphthalein as the indicator. Kinetics of acidification was modelled according to the Gompertz equation as modified by Zwietering *et al.* (1990).

Organic acid determination by cITP. For the measurements of lactic and acetic acid, the capillary isotachophoretic (cITP) method according to Kohajdová *et al.* (2006) and the isotachophoretic analyser ZKI 01 (Villa Labeco, Spišská Nová Ves, Slovakia) with conductivity detector and two-line recorder TZ 4200 (Laboratorní přístroje, Prague, Czech Republic) were used. Validation of the cITP method was also carried out. Determination of validation parameters (selectivity, linearity, limit of detection, limit of quantification, precision and recovery) was performed according to Karovičová *et al.* (2003) and Lehman *et al.* (2011).

HPLC analysis of saccharides. Concentrations of glucose and fructose in water-soluble extracts of samples were measured by high-performance liquid chromatography (HPLC) according to a modified method of Xiong *et al.* (2014) using a DeltaChrom™ SDS 030 apparatus (Watrex, Bratislava, Slovakia) equipped with a Polymer IEX column in H^+ form (250 × 8 mm, 8 µm) (Watrex, Bratislava, Slovakia) and an RI K-2301 refractive index detector (Knauer, Berlin, Germany). Elution was at 50°C, with a flow rate of 1 ml/min, using 0.9 mM H_2SO_4 as the mobile phase.

Enumeration of lactic acid bacteria. A 10-g sample was taken aseptically and blended in 90 ml of sterile NaCl solution (0.85 w/v) in a 500-ml Erlenmeyer flask using a lab-blender for 2 min at medium speed, and then a 1 : 10 dilution was made. Sequential decimal dilutions of the homogenate were made. Dilutions were then plated onto MRS agar (Merck, Darmstadt, Germany) and incubated at 37°C for 48–72 hours. The colonies that appeared on the selected plates (50–300 colonies per plate) were counted as colony-forming units (CFU) per ml of the sample (Xiong *et al.* 2012).

Viscosity measurements. The apparent viscosity of fermented beverages was measured on the Haake VT 550 (Champlan, France) rotation viscometer and was performed at a constant shear rate of 10 s^{-1} at 24 ± 0.2°C. Obtained data were processed and evaluated by using the software RheoWin Job Manager and RheoWin Data Manager (Haake, Karlsruhe, Germany). For the evaluation of flow behaviour, a modified method of Espirito-Santo *et al.* (2014) was used and the dependence of viscosity on the shear rate was measured.

Sensory evaluation. For evaluation of fermented rice beverage sensory parameters (aroma, taste, consistency, and sourness), a 9-point hedonic scale from 0 (lowest) to 9 (highest) and the method according to Faccin *et al.* (2009) and Coda *et al.* (2012) were used. Overall acceptability was expressed as percentages by using a 100-mm non-structured straight line. A panel of 11 trained members was chosen.

Statistical analysis. All analyses were carried out in triplicate, and average values were calculated. The results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Fisher's least-significant difference (*LSD*) multiple range test was applied to data to establish the significance

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Table 1. Parameters of the kinetics of acidification and parameters of lactic acid bacteria growth curves during fermentation of rice beverage media

Parameter	RB *Lbp*	RB *Lbb*	RB *Lbf*	RB *Lbp* + *B. long*
ΔpH	1.40 ± 0.03	0.85 ± 0.01*	0.90 ± 0.02*	1.18 ± 0.01*
v_A (dpH/h)	0.14 ± 0.01	0.08 ± 0.00*	0.06 ± 0.00*	0.24 ± 0.01*
λ_A (h)	0.30 ± 0.01	2.35 ± 0.04*	5.22 ± 0.02*	2.72 ± 0.03*
v_{LAB} (Log_{10} CFU/ml/h)	0.21 ± 0.01	0.44 ± 0.02*	0.22 ± 0.00	0.35 ± 0.01*
λ_{LAB} (h)	2.14 ± 0.03	1.62 ± 0.01*	3.05 ± 0.08*	nd

ΔpH – difference in pH between inoculation and the stationary phase; v_A – maximum acidification rate; λ_A – length of the lag phase of acidification; v_{LAB} – maximum specific growth rate of lactic acid bacteria in an exponential growth phase; λ_{LAB} – length of the lag phase for lactic acid bacteria growth; *significant differences between RB *Lbp* and other RB samples at $P < 0.05$ (Fisher's *LSD* test); nd – not determined

of differences at the level of $P < 0.05$. Statgraphic Plus, Version 3.1 (Statistical Graphic Corporation, Princeton, USA), was used as the statistical software.

RESULTS AND DISCUSSION

pH, titratable acidity and acidification kinetics. The initial values of pH were in the range from 5.04 to 5.14 and dropped to 3.74–4.35 after 24 h of fermentation at 30°C (Figure 1A). All the strains used were tested for the extent of the pH decrease (ΔpH), the rate of pH reduction (v_A) and the length of the acidification lag phase (λ_A) (Table 1). The highest acidification rate was observed in RB *Lbp* sample and in beverage with mixed culture RB *Lbp* + *B. long*. In particular, the above-mentioned isolates were characterised by values that coincided with or

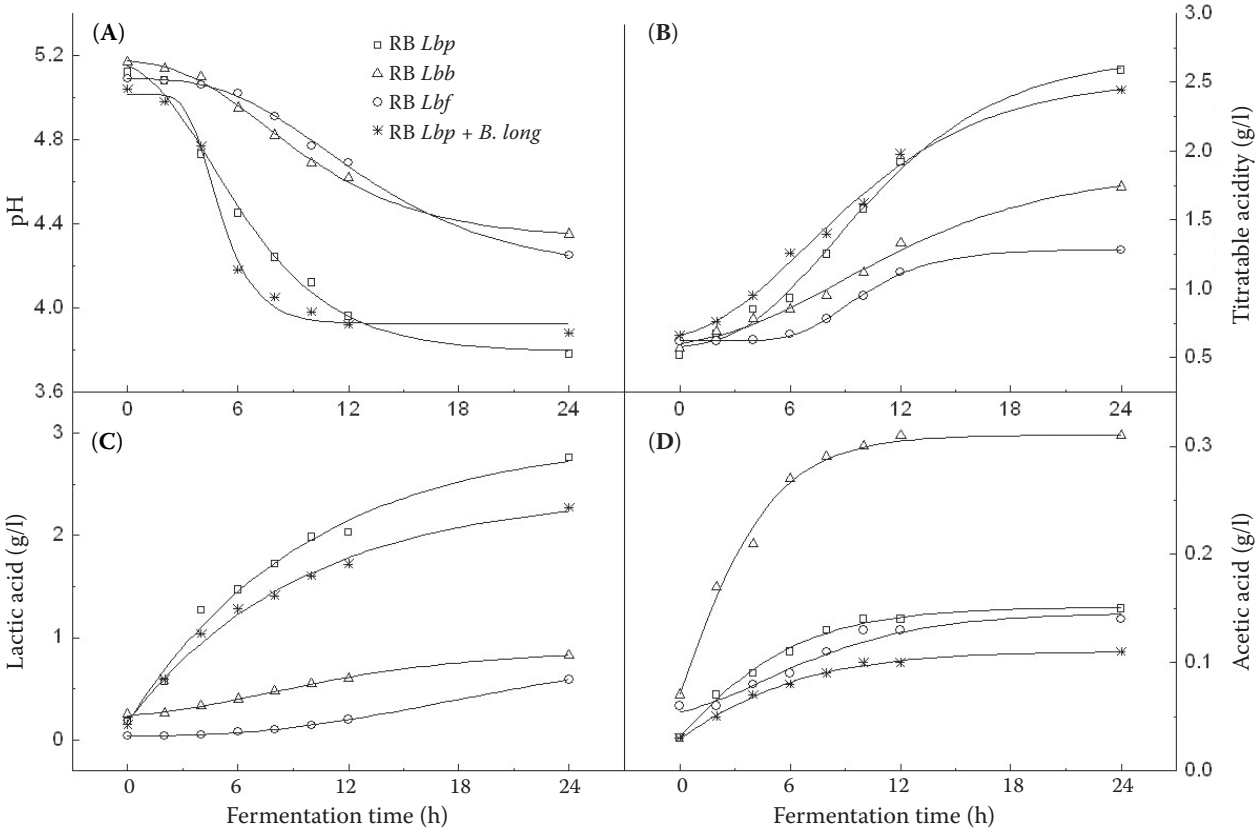

Figure 1. Effect of fermentation on the pH value of rice media (A), titratable acidity (B) and changes in lactic acid (C) and acetic acid (D) concentration

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Table 2. Validation parameters for organic acid analysis using the cITP method

Analyte	Selectivity	Linearity	LOD/LOQ (mg/l)	Precision (RSD in %)	Recovery (%)
Lactic acid	RSH value for analysed acids did not change	0.9975–0.9999	2.03/10.84	2.38–3.72	95.16–107.55
Acetic acid		0.9973–0.0094	8.98/24.10	2.91–4.18	97.28–106.35

RSH – relative step height; LOD – limit of detection; LOQ – limit of quantification; RSD – relative standard deviation

almost approached the highest v_A and the lowest λ_A. The rapid increase in acidity minimises the influence of spoilage bacteria (Kohajdová *et al*. 2007). The acidity of rice beverage samples increased during fermentation and after 24 h it reached values in the range of 1.28–2.59 g/l (Figure 1B).

Organic acids and saccharide analysis. The organic acids, mainly lactic acid and acetic acid, produced by lactic acid bacteria are effective antimicrobial agents, and they reduce the pH in the foods to prevent the growth of hazardous food microorganisms (Lee 1997). Fermentation using lactic acid bacteria caused an increase in lactic acid concentration (Figure 1C) and at the end of fermentation, the beverages contained between 0.59 (RB *Lbf*) and 2.76 g/l (RB *Lbp*) of lactic acid. Nionelli *et al*. (2014) reported that the concentration of lactic acid after 12-h fermentation of cereal beverage at 30°C with *Lb. plantarum* LP09 was 2.46 g/l. At the end of fermentation, the beverages contained between 0.11 (RB *Lbp* + *B. long*) and 0.30 g/l (RB *Lbb*) of acetic acid (Figure 1D). In the study of Rathore *et al*. (2012), during fermentation of barley-malt media inoculated with a mixture of *Lb. plantarum* and *Lb. acidophilus* (1 : 1), the maximum concentration of acetic acid was 0.25 g/l. The results of cITP validation parameters for lactic and acetic acid determination in fermented rice beverage samples are listed in Table 2. Carbohydrates are consumed during fermentation due to the microbial growth (Costa *et al*. 2013). Glucose was utilised more rapidly compared to fructose (Figure 2A and B). The concentration of glucose decreased from initial

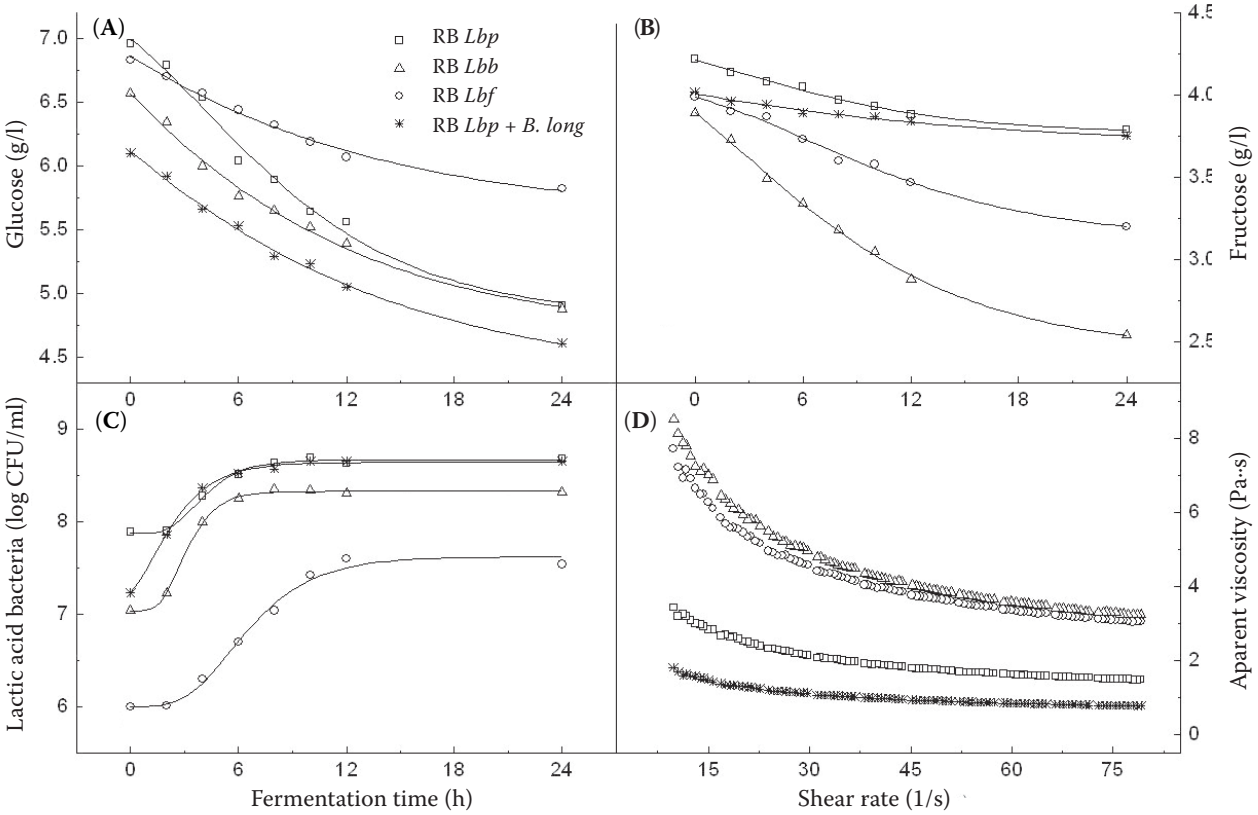

Figure 2. Concentration of glucose (A), fructose (B), lactic acid bacteria cell count (C) during fermentation of rice beverages, and the dependence of apparent viscosity on shear rate values (D)

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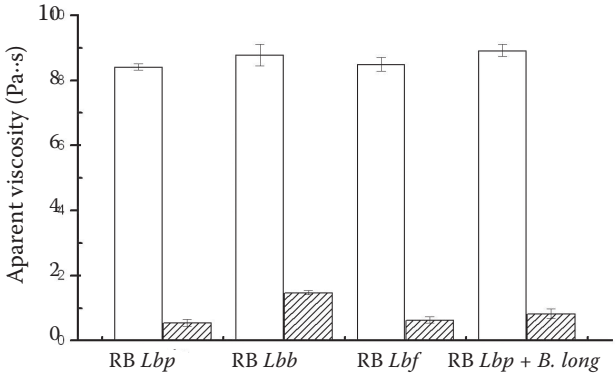

Figure 3. Changes in apparent viscosity values before and after fermentation of rice beverages

values of 6.10–6.96 g/l to 4.61–5.86 g/l after 24-h incubation. Fermentation also led to a reduction in fructose content from 3.89–4.22 to 2.54–3.79 g/l.

Enumeration of lactic acid bacteria. During fermentation, the population of presumptive lactic acid bacteria increased (Figure 2C). The highest cell counts of lactic acid bacteria were reached in RB *Lbp* and in a sample fermented with a mixed culture of *Lb. plantarum* CCM 7039 and *Bifidobacterium longum* CCM 4990 (8.6 Log_{10} CFU/ml after 8 h). From the growth curve parameters (Table 1), the highest rate of lactic acid bacteria growth was observed in beverage RB *Lbp* + *B. long* and in RB *Lbb* sample, which had the highest values of ν_{LAB} and a short lag phase (λ_{LAB}). Rathore *et al*. (2012) reported an increase in cell counts of lactic acid bacteria from 7.2 to 8.4 Log_{10} CFU/ml after 30-h fermentation of barley and malt flour media by the mixed culture of *Lb. acidophilus* NCIMB 8821 and *Lb. plantarum* NCIMB 8826 (1 : 1).

Viscosity of beverages. Texture and consistency are considered as important for the development of new functional fermented non-dairy beverages (Mårtensson *et al*. 2000). When the apparent viscosity and the shear rate were plotted (Figure 2D), it was observed that the apparent viscosity decreased with an increase in the rate of shear. This shows the behaviour of non-Newtonian fluid (shear thinning or pseudoplastic) (Genç *et al*. 2002). Before fermentation, the viscosity of the beverages (Figure 3) made with rice flour was in the range of 8.41–8.92 Pa·s. Following 24-h fermentation, viscosity decreased gradually by about 83–94% in all the beverage samples.

Sensory properties of rice beverages. Sensory evaluation of fermented rice beverages (Table 3) showed that samples RB *Lbp* and RB *Lbp* + *B. long* reached the highest values of flavour, taste and consistency. The most acceptable sourness was found in the rice beverage fermented with *Lb. brevis* CCM 1815. Evaluation of overall acceptability showed that samples fermented with a single culture of *Lb. plantarum* CCM 7039 and samples with a mixed culture of *Lb. plantarum* CCM 7039 and *Bifidobacterium longum* CCM 4990 reached the highest overall acceptability and these rice beverages could be considered as the most acceptable to consumers.

CONCLUSION

The results showed that during fermentation of rice beverages, pH decreased and, at the same time, total acidity increased as a consequence of lactic and acetic acid production. Fermentation also led to a decrease in glucose and fructose concentrations due to their consumption as a source of energy for growth and metabolism of lactic acid bacteria. The determination of lactic acid bacteria cell counts showed that lactic acid bacteria proliferated in the initial 8 h of the process and then remained stable. Viscosity of beverages decreased significantly after 24 h, and prepared beverages showed the behaviour of non-Newtonian fluid. The highest values of sensory parameters were found in beverages RB *Lbp* and samples with a mixed culture of *Lb. plantarum* CCM 7039 and *Bifidobacterium longum* CCM 4990.

Table 3. Sensory parameters of rice beverages

Sample	Flavour	Taste	Sourness	Consistency	Overall acceptability (%)
RB *Lbp*	8.3 ± 0.4	7.6 ± 0.3	7.5 ± 0.3	8.6 ± 0.3	86.8 ± 3.7
RB *Lbb*	6.7 ± 0.3*	5.8 ± 0.2*	7.8 ± 0.4*	7.8 ± 0.1*	79.6 ± 2.9*
RB *Lbf*	7.3 ± 0.4*	6.2 ± 0.4*	7.6 ± 0.2*	7.6 ± 0.3*	76.8 ± 3.1*
RB *Lbp* + *B. long*	8.1 ± 0.5	7.5 ± 0.1	7.2 ± 0.2	8.5 ± 0.5	85.7 ± 3.9

*indicates significant differences between RB *Lbp* and other RB samples at $P < 0.05$ as determined using Fisher's multiple range test

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