THE SAFETY, TECHNOLOGY AND SENSORY ASPECTS OF PASTEURIZED AND RAW MILK TREATED BY SOLID-STATE FERMENTED GRAIN EXTRUDATES INOCCULATED WITH CERTAIN LACTOBACILLI

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Abstract. The study mainly focused on the influence of *Lactobacillus sakei* KTU05-6 and *Pediococcus pentosaceus* KTU05-8 fermentations, after their cultivation on a solid-state medium substrate (pre-ferment) of extruded grain (corn and rice), and on the safety, technology and sensory properties of raw and pasteurized milk products treated with fermented grain extrudates.

The strains investigated showed different behavior in pre-fermentation processes: *P. pentosaceus* KTU05-8 multiplied more actively and *L. sakei* KTU05-6 produced more organic acids. Both strains were able to reduce the numbers of coliform in fermented raw milk. *P. pentosaceus* and *L. sakei* produced more (from 2.2 till 2.8 times) L(+) than D(-) isomer (in milk samples fermented with *P. pentosaceus* cultivated in extruded corn substrate and milk samples fermented with *L. sakei* cultivated in extruded rice substrate, respectively).

The results show that certain lactobacilli such as *L. sakei and P. pentosaceus* in a pre-ferment of extruded grain (corn and rice), added to pasteurized and/or raw milk, lower pH and fat content of the final product, improve the acceptability of the end product and reduce coliform bacteria in the final product derived from raw milk.

Keywords: *Lactobacillus sakei* KTU05-6, *Pediococcus pentosaceus* KTU05-8, extruded grain, milk products.

Introduction. Foods derived from fermentation are major constituents of the human diet all over the world (Motarjemi, 2002). Fermented dairy products are manufactured using a wide range of microorganisms incorporated in starter culture. The major functions of starter cultures are: biopreservation of the product resulting in prolonged shelf-life and enhanced safety; improvement of rheological and sensory properties; multifunctional positive effect to human health and bacteriocins production as potential food preservatives (Spasenija et al., 2012; Tamime, 2006; Bhullar et al., 2002; De Vuyst and Vandamme, 1994). Lately, a consumers' demand for the food not only healthy, but also with unique sensory properties has been observed. Most of the sensory properties such as odor, taste, and texture for the fermented milk products are provided by lactic acid bacteria (LAB) (Sobrino-López and Martín-Belloso, 2008; Gomes et al., 2011). The aroma, body, and taste of yogurt and other cultured dairy products can vary depending on the type of culture and milk, amount of milk fat and nonfat milk solids, fermentation processes, and temperature used (Wouters et al., 2002; Routray and Mishra, 2011). One of the main priorities of scientific research worldwide, aimed at proving the association between diet and well-being, is to improve the balance of intestinal microflora and intestinal passability by the production of pathogen-inhibiting substances (Hosono, 2001; Danone Nutritopics, 2002; Danone Nutritopics, 2004; Pernoud et al., 2005). LAB can not only easily be grown on inexpensive media but also produce secondary metabolites such as bacteriocin-like substances (BLIS) that have the ability to inhibit spoilage and foodborne pathogens (Simova et al., 2009). Non-thermal treatments are attracting interest of the food industry due to their capability of assuring the quality and safety of food (Sobrino-López and Martín-Belloso, 2008). Therefore, lactic acid bacteria producing BLIS may be potentially useful for the dairy industry.

Due to the ability of LAB to produce amylolytic enzymes the best substrate for their cultivation are starchy grain products. To minimize the substrate microbial contamination, extruded grains can be used for that purpose. It is also known that extruded grains get better nutritional and sensory properties (Glencross et al., 2011).

Lactobacillus sakei is characterized by its specific features to produce BLIS that inhibit the growth of certain bacteria (Digaitiene et al., 2012). Due to the exceptional versatility which can be partially explained by its ability to survive and grow in adverse conditions *L. sakei* is very widely used in many food industries.

Pediococcus pentosaceus is also categorized as a LAB because the end product of its metabolism is lactic acid. It is a unique bacterium because it can survive and operate in very acidic medium, where the majority of other cannot survive (Xu et al., 2010). This quality and its ability to inhibit the activity of pathogens (Agrawal and Dharmesh, 2011) allows to use *P. pentosaceus* in a majority of areas in the food industry, especially for production of various cheeses (Carraro et al., 2011).

On the other hand, microbially produced lactic acid is usually a mixture of the L(+) and D(-) isomers of lactic acid. As the latter can not be metabolized by humans,

excessive intake can result in acidosis, which is a disturbance in the acid-alkali balance in the blood. The potential toxicity of D-(-) lactic acid is of particular concern for malnourished and sick people (Motarjemi and Nout, 1996). So research is needed with regard to the content of D-(-) lactic acid in fermented foods.

Assuming the abilities of those LAB strains and extruded grain products mentioned above, the idea of the study was to use those LAB strains cultivated in extruded grain substrate, and introduce these fermented products for milk fermentation with the aim to improve the sensory properties of fermented milks due to extruded grain supplements and at the same time enhancing the safety of the final product due to the antimicrobial properties of the LAB and the absence of microbial contaminants in the extruded cereals.

Thus, the aim of the study was to assess the influence of *Pediococcus pentosaceus* KTU05-8 and *Lactobacillus sakei* KTU05-6 after their solid-state cultivation in extruded grain (corn and rice) substrates on the safety, technology and acceptability of milk products fermented with extruded grain.

Materials and methods

Materials

Extruded corn and rice wholemeal flour were obtained from the Ltd 'Uštukių malūnas' (Pasvalys, Lithuania) in 2012. The wholemeal extruded corn (protein 1.01 %, moisture 6.5 %, fat 4.7 %, starch 57.8 %) and rice (protein 11.6 %, moisture 6.3%, fat 2.3, starch 59.5 %) flours were used as the starting material for *Lactobacillus sakei* KTU05-6 and *Pediococcus pentosaceus* KTU05-8 multiplying.

Preparation of extruded corn and rice wholemeal flours fermented products (pre-ferment)

BLIS producing *L. sakei* KTU05-6 and *P. pentosaceus* KTU05-8 have been isolated from fermented rye (Digaitiene et al., 2012). Microorganisms were stored at -70°C (PRO-LAB Diagnostics), and cultured in MRS broth (CM0359, Oxoid Ltd, Hampshire, UK) maintaining: *L. sakei* at 30°C, and *P. pentosaceus* at 35°C for 48 hours prior to be used for flour fermentation. Before use, 40 mM fructose and 20 mM maltose was added to the enrichment broth. After enrichment, strains were diluted with saline to the concentration of 10^8 (CFU ml^{-1}) for the fermentation in the substrates of the extruded grains. 300 g of extruded flour was mixed with 450 ml of water and 5 ml of a suspension of pure microorganism cultures (65% moisture content, 9.2 CFU ml^{-1}) and left for fermentation for 29 hours at 30°C (*L. sakei)* and at 35°C (*P. pentosaceus*).

Pre-ferment analyses

PH, mesophilic bacteria counts and amylolitic activity of LAB were assessed in the extruded flours fermented products. PH was measured by pH - meter 'Sartorius Professional Meter PP – 15'. Total mesophilic bacteria count (CFU g^{-1}) was determined in accordance with ISO15214: 2009. To assess the number of LAB, 10 g of pre-ferment was homogenized in 90 ml saline (0.9 %). The suspension was diluted to achieve the concentration 10^{-4} and 10^{-8}, and placed in Petri plates with MRS agar and incubated for 72 hours under anaerobic conditions at 30°C for *L. sakei* and at 35°C for *P. pentosaceus*.

The amylase levels excreted by single LAB were determined by the starch-iodine method described by Nguyen et al., (2002). The dough sample (5 g) was homogenized with 50 mL of distilled water and centrifuged at 5000g for 10 minutes. The reaction mixture containing 1 mL of 1% (w/v) soluble starch as substrate in 1/15 M phosphate buffer (pH 6) and sample extract (0.5 mL) was incubated for 10 minutes at 30 ºC. The reaction was stopped and the colour was developed by addition of 1.5 mL of diluted iodine reagent [2 mL of iodine (0.25% w/v) with KJ (2.5% w/v) solution diluted with 0.5 M HCl to 100 mL]. Absorbance was measured at 670 nm using a Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc., Langensenbold, Germany). One unit of α-amylase activity (1 AU) was defined as an amount of enzyme that catalyzes 1 g soluble starch hydrolysis to dextrins in 10 minutes at 30 °C temperature.

Fermented products were frozen and kept at -70°C temperature for the further fermentation of and with milk.

Preparation of milk/flour mixtures for fermentation

Pasteurized milk samples "Marge" (from local supermarket) with different fat percentages (1; 2.5; 3.2; 3.5 %) as well as raw milk (1 % fat; was obtained from a small farm (Jonava, Lithuania)) were chosen for the experiment. For the fermentation 5 % (w/v) of different fermented flours were added to the samples of raw and pasteurized milk. Then, milk samples were kept in the thermostat for 19 hours at 30°C (*L. sakei*) and at 35°C (*P. pentosaceus*).

Methods of analysis of fermented milk/flour samples

To assess the impact of *L. sakei* and *P. pentosaceus* fermented flour on safety of fermented milk coliform bacteria were counted in raw milk before and after its fermentation (ISO 4832:2006, samples were cultured in Eosine Methylene Blue (EMB) agar and incubated for 24 h at 37°C).

Fat content (%) was determined in accordance with ISO 488:2008 (IDF 105:2008; Milk - Determination of fat content - Gerber butyrometers) method.

Texture (hardness) of the fermented milk samples was measured using the Stevens-LFRA Texture Analyzer (Voland Corp., New York, NY, USA) with a 10 mm cylindrical probe at test speed of 1 mm/s.

Acceptability analysis of fermented milk was carried out by twelve panelists according to the ISO 8586-1 method (ISO, 1993) for using a 150 mm hedonic line scale ranging from 150 (extremely like) to 0 (extremely dislike).

Also, L(+) and D(-) lactic acid concentrations in fermented milk/flour samples were determined by an enzyme test kit (R-biopharm AG - Roche, Darmstadt, Germany), as reported elsewhere (Yun et al., 2003; De Lima et al., 2009).

At least three samples from one batch were analysed, and analysis was repeated three times.

Statistical analysis

Statistical data analysis was conducted using a Microsoft Excel spreadsheet, and a SPSS program

(Ver.17.0, 2006; SPSS Inc., Chicago, IL, USA) was used for the descriptive analysis (N, mean ± standard deviation), GLM and ANOVA. Calculated mean values were compared using Bonferroni's multiple range test with significance defined at $p < 0.05$.

Results. Pre-ferment analysis (Table 1) showed high count of *P. pentosaceus* in both substrates ($p \leq 0.001$). Substrates also influenced the growth of strains: *P. pentosaceus* was more prone to survive in extruded corn media, *L. sakei* – in extruded rice. Both strains showed the ability to acidify the substrate ($p \leq 0.05$), *L. sakei* however reduced pH more efficiently, regardless to what kind of preferment – extruded corn or rice – it was added to. The results indicate differences in behavior of the strains in the pre-ferment: *P. pentosaceus* multiply more actively, and *L. sakei* produces more organic acids.

Table 1. **Total mesophilic LAB count and pH in pre-ferments of extruded corn and rice**

Pre-ferment	Total mesophilic LAB count (CFU/g)	pH	
		After 24 h	After 29 h
P. pentosaceus in extruded rice substrate	$3.2x10^8 \pm 1.0x10^{8\,a}$	4.18 ± 0.01^a	3.86 ± 0.19^a
P.pentosaceus in extruded corn substrate	$4.0x10^8 \pm 0.8x10^{8\,b}$	4.23 ± 0.12^a	3.95 ± 0.21^b
L. sakei in extruded rice substrate	$5.6x10^7 \pm 0.94x10^{7\,c}$	3.9 ± 0.11^b	3.71 ± 0.05^c
L. sakei in extruded corn substrate	$5.0x10^5 \pm 0.87x10^{5\,d}$	3.88 ± 0.02^b	3.69 ± 0.16^c

Differences $^{a, b, c, d}$ among pre-ferments are statistically significant ($p \leq 0.05$, no difference between the same letters)

A wide range of raw materials are used as substrates and a panoply of products is concocted (Motarjemi, 2002). Recently, considerable interest has arisen in the application of new fermentation media composed of extruded products possessing specific physical properties (Juodeikiene et al., 2011). Extrusion cooking causes gelatinization of starch among the other physicochemical and functionality changes the grain components undergo; moreover, enhances the amount of dietary fibre and eliminates the bacterial contamination of the cereal material. Extruded material is microbiologically safe, therefore a very suitable medium for the cultivation of LAB. The appropriate combination of prebiotics and probiotics manifest higher potential for a synergistic effect (Ranadheera et al., 2009). According to Kedia et al., (2008), different LAB, depending on the substrate composition, behaves differently. In some substrates they may produce more metabolites, in others they multiply more. In addition they produce lactic acid and lactobacilli also have the ability to produce hydrogen peroxide through oxidation of reduced nicotinamide adenine dinucleotide (NADH) by having a nucleotide which reacts rapidly with gaseous oxygen. Flavoproteins, such as glucose oxidase, also generate hydrogen peroxide and produce an antibiotic effect on other organisms that might cause food spoilage; the lactobacilli themselves are relatively resistant to hydrogen peroxide.

One of the main tasks of the study was to determine and compare the antimicrobial activity of strains. Since the dairy industry is keen to explore new possibilities for enhancing the diversity of its product range, there is a new interest nowadays in searching for potential starter organisms from the pool, which existed at the time of raw milk fermentation (Wouters et al., 2002). To find out the difference, coliforms were counted in raw milk and in fermented products made from the raw milk (Table 2).

Table 2. **Coliform count in raw milk befor and after fermentation**

Indicator	Raw milk	Raw milk with pre-ferment of extruded corn	Raw milk with pre-ferment of extruded rice
Coliform count (cfu g^{-1})	$4.8x10^4$	*Pediococcus pentosaceus*	
		$2.5x10^4 \pm 0.6x10^{4\,b}$	$1.4x10^3 \pm 0.14x10^{3\,a}$
		Lactobacillus sakei	
		$1.8x10^3 \pm 0.2x10^{3\,a}$	$3.7x10^3 \pm 0.39x10^{3\,c}$

Differences $^{a, b, c, d}$ among pre-ferments are statistically significant ($p \leq 0.05$, no difference between the same letters)

The results showed that both strains are able to reduce the number of coliform bacteria in fermented raw milk ($p \leq 0.05$), but the effect is substrate-dependent: *L. sakei* is more effective when fermented with extruded corn, *P. pentosaceus* – conversely, with rice. No deference was found between the strains in their ability to reduce coliform count.

According to our research results, at lower substrate pH, coliform bacteria count was reduced effectively. The formation of lactic acid is obtained by acidification below pH 4.2 which is a safety factor and gives a nice taste to whey products (Kedia, Antonio, & Pandiella, 2008). Also, we can say, that in all fermented products the pH after 29 hours fermentation was less than 4.2, so from the results we can assume, that a lower pH of the fermented products ensures a bigger product safety.

Impact of strain, substrate, sample, and pre-ferment on the technology parameters and acceptability of fermented milk/flour mixtures are presented in the Tables 3–5.

Only the pH of the fermentation end-product, texture

and fat loss were affected by strain (Table 3). The effect of substrate was significant only for the acidity parameter. Differences in the amount of milk fat samples meant also differences in texture and fat loss of the fermented milk samples (Table 3).

Table 3. **Significance** (p) **of the impact of different factors** (strain, substrate, sample, and pre-ferment) **on technological and sensory properties of fermented milk/flour mixtures**

Factors and their interactions	Parameters				
	pH (7 h)	pH (19 h)	Acceptability	Texture	Fat loss
Strain	n	0.002	n	0.029	0.026
Substrate	0.0001	0.0001	n	n	n
Sample	n	n	n	0.0001	0.0001
Pre-ferment (Strain* Substrate)	0.0001	n	n	n	n
Pre-ferment * Sample	0.0001	0.0001	0.004	0.001	0.0001

Factors were considered significant, when $p \leq 0.05$; n – not significant, $p \geq 0.05$

Table 4. **Impact of strain and substrate on technological parameters and acceptability of fermented milk/flour mixture samples** (N=48)

Parameter	Strain		Substrate	
	P. pentosaceus	*L. sakei*	Extruded rice flour	Extruded corn flour
pH after 7 h	6.44±0.12	6.47±0.18	6.37±0.13□	6.56±0.11□
pH after 19 h	5.24±0.28*	5.45±0.44*	5.44±0.37□	5.17±0.32□
Acceptability	62.87±52.68	80.64±52.12	78.98±54.00	64.57±51.26
Texture (TAU)	7.66±6.04*	5.13±5.16*	5.94±6.16	6.83±5.27
Fat loss (%)	18.85±16.16*	10.96±8.14*	12.23±10.71	16.98±19.66

Differences between strains* and substrates□ are statistically significant ($p \leq 0.05$)

Fermented milk/flour mixture samples with inoculated *P. pentosaceus* were more acid, solid, and lost most of the fat (Table 4). After 7 h of fermentation, the pH was lower in samples with rice substrate, at the end of the fermentation the pH was reduced significantly in samples with corn.

This may be influenced by several factors. Rice and corn starch have different structures as is seen by using a scanning electron microscopy (SEM): the rice starch is an aggregation (n = 20–60) of smaller granules (3–8 µm in diameter), whereas corn starch is composed of larger (5–15 µm in diameter), single granules (Cheng and Lai, 2000). Also, *P. pentosaceus* is an amylolytic, lactic acid bacterium with a higher potential to produce lactic acid in a different substrate than *L. sakei*. Additional investigations of LAB amylolytic activities in fermentation media (*P. pentosaceus* in rice substrate – 159.21 AU g^{-1}, in corn substrate – 175.35 AU g^{-1}; *L. sakei* in rice substrate 122.51 AU g^{-1}, in corn substrate – 131.52 AU g^{-1}) have revealed that amylase profiles in pre-ferment could be related to the pH decreasing in the fermented milk/four mixture samples (Fig. 1).

The amount of fat in fermented samples was directly proportional to the solidity of the fermented milk product. The samples with the most fat (3.5%) were found to be firmer ($p \leq 0.001$) than others. Biggest fat lost was also registered in the same samples (Table 5). Also, the pre-ferment had a significant impact on pH of fermented milk samples. The lowest pH was found in samples with corn substrate added to it. This could be explained by increased of both LAB amylolytic activity in corn substrate than in rice.

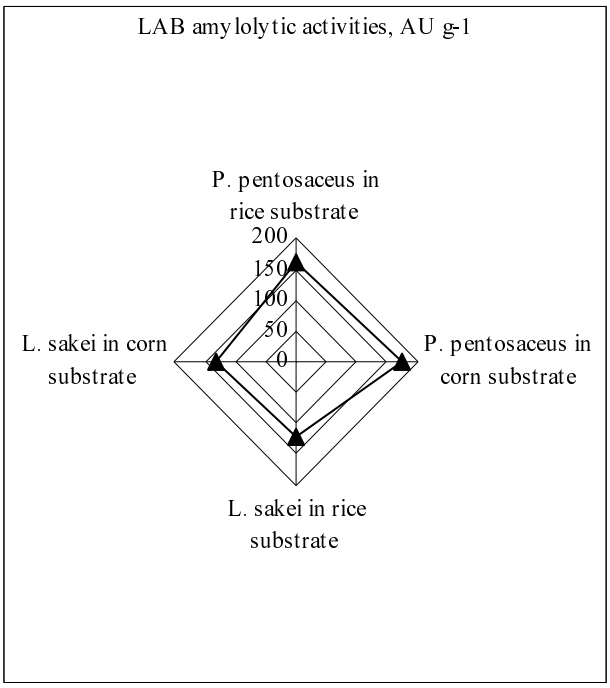


Fig. 1. **LAB amylolytic activities in fermentation media** (AU g^{-1})

LAB in fermented milk products can produce various lipolytic agents and their influence on lipolysis, while free fatty acids (FFA) liberated during lipolysis directly affect fermented milk products flavour, they are also metabolized to other highly flavoured compounds, including methyl ketones and lactones (Collins et al., 2003), and may have an effect on fat loss in fermented milk products.

The effect of a single LAB strain used for milk/flour fermentation on the lactic acid isomers D(-)/L(+) formation in samples was analysed (Table 6).

Table 5. **Impact of sample and pre-ferment** (Strain*Substrate) **on technology parameters and acceptability of fermented milk/flour mixture samples**

Parameters	Sample	Parameters	Pre-ferment	Parameters
pH after 7 h	1%	6.48±0.14	*P. pentosaceus* in extruded rice substrate	6.40±0.12
	2.5%	6.43±0.15	*P. pentosaceus* in extruded corn substrate	6.48±0.11
	3.2%	6.44±0.15	*L. sakei* in extruded rice substrate	6.34±0.13
	3.5%	6.47±0.16	*L. sakei* in extruded corn substrate	6.62±0.05
pH after 19 h	1%	5.39±0.36	*P. pentosaceus* in extruded rice substrate	5.34±0.25 [b]
	2.5%	5.21±0.13	*P. pentosaceus* in extruded corn substrate	5.16±0.28 [a]
	3.2%	5.57±0.42	*L. sakei* in extruded rice substrate	5.55±0.44 [b]
	3.5%	5.23±0.41	*L. sakei* in extruded corn substrate	5.18±0.36 [a]
Acceptability	1%	57.47±50.58	*P. pentosaceus* in extruded rice substrate	67.75±59.21
	2.5%	71.25±46.65	*P. pentosaceus* in extruded corn substrate	57.78±45.65
	3.2%	51.50±32.22	*L. sakei* in extruded rice substrate	90.21±46.78
	3.5%	88.22±59.92	*L. sakei* in extruded corn substrate	71.08±56.31
Texture (TAU)	1%	4.43±3.07[a]	*P. pentosaceus* in extruded rice substrate	7.88±7.15
	2.5%	5.25±2.49[a]	*P. pentosaceus* in extruded corn substrate	7.43±4.76
	3.2%	1.50±0.52[a]	*L. sakei* in extruded rice substrate	4.00±4.30
	3.5%	10.03±7.07[b]	*L. sakei* in extruded corn substrate	6.25±5.77
Fat loss (%)	1%	10.32±7.53 [a]	*P. pentosaceus* in extruded rice substrate	16.23±13.51
	2.5%	10.09±6.63 [a]	*P. pentosaceus* in extruded corn substrate	20.77±19.72
	3.2%	5.46±5.95 [a]	*L. sakei* in extruded rice substrate	8.23±4.305
	3.5%	23.34±21.65[b]	*L. sakei* in extruded corn substrate	13.36±19.31

Differences [a, b] among samples and pre-ferments are statistically significant ($p \leq 0.05$, no difference between the same letters)

Table 6. **Lactic acid isomers D(-)/L(+) amount** (%) **in fermented milk/flour mixture samples**

Milk samples	*P. pentosaceus*				*L. sakei*			
	In extruded rice substrate		In extruded corn substrate		In extruded rice substrate		In extruded corn substrate	
	D(-),	L(+),	D(-),	L(+),	D(-),	L(+),	D(-),	L(+),
	%							
Raw milk, 1%	0.81±0.09	2.11±0.24	1.03±0.11	2.51±0.14	0.41±0.09	1.11±0.06	0.21±0.03	1.12±0.11
1%	0.64±0.11	4.13±0.27	0.71±0.07	3.24±0.32	0.63±0.06	3.10±0.13	0.37±0.05	2.48±0.31
2.5%	2.43±0.21	4.07±0.19	2.56±0.16	3.51±0.26	1.03±0.11	2.17±0.16	1.27±0.14	2.16±0.36
3.2%	1.85±0.12	3.92±0.14	1.23±0.12	4.01±0.35	0.45±0.05	1.39±0.24	0.98±0.23	2.13±0.14
3.5%	2.24±0.13	3.89±0.32	1.91±0.23	3.22±0.21	1.23±0.13	2.81±0.33	1.31±0.24	2.43±0.27

Data are the mean ± SD. Means within a row with different letters are significantly different ($p \leq 0.05$).

Lactic acid isomer D(-) concentration in samples ranged from 0.21 ± 0.03 to 4.07 ± 2.56 % (in raw milk samples treated with *L. sakei* cultivated in extruded corn substrate and in 2.5 % fat milk samples treated with *P. pentosaceus* cultivated in extruded corn substrate , respectively). The results showed that the levels of isomer D(-) in fermented milk samples in all cases were lower than the levels of isomer L(+). Lactic acid isomer L(+) concentration in fermented milk samples ranged from 1.11 ± 0.06 till 4.13 ± 0.27 % (in 1 % fat milk samples treated with *L. sakei* cultivated in extruded rice substrate and in 1 % fat milk samples treated with *P. pentosaceus* cultivated in extruded rice substrate, respectively).

We found that LAB used in the experiment produced more L(+) than D(-) isomer (from 2.2 till 2.8 times more, in milk samples fermented with *P. pentosaceus* cultivated in extruded corn substrate and milk samples fermented with *L. sakei* cultivated in extruded rice substrate,

respectively) (Fig. 2).

The organisms that predominantly yield the L(+) isomer are *Lactobacilli amylophilus, L. bavaricus*, *L. casei*, *L. maltaromicus*, and *L. salivarius*. Strains such as *L. delbrueckii*, *L. jensenii*, or *L. acidophilus* yield the D(-) isomer or mixtures of both (Garlotta, 2002). Hang (1990) and Yu and Hang (1989) reported that *R. oryzae* is capable of saccharifying and simultaneously fermenting corn to L(+) lactic acid. However in industrial fermentations, the use of various species of *Lactobacillus* is preferred owing to higher rates of metabolism and increased yields.

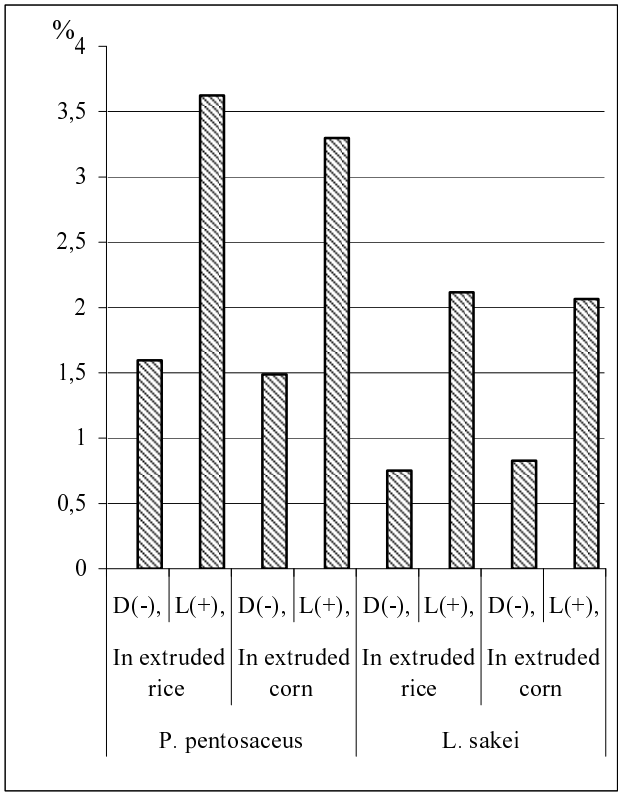
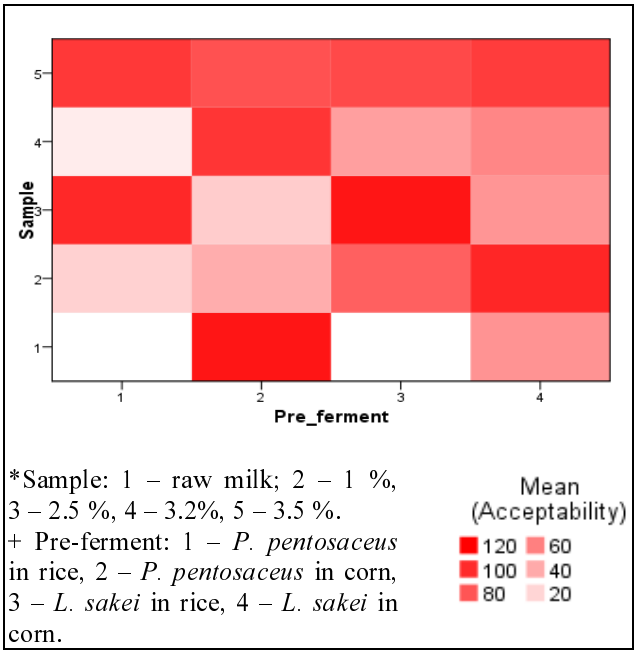


Fig 2. **L(+)and D(-) isomers quantity average (%) in fermented with different LAB, cultivated in different substrate, milk samples**

Sensory analysis revealed that the acceptability of fermented milk/flour mixed samples varied within a wide range and were influenced by the interaction of many factors. These results suggest that the acceptability of fermented dairy products depend not only on the sample properties and pre-treatment, but also on pre-ferment compositions and characteristics (Fig. 3).

The quality of fermented dairy products is influenced by conversion of milk components during fermentation. Metabolic activities of starter culture during the gelation process of milk are of particular importance. Type and ratio of microorganisms in starter culture contribute to different physico-chemical and sensory characteristics of fermented dairy products (Tamime and Robinson, 2004). It can be seen in the Fig. 2 that samples more rich in fat and inoculated with *L. sakei* was found more acceptable.



*Sample: 1 – raw milk; 2 – 1 %, 3 – 2.5 %, 4 – 3.2%, 5 – 3.5 %.
+ Pre-ferment: 1 – *P. pentosaceus* in rice, 2 – *P. pentosaceus* in corn, 3 – *L. sakei* in rice, 4 – *L. sakei* in corn.

Fig 3. **Impact of sample* and pre-ferment+ on acceptability of fermented milk products**

Conclusions

1. The strains investigated showed different behavior in pre-ferment: *P. pentosaceus* multiply more actively, *L. sakei* produces more organic acids.

2. Both strains are able to reduce the number of coliform in fermented raw milk/flour mixtures, but the effect is substrate-dependent: *L. sakei* is more effective when fermented with extruded corn, *P. pentosaceus* – with extruded rice.

3. Strain influenced pH of fermented milk/flour mixture samples, their texture and fat loss. Fermented milk samples with inoculated *P. pentosaceus* were more acid, solid, and lost most of the fat. Substrate influenced only acidity of fermented milk samples: during the fermentation pH was lower in samples with rice substrate; at the end of it pH fell down significantly in samples with corn.

4. Fatness of milk had an influence on texture and fat loss of fermented milk/flour mixture samples, the richer was the sample the more solid was the fermented milk/flour mixture product, and the biggest fat loss was found.

5. *P. pentosaceus* and *L. sakei* produce more L(+) than D(-) isomer: from 2.2 till 2.8 times more, in milk samples fermented with *P. pentosaceus* cultivated in extruded corn substrate and milk samples fermented with *L. sakei* cultivated in extruded rice substrate, respectively.

6. Samples more rich in fat and inoculated with *L. sakei* waere more acceptable: acceptability of fermented milk/flour mixed samples varied within a wide range and are influenced by the interaction of sample and pre-ferment (≤ 0.05).

The results show that certain lactobacilli such as *L. sakei and P. pentosaceus* in a pre-ferment of extruded

grain (corn and rice) added to pasteurized and/or raw milk, lower the pH (produce more L(+) than D(-) isomer) and fat content of the final product and improve the nutritional properties and acceptability of the end product and reduce coliform bacteria in the final product derived from raw milk.

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References

1. Agrawal R., Dharmesh S. An anti-*Shigella dysenteriae* bacteriocin from *Pediococcus pentosaceus* MTCC 5151 cheese isolate. The Turkish Journal of Biology. 2011. 35. P. 1010–1042.

2. Bhullar Y. S., Uddin M. A., Shah N. P. Effects of ingredients supplementation on textural characteristics and microstructure of yoghurt. Milchwissenschaft. 2002. 57. P. 328–332.

3. Carraro L., Maifreni M., Bartolomeoli I., Martino M.E., Novelli E., Frigo F., Marino M., Cardazzo B. Comparison of culture-dependent and and – independent methods for bacterial community monitoring, during Montasio cheese manufacturing. Research in Microbiology. 2011. 162. P. 231–239.

4. Cheng H. H., Lai M. H. Fermentation of Resistant Rice Starch Produces Propionate Reducing Serum and Hepatic Cholesterol in Rats. The Journal of Nutrition. 2000. 130. P. 1991–1995.

5. Collins Y. F., Mc Sweeney P. L. H., Wilkinson M. G. Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. International Dairy Journal. 2003. 13. P. 841–866.

6. Danone Nutritopics. Effect of Probiotics on Body's Natural Defenses. Special Issue. 2002.

7. Danone Nutritopics. The Health Benefits of Probiotics. N. 29. 2004.

8. De Lima C.J.B., Coelho L.F., Blanco K.C., Contiero J. Response surface optimization of D(-)-lactic acid production by *Lactobacillus* SMI8 using corn steep liquor and yeast autolysate as an alternative nitrogen source. African Journal of Biotechnology. 2009. 8. P. 5842–5846.

9. De Vuyst L., Vandamme E.J. Antimicrobial potential of lactic acid bacteria. In Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications ed. De Vuyst, L. & Vandamme, E. J. London: Blackie Academic and Professional. 1994. P. 91–142.

10. Digaitiene A., Hansen A. S., Juodeikiene G., Eidukonyte D., Josephsen J. Lactic acid bacteria isolated from rye sourdoughs produce bacteriocin-like inhibitory substances active against *Bacillus subtilis* and fungi. Journal of Applied Microbiology. 2012. 112. P. 732–742.

11. Glencross B., Hawkins W., Evans D., Rutherford N., Mc Cafferty P., Dods K., Hauler R. A comparison of the effect of diet extrusion or screw-press pelleting on the digestability of grain protein products when fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2011. 312. P. 154–161.

12. Gomes A.A., Braga S.P., Cruz A.G, Cadena R.S., Lollo P.C.B., Carvalho C., Amaya-Farfán J., Faria J.A.F., Bolini H.M.A. Effect of the inoculation level of *Lactobacillus acidophilus* in probiotic cheese on the physicochemical features and sensory performance compared with commercial cheeses. *Journal of Dairy Science*. 2011. 94. P. 4777–4786.

13. Hosono A. Nutritional and Physiological Properties of Lactic Acid Bacteria. 2001. P. 64 Japan: JICA.

14. Yun J.S, Wee Y.J. Ryu H.W. Production of optically pure L(+)-lactic acid from various carbohydrates by batch fermentation of *Enterococcus faecalis* RKY1. Enzyme Microbiology Technology. 2003. 33. P. 416–423.

15. Juodeikiene G., Salomskiene J., Eidukonyte D., Vidmantiene D., Narbutaite V., Vaiciulyte-Funk, L. Impact of novel fermented products on the base of extruded wheat material on the quality of wheat bread. Food Technology and Biotechnology. 2011. 49. P. 502–510.

16. Kedia G., Antonio V.J., Pandiella S.S. Fermentability of whole oat flour, PeriTec flour and bran by *Lactobacillus plantarum*. Journal of Food Engineering. 2008. 89. P. 246–249.

17. Motarjemi Y. Impact of small scale fermentation technology on food safety in developing countries. International Journal of Food Microbiology. 2002. 75. P. 213–229.

18. Motarjemi Y., Nout M.J.R. On behalf of the Joint FAO/WHO Workshop on Assessment of Fermentation as a Household Technology for Improving Food Safety 3 Food fermentation: a safety and nutritional assessment. Bulletin of the World Health Organization. 1996. 74. P. 553–559.

19. Nguyen Q.D., Rezessy-Szabó J.M., Claeyssens M., Stals I., Hoschke Á. Purification and characterisation of amylolytic enzymes from thermophilic fungus *Thermomyces lanuginosus* strain ATCC 34626. Enzyme and Microbial Technology. 2002. 31. P. 345–352.

20. Pernoud S.N., Schneid-Citrain V., Agnetti S., Breton J.M., Faurie L., Marchal D., Obis D., Oudot D., Paquet D., Robinson T. Application des bactéries lactiques dans les produits laitiers frais et effets des probiotiques. In: F.-M. Luquet and G. Corrieu (Eds), Bactéries Lactiques et Probiotiques, Éditions: Technique & Documentation-Lavoisier, Cachan cedex, France. 2005. P. 306.

21. Ranadheera R.D.C.S., Baines S.K., Adams M.C. Importance of food in probiotic efficacy. Food Research International. 2010. 43. P. 1–7.

22. Robert G., Ann J., Ferris M., Lammi-Keefe C. J. The Composition of Milk Fat. *Journal of Dairy Science*. 1991. 74. P. 3228–3243.

23. Routray W., Mishra H.N. Scientific and Technical Aspects of Yogurt Aroma and Taste: A Review. Food Science and Food Safety. 2011. 10. P. 208–220.

24. Savadago A., Quattara C.A.T., Bassole I.H.N., Traore S.A. Bacteriocins and lactic acid bacteria - a minireview. African Journal of Biotechnology. 2006. 5. P. 678-683.

25. Simova E.D., Beshkova D.B., Dimitrov Z.P. Characterization and antimicrobial spectrum of bacteriocins produced by lactic acid bacteria isolated from traditional Bulgarian dairy products. Journal of Applied Microbiology. 2009. 106. P. 692–701.

26. Sobrino-López A., Martín-Belloso O. Use of nisin and other bacteriocins for preservation of dairy products. International Dairy Journal. 2008. 18. P. 329–343.

27. Spasenija M., Katarina K., Vukić V., Hrnjez D., Iličić M., Ranogajec M., Milanović M. Physicochemical and textural properties of kombucha fermented dairy products. African Journal of Biotechnology. 2012. 11. P. 2320–2327.

28. Tamime A.Y. Fermented Milks. Blackwell Science Ltd, UK. 2006. P. 262.

29. Wouters J.T.M, Ayad E.H.E, Hugenholtz J., Smit G. Microbes from raw milk for fermented dairy products. International Dairy Journal. 2002. 12. P. 391–109.

30. Xu Y., Wenshui X., Fang Y., Moon K.J., Xiaohua N. Effect of fermentation temperature on the microbial and physicochemical properties of silver carp sausages inoculated with Pediococcus pentosaceus. Food Chemistry. 2010. 118. P. 512–518.

31. Tamime A.Y., Robinson R.K. Yoghurt, Science and Technology. Woodhead Publishing Ltd, Cambridge, England. 2004. P. 618.

32. Tverdochleb G. V., Tamanauskas R. I. Chemistry and Physic of Milk products. M.: DeLi print. 2006. P. 360.

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