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Application of fermented soya as a bacterial starter for production of fermented milk

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Abstract: In order to improve the quality and health safety of fermented milk, soya fermented by different lactic acid bacteria (LAB) was used. It was found that soya fermented by solid state (SSF) and submerged (SmF) fermentation can be used for the processing of innovative fermented milk, because the final product is enriched with soya proteins and LAB. *Lactobacillus sakei* in milk-soya samples, treated with submerged fermentation, was responsible as the strain for low active acidity (pH 4.62), high titratable acidity (99.0 T) and degradation of lactose (up to 4.18%) $P \leq 0.05$, lower amounts of D(–) lactic acid isomers, biogenic amines and high acceptability of the final product. The results showed a possibility for innovations to use LAB, especially *L. sakei,* in soya fermentation and production of fermented milk products of valuable composition.

Keywords: *Glycine max* L.; solid state and submerged fermentation; lactic acid bacteria; biogenic amines; quality; health safety

In recent years health-conscious consumers have been looking for new healthier foods with prolonged expiry date, processed without chemical preservatives, suitable to their lifestyle. To harmonize consumer demands, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by a biopreservation technique, using combinations of innovative technologies that include biological antimicrobial systems such as LAB and/or their metabolites (Nath *et al.* 2013).

One of the possibilities of the extension of food shelf life is biopreservation by natural or controlled microbiota. Fermentation, as a process for manufacturing foods, has traditionally been used to preserve perishable products and enhance their nutritional value. However, some authors propose that solid state fermentation (SSF) is more effective than liquid-phase submerged fermentation (SmF) because of lower contents of water and energy used for product stabilization by dehydration (Bartkiene e*t al.* 2015).

LAB are capable not only to prolong the food shelf life, but also to increase safety because of antimicrobial properties and to form the unique flavour and texture of food (Singh 2018). However LAB are also related with formation of undesirable compounds (*e.g.* D-lactate and biogenic amines). D(–) lactic acid is toxic to humans (Bianchetti *et al.* 2018) and should be controlled. Biogenic amines (BA) in food constitute a potential public health risk due to their physiological and toxicological effects. They are responsible for vasoactive, psychoactive, and toxicological properties and may potentiate the toxicity of the others (Capillas & Herrero 2019).

Fermented soybean foods are of public interest and they are consumed more frequently (Mah *et al.* 2019). Soya (*Glycine max* L.) is extremely versatile and can be used for development of new products, such as vegetarian diets (Rizzo & Baroni 2018), breakfast cereals (Mbaeyi-Nwaoha & Uchendu 2016) and meat analogues (Joshi & Kumar 2015). However, there is a lack of data on soya incorporation into milk products. Therefore the aim of the present study was to evaluate the impact of two fermentation types, used for fermentation of *Glycine max* L. with

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Pediococcus pentosaceus KTU05-9, *Pediococcus acidilactici* KTU05-7 and *Lactobacillus sakei* KTU05-6 strains, which were later used for milk fermentation, on quality and safety parameters of newly created fermented milk.

MATERIALS AND METHODS

Fermentation of soya with selected LAB using SSF and SmF types. Soya (*Glycine max* L.) (G) flour was purchased at a pharmacy in Kaunas (LTU) and used for fermentation with three different LAB (*P. pentosaceus* KTU05-9, *P. acidilactici* KTU05-7, *L. sakei* KTU05-6), isolated from fermented rye according to Digaitiene *et al.* (2005). The soya fermentation process was carried out for 24 h at the temperatures of 35°C (*P. pentosaceus* KTU05-9), 32°C (*P. acidilactici* KTU05-7) and 30°C (*L. sakei* KTU05-6). Fermented soya was further used for milk fermentation.

***Fermentation of milk with fermented Glycine max* L.** Ten percent of each soya sample, fermented by SSF and SmF, calculated by weight, were added into the samples of ultra-high temperature (UHT) milk and were homogenised well (Micra D-1 homogenizer; Micra GmbH, Germany). The prepared milk and soya (milk-G) samples (50 g each) were incubated for 24 h at temperatures of 35°C (*P. pentosaceus* KTU05-9), 32°C (*P. acidilactici* KTU05-7) and 30°C (*L. sakei* KTU05-6) till gel (yogurt-like) consistence was formed.

Acidity parameters of fermented milk. Active acidity (AA) (pH) (PP-15 pH-meter; Sartorius, Germany), titratable acidity (TTA) (according to the ISO 11869:2013 standard), lactose concentration (using the high-pressure liquid chromatography method) (Zeppa *et al.* 2001), L(+) and D(–) lactic acid concentrations (by an enzyme test kit R-biopharm; Roche, Germany) according to de Lima *et al.* (2009) were tested.

Microbiological analysis of LAB. Tested LAB strains (at dilutions of 10^{-4} to 10^{-8}) were inoculated on MRS Agar (DeMan-Ragosa-Sharpe, UK), cultivated for 72 h at a temperature of 30°C for *L. sakei* KTU05-6, at 32°C for *P. acidilactici* KTU05-7 and at 35°C for *P. pentosaceus* KTU05-9 under anaerobic conditions and the number of LAB (CFU/g) was calculated.

Determination of BA content. Content of BA was determined according to Gigirey *et al.* (1999).

Sensory analysis of milk fermented by LAB pregrown in soya. Sensory analysis was carried out by 10 expert panellists at 30–55 years of age, according to the ISO 8586:2012 standard. Preliminary acceptability of the products was scored using a 9-point hedonic scale. The sensory profile test was performed according to the ISO 13299:2016 standard. Attributes such as intensity of external taste, taste pleasure and smoothness were evaluated.

Statistical analysis. Statistical data analysis was conducted using the Microsoft Excel 2007 (Microsoft Corporation, USA). The SPSS 17.0 (SPSS Inc.; USA) was used for descriptive analysis (N, mean ± standard deviation), GLM (General Linear Modelling) and ANOVA. Calculated mean values were compared using Bonferroni multiple range tests. In total 18 fermented milk-G samples were tested, performing all analyses in triplicate. For all statistical analyses $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Analysis of AA, TTA, lactose and lactic acid isomers. The AA (pH) of all milk-G samples decreased with fermentation time (Table 1). The higher drop after 24 h of fermentation was seen in SmF samples, especially using *L. sakei*. The pH in these samples dropped to the value 4.6 meaning that the isoelectric point of casein was reached and desired structural characteristic of sour milk was formed.

In all milk-G samples, the L(+) lactic acid isomer was the major formed isomer. In SmF the amount of the L(+) lactic acid isomer was more constant and varied between 6.06 (*P. pentosaceus*) and 9.08 g/kg (*P. acidilactici*). Whereas the differences in L(+) isomers using different LAB were higher in SSF and varied from 1.0 g/kg (*P. pentosaceus*) to 15.1 g/kg (*P. acidilactici*). The amounts of the D(–) lactic acid isomers depended on the fermentation type and were lower in SmF compared with SSF. Similar results were obtained by Slapkauskaite *et al.* (2016), when milk fermented with *P. acidilactici* and *L. sakei* (in SSF) produced more L(+) than D(–) isomers ($P \leq 0.01$) compared to *P. pentosaceus.*

The lactose content in milk-G samples after 24-hour fermentation varied from 4.18% to 4.48% ($P \leq 0.05$) (Table 1). *L. sakei* was the most suitable strain for lactose fermentation. The values differed from those of *P. pentosaceus* and *P. acidilactici* by 0.21 and 0.07% (in SSF) and by 0.28 and 0.23% (in SmF), respectively. Only the samples with *L. sakei* were fully fermented after 24 h and the isoelectric point of casein (pH 4.6) was reached. This fact is important for humans who are lactose intolerant (Solomons *et al.* 2002).

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Table 1. Monitored values of milk-G samples after 24 hours of fermentation (SSF and SmF) using different LAB

LAB	AA initial pH (–)	AA pH after 24 h (–)	TTA after 24 h (–)	Lactic acid isomer D(–) (g/kg)	Lactic acid isomer L(+) (g/kg)	Lactose after 24 h (%)
Milk-G samples treated with SSF						
P. pentosaceus KTU05-9	5.31 ± 0.06*	5.27 ± 0.07*	58.62 ± 4.70*	5.15 ± 0.07	1.00 ± 0.01	4.42 ± 0.36*
P. acidilactici KTU05-7	5.57 ± 0.03*	5.34 ± 0.12*	57.11 ± 6.90*	5.00 ± 0.05	15.12 ± 0.15	4.28 ± 0.42*
L. sakei KTU05-6	5.09 ± 0.05*	4.93 ± 0.34*	78.00 ± 1.70*	1.03 ± 0.10	9.20 ± 0.11	4.21 ± 0.78*
Milk-G samples treated with SmF						
P. pentosaceus KTU05-9	6.19 ± 0.07*	5.10 ± 0.07*	46.00 ± 17.00*	2.10 ± 0.03	6.06 ± 0.02	4.46 ± 0.01*
P. acidilactici KTU05-7	5.78 ± 0.05*	5.28 ± 0.03*	53.20 ± 0.10*	2.12 ± 0.25	9.08 ± 0.09	4.41 ± 0.14*
L. sakei KTU05-6	5.94 ± 0.02*	4.62 ± 0.04*	89.00 ± 0.10*	2.09 ± 0.04	8.01 ± 0.18	4.18 ± 0.10*

*Differences between samples, bacteria and fermentation are statistically significant ($P \leq 0.05$); LAB – lactic acid bacteria; AA – active acidity; TTA – titratable acidity

Microbiological analysis of LAB. The total amount of LAB ranged from 6.77 to 8.34 $\log_{10}$ CFU/g (in SSF) and from 7.04 to 9.41 $\log_{10}$ CFU/g (in SmF) and depended on the LAB strain and fermentation type used (Figure 1). The lowest LAB count was found in milk-G-SSF treated with *P. pentosaceus,* whereas the highest in milk-G-SmF treated with *P. acidilactici* and *L. sakei,* and it was higher by 1.02 and 1.09%, respectively, compared to the results of SSF fermentation.

It is proposed that the application of LAB for fermentation is effective because the LAB become predominant and are responsible for lowering the amount of spoilage bacteria and those who are responsible for induction of oxidative processes (Widyastuti *et al.* 2014).

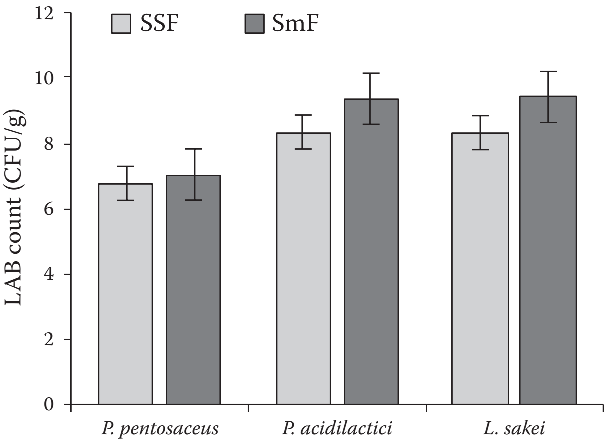

Figure 1. The difference in LAB counts (CFU/g) in fermented milk after 24 h of SSF and SmF.

SSF – fermented by solid state; SmF – submerged fermentation

Content of BA. During the study, 6 types of BA were identified in fermented milk samples (Table 2). The influence of fermentation on BA formation was not significant whereas some differences in milk-G samples could be seen. The lowest total BA concentration was found using *P. pentosaceus* in SmF. These values were lower by 43.75 and 26.91 mg/kg compared to *P. acidilactici* and *L. sakei*. Different concentrations of BA in fermented milk samples can be explained by different properties of fermented milk and by the LAB which have diverse biochemical potentials regarding the metabolism of amino acids.

According to Parente *et al.* (2001), histamine intake higher than 100 mg/kg can cause intermediate or intensive poisoning. Despite soya used in our research is rich in protein concentration and a possibility of BA formation is higher, we found that BA concentrations in all analysed milk-G samples were far below those levels associated with a health risk.

Sensory evaluation of fermented milk. The results of sensory evaluation of milk-G samples showed that the addition of fermented soya was acceptable to consumers (Figure 2). The mean score for overall acceptability of milk-G samples on a 9-point hedonic scale ranged from 4.2 (in SSF) to 9.0 (in SmF). Two of three milk-G samples treated with SmF were more acceptable compared to milk-G samples treated with SSF. However, the influence of fermentation was not

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Table 2. Contents of Ba (mg/kg) in fermented milk after 24 h of storage at a temperature of 4°C

BA	*P. pentosaceus* KTU05-9		*P. acidilactici* KTU05-7		*L. sakei* KTU05-6	
	SSF	SmF	SSF	SmF	SSF	SmF
Phenylethylamine	43.39 ± 0.11*	12.34 ± 0.15*	43.39 ± 0.04*	12.64 ± 0.06*	68.36 ± 0.07*	13.01 ± 0.17*
Putrescine	–	–	–	–	49.85 ± 0.11*	–
Cadaverine	–	76.08 ± 0.16*	–	58.83 ± 0.13*	88.8 ± 0.01*	55.17 ± 0.05*
Histamine	–	–	–	37.58 ± 0.10*	–	30.97 ± 0.08*
Tyramine	–	–	–	–	–	–
Spermidine	46.09 ± 0.09*	34.75 ± 0.09*	46.09 ± 0.01*	34.85 ± 0.17*	73.01 ± 0.09*	35.12 ± 0.09*
Spermine	46.73 ± 0.06*	–	46.70 ± 0.01*	23.02 ± 0.02*	46.73 ± 0.02*	15.81 ± 0.01*
Tryptamine	–	–	–	–	–	
Total	136.21	123.17	136.18	166.92	326.75	150.08

SSF – fermented by solid state; SmF – submerged fermentation; *Differences between samples, bacteria and fermentation are statistically significant

significantly different. Milk-G-SSF samples treated with *P. pentosaceus* and milk-G-SmF samples treated with *P. acidilactici* and *L. sakei* showed the highest acceptability. In general, a product is considered to be acceptable when the overall acceptability value is higher than or equal to 7.0 points (Ryland *et al.* 2018). As the average values of overall acceptability of those three milk-G samples were above 8.0, it could be concluded that sensory properties of these samples were excellent.

In all cases the taste of milk-G samples was acceptable to consumers. However, the overall taste acceptability of milk-G treated with *L. sakei* and SmF was evaluated by higher scores (8.0 points) than that of the others. It seems likely that the acceptability of the overall taste was mostly influenced by the highest taste pleasure (9.5 points) and the lowest external taste (1.0 point) of these products (Figure 3).

The overall appearance of milk-G samples treated with *L. sakei* and SmF was highly acceptable (9.5 points) and was related with the evaluation of smoothness. The higher count of *L. sakei* together with the lower values of pH and high acidity could be responsible for the sour taste, flavour and unique aroma of fermented milk. The production of lactic acid provides an acceptable sour taste and influences the formation of the fermented product texture.

Food flavour (taste plus odour) is a characteristic not only related with the acceptance of the product but also associated with the feeling of consumer satisfaction (Routray & Mishra 2011). Consumers usually chose food according to the whole acceptance, not accentuating different characteristics. However, we can accentuate the milk-G sample, treated with *L. sakei* and SmF, which showed not only the highest overall acceptability but also the separate characteristics (overall taste acceptability, taste pleasure, low external taste and smooth consistence) were the most acceptable to the panellists.

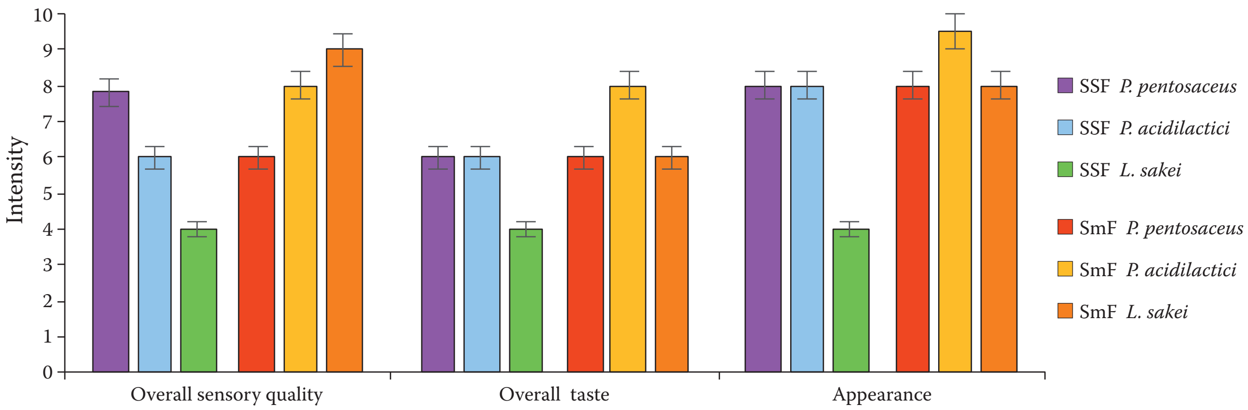

Figure 2. The difference in overall sensory quality of fermented milk after 24 hours of (SSF and SmF) using different LAB

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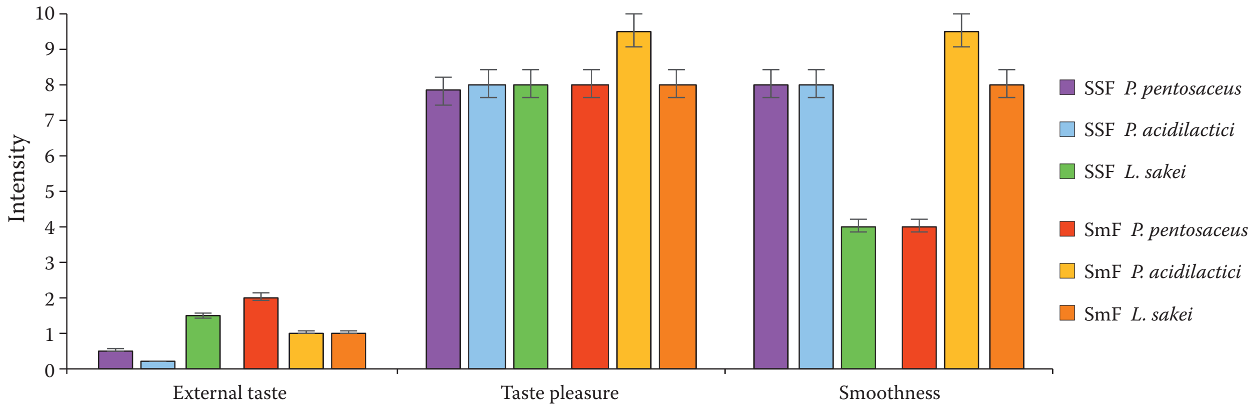

Figure 3. The difference in sensory attributes of fermented milk after 24 hours of (SSF and SmF) using different LAB

CONCLUSION

Three LAB strains *(P. pentosaceus, P. acidilactici, L. sakei)* were used for soya fermentation (SSF and SmF). The fermented soya was used as a starter in order to get new fermented milk products, rich in proteins and other biologically active compounds originating from soya. The results showed a different influence of the fermentation type and tested LAB strains. *L. sakei* strain, which was used for the SmF fermentation, was responsible for optimal AA (pH 4.6), higher TTA, intensive degradation of lactose, lower amounts of the D(–) lactic acid isomers and BA content compared to the other LAB. Besides, the acceptability of milk-G samples, threated with *L. sakei* by SmF fermentation, was the highest. The other LAB (excluding *L. sakei*) produced small amounts of acids and pH of the fermented milk did not reach the value 4.6, what is necessary for the formation of usual texture. The used LAB are not mostly analysed in milk products, but in other carbohydrate-rich food matrices. Our research results indicated that these LAB (especially *L. sakei)* can be responsible for milk fermentation, however for the formation of full aroma of the final product it is recommended to combine them with other suitable mesophilic LAB. The results of this study open possibilities for innovations to use LAB in fermentation of plants (soya in this case) and enrichment of milk with plant proteins and biologically active soya compounds together with additional analysis of lactose fermentation which has been little analysed worldwide. Therefore further development of a technology for new fermented milk products with functional properties is a crucial issue.

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