Research Article

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Solid state fermentation with lactic acid bacteria to improve the nutritional quality of lupin and soya bean

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Abstract

BACKGROUND: The ability of bacteriocin-like inhibitory substance (BLIS)-producing lactic acid bacteria (LAB) to degrade biogenic amines as well as to produce L(+) and D(−)-lactic acid during solid state fermentation (SSF) of lupin and soya bean was investigated. In addition, the protein digestibility and formation of organic acids during SSF of legume were investigated.

RESULTS: Protein digestibility of fermented lupin and soya bean was found higher on average by 18.3% and 15.9%, respectively, compared to untreated samples. Tested LAB produced mainly L-lactic acid in soya bean and lupin (D/L ratio 0.38–0.42 and 0.35–0.54, respectively), while spontaneous fermentation gave almost equal amounts of both lactic acid isomers (D/L ratio 0.82–0.98 and 0.92, respectively). Tested LAB strains were able to degrade phenylethylamine, spermine and spermidine, whereas they were able to produce putrescine, histamine and tyramine.

CONCLUSIONS: SSF improved lupin and soya bean protein digestibility. BLIS-producing LAB in lupin and soya bean medium produced a mixture of D- and L-lactic acid with a major excess of the latter isomer. Most toxic histamine and tyramine in fermented lupin and soya bean were found at levels lower those causing adverse health effects. Selection of biogenic amines non-producing bacteria is essential in the food industry to avoid the risk of amine formation.

Keywords: legume; fermentation; D-, L-lactic acid; biogenic amines

INTRODUCTION

The importance of lupin as valuable source of nutrients to be used in human or animal nutrition has increased in recent years. Lupin seeds contain significant amounts of protein, fat, minerals and dietary fiber.[1–3] Lupin is increasingly used as a protein source in European countries as a replacement for potentially genetically modified soya products[4,5] and as an additive to the human diet because of its high protein and low oil content.[6] The dietary value of lupin proteins is higher than that of beans or peas, mainly because of the high concentrations of the essential amino acids lysine, leucine and threonine, which are higher only in soya beans.[7] Therefore, fermented soya bean products with high nutrition and health benefits have gained more attention.[8]

The reduction of protein digestibility in soya and lupin seeds has been associated with the presence of protease inhibitors,[9,10] as well as antinutritionals such as fibre and oligosaccharides.[11] Sweet lupin seeds (*Lupinus albus* and *L. angustifolius*) have a much lower amount of inhibitor (<0.1 mg g^{-1}) than soya beans (26.2 mg g^{-1}).[9]

Fermentation is an ancient technology for enhancing the shelf life, nutritional and organoleptic quality of food.[12–14] Recently, this bioprocess has been applied to the production and extraction of bioactive compounds in food.[15] In the last few years, fermentation has been performed to increase the content of bioactive phenolic compounds in legumes, thus enhancing their antioxidant activity.[16,17]

Lactic acid bacteria (LAB) play a key role in food fermentation, where they contribute not only to the development of the desired sensory properties in the final product but also to their microbiological safety.[18] LAB has a GRAS (Generally Recognized As Safe) status and it has been estimated that 25% of the European diet and 60% of the diet in many developing countries consist of fermented foods.[19,20]

However, the demand for safer foods has promoted more research into biogenic amines and lactic acid isomers in recent years.[21–26] Food-fermenting LAB are generally considered to be non-toxic and non-pathogenic. However, the ability of microorganisms to decarboxylate amino acids is strain specific, and therefore the detection of bacteria possessing amino acid decarboxylase activity is important in estimating the likelihood that foods contain biogenic amines (BA) and in preventing their accumulation in food products.[21] The optimum temperature for

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histidine decarboxylase enzyme activity has been reported as 30 °C,[22] which is also optimal for LAB. The amount and types of BA formed in fermented food products are strongly influenced by the food composition, microbial flora and other parameters which allow bacterial growth during food processing and storage.[23] Low levels of BA in food are not considered a serious risk. However, when consumed in excessive amounts, they can cause severe toxicological effects in human beings.[24]

In addition, LAB naturally produce D- and L-lactic acid, which is found in many fermented milk products and also in pickled vegetables and cured meats and fish.[25] L-Lactic acid is the only optical isomer for use in the food industry because the human body is adapted to assimilate only this form.[25] Moreover, the potential toxicity of D-lactic acid is of particular concern for malnourished and sick people.[26]

The protein digestibility and formation of organic acids were determined to evaluate the effect of solid state fermentation (SSF) of different types of lupin (*L. luteus* and *L. albus*) and soya bean (Rudoji and Progress). In addition, the present paper aimed to study the amine-degrading as well as L(+) and D(−) isomers of lactic acid production ability of bacteriocin-like inhibitory substance (BLIS)-producing LAB (*Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8) used for SSF of legumes, with the purpose of improving the nutritional quality and safety of legume products.

MATERIALS AND METHODS

Flours and lactic acid bacteria

Lupinus luteus and *L. albus* seeds with low alkaloid content (<0.01%) were obtained from the Lithuanian Institute of Agriculture (Voke, Lithuania) after the year 2012 harvest. Soya beans (var. Rudoji and Progress) were obtained from the Aleksandras Stulginskis University (Kaunas, Lithuania) pilot farm after the year 2012 harvest.

BLIS-producing *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8 strains previously isolated from spontaneous rye sourdough[27] were cultured at 25–35 °C for 48 h in MRS broth (CM0359; Oxoid Ltd, Basingstoke, UK) prior to use.

Preparation of fermented lupin and soya bean products

Lupin seeds and soya beans were ground and the wholemeal (200 g) and tap water (10 g) were mixed. A LAB cell suspension (10 g) containing 8.9 $\log_{10}$ colony-forming units (CFU) mL^{-1} of the above individual LAB strains was added. Fermentation of legume products was carried out for 24 h at 30 °C for *Lact. sakei*, at 32 °C for *P. acidilactici* and at 35 °C for *P. pentosaceus*. At the end of the fermentation the colony number in the fermented lupin and soya bean products averaged 7.28 $\log_{10}$ CFU g^{-1} and the final moisture content of solid state fermented products averaged 45%.

Analysis

Moisture content was determined by drying the sample at a temperature of 105 °C to constant weight. The pH value of lupin and soya bean products was measured and recorded using a pH electrode. Total titratable acidity (TTA) was determined on a 10 g sample homogenized with 90 mL distilled water and expressed as the amount (mL) of 0.1 mol L^{-1} NaOH to obtain a pH value of 8.2.

Determination of L- and D-lactic acid

Concentrations of L- and D-lactic acid in fermented lupin and soya bean products were determined using a rapid Megazyme test kit (Megazyme International Ireland, Wicklow, Ireland) for D-lactate and L-lactate determination.

Determination of *in vitro* protein digestibility

Determination of protein digestibility was carried out according to Lqari *et al.*[28] Samples containing 62.5 mg protein were suspended in 10 mL water and the pH was adjusted to 8 with 0.1 mol L^{-1} NaOH. An enzymatic solution containing 1.6 mg trypsin (18 U mg^{-1}), 3.1 mg α-chymotrypsin (40 U mg^{-1}) and 1.3 mg protease (15 U mg^{-1}) mL^{-1} was added to the protein suspension in a 1:10 (v/v) ratio. The pH of the mixture was measured exactly after 10 min and the *in vitro* digestibility calculated as a percentage of digestible protein (DP) using the equation $DP = 210.464 - 18.103 \times pH$.[28]

Biogenic amine analysis

Extraction and determination of BA were carried out according to the procedures developed by Ben-Gigirey *et al.*[29] Perchloric acid (0.4 mol L^{-1}, 10 mL) containing a known amount of 1,7-diaminoheptane used as an internal standard was added to a 3 g sourdough sample, and the mixture was homogenized with Ultra-Turrax (IKA Labortechnik, Staufen, Germany) and centrifuged at 3000 × *g* for 10 min. The residue was extracted again with an equal volume of 0.4 mol L^{-1} perchloric acid. Both supernatants were combined, and the final volume was adjusted to 30 mL with 0.4 mol L^{-1} perchloric acid. The extract was filtered through Whatman No. 1 paper. One millilitre of extract or standard solution was mixed with 200 mL of 2 mol L^{-1} sodium hydroxide and 300 mL saturated sodium bicarbonate. 5-(Dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride reagent) (10 mg mL^{-1}, 2 mL) prepared in acetone was added to the mixture and incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 100 mL of 25 mg L^{-1} ammonium hydroxide. After incubation at room temperature for 30 min, the mixture was adjusted to 5 mL with acetonitrile. Finally, the mixture was centrifuged at 3000 × *g* for 5 min; the supernatant was filtered through 0.2 µm filters (Millipore Co., Bedford, MA, USA) and stored at 225 °C until analysis by high-performance liquid chromatography (HPLC).

An Agilent 1200 HPLC instrument (Carlsbad, CA, USA) equipped with diode-array detector and Chemstation LC software was employed. A Chromolith C_{18} HPLC column (100 mm × 4.6 mm × 4 mm, Merck KGaA/EMD Chemicals, Darmstadt, Germany) was used. Ammonium acetate (0.1 mol L^{-1}) and acetonitrile were used as the mobile phases, with a flow rate of 0.45 mL min^{-1}. The sample volume injected was 10 mL and amines were monitored by at nm. The detection limit for standard amine solutions was approximately 0.1 mg kg^{-1}.

Statistical analysis

All analytical determinations were performed at least in triplicate. Data obtained were analysed using the statistical package SPSS for Windows XP V15.0 (2007; SPSS Inc., Chicago, IL, USA). The significance of differences between treated samples was evaluated using Duncan's multiple range test. The confidence interval was 95% ($P < 0.05$).

RESULTS

Influence of solid state fermentation on acidity of lupin and soya bean products

The results pertaining to the effects of a single LAB strain on the pH, total titratable acidity (TTA) and D- and L-lactic acid formation during SSF of lupin and soya bean are presented in Tables 1 and 2.

The experiment showed that the formation of organic acids depended on the type of fermentation and LAB strain used, and slightly on lupin genotype. Most intensive formation of organic acids was observed in lupin during SSF treatment. The highest TTA (average 23.56) was observed in both lupin samples fermented with *P. pentosaceus*, followed by *Lact. sakei* (21.56) and *P. acidilactici* (20.67) (Table 1). pH values measured in lupin products after SSF varied between 4.10 and 4.24. Generally, SSF gave pH values lower on average by 4.3% and TTA higher on average by 14.5% than that of spontaneous treatment (Table 1).

Analysis of fermented soya bean products showed similar trends. The higher acidity in soya bean fermented products was measured after SSF compared to spontaneously treated samples (Table 2). SSF using *P. pentosaceus* gave the lowest pH and highest TTA values (average 4.29 and 22.83, respectively). The lower TTA values were found in soya bean fermented with *P. acidilactici* (average 21.03), followed by *Lact. sakei* (19.07). Mean TTA values in soya bean after SSF with *P. pentosaceus*, *P. acidilactici* and *Lact. sakei* were found to be lower on average by 20.4%, 13.6 and 4.7%, respectively, compared to spontaneously treated (Table 2). It could be mentioned also that the pH and TTA values between genotypes of soya bean were not significantly different ($P > 0.05$).

It could be said that after SSF with pure LAB as well as spontaneous fermentation slightly higher acidity was found in white lupin (*L. albus*). TTA of these samples was higher by 6.7% (spontaneous), 5.4% (*P. acidilactici* and *Lact. sakei*) and 1.2% (*P. pentosaceus*) compared to yellow lupin (*L. luteus*) (Table 1).

Formation of lactic acid during solid state fermentation of lupin and soya bean

The highest mean concentrations of lactic acid were determined in lupin samples fermented with *P. pentosaceus* (180.2 mg kg^{-1}), followed by *P. acidilactici* (160.4 mg kg^{-1}) and *Lact. sakei* (138.4 mg kg^{-1}). Generally, fermentation of *L. albus* gave higher contents of LA than appropriate treatment of *L. luteus*, by 8.6% (spontaneous) and by 13.8% (*P. acidilactici*), 6.8% (*Lact. sakei*) and 3.2% (*P. pentosaceus*).

All tested LAB produced a mixture of L- and D-lactic acid in both lupin species (Table 1). Results indicated that genotype of lupin had no influence on L- and D-lactic acid isomer ratio, which strongly ($P < 0.05$) depended on type of fermentation. The lowest mean contents of D-lactic acid were measured in lupin samples after SSF with *L. sakei* (40.6 mg kg^{-1}), while fermentation with *P. acidilactici* and *P. pentosaceus* slowly yielded higher contents of the latter isomer (46.5 and 50.8 mg kg^{-1}, respectively). The highest contents of D-lactic acid were measured in lupin samples after spontaneous fermentation (average 62.0 mg kg^{-1}).

The highest mean levels of lactic acid were determined in products of soya bean Rudoji fermented with *P. pentosaceus* (185.1 mg kg^{-1}), followed by *P. acidilactici* (160.2 mg kg^{-1}) and *Lact. sakei* (136.2 mg kg^{-1}).

Results indicated that tested LAB produced a mixture of L- and D-lactic acid in soya bean (Table 2) depending on genotype, type of fermentation and LAB strain used. Content of L-lactic acid was higher by 29.2%, 19.2% and 34.3% in soya bean after SSF compared to spontaneously fermented samples (7.82 mg kg^{-1}). The highest content of D-lactic acid was measured in spontaneously fermented Progress samples (78.6 mg kg^{-1}), whereas in Rudoji samples the content of the latter isomer was lower by 17.9%. The lowest content of D-lactic acid was measured in soya bean Rudoji (32.9 mg kg^{-1}) and Progress (41.7 mg kg^{-1}) after SSF with *Lact. sakei*. Most intensive production of L-lactic acid was observed in soya bean samples fermented with *P. pentosaceus* (mean 11.91 mg kg^{-1}).

In summary, tested LAB produced mainly L-lactic acid (D/L ratio varied between 0.42 and 0.38 in lupin and between 0.35 and 0.54 in soya bean), whereas spontaneous fermentation gave almost equal amounts of both lactic acid isomers: D/L ratio calculated was 0.92 in both lupin species, 0.82 in soya bean Rudoji and 0.98 in soya bean Progress).

Results of our study indicated that the use of pure LAB cultures significantly ($P < 0.05$) reduced the content of D-lactic acid in lupin and soya bean products (reduction by 23.9% and 38.6%, respectively) compared to the spontaneously fermented ones (Tables 1 and 2). The reduction on average by 45.7% of the latter isomer was observed after fermentation with *P. acidilactici* and *Lact. sakei*.

The *in vitro* protein digestibility of fermented lupin and soya bean

In vitro protein digestibility (Table 3) depended on type of fermentation and type of legume. In all cases lupin and soya bean protein digestibility was found to be higher by 18.9% (*L. luteus*) and 17.7% (*L. albus*), and by 16.9% (soya bean Rudoji) and 14.9% (soya bean Progress) in fermented samples compared to untreated (Table 3). It was noticed that protein digestibility of SSF soya bean (average 88%) was slightly (2.6 %) higher than that of SSF lupin. The highest protein digestibility was found in lupin and soya bean fermented with *P. pentosaceus* (average 86.9% and 89.3%, respectively). The lowest *in vitro* protein digestibility was determined in *L. albus* fermented with *P. acidilactici* (83.4%) and in soya bean Progress fermented with *Lact. sakei* (87.2%). Differences between protein digestibility of different types of lupin as well as soya bean were not significant. Further, fermentation using pure LAB resulted in a 6.5% higher protein digestibility than spontaneous fermentation.

Degradation of biogenic amines during fermentation of lupin and soya bean

Results of biogenic amine analysis showed that phenylethylamine was the predominant amine in both untreated and spontaneously fermented lupin (Table 4) and soya bean (Table 5). During spontaneous fermentation phenylethylamine degradation activity in soya bean medium was low (5.4%), whereas in lupin medium this amine was degraded by up to 43.2% from its initial concentration. SSF treatment of lupin (Table 4) and soya bean (Table 5) allowed phenylethylamine degradation of up to 4.8-fold and 3.4-fold, respectively.

During SSF treatment of soya bean with *P. acidilactici* and *P. pentosaceus*, putrescine concentration increased up to 8.0% (soya bean Rudoji) and by 58.5% (soya bean Progress) from their initial concentrations (Table 5). Conversely, during spontaneous fermentation putrescine-degrading activity was found to be significantly higher in soya bean (mean decrease 87.4% compared to initial concentration), as well as in lupin (24.0%) (Table 4). Putrescine concentration was found considerably increased, reaching levels up to 43-fold higher after SSF with *P. pentosaceus* of *L. albus*.

Table 1. pH, total titratable acidity (TTA), and L- and D-lactic acid concentrations (mg kg^{-1}) of fermented lupin (*L. luteus* and *L. albus*)

Lupin products	pH	TTA	L-lactic acid	D-Lactic acid	D/L ratio
Spontaneously fermented					
L. luteus	4.30 ± 0.02b	18.01 ± 1.04a	64.3 ± 7.8a	59.1 ± 6.5d	0.92c
L. albus	4.41 ± 0.02b	19.34 ± 1.31b	70.2 ± 5.3b	64.8 ± 7.1e	0.92c
Fermented with *P. acidilactici*					
L. luteus	4.21 ± 0.01a	20.05 ± 1.27b	105.8 ± 4.7c	42.7 ± 8.3b	0.40a
L. albus	4.15 ± 0.01a	21.29 ± 0.83c	122.0 ± 9.6d	50.3 ± 7.9c	0.41a
Fermented with *Lact. sakei*					
L. luteus	4.24 ± 0.01a	21.00 ± 1.08c	94.3 ± 7.2c	38.2 ± 4.8a	0.40a
L. albus	4.19 ± 0.01a	22.13 ± 1.17d	101.2 ± 9.3c	43.1 ± 5.1b	0.42ab
Fermented with *P. pentosaceus*					
L. luteus	4.13 ± 0.01a	23.42 ± 1.00e	128.4 ± 5.4de	48.9 ± 5.5c	0.38a
L. albus	4.10 ± 0.01a	23.71 ± 1.17e	130.5 ± 3.9e	52.7 ± 8.1 cd	0.40a

Data are the mean ± SD ($n = 3$).
Means within a column with different letters are significantly different ($P \leq 0.05$).

Table 2. pH, total titratable acidity (TTA), and L- and D-lactic acid concentrations (mg kg^{-1}) of the fermented soya bean (Rudoji and Progress)

Soya bean products	pH	TTA	L-Lactic acid	D-Lactic acid	D/L ratio
Spontaneously fermented					
Rudoji	4.51 ± 0.02b	17.93 ± 0.99a	76.1 ± 3.7a	62.4 ± 4.2d	0.82e
Progress	4.49 ± 0.02b	18.41 ± 1.02a	80.4 ± 4.2b	78.6 ± 10.2e	0.98f
Fermented with *P. acidilactici*					
Rudoji	4.36 ± 0.01a	20.30 ± 0.93bc	114.2 ± 6.1e	50.3 ± 6.8bc	0.44b
Progress	4.34 ± 0.01a	21.76 ± 0.72c	106.7 ± 8.7d	49.1 ± 7.2b	0.46bc
Fermented with *L. sakei*					
Rudoji	4.41 ± 0.01b	19.38 ± 0.46ab	93.6 ± 2.2c	32.9 ± 5.5a	0.35a
Progress	4.48 ± 0.02b	18.76 ± 0.85a	100.1 ± 3.4d	41.7 ± 9.3b	0.42b
Fermented with *P. pentosaceus*					
Rudoji	4.30 ± 0.01a	23.02 ± 1.14d	120.3 ± 5.8e	64.8 ± 8.1d	0.54d
Progress	4.29 ± 0.01a	22.64 ± 1.00 dc	117.9 ± 6.4e	56.2 ± 7.2c	0.48c

Data are the mean ± SD ($n = 3$).
Means within a column with different letters are significantly different ($P \leq 0.05$).

After SSF treatment of lupin and soya bean, lower contents of cadaverine were measured compared to spontaneous fermentation, whereas cadaverine levels after spontaneous fermentation were higher by 61.4% in soya bean and by 32.9% in lupin compared to untreated samples.

Concentrations of histamine showed a rising trend during the spontaneous fermentation as well as SSF, reaching levels 8-fold and 16-fold higher (Table 4) compared to soya bean (the latter amine was degraded by up to 50.6% (SSF) and 15.3% (spontaneous fermentation) from its initial concentration) (Table 5).

It should be mentioned that during spontaneous fermentation tyramine production was comparatively low. Tyramine concentrations varied between 32.6 and 69.1 mg kg^{-1} in lupin and between 27.8 and 30.1 mg kg^{-1} in soya bean products. SSF significantly increased tyramine concentrations, reaching levels up to 215.8 mg kg^{-1} in *L. albus* (Table 4) and up to 416.1 mg kg^{-1} in soya bean Rudoji (Table 5).

Generally, the lowest concentrations of putrescine, histamine and tyramine were measured in lupin and soya bean products after fermentation with *Lact. sakei*, and the highest after fermentation with *P. acidilactici*.

After spontaneous fermentation and SSF, concentrations of spermidine were lower by 71% and 50%, respectively, in soya bean samples (Table 5) and by 43.6% and 39%, respectively, in lupin samples (Table 4). After spontaneous fermentation spermine content in lupin and soya bean products was lower by 71.4% and 76.2%, respectively, compared to initial concentrations, whereas after SSF a lower decrease in spermine content was observed (24.5% and up to 29.7%, respectively).

DISCUSSION

LAB are generally fastidious on artificial media but they grow readily in most food substrates and lower the pH rapidly to a point where other competing organisms are no longer able to grow. *Leuconostoc* species and lactic streptococci generally lower the pH to about 4.0–4.5 and some of the lactobacilli and pediococci to about 3.5 before inhibiting their own growth.[20] Some of the LAB are homofermentative and produce lactic acid as the main product of glucose fermentation, whereas others are heterofermentative and produce carbon dioxide and ethanol in addition to lactic acid.[30] Formation of organic acids in fermented legume depends on existent microbiota, while concentrations of lactic

Table 3. *In vitro* protein digestibility (%) of lupin (*L. luteus* and *L. albus*) and soya bean (Rudoji and Progress): untreated, spontaneously fermented and fermented with lactic acid bacteria (*P. acidilactici*, *Lact. sakei*, *P. pentosaceus*)

	Lupin		Soya bean	
Samples	*L. luteus*	*L. albus*	Rudoji	Progress
Untreated	72.56 ± 1.16a	74.38 ± 1.87a	76.12 ± 1.19a	78.02 ± 0.73a
Spontaneously fermented	78.19 ± 2.03b	77.01 ± 1.04b	83.07 ± 1.04b	85.92 ± 1.44b
Fermented with *P. acidilactici*	84.98 ± 1.13c	83.42 ± 0.94c	88.03 ± 1.18c	87.92 ± 0.80b
Fermented with *Lact. sakei*	84.70 ± 0.87c	85.32 ± 1.10c	86.31 ± 1.00c	87.24 ± 1.23b
Fermented with *P. pentosaceus*	86.25 ± 1.25c	87.53 ± 1.44c	89.01 ± 1.84c	89.67 ± 1.56bc

Data are the mean ± SD ($n = 3$).
Means with different letters within a column are significantly different ($P \leq 0.05$).

Table 4. Biogenic amine contents (mg kg^{-1}) in lupin (*L. luteus* and *L. albus*): untreated, spontaneously fermented and fermented with lactic acid bacteria (*Pediococcus acidilactici*, *Lact. sakei*, *Pediococcus pentosaceus*)

Lupin products	Total	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
Non treated								
L. luteus	392.4	240.5 ± 6.5e	45.6 ± 0.2b	28.4 ± 0.6b	6.2 ± 1.0a	–	55.5 ± 1.5d	16.2 ± 0.0d
L. albus	400.1	240.4 ± 5.6e	45.3 ± 0.1b	37.3 ± 1.3b	5.9 ± 0.7a	–	55.2 ± 1.1d	16.0 ± 0.7d
Spontaneously fermented								
L. luteus	293.5	128.8 ± 10.1c	33.7 ± 5.2a	54.7 ± 7.4d	20.8 ± 3.2b	32.6 ± 4.9a	17.8 ± 3.1a	5.1 ± 0.0b
L. albus	393.7	144.1 ± 23.4d	35.3 ± 1.2a	43.7 ± 5.8c	52.7 ± 4.9c	69.1 ± 15.2c	44.4 ± 3.6c	4.4 ± 0.1a
Fermented with *P. acidilactici*								
L. luteus	1066.4	41.3 ± 5.3a	824.4 ± 5.7c	–	67.5 ± 2.6d	92.5 ± 6.0b	34.1 ± 1.9b	6.6 ± 1.0c
L. albus	2455.5	81.2 ± 10.3b	1951.9 ± 36d	17.1 ± 0.0a	96.7 ± 0.3e	215.8 ± 9.2d	69.7 ± 2.9e	23.1 ± 0.8e
Fermented with *L. sakei*								
L. luteus	1006.4	48.4 ± 3.2a	701.7 ± 11.0c	21.4 ± 4.1b	74.3 ± 7.1d	104.1 ± 5.1b	48.7 ± 3.2a	7.8 ± 3.1c
L. albus	1179.0	51.9 ± 5.0a	894.315.2c	32.9 ± 3.8b	68.7 ± 8.2d	90.8 ± 3.9b	31.2 ± 1.0b	9.2 ± 2.7c
Fermented with *P. pentosaceus*								
L. luteus	1189.6	64.3 ± 4.8d	901.3 ± 9.4c	28.3 ± 4.0b	69.1 ± 4.1d	87.5 ± 8.2b	24.8 ± 5.3b	14.3 ± 4.0d
L. albus	1388.9	61.8 ± 2.9d	1120.8 ± 7.3c	34.7 ± 4.2b	50.2 ± 5.8d	80.7 ± 4.9d	29.1 ± 4.7b	11.6 ± 3.5d

Data are the mean ± SD ($n = 3$).
Means with different letters within a column are significantly different ($P \leq 0.05$).

acid, succinic acid, mandelic acid, propionic acid and glutaric acid increase rapidly with fermentation time; concentration of citric acid gradually decreases upon fermentation.[31] Resulting from the accumulation of lactic and acetic acids in fermented products, pH decreases to values lower than 4.5, when the growth of foodborne pathogens is inhibited.[32] In the current study pH values measured after SSF of lupin and soya bean were lower than 4.4.

In mammals, L-lactate is the major stereoisomer formed in intermediary metabolism and is present in blood. D-Lactate is also present but only at about 1–5% of the total lactate concentration.[33] D-Lactate found in human physiological fluids originates from bacterial production in the intestinal tract.[34,35] L-Lactic acid is normally found in the blood and interstitial fluid of humans at a level of 10 mg dL^{-1}.[36] L-Lactate is the natural form metabolized by being first converted to glucose by the liver before metabolism. Lactic acidosis is characterized by lactate levels > 5 mmol L^{-1}.[36] The unnatural D- form is diluted little, if at all, by metabolism in the body. Animals can oxidize a definite, though limited, amount of the D- isomer. Part of the rise in plasma lactate concentration must have been due to accumulation of the D- isomer.[37]

It was found that soya bean and lupin products after SSF had higher L-lactic acid content, by 35.2% and 40.1%, respectively, compared to spontaneously fermented samples. Generally, tested *Lact. sakei*, *P. acidilactici* and *P. pentosaceus* strains were able to produce L-lactate content higher by 52.4 – 60.1% (in lupin) and by 45.5 – 61.5% (in soya bean) compared to the D- isomer (Table 2).

Low digestibility of plant proteins, such as those from legumes and cereals, together with a limiting content of essential amino acids, represents a major issue because of their low nutritional value compared with animal proteins.[38] According to Carbonaro *et al.*,[38] soya bean is the legume species with the highest digestibility (79.9%), followed by lentil (79.3%), chickpea (77.0%) and common bean (73.5%). There is a relationship between the structure of food proteins and their digestibility behaviour, and β sheets of proteins were shown to be the main components in legume proteins, followed by cereal, milk product and chicken meat proteins, and these secondary structure elements were found to play a major role in decreasing protein digestibility.[38] In the other studies it was shown that *Bacillus* species were able to degrade soya bean macromolecules to a large extent, resulting in water-soluble low-molecular-weight compounds. *In vitro* digestion of *Bacillus*-fermented soya bean using gastrointestinal enzymes only slightly increased the amount of dialysable matter, which clearly demonstrated the beneficial effect of *Bacillus* fermentation on food nutrient availability.[39] Regarding protein

Table 5. Biogenic amine content (mg kg^{-1}) in soyabean (Rudoji and Progress): untreated, spontaneously fermented and fermented with lactic acid bacteria (*Pediococcus acidilactici, Lactobacillus sakei, Pediococcus pentosaceus*)

Soya bean products	Total	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
Non-treated								
Rudoji	549.1	239.8 ± 9.3d	135.5 ± 4.1d	–	30.5 ± 1.5d	11.7 ± 1.1b	114.4 ± 6.3f	17.2 ± 1.4 cd
Progress	488.2	251.4 ± 5.9a	64.9 ± 4.6c	33.8 ± 4.2b	25.5 ± 0.8a	6.4 ± 1.3a	89.8 ± 5.3c	16.4 ± 2.1c
Spontaneously fermented								
Rudoji	432.1	230.1 ± 8.6d	14.2 ± 3.1b	87.2 ± 5.6d	41.5 ± 3.2e	30.1 ± 3.1d	25.3 ± 4.1a	3.7 ± 0.9a
Progress	379.6	234.5 ± 12.3d	9.6 ± 2.1a	33.8 ± 3.6b	35.5 ± 0.9a	27.8 ± 3.2c	34.2 ± 4.1b	4.2 ± 0.2b
Fermented with *P. acidilactici*								
Rudoji	752.6	68.2 ± 65.3b	153.2 ± 10.2e	15.9 ± 3.2a	10.9 ± 1.7b	416.1 ± 15.3f	71.1 ± 5.7d	17.2 ± 3.1 cd
Progress	676.6	76.1 ± 5.3c	177.5 ± 10.8f	43.8 ± 4.8c	14.5 ± 2.1c	268.3 ± 25.3e	80.5 ± 3.2e	15.9 ± 1.8c
Fermented with *Lact. sakei*								
Rudoji	464.3	114.7 ± 4.2f	124.8 ± 7.2f	28.1 ± 4.8d	10.8 ± 3.1c	141.7 ± 9.3f	32.4 ± 4.2d	11.8 ± 3.1c
Progress	471.7	121.3 ± 7.1f	131.2 ± 6.9d	34.9 ± 7.3d	11.2 ± 2.0c	124.2 ± 7.1f	36.9 ± 3.8d	12.0 ± 2.7c
Fermented with *P. pentosaceus*								
Rudoji	505.4	138.4 ± 5.0f	142.0 ± 8.1f	20.4 ± 5.8c	15.4 ± 1.9c	131.8 ± 6.0f	41.7 ± 5.1b	15.7 ± 1.9c
Progress	521.9	142.0 ± 8.9f	139.8 ± 5.6f	19.2 ± 8.0c	20.1 ± 2.3b	139.7 ± 8.4f	42.9 ± 3.0b	18.2 ± 2.0c

Data are the mean ± SD ($n = 3$).
Means within a column with different letters are significantly different ($P \leq 0.05$).

quality, the fermentation process affects the nutritional quality of legumes by improving protein digestibility as a consequence of the partial degradation of complex stored proteins into more simple and soluble products.[40]

In our study, the formation of higher contents of L-lactic acid and higher protein digestibility of SSF legume products compared to spontaneous fermentation indicates that fermentation with pure LAB is more effective; moreover, such legume products could be considered to have higher nutritional quality.

Biogenic amine formation through the microbial decarboxylation of amino acids is dependent on the specific bacterial strain(s) present, the level of decarboxylase activity and availability of the amino acid.[41,42] The most common biogenic amines found in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine and putrescine.[43] Putrescine, spermine, spermidine and cadaverine have no adverse health effects, but they may react with nitrite to form carcinogenic nitrosoamines and also have been proposed as indicators of spoilage.[44] Concentrations of histamine and tyramine above 500–1000 mg kg^{-1} and 100–800 mg kg^{-1} of food, respectively, are regarded as potentially dangerous for human health.[45] The level for no observed adverse effect was 2000 mg kg^{-1} for cadaverine and putrescine, 1000 mg kg^{-1} for spermidine and 200 mg kg^{-1} for spermine.[45] Tyramine alone at high levels can cause an intoxication known as the cheese reaction, which has similar symptoms to histamine poisoning.[43] Some LAB strains have the ability to simultaneously produce different amines; some strains might possess more than one amino acid decarboxylase activity under specific culture conditions.[46]

A qualitative risk assessment of BA in fermented foods was conducted using data from the scientific literature, as well as from European Union-related surveys, reports and consumption data .[47]

The study indicated that fermentation of lupin and soya bean with tested LAB strains have the ability to degrade BA such as phenylethylamine, spermine and spermidine. Moreover, putrescine, tyramine and histamine in lupin and soya bean products fermented with *Lact. sakei*, *P. acidilactici* and *P. pentosaceus* were found at levels lower those causing a health risk.[43–45]

CONCLUSIONS

Digestibility of lupin and soya bean protein could be improved by using pure LAB cultures, and in this case SSF is more effective than spontaneous fermentation. During fermentation of lupin and soya bean, *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-07 and *Pediococcus pentosaceus* KTU05-8 produced a mixture of D- and L-lactic acid, with a major excess of the latter isomer.

The most important increase in biogenic amines (putrescine, histamine and tyramine) takes place during lactic fermentation, which are, for the most part, associated with amino acid decarboxylation and biogenic amine formation. However, the content of the most toxic biogenic amines – histamine and tyramine – in fermented lupin and soya bean products was found at levels lower than those causing adverse health effects. The highest amine-degrading (phenylethylamine, spermine and spermidine) ability was shown by *L. sakei* KTU05-6 strain.

Selection of LAB which have ability to degrade BA is essential in the food industry to avoid the risk of amine formation. BA formation in fermented legume is most likely to be prevented by inhibition of indigenous bacteria and other spoilage microorganisms that could possess decarboxylase activity. Antimicrobial peptides produced by tested LAB have an inhibition effect on growth of spoilage bacteria. Thus lacto-fermentation with BLIS-producing LAB such as *Lact. sakei* KTU05-6 could be recommended for legume treatment for the purpose of improving their nutritional value.

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