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ORIGINAL ARTICLE

Amino acids profile and antioxidant activity of different *Lupinus angustifolius* seeds after solid state and submerged fermentations

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Abstract The objective of this study was to investigate the amino acids profile, total phenolic compounds (TPC) content, antioxidant activity after submerged (SmF) and solid state (SSF) fermentations of different *Lupinus angustifolius* seeds by the *Lactobacillus sakei* KTU05-6. Additionally, the impact of different lupin seeds as fermentation media for LAB biomass and D/L-lactic acid production was analysed. The D/L ratio for SmF and SSF treated lupin samples varied from 0.15 to 0.45 and from 0.12 to 0.46, 16 respectively. Nutritional analysis highlighted a substantial increase in the TPC content and antioxidant activity up to 31.5–48.8% for SSF treated *L. angustifolius* samples compared to unfermented. The interaction between analysed factors (lupin variety and fermentation conditions) had a significant influence on essential and nonessential amino acids profile.

Keywords *Lupinus angustifolius* · *Lactobacillus sakei* · Amino acids · Phenolic compounds · Antioxidant activity

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Introduction

In most parts of the world lupin has traditionally been used primarily as feedstock, and an interest in unique nutritional values of lupin components as food ingredients has increased as people have become more aware of their health benefits. The lupin have a high content of essential amino acids, protein, fibre and low fat content compared to soya bean (Bähr et al. 2015; Thambiraj et al. 2015), that provide the huge industrial potential for this legume. The consumption of lupin may have cholesterol lowering effect and has high antioxidant properties. The potential health benefits of lupin nutritional components increased the potential of lupin incorporation as additive in various food products including pasta (Jayasena and Nasar-Abbas 2012), bakery products (Abdelrahman 2014), wheat bread (Villarino et al. 2015), muffins (Rumiyati et al. 2015).

Legume crops, which represent the major food and feed sources worldwide, contains limiting levels of some essential amino acids (EAA), particularly lysine and methionine (Galili and Amir 2013). The EAA could be complemented from the animal food (meat, eggs and milk) as well as from a variety of cereals and legumes that provide optimal levels of EAA (Galili 2011). Amino acids such as lysine, methionine, threonine, phenylalanine, tryptophan, valine, isoleucine, leucine, and histidine are

covered in this definition (Galili et al. 2016). Lupin is considered as a cheap alternative stock to other legume crops such as soya beans as it contains comparable quantity of proteins with similar AA profile.

Legume proteins are resistant to proteolysis because of specific structural properties. Their structural stability affects in vivo digestibility and availability of the EAA, and also the production of bioactive compounds with functional properties (Orlovskaya et al. 2010). The possibility to modulate protein structure during technological processing may be used as a novel strategy to improve the nutritional potential of protein rich plant foods (Carbonaro et al. 2015).

In addition to their favourable nutritional profile, lupin seeds contain significant amounts of polyphenols, carotenoids, phytosterols, tocopherols and peptides with antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities (Khan et al. 2015; Rumiyati et al. 2013). Phenolic compounds were characterized to have health benefits because of their high antioxidant capacity and protection against highly prevalent diseases (Van Hung 2016).

The fermentation of legumes enriched food products with high value proteins improved protein digestibility, changed an amino acid profile, reduced the concentration of antinutritional factors, increases antioxidant activity, thus improves nutritional characteristics (Yabaya et al. 2009; Curiel et al. 2015). Lactic acid bacteria (LAB) are important in various fermentation processes, implying that they have been proven to be safe for human consumption. *Lactobacilli* and pediococci prevent growth of spoilage and pathogenic microorganisms by acidification and production of antimicrobial compounds, contributing to improved food safety and quality (Cizeikiene et al. 2013).

Solid state fermentation (SSF) presents less energy requirements, lower potential of bacterial contamination, simple technology and equipment for fermentation compared to submerged fermentation (SmF) (Pandey et al. 2000).

Our previous studies showed, that fermentation improved nutritional properties of legume protein (Bartkiene et al. 2015). Diets to Wistar rats supplemented with fermented lupin products improved their gut environment (Bartkiene et al. 2013a). The use of fermented lupin flour additives reduce the acrylamide content in baked goods (Bartkiene et al. 2011, 2013b).

The object of this study was to evaluate the effect of submerged (SMF) and solid state fermentation (SSF) with *Lactobacillus sakei* KTU05-6 strain on the amino acids (AA) profile, total phenolics (TPC) content, and antioxidant activity of different *Lupinus angustifolius* seeds. Additionally, the impact of different lupin as fermentation media for LAB biomass and D/L-lactic acid production was analysed.

Materials and methods

Materials and microorganisms

Seeds of narrow-leaved lupin (*Lupinus angustifolius* L.) var. 'Vilniai' and six new hybrid lines (Nos. 1700, 1701, 1703, 1072, 1734, 1800) were obtained from the Voke Branch of Lithuanian Institute of Agriculture (Voke, Lithuania) in 2015.

The LAB strain *Lactobacillus sakei* KTU05-6, previously isolated from spontaneous rye sourdoughs (Digaitiene et al. 2012), were cultured at 30 °C temperature for 48 h in MRS medium (CM0359, Oxoid, Hampshire, United Kingdom) prior to be used.

Fermentation of lupin material

Lupin seeds were ground in a laboratory mill and obtained wholemeal was mixed with a LAB suspension, containing 8.9 $\log_{10}$ colony-forming units (cfu) per mL, followed by a fermentation in an anaerobic chamber (LabXMedia Group, Midland, Canada). The water content of samples was calculated with a reference to the moisture content of raw material and the required humidity of the end product [45% moisture content for solid state fermentation (SSF) and 65% for submerged fermentation (SMF)] Fermentation was carried out at 30 °C for 48 h. Each sample was analysed in triplicate for acidity, D- and L-lactic acid content and bacteria count. Unfermented lupin sample was analysed as a control.

Determination of acidity parameters

The pH values were measured and recorded with a pH electrode (Sartorius, Gottingen, Germany). The total titratable acidity (TTA) was determined by homogenizing a 10 g sample with 90 mL of distilled water and expressed as milliliters of 1 mol L^{-1} NaOH solution used to neutralise organic acids in 100 g of sample using the phenolphthalein as the indicator. The L(+)/D(−)-lactic acid concentrations in lupin samples were determined by an enzyme test kit (R-biopharm AG-Roche, Darmstadt, Germany).

Microbiological analysis

Ten grams of sample were homogenised with 90 mL of 9 g L^{-1} NaCl solution in distilled water. Samples after serial dilutions of 10^{-4}–10^{-8} were sowed on sterile MRS agar (CM0361, Oxoid) on Petri plates. Samples were

incubated under anaerobic conditions at 30 °C for 72 h. The number of LAB colonies was calculated and expressed as a log10 cfu g^{-1} of sample. All analysis were carried out in triplicate.

Preparation of lupin extracts

Lupin wholemeal (10 g) was transferred to dark-coloured flasks and mixed with 200 mL of methanol and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvent. Combined supernatants were evaporated to dryness under vacuum at 40 °C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 ± 1 °C.

Determination of phenolic compounds

The total content of phenolic compounds (TPC) were determined by spectrophotometric method (Vaher et al. 2010). Methanolic solution of the sample extract in the concentration of 1 mg mL^{-1} was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic lupin extract, 2.5 mL of 10% Folin–Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank sample contained 0.5 mL methanol, 2.5 mL 10% Folin–Ciocalteu's reagent dissolved in a water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were incubated at 45 °C for 40 min. The absorbance was measured at 765 nm using UV–Vis spectrophotometer (Genesys 10, Thermo Fisher Scientific Inc., Germany). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The concentration of phenolics (mg mL^{-1}) was calculated from the calibration curve, and the content of phenolics in extracts was expressed as gallic acid equivalent (mg of GA g^{-1} of extract).

Antioxidant activity determination

The ability of the lupin extract to scavenge DPPH free radicals was assessed by the standard method (Zhu et al. 2011). The stock solutions of extracts (1 mg/mL) were prepared in methanol. This solution (1 mL) was added to 3 mL of each extract in methanol at different concentrations (125, 250, 500 and 1000 μg mL^{-1}). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Genesys 10, Thermo Fisher Scientific Inc., Germany) α-Tocopherol was used as the reference. The capability of scavenging the DPPH free radical was calculated by the following formula: DPPH scavenging effect (% inhibition) = $(A_0 - A_1) \times 100/A_0$ where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference.

Determination of the amino acids content

Amino acids (AA) contents in lupin samples were analysed by gas chromatography (GC) with flame ionization detection after the ion-exchange solid phase extraction and chloroformate derivatization using EZ-Fast technology (Phenomenex, Torrance, CA, USA). Standard solutions of the amino acids alanine (ALA), glycine (GLY), valine (VAL), leucine (LEU), isoleucine (ILE), threonine (THR), serine (SER), proline (PRO), asparagine (ASP), methionine (MET), glutamine (GLU), phenylalanine (PHE), lysine (LYS), histidine (HIS), and tyrosine (TYR) were analysed, in addition to the internal standard (NVAL). Samples (1.00 g) were weighed in 15 mL polypropylene test tubes with screw caps and mixed with 7.5 mL of 0.1 M HCl, and subjected to ultrasonification in a water bath (t = 40 °C) for 15 min. The mixture was shaked and then centrifuged (3000 *g*, 15 min). An 2.5 mL aliquot of the mixture was transferred into another 15 mL polypropylene screw cap test tube and 7.5 mL of deionized water was added to 10 mL volume. Samples were then stored at −80 °C until analysis. The derivatized AA were analysed using a GC-FID instrument (6890 N, Agilent Technologies Inc., USA) equipped with an auto-sampler (7683 Series, Agilent Technologies Inc., USA). Aliquots of the derivatized amino acids (2 μL) were injected (1:15 split ratio) at 250 °C into a Zebron column (ZB-AAA, 10 m × 0.25 mm). Five different standard solutions with different concentrations (from 50 to 200 nmol μL^{-1}) of AA standards were used for the calibration of gas chromatograph.

Statistical analysis

All analytical experiments were carried out in triplicate. In order to evaluate an influence of different factors (fermentation conditions and lupin variety) on acidity parameters, LAB count, AA profile, TPC content and antioxidant activity, data were subjected to analysis of variance (ANOVA) using statistical package SPSS for Windows (Ver.15.0, SPSS). The calculated mean values were compared using Tukey multiple range test with significance defined at $p \leq 0.05$.

Results and disscussion

Effect of lupin seeds fermentation

Acidity parameters and viable LAB counts in lupin samples after SmF and SSF are presented in Table 1. The pH

values in SmF and SSF lupin samples ranged from 3.67 to 3.95 and from 3.99 to 4.84. In all by SmF treated lupin samples pH was lower (8.5 to 9.3%) compared to SSF. The total titratable acidity (TTA) of fermented lupin ranged from 8.14 to 11.05 for SmF samples, and from 9.97 to 12.92 for SSF samples.

The viable cell number of tested LAB (in 48 h lupin fermentation) ranged from 9.0 to 9.9 log10 cfu g^{-1} in SmF samples and from 9.15 to 10.04 log10 cfu g^{-1} in SSF samples (Table 1). Because the growth of LAB in the fermentable substrate was limited by pH, the selection of substrates with strong buffer capacity allowed the production of products with high concentrations of organic acids and correspondingly high TTA values. In our study, there was not significant correlation obtained between the LAB cell count and pH, and between the LAB cells and TTA ($r = 0.0134$ and $r = 0.0117$, respectively) in the fermented lupin samples.

The TTA and pH are the most important parameters during fermentation processes (Yin et al. 2015). The LAB are a large group of closely related bacteria that have similar properties such as lactic acid production, which is an end product of the fermentation (Opere et al. 2012). Lactic acid produced by microbial fermentation usually is a mixture of L- and D-lactic acid. L-lactic acid was easily metabolized by humans, whereas D-lactic acid could be harmful when present in food in high concentrations (Reddy et al. 2008). According to Bartkiene et al. (2015), *Lactobacillus sakei* and *Pediococcus pentosaceus* strains 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). In our study, the D/L ratio in fermented lupin varied from 0.15 to 0.45 (SmF) and from 0.12 to 0.46 (SSF), thus the *L. sakei* used for lupin fermentation could be indicated as a L-lactic acid producing bacteria.

Results of ANOVA test indicated a significant effect of lupin variety on TTA, L-lactate content, and LAB cell count. Also, it was found a significant influence of fermentation conditions (SmF and SSF) on substrate pH, TTA, and L-lactic acid concentration in lupin.

Total phenolic compounds and antioxidant activity of fermented lupin

Table 2 presents the total phenolic compounds (TPC) contents and antioxidant activity of fermented lupin. The TPC in unfermented lupin samples ranged from 318.21 (No. 1072) to 557.82 mg 100 g^{-1} d.w. (No. 1800). Fermented lupin have significantly ($p < 0.05$) higher TPC content than the unfermented seeds. Nutritional analysis highlighted a

Table 1 pH, total titratable acidity (TTA) values, D- and L-lactic acid contents (g 100 g^{-1} d.w.), and LAB counts (log_{10} cfu g^{-1}) in *L. angustifolius* seeds after fermentation (48 h) with *L. sakei*

Samples	'Vilniai'	No. 1072	No. 1734	No. 1700	No. 1701	No. 1800	No. 1702
pH							
SmF	3.90 ± 0.03b	3.67 ± 0.04a	3.64 ± 0.01a	3.70 ± 0.02a	3.72 ± 0.02a	3.67 ± 0.01a	3.76 ± 0.02a
SSF	4.02 ± 0.03b	4.02 ± 0.02b	4.41 ± 0.03c	4.84 ± 0.03	4.20 ± 0.02b	4.37 ± 0.03bc	3.99 ± 0.02b
TTA							
SmF	8.75 ± 0.03b	8.89 ± 0.04b	8.14 ± 0.03a	11.05 ± 0.06d	9.82 ± 0.05c	11.04 ± 0.08d	9.52 ± 0.07c
SSF	12.34 ± 0.05e	11.18 ± 0.05d	9.97 ± 0.04c	12.62 ± 0.11ef	10.80 ± 0.08	11.20 ± 0.08d	11.13 ± 0.08d
L(+)-lactate							
SmF	7.11 ± 0.10c	6.87 ± 0.27c	7.04 ± 0.22c	8.01 ± 0.15e	6.20 ± 0.20a	7.87 ± 0.06e	6.88 ± 0.22c
SSF	7.52 ± 0.14d	7.61 ± 0.15d	7.88 ± 0.12e	9.01 ± 0.14g	6.41 ± 0.07b	8.27 ± 0.05f	7.67 ± 0.26d
D(−)-lactate							
SmF	1.14 ± 0.09a	3.12 ± 0.10 k	1.32 ± 0.19c	2.40 ± 0.14f	2.01 ± 0.22e	1.21 ± 0.18b	2.29 ± 0.12f
SSF	2.87 ± 0.15g	1.41 ± 0.18d	1.23 ± 0.18b	1.09 ± 0.15a	2.97 ± 0.26h	2.89 ± 0.11g	2.88 ± 0.09g
LAB count							
SmF	9.12 ± 0.04a	9.21 ± 0.03a	9.38 ± 0.04a	9.78 ± 0.02c	9.46 ± 0.03b	9.00 ± 0.03a	9.90 ± 0.02c
SSF	9.18 ± 0.03a	9.44 ± 0.02b	9.43 ± 0.04a	9.15 ± 0.03a	10.02 ± 0.04c	10.02 ± 0.02c	10.04 ± 0.02c

Data expressed as mean values (n = 3) ± SD

LAB lactic acid bacteria; *SmF* submerged fermentation; *SSF* solid state fermentation; *SD* standard deviation

[a–h] Values in the same row followed by different letters differ by Tukey test with significance defined at $p \leq 0.05$

relevant increase in TPC content from 13.8–24.5% (No. 1702, No. 1800, 'Vilniai' and No. 1700) to 39.0–65.2% (No. 1734, No. 1701 and No. 1072) in by SSF treated *L. angustifolius* seeds, compared to unfermented samples. ANOVA test indicated a significant effect of lupin variety and fermentation type (SmF and SSF) on TPC content in lupin seeds.

The results of analysis of antioxidant activity showed that unfermented lupin have antioxidant activity varying between 59.28 and 74.24% in *L. angustifolius* seeds. The increase in antioxidant activity after 48 h fermentation was noticed in all lupins ranging from 62.98 (No. 1072) to 84.12% (No. 1700) in SmF samples, and from 62.05 (No. 1800) to 88.18% ('Vilniai') in SSF samples. There was found a significant effect of lupin variety on the antioxidant activity, however the significant impact of fermentation type (SmF and SSF) on the antioxidant activity of lupin seeds was not observed. Furthermore, there was not a significant relation between the TPC and antioxidant activity of fermented lupin.

Fermented lupin showed higher antioxidant activities compared to unfermented lupin at the concentrations tested. In the literature has been reported that fermentation enhances micro nutrient bioavailability and aids in degrading antinutritional factors (Oboh and Rocha 2007). Fermentation also improved the phenolic content and antioxidant properties of legume seeds (Ademiluyi and Oboh 2011). Since lupin seeds have been found to be of high protein content, the breakdown of protein to free amino acids and peptides by microbial protease activity could also increase the antioxidant activity. Watanabe et al. (2007) after modification of the fermentation conditions observed that isoflavoneaglycones, free amino acids and peptides were responsible for the antioxidant activity of tempeh. Basically, the improvement in antioxidant activity occurs when microorganisms start breaking down the linkage of phenolic and flavonoid compounds, which free radicals actively play the role of antioxidants (Moktan et al. 2008; Ademiluyi and Oboh 2011).

Amino acids profile in fermented and nonfermented lupin

Tables 3 and 4 present the EAA and NEAA content of unfermented and fermented with *L. sakei* lupin, respectively. Among all EAA, the LEU was found to be of the highest content while MET (0.15–1.38% from total AA) was the one of lowest content in all lupins (Table 3). The results showed that *L. angustifolius* seeds contained the highest amounts of LEU (7.05–9.34% from TTA), LYS (3.63–7.66% from TAA), PHE (4.75–6.41% from TAA) depending on lupin variety (Table 3). Our results were similar to Iqbal et al. (2006) which reported that legumes were deficient in amino acids such as methionine, cystine and cysteine.

The use of the SmF as well as SSF significantly increased the EAA contents in *L. angustifolius* samples due to increased VAL [6.48–8.76% (SmF) and 4.79–6.83% (SSF) from TAA], LEU [7.50–9.67% (SmE) and 8.19–15.41% (SSF) from TAA], MET (0.94–2.08% (SmE) and 0.67–1.54% (SSF) from TAA), THR [4.36–6.39% (SmE) and 3.98–6.54% (SSF) from TAA] contents with the exeption of hybrid No. 1700.

The amounts of other EAA were found to vary among the lupin varieties. Statistical analysis showed a significant effect of lupin variety on different EAA contents.

Table 2 Total phenolic compounds (TPC) (mg 100 g^{-1} d.w.) and antioxidant activity (%) of *L. angustifolius* seeds before and after fermentation with *L. sakei*

Samples	'Vilniai'	No. 1072	No. 1734	No. 1701	No. 1700	No. 1800	No. 1702
TPC							
Control	500.38 ± 4.89d	318.21 ± 3.54a	466.31 ± 4.58d	408.87 ± 3.79c	419.29 ± 3.86c	557.82 ± 5.22f	552.47 ± 5.16f
SmF	510.61 ± 3.49d	382.69 ± 4.02b	513.05 ± 4.01d	469.69 ± 4.82d	401.82 ± 3.60bc	553.88 ± 5.20f	524.31 ± 5.03e
SSF	586.54 ± 5.32g	525.32 ± 5.16e	648.20 ± 6.15j	596.95 ± 5.34g	522.06 ± 4.93e	650.54 ± 5.00j	628.58 ± 4.77h
Antioxidant activity (DPPH)							
Control	59.28 ± 0.98a	61.04 ± ± 0.97a	68.51 ± 1.02c	64.54 ± 0.97b	70.27 ± 1.09d	74.24 ± 1.16d	72.21 ± 1.12c
SmF	65.65 ± 1.01b	62.98 ± 0.98ab	66.85 ± 1.04c	74.15 ± 1.16d	84.12 ± 1.36e	83.29 ± 1.34e	75.62 ± 1.14d
SSF	88.18 ± 1.41f	65.37 ± 0.52b	74.24 ± 0.97d	71.19 ± 1.08c	71.93 ± 1.10c	62.05 ± 0.88a	83.65 ± 1.31e

Data expressed as mean values (n = 3) ± SD

Control—unfermented lupin. *SmF* submerged fermentation; *SSF* solid state fermentation; *SD* standard deviation

[a–j] Values in the same row followed by different letters differ by Tukey test with significance defined at $p \leq 0.05$

Table 3 Essential amino acids (EAA) content in percentage from the total amino acids in *L. angustifolius* seeds before and after fermentation with *L. sakei*

Samples	VAL	ILE	LEU	THR	MET	PHE	LYS	HIS
Unfermented (controls)								
'Vilniai'	4.32 ± 0.05a	4.83 ± 0.04a	7.05 ± 0.04a	3.45 ± 0.04a	0.15 ± 0.01a	4.75 ± 0.03a	7.16 ± 0.09d	4.34 ± 0.05a
No. 1072	4.56 ± 0.05b	5.10 ± 0.05b	7.39 ± 0.04b	3.75 ± 0.03b	0.15 ± 0.01a	5.10 ± 0.05b	7.66 ± 0.09e	4.40 ± 0.05a
No. 1734	4.69 ± 0.06b	5.12 ± 0.03b	8.43 ± 0.06c	4.26 ± 0.04c	0.17 ± 0.02c	5.58 ± 0.05c	5.83 ± 0.06b	4.30 ± 0.04a
No. 1701	4.74 ± 0.04c	5.05 ± 0.05b	7.36 ± 0.04a	3.87 ± 0.03b	0.15 ± 0.01a	5.06 ± 0.05b	6.40 ± 0.06c	4.47 ± 0.05a
No. 1700	6.07 ± 0.08d	6.04 ± 0.07c	9.34 ± 0.07a	4.36 ± 0.05c	1.38 ± 0.02d	6.41 ± 0.06d	3.63 ± 0.04a	9.27 ± 0.05d
No. 1800	4.50 ± 0.04a	4.89 ± 0.04a	7.32 ± 0.05a	3.76 ± 0.03b	0.17 ± 0.01c	4.85 ± 0.05a	6.51 ± 0.07c	4.37 ± 0.04a
No. 1702	4.54 ± 0.04b	5.09 ± 0.05b	7.47 ± 0.05b	3.69 ± 0.03b	0.15 ± 0.01a	5.17 ± 0.06b	6.47 ± 0.07c	4.78 ± 0.05b
Fermented (SmF)								
'Vilniai'	6.50 ± 0.04c	4.39 ± 0.04a	8.11 ± 0.05c	6.33 ± 0.02d	2.08 ± 0.0e	4.62 ± 0.05a	7.95 ± 0.08g	4.18 ± 0.04d
No. 1072	6.48 ± 0.04c	4.53 ± 0.04a	7.50 ± 0.05b	6.14 ± 0.02d	1.58 ± 0.03c	4.77 ± 0.04b	6.39 ± 0.07f	3.97 ± 0.03d
No. 1734	6.75 ± 0.05d	4.64 ± 0.04b	7.52 ± 0.05b	6.39 ± 0.04d	1.76 ± 0.01d	4.53 ± 0.04a	5.24 ± 0.05d	3.69 ± 0.03c
No. 1701	8.76 ± 0.06f	5.69 ± 0.06e	8.64 ± 0.06d	4.15 ± 0.02b	1.43 ± 0.02b	5.63 ± 0.06d	4.90 ± 0.05c	4.54 ± 0.04e
No. 1700	5.22 ± 0.05a	9.34 ± 0.08g	6.04 ± 0.06a	4.36 ± 0.04c	0.94 ± 0.01a	6.41 ± 0.06e	3.63 ± 0.03a	4.84 ± 0.04f
No. 1800	7.68 ± 0.05e	6.43 ± 0.07f	9.67 ± 0.08e	3.77 ± 0.04a	1.97 ± 0.02e	6.59 ± 0.07e	5.73 ± 0.06e	5.73 ± 0.06g
No. 1702	8.42 ± 0.05f	5.33 ± 0.06d	8.79 ± 0.06d	4.30 ± 0.04b	1.62 ± 0.04c	5.32 ± 0.05c	4.20 ± 0.04b	2.87 ± 0.02a
Fermented (SSF)								
'Vilniai'	5.32 ± 0.03d	4.65 ± 0.04a	9.42 ± 0.08c	6.54 ± 0.04f	0.81 ± 0.05b	5.92 ± 0.06b	6.10 ± 0.04e	5.75 ± 0.07d
No. 1072	4.83 ± 0.05b	5.39 ± 0.05b	9.32 ± 0.08b	5.83 ± 0.05e	0.90 ± 0.04c	6.55 ± 0.07d	4.29 ± 0.04b	6.36 ± 0.07e
No. 1734	4.79 ± 0.05b	5.28 ± 0.05b	8.30 ± 0.07a	4.23 ± 0.04b	0.84 ± 0.04b	5.31 ± 0.05a	3.99 ± 0.03a	4.90 ± 0.04c
No. 1701	5.08 ± 0.05c	6.15 ± 0.07d	15.41 ± 0.06e	3.98 ± 0.03a	0.89 ± 0.03c	6.92 ± 0.07e	4.31 ± 0.04b	4.75 ± 0.04c
No. 1700	6.83 ± 0.07e	5.74 ± 0.06c	11.78 ± 0.08d	5.50 ± 0.04d	1.54 ± 0.04e	5.81 ± 0.06b	5.31 ± 0.04d	3.36 ± 0.02a
No. 1800	5.01 ± 0.05c	5.66 ± 0.06c	8.92 ± 0.07b	5.51 ± 0.04d	0.67 ± 0.04a	6.07 ± 0.06c	4.30 ± 0.03b	3.44 ± 0.03a
No. 1702	5.39 ± 0.05d	5.03 ± 0.04a	8.19 ± 0.06a	5.28 ± 0.05c	1.07 ± 0.05d	5.83 ± 0.06b	4.76 ± 0.04c	3.34 ± 0.03a

Data expressed as mean values (n = 3) ± SD

SmF submerged fermentation; *SSF* solid state fermentation; *SD* standard deviation

[a–g] Values in the same column followed by different letters differ by Tukey test with significance defined at $p \leq 0.05$

Furthermore, data analysis showed a significant effect of fermentation conditions on EAA content in lupin. Either, the significant interaction of these factors (seed variety and fermentation conditions) was obtained on total EAA content in lupin. The hydrolytic breakdown of the nutrient components during fermentation may have caused the increase in AA content (Ferial and Esmat 2011).

The amount of remaining non-essential amino acids (NEAA) varied depending on lupin variety. The amino acids such as ALA, followed by GLY and PRO was found to be in lowest contents (Table 4). The results indicate that in most cases (aprox. 50% of samples) the SSF reduces the NEAA contents (Table 4), while the SmF conditions has lower impact on changes in the NEAA profile. The reduction in NEAA content on average by 6.6% was observed in *L. angustifolius* var. 'Vilniai'and hybrids No. 1072, 1734 and 1701 after SSF, and by 6.1% in hybrids Nos. 1701, 1700 and 1800 after the SmF due to lower contents of TYR (from 3.58–10.61 to 1.97–7.5%) and GLU (24.86–27.80 to 18.57–30.43%) compared to unfermented lupin.

The SmF as well as SSF with *L. sakei* strain increased the ALA content in all lupin samples (from 2.92–3.99 to 3.37–4.79%) with the exception of lupin No. 1734. Also, in fermented lupin samples higher contents of GLY (from 3.79–4.59 to 3.98–5.75%), SER (5.02–6.11 to 5.53–7.68%), PRO (4.35–5.08 to 4.56–6.76%), and ASP (10.39–12.16 to 10.39–13.60%) were determined compared to controls.

Results of ANOVA indicated a significant effect of lupin variety on NEAA content ($p \leq 0.05$), with the exception of GLY, SER and TYR. Fermentation type had a significant influence on all NEAA contents ($p < 0.0001$), and statistically significant interaction of this factors on the NEAA in lupin was observed. The NEAA should be taken into consideration in revising the ideal protein concept and formulating balanced diets to

Table 4 Non-essential amino acids (NEAA) content in percentage from the total amino acids in *L. angustifolius* seeds before and after fermentation with *L. sakei*

Samples	ALA	GLY	SER	PRO	ASP	GLU	TYR
Unfermented (controls)							
'Vilniai'	3.14 ± 0.04a	3.83 ± 0.04a	5.02 ± 0.04a	4.35 ± 0.04a	10.43 ± 0.10a	26.58 ± 0.16b	10.61 ± 0.10e
No. 1072	3.37 ± 0.03c	4.06 ± 0.04b	5.53 ± 0.06c	4.67 ± 0.05b	11.34 ± 0.12b	27.34 ± 0.15c	5.57 ± 0.04b
No. 1734	3.99 ± 0.04e	4.59 ± 0.05d	6.11 ± 0.07d	4.70 ± 0.06b	10.39 ± 0.11a	26.20 ± 0.17b	5.64 ± 0.06b
No. 1701	3.26 ± 0.03b	4.19 ± 0.04b	5.72 ± 0.05c	4.72 ± 0.04b	11.77 ± 0.12c	24.86 ± 0.16a	8.40 ± 0.07d
No. 1700	3.64 ± 0.02d	4.38 ± 0.05c	5.90 ± 0.06d	5.08 ± 0.05c	12.16 ± 0.13d	26.25 ± 0.18b	3.58 ± 0.02a
No. 1800	3.27 ± 0.03b	3.99 ± 0.04b	5.63 ± 0.06c	4.53 ± 0.05b	11.31 ± 0.11b	27.80 ± 0.17c	7.00 ± 0.06c
No. 1702	3.22 ± 0.04b	4.05 ± 0.05b	5.38 ± 0.04b	4.69 ± 0.04b	11.04 ± 0.12a	27.54 ± 0.19c	6.74 ± 0.05c
Fermented (SmF)							
'Vilniai'	3.58 ± 0.03a	3.98 ± 0.03a	7.29 ± 0.07f	4.56 ± 0.04a	11.37 ± 0.12b	30.43 ± 0.18e	2.32 ± 0.02a
No. 1072	3.37 ± 0.03a	4.06 ± 0.04a	5.53 ± 0.05a	4.67 ± 0.05a	11.34 ± 0.10b	27.34 ± 0.16d	5.57 ± 0.05e
No. 1734	3.99 ± 0.04b	4.59 ± 0.05c	6.11 ± 0.06b	4.70 ± 0.04a	10.39 ± 0.11a	26.20 ± 0.15d	5.64 ± 0.05e
No. 1701	4.79 ± 0.05d	5.36 ± 0.06e	6.96 ± 0.07e	5.56 ± 0.06d	12.61 ± 0.13c	21.40 ± 0.13b	3.97 ± 0.03b
No. 1700	4.18 ± 0.04c	5.27 ± 0.05e	6.65 ± 0.06d	6.43 ± 0.07f	12.95 ± 0.14c	19.85 ± 0.17a	3.88 ± 0.04b
No. 1800	3.80 ± 0.04b	4.63 ± 0.05c	6.32 ± 0.06c	5.95 ± 0.07e	11.45 ± 0.11b	19.36 ± 0.16a	4.71 ± 0.04c
No. 1702	4.63 ± 0.05d	4.87 ± 0.04d	6.55 ± 0.06d	5.37 ± 0.06c	12.86 ± 0.12c	23.76 ± 0.18c	5.23 ± 0.05d
Fermented (SSF)							
'Vilniai'	3.91 ± 0.02b	5.39 ± 0.05c	7.23 ± 0.06b	5.85 ± 0.05a	12.12 ± 0.11a	23.38 ± 0.14d	1.97 ± 0.01a
No. 1072	3.88 ± 0.03b	5.75 ± 0.06d	7.12 ± 0.05b	6.76 ± 0.06c	13.33 ± 0.13b	18.57 ± 0.17a	2.37 ± 0.02c
No. 1734	3.82 ± 0.04b	5.09 ± 0.05a	7.67 ± 0.07c	5.78 ± 0.05a	13.04 ± 0.12b	25.43 ± 0.18e	2.11 ± 0.01b
No. 1701	3.40 ± 0.02a	5.07 ± 0.04a	6.18 ± 0.06a	6.67 ± 0.07c	12.89 ± 0.11b	20.53 ± 0.19b	3.87 ± 0.03d
No. 1700	4.25 ± 0.04c	4.84 ± 0.03a	7.27 ± 0.07b	5.78 ± 0.05a	13.60 ± 0.13c	19.28 ± 0.17a	6.50 ± 0.06f
No. 1800	3.79 ± 0.03b	4.91 ± 0.04a	6.40 ± 0.05a	6.15 ± 0.05b	13.53 ± 0.13c	21.91 ± 0.15c	4.93 ± 0.04e
No. 1702	4.27 ± 0.04c	5.16 ± 0.05b	7.68 ± 0.06c	6.24 ± 0.06b	12.43 ± 0.11a	19.23 ± 0.14a	7.50 ± 0.05g

Data expressed as mean values (n = 3) ± SD

SmF submerged fermentation; *SSF* solid state fermentation; *SD* standard deviation

[a–g] Values in the same column followed by different letters differ by Tukey test with significance defined at $p \leq 0.05$

improve protein accretion, food nutritional value, and human health (Wu et al. 2013).

Conclusion

The present study represent amino acids profile and antioxidant activity of seeds of *L. angustifolius* a wild plant of Baltic Sea region. The results demonstrated that fermentation caused a marked increase in total phenolic contents and antioxidant activity of lupin which enhanced DPPH radical-scavenging ability. Fermentation for 48 h was applicable as exemplified by the DPPH radical-scavenging ability of the lupin seeds. The interaction of the lupin variety and fermentation conditions had a significant effect on the EAA and NEAA contents. Considering total phenolics, DPPH radical-scavenging ability and improved EAA profile, fermented lupin showed greater nutritional quality compared to unfermented.

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