DESIGN OF LUPIN SEEDS LACTIC ACID FERMENTATION – CHANGES OF DIGESTIBILITY, AMINO ACID PROFILE AND ANTIOXIDANT ACTIVITY

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Abstract. Lupin seeds contain significant amounts of protein, fat, minerals and dietary fibre. The importance of lupin as a valuable source of nutrients to be used in food and feed production has increased in recent years. However, the use of legumes as a source of protein is somewhat limited because of low digestibility of most plant proteins. The digestibility of lupin protein could be improved by using lactic acid fermentation.

The aim of this study was to evaluate the influence of solid state fermentation (SSF) with *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8 strains on *in vitro* protein digestibility, changes of total amino acids (TAA) profile, total phenolic compounds (TPC) content, and antioxidant activity of *Lupinus luteus* L. and *Lupinus albus* L. lupin seeds.

Lupin variety and lactic acid bacteria (LAB) used for fermentation have a significant influence on acidity parameters, digestibility, amino acid profile, total phenolic compounds content and antioxidant activity of lupin wholemeal. Optimisation of lupin fermentation conditions could increase the possibility to produce new higher value food/feed products, which are of great interest for the design of functional foods/feeds and nutraceuticals.

Keywords: Lupin, fermentation, lactobacilli, biogenic amine, amino acids

Introduction

Legume grains, such as beans, peas and lupins, are valuable feedstuffs because of relatively high energy and protein contents and an attractive protein quality. However, they also contain anti-nutritional substances, such as alkaloids, oligosaccharides (e.g. raffinose, stachyose and verbascose) and tannins, which may reduce feed intake and digestibility of individual nutrients (Gefrom et al., 2013; Day, 2013; Bora, 2014; Stanek et al., 2015). Due to the quantities of alkaloids, oligosaccharides and tannins, legume grains can only be used in limited amounts in the animals' diet (Gefrom et al., 2013). The use of legumes depends on the target animal species and its age. In diets for pigs and hens, the recommended percentage varies between >5–25 and 10–30%, respectively (Martens et al., 2013; Zdunczyk et al., 2014; Messad et al., 2015). Various studies have demonstrated a reduction in several anti-nutritional factors and increase in the nutritive value of legumes during fermentation (Stahl, 2014).

The importance of lupin as a valuable source of nutrients to be used in food/feed production has increased in recent years. Lupin seeds contain significant amounts of protein, fat, minerals and dietary fibre (Bartkienė et al., 2016). However, the use of legumes as a source of protein is somewhat limited because of low digestibility of most plant proteins. The reduction of protein digestibility in lupin seeds has been associated with the presence of protease inhibitors (Palliyeguru et al., 2011), as well as anti-nutritionals, such as fibres and oligosaccharides (Glencross, 2009). Regarding protein 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 (Shekib, 1994). A further benefit of fermentation with LAB is that many species have been referred to the European Food Safety Authority (EFSA) for safety assessment without raising safety concerns. As a result, they have been included in the QPS (Qualified Presumption of Safety) list for authorised use in the food and feed chain within the European Union (EFSA, 2012). The same applies to the United States of America, where they enjoy the Generally Regarded as Safe (GRAS) status assigned by the U.S. Food and Drug Administration.

Legume crops represent the major food/feed sources for humans and livestock worldwide; they possess limiting levels of some of these essential amino acids, particularly lysine and methionine (Galili and Amir, 2013). In addition to their favourable nutritional profile, legumes also contain a range of bioactive compounds, such as phenolic compounds and phytosterols, which may protect against chronic diseases, including cancer and cardiovascular disease (Rumiyati et al., 2013). Phenolic compounds have been widely studied as antioxidants due to their ability in quenching free radicals contributing to total antioxidant capacity and their protection role against highly prevalent diseases (Dueñas et al., 2009). Lupin would be a good alternative source of protein, enabling affordable nutritional enrichment of food/feed and

providing better access to protein for underserved populations (Monteiro et al., 2014).

The digestibility of lupin protein could be improved by using lactic acid fermentation. Structural modification occurring during technological processing drives novel strategies aimed at the improvement of higher value food/feed potential of protein-rich plant foods (Carbonaro et al., 2015).

The aim of this study was to evaluate the influence of solid state fermentation (SSF) with *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8 strains on *in vitro* protein digestibility, changes of total amino acids (TAA) profile, total phenolic compounds (TPC) content, and antioxidant activity of *Lupinus luteus* L. and *Lupinus albus* L. lupin seeds.

Material and methods

Lupin seeds and lactic acid bacteria

The lupin seeds *Lupinus luteus* L. and *Lupinus albus* L. with low alkaloid content (<0.01%) were obtained from the Lithuanian Institute of Agriculture (Vokė, Lithuania) after harvest of 2014. The bacteriocin-like inhibitory substances (BLIS) producing *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8 strains previously isolated from spontaneous rye sourdough (Digaitienė et al., 2005) were cultured at 25–35°C for 48 h in MRS broth (CM0359, Oxoid Ltd, Hampshire, UK) prior to be used.

Preparation of fermented lupin products

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

Determination of acidity parameters

The pH value of lupin products was measured and recorded by a pH electrode. Total titratable acidity (TTA) was determined on 10 g of the sample homogenized with 90 mL of distilled water, and expressed as the amount (mL) of 0.1 M NaOH to get pH 8.2.

Determination of in vitro protein digestibility

Determination of protein digestibility was carried out according to Lqari et al. (2002). The samples containing 62.5 mg of protein were suspended in 10 mL of water, and the pH was adjusted to 8 with 0.1 mol L^{-1} NaOH. An enzymatic solution containing 1.6 mg of trypsin (18 U mg^{-1}), 3.1 mg of α-chymotrypsin (40 U mg^{-1}) and 1.3 mg of protease (15 U mg^{-1}) per millilitre was added to the protein suspension in a 1:10 (v/v) ratio. The pH of the mixture was measured exactly after 10 minutes and the *in vitro* digestibility was calculated as a percentage of digestible protein (DP) using the equation DP = 210.464 – 18.103 × pH (Lqari et al., 2002).

Determination of free amino acids (FAA)

Free amino acids (FAA) were extracted using 0.1 M HCl. The extracts were processed by ion-exchange solid phase extraction and chloroformate derivatisation using EZ:faast® technology (Phenomenex) and then analysed by gas chromatography with flame ionisation detection. 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). All eluting and derivatisation agents were provided in an inclusive kit (EZ-Faast amino acid analysis kit for protein hydrolysates by GC-FID or GC-NPD). Hydrochloric acid (25%) and thioglycolic acid were purchased from Sigma-Aldrich (Cat. No: T3758). The samples were milled using Cross Beater Mill Pulverisette 16 (Idar-Oberstein, Germany), weighed (1.00 g) in 15 mL polypropylene test tubes with screw caps, mixed with 7.5 mL of 0.1 M HCl, and subjected to sonication in a water bath (t = 40°C) for 15 minutes. The mixture was shaken and then centrifuged (3000 rpm, 15 min). And 2.5 mL aliquot of the mixture was transferred into another 15 mL polypropylene screw cap test tube and 7.5 mL of deionised water was added to 10 mL volume. The samples were then stored at -80°C until analysis.

The derivatised amino acids were analysed using a GC-FID instrument (Agilent; 6890N) equipped with an auto-sampler (Agilent; 7683 Series). Aliquots of the derivatised amino acids (2 µL) were injected using a 1:15 split ratio at 250°C into a Zebron column (ZB-AAA, 10 m, 0.25 mm in diameter) programmed from 110−320°C at 32°C/min. Helium was used as the carrier gas at a constant 1.5 mL/min flow, and nitrogen was used as the make-up gas. The detector temperature was 320°C. Five standard solutions with different concentrations (from 50 to 200 nmol/µL) of amino acid standards were used for the calibration of gas chromatograph.

Determination of total content of phenolic compounds (TPC) and antioxidant activity of lupin samples

The total content of phenolic compounds (TPC) in the fermented lupin samples was determined by the spectrophotometric method, as reported elsewhere (Vaher et al., 2010). The absorbance of the samples was measured at 765 nm using spectrophotometer J.P. SELECTA S.A. V-1100D (Barcelona, Spain). Antioxidant activity of the lupin samples was evaluated according to the method reported by Zhu et al. (2011).

Statistical analysis

All analytical determinations were performed at least in triplicate. The data obtained were analysed using statistical package SPSS for Windows XP V15.0 (SPSS Inc. Chicago, IL, USA, 2007). Significance of differences between the treated samples was evaluated using the Duncan multiple range tests. The confidence interval was 95% ($P < 0.05$). In order to evaluate the influence of different factors (different lupin variety and application of several microorganisms) and their interaction on the parameters of the fermented lupin wholemeal, the statistical analysis was performed using the one-way

analysis of variance (ANOVA) and the Tukey HSD test as post-hoc test (statistical program R 3.2.1 (R Core Team 2015)).

Results

Acidity parameters of lupin seeds

The results pertaining to the effects of a single LAB strain on the pH and total titratable acidity (TTA) during SSF of lupin wholemeal are presented in Table 1. pH values measured in SSF lupin wholemeal varied from 4.10 ± 0.01 to 4.24 ± 0.01 (*L. albus* fermented with *P. pentosaceus* and *L. luteus* fermented with *L. sakei*, respectively). The lowest TTA was observed in *L. luteus* lupin seeds fermented with *P. acidilactici* (20.05 ± 1.27 ºN), and the highest TTA in *L. albus* fermented with *P. pentosaceus* (23.71 ± 1.17 ºN). A significant negative moderate correlation between pH and TTA in lupin seeds was determined (R = -0.565, P = 0.015).

Table 1. **pH and total titratable acidity (TTA) of the fermented lupin (*L. luteus* and *L. albus*)**

Lupin products	pH	TTA, ºN
Fermented with *P. acidilactici*		
L. luteus	4.21±0.01[a]	20.05±1.27[b]
L. albus	4.15±0.01[a]	21.29±0.83[c]
Fermented with *L. sakei*		
L. luteus	4.24±0.01[a]	21.00±1.08[c]
L. albus	4.19±0.01[a]	22.13±1.17[d]
Fermented with *P. pentosaceus*		
L. luteus	4.13±0.01[a]	23.42±1.00[e]
L. albus	4.10±0.01[a]	23.71±1.17[e]
Data are the mean ± SD (n = 3); SD – standard deviation. Means within a column with different letters are significantly different ($P \leq 0.05$).		

In vitro protein digestibility of lupin seeds

The *in vitro* protein digestibility of lupin wholemeal is presented in Table 2. In all the cases, fermentation increased digestibility of lupin seeds. In comparison with *L. luteus* wholemeal, the highest digestibility was found in the sample fermented with *P. pentosaceus* (86.25 ± 1.25%). The same tendencies were found in *L. albus* samples (the highest digestibility was found in the samples fermented with *P. pentosaceus* strain, 87.53 ± 1.44%). In comparison with the non-fermented samples, digestibility increased 17.12%, 16.73% and 18.87% in *L. luteus* and 12.15%, 14.71% and 17.68% in *L. albus* fermented with *P. acidilactici*, *L. sakei* and *P. pentosaceus*, respectively.

Table 2. ***In vitro* protein digestibility (%) of the untreated and fermented lupin (*L. luteus* and *L. albus*) with lactic acid bacteria (*P. acidilactici*, *L. sakei*, *P. pentosaceus*)**

Samples	In vitro protein digestibility (%)	
	L. luteus	*L. albus*
Non-fermented	72.56±1.16[a]	74.38±1.87[a]
Fermented with *P. acidilactici*	84.98±1.13[c]	83.42±0.94[c]
Fermented with *L. sakei*	84.70±0.87[c]	85.32±1.10[c]
Fermented with *P. pentosaceus*	86.25±1.25[c]	87.53±1.44[c]
Data are the mean ± SD (n = 3); SD – standard deviation. Means within a column with different letters are significantly different ($P \leq 0.05$).		

Essential and non-essential amino acid profile of fermented and non-fermented lupin seeds

Essential free amino acids (EFAA) content (%) from extracted amino acids in lupin wholemeal is presented in Table 3. The comparison of valine content in fermented and non-fermented samples demonstrated that in most fermented samples valine increased (except in *L. luteus* fermented with *P. acidilactici*). Different tendencies were found for leucine content: an increase was observed in all the fermented *L. luteus* samples, and a decrease in all the fermented *L. albus* samples.

Isoleucine increased in 2 samples of 6 (in *L. luteus* fermented with *P. acidilactici* and in *L. luteus* fermented with *P. pentosaceus*), and threonine increased in all the samples, except in *L. luteus* fermented with *P. acidilactici*. Methionine increased in all the fermented samples. Lysine increased in all the fermented *L. luteus* samples; however, in all *L. albus* samples, lysine decreased. The same tendencies were found for histidine, which increased in all the fermented *L. luteus* samples, but decreased in all the fermented *L. albus* samples.

Non-essential free amino acids (EFAA) content (%) from extracted amino acids in lupin seeds is presented in Table 4. Alanine, glycine, serine, proline and asparagine content increased in all the fermented lupin wholemeal samples. Glutamine increased in all the fermented *L. luteus* and decreased in all *L. albus* samples. Tyrosine

increased in *L. luteus* fermented with *P. acidilactici* and was found to decrease in other analysed samples.

Table 3. **Essential free amino acids (FAA) content (%) in the extracted amino acids from SSF lupin**

Samples	Essential free amino acids (FAA) content (%) among the extracted amino acids							
	Val	Leu	Ile	Thr	Met	Phe	Lys	His
	Untreated							
L. luteus	4.35±0.03	2.93±0.02	2.93±0.02	3.79±0.02	0.35±0.03	3.79±0.02	4.35±0.03	2.93±0.02
L. albus	4.32±0.03	7.05±0.03	4.83±0.04	3.45±0.02	0.15±0.01	4.75±0.03	7.16±0.06	4.34±0.04
Fermented with *P. acidilactici*								
L. luteus	4.21±0.04	9.45±0.09	5.08±0.08	3.20±0.03	0.78±0.01	5.72±0.05	7.61±0.08	4.96±0.05
L. albus	4.73±0.04	3.90±0.04	2.44±0.02	6.92±0.08	0.83±0.02	1.11±0.03	5.98±0.07	3.13±0.02
Fermented with *L. sakei*								
L. luteus	5.23±0.06	3.99±0.05	2.81±0.02	6.54±0.08	0.56±0.01	0.88±0.02	6.10±0.05	3.72±0.03
L. albus	5.23±0.06	3.99±0.05	2.81±0.02	6.54±0.08	0.56±0.01	0.88±0.02	6.10±0.05	3.72±0.03
Fermented with *P. pentosaceus*								
L. luteus	4.81±0.05	8.39±0.08	5.53±0.06	4.23±0.04	0.78±0.01	5.43±0.05	6.25±0.06	3.94±0.04
L. albus	4.86±0.04	4.90±0.05	2.59±0.03	6.31±0.07	0.87±0.01	2.43±0.02	3.02±0.02	3.23±0.02

Data expressed as mean values (n = 3) ± SD; SD – standard deviation; Pa – *Pediococcus acidilactici*; Pp – *Pediococcus pentosaceus*; Ls – *Lactobacillus sakei*; Val – valine; Leu – leucine; Ile – isoleucine; Thr – threonine; Met – methionine; Phe – phenylalanine; Lys – lysine; His – histidine.

Table 4. **Non-essential free amino acids (FAA) content (%) in the extracted amino acids from SSF lupin**

Samples	Non-essential free amino acids (FAA) content (%) among the extracted amino acids						
	Ala	Gly	Ser	Pro	Asp	Glu	Tyr
	Untreated						
L. luteus	2.93±0.02	3.79±0.02	4.35±0.02	2.93±0.02	3.79±0.02	20.93±0.02	3.79±0.03
L. albus	3.14±0.03	3.83±0.03	5.02±0.05	4.35±0.05	4.43±0.11	26.58±0.15	10.61±0.10
Fermented with *P. acidilactici*							
L. luteus	3.15±0.02	3.72±0.02	5.92±0.05	4.61±0.04	10.06±0.12	27.56±0.18	4.24±0.04
L. albus	7.85±0.05	6.06±0.05	21.98±0.12	6.05±0.05	5.88±0.04	21.84±0.13	1.32±0.01
Fermented with *L. sakei*							
L. luteus	3.51±0.03	4.46±0.03	7.04±0.08	5.03±0.05	12.86±0.13	27.04±0.14	2.77±0.02
L. albus	8.71±0.06	6.06±0.05	25.75±0.18	7.08±0.10	7.38±0.10	12.57±0.11	2.63±0.02
Fermented with *P. pentosaceus*							
L. luteus	3.48±0.03	4.55±0.04	6.84±0.06	5.33±0.04	12.31±0.08	24.69±0.12	3.45±0.03
L. albus	7.59±0.07	5.53±0.05	26.15±0.11	7.03±0.05	6.67±0.06	11.41±0.09	7.40±0.06

Data expressed as mean values (n = 3) ± SD; SD – standard deviation; Pa – *Pediococcus acidilactici*; Pp – *Pediococcus pentosaceus*; Ls – *Lactobacillus sakei*; Ala – alanine; Gly – glycine; Ser – serine; Pro – proline; Asp – asparagine; Glu – glutamine; Tyr – tyrosine.

Total phenolic compounds (TPC) content and antioxidant activity of lupin seeds

Total phenolic compounds content and antioxidant activity of SSF lupin samples are presented in Table 5. In all the cases, fermentation increased the TPC content. In comparison with non-fermented samples, in the samples fermented with *P. acidilactici*, *L. sakei* and *P. pentosaceus*, *L. luteus* wholemeal TPC increased by 8.1, 5.7 and 6.7 mg/100 g d.m., and *L. albus* wholemeal TPC by 12.1, 7.9 and 9.1 mg/100 g d.m., respectively.

Besides, fermentation increased free radical (DPPH) scavenging activity (%) of the tested samples. In comparison with the non-fermented samples, in *L. luteus* wholemeal fermented with *P. acidilactici*, *L. sakei* and *P. pentosaceus,* free radical scavenging activity increased by 36.7%, 32.1% and 41.8%, and in *L. albus* wholemeal by 13.6%, 8.2% and 19.7%, respectively. A strong significant correlation between total phenolic compounds content and antioxidant activity of SSF lupin samples was found (R = 0.857, P = 0.0001).

Discussion

Lactic acid bacteria (LAB) are generally fastidious on artificial media, but they grow readily in most plant substrates and lower pH rapidly to a point where other competing organisms are no longer able to grow. *Leuconostocs* and lactic *streptococci* generally lower pH to about 4.0–4.5 and some of the *lactobacilli* and *pediococci* to about 3.5 before inhibiting their own growth (Steinkraus, 1983). Lupin variety was found to have a significant influence on the pH of the fermented samples (P = 0.044); therefore, the influence of lupin variety on TTA was not significant (P = 0.264). LAB

used in the experiment, had a significant influence on both analysed parameters (pH P = 0.0001, TTA P = 0.002). Interaction of these factors (different lupin variety and application of several microorganisms) had a significant influence on pH and TTA of lupin wholemeal (P = 0.0001).

Table 5. **Total phenolic compounds content and antioxidant activity of solid state fermented (SSF) lupin samples**

	L. luteus	*L. albus*
Total phenolic compound content, mg/100 g d.m.		
Control	524.61±3.29	487.27±3.29
Fermented with *P. acidilactici*	567.10±4.13	546.32±4.09
Fermented with *L. sakei*	554.23±2.09	525.89±2.85
Fermented with *P. pentosaceus*	559.68±3.84	531.63±1.96
Free radical (DPPH) scavenging activity, %		
Control	59.47±1.14	52.84±1.18
Fermented with *P. acidilactici*	81.31±1.48	60.01±1.00
Fermented with *L. sakei*	78.57±1.03	57.15±1.12
Fermented with *P. pentosaceus*	84.33±1.73	63.24±1.52

SSF – solid state fermentation; Control – non-fermented lupin samples.
Data expressed as mean values (n = 3) ± SD; SD – standard deviation.

Nutritive value of lupin proteins is comparable with that of soy proteins widely used for nutritional purposes. Among all legumes, seeds of low-alkaloid lupins contain a very limited amount of anti-nutritional substances (Gorecka et. al., 2000). Therefore, 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 for their low nutritional value compared with animal proteins (Carbonaro et al., 2012). Different technological methods for improvement of nutritional value of legumes are used: thermal treatment (coking, extrusion), germination, fermentation, etc. (Kohajdová et al., 2011). We found that lupin variety (P = 0.002) and LAB used in the experiment (P = 0.0001) had a significant influence on its digestibility, but the interaction of these factors was not significant for digestibility (P = 0.300).

Seed legumes are strategically important not only because they decrease the marked deficit of high-protein feedstuff but also because they increase the sustainability of crop–livestock systems through the safeguarding of soil fertility, the reduction of greenhouse gas emission and the reduction of nitrogen fertiliser use. Recently, *Leguminosae* seeds have been considered as an alternative protein source to soybean meal in animal feeding owing to the controversy related to the use of genetically modified organisms (GMOs). Among legumes, lupin appears an interesting and promising crop since it represents a resource for agriculture in human and animal nutrition as well as a solution for both challenges. In fact, this plant has some traits that make it a valuable alternative crop: it has a winter cycle, a high grain productivity for food and feed destination, a limited phosphorus requirement compared with other crops, a high content of protein deriving from nitrogen fixed from the atmosphere compared with other winter legumes, and it is also an excellent rotation crop able to enrich soil with nitrogen. Lupin seeds are a valuable nitrogen and energy source owing to their high content of crude protein (300–500 g/kg) and oil (50–100 g/kg), which vary as a function of species and variety (Calabrò et al., 2015). Lupin protein has a relatively good amino acid profile with high content of arginine (4.1–11.2%), leucine (7.5–9.4%), lysine (4.3–5.2%), and phenylalanine (3.0–6.8%) (Bähr et al., 2015). Adequate provision of dietary amino acids (AA) is essential for health, growth, development and survival of animals and humans (Ren et al. 2012; Wu, 2009). Based on growth or nitrogen balance, AA have traditionally been classified as nutritionally essential (indispensable) or non-essential (dispensable) for mammals, birds and fish (Le Ple'nier et al., 2012; Liu et al., 2012; Obayashi et al., 2012). Although both animal and plant-based proteins can provide the required essential amino acids for health, animal proteins generally contain a higher proportion of leucine. This amino acid plays a key role in stimulating translation initiation and muscle protein anabolism and is the focus of ongoing research (Paddon-Jones et al., 2015). Based on new research findings, NEAA should be taken into consideration in revising the classical 'ideal protein' concept and formulating balanced diets to improve protein accretion, food efficiency, and health in animals and humans (Wu, 2013). We find that lupin variety and LAB used for fermentation have a significant influence on all AA content (P = 0.0001) (except valine). Besides, the interaction of these factors (different lupin variety and application of several microorganisms) has a significant influence on AA profile in lupin wholemeal (P = 0.0001), except valine.

Phenolic compounds are secondary metabolites essential for growth and reproduction of plants and act as protective agents against pathogens, being secreted as a defence mechanism during stress conditions, such as infections and UV radiation, among others (Wink, 2013). These hydrophilic phytochemicals occurring in lupin seeds may be divided into phenolic acids, flavonoids and

tannins. Antioxidant activity of phenolic compounds depends on many factors. The most significant is their structure in which the number and position of hydroxyl groups are important. Furthermore, phenolic compounds may occur in combination with other compounds present in substrate, which can significantly affect their bioactivity (Dueñas et al., 2009). We find that lupin variety and LAB used for fermentation have a significant influence on TPC content and antioxidant activity of lupin seeds ($P = 0.0001$), and interaction of these factors is significant ($P = 0.0001$).

Conclusions

Lupin variety and LAB used for fermentation have a significant influence on acidity parameters, digestibility, amino acid profile, total phenolic compounds content and antioxidant activity of lupin wholemeal. Optimisation of lupin fermentation conditions could increase the possibility to produce new higher value food/feed products, which are of great interest for the design of functional foods/feeds and nutraceuticals.

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