

# The Effect of Dry Yeast Fermentation on Chemical Composition and Protein Characteristics of Blue Lupin Seeds

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## Summary

The effect of 24-hour fermentation of lupin seeds by different yeast strains on their chemical composition was determined. After fermentation, the mass fraction of proteins increased and their *in vitro* digestibility and biological activity significantly improved. The amino acid profile of fermented products was similar to that of raw lupin seeds. The significant reduction in the mass fraction of oligosaccharides and phytate, but not of alkaloids was found. The pH level of fermented products decreased as a consequence of the increase of lactic and propionic acid mass fractions. The most favourable changes in the chemical composition of blue lupin seeds were obtained in fermentation with *Saccharomyces cerevisiae* baker's yeast and Fermivin 7013 strain.

*Key words:* yeast, fermentation, lupin, nutritional value, antinutritional factors

## Introduction

The last decade has seen an increased interest in the cultivation of blue lupin after the discovery of its anthracnose resistance and high protein content, but the presence of antinutritional factors (ANF) adversely affects the palatability of seed (alkaloids) and utilization of nutrients ( $\alpha$ -galactosides and phytates) by monogastric animals (1). Blue lupin seeds also have a lower energy level (*e.g.* metabolizable energy in diets for pigs) than the other lupin species, because of the higher content of crude fibre, fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)) and non-starch polysaccharides in seeds, which are partially non-digestible (2). For this reason, attempts are being made to improve the nutritional value of blue lupin seeds, especially by reducing the ANF content and increasing protein and carbohydrate utilization. There are many methods for removing undesirable substances from the seeds of leguminous plants (3). Ex-

tractions with different solvents yield the seeds with a high protein content, without non-nutritive substances (4). Sprouting is a very simple method, but it causes many changes in the chemical composition and microbiological quality, which can be potentially undesirable in some conditions (5–7). Fermentation is an easy and cheap method of improving nutritional value of feed and food. Fermentation of lupin was successfully carried out with the use of fungi (*Aspergillus oryzae* and *Rhizopus oryzae*), bacteria (*Bacillus subtilis* and *Lactobacillus brevis*) or by natural fermentation. It also contributed to the improvement of sensory qualities, increasing the availability of minerals and reducing the concentrations of phytates (2–4). There are no studies available concerning lupin seeds fermented by yeast. Yeast fermentation is generally used in practice to increase protein levels in feed with low protein content and rich in starch. Therefore, more studies focus only on starch-containing components (pea, faba bean, *etc.*). Lupin seeds store energy in the form of non-starch polysac-

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charides, mostly oligosaccharides of the raffinose family, glucose, galactose, arabinose and xylose. Yeast fermentation of starch substrates in aerobic conditions leads to the production of mainly carbon dioxide and water, and stimulates appropriate propagation of yeast, generating large amounts of biomass formation (8). However, depending on the species and strain, yeast can utilize various sources of nutrients, such as oligosaccharides, simple sugars, amino acids or industrial waste (8–10). Research provided by Trojanowska *et al.* (11) shows that the lupin extract, containing mainly oligosaccharides and alkaloids, can be a cultural medium for the development of certain strains of yeast, especially *Torula* sp. and *Saccharomyces* sp. During yeast fermentation, the product is enriched with high-value protein of microbial origin and it can also improve the digestibility of protein and amino acid profile and reduce the concentration of antinutritional factors (12–16). Fermented products, in addition to the nutrient content (protein and vitamins), usually include live, dried or lyophilized cells of lactic acid bacteria or yeast, which can work probiotically or prebiotically on digestive tract microflora (8,10,17–19).

The innovation of the presented work is an attempt of nutritive improvement of blue lupin seeds by fermentation with different species of yeast. The hypothesis has been put forward that products obtained by yeast fermentation of lupin seeds will be characterized by a higher nutritional value than unprocessed seeds, and could be a potential new alternative protein source in human and animal nutrition with reduced antinutritional compounds and a higher energy value. Therefore, the aim of the study is to determine the effect of aerobic fermentation of lupin seeds using different strains of active dry yeast on the chemical composition of the obtained lupin products.

## Materials and Methods

### Lupin seeds and yeast strains

*Lupinus angustifolius* cv. Neptun (registered in 2009) was chosen for the study. Seeds were obtained from the Plant Breeding Smolice Ltd., IHAR Przebedowo Branch, Przebedowo, Poland. Active dry yeasts *Saccharomyces cerevisiae*: baker's yeast (Dr. Oetker, Bielefeld, Germany), Bayanus G-995 (Starowar, Sulejówek, Poland), Fermivin® 7013 strain (Biovin, Łódź, Poland) and *Saccharomyces carlsbergensis* Fermentis (Lesaffre, Wołczyn, Poland) were used for fermentation. The number of active yeast cells and saccharolytic activity were: of baker's yeast  $1.8 \cdot 10^{10}$  cells/g and 125 mL of CO<sub>2</sub> per 1 g of yeast per h, of Bayanus 2.8·10<sup>9</sup> cells/g and 28 mL of CO<sub>2</sub> per 1 g of yeast per h, of Fermivin® strain 7013  $1.4 \cdot 10^{10}$  cells/g and 11 mL of CO<sub>2</sub> per 1 g of yeast per h, and of *Saccharomyces carlsbergensis*  $1.6 \cdot 10^{10}$  cells/g and 11 mL of CO<sub>2</sub> per 1 g of yeast per h, respectively.

### Fermentation

Seeds were soaked in 2.5 g/L of sodium hypochlorite for 10 min to reduce natural microbial activity before fermentation by yeast and then washed with distilled water to obtain neutral pH, dried and ground in a laboratory mill. Samples of seeds (100 g) were weighed into glass ves-

sels and mixed with 400 mL of water. Dishes were placed on magnetic stirrers and after the initial mixing for 30 min, 1 % of each of the dry yeasts listed above was added. Fermentations were carried out under aerobic conditions (natural pH=5.5) for 24 h in a continuous mixing system. After that, the yeast enzymes were deactivated for 10 min at 70 °C, and the material was dried at 55 °C. Each product was obtained in four replications.

### Chemical analyses

For chemical analysis, the samples were ground to pass through a 0.5-mm sieve. Dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), crude ash (CA), acid detergent fibre (ADF) and neutral detergent fibre (NDF) of raw seeds and fermented products were analyzed in duplicate (20–25). Nitrogen-free extracts (NFE) were calculated as follows:

$$\text{NFE} = \text{DM} - (\text{CP} + \text{CA} + \text{CF} + \text{EE}) \quad /1/$$

The amino acid content was determined using an amino acid analyzer type AAA-339 (Mikrotechna, Prague, Czech Republic) using ninhydrin for post-column derivatization. Before analysis, the samples were hydrolyzed with 6 M HCl for 24 h at 110 °C (26). The phytate content was analyzed according to AOAC method 986.11 (27). The protein biological value was determined by the following indices: the chemical score was calculated using Mitchell and Block's method (28), the essential amino acid index (EAAI) was calculated with Oser's method (29), the tryptophan concentration was not determined chemically and it was assumed to be 0.72 g per 100 g of protein in raw and fermented lupin seeds, and digestible protein was determined by the enzymatic method.

Metabolizable energy in diets for pigs was calculated according to the recommendations of German Society of Nutrition Physiology (30) using the same digestibility coefficients for lupin and lupin products.

Lupin alkaloids were extracted from flour by trichloroacetic acid and methylene chloride, and determined with gas chromatograph model GC-17A (Shimadzu Corp., Kyoto, Japan) with a capillary column (Phenomenex, Torrance, CA, USA). Raffinose family oligosaccharides were extracted and analyzed by high-resolution gas chromatography as described previously by Zalewski *et al.* (31). The pH was measured in 10 % water extract by a pH meter inoLab® (WTW, Weilheim, Germany). For organic acid determination, the extract was centrifuged at 10 000×g for 8 min. All samples were filtered through a 0.20-mm filter before HPLC analysis. The supernatant was analysed directly by the HPLC method using a UV detector (Waters Corp., Milford, MA, USA). Organic acids were separated on an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C using 5 mmol/L of H<sub>2</sub>SO<sub>4</sub> as the eluent, at a flow rate of 0.5 mL/min.

### Statistical analyses

One-way analysis of variance was performed. The significance of differences between control and experimental groups was calculated by Duncan's global test, and an alpha level of p<0.05 was used to assess the significance among the mean values. The statistical analysis was performed using STATGRAPHICS, v. 5.0 (Statpoint Technologies, Inc., Warrenton, VA, USA).

## Results

All the products were characterized by a similar fresh and final dry matter content (Table 1). In comparison with raw lupin seeds, the content of crude ash and acid detergent fibre (ADF) significantly increased ( $p < 0.05$ ) in all fermented products, whereas the ether extract and nitrogen-free extract (NFE) contents significantly decreased ( $p < 0.05$ ).

The metabolizable energy was similar in all the samples. A significant ( $p < 0.05$ ) increase in the mass fractions of alanine in the protein of the products fermented with baker's yeast, Bayanus G-995 and Fermivin 7013, and lysine in products fermented with Fermivin 7013 and *S. carlsbergensis* was found in comparison with unprocessed seeds (Table 2). The arginine level decreased significantly

Table 1. Composition of lupin seeds and fermented products expressed on dry matter basis

$w/(g/kg)$	Lupin seeds	Fermented product				SEM	p-value
		Baker's yeast	Bayanus G-995	Fermivin 7013	<i>S. carlsbergensis</i>		
Fresh dry matter	–	203.1	203.7	210.6	199.6	2.1	0.350
Final dry matter	957.1	940.4	956.4	963.5	956.4	3.7	0.359
Crude protein	330.3	362.9	358.8	360.0	358.5	2.2	0.093
Digestible protein	262.1 <sup>b</sup>	304.8 <sup>a</sup>	290.8 <sup>a</sup>	300.5 <sup>a</sup>	296.1 <sup>a</sup>	0.2	0.012
Crude ash	38.8 <sup>b</sup>	43.7 <sup>a</sup>	43.9 <sup>a</sup>	44.6 <sup>a</sup>	45.6 <sup>a</sup>	3.7	0.028
Crude fibre	174.1	184.1	189.0	177.7	185.4	1.6	0.103
Ether extract	46.4 <sup>a</sup>	40.3 <sup>b</sup>	40.1 <sup>b</sup>	41.3 <sup>b</sup>	41.9 <sup>b</sup>	4.3	0.018
NFE	367.6 <sup>a</sup>	309.5 <sup>c</sup>	324.6 <sup>bc</sup>	339.9 <sup>b</sup>	325.0 <sup>bc</sup>	3.2	0.009
ADF	188.7 <sup>c</sup>	240.2 <sup>ab</sup>	243.7 <sup>a</sup>	229.7 <sup>b</sup>	226.9 <sup>b</sup>	2.2	0.049
NDF	246.1	266.0	272.7	257.6	253.7	3.1	0.204
ME/(MJ/kg)	14.71	15.09	14.83	14.71	14.70	0.6	0.211

Values in the same row with different letters in superscript differ significantly at  $p < 0.05$

SEM=standard error of the mean, NFE=nitrogen-free extract, ADF=acid detergent fibre, NDF=neutral detergent fibre, ME=metabolizable energy

Table 2. Amino acid content in proteins and biological values of raw lupin seeds and fermented products

$w(\text{amino acid})$ g/100 g	Lupin seeds	Fermented product				SEM	p-value
		Baker's yeast	Bayanus G-995	Fermivin 7013	<i>S. carlsbergensis</i>		
<i>Essential</i>							
Arginine	12.17 <sup>a</sup>	8.17 <sup>b</sup>	8.17 <sup>b</sup>	8.75 <sup>b</sup>	10.18 <sup>ab</sup>	0.26	0.050
Histidine	3.75	3.02	3.70	2.98	2.38	0.17	>0.05
Isoleucine	3.54	3.86	3.83	3.81	3.78	0.03	>0.05
Leucine	5.90	6.51	6.39	6.48	6.33	0.04	>0.05
Lysine	5.26 <sup>bc</sup>	5.52 <sup>ab</sup>	5.14 <sup>c</sup>	5.72 <sup>a</sup>	5.64 <sup>a</sup>	0.03	0.019
Methionine	0.53	0.50	0.58	0.58	0.59	0.03	>0.05
Phenylalanine	3.48	3.87	4.40	3.75	3.78	0.15	>0.05
Threonine	3.27 <sup>a</sup>	3.32 <sup>a</sup>	3.31 <sup>a</sup>	3.10 <sup>b</sup>	3.21 <sup>ab</sup>	0.02	0.029
Valine	3.57	3.78	3.83	3.77	3.74	0.03	>0.05
Cystine	1.38	1.70	1.46	1.38	1.36	0.05	>0.05
<i>Non-essential</i>							
Alanine	3.03 <sup>c</sup>	3.41 <sup>ab</sup>	3.36 <sup>ab</sup>	3.50 <sup>a</sup>	3.21 <sup>bc</sup>	0.03	0.032
Aspartic acid	8.95	9.42	9.20	9.25	9.15	0.07	>0.05
Glutamic acid	22.74	22.19	22.47	22.62	21.85	0.07	>0.05
Glycine	3.98	4.25	4.16	4.20	4.05	0.03	>0.05
Proline	5.58	5.64	5.96	5.79	5.87	0.07	>0.05
Serine	4.39	4.34	4.44	4.19	4.24	0.02	>0.05
Tyrosine	3.47	5.46	4.50	5.12	5.60	0.20	>0.05
Chemical score	33	38	35	34	34	0.45	0.165
EAAI	62 <sup>b</sup>	69 <sup>a</sup>	68 <sup>a</sup>	68 <sup>a</sup>	69 <sup>a</sup>	0.40	0.007
TEAA	42.85 <sup>a</sup>	40.25 <sup>c</sup>	40.81 <sup>bc</sup>	40.32 <sup>bc</sup>	40.99 <sup>b</sup>	0.26	0.001

Values in the same row with different letters in superscript differ significantly at  $p < 0.05$

SEM=standard error of the mean, EAAI=essential amino acid index, TEAA=total essential amino acids (100 g per g of protein)

( $p < 0.05$ ) in the products fermented with baker's yeast, Bayanus G-995 and Fermivin 7013 and threonine in those with Fermivin 7013. Chemical score increased ( $p > 0.05$ ) from 33 in raw lupin seeds to 34 in the products obtained with Fermivin 7013 and *S. carlsbergensis*, to 35 in those with Bayanus G-995 and 38 with baker's yeast, whereas essential amino acid index (EEAI) increased ( $p < 0.05$ ) from 62 in lupin seeds to 68 in those fermented with Bayanus G-995 and Fermivin 7013 or to 69 in those with baker's yeast and *S. carlsbergensis*. In each case, methionine and cystine were the limiting amino acids. The total essential amino acid (TEAA) index was lower ( $p < 0.05$ ) in all the fermented products than in raw seeds. No significant effect of yeast fermentation ( $p > 0.05$ ) on the total alkaloid mass fraction and structure was found (Table 3). The mass fraction of phytate was significantly lower in the products fermented with Fermivin 7013 and *S. carlsbergensis* than in raw seeds. During fermentation, the reduction of total raffinose family oligosaccharides ( $p < 0.05$ ) was found in all the samples. The pH of the starting material was about 5.5. In fermented products, the pH decreased (Table 4) and ranged from 5.1 to 4.3. The pH of the seeds fermented with Bayanus G-995 was significantly lower than in other preparations. The mass fraction of lactic acid (in g/kg) ranged from 13.45 to 35.05, of butyric acid from 0 to 0.70, of propionic acid from 14.05 to 18.35 and of acetic acid from 4.70 to 5.21. The mass fraction of lactic acid in the seeds fermented with baker's yeast was significantly lower

( $p < 0.05$ ) and acetic acid in the seeds fermented with *S. carlsbergensis* was significantly higher ( $p < 0.05$ ) than in other preparations. The mass fraction of propionic acid in seeds fermented with *S. carlsbergensis* and Fermivin 7013 was significantly higher ( $p < 0.05$ ) than in those with Bayanus G995 and baker's yeast.

## Discussion

Fermentation conditions for optimum biomass increase in the first 24 h were described in other studies (16,32,33). The dry matter loss did not exceed 10 %. All yeast strains used in the experiment showed the tendency (at  $p = 0.09$ ) to increase the content of digestible proteins in dry matter (on average by 10 %), which was correlated with a decrease in NFE content, used probably as a carbon source by the yeast. A similar increase in the protein content was observed in the study by Nguyen *et al.* (34) during fermentation of pea seeds and wheat grains using *Saccharomyces boulardii*. The increase in protein content was associated with biomass production (8,31), which was confirmed by higher protein digestibility ( $p = 0.01$ ). Yeast uses available sources of nitrogen as free amino acids, dipeptides or proteins for growth (35). In the fermented products a negligible increase in the mass fractions of some essential amino acids was observed, which contributed to the improve-

Table 3. The mass fractions of total alkaloids, total oligosaccharides and phytate, and alkaloid and oligosaccharide compositions in lupin seeds and fermented products

Compound	Lupin seeds	Fermented product				SEM	p-value
		Baker's yeast	Bayanus G-995	Fermivin 7013	<i>S. carlsbergensis</i>		
$w(\text{total alkaloids})/(\text{g/kg})$	0.24	0.23	0.19	0.22	0.24	0.01	>0.05
$w(\text{angustifoline in TA})/\%$	11.35	11.77	9.31	10.69	10.98	0.29	>0.05
$w(\text{isolupanine in TA})/\%$	4.07	3.99	4.23	3.98	3.94	0.03	>0.05
$w(\text{lupanine in TA})/\%$	61.34	62.19	63.34	62.71	62.01	0.35	>0.05
$w(\text{13-OH-lupanine in TA})/\%$	23.24	22.06	23.13	22.62	23.08	0.28	>0.05
$w(\text{phytate})/(\text{mg/kg})$	1.90 <sup>a</sup>	1.05 <sup>ab</sup>	0.96 <sup>ab</sup>	0.71 <sup>b</sup>	0.39 <sup>b</sup>	0.12	0.008
$w(\text{total oligosaccharides})/(\text{g/kg})$	73.6 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	32.2	0.010
$w(\text{raffinose in TO})/\%$	12.2 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00	0.010
$w(\text{stachyose in TO})/\%$	41.5 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00	0.010
$w(\text{verbascose in TO})/\%$	19.9 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.0 <sup>b</sup>	0.00	0.010

Values in the same row with different letters in superscript differ significantly at  $p < 0.05$   
SEM=standard error of the mean, TA=total alkaloids, TO=total oligosaccharides

Table 4. The pH value and organic acid concentration on dry matter basis in fermented products

$w(\text{organic acid})/(\text{g/kg})$	Fermented products				SEM	p-value
	Baker's yeast	Bayanus G-995	Fermivin 7013	<i>S. carlsbergensis</i>		
Lactic	13.45 <sup>b</sup>	30.90 <sup>a</sup>	35.05 <sup>a</sup>	27.00 <sup>a</sup>	1.39	0.018
Acetic	5.21 <sup>b</sup>	5.06 <sup>b</sup>	4.70 <sup>b</sup>	6.00 <sup>a</sup>	0.08	0.009
Propionic	14.05 <sup>b</sup>	13.55 <sup>b</sup>	18.35 <sup>a</sup>	17.50 <sup>a</sup>	0.35	0.022
Butyric	0.05	0.00	0.70	0.05	0.18	>0.05
pH	5.1 <sup>a</sup>	4.3 <sup>c</sup>	4.7 <sup>b</sup>	4.7 <sup>b</sup>	0.03	0.012

Values in the same row with different letters in superscript differ significantly at  $p < 0.05$   
SEM=standard error of the mean

ment of seed protein indices: chemical score (up 1 to 5 units) and EAAI (up 6 to 7 units), as was also confirmed by Trojanowska *et al.* (11), Khattab *et al.* (14) and Yabaya *et al.* (16). Only the arginine content decreased significantly ( $p < 0.05$ ) in the seeds fermented with baker's yeast, Bayanus G-995 and Fermivin 7013, which had a negative impact on the TEAA index. Arginine is used by yeast as a precursor of other amino acids, and this phenomenon was also observed by Yabaya *et al.* (16). The most preferred indices of the protein were found in the product fermented by baker's yeast, which also contained more cystine ( $p > 0.05$ ) than the other products.

Lupin seeds are a difficult material for fermentation by yeast because of the lack of easily accessible starch (10). The carbohydrates found in lupin seeds consist mainly of simple sugars (about 30 g/kg), and raffinose family sugars (about 76 g/kg) (9). Carbohydrates of the raffinose family have been used completely by the yeast during 24 h of fermentation (36). On the other hand, the total use of available sugars (NFE) was relatively low and did not exceed 17 %, which could be partly a result of short fermentation time. Structural sugars proved to be resistant to indirect digestion by the used yeast, which is confirmed by high levels of carbohydrate complexes (as crude fibre, ADF and NDF) in the fermented seeds (37). Moreover, the increase in the NDF content in some technological treatments is usually accompanied by an increase in NDF-bound protein and reduced availability (38). It can be assumed that the increase in the ADF and NDF fractions in fermented products can indicate a higher degree of protein binding by the fibre.

Yeasts are also a rich source of minerals and, depending on the species and strain, may introduce into the fermented mass from 4 up to 10 % of ash (10), which was confirmed by our research. The level of fat in the fermented products was lower than in raw seeds, which was also found by Yabaya *et al.* (16), Mbata *et al.* (32) and Hassan *et al.* (37). The yeast used fat as an energy source to produce cell biomass.

Generally, fermentation caused a decrease in the fat and carbohydrate content in the seeds, which can lead to changes in the metabolic energy value. However, metabolizable energy, calculated on the basis of the chemical composition, indicates that fermentation did not reduce it. It should be noted that digestibility coefficients of nutrients in lupin seeds were taken into account in the metabolizable energy calculation, while fermentation may really affect the digestibility of protein (*e.g. in vitro* digestibility of protein was improved by about 13 %) or carbohydrates. These results should therefore be treated as rough approximation.

No significant effect of yeast fermentation ( $p > 0.05$ ) on total alkaloid mass fraction and structure was found. Trojanowska *et al.* (11) observed that the development of different yeast strains on lupin extract can lead to a reduction in the alkaloid content by up to 20 % (as was confirmed in the case of seeds fermented with Bayanus G-995). It should be noted, however, that the lupin extract contained mainly alkaloidal nitrogen (about 10 % dry matter), free amino acids or peptides (about 7 % dry matter), and only small amounts of protein. Due to the unavaila-

bility of a more absorbable form of nitrogen, the yeast may use the nitrogen bound in the form of alkaloids. In contrast, the lupin seeds contain significant amounts of protein, but low mass fractions of alkaloids (2.4 mg/kg in raw seeds), which promotes nitrogen utilization for biomass production.

In all the fermented products, the phytate mass fraction was reduced, which was confirmed by other studies (15,37,39). The *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* strain 7013 proved to be the most effective as they reduced the phytate content by approx. 80 and 63 %, respectively ( $p < 0.05$ ). The observed changes confirm the opinion that fermentation increases the activity of native phytase, which can disintegrate insoluble organic complexes with minerals (13,40).

Raffinose family oligosaccharides are considered to be antinutritional factors responsible for excessive formation of gases and diarrhoea after feeding animals with raw legume seeds. These carbohydrates were used during fermentation by yeast due to their availability resulting from the enzymatic activity of microorganisms. Yeast produces different types of glycosyl hydrolases, such as  $\alpha$ -D-galactosidase, which degrade the oligosaccharides into simple sugars (36). Trojanowska *et al.* (11) found that different yeast strains are able to degrade up to 70 % of oligosaccharides, with approx. 50 % of their participation in the lupin extract.

During fermentation, yeast also produces organic acids, as a result of sugar and glycerol degradation, which reduces the pH (19,33). Yabaya *et al.* (16) observed a similar reduction in the initial pH from 5.6 to 5.1, and a significant increase in the yeast count during the fermentation of soya cake by *Saccharomyces cerevisiae* for 24 h. Mbata *et al.* (32) found a decrease in the pH from 6.5 to 4.5 during fermentation of bambara groundnut. The composition of organic acids was not given by the authors cited above. In the present study mass fractions of lactic, acetic, propionic and butyric acids in the final products were investigated. The short-chain fatty acids, such as acetic, propionic and butyric, are by-products of fermentation, although the presence of propionic and butyric acids may also be associated with the activity of bacteria (41). Sriprya *et al.* (35) observed that lactic acid appeared in a significant amount from the 6th h of fermentation and steadily increased up to 37 g/kg in 48 h, while in this study, the lactic acid mass fraction ranged from 13 to 35 g/kg. The lactic acid mass fraction was the highest in the seeds fermented with Fermivin 7013, in which the highest mass fraction of butyric acid was also found. Acetic acid formation by *Saccharomyces cerevisiae* strains is affected by sugar concentration, pH and nitrogen (41). Van Winsen *et al.* (42) pointed out that low pH level and especially the high lactic acid concentration are the main factors responsible for the antimicrobial effect of fermented feed and can have a beneficial effect on feed intake, daily gain and feed/gain ratio (42,43).

Generally, for better fermentation effects, the time of the process should be extended and the initial hydrolysis of the material should be applied. Moreover, in our opinion, yeast strains that degrade structural carbohydrates should be used. For this reason, the study should be continued.

## Conclusions

Yeast fermentation of lupin seeds allows formation of valuable feed or food products. The benefit of the process is primarily a reduction of some antinutritional factors, as well as lowering the pH, and favouring the formation of probiotic lactic acid bacteria in the products. Fermentation of lupin seeds increased their nutritional value, especially by increasing the protein content and improving the amino acid profile. The *Saccharomyces cerevisiae* baker's yeast and Fermivin 7013 strain proved to be the most effective for direct fermentation of blue lupin seeds.

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