CHANGES IN THE ALKALOID, α-GALACTOSIDE AND PROTEIN FRACTIONS CONTENT DURING GERMINATION OF DIFFERENT LUPIN SPECIES

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ABSTRACT

The objective of our studies were seeds of two lupin species *Lupinus luteus* L. and *Lupinus angustifolius* L. cvs. Lord and Graf respectively. Lupin seeds were germinated at 15 and 24°C and during two, three and four days. In the lupin sprouts antinutritional factors: alkaloids and raffinose family oligosaccharides (RFOs) and five nitrogen fractions: non protein (Nnp), albumin (A), globulin (G), glutelin and prolamin (Gt+P) and nitrogen residue fraction (Nr) were determined. The level of these compounds was compared with the proper ones of initial material (not germinated seeds). These studies showed that the germination process clearly affects the decrease of antinutritional factors: RFOs and alkaloids. The decrease level of these compounds depended on such factors like, lupin species and used germination conditions. It was found on the base of nitrogen analysis of particular protein fractions that the germination process of lupin seeds causes deep quantitative and qualitative changes in fractional composition of lupin proteins. It especially concerns the decrease of globulin and residual fraction content and distinct increase of Nnp fraction. The changes in other fractions were not so unequivocal in comparison with the mentioned above and depended on lupin species, temperature and time of germination. Qualitative changes of A, G and Gt+P fractions caused by germination were confirmed by gel electrophoresis (SDS-PAGE). The amino acid analysis of seeds and sprouts of Nnp fractions showed an increased content of Asp, Ser, Ala, Pro – non essential amino acids (NEAA), and Val, Met, iLeu, Leu, Thr – essential amino acids (EAA). Simultaneously a decrease of Glu, Arg (NEAA), Phe, Lis, Cys (EAA) contents was observed. Generally the germination process causes the decrease of total NEAA and an increase of total EAA in Nnp fractions of both lupin species.

KEY WORDS: lupin, alkaloids, α-galactosides, protein, protein fractions, amino acids.

INTRODUCTION

The features which distinguish lupin from among other plants may be determined as follows: i – belongs to the highest protein plants; ii – is characterised by modest soil and climatic demands; iii – leaves big organic mass in soil; iv – improves air-water conditions of soil; v – makes accessible macro- and microelements eluted to sublayer of soil (Gulewicz et al. 1994). In spite of such great significance the utilization of lupin in human and animals nutrition has been up to now on unsatisfactory level. The limiting role in the utilization of lupin seeds as protein source for purposes of human and animals nutrition is played by antinutritive factors: alkaloids and α-galactosides. The first ones are toxic and their presence in fodder results in a bitter taste and limits its consumption by animals, whereas the RFOs after consumption cause the arduous flatulence (Culvenor and Petterson 1986; Price et al. 1988; Frias et al. 1995).

Among many technological processes that decrease of antinutritive factors, increasing some nutrients and improving the availability of legumes the germination process should be specified (Vidal-Valverde et al. 2002; Urbano et al. 2005). During germination process the extensive breakdown of seed storage proteins and the improvement of protein digestibility and the essential amino acid content are observed, thus enhancing the nutritional value of legumes (Rozan et al. 2001; Kuo et al. 2004). Little information, however, is available about the influence of germination on the protein fractions content and amino acid composition in legume seeds, which might show that lupin sprouts are an unexploited potential source of protein. To determine the effect of a different technological treatment on nutritional value of plants simple chemical analysis proposed by Osborne in 1891 was applied. This way is based on differential solubility of protein fractions of the biological material (Nelson 1969). Osborne distinguishes four protein fractions present in seeds according to their solubility. They are: albumins (1.6S–2S) – water soluble, globulins (7S–13S) – salt soluble, prolamins – alcohol soluble and glutelins – acid and alkali soluble (Mandal and Mandal 2000). In cereal seeds proteins from prolamin and glutelin groups compose up to 90% of all storage proteins, except oat, where globulins are the main protein fraction (Restani et al. 1981; Oomah and Bushuk 1983). Lupin seed protein is composed dominantly by globular proteins, and their quality depends on lupin species and biotype (Peretiatkowicz et al. 1988a, b). The main components of lupin storage proteins are globulins and sometimes albumins, while the prolamins and glutelins are detected in small amounts and sometimes they are simply neglected (Blagrove and Gillespie 1975; Peretiatkowicz et al. 1988a, b; Duranti et al. 1990).

The main aim of our studies was to answer how germination of lupin seeds affects on the level of antinutritive factors and fractional protein composition connected with the improvement or worsening of its nutritional value. The objective of our studies were two lupin species cultivated in Poland: *Lupinus luteus* cv. Lord and *Lupinus angustifolius* cv. Graf.

MATERIALS AND METHODS

Samples and Chemicals

Seeds of *Lupinus angustifolius* cv. Graf and *Lupinus luteus* cv. Lord were kindly supplied by Dr. Stanisław Stawiński from Breeding Station IHAR in Przebędowo, near Poznań (Poland).

Germination

10 g of both lupin seeds species were soaked for 30 min with 50 mL of 0.25% sodium hypochlorite. Next, the seeds were drained and washed with distilled water toward neutral pH. Afterwards, seeds were soaked with 50 mL of destilled water for six hours shaken every 30 minutes. The imbibed seeds were germinated in darkness for 48, 72 and 96 hours at temperatures 15˚C and 24˚C. Germination rate was higher than 96% for both used lupin seeds species. Sprouted seeds were lyophylized and stored under vacuum in plastic bags.

Analysis of α-galactosides

α-Galactosides were isolated from seeds according to the procedure described previously by Muzquiz et al. 1992 with some modifications. Lupin flour (0.5 g) was homogenized in 5 mL of 50% of ethanol for one minute at room temperature using the Ultraturrax homogenizer. The mixture was centrifuged for 5 min at 3000 g, the supernatant was decanted and the procedure repeated twice. The sample extract was purified using the C_{18} cartridges (500 mg/6 mL) connected with a vacuum system. The effluent was evaporated to dryness, redissolved in deionized water (1 mL) and centrifuged for 8 min at 6000 g. The analysis of RFOs was carried out in the supernatant by high performance liquid chromatography using a Merck Hitachi HPLC with a refraction index detector. For separation of oligosaccharides a Spherisorb-5-NH_2 column (250×4.6 mm i.d.) and acetonitrile/water 60:40 (v/v) as mobile phase were used. Solvents were filtered through a Millipore FH (0.45 mm) membrane and degasified under vacuum. Injection volume was 20 µL. Quantification of each sugar was accomplished by comparing the peak areas of the samples with those of the standard solutions over the range 0-4 mg/mL and coefficients of determination above 0.99.

Analysis of alkaloids

Extraction of alkaloids from seeds and sprouts was performed according to the method described by Muzquiz et al., 1994. 0.5 g of grounded analyzed material were homogenized with 5mL of 5% trichloroacetic acid (TCA) for 1 min. The mixture was then centrifuged for 15 minutes at 10.000 g and the supernatant separated. The extraction was repeated twice. The supernatants were collected in a decantation funnel and 0.8 mL of 10M NaOH were added. Three extractions with 15 mL of dichloromethane were performed and the organic phase was evaporated to dryness at room temperature. The residue was dissolved in 1mL of methanol and codeine solution was added as an internal standard (final concentration of codeine, 1 mg/mL). For the analysis of alkaloids a System GC ToF Waters firm, model GCT Premier equipped with a NPD (nitrogen – phosphorus detector) and a SPB-1 (30 m × 0.25 mm i.d.) column was used. Helium was the carrier gas. The temperatures of the injector and detector were 240˚C and 300˚C, respectively. The oven temperature was 150˚C, increased in 5˚C/min to 235˚C and final hold time of 23 min at 235˚C. Calibration curve was performed for lupanine with linear response over the range 0-1.250 mg/mL and coefficients of determination above 0.99.

Determination of protein

The crude protein in seeds was determined by Kjedahl method using a Kjeltec Auto Distillation 2200 apparatus (FOSS TECATOR).

Determination of nitrogen fractional composition of seeds and sprouts

Nitrogen fractionation of seeds and sprouts was generally supported on the method described by Michael and Blume 1960 with some modification of Peretiatkowicz et al. 1988a, b; Ciesiołka et al. 2008. The method modification was based on: i – application of ultrasonification in processes of extraction; ii – fractions of glutelin and prolamin (Gt+P) were determined together; iii – fraction Nr (nitro-

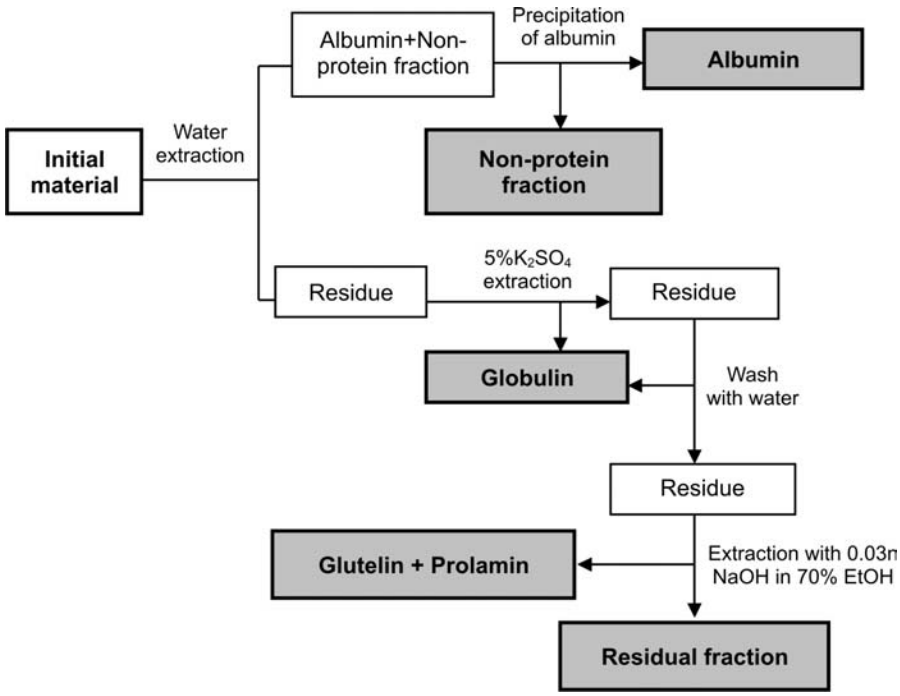

Fig. 1. Ideological scheme of protein fractionation.

gen residue fraction) was determined for calculation of total nitrogen balance. The general scheme of fractionation is showed in Figure 1. The total nitrogen of initial material (seeds used to germination) and fractions marked in Figure 1 in shading thicken frame were determined by Kjeldahl method using the Kjeltec Auto Distillation 2200 apparatus (FOSS TECATOR, Foss, Hillerod, Denmark).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAG) of nitrogen fraction

The precipitated albumin fraction obtained according to the scheme (Fig. 1) was resolved in TBS buffer (25 mM NaCl and 25 mM TRIS pH 7.5). Then, this fraction was passed through the Amicon filter (10 kD) in the presence of TBS buffer. In the purified albumin and other fractions (globulins and glutelins + prolamins), the protein contents were determined according to the Bradford method (Bradford 1976). The samples of protein fractions containing 20 µg protein were applied onto 10% acrylamide gel. The SDS PAGE was performed on the Biometra Power Pack P25 apparatus at 7.5 mA (concentrating gel), 14 mA (developing gel) for three hours (Laemmli 1970). Then, the gels were passed to the fixing agent (10% acetic acid in 50% ethanol) and dyed with Coomassie Brillant Blue.

Isolation of non protein fractions (Nnp) and analysis of their amino acid composition.

To separate lupin seeds and sprouts Nnp fractions from other ones (Fig. 1) the acetone (4:1 v/v) was used. Hydrolysis of fractions was done in 6M HCl, 105˚C for 24 h in sealed vials under nitrogen. Next, the hydrolysates were dried under vacuum and purified on 50WX8 Sephadex. The amino acid composition of protein fractions was determined by mean of Amino Acid Analyzer type AAA-339 Mikrotechna Praha firm. The following gradient temperature programm was used: T_1 – 43˚C (0-50 min), T_2 – 58˚C (51-113 min). Citrate bufors pH 3.5, 4.25, 9.45 were used. The separation of amino acids on column 38×0.4 cm filled with OSTION LG ANB.

Statistical analysis

Multi-way analysis of variance (ANOVA) was conducted using MATLAB program, version 6.5 (MathWorks, Inc.) available at Poznań Supercomputing and Networking Center of the Institute of Bioorganic Chemistry, PAS, Poznań, Poland.

RESULTS

Tables 1A and 1B present result of effect of temperature and time germination on fresh and dry weight and length of lupin sprouts in both lupin species. The time of germination of both lupin species have significant effect on increase of fresh mass and length of sprouts in both the used temperatures. Dry mass for lupin cv. Graf at 15˚C decrease in forth day of germination. This effect at 24˚C is observed already after third day of germination. In general, the germination of lupin cv. Graf in higher temperature (24˚C) results in the increase of fresh mass and length of sprouts in comparison to the lower temperature (15˚C). The higher temperature had no clear influence on yield of dry mass of sprouts. In the case of lupin cv. Lord (Table 2B) dry mass at 15˚C decreased after third day of germination while at 24˚C slightly increased. In contrast to *L. angustifolius* higher temperature had not so clear influence on yield of fresh and dry mass while only on length of sprouts.

The content of sugars in seeds of *L. angustifolius* cv. Graf and *L. luteus* cv. Lord and their sprouts after germination at 15˚C and 24˚C three and four days are presented in Tables 2A and 2B. Both lupin species are different with regard to sugar composition and content of RFOs. In the case of *L. angustifolius* (Table 2A) the content of total sugars in sprouts after 4th germination at 15˚C decreased ca. 30% from 132.5 to 91.8 mg/g, while at 24˚C this effect was not so clear (from 132.5 to 128.2 mg/g). The time of germination and temperature had significant effect on total content of raffinose family oligosaccharides (RFOs). For temperatures 15˚C and 24˚C the content of RFOs in sprouts after

TABLE 1A. Effect of temperature and time germination on yield of fresh and dry weight and length of lupin sprouts in *Lupinus angustifolius* cv. Graf*.

Day of germination	15°C			24°C		
	Fresh weight (g)	Dry weight (g)	Length of sprouts (cm)	Fresh weight (g)	Dry weight (g)	Length of sprouts (cm)
2	26.401±0.403aA	9.087±0.003aA	1.0±0.2aA	28.095±0.905aB	8.993±0.013aA	1.5±0.3aB
3	33.095±0.105bA	9.066±0.016aA	2.8±0.4bA	34.232±0.132bB	8.848±0.002bB	3.6±0.6 bA
4	37.258±0.258cA	8.937±0.129bA	4.1±0.4cA	41.455±0.130cB	8.621±0.079cB	7.0±0.6cB

* To the germination 10 g (9.268 g d.w.) of lupin seeds were used.
The letters a-c indicate significance in the same temperature and different days of germination, p<0.05.
The letters A-B indicate significance in the same day of germination and different temperature, p<0.05.

TABLE 1B. Effect of temperature and time germination on yield of fresh and dry weight and length of lupin sprouts in *Lupinus luteus* cv. Lord*.

Day of germination	15°C			24°C		
	Fresh weight (g)	Dry weight (g)	Length of sprouts (cm)	Fresh weight (g)	Dry weight (g)	Length of sprouts (cm)
2	27.712±0.2aA	9.164±0.07aA	0.9±0.2aA	28.181±0.18aB	8.811±0.021aB	1.0±0.4aA
3	33.465±0.035bA	9.004±0.012bA	2.7±0.6bA	32.542±0.222bB	8.891±0.005bB	3.2±0.7bA
4	39.793±0.103cA	8.699±0.001cA	3.9±0.5cA	36.994±0.094cB	9.234±0.006cB	4.9±0.4cA

* To the germination 10 g (9.289 g d.w.) of lupin seeds was used.
The letters a-c indicate significance in the same temperature and different days of germination, p<0.05.
The letters A-B indicate significance in the same day of germination and different temperature, p<0.05.

TABLE 2A. Contents of sugars in seeds of *Lupinus angustifolius* cv. Graf and sprouts after germination in 15 and 24°C three and four days (mg/g d.w.).

Sugars	Seeds	Sprouts (15°C)		Sprouts (24°C)	
		3rd day	4th day	3rd day	4th day
ramnose	1.072	1.212	1.102	0.757	1.909
G+F+Ga	8.937	21.487	25.872	33.563	41.902
Sucrose	39.487	52.919	38.689	50.272	40.168
n.i*.	0.000	2.298	0.519	0.000	0.764
raffinose	9.952	5.631	1.926	3.009	2.108
n.i	2.437	24.735	14.875	31.806	36.296
stachyose	55.780	9.595	8.184	9.513	4.976
verbascose	14.854	1.011	0.667	1.931	0.091
Total	132.519	118.888	91.834	130.851	128.214
Total RFOs	**80.586**	**16.237**	**10.777**	**14. 453**	**7.175**

Legend: G – glucose; F – fructose; Ga – galactose
* not identified

TABLE 2B. Contents of sugars in seeds of *Lupinus luteus* cv. Lord and sprouts after germination in 15 and 24°C three and four days (mg/g d.w.).

Sugars	Seeds	Sprouts (15°C)		Sprouts (24°C)	
		3rd day	4th day	3rd day	4th day
ramnose	1.220	1.611	0.137	1.599	0.793
G+F+Ga	9.040	24.071	11.360	17.619	17.513
Sucrose	19.684	59.568	61.395	49.856	29.170
n.i*	0.000	0.000	0.000	0.000	0.361
raffinose	10.906	6.600	4.899	5.492	5.094
n.i	0.000	33.686	11.898	36.312	37.695
stachyose	74.153	20.478	11.576	16.456	15.039
verbascose	45.847	4.526	0.945	2.466	1.553
Total	160.850	150.540	102.210	129.800	107.218
Total RFOs	**130.906**	**31.604**	**17.420**	**24.414**	**21.686**

Legend: G – glucose; F – fructose; Ga – galactose
* not identified

four days germination decreased about 87% and 91% respectively. Among all sugars belonging to this group the highest decrease of verbascose content was observed. Similarly, in the case of *L. luteus* (Table 3B) germination

TABLE 3A. Contents of alkaloids in seeds of *Lupinus angustifolius* cv. Graf and sprouts after germination at 15 and 24°C during three and four days (% in total alkaloids).

Alkaloids	Initial material (seeds)	15°C – Day of germination		24°C – Day of germination	
		3	4	3	4
Ammodendrine	0.34	0.45	0.43	0.35	0.32
Angustifoline	0.66	5.58	4.34	2.34	1.76
iso-Lupanine	5.79	4.78	4.04	4.93	4.84
Lupanine	68.79	50.3	50.40	52.00	52.10
3 OHLupanine	0.36	2.80	3.90	3.30	5.20
13OHLupanine	24.06	22.51	14.48	24.05	18.21
13alpha-Tigloyloxylupanine	0.00	13.58	22.41	13.03	17.57
Total alkaloids mg/100 mg	**0.145**	**0.109**	**0.106**	**0.107**	**0.098**

TABLE 3B. Contents of alkaloids in seeds of *Lupinus luteus* cv. Lord and sprouts after germination at 15 and 24°C during three and four days (% in total alkaloids).

Alkaloids	Initial material (seeds)	15°C – Day of germination		24°C – Day of germination	
		3	4	3	4
Lupinine	26.83	20.95	22.10	28.68	29.00
Gramine	0.00	7.05	6.78	9.35	8.11
Sparteine	57.77	57.45	57.41	53.16	53.90
Ammodendrine	3.66	5.02	3.86	1.62	2.59
Lupanine	2.87	1.53	1.49	0.69	0.08
17oxoSparteine	2.53	2.40	2.86	1.50	1.40
13OHLupanine	6.34	5.60	5.50	5.00	4.92
Total alkaloids mg/100 mg	**0.102**	**0.094**	**0.085**	**0.079**	**0.070**

caused a clear decrease in contents of total sugars and RFOs. This effect was dependent from germination time and temperature. The germination process caused the highest decrease in content of verbascose.

The results of effect of seed germination on alkaloid composition of sprouts in both tested lupin species are presented in the Tables 3A and 3B. Apart from the studied lupin species with different alkaloid content and composition, the germination caused a clear decrease of total alkaloids contents in both the cases. However, the contents of the same alkaloids during germination increased. For example, in the case of *L. angustifolius,* the increase of tigloyloxylupanine, 3OHlupanine, angustifoline is noted. For *L. luteus* the increase of gramine content is observed. Generally, the alkaloid content in sprouts is dependent on the lupin species, temperature and time of germination.

The Tables 4, 4A, 5 and 5A showed the influence of germination on the protein content and protein fraction composition. As shown (Tables 4 and 5) the germination causes in both the lupin species an increase of total protein in sprouts in comparison to the initial material. The main protein fractions in the seeds of *L. angustifolius* (Tables 4 and 4A) and *L. luteus* (Tables 5 and 5A) are globulins and albumins. During germination the contents of globulins of both the lupin species clearly decreased whereas the contents of Nnp and R fractions increased. The content of albumin fraction of *L. luteus* decreased, while this fraction in *L. angustifolius* increased. The Gt+P fraction of *L. luteus* shows the highest stability during germination. However, this dependence is not observed in the case of *L. angustifolius*. Generally, the changes of protein fractions depend on lupin species, time and temperature of germination. It is also emphasized that the choice of protein fractionation method based on different solubility of protein seemed to be very correct, what guarantees a high level of recovery from 97.86 to 103.0% (Tables 4 and 5).

The qualitative changes of A, G and Gt+P fractions of seeds before and after germination of both studied lupin species are showed on SDS-PAGE electrophoregrams (Figs 2 and 3). The electrophoregrams show a very great difference in the chemical composition of particular protein fraction seeds and sprouts within the framework of this same lupin species. For example, the seeds albumin, globulin and Gt+P fractions of *L. luteus* (Fig. 2 paths 1, 6, Fig. 3 path 1 respectively) are different from albumin, globulin and Gt+P fractions of sprouts (Fig. 2 path 3, 8, Fig. 3 path 3 respectively). This concern also *L. angustifolius* where these difference between seeds protein fractions and proper sprouts fractions are very clear (Fig. 2 paths 2, 7, Fig. 3 path 2) and (Fig. 2 paths 4, 9, Fig. 3 path 4). These deep changes proceed during germination of lupin seeds consist in decay of some protein bands and appear also in other ones.

Table 6 presents the amino acid compositions of seeds and sprouts of Nnp fractions in both lupin species. These results shows that the germination process causes not only the increase of Nnp fractions (Tables 4 and 5) but also changes the amino composition. In Nnp fractions of sprouts the increase of contents of such amino acids like: Asp, Ser, Ala, Pro (NEAA), Val, Met, iLeu, Leu, Thr (EAA) and decrease of the contents Glu, Arg (NEAA), Phe, Lis, Cys (EAA) are observed. In general, the germination process causes the decrease of total NEAA and increase of total EAA in Nnp fractions of both lupin species.

TABLE 4. The nitrogen fractional composition of lupin seeds (*Lupinus angustifolius* cv. Graf) and their sprouts after 3 and 4 days germination at 15 and 24˚C (in mgN/g d.w.).

Nitrogen fraction	Seeds	Sprouts (15˚C)		Sprouts (24˚C)	
		3rd day	4th day	3rd day	4th day
A	10.08±0.92a	12.52±0.22b	12.28±0.46b	11.12±0.02c	11.86±0.18d
Nnp	5.62±0.80a	9.81±0.07b	10.81±0.50c	14.74±0.06d	20.85±0.69e
G	32.36±1.64a	28.07±0.16b	27.06±0.16c	22.53±0.97d	15.40±0.04e
Gt+P	3.07±0.72a	1.75±0.13b	1.87±0.11b	2.45±0.01c	2.95±0.95a
R	2.94±0.49a	5.74±0.18b	6.01±0.29b	4.43±0.17c	5.30±0.10d
Total	54.07	57.89	58.03	55.27	56.36
IM*	54.37	56.72	56.28	56.48	56.48
Recovery (%)**	99.45	102.06	103.10	97.86	99.79
Protein content (%)	33.98	35.45	35.17	35.20	35.30

± standard deviation (SD)
* IM initial material (seeds or sprouts used to fractionation)
** in relation to IM
The letters a-e indicate significance within the same fraction; $p<0.05$

TABLE 4A. The percentage participation of nitrogen fraction in protein composition of lupin seeds (*Lupinus angustifolius* cv. Graf) and their sprouts after 3 and 4 days germination at 15 and 24˚C.

Nitrogen fraction	Seeds	Sprouts (15˚C)		Sprouts (24˚C)	
		3rd day	4th day	3rd day	4th day
A	18.64	21.63	21.16	20.12	21.04
Nnp	10.39	16.95	18.63	26.67	37.00
G	59.85	48.49	46.63	40.76	27.33
Gt+P	5.68	3.02	3.22	4.43	5.23
R	5.44	9.91	10.36	8.02	9.40

TABLE 5. The nitrogen fractional composition of lupin seeds (*Lupinus luteus* cv. Lord) and their sprouts after 3 and 4 days germination at 15 and 24˚C (in mgN/g d.w.).

Nitrogen fraction	Seeds	Sprouts (15˚C)		Sprouts (24˚C)	
		3rd day	4th day	3rd day	4th day
A	12.76±0.76a	10.50±0.10b	10.71±0.29b	8.89±0.25c	8.46±0.25c
Nnp	8.67±0.38a	13.71±0.21b	13.82±0.01b	16.10±1.14c	20.10±0.20d
G	36.24±1.76a	36.05±2.02a	33.71±0.01b	33.21±0.21b	29.37±0.24c
Gt+P	3.56±1.19ab	4.50±0.10a	4.61±0.01a	3.46±0.10b	4.70±0.63c
R	3.61±0.85a	4.06 ± 0.01a	4.06±0.94a	6.20±0.01b	6.03±0.33b
Total	64.84	68.82	66.91	67.86	68.66
IM*	65.53	67.47	67.47	66.10	68.52
Recovery (%)**	98.94	103.00	99.17	102.66	100.20
Protein content (%)	40.96	42.17	42.17	42.56	42.82

± standard deviation (SD)
* IM initial material (seeds or sprouts used to fractionation)
** in relation to IM
The letters a-e indicate significance within the same fraction; $p<0.05$

TABLE 5A. The percentage participation of nitrogen fraction in protein composition of lupin seeds (*Lupinus luteus* cv. Lord) and their sprouts after 3 and 4 days germination at 15 and 24˚C.

Nitrogen fraction	Seeds	Sprouts (15˚C)		Sprouts (24˚C)	
		3rd day	4th day	3rd day	4th day
A	19.68	15.26	16.01	13.10	12.32
Nnp	13.37	19.92	20.65	23.72	29.27
G	55.89	52.38	50.38	48.94	42.78
Gt+P	5.49	6.54	6.89	5.10	6.85
R	5.57	5.90	6.07	9.14	8.78

DISCUSSION

Until now, soybean has been the most studied and utilized legume. The great success of soybean as a source of protein initiated intensive investigations on other legumes, particularly lupin, that, except for high protein content, belongs to unique ecological plants (Gulewicz et al. 1994). Moreover, lupin protein has good functional properties, i.e.

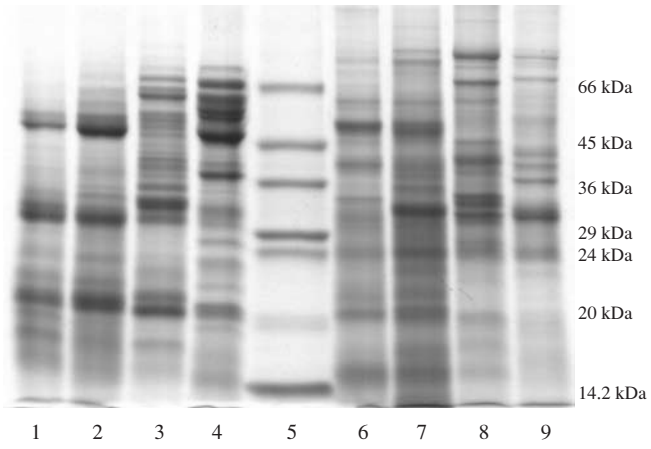

Fig. 2. SDS-PAGE electrophoregrams of albumin and globulin fractions of seeds and sprouts of *Lupinus luteus* cv. Lord and *Lupinus angustifolius* cv. Graf. **1** – albumin of *L. luteus* seeds; **2** – albumin of *L. angustifolius* seeds; **3** – albumin of *L. luteus* sprouted seeds; **4** – albumin of L. *angustifolius* sprouted seeds; **5** – molecular weight marker; **6** – globulin of *L. luteus* seeds; **7** – globulin of *L. angustifolius* seeds; **8** – globulin of *L. luteus* sprouted seeds; **9** – globulin of L. *angustifolius* sprouted seeds.

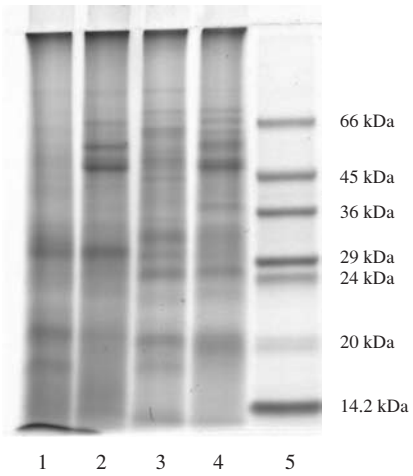

Fig. 3. SDS-PAGE electrophoregrams of glutelin+prolamin fractions of seeds and sprouts of *Lupinus luteus* cv. Lord and *Lupinus angustifolius* cv. Graf. **1** – glutelin + prolamin fractions of *L. luteus* seeds; **2** – glutelin+prolamin fractions of *L. angustifolius* seeds; **3** – glutelin + prolamin fractions of *L. luteus* sprouted seeds; **4** – glutelin + prolamin fractions of L. *angustifolius* sprouted seeds; **5** – molecular weight marker.

TABLE 6. Contents of amino acids of seeds and sprouts Nnp fractions of *L. luteus* cv. Lord and *Lupinus angustifolius* cv. Graf (in g/100 g of protein).

Amino acids	*Lupinus luteus* cv. Lord		*Lupinus angustifolius* cv. Graf	
	seeds	sprouts	seeds	sprouts
Non essential amino acids (NEAA)				
Asp	3.16	36.96	4.30	18.78
Glu	5.03	4.09	9.36	3.37
Ser	0.90	3.08	1.58	3.33
Gly	1.89	1.16	1.41	1.31
Arg	59.89	20.26	53.28	27.09
Ala	0.85	3.89	1.59	3.74
Pro	1.59	2.59	3.39	15.15
Essential amino acids (EAA)				
His	3.21	4.10	3.05	2.52
Val	0.73	1.76	1.32	6.36
Met	0.22	0.37	0.20	0.24
Cys	0.92	ND*	0.73	ND
Ile	0.47	1.04	0.73	2.12
Leu	0.70	1.62	1.31	2.43
Phe	10.11	8.02	5.41	3.36
Tyr	0.62	1.88	2.43	0.24
Lys	3.84	2.08	3.57	2.31
Thr	0.87	2.10	1.34	2.65
Total NEAA	**73.31**	**72.03**	**74.91**	**72.77**
Total EAA	**21.69**	**22.97**	**20.09**	**22.23**

* ND – not detected

emulsifying power, binding, and foaming properties (Edwin 1974; Mohamed and Rayas-Duarte 1995). In spite of these advantages, its utilization in human nutrition has been up to now not satisfied. One of the main reasons limiting their wide utilization is the presence in lupin seeds of quinolizidine alkaloids and the raffinose family oligosaccharides, that are considered as antinutritional factors. Among many methods used for removing these compounds (Ciesiołka et al. 2005), the germination of seeds seems to be the best, leading to their degradation and obtaining high quality products in human and animal nutrition (Snauwaert and Markakis 1976; Wojtaszek 1992; Vidal-Valverde and Frias 1992; Frias et al. 1995; Prodanov et al. 1997; Sierra and Vidal-Valverde 1999; de Cortes Sanchez et al. 2005). Hence, the choice of germination as the processing method was not accidental but well-grounded. In spite of using the same germination conditions for both lupin species the decrease of RFOs content was different. In case of *L. angustifolius* the highest decrease of RFOs at 24˚C after four days (from 80.6 to 7.2 mg/g) was observed (Table 2A). The highest decrease of RFOs content for *L. luteus* at 15˚C after four days (from 130.9 to 17.4 mg/g)

was noted (Table 2B). This phenomenon may by explained by different qualitative and quantitative compositions of both lupin species (Tables 2A and 2B). The decrease of RFOs content in the sprouts of both lupin species is in agreement with previous results (Vidal-Valverde and Frias 1992) and is caused by degradation of these sugars and utilization as carbon source in biosynthesis of new compounds. According to Vidal-Valverde et al. (2002), the decrease of RFOs content by germination of lentil, bean and pea seeds directly affect a the nutritive value of legumes.

The main limiting factor of using lupin as protein source are alkaloids which in toxic doses cause neuromuscular blockage, respiratory depression, cyanosis, cramps and cardiac arrest (Culvenor and Petterson 1986). For that reason, many studies on decrease of alkaloid content in lupin were carried out (Gulewicz 1988). Among many technological methods used for decrease of alkaloid content in lupin seeds germination has been recently considered as particularly promising (Trugo 1993; de la Cuadra et al. 1994; Cunha-Queda and Beirao da Costa 1994; de Cortes Sanchez et al. 2005). The results of our paper show that during germination in controlled conditions the total of lupin alkaloids are clearly reduced. However, the content of not all alkaloids is decreased. Similar results were obtained by other researches for these lupin species (Trugo 1993; de la Cuadra et al. 1994; Cunha-Queda and Beirao da Costa 1994). During germination, some degree of transformation of alkaloids into other more bioactive compounds, of such esters was observed. For example the ester 13-tigloyloxylupanine increased progressively during germination of *L. angustifolius* from 0 to 22.41% (Table 3A). In case of *Lupinus luteus*, in the third day of germination at 15 and 24˚C gramina appeared. These results are in full agreement with other studies (de Cortes Sanchez et al. 2005).

During germination deep physiological and biochemical changes are taking place. The hydration of seeds intensifies the activity of numerous enzymes starting a complex transformation of the matter. At this time the protein biosynthesis is on a very low level. Disintegration of the endosperm storage proteins to low molecular compounds like peptides and amino acids, begins at the moment of seed cover puncture, that is after ca. 24 h of imbibition (Grzesiuk and Kulka 1988). In this respect storage protein plays an important function in developing seedlings as a source of nitrogen and amino acids.

In our present studies we also concentrate our attention on the assessment of germination effect on protein fractional composition of *Lupinus luteus* and *Lupinus angustifolius* seeds. This approach allows the determination of nutritive value of lupin seeds and sprouts on the basis of chemical analysis. Obviously, in comparison to the method based on the nutritional experiment, this method gives not the most reliable results, but is not so expensive and time-consuming and is suitable for wide use. The chemical method of evaluating the nutritive value of proteins is based on determination of total nitrogen that may be converted into so-called "crude protein", and may define its fractional composition. Certainly, we are aware of possible critical points of view connected with the term "crude protein" (Tkachuk 1969; Cherry 1977; Ewart 1981; Boisen et al. 1987; Haque et al. 1989). However, the interpretation of such analysis is based on the assumption that structural protein, e.g. albumins, as a rule have a richer amino acid composition and higher biological value than storage protein. Moreover, it may be accepted that lack of higher aberration in the composition of protein fractions indirectly certifies the lack of changes in amino acid composition of total protein. This assumption results from the statement saying that a composition of "individual" protein is conditioned by genetic features and outward factors have no impact on it. Naturally, protein fractions are not "individual" proteins, but they may be considered as groups of related substances with similar properties and relatively poorly diversified amino acid composition. The choice of a fractionation method for the studies appeared to be very accurate. The values of recovery presented in Table 4 and 5 show that the used method gave reproductive and reliable results. The comparison of total mg N of fractions with total mg N in the seeds, showed that the used method was very accurate, which is confirmed by the high level of recovery.

The results of fractional protein composition of initial material (IM) and the germinated, presented in Tables 4, 4A, 5 and 5A show deep changes in protein fractional composition. It concerns the clear decrease of globulins and residual fractions and the increase Nnp in both the tested lupin species. These results are in full conformity with the results of Gulewicz et al. (2008) for other lupin varieties. The decrease of Nr fraction may prove that the germination process causes degradation of protein cell wall to low molecular compounds peptide and amino acids. These proteins are not soluble in the used fractionation solvents, and except for nucleic acids compounds (purines, pyrimidines), poliamines are a part of nitrogen residue fraction (Nr). The degradation of cell-wall proteins and decrease of globulin fraction content during germination results in the increase of Nnp fraction may be of considerable importance from nutritional point of view (Hill 1977). Moreover, amino acid analysis of Nnp fraction showed that germination caused an increase of EAA and the decrease of NEAA, what is also beneficial from nutritional point of view.

In the light of the obtained results it seems to be purposeful to pay attention to obtaining nitrogen balance (total nitrogen of protein fractions) presented in Tables 4 and 5. As we mentioned above in both lupin species the total nitrogen is significantly higher for sprouts than in the initial material. Here the question comes why increase of nitrogen content is observed in the sprouts. This questions is much more interesting because under the used experimental conditions of the germination no additional nitrogen source was used and the total nitrogen of seeds and sprouts should be the same. Unfortunately, the used Kjeldahl method for nitrogen determination does not determine the nitrite (III) and nitrate (V) contents. According to the results of Okafor et al. (2002), the content of these compounds in different legume species ranges from 49 to 239 mg/100 g of seeds. Hence, we may conclude that during germination these nitrogen forms are included in biosynthesis of proteins and other nitrogen compounds. Involving the nitrites (III, V) into biosynthesis is especially beneficial from nutritional point of view because they negatively affect a human health (Pannala et al. 2003; Chen et al. 2004).

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