ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 8(3) 2009, 87-96*
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

EFFECT OF FERMENTATION AND EXTRUSION ON THE RELEASE OF SELECTED MINERALS FROM LUPINE GRAIN PREPARATIONS*

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Introduction. Antinutritional factors in legumes lower the nutritional value of foods by lowering the digestibility or bioavailability of nutrients. Technological processes applied in food production (e.g. extrusion, fermentation, germination) may influence the leguminous seeds matrix composition which in consequence may affect (improve or decrease) mineral bioaccessibility and uptake in animals and humans. The aim of this study was to determine the effect of fermentation and extrusion processing, as well as their combination, on the potential availability of Fe, Cu, and Zn from lupine grain preparations.
Materials and methods. The content and the release of Fe, Cu and Zn from three different lupine species (*Lupinus albus, Lupinus luteus, Lupinus angustifolius*) was determined. The samples were subjected to enzymatic digestion under *in vitro* conditions. The content of minerals in lupine grains before and after enzymatic digestion was determined by the flame atomic absorption spectrometry.
Results. The degree of release of Fe, Cu, and Zn from dehulled lupine grains was higher than from whole grains. Fermentation processing increased the degree of Fe release, extrusion decreased the degree of Cu release, while the extrusion after fermentation processing increased the degree of Fe release from lupine grain preparations.
Conclusions. The degree of mineral release from lupine grains depends on the hull content, technological processing applied and the lupine variety.

Key words: lupine, fermentation, extrusion, iron, copper, zinc

*The experiment was an integral part of the research grant nr 2PO6T08628 financed by the Ministry of Science and Informatisation of Poland, in the years 2005-2008.

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INTRODUCTION

Lupine species have been cultivated by humans since ancient times. At the end of the 1920s, the development of new lupine varieties containing low levels of alkaloids resulted in a renewed interest in this legume. The importance of lupine as valuable source of nutrients to be used in human or animal nutrition has increased in recent years [Egana et al. 1992].

Lupine grains contain significant amounts of protein, fat, minerals and dietary fiber [Barneveld 1994, Dupont et al. 1994, Rahman et al. 1997]. However, the leguminous grains also contain antinutritional factors, such as proteinase inhibitors, lectins, saponins and phytates. Some of these substances may reduce the bioavailability of minerals, either due to formation of extremely insoluble salts, or very poorly dissociated chelates [Alonso et al. 2000 a, Hall et al. 2005, Lampart-Szczapa et al. 2003]. With the aim of decreasing the amount of non-nutritional components that interfere with an optimal nutritive utilisation of legumes, different technological processes have been applied, including fermentation, germination, soaking at different pH levels, cooking and enzyme supplementation [Porres et al. 2006]. A benefit derived from heat and pressure treatment is a partial or total destruction of potentially antinutritional factors, especially protease inhibitors, haemaglutinins, tannins and phytates, which limit bioavailability of minerals [Alonso et al. 2000 b].

The aim of this study was to determine the effect of fermentation and extrusion processing, as well as their combination, on the potential availability of Fe, Cu, and Zn from lupine grain preparations.

MATERIALS AND METHODS

The experimental material comprised various kinds of lupine grains of yellow lupine (*Lupinus luteus*, Juno and Parys var.), white lupine (*Lupinus albus*, Butan and Boros var.) and narrow-leaved lupine (*Lupinus angustifolius*, Cesar and Baron var.). Materials came from The Plant Breeding Acclimatization Experimental Station in Przebędowo near Murowana Goślina in Poland. All kinds of lupines were grown in the equal cultivation conditions in the year 2001. Lupine grains (whole and dehulled) were ground under laboratory conditions with a Rekord impact mill and divided with proper sieves into fractions with particles having maximal diameters below 2 mm. The lupine preparations (obtained from whole and dehulled grains) were subjected to technological processing: fermentation, extrusion and fermentation followed by extrusion.

The process of fermentation relied on lactic acid fermentation performed by strains of *Leukonostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis* bacteria at 37°C for 20-24 h at moisture 60%, range of pH: 4.0-4.2. The process of extrusion was performed in the snail extruder Krupp Werner & Pheidere type ZSK 25P.8.2, at moisture 35% and temperature in successive parts of the extruder: 95/120/140/130°C.

The *in vitro* sample digestion was conducted by the method described by Skibniewska et al. [2002] with appropriate modifications. The comminuted sample (2 g) was mixed for 10 min with 20 cm^{-3} deionised water. The solution was brought to pH = 2 using 0.1 $mol \cdot dm^{-3}$ HCL and pepsin solution (16 g pepsin in 100 cm^{-3} 0.1 $mol \cdot dm^{-3}$ HCl) in the amount of 0.5 cm^{-3} per 100 cm^{-3}. The homogenate was added to the sample which was then incubated for 2 h at the temperature of 37°C shaking it in a water bath. The homogenate's pH was controlled throughout the incubation process correcting it with 6 mol·dm^{-3} HCl. After the incubation the pH of the solution was brought to the level of 6.8-7.0 using 6% $NaHCO_3$ and then a solution of pancreatin was added (4 g pancreatin in l dm^{-3} 0.1 mol·dm^{-3} $NaHCO_3$) in the amount of 10 cm^{-3} per 40 cm^{-3} of the sample and incubated for 4 h. The digested sample was filtered through a filter into quartz flasks. The supernatant was mineralized with concentrated 10 cm^{-3} nitric acid (Suprapure, Merck) and 5 cm^{-3} perchloric acid (Suprapure, Merck) in a heating block (Mineralizator TH2 digital).

The Fe, Cu and Zn contents in the crude lupine preparations (non-modified and after processing), was determined after dry ashing of samples (in a muffle furnace at 450°C), while in the digested samples, after wet mineralization, by the flame ASA method, using the Zeiss AAS-3 spectrophotometer with BC. The accuracy of the method was assured on the basis of the simultaneous analysis of the reference material (CTA-VTL--2, *Virginia Tobacco Leaves, Poland*) that reached: 96.7%, 102.2% and 94.2% for Fe, Cu and Zn, respectively.

The release of a mineral from lupine grains (expressed as the percentage of the total mineral content) was calculated using the following formula: the amount of a mineral released in enzymatic digestion (mg/100 g d.m.)/the total mineral content in lupine grain preparation (mg/100 g d.m.) · 100%.

The statistical analysis was carried out employing the Statgrafics ver 7.0 computer programe (Manugistic Inc. USA).

RESULTS AND DISCUSSION

The results of the content of Fe, Cu and Zn in the lupine grains and the release of these minerals after enzymatic digestion is presented in Tables 1-6. It was found that the content of Fe, Cu and Zn in lupine grains depends on the kind of grain preparation (both lupine variety and the hull content).

As can be seen in Tables 1-3, a removal of hull from the whole lupine grains resulted in a significant reduction of the content of Fe in the yellow and white lupine, while a significant increase of the content of Cu in the yellow lupine and the content of Zn in all kinds of lupine grains. Besides, the conditions of cultivation e.g. soil fertilisation, atmospheric conditions and herbicides used during the growth period could influence on the content of minerals in lupine grains, but these conditions were equal for all the plant materials.

Technological processing applied also affected in various ways the content of these minerals in lupine grain preparations. The content of Fe and Cu in the processed lupine grains was significantly higher (in most cases) after extrusion and extrusion and fermentation, while fermentation alone in some cases (the white and narrow-leaved lupine) increased the content of Cu (both in whole and dehulled grains), however it did not affect the yellow lupine grains. Generally the strongest effect on the content of Fe and Cu in lupine grains was observed after extrusion processing. Besides, independently on the grain type (whole and dehulled) and processing applied, the highest content of Fe and Cu was determined in the yellow lupine vs the white and narrow-leaved lupine grains.

Table 1. The mean content of iron in lupine grains, mg/100 g d.w.

Process	Yellow lupine		White lupine		Narrow-leaved lupine	
Whole grains		B		B		A
Without modification	8.72 ±0.31	a	3.66 ±0.12	a	4.80 ±0.15	a
After fermentation	8.21 ±0.83	a	4.22 ±0.16	a	5.08 ±0.10	a
After extrusion	8.43 ±0.20	a	7.98 ±0.20	c	6.75 ±0.23	c
Extrusion after fermentation	10.49 ±0.62	b	5.31 ±0.09	b	5.96 ±0.12	b
	b1		a1		a1	
Dehulled grains		A		A		A
Without modification	5.25 ±0.11	a	2.84 ±0.08	a	4.10 ±0.18	a
After fermentation	5.50 ±0.14	a	4.36 ±0.19	b	4.82 ±0.18	a
After extrusion	6.01 ±0.44	a	6.43 ±0.37	c	8.82 ±0.66	c
Extrusion after fermentation	8.95 ±0.71	b	5.89 ±0.35	c	8.32 ±0.58	b
	b1		a1		a1	
	B1		A1		A1	

The mean ±SD content of the mineral taken from three parallel samples: A, B – significant differences between whole and dehulled grains, a, b – significant differences between technological modifications, a1, b1 – significant differences between kinds of lupine within whole or dehulled grains, A1, B1 – significant differences between kinds of lupine.

Table 2. The mean content of copper in lupine grains, mg/100 g d.w.

Process	Yellow lupine		White lupine		Narrow-leaved lupine	
Whole grains		A		A		A
Without modification	0.98 ±0.02	a	0.51 ±0.01	a	0.47 ±0.01	a
After fermentation	1.13 ±0.05	b	0.56 ±0.02	b	0.61 ±0.02	a
After extrusion	1.16 ±0.03	b	0.67 ±0.02	c	0.78 ±0.02	a
Extrusion after fermentation	0.99 ±0.02	a	0.57 ±0.01	b	0.63 ±0.03	a
	b1		a1		a1	
Dehulled grains		B		A		A
Without modification	1.51 ±0.06	a	0.46 ±0.01	a	0.58 ±0.05	a
After fermentation	1.39 ±0.04	a	0.62 ±0.03	b	0.76 ±0.04	b
After extrusion	1.51 ±0.03	a	0.62 ±0.03	b	0.83 ±0.02	b
Extrusion after fermentation	1.39 ±0.05	a	0.60 ±0.02	b	0.82 ±0.03	b
	b1		a1		a1	
	B1		A1		A1	

Description as in Table 1.

Table 3. The mean content of zinc in lupine grains, mg/100 g d.w.

Process	Yellow lupine		White lupine		Narrow-leaved lupine	
Whole grains		A		A		A
Without modification	8.26 ±0.70	a	5.18 ±0.22	a	4.84 ±0.28	a
After fermentation	6.84 ±0.63	a	4.64 ±0.17	a	4.08 ±0.17	a
After extrusion	8.64 ±0.74	a	5.31 ±0.14	a	4.76 ±0.22	a
Extrusion after fermentation	6.87 ±0.30	a	5.11 ±0.19	a	4.55 ±0.16	a
	b1		a1		a1	
Dehulled grains		B		B		B
Without modification	10.46 ±0.79	a	5.43 ±0.33	a	5.02 ±0.31	a
After fermentation	8.98 ±0.54	a	5.56 ±0.27	a	5.91 ±0.16	a
After extrusion	9.11 ±0.63	a	5.88 ±0.22	a	5.24 ±0.50	a
Extrusion after fermentation	8.76 ±0.70	a	5.68 ±0.27	a	5.75 ±0.29	a
	c1		b1		a1	
	B1		A1		A1	

Description as in Table 1.

Interestingly enough, the content of Zn in the lupine preparations did not depend on the hull content and technological processing. On the other hand, the content of this element was significantly higher in the yellow lupine grains *vs*. the white and narrow--leaved lupine grains, independently on the processing applied.

The release of minerals (Fe, Cu and Zn) from lupine grains preparations, which may reflect (to some extend) the potential availability after enzymatic digestion, depended on the type of grains, lupine kind and technological processing is shown in Tables 4-6. The release of these nutrients was highly variable, however some rules can be drawn from the obtained data. The release of Fe from the plant material, in most cases, did not depend of the hull content, except the yellow lupine material, which was significantly higher from the dehulled grains *vs.* the whole grains. The release of Cu was not affected by the hull content, but in the narrow-leaved lupine which was markedly higher in the dehulled grains. On the contrary, the release of Zn from the lupine grains was significantly higher after dehulling, except the narrow-leaved lupine grains.

Technological processing also influenced in various ways the release of Fe, Cu and Zn from lupine grains. Generally the release of Fe from lupine preparations was significantly higher after fermentation and fermentation after extrusion, however in different degree, depended on the lupine variety (Table 4).

The release of Cu and Zn from the plant materials was appreciably elevated after fermentation, and fermentation after extrusion only in the case of the yellow lupine (both whole and dehulled grains), while not for the white and the narrow-leaved lupine grains (Table 5-6). On the other hand, unlike in the case of Fe, the release of these microelements was significantly higher for the white and the narrow-leaved lupine grains (independently on the hull content and technological processing applied.

Table 4. Influence of experimental factors on the release of iron from lupine grains, %

Process	Yellow lupine		White lupine		Narrow-leaved lupine	
Whole grains		A		A		A
Without modification	9.05	a	18.43	a	13.95	a
After fermentation	19.18	b	14.51	a	22.00	b
After extrusion	9.88	a	11.91	a	12.46	a
Extrusion after fermentation	20.37	b	19.37	b	14.55	b
	a1		a1		a1	
Dehulled grains		B		A		A
Without modification	15.38	b	15.37	a	10.67	a
After fermentation	27.24	c	17.99	b	25.63	d
After extrusion	6.31	a	21.16	b	13.66	b
Extrusion after fermentation	31.32	c	24.70	c	18.45	c
	b1		b1		a1	

Description as in Table 1.

Table 5. Influence of experimental factors on the release of copper from lupine grains, %

Process	Yellow lupine		White lupine		Narrow-leaved lupine	
Whole grains		A		A		A
Without modification	53.99	b	46.28	b	56.53	b
After fermentation	48.55	b	34.04	a	56.45	b
After extrusion	43.18	a	46.41	b	34.68	a
Extrusion after fermentation	59.21	b	30.92	a	37.10	a
	b1		a1		a1	
Dehulled grains		A		A		B
Without modification	68.04	b	53.97	a	58.32	b
After fermentation	57.78	b	50.61	a	44.95	a
After extrusion	32.85	a	47.14	a	36.61	a
Extrusion after fermentation	78.10	c	50.15	a	34.00	a
	b1		a1		a1	

Description as in Table 1.

Table 6. Influence of experimental factors on the release of zinc from lupine grains, %

Process	Yellow lupine		White lupine		Narrow-leaved lupine	
Whole grains		A		A		A
Without modification	9.43	a	26.55	a	36.70	a
After fermentation	14.48	b	33.68	a	34.75	a
After extrusion	12.84	b	31.74	a	28.38	a
Extrusion after fermentation	21.41	c	23.14	a	26.37	a
	a1		b1		b1	
Dehulled grains		B		B		A
Without modification	11.95	a	27.43	a	36.09	b
After fermentation	15.75	b	25.20	a	23.41	a
After extrusion	4.08	a	23.59	a	27.63	a
Extrusion after fermentation	23.56	b	30.65	a	26.08	a
	a1		b1		b1	

Description as in Table 1.

Similar tendencies were observed in our previous studies on different types of lupine grains [Krejpcio et al. 2007 a, b, Lampart-Szczapa et al. 2003]. These changes are probably due to the higher content of antinutritive compounds, like: phytates, tannins and fibre in the whole grains, *vs* the dehulled grains, that have strong affinity to bind minerals, depressing their liberation during *in vitro* enzymatic digestion [Funatsuki et al. 2000, Sandberg 2002]. Hung et al. [2006] found significantly higher contents of minerals in the endosperm than in the hull. However, in legume grains minerals bound with phytatic acid are also located in the protein of the endosperm, these forms are insoluble at the physiological pH of the intestine [Sandberg 2002]. Gąsiorowski [1997] found that some hydrated fibre fractions may reduce the rate of interaction between enzymes and foods which could also be a factor responsible for poor digestibility of nutrients from leguminous and cereal grains. Rahman et al. [1997] reported that plant proteins, apart from phytates and fibre, can play an important role in binding minerals in lupine grains.

As can be seen from Tables 4 and 6, fermentation and extrusion after fermentation processing markedly increased the degree of Zn and Fe release from the whole and dehulled yellow lupine grains. In the case of Fe, the same shift was observed also in the whole and dehulled white and narrow-leaved lupine grains (Table 4). In this study the extrusion increased the release of Zn from the whole yellow lupine grains and the release of Fe from the dehulled white and narrow-leaved lupine grains (Tables 4 and 6).

The observed changes of the mineral release from processed lupine grains seem to confirm the opinion that fermentation by increasing the activity of native phytase, and extrusion (application of high temperature and pressure) can disintegrate insoluble organic complexes with minerals [Bhatia and Khetarpaul 2002]. The heat treatment may reorganise dietary fibre structure, this changing its chelating affinity. Brzozowska [1996] reported that fermentation, in contrast to extrusion, increases the phytase activity that in consequence increases availability of minerals. Other Authors [Bhatia and Khetarpaul 2002, Camacho et al. 1991, Lopez et al. 2001] showed that fermentation of bean, lupine and cereal grains diminished concentration of phytic acid in grains and increased bioavailability of Fe and Zn. However, phytase is sensitive to temperature and the length of time of fermentation, therefore temperature and time conditions applied in the present experiment could have an impact on the obtained results [Adeola et al. 1995, Brzozowska 1996].

According to Rahman et al. [1997] lupine grains (*Lupinus angustifolius*) contain a relatively small amounts of phytates therefore these compounds play rather a minor role in mineral binding. Fermentation and extrusion could also (except phytates and fibre) liberate minerals from complexes with protein fraction (highly effective as minerals binder).

In this study, the extrusion processing significantly decreased the release of Cu from the yellow and narrow-leaved lupine grains independently of the hull content (Table 5). The inhibitory effect of extrusion on the release of Fe in the dehulled yellow lupine grains and the release of Zn from the dehulled narrow-leaved lupine grains was also observed (Tables 4 and 6).

The data obtained in the present study are in agreement with those reported by Skibniewska et al. [2002], who found lower release of Fe and Zn from extruded oat *vs* crushed oat. Extrusion, depending on the type of material and technological process conditions (temperature, pressure, moisture) could probably change the structure of organic complexes with minerals into insoluble salts or poorly bioavailable chelates [Skibniewska et al. 2002].

The release of Cu was markedly higher from the yellow lupine than from the white and the narrow-leaved lupine grains, while the inverse relationship was observed in the case of the realease of Zn (Tables 5 and 6). The release of Fe from the dehulled narrow-leaved lupine grains was significantly lower than from the yellow and white lupine grains (Table 4). Porres et al. [2007] also fund that the effect of the α-galactoside extraction process on mineral content varied with the different lupine species.

CONCLUSIONS

1. The degree of mineral release from lupine grains depends on the hull content, technological processing applied, and the analysed lupine species.
2. The degree of release of Fe, Cu and Zn in *in vitro* enzymatic digestion from the whole and dehulled grains depends on the lupine variety.
3. Fermentation and extrusion after fermentation increase the degree of release of Fe from lupine grain preparation.
4. Extrusion processing decreases the degree of Cu release from lupine grain preparation.

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WPŁYW FERMENTACJI I EKSTRUZJI NA STOPIEŃ UWOLNIENIA WYBRANYCH SKŁADNIKÓW MINERALNYCH Z PREPARATÓW NASION ŁUBINU

Wstęp. Czynniki antyodżywcze zawarte w roślinach strączkowych zmniejszają właściwości odżywcze żywności poprzez zmianę trawienia i bioprzyswajalności składników pokarmowych. Procesy technologiczne stosowane w produkcji żywności (np. ekstruzja, fermentacja, obłuszczanie) mogą oddziaływać na zmianę składu i właściwości matrycy, a poprzez to wpływać (zwiększać lub zmniejszać) na biodostępność i wchłanianie składników mineralnych u ludzi i zwierząt. Celem badań była ocena wpływu fermentacji i ekstruzji oraz ich kombinacji na potencjalną dostępność Fe, Cu i Zn z preparatów nasion łubinu.
Materiał i metody. Określono zawartość i uwalnianie Fe, Cu i Zn z trzech różnych gatunków łubinu (*Lupinus albus*, *Lupinus luteus*, *Lupinus angustifolius*). Próbki śruty łubinowej poddano trawieniu enzymatycznemu *in vitro*. Zawartość składników mineralnych przed i po trawieniu enzymatycznym oznaczono po mineralizacji próbek na sucho lub na mokro metodą płomieniowej spektrofotometri atomowo-absorpcyjnej.
Wyniki. Stopień uwolnienia Fe, Cu i Zn z nasion obłuszczonych na ogół był wyższy aniżeli z nasion z łuską. Proces fermentacji wpływał na wzrost stopnia uwalniania Fe, a proces ekstruzji na spadek uwalniania Cu z preparatów łubinowych. Zastosowanie ekstruzji po fermentacji wpłynęło na wzrost uwalniania Fe z nasion łubinu.
Wnioski. Stwierdzono, że stopień uwolnienia składników mineralnych zależy od zawartości łuski, procesu technologicznego i gatunku łubinu.

Słowa kluczowe: łubin, fermentacja, ekstruzja, żelazo, miedź, cynk

Accepted for print – Zaakceptowano do druku: 4.06.2009

For citation – Do cytowania: Suliburska J., Krejpcio Z., Lampart-Szczapa E., Wójciak R.W., 2009. Effect of fermentation and extrusion on the release of selected minerals from lupine grain preparations. Acta Sci. Pol., Technol. Aliment. 8(3), 87-96.