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CONTENT OF AMINO ACIDS AND MINERALS IN SELECTED SORTS OF LEGUMES

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ABSTRACT

The aim of this study was to determine amino acid composition and mineral content in selected legume samples. All analyses were carried out at the laboratory temperature of 21±2 °C in triplicate. Amino acid composition was determined using the automatic amino acid analyzer AAA 400 with post-column derivatization. To assess the nutritional value of protein, index of essential amino acids (EAAI) was calculated. Minerals were determined using the atomic absorption spectrometer AA 30. All results were statistically evaluated. The highest content of Cys, Glu, Asp, Leu, Lys and Arg was determined in seeds of *G. max*; only the content of Cys and His was lower than 10 g kg^{-1}. The greatest total content of essential amino acids (EAA) was discovered in soybeans, almost 128 g kg^{-1}. The majority (Na, K, Mg, and Ca), trace (Fe, Zn, and Cr) and toxic elements (Pb, Cd) were determined. Legumes were rich in Mg and Ca-mainly *G. max* and *Ph. vulgaris*. The content of Mg in was 2.1 g $1000g^{-1}$ in soybeans and 1.6 g $1000g^{-1}$ in common beans. Also in these two legumes the greatest concentration of toxic Pb was found. Values obtained during the determination of the chemical composition in samples of legumes and buckwheat products can be influenced by many factors, e.g. climatic conditions, location etc.

Keywords: legumes; amino acid; protein; minerals; statistics

INTRODUCTION

Legumes are dry edible seeds of some plants from the family of *Fabaceae*. The nutritional potential of seeds from this group of plants is based on their high level of proteins. Legume seeds are the richest and cheapest alternative sources of protein among all foods of plant origin. Protein content in legume grains ranges from 17 to 40%, being equal to the protein contents of meat (18-25%). However, the legumes also contain antinutritional factors, such as proteinase inhibitors, lectin, rafinose oligosaccharides, saponins, polyphenols and phytate **(Urbano et al., 2000; Sandberg, 2002; De Almeida Costa et al., 2006)**.

Grain legumes are commonly subdivided into pulses which, in addition to protein, store high levels of carbohydrate and low amount of lipids in their dry seeds, and leguminous oilseeds which boast higher lipid, but lower carbohydrate levels than pulses. Pulses also contain high levels of dietary fiber **(Michaels, 2004)**.

Legumes provide a large amount of proteins, carbohydrates, dietary fibre, minerals and water-soluble vitamins in human diets. They can be considered as food with health benefits, but their phytate content can limit the availability of minerals **(Frias et al., 2003)**.

Low digestibility hampers full utilization of pulse protein. Antinutritional factors in pulses also play a major role in restricting dietary utilization in some pulses species. These compounds usually include proteinaceous molecules such as protease inhibitors, and lectins, and also nonproteinaceous compounds such as tannins. Most of the wild relatives of pulses contain toxins and antimetabolites. Tannins can form strong cross-linked complexes with dietary proteins and enzymes **(Michaels, 2004)**.

Incorporation of leguminous seeds into the human diet in developing countries can offer protective effects against chronic diseases. Legumes contain a number of bioactive substances including phenolics that can diminish protein digestibility and mineral bioavailability **(Chung et al., 1998; Sandberg, 2002).** On the other hand, phenolic compounds such as flavonoids, phenolic acids, lignans and tannins have antioxidant properties. They are very important from the nutritional and technological point of view **(Amarowicz & Pegg, 2008).**

Grain legumes are used as pulses with cereals, grown in both tropical and temperate regions of the globe. They enhance the protein content of cereal-based diets and may improve the nutritional status of the cereal-based diets. Cereals are deficient in lysine. Legumes contain adequate amounts of lysine, but are deficient in S-containing amino acids, methionine and cysteine **(Tharanathan & Mahadevamma, 2003; Iqbal et al., 2006).**

Minerals are essential nutrients for human well-being and they play a vital role in the effective functioning of the body activity. Currently, mineral malnutrition is considered to be one of the most serious global challenges for mankind **(Copenhagen Consensus, 2004).** Over three billion people suffer from micronutrient malnutrition worldwide, leading to poor health, anaemia, lower productivity, increased morbidity, and mortality rates. The most prevalent micronutrient deficiencies are Fe, Zn and I, which occur particularly among children and women in developing countries. Phytic acid chelating essential minerals such as Fe, Zn and Ca can have serious negative impact on the utilization of mineral nutrients and lead to malnutrition in humans. Now, breeding for staple micronutrient-enriched food crops with low phytic acid

content is considered as a cost-effective and promising approach to alleviate malnutrition and other related health problems **(WHO, 2002; Welch & Graham, 2004; Liu et al., 2005; Tang et al., 2008; Chatzav et al., 2010; Wang et al., 2011).**

Minerals are important for various physiological functions in the human body. In many metabolic processes in the human body, many minerals have an irreplaceable role. Regular supply of minerals in appropriate amounts is very important for the body. The surplus and deficiency can have very serious consequences. The proportions of individual elements can greatly influence the final effect in the body **(Turek, 2007).**

The aim of this study was to determine content of minerals and amino acid composition in selected sorts of legumes.

MATERIAL AND METHODOLOGY

In this study selected legume samples, common beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), soybeans (*Glycine max*) and lentil (*Lens esculenta*), were used for the analysis. All legume samples were purchased in the trade network.

SAMPLE PREPARATION

Samples were packaged in consumer wrapping. A package of dry samples was ground to a fine powder and sieved through 1 mm mesh. After 24 hours of resting, the powder was poured into sample containers and subsequently, particular analyses were performed. They were realized according to the Official Journal of the European Union **(Commission Regulation, 2009)**. All analyses were carried out at the laboratory temperature of 21±2 °C in triplicate. All used reagents were of the analytical grade, they were from the company PENTA, Chrudim, Czech Republic, unless stated otherwise.

AMINO ACIDS

Before determination of total amino acid composition, amino acids were released from proteins and peptides by acid hydrolysis (6 mol L^{-1} HCl, 115 °C, 23 h). Sulfur amino acids (cysteine and methionine) were, prior to acid hydrolysis, oxidized by mixture of formic acid and hydrogen peroxide (9:1; 6±2 °C, 16h), because acid hydrolysis would cause their degradation. After hydrolysis, HCl was evaporated on vacuum evaporator RVO 400 (INGOS, Prague, CZ) to the consistency of syrup, the residue was dissolved with sodium citrate buffer (pH 2.2) and filtered through 0.45 μm filter (Millipore, USA) before analysis. Amino acids were analyzed by ion-exchange liquid chromatography on an automatic amino acid analyzer AAA 400 (INGOS, Prague, CZ) with post-column ninhydrin derivatization and spectrophotometric detection (440 nm for proline and 570 nm for other amino acids) **(Buňka et al., 2004; Lazárková et al., 2011)**. Chromatographic column 250x4 mm (Polymer AAA 8u; ion exchanger Ostion LG ANB) was used.

Cysteine was determined as cysteic acid, methionine as methioninsulfone. Sodium system is faster, but does not allow separation of amides (asparagine, glutamine) **(Anonym, 2007)**.

To assess the nutritional value of protein, index of essential amino acids (EAAI) was calculated. As reference file, egg white protein was chosen and to compare, the standard protein designated by WHO/FAO was used (**Table 1**).

Essential Amino Acid Index is a geometric mean of ratios of essential amino acids expressed in percentage in studied protein food to the same standard amino acids in egg protein. EAAI provides more accurate data than the amino acid score **(Kráčmar et al., 1981)**.

Table 1 Content of essential amino acids in standard (FAO/WHO) and egg protein **(Velíšek, 1999; Dvořáčková et al., 2011)**

Amino acid	FAO/WHO (g $16gN^{-1}$)	Egg protein (%)
Val	5.0	7.3
Leu	7.0	8.7
Ile	4.0	6.6
Met + Cys	3.5	5.7
Thr	4.0	5.1
Lys	5.4	6.9
Phe + Tyr	6.1	9.8

MINERAL ELEMENTS

Samples (0.3 to 0.5±0.01g) were decomposed in a microwave device Ethos SEL (Milestrone, Sorisole, Italy) using concentrated HNO_3 (5 ml conc. HNO_3 + 5 ml of deionised H_2O) at a temperature of 210 °C for 30 min. The final was transferred into 25 ml volumetric flasks after cooling to 80 °C. Flasks were refilled to the mark after cooling to a room temperature. Mineralisation solutions were processed on the atomic absorption spectrometer AA 30 (Varian A.G., Australia).

Na, K, Ca, Mg, Fe, Zn and Cu were determined by flame AAS (acetylene-air). Strontium nitrate at a concentration of 1000 mg L-1 was used as a spectral buffer to suppress the emission in the case of Ca, Mg. Cu, Fe, Zn, Ca and Mg were measured in absorption mode while Na and K in emission mode. Pb, Cd and Cr were measured in absorption mode with electrothermal atomization in the graphite cuvette. For protection, the N_2 gas was elected in a purity of 5.0. A matrix modifier (10 g L^{-1} solution $NH_4H_2PO_4$ + 10 g L^{-1} solution of Mg $(NO_3)_2$ (Sigma Aldrich, USA) and a deuterium lamp background correction was used in the case of Pb and Cd. A 10 g L^{-1} solution of ascorbic acid (reduced formation of CrO_2Cl_2) was selected as a matrix modifier for Cr determination. Evaluation of concentration in all elements was performed by the calibration curve method and the integration of peak area.

STATISTICAL EVALUATION

All results were statistically evaluated using the variation statistics (ANOVA). Correlation matrices and regression functions were calculated according to **Snedecor and Cochran (1967)** using the statistical package Unistat, v. 5.5 (Unistat Ltd., England, UK).

RESULTS AND DISCUSSION

AMINO ACIDS

Amino acid composition was determined using the analyzer AAA 400 with post-column derivatization.

In total, 17 amino acids (glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, glutamic acid, lysine, arginine, histidine, phenylalanine, tyrosine, proline, methionine and cysteine) were determined. Tryptophan was not determined, because it is destroyed during acid hydrolysis and requires alkaline hydrolysis.

As can be seen from **Table 2 and 3**, all studied samples contain all 17 amino acids (AA). The highest content of Cys, Glu, Asp, Leu, Lys and Arg was determined in all legumes. The greatest concentration of almost all amino acids was discovered in soybeans; only the content of Cys and His was lower than 10 g kg^{-1}. **Jezierny et al. (2010)** reported the amino acid composition of *P. sativum* in their study. Values for almost all studied amino acids were higher then those in this experiment; only for Met they presented a value of 2.2 g kg^{-1} DW which is lower then the one in the experiment and value for Cys (3.5 g kg^{-1}) which is close to the value in the study (3.6 g kg^{-1}).

Table 2 Amino acid composition of *G. max* and *P. sativum* (mean±S.D.) in g kg^{-1} DW

AA	*G. max*			%CV	*P. sativum*			%CV
Cys	6.6	±	0.55	8.0	3.6	±	0.02	1.0
Glu	51.9	±	4.11	8.0	22.0	±	2.68	12.0
Asp	33.5	±	1.22	4.0	16.0	±	1.66	10.0
Tyr	10.1	±	0.42	4.0	4.3	±	0.42	10.0
Ser	14.1	±	0.81	6.0	6.1	±	0.71	12.0
Pro	16.7	±	1.79	11.0	5.8	±	0.76	13.0
Gly	12.6	±	0.96	8.0	6.3	±	0.65	10.0
Ala	12.7	±	0.94	7.0	6.1	±	0.68	11.0
Val	15.5	±	1.47	10.0	7.2	±	0.73	10.0
Leu	23.1	±	1.29	6.0	10.4	±	0.79	8.0
Ile	14.7	±	1.12	8.0	6.4	±	0.50	8.0
Thr	11.1	±	0.72	6.0	5.1	±	0.57	11.0
Met	28.6	±	2.18	8.0	8.2	±	1.16	14.0
Lys	18.9	±	1.82	10.0	10.4	±	1.01	10.0
Phe	16.0	±	1.05	7.0	7.5	±	0.68	9.0
His	7.9	±	0.51	6.0	4.0	±	0.41	10.0
Arg	27.4	±	2.29	8.0	12.5	±	0.94	7.0

%CV - Coefficient of Variation; DW - Dry Weight; S.D.- Standard Deviation

Table 3 Amino acid composition of *Ph. vulgaris* and *L. esculenta* (mean±S.D.) in g kg^{-1} DW

AA	*Ph. vulgaris*			%CV	*L. esculenta*			%CV
Cys	2.8	±	0.02	1.0	2.6	±	0.09	3.0
Glu	23.1	±	0.52	2.0	24.7	±	1.07	4.0
Asp	20.1	±	0.12	1.0	17.9	±	0.15	1.0
Tyr	5.3	±	0.22	4.0	4.5	±	0.25	6.0
Ser	9.1	±	0.18	2.0	7.4	±	0.16	2.0
Pro	6.6	±	0.49	7.0	6.9	±	0.05	1.0
Gly	6.9	±	0.16	2.0	6.7	±	0.22	3.0
Ala	7.0	±	0.16	2.0	6.9	±	0.23	3.0
Val	9.6	±	0.25	3.0	8.6	±	0.02	0.0
Leu	13.9	±	0.65	5.0	12.2	±	0.40	3.0
Ile	8.3	±	0.29	3.0	7.5	±	0.13	2.0
Thr	7.4	±	0.02	0.0	5.7	±	0.08	1.0
Met	6.3	±	0.51	8.0	4.1	±	0.13	3.0
Lys	12.0	±	0.96	8.0	11.6	±	0.88	8.0
Phe	10.3	±	0.75	7.0	9.0	±	0.32	4.0
His	5.2	±	0.26	5.0	4.7	±	0.11	2.0
Arg	13.4	±	0.56	4.0	14.9	±	0.66	4.0

%CV - Coefficient of Variation; DW - Dry Weight; S.D.- Standard Deviation

The evaluation of total essential amino acids (EAA) content (**Table 4**) in individual samples was also performed. All legume samples contained more than 50 g kg^{-1} of EAA.

Protein quality of studied samples was evaluated by the essential amino acid index (EAAI). Calculated values are presented in **Table 4**.

This method of evaluation is more objective than using chemical score assessment, because it includes all essential amino acids. **Kráčmar et al. (1981)** stated that chemical evaluation of protein quality is only an approximate expression of their real quality as it disregards the digestibility, the influence of inhibitors and other factors that determine the actual use of essential amino acids in the body.

Table 4 Content of essential amino acids (EAA) and essential amino acid index (EAAI)

	∑EAA (g kg^{-1})	EAAI (%)
G. max	127.8	19.9
P. sativum	55.1	12.3
Ph. vulgaris	67.7	15.0
L. esculenta	58.9	12.9

MINERALS

Minerals were determined on the atomic absorption spectrometer AA 30. The majority (Na, K, Mg, Ca), trace (Fe, Zn, Cr) and toxic elements (Pb, Cd) were determined.

A quantity of minerals in lentil and peas was studied by **Iqbal et al. (2006)** who reported contents of Fe and Zn as 3.1 and 4.4 mg 100 g^{-1}, resp. in lentil and 2.3 and 3.2 mg 100 g^{-1}, respectively in peas. **Table 5** and **Table 6** present content of minerals in legume samples. Values for peas and lentil are higher than those reported by **Iqbal et al. (2006)**. From these results, it can be concluded that legumes are rich in Mg and Ca, mainly soybeans and common beans. Also in these two legumes the greatest concentration of toxic Pb was found.

Table 5 Content of minerals in *G. max* and *P. sativum* (mean±S.D.) in 1000 g of DW

		G. max			*P. sativum*		
Pb	µg	422.0	±	4.22	146.0	±	1.46
Cd	µg	78.0	±	0.78	27.0	±	0.27
Cr	µg	347.0	±	3.46	405.0	±	4.05
Zn	mg	40.7	±	0.40	23.8	±	0.23
Cu	mg	12.9	±	0.12	4.4	±	0.04
Na	mg	3.7	±	0.04	22.4	±	0.22
Fe	mg	70.2	±	0.70	40.6	±	0.40
Ca	mg	1807.3	±	9.04	688.4	±	3.44
Mg	g	2.1	±	0.02	1.2	±	0.01
K	g	17.3	±	0.17	9.8	±	0.10

DW - Dry Weight; S.D.- Standard Deviation

Table 6 Content of minerals in *Ph. vulgaris* and *L. esculenta* (mean±S.D.) in 1000 g of DW

		Ph. vulgaris			*L. esculenta*		
Pb	µg	447.0	±	4.47	166.0	±	1.64
Cd	µg	30.0	±	0.30	21.0	±	0.21
Cr	µg	365.0	±	3.64	286.0	±	2.85
Zn	mg	32.1	±	0.32	28.2	±	0.28
Cu	mg	7.5	±	0.07	7.1	±	0.07
Na	mg	3.0	±	0.03	8.8	±	0.08
Fe	mg	76.9	±	0.77	78.3	±	0.78
Ca	mg	1718.3	±	8.59	695.5	±	3.48
Mg	g	1.6	±	0.02	1.1	±	0.01
K	g	14.8	±	0.15	9.4	±	0.09

DW - Dry Weight; S.D.- Standard Deviation

CONCLUSION

Legumes, due to the high content of proteins, can be used instead of animal proteins, particularly in developing countries, where the lack of meat is frequent.

Legumes contained all seventeen amino acids. All legume samples were rich in Cys, Glu, Asp, Leu, Lys and Arg. Results from the experiment showed that legumes contained more than 50 g kg^{-1} of essential amino acids (EAA).

The study proved that legumes are rich in K, Mg and Ca. The highest content of K and Mg was found in *G.max*, 17.3 and 2.1 g 1000 g^{-1}, respectively. Also the amount of Ca was significant. Its level was the greatest in *G. max* and *Ph. vulgaris*, 1807 and 1718 mg 1000 g^{-1}, respectively.

Values obtained during the determination of the chemical composition in samples of legumes and buckwheat products can be influenced by many factors, e.g. climatic conditions, location, type of soil, different varieties of plants, irrigation, type of soil and used fertilizers, different crop period, using different, modified methods of determination, chemicals from different producers, etc.

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