

CRUDE PROTEIN, FIBRE AND PHYTIC ACID IN VITRO DIGESTIBILITY OF SELECTED LEGUME AND BUCKWHEAT SAMPLES

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Abstract

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The aim of this study was to determine crude protein, fibre and phytic acid *in vitro* digestibility of selected legumes and buckwheat products. All analyses except the phytic acid contents were performed in the line with the Commission Regulation (EC) No. 152/2009. A modified version of Holt's Method was used for phytic acid (phytate) determination. None of all samples contained more than 11% of moisture. Soybeans are rich in crude protein; they contain nearly 40% of this compound. The content of crude protein in buckwheat flours was about 14%. The highest amount of phytate was found in common beans and soybeans-about 2 g/100g of dry matter. On the other hand, the lowest phytate content was observed in buckwheat pasta (< 1 g/100 g). The quantification of phytate in *F. esculentum* groats was 1.9g per 100g of dry matter. *In vitro* digestibility was determined using an incubator Daisy and pepsin enzymes and the combination of pepsin and pancreatin. The highest coefficient of crude protein digestibility was discovered to be in peels and wholemeal flour. The greatest fibre digestibility coefficients were obtained for peels, which contain about 65% of fibre in their dry matter. When pepsin was used, a higher phytic acid digestibility coefficient for *G. max*, *Ph. vulgaris*, peels, flour, groats and broken groats was observed; while when the combination of pepsin and pancreatin was used, higher phytic acid digestibility coefficients for peas, lentil and wholemeal flour were observed.

legumes, *Fagopyrum esculentum* Moench, buckwheat products, phytic acid, digestibility

Legumes, dry seeds from the family *Fabaceae*, contain a large amount of proteins, carbohydrates, dietary fibre, minerals and water-soluble vitamins. They can be considered as food with health benefits, but their phytate content can limit the availability of minerals (Frias *et al.*, 2003). 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% (De Almeida Costa *et al.*, 2006; Iqbal *et al.*, 2006). However, legumes also contain antinutritional factors, such as proteinase inhibitors, lectin, raffinose oligosaccharides, saponins, polyphenols and phytate (Sandberg, 2002).

Common buckwheat (*Fagopyrum esculentum* Moench) is the most commonly grown species. It

is one of the traditional crops cultivated in Asia, central and Eastern Europe (Wijngaard and Arendt, 2006). Buckwheat is categorized as a pseudocereal from the family of *Polygonaceae* (Skrabanja *et al.*, 2004). The buckwheat fruit contains proteins, saccharides, lipids, fibre, vitamins and minerals as basic components (Ikeda and Yamashita, 2004). Buckwheat seeds do not contain any gluten so they are safe for people with celiac disease. Buckwheat also contains rutin, a bioflavonoid which improves cardiovascular health (Halbrech *et al.*, 2005).

The term "dietary fibre" is widely accepted to include the complex mixture of indigestible polysaccharides, waxes and lignin found in plants, mainly plant cell wall material. Pulses, edible seeds of leguminous crop, are rich food source of fibres that promote various beneficial physiological effects

for human health (Tosh and Yada, 2010). Fibre acts in the prevention of many mass occurrence non-infectious diseases, such as colon cancer, cardiovascular diseases, diabetes, obesity, chronic constipation, etc. However, dietary fibre can also have negative effect, it may bind minerals and proteins, inhibit digestive enzymes and thereby lower absorption or digestibility. (Blatná *et al.*, 2005). Bonafaccia *et al.* (2003) reported a content of dietary fibre of 27.4% in buckwheat seeds.

Phytates are gaining increasing attention from researchers as antinutritional factors because of modern trends towards consumption of increasing amounts of vegetable fibre and fibre-rich cereal and oil seed products (Molins, 1991). Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate, IP₆) represents a major antinutrient in food and feed. It accumulates during seed development until the seeds reach maturity and accounts for 60–90% of total phosphorus content in cereals, legumes, nuts and oil seeds (Lott *et al.*, 2000). Six phosphates groups in the molecule of IP₆ make it a strong chelating agent, which binds minerals such as Ca²⁺, Mg²⁺, Fe³⁺ and Zn²⁺. Under gastrointestinal pH conditions, insoluble metal-phytate complexes are formed. They make the metal unavailable for absorption from the gastrointestinal tract of animals and humans (Zhou and Erdman, 1995). In literature, the name phytic acid has been used interchangeably with the term phytate, which is a salt (Reddy *et al.*, 1989).

Determination of nutritional value of specific food is also necessary for providing their utilization by the body – digestibility. The coefficient of digestibility expresses the percentage of digested nutrient from the total content of the nutrient in food or feed. *In vitro* method is carried out under laboratory conditions and uses pepsin and pancreatic proteases to simulate digestive functions *in vivo* (Qiao *et al.*, 2004; Pajtáš *et al.*, 2009). Digestibility may be used as an indicator of protein availability (Duodu *et al.*, 2003). Digestion and absorption are considered to be inseparable parts of protein quality. The quality of protein can be evaluated on the basis of its amino score, digestibility and bioavailability of amino acids in the protein source (Sarwar-Gilani and Sepehr, 2003).

MATERIAL AND METHODS

Samples

Legumes for this study, *Glycine max*, *Pisum sativum*, *Phaseolus vulgaris* and *Lens esculenta*, were obtained from the Food Research Institute in Bratislava, Slovakia and buckwheat products, made from common buckwheat (*Fagopyrum esculentum* Moench), were obtained directly from the Czech producer Pohankový mlýn Šmajstrla, s.r.o., Frenštát pod Radhoštěm, Czech Republic. Dry samples were grounded in a mill to a fine powder, sieved through

1 mm mesh and thoroughly mixed. They were stored at laboratory temperature of 21 ± 2 °C.

Analysis

First, the moisture of samples was determined according to the Commission Regulation (2009). The crude protein content was determined according to the Kjeldahl method using the Pro-Nitro 1430 apparatus (BIO PRO, Prague, CZ). Results were recalculated to the sample weight and by multiplying it with the factor 6.25 for legumes, or 5.7 for buckwheat products, the percentage of crude protein was obtained.

Total fibre content in studied samples was determined using the apparatus Ancom²²⁰ Fibre Analyzer (ANCOM Technology, New York, USA). For the analysis, filter bags F57 with pore size 50 µm were used. From obtained values, fibre content in original mass of individual samples, in % (w/w), was calculated (Kráčmar *et al.*, 1981).

The determination of phytate was realized by modified Holt's method (Holt, 1955). After the treatment of samples, the intensity of the colour in the amyl layer was determined at 465 nm using a spectrophotometer (Biochrom Libra S6, Cambridge, England) against an amyl alcohol "blank" exactly 15 minutes after addition of NH₄CNS (Davies and Reid, 1979). Standard curve was determined the same way using Na phytate standard solution (0.2 mM; Sigma Aldrich, USA) instead of the filtrate (Vojtíšková *et al.*, 2010). The equation from the standard curve was used for the calculation of the amount of phytate in studied samples.

Digestibility of legumes and buckwheat products was determined using the enzymatic-gravimetric method *in vitro*. Two enzymes were used; pepsin (from porcine gastric mucosa; 0.7 FIP-U/mg; Merck, KGaA, Darmstadt, Germany) and pancreatin (from porcine pancreas; protease activity 350 FIP-U/g; lipase activity 6000 FIP-U/g; amylase activity 7500 FIP-U/g; Merck, KGaA, Darmstadt, Germany). Hydrolysis with pepsin and pancreatin were performed (Mišurcová, 2008). Results of digestibility were expressed as coefficient of digestibility (X). It is a ratio of amount of compound after digestion (C₁) to amount of compound before digestion (C₂) multiplied by 100 and expressed in %.

Statistical analysis

All results were 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

The crude protein, fibre and phytic acid *in vitro* digestibility in legumes and buckwheat products was studied.

Content of moisture (Tab. I) in peas, beans and lentil is about 9%, only soybeans contain lower

amount of moisture, about 6.5%. In buckwheat products content of moisture ranges from 6.5% in crunchy products cocoa to almost 11% in buckwheat groats.

Content of crude protein (Tab. I) in dry matter of legumes is the highest from all examined samples. Soybeans contain nearly 40% of this compound. Khattab *et al.* (2009) presented in their study a crude protein content in common beans as 24.9%. It is only a little bit more than the content determined in the experiment; it was almost 24% in dry matter. Zeman *et al.* (1995) present the content of crude protein in soybeans, peas and lentil as 36.8, 22.9 and 29.0%, respectively. The crude protein content determined in *G. max*, 37.8%, was similar to the reported value. The rich sources of crude protein are both flours, they contain about 14% of crude protein in dry matter. These observations confirmed that legumes are valuable potential source of proteins, mainly in developing countries.

Fibre was detected only in legumes, peels and products containing peels like whole seeds and wholemeal flour; in other products, fibre content was so low that it was not possible to determine it by this method. Peels contained more than 65% of fibre (Tab. I). Bonafaccia and Fabjan (2003) reported the fibre content in flour from common buckwheat as 6.5%. Results from the experiment are in concordance with this study. Dalgetty and Baik (2003) studied content of fibre in *Ph. vulgaris* and *L. esculenta*. Their results were 14–26% of fibre in common beans and 6.8% in lentil. When compared with values from the experiment it can be concluded that Dalgetty's team determined higher contents of fibre. In the laboratory experiment, determined amounts of fibre were 11.1% for peas and 5.6% for lentil (Tab. I).

Phytic acid extracted from samples formed, with added ferric ammonium sulphate, a compound called phytate. Ferric ions, which were not bound to phytic acid, reacted with ammonium thiocyanate

II: Content of phytic acid (mean \pm S.E.) in g 100 g⁻¹

	Phytic acid
Peels	1.1 \pm 0.01
Groats	1.9 \pm 0.01
Broken groats	2.0 \pm 0.01
Flour	1.7 \pm 0.00
Wholemeal flour	2.0 \pm 0.00
Pasta	0.9 \pm 0.00
<i>G. max</i>	2.0 \pm 0.01
<i>P. sativum</i>	1.7 \pm 0.00
<i>Ph. vulgaris</i>	2.0 \pm 0.00
<i>L. esculenta</i>	1.7 \pm 0.01

and created pink-red colour of the solution. The intensity of the colour was determined at 465 nm using the spectrophotometer.

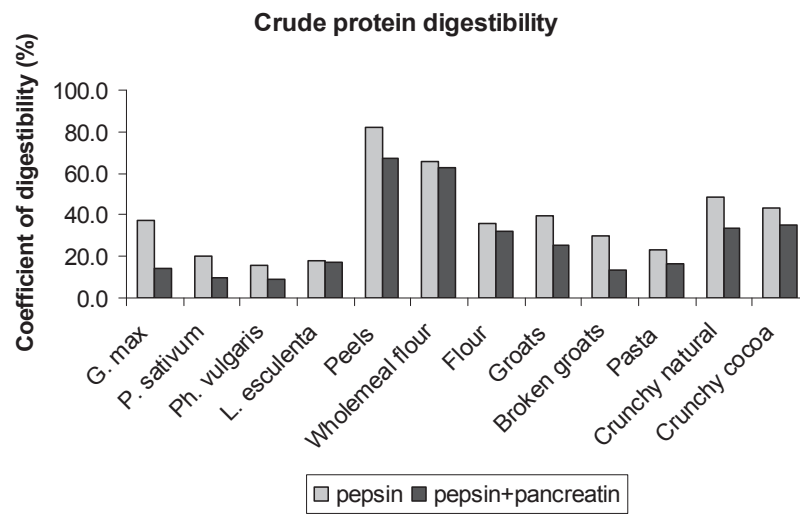
Table II shows that the amount of phytate in dry matter of soybeans was about 2.0 g/100g. Hídvégi and Lásztity (2002) assigned the content of phytate in soybeans in the range of 1.2–1.8g/100g and 0.7–1.2 g/100g in peas. These values are lower than those presented in Tab. II. For common beans, Hídvégi and Lásztity (2002) stated the range of phytate content as 0.6–1.7 g/100g. Data for common beans from this experiment does not suit to this extent. Amount of 2.0 and 1.8 g/100g is higher.

The highest amount of phytate was found in common beans, soybeans, broken groats and wholemeal flour, about 2 g/100g. On the other hand, the lowest content of phytate was observed in buckwheat pasta, less than 1 g/100g. Also Campos-Vega *et al.* (2010) studied content of phytate in legumes. They presented amount of phytate in *Ph. vulgaris*, *L. esculenta* and *P. sativum* as 0.2–1.9, 0.2–2.3 and 0.2–1.3%, respectively. *P. sativum* and *Ph. vulgaris* in the experiment contain higher amounts of phytate; results for *L. esculenta* were in the range of values reported by Campos-Vega *et al.* (2010). The

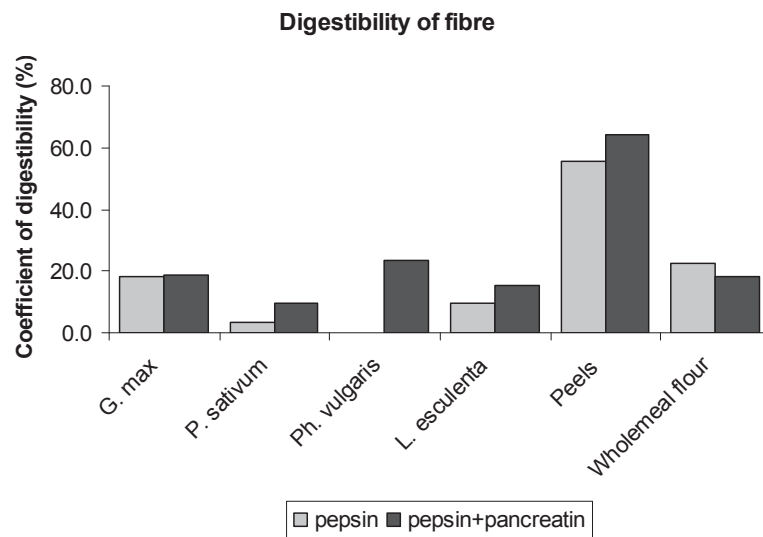
I: Content of moisture, crude protein and fibre in studied samples (mean \pm S.E.) in %

	Moisture	Crude protein	Fibre
Peels	8.0 \pm 0.04	3.5 \pm 0.24	65.9 \pm 1.17
Whole seed	10.4 \pm 0.04	10.2 \pm 0.23	14.8 \pm 1.05
Groats	10.7 \pm 0.06	12.9 \pm 0.00	ND
Broken groats	9.1 \pm 0.04	9.2 \pm 0.02	ND
Crunchy natural	7.5 \pm 0.02	6.8 \pm 0.34	ND
Crunchy cocoa	6.5 \pm 0.03	6.5 \pm 0.18	ND
Flour	10.1 \pm 0.01	13.8 \pm 0.54	ND
Wholemeal flour	9.5 \pm 0.07	13.8 \pm 0.27	6.7 \pm 0.19
Pasta	9.4 \pm 0.04	8.1 \pm 0.37	ND
<i>G. max</i>	6.4 \pm 0.01	37.8 \pm 1.12	13.3 \pm 0.14
<i>P. sativum</i>	9.0 \pm 0.02	18.4 \pm 0.05	15.3 \pm 0.21
<i>Ph. vulgaris</i>	8.4 \pm 0.02	24.0 \pm 0.09	11.1 \pm 0.72
<i>L. esculenta</i>	8.8 \pm 0.01	22.5 \pm 0.78	5.6 \pm 0.29

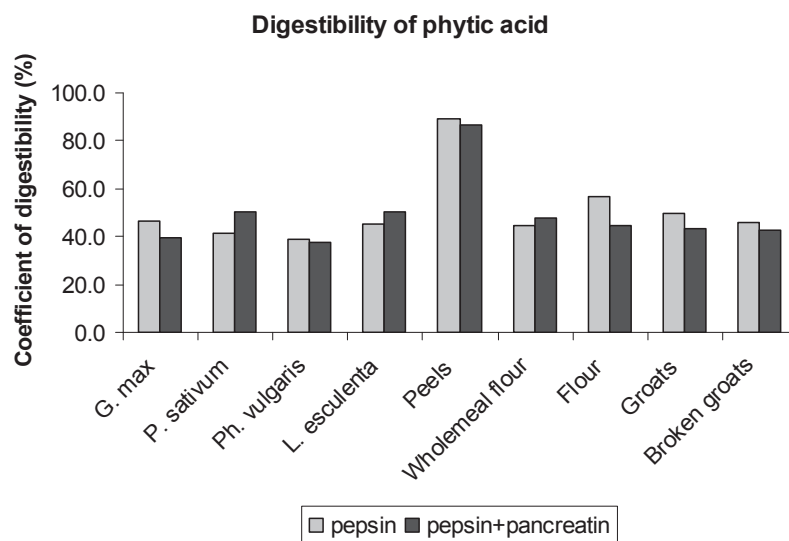
S.E. – standard error; ND – fibre was not detected



1: Digestibility of crude protein



2: Digestibility of fibre



3: Digestibility of phytic acid

quantification of phytate in *F. esculentum* groats was 1.9 g per 100 g of dry matter.

In vitro digestibility was determined using incubator and enzymes pepsin and the combination of pepsin and pancreatin. Coefficients of digestibility for particular samples are presented in Fig. 1, 2 and 3. The highest coefficient of crude protein digestibility (Fig. 1) was discovered in peels and wholemeal flour. The highest coefficients of digestibility in all samples were obtained when using pepsin. For the combination of pepsin and pancreatin, lower values were obtained. In Fig. 2, coefficients of fibre digestibility are shown. From these data, it can be concluded that the greatest fibre digestibility coefficients were obtained for peels, which contain about 65% of fibre in dry matter. Value for calculating the digestibility coefficient for common beans (when using pepsin) was not detected. Finally, coefficients of phytic acid digestibility are presented in Fig. 3. When pepsin was used, higher digestibility coefficients for *G. max*, *Ph. vulgaris*, peels, flour, groats and broken groats were found out. On the other hand, when the combination of pepsin and pancreatin was used, higher digestibility coefficients for phytic acid in

P. sativum, *L. esculenta* and wholemeal flour were discovered.

CONCLUSIONS

The main emphasis in this study was put on the determination of phytic acid and its subsequent digestibility. Phytates reduce the nutritional value of plant foods, especially when their content is high. Digestibility can be influenced by many factors. Mainly digestibility of crude protein may be affected by the concentration of phytic acid. As stated by Fredlund *et al.* (2006) phytate forms with minerals (Fe, Zn and Mg) a complex which is insoluble at the physiological pH of the intestine and can reduce digestibility of proteins, starch and lipids. 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.

SUMMARY

This study was carried out to study crude protein, fibre and phytic acid digestibility in selected samples of legumes and buckwheat products. All analyses were performed in the line with the Commission Regulation (EC) No. 152/2009; only for phytic acid (phytate) determination a modified version of Holt's Method was used. Soybeans are rich in crude protein and with common beans they contain the highest amount of phytate (2 g/100 g of dry matter). The lowest phytate content was found in buckwheat pasta. *In vitro* digestibility was determined using an incubator Daisy and pepsin enzymes and the combination of pepsin and pancreatin. The highest coefficient of crude protein digestibility was discovered to be in peels and wholemeal flour. The greatest fibre digestibility coefficients were obtained for peels. The value of phytic acid digestibility coefficients was dependent on the enzyme. All results were statistically evaluated using the statistical package Unistat, v. 5.5.

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