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CONTENT OF PHYTIC ACID IN SELECTED SORTS OF LEGUMES

P. Vojtíšková, S. Kráčmar, I. Hoza

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Abstract

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The aim of this study was to determine the content of phytic acid (phytate) in soybeans (*Glycine max)*, yellow shelled peas (*Pisum sativum)* and lentil *(Lens esculenta)*. Dry seeds were grounded to a fine powder. The moisture of samples was determined according to the Official Journal of the European Union (2009). The moisture of the samples was 8.42% in soybeans, 11.19% in yellow shelled peas and 10.07% in lentil. The content of phytic acid was determined by the modified Holt's method (1955) using the spectrophotometer. Standard curve was measured using the Na phytate standard solution (0.2 mM). The phytate content in *G. max* varied from 1.28 to 1.86% in dry matter and from 1.17 to 1.70 g per 100 g of the sample. The content of phytate in *P. sativum* ranged from 0.49 to 0.86% and from 0.43 to 0.77 g per 100 g of the sample. The phytate content in *L. esculenta* varied from 0.45 to 1.39% in dry matter and from 0.40 to 1.25 g per 100 g of the sample. These obtained values could be influenced by many factors, e.g. climatic conditions, location, variety, etc.

phytic acid, phytate, *Glycine max*, *Pisum sativum*, *Lens esculenta*

Legumes are dry edible seeds of some plants from the family *Fabaceae*, as beans, lupine, peas and lentil are. The nutritional potential of the seeds from this group of plants is based on their high level of proteins. However, the legumes also contain antinutritional factors, such as proteinase inhibitors, lectin, rafinose oligosaccharides, saponins, polyphenols and phytate (Sandberg, 2002).

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexa*kis*-phosphate, IP_6) represents a major antinutrient in food and feed. In seeds, it is stored as mixed salts of cations, mainly K and Mg, together with minor amounts of Ca, Fe, Zn and Mn (Raboy, 1997).

Inositol phosphates consist of an inositol ring and at least one phosphate group. Breaking the name into its separate parts describes the exact structure and appearance: the prefix "*myo*" refers to the conformation of the hydroxyl groups on the inositol ring. There are nine stereoisomers of inositol, of which seven are meso structures and two form a chiral pair. They are (1) *cis*-, (2) *epi*-, (3) *allo*-, (4) *neo*-, (5) *myo*-, (6) *muco*-, (7) 1L-*chiro*-, (9) 1D-*chiro*-, and (9) *scyllo*-inositol (Loewus and Loewus, 1980). The *myo*-inositol is common in plants (Cosgrove, 1980). The nine possible configurations of the inositol ring have been annotated in a number of ways, but the adopted nomenclature is according to the set of rules suggested by Posternak (1965).

The conformation *myo*-inositol thus has one plane of symmetry, going directly from the most left to the most right atom. The D/L-prefixes specify the numbering direction of carbons in the inositol ring, where the D annotates counterclockwise and L clockwise counting, respectively. In general chemistry, numbering of the atoms should always follow the lowest possible route. Confusions regarding *myo*-inositols and enzymes related to them have led the International Union of Biochemistry (IUPAC-IUB, 1989) to recommend that the atoms in the *myo*-inositol ring should always be numbered according to the D configuration.

Myo-inositol is the major nutritionally relevant form of inositol, and although some of the other stereoisomers are also found in nature. *Myo*-inositol (1,2,3,4,5,6) hexa*kis*phosphate has six groups of phosphates attached to the inositol ring. Using

the prefix "hexa*kis*" instead of "hexa" indicates that the phosphates are not internally connected and the compound is consequently a polydentate ligand, which is a chelator that can bind to more than one coordination site of the metal atom. Each of the phosphate groups is esterified to the inositol ring and together they can bind up to 12 protons in total. The acidity of the protons varies from very strong acids to very weak although ionic strength of the solution and temperature influence these values (Torres *et al*., 2005; He *et al*., 2006).

Cereals and legumes contain significant amounts of phosphorus in the form of phytic acid (Tables I and II). It serves several physiological functions and also significantly influences the functional and nutritional properties of cereals and legumes and their derived foods by complexing with proteins and essential minerals. The terms phytic acid, phytate and phytin refer to free acid, salt and calcium/magnesium salt, respectively. In the literature, the name phytic acid has been used interchangeably with the term phytate, which is a salt (Reddy *et al*., 1989).

I: *Content of phytic acid in dry matter of cereals and legumes [%] (Reddy, 1982)*

Cereal / Legume	Amount of phytic acid
Wheat	0.62–1.35
Rye	0.97
Barley	0.97–1.16
Corn (Maize)	0.89–0.99
Oat	0.79–1.01
Soya	1.00–1.47
Peas	1.20
Beans	0.55–0.75
Lentil	0.51

II: *Content of phytic acid in some legume seeds [g per 100 g] (Hídvégi and Lásztity, 2002)*

Legume	Amount of phytic acid
Soybeans	1.20–1.75
Peas	0.72–1.23
Common bean	0.55–1.69

Phytic acid 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). The primary functions of phytic acid in seeds are storage of phosphates as energy source and antioxidant for the germinating seed (Raboy, 2003).

Legume seeds are the richest and cheapest alternative sources of protein among all foods of plant origin (Urbano *et al*., 2000). The chelating effect of the phosphate groups causes phytic acid to bind readily to mineral cations, especially to Cu^{2+} and Zn^{2+} which appear to have a high affinity for inositol phosphates. The order of the ability of the mineral cations to form complexes *in vitro* with inositol phosphates has been found to be $Cu^{2+}>Zn^{2+}>Cd^{2+}$ for all $InsP_3$-$InsP_6$ at pH 3-7, but binding strength is weaker for the lower inositol phosphates (Persson *et al*., 1998). Recent finding shows that phytic acid is stored *in vivo* in complexes not only with these minerals, but to a much larger extents with Mg, Ca and K (Bohn *et al*., 2007).

In presence of excess phytic acid, formation of soluble complexes between phytic acid and metal ion displaying 1 : 1 stoichiometries predominates. However, when metal ions are in excess, an insoluble solid called phytate is formed (Torres *et al*., 2005). Phytate as the mineral bound salt of phytic acid is also an important mineral reserve in seeds, and it is stored in protein storage vacuoles in the aleurone cell layer or the embryo of the seed (Storcksdieck *et al*., 2007).

MATERIALS AND METHODS

Legume sampling

The content of phytic acid in selected sorts of legumes was determined. Legumes for this study were obtained from the Food Research Institute in Bratislava, Slovakia. Dry seeds were grounded in a mill to a fine powder and thoroughly mixed. They were stored at laboratory temperature.

Analysis

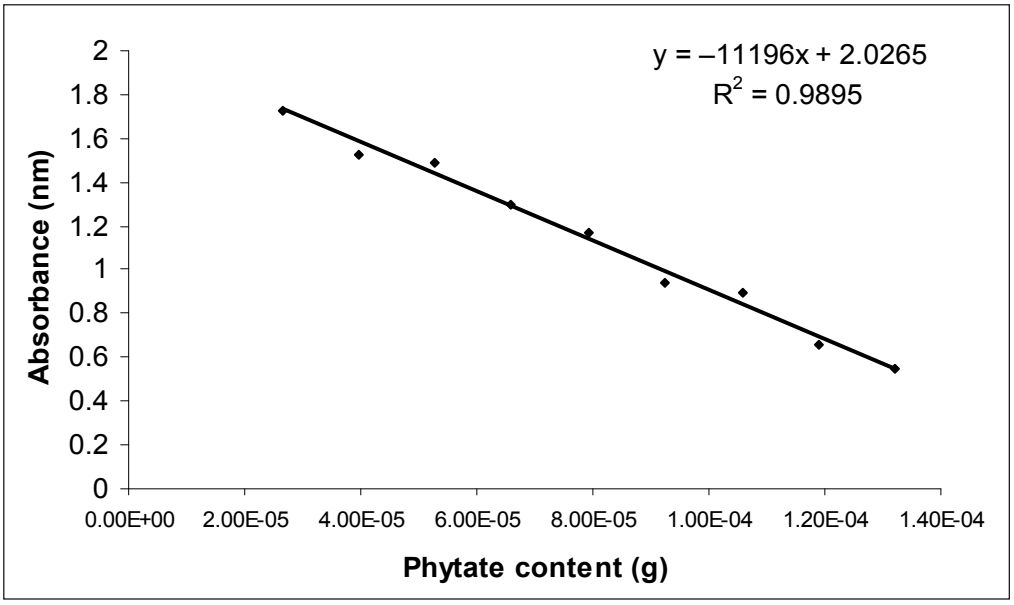
First, the moisture of samples was determined (Official Journal of the European Union, 2009).

The determination of phytate was realized by modified Holt's method (Holt, 1955). Triplicate 1.0 g of samples was extracted with 40 ml 0.5M HNO_3 for 3 hours with continuous shaking. The extract was filtrated through the filter FILTRAK, No. 390, ∅ 12.5 cm.

Next, 0.2–1.0 ml of filtrate was diluted with distilled water to a final volume of 1.4 ml in polypropylene test-tubes; after that 1 ml of a ferric ammonium sulphate solution (containing 50 µg of Fe) was added, test-tubes were stoppered and mixed. Then, they were put into a boiling water bath (Memmert, Germany) for 20 minutes and after that cooled to a room temperature.

Five millilitres of amyl alcohol and 0.1 ml of ammonium thiocyanate solution (100 $g.l^{-1}$) was subsequently added to each test-tube. They were stoppered and immediately mixed by inversion and shaking. After centrifugation for a short time at low speed, 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 NH_4CNS (Davies and Reid, 1979).

Standard curve was determined the same way using Na phytate standard solution (0.2mM; Sigma Aldrich, USA) instead of the filtrate. The equation from the standard curve was used for the calculation



1: *Standard curve for phytate determination*

of the amount of phytate in *G. max*, *P. sativum* and *L. esculenta* (Fig. 1).

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.

RESULTS AND DISCUSSION

The content of phytic acid in selected sorts of legumes, *Glycine max*, *Pisum sativum* and *Lens esculenta*, was studied. First of all, the moisture of the samples was determined. As can be seen from Table III, the content of moisture in *G. max* was 8.42%, in *P. sativum* 11.19% and in *L. esculenta* 10.07%.

Consequently, the legume samples were analysed using the modified Holt's method.

Values in Table IV show that the amount of phytate in dry matter of *G. max* varies from 1.28 to 1.86%. These values are slightly higher than those reported by Reddy *et al.* (1982; Table I) who presented a range of 1.00–1.47% of phytate content in dry matter of soybeans. Hídvégi and Lásztity (2002; Table II) assigned the content of phytate in soybeans in the range of 1.20–1.75 g per 100 g of the sample. The quantification of phytate in *G. max* (Table V) varies from 1.17 to 1.70 g per 100 g of the sample. As can be seen, these values are almost the same as those reported values are.

The content of phytate in dry matter of *P. sativum* is lower than in reports by Reddy (1982) and Hídvégi and Lásztity (2002). Reddy (1982; Table I) assigned the value of 1.20% of phytate in dry matter of yellow shelled peas, and Hídvégi and Lásztity (2002; Table II) presented the phytate content in the range of 0.72–1.23 g per 100 g of the sample. The quantification of phytate in *P. sativum* varies from 0.49 to 0.86% in dry matter of the sample (Table IV) and from 0.43 to 0.77 g per 100 g of the sample (Table V). These determined values are almost by half lower than those reported by Reddy (1982) and Hídvégi and Lásztity (2002).

The content of phytate in dry matter of *L. esculenta* (Table IV) is in the range of 0.45–1.39%. Reddy (1982; Tab. I) assigned the value of 0.51% in dry matter of lentil. The content of phytate is higher than in reports by Reddy (1982). The amount of phytate in *L. esculenta* varied from 0.40 to 1.25 g per 100 g of the sample.

Current data obtained by the determination of phytate content in *G. max*, *P. sativum* and *L. esculenta* using the modified Holt´s method can differ from

III: *Moisture and dry matter in legume samples (mean ± S.E.) [%]*

Sample	Average moisture	Average dry matter
Glycine max	8.42 ± 0.042	91.58 ± 0.042
Pisum sativum	11.19 ± 0.064	88.81 ± 0.064
Lens esculenta	10.07 ± 0.019	89.93 ± 0.019

IV: *Content of phytate in dry matter of selected legumes (value ± S.E.) [%]*

Sample	Minimum	Average	Maximum
Glycine max	1.28 ± 0.033	1.60 ± 0.033	1.86 ± 0.033
Pisum sativum	0.49 ± 0.022	0.62 ± 0.022	0.86 ± 0.022
Lens esculenta	0.45 ± 0.046	0.85 ± 0.046	1.39 ± 0.046

V: *Content of phytate in selected legumes (value ± S.E.) [g per 100g of the sample]*

Sample	Minimum	Average	Maximum
Glycine max	1.17 ± 0.030	1.46 ± 0.030	1.70 ± 0.030
Pisum sativum	0.43 ± 0.020	0.55 ± 0.020	0.77 ± 0.020
Lens esculenta	0.40 ± 0.042	0.55 ± 0.042	1.25 ± 0.042

those obtained previously because of many factors, e.g. climatic conditions, location, type of soil, different varieties of legumes, different crop period, using different, modified method of determination, chemicals from different producers, etc.

Not many authors dealt with phytic acid content in legumes. More of them studied the content of phytate in cereals and cereal bran, for example in wheat (Egli *et al*., 2003), millet (Talamond *et al*., 1998), barley (Harland and Morris, 1995), wheat, rye, oat (Reddy and Sathe, 2002) and sorghum (Deshpande, 2002).

Content of phytic acid, and also phytate is very important because of its chelating properties. Cations, as Ca^{2+}, Zn^{2+}, Fe^{3+}, Cu^{2+} and others, can be bound by the phytate anion and form indigestible compounds. The capacity of binding minerals makes phytic acid an antinutritional factor. On the other hand, phytic acid has also positive properties. It can act as an antioxidant. Phytic acid can also participate in lowering of cholesterol and triglyceride level in blood, kidney stone formation, and maybe in cancer therapy. As Golian (2009) brings out, good nutrition can lead to an impressive range of benefits, for example improved health and greater resilience to shocks induced by social, economic and natural causes.

Soybeans, peas and lentil are rich in proteins, saccharides, vitamins and other compounds; phytate is one of them. Therefore, this study was carried out to determine the content of phytate in *G. max*, *P. sativum* and *L. esculenta*.

SOUHRN

Obsah kyseliny fytové ve vybraných druzích luštěnin

V práci byl sledován obsah kyseliny fytové (fytátu) ve vybraných druzích luštěnin, sójových bobů *(Glycine max)*, žlutého loupaného hrachu *(Pisum sativum)* a čočky *(Lens esculenta)*. Zkoumané vzorky byly homogenizovány. Vlhkost vzorků byla stanovena podle Nařízení komise (ES) č. 152/2009 uvedeného v Úředním věstníku Evropské Unie. Vlhkost sójových bobů byla 8,42 %, žlutého loupaného hrachu 11,19 % a čočky 10,07 %. Obsah kyseliny fytové byl stanoven modifikovanou metodou podle Holta jako množství fytátu. Standardní křivka byla proměřena pomocí 0,2mM standardního roztoku fytátu sodného v rozmezí koncentrací 40 až 200 nmol. Obsah fytátu v sójových bobech (*G. max*) kolísal mezi 1,28 až 1,86 % v sušině a mezi 1,17 až 1,70 g ve 100 g vzorku, ve žlutém loupaném hrachu (*P. sativum*) v rozmezí od 0,49 do 0,86 % v sušině a od 0,43 do 0,77 g ve 100 g vzorku a u čočky (*L. esculenta*) v rozmezí 0,45–1,39 % v sušině a 0,40–1,25 g ve 100 g vzorku.

kyselina fytová, fytát, *Glycine max*, *Pisum sativum*, *Lens esculenta*

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Address

Ing. Petra Vojtíšková, prof. Ing. Stanislav Kráčmar, DrSc., prof. Ing. Ignác Hoza, CSc., Univerzita Tomáše Bati ve Zlíně, Fakulta technologická, Ústav biochemie a analýzy potravin, nám. T. G. Masaryka 275, 762 72 Zlín, Česká republika, e-mail: vojtiskova@ft.utb.cz