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THE INFLUENCE OF PHYTIC ACID ON THE NUTRITIONAL VALUE OF FOODSTUFFS

VLIV KYSELINY FYTOVÉ NA NUTRIČNÍ HODNOTU POTRAVIN

DOCTORAL THESIS

Study programme: P2901 Chemistry and Food Technology

Course: 2901V013 Food Technology

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Zlín 2012

ABSTRACT

The aim of this Doctoral thesis was to determine the basic chemical composition and phytic acid content in selected legumes and buckwheat products, made from common buckwheat (*Fagopyrum esculentum* Moench). During this one-year storage experiment, samples were stored at a room temperature of 21±2 °C; and four samplings were performed. Moisture, ash, total fat, crude protein, fibre, phytic acid, mineral, starch and rutin (for buckwheat products) contents, amino acid composition and digestibility were all determined. All analyses except the rutin and phytic acid contents were performed in the line with Commission Regulation (EC) No. 152/2009. A modified version of Holt´s Method was used for phytic acid (phytate) determination. The rutin concentration was determined using a modified HPLC Method.

None of all samples contained more than 11% of moisture. During storage, the content of ash increased in almost all samples-only in lentils a small decrease was observed. Of all these samples, soybeans were the most energy abundant foodstuff. Their energy value was about 22 MJ/kg. Energy values in buckwheat products ranged from 16 to 18 MJ/kg. The richest source of fat were soybeans, the amount was almost 17% in samples after receiving them; others contained markedly lower amounts of fat. In general, buckwheat products are low fat products. The content of crude protein in the dry matter of legumes was the highest from all the samples examined. Soybeans are rich in crude protein; they contain nearly 40% of this compound. The content of crude protein in buckwheat products was the highest in both flours (about 14%). Peels contained the lowest amounts of all amino acids (AA). On the other hand, the highest contents of AA were found in both flours and groats. All buckwheat products were rich in Glu, Asp and both flours were also rich in Arg. The highest content of Cys, Glu, Asp, Leu, Lys and Arg was determined in all legumes in both samplings. The highest concentration of almost all amino acids was discovered in soybeans. Total content of essential amino acids (EAA) changed during storage. All the legume samples contained more than 50 g kg^{-1} of EAA in both samplings. In buckwheat products, the content of starch was higher than 50% in the dry matter, with the exception of peels. Fibre was detected only in legumes, peels and products containing peels like whole seeds and wholemeal flour. Peels, after receipt of the samples contained more than 65% of fibre. The majority (Na, K, Mg, and Ca), trace (Fe, Zn, and Cr) and toxic elements (Pb, Cd) were only determined at the beginning of the experiment-not during the storage period. Wholemeal flour is a very rich source of Ca, Fe and Zn. Peels are also a good source of Ca. Legumes are rich in Mg and Ca-mainly soybeans and common beans. The highest concentration of rutin in both samplings was found in wholemeal flour, almost 703 μg g^{-1} upon delivery. The highest amount of

phytate was found in common beans and soybeans-about 2 g/100 g prior to storage. 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 1.9 g per 100 g of dry matter pre-storage.

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 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.

Keywords: legumes, *Fagopyrum esculentum* Moench, buckwheat products, chemical composition, phytic acid, digestibility

ABSTRAKT

Cílem disertační práce bylo stanovit základní chemické složení a obsah kyseliny fytové ve vybraných vzorcích luštěnin a pohankových produktů vyrobených z pohanky seté (*Fagopyrum esculentum* Moench). Během jednoletého skladovacího pokusu byly vzorky skladovány při teplotě 21±2 °C a byly odebrány čtyřikrát. U vzorků byl stanoven obsah vlhkosti, popelovin, celkový obsah tuku, dusíkatých látek, vlákniny, kyseliny fytové, minerálních látek, škrobu a rutinu (u pohankových výrobků), dále aminokyselinové složení a stravitelnost. Všechny analýzy, kromě stanovení rutinu a kyseliny fytové, byly provedeny podle Nařízení Evropské komise č. 152/2009. Pro stanovení kyseliny fytové byla použita modifikovaná metoda podle Holta. Koncentrace rutinu byla stanovena modifikací HPLC metody.

Žádný ze vzorků neobsahoval více než 11 % vlhkosti. Během skladování vzrostl obsah popelovin téměř u všech vzorků, pouze u čočky byl pozorován mírný pokles. Ze všech zkoumaných vzorků byly sójové boby nejvydatnějším zdrojem energie. Jejich energetická hodnota se pohybovala okolo 22 MJ/kg. U pohankových výrobků se energetické hodnoty pohybovaly v rozmezí 16-18 MJ/kg. Nejbohatším zdrojem tuku byly sójové boby s jeho obsahem téměř 17 % ve vzorcích po jejich obdržení; ostatní luštěniny obsahovaly výrazně nižší množství tuku. Pohankové výrobky jsou obecně považovány za potraviny s nízkým obsahem tuku. Ze všech zkoumaných vzorků bylo nejvyšší množství dusíkatých látek v sušině luštěnin. Sójové boby jsou bohatým zdrojem dusíkatých látek; jejich obsah je téměř 40 %. Nejvíce dusíkatých látek bylo zjištěno v obou pohankových moukách (téměř 14 %). Nejnižší množství všech aminokyselin bylo stanoveno ve slupkách. Na druhé straně nejvyšší obsah aminokyselin byl zjištěn v obou pohankových moukách a kroupách. Všechny výrobky z pohanky jsou bohaté na Glu, Asp a obě mouky také na Arg. Nejvíce Cys, Glu, Asp, Leu, Lys a Arg bylo zjištěno ve všech luštěninách u obou odběrů vzorků. Nejvyšší množství všech aminokyselin bylo ve vzorcích sóji. Celkový obsah esenciálních aminokyselin se v průběhu skladování měnil. Všechny vzorky luštěnin obsahovaly více než 50 $g.kg^{-1}$ EAA v obou odběrech vzorků. U pohankových výrobků byl obsah škrobu v sušině vyšší než 50 %, kromě slupek. Vláknina byla zjištěna pouze v luštěninách, slupkách a výrobcích obsahujících slupky (celá zrna a celozrnná mouka). Slupky po obdržení vzorků obsahovaly více než 65 % vlákniny. Majoritní (Na, K, Mg, Ca), stopové (Fe, Zn, Cr) a toxické prvky (Pb, Cd) byly stanoveny pouze na začátku experimentu. Celozrnná mouka je bohatým zdrojem Ca, Fe a Zn. Také slupky jsou dobrým zdrojem vápníku. Luštěniny, zejména sójové boby a fazole, jsou bohaté na hořčík a vápník. Nejvíce rutinu v obou vzorkováních bylo zjištěno v celozrnné mouce, téměř 703 $\mu g.g^{-1}$ po obdržení vzorků. Nejvyšší množství fytátu bylo stanoveno ve vzorcích fazolí a sójových bobů, téměř 2 g/100 g před

skladováním. Na druhé straně nejnižší obsah fytátu byl pozorován u pohankových těstovin (< 1 g/100 g). Množství fytátu v kroupách pohanky seté bylo 1,9 g/100 g sušiny před skladováním.

Stravitelnost *in vitro* byla stanovena pomocí inkubátoru Daisy a enzymu pepsinu a kombinace enzymů pepsinu a pankreatinu. Nejvyšší koeficient stravitelnosti dusíkatých látek byl zjištěn u slupek a celozrnné mouky. Nejvyšší koeficient stravitelnosti vlákniny byl zjištěn u slupek, které obsahují zhruba 65 % vlákniny v sušině. Pokud byl použit pouze pepsin, byly zjištěny nejvyšší koeficienty stravitelnosti u *G. max*, *Ph. vulgaris*, slupek, mouk, krup a lámanky; při použití kombinace pepsinu a pankreatinu byl nejvyšší koeficient stravitelnosti kyseliny fytové u hrachu, čočky a celozrnné mouky.

Klíčová slova: luštěniny, *Fagopyrum esculentum* Moench, pohankové výrobky, chemické složení, kyselina fytová, stravitelnost

Motto:

„Alea iacta est.“

Gaius Iulius Caesar

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my supervisor, prof. Ing. Stanislav Kráčmar, DrSc. for his valuable advice, comments and professional support throughout the whole period of my doctoral studies. He also introduced me into the world of conferences and scientific meetings. At any time throughout my doctoral studies I could turn to him for advice and always he took time for me. For all he deserves great thanks.

Many thanks also belong to the staff of Department of Food Analysis and Chemistry, especially to the laboratory assistant Mrs. Živocká, for her kind assistance during the analyses, as well as to employees of Department of Food Technology and Microbiology and Department of Fat, Surfactant and Cosmetics Technology from Tomas Bata University in Zlín. For some types of determination cooperation with other universities was inevitable so I would like to thank to the scientific employees of Slovak Agricultural University in Nitra, Mendel University in Brno and Masaryk University in Brno for their kind assistance in some analyses.

Also I would like to thank to Mr. Šmajstrla from Pohankový mlýn s.r.o., Frenštát pod Radhoštěm for providing samples of buckwheat and its products.

Last but not least thanks go to my family and friends who supported me throughout my studies.

This work was kindly supported by the internal grant of TBU in Zlín No. IGA/14/FT/10/D funded from the resources of specific university research and by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 7088352101).

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LIST OF ABBREAVIATIONS

AA	Amino Acid
AAA	Amino Acid Analyzer
AAS	Atomic Absorption Spectrometry
ANOVA	Analysis of Variance
%CV	Coefficient of Variation
CZ	Czech Republic
DAD	Diode Array Detector
DW	Dry Weight
EAA	Essential Amino Acids
EAAI	Essential Amino Acids Index
EC	European Commission
FAO	Food and Agricultural Organization
FIP-U	Fédération Internationale Pharmaceutique (International Commission on Pharmaceutical) Unit
HPLC	High Performance Liquid Chromatography
IDF	Insoluble Dietary Fibre
InsP	Inositol Phosphate
IP_6	Inositol Hexaphosphate
ND	Not Detected
SCFA	Short Chain Fatty Acids
S.D.	Standard Deviation
S.E.	Standard Error
SDF	Soluble Dietary Fibre
WHO	World Health Organization

1 INTRODUCTION

Naturally occurring phosphorous compounds are phytic acid and phytates [1]. Phytic acid is a natural plant compound. It is a simple ringed carbohydrate with six phosphate molecules attached to each carbon. This unique structure, with twelve replaceable protons and high density of negatively charged phosphate groups, is responsible for its characteristic properties [2].

1.1 Phytic acid

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate, IP_6) represents a major antinutrient in food and feed [3]. Phytic acid was first identified in 1855 [4]. In nature it can be found in the form of mixed salt called phytin which, instead of calcium and magnesium, contains also smaller amount of zinc, copper, iron and other elements [5].

1.1.1 Structure

Inositol phosphates consist of an inositol ring and at least one phosphate group (Fig.1). 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 stereo isomers 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. The *myo*-inositol is common in plants [6,7].

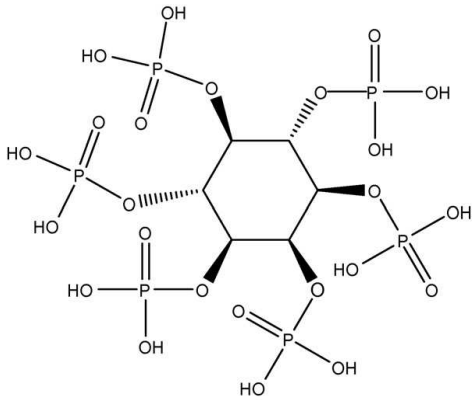

Figure 1: Structure of phytic acid [3]

The conformation of *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

counter clockwise 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 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 stereo isomers 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 [8-10].

Phytates are gaining increasing attention from researchers as antinutritional factors because of modern trends toward consumption of increasing amounts of vegetable fibre and fibre-rich cereal and oil seed products. Phytates also interfere with digestion of proteins and carbohydrates [1].

1.1.2 Occurrence

Phytic acid occurs naturally in many foods derived from plants [7]. It is a typical component of mature plant seeds, but it is also found in the roots and tubers of many species and has been detected in pollen and spores. Besides cereals, legumes, oil plants and nuts which are characterized by high content of phytic acid, there exist also plants with low content (potatoes, artichoke, carrot, broccoli, strawberries, blackberries and figs) and plants which does not contain phytic acid (lettuce, spinach, onion, celery, mushrooms, apples, bananas, pineapple and citrus fruits) [11,12]. Content of phytic acid in different foodstuffs is presented in Table 1.

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 [13]. Its content in endosperm is low, but it is higher in surface layers. A lot of phytic acid is in soy and pea seeds (more than 2%), also in sunflower and rape seeds [5].

The primary functions of phytic acid in seeds are storage of phosphates as energy source and antioxidant for the germinating seed [14].

Table 1: Phytic acid content in some crops and foodstuffs (g kg^{-1}) [11]

Foodstuff	Phytic acid
Wheat	3.9-13.5
Wholemeal wheat bread	4.3-8.2
Rye	5.4-14.6
Barley	7.5-11.6
Oat	7.0-11.6
Corn	8.3-22.2
Non-peeled rice	8.4-8.9
Peeled rice	3.4-5.0
Soybeans	10.0-22.2
Soy flour defatted	15.2-25.2
Lentil	2.7-10.5
Peas	2.2-12.2
Almonds	12.9-14.6
Peanuts	17.6
Walnuts	6.5-7.7
Cocoa	0.9
Carrot	0.2-0.3
Potatoes	0.2-0.5

1.1.3 Properties

The terms phytic acid, phytate and phytin refer to free acid, salt and calcium/magnesium salt, respectively. In literature, the name phytic acid has been used interchangeably with the term phytate, which is a salt [15].

Six phosphate groups in the molecule of IP_6 make it a strong chelating agent, which binds minerals such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Zn^{2+}. 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 [16].

Stability and solubility of the metal cation-phytate complexes depend on the individual cation, the pH value, the phytate:cation molar ratio and the presence of other compounds in the solution. A cation can bind to one or more phosphate groups of a single phytate molecule or bridge two or more phytate molecules. Most phytates tend to be more soluble at lower pH values. Solubility of phytates increases at pH values lower than 5.5-6.0 with Ca^{2+}, 7.2-8.0 with Mg^{2+} and 4.3-4.5 with Zn^{2+} as the counter ion. Ferric ion is insoluble at pH values in the range of 1.0-3.5 and the solubility increases above pH 4 [17].

Phytic acid is a strong acid with particularly great ability to form complexes with different cations. Cation binding is influenced by its concentration, phytic

acid concentration, presence of other cations and the pH value. The phytic acid affinity to cations falls in this sequence: $Cu^{2+}>Zn^{2+}>Co^{2+}>Mn^{2+}>Fe^{3+}>Ca^{2+}$. Proportional coupling between phytic acid and metal cations makes their absorption difficult. Phytic acid binds also cations of heavy metals. It could be supposed that phytic acid lowers the heavy metals toxicity, their absorption and accumulation in tissues [5].

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 [18].

Recent findings show 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 [19].

Phytic acid is also able to form complexes with proteins. At low pH it electrostatically binds to alkali amino acids (Arg, Lys, His). This complex is broken in the isoelectric point, but the new one, in which the binding between phytic acid and protein is intermediated by divalent cations, especially Ca^{2+}, is formed. Complexes of phytic acid with proteins are insoluble and are more resistant to the proteolytic cleavage than the initial protein. Phytic acid lowers the activity of digestive enzymes, pepsin, trypsin, α-amylase and lipase. It can be caused by the non-specific interaction with protein enzyme or with the withdrawal of Ca^{2+} ions which are necessary for some of the enzyme effect. On the other hand, interactions with proteins moderate the adverse effect of phytic acid on the Ca and Zn absorption [5].

Phytic acid has some properties of antioxidants. It defends the Fenton reaction (1.1), in which hydroxyl radicals are formed, and the oxidative damage of stored foodstuffs. Hydroxyl radicals are very reactive and can damage all biologically significant molecules [5].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} - OH' - OH^- \qquad (1.1)$$

Phytate is remarkably unreactive and extraordinarily stable. All of the antioxidant properties of phytic acid likely derive from its relatively high binding affinity for iron [20].

Phytic acid acts as an antioxidant due to its ability to bind iron, which is involved in the generation of iron-catalyzed hydroxyl radicals as free iron or chelated iron. Phytic acid is one of the few chelators which, while preserving the solubility of iron, make this metal completely unreactive. Phytic acid may prevent the formation of radicals in food or in the alimentary tract [12,16,20]**.**

Phytic acid could be considered to be a food additive which protects ascorbic acid and lipids against oxidation. It also defends the enzymatic browning of fruit and vegetables where it inhibits polyphenoloxidase [5].

1.1.4 Interactions with proteins

Phytic acid forms strong electrostatic linkages with basic amino acyl residues at low pH and thereby precipitates most proteins below pH 5.0. At neutral and alkaline pH both phytate and proteins have a negative charge which leads to their dissociation from each other. Polyvalent cations form metal bridges between phytic acid and proteins and promote their association at neutral pH. By virtue of binding proteins, phytic acid has been found to inhibit polyphenol oxidase, α-amylase, alcohol dehydrogenase, trypsin and other enzymes. A unique type of protein-phytate interaction is the high affinity of phytic acid for the 2, 3-diphosphoglycerate site in haemoglobin. In human haemoglobin A, eight basic amino acyl residues form electrostatic bridges and two hydrogen bonds with the six phosphate moieties of phytic acid. The binding of phytic acid modifies the heme iron-O_2 interaction which facilitates dissociation of oxygen from haemoglobin. Phytic acid can be incorporated into erythrocytes irreversibly to give functionally intact cells with improved O_2 transport capabilities. These phytate-laden erythrocytes may prove useful in the treatment of organ ischemia, haemolytic anemia and pulmonary insufficiency [20].

1.1.5 Interactions with metals

The unique structure of phytic acid suggests tremendous chelation potential. By virtue of its high calcium affinity, phytic acid also adsorbs tightly to hydroxypatite, a complex crystalline calcium phosphate ($Ca_5\,[PO_4]_3\,OH$), which is the chief structural element of vertebrate bones and teeth. Metal phytate complexes have long been known to be highly insoluble over a wide pH range, which forms the basis for the highly publicized concern over dietary phytate [20].

1.1.6 Influence on human health

Alimentary intake of phytic acid in human fluctuates depending on the food composition. High intake is in vegetarians and microbiotics. The daily intake of phytate for humans on vegetarian diets, on an average, is 2000-2600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150-1400 mg [21,22]. Consumption of phytate, however, seems not to have only negative aspects on human health. Dietary phytate could prevent kidney stone formation, protect against *diabetes mellitus*, caries, atherosclerosis, serum cholesterol level and coronary heart disease as well as against a variety of

cancer. Phytate has also been found to inhibit platelet aggregation. Inhibition of α-amylase also lowers the blood glucose response and may prove useful in the clinical management of hyperlipidemia and diabetes [12,17,20].

The formation of insoluble metal cation-phytate complexes at physiological pH values is regarded as the main reason for a poor mineral availability, because these complexes are essentially non-absorbable from the gastrointestinal tract [23].

Binding of phytate with minerals or proteins depends on pH value, which changes from low pH in the stomach to about neutral in the upper small intestine, dietary phytate complexes may dissociate and phytate may form other chelates during its passage through the gastrointestinal tract [24].

While intestinal mucosa and bacteria have been shown to contain some phytase activity, the majority of the ingested phytate passes through the gastrointestinal tract undegraded [20].

1.2 Phytases

Phytases (*myo*-inositol (1,2,3,4,5,6) hexa*kis*phosphate phosphohydrolases) are a class of phosphatases with the *in vitro* capability to release at least one phosphate from phytic acid. Phytases are able to hydrolyse phytate to a series of lower phosphate esters of *myo*-inositol and phosphate. The earliest reports of a phytase activity are from the blood of calves and rice bran indicating from its discovery [25-28]. Phytases can be produced by fungi, bacteria, yeasts and higher plants [12]. Most monogastric animals, including humans, lack the enzyme in their digestive system, making phytic acid hydrolysis dependent on mucosal or bacterial enzymes or on non-enzymatic hydrolysis by gastrointestinal acidity [29-31].

Phytases are phosphatases and can be divided according to the phosphate group in phytic acid they can cleave. There are two types of phytase: 3-phytase (EC 3.1.3.8), which is considered to be typical for microorganisms, and 6-phytase (EC 3.1.3.26), which is typical for higher plants. Phytases of different origin have different pH and temperature optima [32,33]. The phytases of many important crops are active from pH 4 to 6, with an optimum at about pH 5. Türk *et al*. (1996) reported that the activity of yeast phytase is fairly high from pH 3.5 to 4.5 and peaks at pH 3.5. The hydrolysis of phytic acid can take place in the digestive tracts of humans and animals or in the food and feed prior to consumption [12,33,34].

Lower inositol phosphates originate gradually by the enzymatic hydrolysis. The final hydrolysis product is six molecules of orthophosphate and *myo*-inositol which is absorbed by the intestinal mucosa. Phytase activity in gastrointestinal tract comes from three sources. It is phytase activity coming

from plant food, activity of endogenous secrets and activity of the microbial origin. Phytase activity of plant origin is significant, for example only in wheat grains [5].

The main significance during phytic acid hydrolysis in the gastrointestinal tract has enteric bacteria. Ruminants, which have large *proventriculi* composed of rumen, second stomach and third stomach, densely colonised with bacteria, protozoa and anaerobic fungi, hydrolyse phytic acid entirely while in other animals (with simple stomach) the phytic acid hydrolysis is only in part. The greatest microbial colonisation in monogastric animals is found in back parts of the gastrointestinal tract. There are the best conditions for phytic acid hydrolysis in these sections. Microbial hydrolysis is insignificant in stomach and small intestine. For greater part of phytases activity *Selenomonas ruminantium* is responsible, in minor rate also *Megasphaera elsdenii*, *Prevotella ruminicola* and *Mitsuokella multiacidus*. Phytase produced by *S. ruminantium* can be inhibited by Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Hg^{2+} and citrate [5].

Ruminant animals make full use of phytic acid-P, because rumen microbes produce large amount of phytase. In contrast, the bioavailability of phytic acid-P is low in non-ruminants such as pigs and poultry, especially when these animals are fed maize and/or soybeans [12].

1.3 Legumes

Legumes are dry edible seeds of some plants from the family of *Fabaceae*, such as beans, lupine, peas and lentil. 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. However, the legumes also contain antinutritional factors, such as proteinase inhibitors, lectin, rafinose oligosaccharides, saponins, polyphenols and phytate [35,36].

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 [37].

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. Phytic acid is a potent inhibitor of iron-catalysed hydroxyl radical formation by chelating free iron and then blocking the coordination site [26].

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. Protease inhibitors, a major class of antinutritional factors in pulses, often inhibit the digestive enzyme trypsin, but may act more broadly by inhibiting chymotrypsin and other serine proteases. Lectins are proteins that bind to carbohydrates or to the molecules containing carbohydrates. This binding capacity allows them to agglutinate red blood cells of different animal species depending on the specific receptors on the cell membrane surface. Tannins can form strong cross-linked complexes with dietary proteins and enzymes [37].

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 [35,38]. 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 [39].

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 [40].

Germinated legumes are rich in vitamin C and in some there is an increase in the riboflavin as well as niacin contents upon germination. The activity of many enzymes such as amylase, protease, phytase and lipase, will increase during germination [41].

1.4 Buckwheat

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 [42]. Buckwheat is categorized as a pseudocereal, so it shows both differences and similarities with cereals. It is an annual, dicotyledonic plant from the family of *Polygonaceae* [43]. Buckwheat does not have too massive root system, but its physiological activity is significant. Buckwheat roots excrete formic, acetic, citric and oxalic acids which help the plant to take nutrients, mainly phosphorus, from hard available forms. The stalks are hollow and their colour is green to red. Leaves stand alternately on the stalk. Buckwheat inflorescence is formed by 7 to 9 blossoms. They are tiny of white, pink or red colour [44]. Its seeds are edible and have triangular shape. The pericarp has a

hard fibrous structure and surrounds the seed coat, endosperm and embryo tightly. The endosperm consists mainly of starch [42].

The buckwheat fruit contains proteins, saccharides, lipids, fibre, vitamins and minerals as basic components. It is a source of dietary minerals like zinc, copper and manganese [45]. It is also rich in dietary fibre which has a positive physiological effect in the gastrointestinal tract and also significantly influences the metabolism of other nutrients. 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 [46].

Buckwheat can act in the prevention and treatment of hypertension and hypercholesterolemia and it could be useful in preventing colon cancer. The preventive effect can be connected with the content of dietary fibre in buckwheat. It has become increasingly apparent that dietary fibre components in food may have a positive physiological effect in the gastrointestinal tract and also significantly influence the metabolism of other nutrients [47]. Similar effects are associated with the inclusion of resistant starch in the diet. Buckwheat groats contain an important amount of resistant starch [48,49]. In different parts of the buckwheat plant and groats, Watanabe (1998), Kreft *et al.* (1999) and Park *et al.* (2000) found appreciable amounts of rutin, a secondary plant metabolite that antagonizes the increase of capillary fragility associated with hemorrhagic disease or hypertension in man [50-52]. It also decreases the permeability of the blood vessels and has an anti-oedema effect, reduces the risk of arteriosclerosis and has antioxidant activity. Rutin (quercetin-3-rutinosid) is a flavonol glycoside synthesized in higher plants as a protection against ultraviolet radiation and diseases [53]. It was firstly detected in *Ruta graveolens* which gave the common name to this pharmaceutically important substance. Among fruits, vegetables and grain crops, grapes and buckwheat are the most important rutin containing food. Most rutin is accumulated in the inflorescence, stalks and upper leaves [54,55].

Besides common buckwheat (*Fagopyrum esculentum* Moench), limited extent of tartary buckwheat (*Fagopyrum tataricum*) is cultivated. It is original plant from Siberia and Central Asia. Because of its higher resistance to low temperatures and simplicity to soil and climatic conditions it replaced common buckwheat at higher altitudes (Tibet, the Himalayas, Nepal, India, etc.) [56].

1.5 Chemical composition

Buckwheat is important as a functional food. Besides various polyphenols, it contains proteins with high biological value and balanced amino acids, relatively high fibre content, retrograded starch in groat products, high content of zinc, copper and manganese and dietary selenium [55].

Legumes 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 [26].

1.5.1 Proteins and amino acids

Protein malnutrition is a major nutritional syndrome affecting more than 170 million preschool children and nursing mothers in developing Afro-Asian countries. Provision of adequate proteins of the animal origin is expensive. The alternative is to supplement the diet with plant proteins. Legumes are the major contributors of proteins in Afro-Asian diets. Protein content in legume grains ranges from 17 to 40%, being equal to the protein contents of meat (18-25%). Legumes contain adequate amounts of lysine, but are deficient in S-containing amino acids (methionine and cysteine) [40,57].

Most of the protein in buckwheat is located in protein bodies. Protein bodies are special cellular organelles with average diameters of 1-10 μm and are bound by a single membrane. The majority of buckwheat proteins consist of globulins and albumins. Buckwheat contains only a little or no prolamine. This is the reason why people with coeliac disease can consume it. Buckwheat protein contains a wide range of various amino acids; 17 of them have been identified. In buckwheat, in contrast to cereals, leucine is sometimes limiting instead of lysine [42].

1.5.2 Minerals

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 [58]. 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 is an effective chelator of many essential mineral nutrients, constituting about 1-5% of the dry matter of many cereals and edible legumes. 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 [59-64].

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. Buckwheat is a richer source of minerals than many cereals, especially in levels of Mg, Zn, K, P and Mn [42,65].

1.5.3 Lipids

In general, lipids comprise a small part of cereals and pseudocereals, but they have an important physiological role. Lipids also play a role in food quality as they may cause deterioration of stored seeds or flours. In buckwheat, lipids are concentrated in embryo. Eighteen fatty acids has been identified in buckwheat, eight of them (oleic, linoleic, palmitic, linolenic, lignoceric, stearic, behenic and arachidic) represent 93% of total fatty acids. From essential fatty acids, linoleic acid is the major one in buckwheat [42].

Except soybeans, legumes are low in lipid content. The lipid component is highly unsaturated and often contains relatively high levels of other constituents such as plant sterols, isoflavones and saponins which may be physiologically active [66].

1.5.4 Starch

Starch, the major biopolymeric constituent of plants, occurs in characteristic granular forms of various shapes and sizes [67]. Starch provides the major source of physiological energy in human diet. It is also functionally very important polysaccharide. Chemically, starch is composed of two main components, amylase and amylopectin and a minor third component known as the intermediate fraction. The properties of these components depend upon the type of starch, its maturity, agro-climatic conditions and the type of cultivars. Starch in buckwheat seed is stored in endosperm where it is, during germination, hydrolysed to simply sugars to provide energy for seedling growth [41,68]. Buckwheat groats contain about 54.5% of starch. In general, buckwheat starch has its own unique characteristics; some properties correspond to tuber starches (high viscosity value) and others correspond more with cereal starches (shape and composition) [42].

1.5.5 Dietary fibre

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**.** The term dietary fibre was first used in 1953. In 1972, the first definition was formulated. There is still no clear, globally accepted definition of dietary fibre [69].

Dietary fibre can be divided in insoluble fibre (IDF) and soluble fibre (SDF). IDF generally includes lignin and cellulose, while SDF includes pectin and gums. SDF especially may contribute positively to human health by reducing levels of blood cholesterol. However, dietary fibre can also have negative effect it may bind minerals and proteins, inhibit digestive enzymes and thereby lower absorption or digestibility [42]. Bonafaccia et al. (2003) reported a content of dietary fibre of 27.4% in buckwheat seeds [70].

The raffinose family of oligosaccharides, which are soluble carbohydrates found in appreciable concentrations in pulses and other legumes, are potential prebiotics. These oligosaccharides resist digestion and absorption in the upper part of the intestinal tract and pass into the large intestine where they are fermented by colon microflora; fermentation products include gases and short chain fatty acids (SCFA). Although the gases may cause digestive discomfort due to flatulence, the SCFA support the health of the intestinal mucosa. Pulses are edible seeds of leguminous crop, are rich food source of fibres that promote various beneficial physiological effects for human health [71].

The energy benefit of fibre is small, it has especially protective function; it acts in the prevention of many mass occurrence non-infectious diseases, such as colon cancer, cardiovascular diseases, diabetes, obesity, chronic constipation, etc. The major sources are mainly cereals, pulses, vegetables, fruit and potatoes and products thereof [72].

1.6 Digestibility

Determination of nutritional value of specific foods 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 feed or food. Methods for digestibility determination can be divided into two basic: *in vivo* and *in vitro*. If the digestibility is determined in experiments with organisms, it is *in vivo* method. *In vitro* method is carried out under laboratory conditions and uses pepsin and pancreatic proteases to simulate digestive functions *in vivo*. Although *in vitro* methods are less expensive and time consuming than *in vivo* methods, using of these findings in human nutrition can have numerous limitations [73,74].

Digestibility may be used as an indicator of protein availability. It is essentially a measure of the susceptibility of a protein to proteolysis. A protein with high digestibility is potentially of better nutritional value than one of low digestibility because it would provide more amino acids for absorption on proteolysis [75]. 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 [76,77].

2 OBJECTIVES OF THE DOCTORAL THESIS

The aim of the Doctoral thesis is to ascertain the basic chemical composition of legumes and products made from common buckwheat (*Fagopyrum esculentum* Moench) during one-year storage, with the stress on subsequent determination of phytic acid content and digestibility in all samples.

Partial aims:

1. Basic chemical composition in particular samples (moisture, ash, total fat, crude protein)
2. Determination of amino acid composition
3. Establishing of starch content in buckwheat products
4. Determination of fibre content
5. Minerals analysis
6. Extraction and determination of rutin in buckwheat products
7. Extraction and determination of phytic acid
8. Finding of digestibility of crude protein, fibre and phytic acid
9. Statistical evaluation of measured data

3 MATERIAL AND METHODS

In order to achieve the aims of the thesis, one-year (2010/2011) storage experiment was carried out. Samples were stored at ordinary room temperature of 21±2 °C, so roughly at the same conditions as in the shop or at home after purchasing them. Every three months sampling and chemical analyses were carried out. In total, four samplings were performed. First sampling was after receiving them, the last one after the best before date. Content of moisture, ash, total fat, crude protein, fibre, phytic acid and starch (for buckwheat products) has always been determined. Amino acid composition and concentration of rutin (in buckwheat products) were determined only in the first and the last sampling. Digestibility and mineral content were determined only at the beginning of the experiment.

3.1. Material

In all experiments, two basic groups of samples were used. From legumes, common beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), soybeans (*Glycine max*) and lentil (*Lens esculenta*), were selected for the analysis. All legume samples were purchased in the trade network.

Buckwheat products (peels, whole seeds, wholemeal flour, broken groats, crunchy products natural and cocoa, flour, groats and pasta) were obtained from Pohankový mlýn Šmajstrla s.r.o., Frenštát pod Radhoštěm, Czech Republic. The products were made from seeds of common buckwheat (*Fagopyrum esculentum* Moench) cultivated in the region of Slezské Rudoltice, Czech Republic.

3.1.1. Sample preparation

All samples were packaged in consumer wrapping. In each sampling, one 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 chemical analyses were performed. All analyses were realized according to the Official Journal of the European Union [78] except rutin and phytic acid analyses. Rutin was performed by modified method using information from Deineka *et al*. (2004) and Gokarn *et al.* (2010) [79,80]. Phytic acid was determined using modified Holt´s method [81]. 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.

3.2 Methodology

All operations during sample preparation were performed carefully in order to avoid the damage of samples. In selected samples following parameters were determined - moisture, ash, crude protein, total fat and mineral contents. Also amino acid composition, fibre, phytic acid contents and *in vitro* digestibility were performed. Rutin concentration and starch amount were ascertained in buckwheat products. In order to compare different samples, all values were converted to 100% dry weight by multiplying with the conversion factor.

3.2.1. Moisture and ash content

Moisture content was determined using drying at 103±2 °C to the constant loss of the weight. Content of moisture was expresses in % (w/w) of original sample. Ash content was determined by burning of the sample at 550±5 °C for 5 hours in the muffle-furnace (mLw Electro, Electric furnaces Svoboda, CZ) [78].

3.2.2. Energy

The energy was determined in an automatic bomb calorimeter PARR 1281 (Parr Instrument Company, Moline, IL, USA). Calorimetry is based on determination of energy released by burning off the food out of body. Brutto energy was determined by absolute burning of feed in oxygen atmosphere and was expressed in MJ kg^{-1} DW (dry weight) [82,83].

3.2.3. Total fat content

Total fat content was determined gravimetrically by the Soxhlet method extraction under reflux. Fat was hot-extracted using n-hexane (LUKEŠ, Uherský Brod, CZ). In the end of the distillation, the solvent was distilled off and the residue was dried and weighed [78].

3.2.4. Crude protein content

Crude protein content was determined according to the Kjeldahl method using the Pro-Nitro 1430 apparatus (BIO PRO, Prague, CZ). From mineralised samples (mineralisation block Digest 12, BIO PRO, Prague, CZ; Fig.2), prepared according to Kjeldahl, the ammonia released from the reaction of ammonium sulphate with heavy solution of sodium hydroxide was distilled with water vapour into boric acid solution (3.1). Incurred ammonium borate was determined by the titration with hydrochloric acid solution, using the Tashiro indicator (Fluka, Germany) (3.2).

$$6NH_3 + H_3BO_3 \rightarrow 2(NH_4)_3BO_3 \quad (3.1)$$

$$2(NH_4)_3BO_3 + 6HCl \rightarrow 6NH_4Cl + 2H_3BO_3 \quad (3.2)$$

From the acid consumption, the amount of nitrogen was calculated. The result was recalculated to the sample weight and by multiplying it with the factor 6.25 for legumes, or 5.7 for buckwheat, the percentage of crude protein was obtained [78].

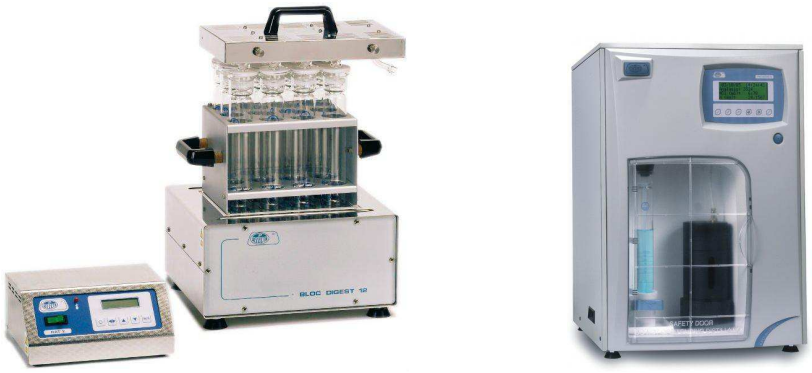

Figure 2: Mineralisation block Digest 12; Pro-Nitro 1430 apparatus [84]

3.2.5. Amino acid composition

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, and 23h). Sulfur amino acids (cysteine and methionine) were, prior to acid hydrolysis, oxidized by mixture of formic acid and hydrogen peroxide (9:1; 16h, 6±2 °C), 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; Fig.3) with post-column ninhydrin derivatization and spectrophotometric detection (440 nm for proline and 570 nm for other amino acids) [85,86]. 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) [87].

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. The amount of individual amino acids in these samples was expressed in g kg^{-1}. Amino acid composition was determined from the initial dry mass of all samples during the storage experiment.

Figure 3: Amino acid analyzer AAA 400 [88]

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 2). 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 [83].

Table 2: Content of essential amino acids in standard (FAO/WHO) and egg protein [89,90]

Amino acid	FAO/WHO (g $16gN^{-1}$)	Egg protein (%)
Valine	5.0	7.3
Leucine	7.0	8.7
Isoleucine	4.0	6.6
Methionine + Cysteine	3.5	5.7
Threonine	4.0	5.1
Lysine	5.4	6.9
Phenylalanine + Thyrosine	6.1	9.8

3.2.6. Starch content

Samples for starch determination were treated with dilute hydrochloric acid. After clarification (30% $ZnSO_4$ and 15% $K_4[Fe(CN)_6]$) and filtration, the optical rotation of the solution was measured by polarimetry (OPTIKA microscopes, Ponteranica, Italy). Content of starch was calculated and expressed in % (w/w). Specific rotation for buckwheat starch was 184.0° [78].

3.2.7. Fibre

Total fibre in legumes and buckwheat products was determined using the apparatus Ancom^{220} Fibre Analyzer (ANCOM Technology, New York, USA). For the analysis, filter bags F57 with pore size 50 μm were used. Samples were weighed into filter bags, sealed, stacked into the stand and put into the analyzer (Fig.4). First, acid solution (5% H_2SO_4) was added and after 30 minute incubation at 100±2 °C, acid solution was drained and bags were rinsed with hot distilled water. Then, alkaline solution (5% NaOH) was added and after incubation (30 minutes, 100±2 °C), the solution of NaOH was launched and bags were washed with hot distilled water. Filter bags were slightly dried on filter paper and rinsed with acetone. After evaporation of the solvent, they were dried in laboratory oven (Venticell, BMT, Brno, CZ) at a temperature of 103±2 °C and after cooling, weighed. Subsequently, bags were burnt in a muffle furnace (mLw Electro, Electric furnaces Svoboda, CZ) at 550±5 °C. From obtained values, fibre content in original mass of individual samples, in % (w/w), was calculated [83].

Figure 4: Ankom220 Fibre Analyzer [91]

3.2.8. Minerals

Samples (0.3 to 0.5 g) 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.

Table 3: Wavelengths for particular elements (nm)

Element	Na	K	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Cr
Wavelength	589.0	766.5	422.7	285.2	213.9	324.7	248.3	217.0	228.8	357.9

3.2.9. Rutin

Rutin concentration was determined in buckwheat products using a modified method according to Deineka *et al.* (2004) and Gokarn *et al.* (2010). Two grams of the sample (rutin hydrate was from Dr. Ehrenstorfer GmbH, Ausburg, Germany) were extracted with methanol:acetic acid:water (100:2:100). After sonification and shaking, test-tubes were centrifuged at 4000 rpm for 5 minutes and filtrated through 0.45 μm filter (Millipore, USA). Subsequent analysis was provided using an HPLC 10 AVP system equipped with a SCL-10 AVP control unit with a control software Class-VP 5.02, two LC-10AVP pumps, a GT-154 degasser, a CTO-10ASVP column thermostat, a Rheodyne 7120 injector valve, Waters C18 column (4.6 x 75 mm, 5 μm pore size) and a SPD-M10AVP diode array detector (all from Shimadzu, Tokyo, Japan). Mobile phase consisted of acetic acid:acetonitrile:methanol (75:15:10), the flow rate was 1 ml per minute,

and the detection was performed at 355 nm. Rutin concentration was expressed in μg g^{-1} [79,80].

3.2.10. Phytic acid content

The determination of phytate was realized by modified Holt´s method [81]. Samples were extracted with 0.5M HNO_3. The extract was filtrated through the filter FILTRAK, No. 390, ø 12.5 cm.

Next, filtrate was diluted with distilled water to a final volume of 1.4 ml. After that, ferric ammonium sulphate solution (containing 50 μg of Fe) was added. After heating in boiling water bath (Memmert, Germany) and cooling to a room temperature, amyl alcohol and ammonium thiocyanate solution (100g L^{-1}) was subsequently added. After centrifugation for 5 minutes at 500 rpm, the intensity of the colour in the amyl layer was determined at 465 nm using a spectrophotometer (Biochrom Libra S6, Cambridge, England, UK) against an amyl alcohol "blank", exactly 15 minutes after addition of NH_4CNS [92].

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 of the amount of phytate in samples (Fig.5). Phytic acid concentration was expressed in g 100 g^{-1}.

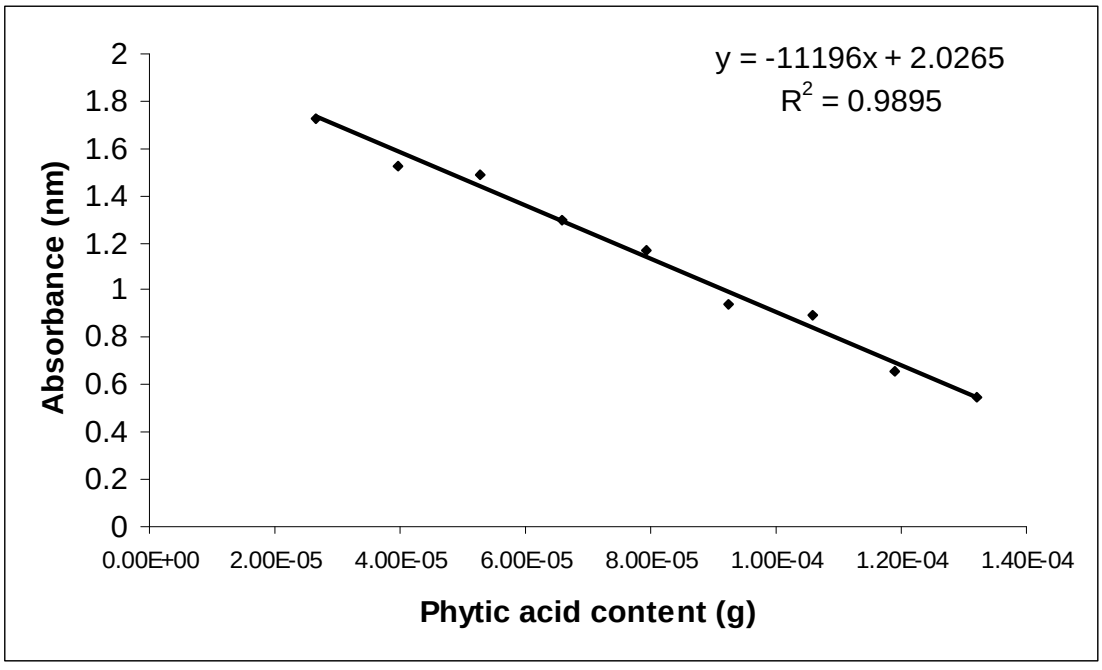

Figure 5: Standard curve for phytic acid determination

3.2.11. Digestibility

Digestibility of legumes and buckwheat products was determined using the enzymatic-gravimetric methods *in vitro*. For digestibility determination 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 combined hydrolysis with pepsin and pancreatin were performed.

Hydrolysis with pepsin

One gram of homogenised sample was weighted into filter bags (F 57, pore size 50 μm, ANCOM Technology, New York, USA) with the accuracy of 0.0001 g. Bags with samples were sealed and together with empty bag (used for correction) were put into incubating bottles in the number of 24 bags to one bottle in maximum. Into each bottle, 1700 ml of incubating solution was added. The solution was prepared by dissolving 3 g of pepsin in HCl solution (0.1M) tempered to 40 °C. Bottles were capped, placed to the *in vitro* incubator Daisy (Fig.6) and were incubated for 24 hours at a temperature of 40±2 °C. After incubation, bags were rinsed with distilled water till it was not clear. Excess water in bags was removed using filtrate paper. Subsequently, bags were dried in laboratory drying machine (Venticell, BMT, Brno, CZ) at 103±2 °C for 24 hours. Then, they were put into a desiccator and after cooling weighted [93].

Figure 6: *In vitro* incubator Daisy [94]

Combined hydrolysis with pepsin and pancreatin

Digestibility of all samples was also determined using combined hydrolysis with pepsin and pancreatin. Pepsin hydrolysis was performed by the method mentioned above. After washing of bags, 1700 ml of incubation solution was poured into bottles with filter bags and the incubation continued in the incubator for next 24 hours. The solution was prepared by dissolving 3 g of pancreatin in phosphate buffer with pH 7.45, tempered to 40 °C. Phosphate buffer was a mixture of KH_2PO_4 (9.078 g L^{-1}) and $Na_2HPO_4.12H_2O$ (23.889 g L^{-1}) in the ratio of 2:8. After finishing the incubation, filter bags were washed with distilled water till it was clear, excess water was removed by the filtrate paper, bags were put into laboratory drying machine, dried at 103±2 °C for 24 hours and after cooling in a desiccator, they were weighted [93].

Results of digestibility were expressed as coefficient of digestibility (X). It is a ratio of amount of compound after digestion (C_1) to amount of compound before digestion (C_2) multiplied by 100 and expressed in % (3.3).

$$X = \frac{C_1}{C_2} \cdot 100 \qquad (3.3)$$

3.3 Statistical evaluation of data

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) [95].

4 RESULTS AND DISCUSSION

It is important to mention that not all studied buckwheat products have been examined before; only flour, groats and in some studies also peels have been tested. Other products therefore can not be compared with any currently available literature.

4.1. Samples

For all experiments, samples of soybeans (*G. max*), peas (*P. sativum*), lentil (*L. esculenta*), common beans (*Ph. vulgaris*) and buckwheat products (peels, whole seeds, wholemeal flour, broken groats, crunchy products natural and cocoa, flour, groats and pasta) made from common buckwheat (*Fagopyrum esculentum* Moench) were used.

4.2. Nutritional composition

The basic chemical analysis of all samples was performed according to the Official Journal [78].

4.2.1. Moisture and ash

First of all, moisture and ash contents were determined according to the method presented in section 3.2.1. Values, expressed in %, are presented in Table 4.

The Regulation of the Ministry of Agriculture of the Czech Republic no. 329/1997 Coll. [96] states the maximum permitted content of moisture in legumes; for peas and beans 16%, soybeans 13% and lentil 15% in maximum. After comparison of these stated values with those obtained in the experiment, it can be concluded that all studied legumes comply with requirements from the Regulation. None of them contain more than 10% of moisture.

The Regulation of the Ministry of Agriculture of the Czech Republic no. 333/1997 Coll. [97] presents the highest possible content of moisture for buckwheat flour as 15% and 13% for pasta. Table 4 shows, that in both buckwheat flours the content of moisture was about 10%. Moisture in pasta after receiving was about 9% and during storage it decreased to almost 7%. From these results, it can be concluded that both buckwheat flours and also buckwheat pasta meet the requirements specified in the Regulation. During the one-year storage experiment, reduction of the moisture content almost in all samples was observed, only the moisture content of soybeans increased slightly. There was a gradual evaporation of water from samples, resulting in the aforementioned

reduction in moisture content. In contrast, the increase of the moisture content in soybeans could indicate that during storage, there was some chemical reaction in which water was formed.

Table 4 also presents the content of ash in examined samples. The amount of ash is related to content of minerals. The content of ash grew during storage almost in all samples, only in lentil, a small decrease was observed. The greatest content of ash was determined in *G. max*, more than 4%. De Costa Almeida *et al*. (2006) presented content of ash in *P. sativum*, *Ph. vulgaris* and *L. esculenta* as 3.0, 3.8 and 2.8%, respectively [57]. Content of ash in buckwheat seed was reported by Bonaffacia *et al.* (2003) as 2.6% in dry matter [98]. If we compare these value with those obtained in the experiment, it can be concluded that all values are similar.

Table 4: Content of moisture and ash (mean±S.E.) in %

	Moisture		Ash	
	Receiving	Best before date	Receiving	Best before date
Peels	8.0 ± 0.04	7.2 ± 0.11	1.6 ± 0.05	2.1 ± 0.01
Whole seed	10.4 ± 0.04	7.8 ± 0.02	2.0 ± 0.01	2.4 ± 0.01
Groats	10.7 ± 0.06	8.7 ± 0.10	2.4 ± 0.03	2.5 ± 0.02
Broken groats	9.1 ± 0.04	8.6 ± 0.14	1.6 ± 0.01	1.8 ± 0.02
Crunchy products natural	7.5 ± 0.02	6.7 ± 0.15	0.8 ± 0.00	1.1 ± 0.02
Crunchy products cocoa	6.5 ± 0.03	5.5 ± 0.01	1.0 ± 0.01	1.3 ± 0.01
Flour	10.1 ± 0.01	9.2 ± 0.08	2.2 ± 0.02	2.3 ± 0.03
Wholemeal flour	9.5 ± 0.07	9.0 ± 0.07	2.8 ± 0.06	3.0 ± 0.02
Pasta	9.4 ± 0.04	6.9 ± 0.10	0.9 ± 0.00	1.2 ± 0.03
G. max	6.4 ± 0.01	8.0 ± 0.12	4.6 ± 0.01	4.8 ± 0.08
P. sativum	9.0 ± 0.02	8.6 ± 0.04	2.6 ± 0.00	3.2 ± 0.05
Ph. vulgaris	8.4 ± 0.02	7.9 ± 0.11	3.7 ± 0.01	4.0 ± 0.01
L. esculenta	8.8 ± 0.01	8.0 ± 0.07	2.5 ± 0.02	2.3 ± 0.11

4.2.2. Energy

Energy was determined according to the method described in section 3.2.2. In Table 5, ascertained energy values of particular samples are presented. From all samples, soybeans are the most energy abundant foodstuff. Their energy value is about 22 MJ/kg. Energy values in buckwheat products range from 16 to 18 MJ kg^{-1}. If values measured in the experiment are compared with values from the literature (presented in Table 5) it is obvious that these values are not too different. Differences may be caused for example by using seeds of a different variety of the plant. Energy is necessary for all life processes in the body. Brutto energy is an important indicator of nutritional value.

Table 5: Energy value (mean±S.E.) in MJ kg^{-1}

	Literature values of energy [82]	**Energy values from the experiment**
Peels	-	18.2 ± 0.18
Whole seed	-	16.9 ± 0.03
Groats	16.6	16.7 ± 0.10
Broken groats	-	16.4 ± 0.10
Crunchy products natural	-	16.6 ± 0.12
Crunchy products cocoa	-	16.7 ± 0.12
Flour	-	16.8 ± 0.08
Wholemeal flour	-	17.4 ± 0.05
Pasta	-	16.4 ± 0.11
G. max	21.3	22.2 ± 0.20
P. sativum	16.3	16.8 ± 0.04
Ph. vulgaris	16.7	17.0 ± 0.05
L. esculenta	17.1	17.0 ± 0.15

4.2.3. Total fat content

Total fat content was determined according to the methodology mentioned in section 3.2.3. Table 6 shows discovered amounts of fat in particular samples. From the results it is obvious that the richest source of fat is *G. max*, the amount is almost 17% in the sample after receiving and a little bit higher in the sample after the best before date. Sometimes, soybeans are included in a group of oilseeds. On the other hand, *P. sativum*, *L. esculenta* and *Ph. vulgaris* contain markedly lower amounts of fat. For comparison with literature values a paper of Iqbal *et al*. (2006) was used. His team found the content of fat in *P. sativum* and *L. esculenta* as 1.5 and 2.2%, respectively [40]. Values for fat content in peas

and lentil are similar. The value for fat content in *Ph. vulgaris* is presented in the book from Zeman *et al.* (1995) where he reported that in common beans the content of fat is about 1.8% [82]. The value from the experiment is lower, but not so different.

Buckwheat products, in general, are low fat products. The content of fat differs from one product to another. Only whole seeds and wholemeal flour contain higher amount of fat, more than 7%. Bonafaccia *et al.* (2003) presented in their study the content of fat as 3.4% [98]. This value is lower than the one from the experiment.

During the storage experiment the fat amount was descending in all samples, except soybeans. Total fat in this pulse increased a little during storage.

4.2.4. Crude protein content

Crude protein content was determined according to the Kjeldahl method as described in section 3.2.4. All results are presented in Table 6. As can be seen from that table, content of crude protein in dry matter of legumes is the highest from all examined samples. Soybeans are rich in crude protein; they contain nearly 40% of this compound. Khattab *et al.* (2009) presented in their study a crude protein content in common beans as 24.9% [99]. 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 [82]. There were observed some differences in the content of crude fat in legumes. In *L. esculenta* and *P. sativum* lower values, 22.5 and 18.4%, respectively, were determined; the crude protein content determined in *G. max*, 37.8%, was similar to the reported value.

Content of crude protein in buckwheat products is the greatest in whole seeds, groats and both flours. Really the richest sources of crude protein are both flours, they contain about 14% of crude protein in dry matter and the amount does not differ so much in the first and the last sampling.

Crude protein content in most samples decreased during storage. There was a slight increase in crude protein content in some samples; but no significant changes were observed. These observations confirmed that legumes are valuable potential source of proteins, mainly in developing countries.

Table 6: Amount of crude protein and fat (mean±S.E.) in %

	Crude protein				Fat			
	Receiving		Best before date		Receiving		Best before date	
Peels	3.5 ± 0.24		3.0 ± 0.05		4.6 ± 0.00		0.7 ± 0.01	
Whole seed	10.2 ± 0.23		10.2 ± 0.13		7.3 ± 0.01		2.0 ± 0.01	
Groats	12.9 ± 0.00		13.0 ± 0.04		4.0 ± 0.00		2.6 ± 0.01	
Broken groats	9.2 ± 0.02		9.2 ± 0.07		6.0 ± 0.02		1.7 ± 0.01	
Crunchy products natural	6.8 ± 0.34		7.1 ± 0.33		2.4 ± 0.00		0.4 ± 0.00	
Crunchy products cocoa	6.5 ± 0.18		6.3 ± 0.14		1.7 ± 0.01		0.5 ± 0.01	
Flour	13.8 ± 0.54		12.4 ± 0.29		3.1 ± 0.01		2.1 ± 0.02	
Wholemeal flour	13.8 ± 0.27		14.5 ± 0.18		7.5 ± 0.02		2.9 ± 0.01	
Pasta	8.1 ± 0.37		7.3 ± 0.06		3.5 ± 0.01		1.3 ± 0.00	
G. max	37.8 ± 1.12		36.6 ± 0.01		16.9 ± 0.01		17.7 ± 0.04	
P. sativum	18.4 ± 0.05		19.1 ± 0.24		1.5 ± 0.01		1.4 ± 0.01	
Ph. vulgaris	24.0 ± 0.09		22.7 ± 0.32		1.5 ± 0.00		1.4 ± 0.00	
L. esculenta	22.5 ± 0.78		21.4 ± 0.14		2.0 ± 0.01		0.8 ± 0.00	

4.2.5. Amino acid composition

Amino acid composition was determined in the first and the last sampling. Samples were treated according to the methodology stated in section 3.2.5.

As can be seen from Tables 7a, 7b, 8a, 8b, 9a and 9b, all studied samples contain all 17 amino acids (AA). From buckwheat products tested after receiving them and also after the best before date, peels contain the lowest amounts of all amino acids. On the other hand, the highest contents of amino acids were found in wholemeal flour, groats, broken groats and light flour. All buckwheat products were rich in Glu, Asp and both flours were also rich in Arg.

Regarding to legume samples, the situation was different. The highest content of Cys, Glu, Asp, Leu, Lys and Arg was determined in all legumes in both samplings. 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} after receiving. After the best before date, also the content of Met declined. During the storage experiment, amounts of amino acids were changing a little; generally some of them grew, some of them decreased.

Jezierny *et al.* (2010) reported the amino acid composition of *P. sativum* in their study [100]. Values for almost all studied amino acids were higher then those in the experiment of this thesis; only for Met they presented a value of 2.2 g/kg DW which is lower then the one in the experiment and value for Cys (3.5 g/kg) which is close to the value in the experiment (3.6 g/kg).

Table 7a: Amino acid composition of buckwheat products after receiving (mean±S.D.) in g kg^{-1} DW

AA	Peels	%CV	Groats	%CV	Broken groats	%CV	Crunchy products natural	%CV
Cys	0.4 ± 0.03	7.0	4.5 ± 0.07	5.0	3.1 ± 0.05	2.0	1.5 ± 0.05	4.0
Glu	1.7 ± 0.06	4.0	14.1 ± 1.60	11.0	14.1 ± 0.12	1.0	7.0 ± 0.34	5.0
Asp	1.8 ± 0.12	7.0	9.8 ± 0.26	3.0	7.6 ± 0.18	2.0	4.4 ± 0.21	5.0.
Tyr	0.7 ± 0.06	9.0	3.1 ± 0.35	11.0	2.3 ± 0.07	3.0	1.4 ± 0.11	8.0
Ser	1.0 ± 0.08	9.0	3.9 ± 0.43	11.0	3.9 ± 0.03	1.0	2.1 ± 0.04	2.0
Pro	1.0 ± 0.07	7.0	4.0 ± 0.42	10.0	3.1 ± 0.26	8.0	2.1 ± 0.11	6.0
Gly	1.9 ± 0.13	7.0	6.4 ± 0.15	2.0	4.6 ± 0.24	5.0	2.9 ± 0.13	4.0
Ala	1.0 ± 0.08	8.0	4.4 ± 0.48	11.0	3.6 ± 0.27	7.0	2.3 ± 0.07	3.0
Val	1.1 ± 0.07	6.0	5.4 ± 0.63	12.0	4.4 ± 0.40	9.0	2.7 ± 0.07	3.0
Leu	1.2 ± 0.10	8.0	5.8 ± 0.35	6.0	4.7 ± 0.26	6.0	3.4 ± 0.05	1.0
Ile	0.7 ± 0.04	6.0	3.4 ± 0.14	4.0	2.8 ± 0.02	1.0	2.0 ± 0.07	4.0
Thr	0.9 ± 0.08	9.0	3.3 ± 0.17	5.0	3.4 ± 0.18	5.0	1.9 ± 0.06	3.0
Met	0.6 ± 0.01	3.0	2.9 ± 0.16	6.0	1.9 ± 0.17	9.0	1.9 ± 0.08	3.0
Lys	1.0 ± 0.04	4.0	6.0 ± 0.63	11.0	4.1 ± 0.37	9.0	2.6 ± 0.11	4.0
Phe	0.9 ± 0.08	9.0	6.1 ± 0.30	7.0	3.6 ± 0.27	8.0	2.4 ± 0.11	4.0
His	0.7 ± 0.04	6.0	2.7 ± 0.35	13.0	2.4 ± 0.14	6.0	1.2 ± 0.12	10.0
Arg	1.0 ± 0.08	9.0	11.5 ± 0.81	7.0	8.8 ± 0.62	7.0	4.6 ± 0.17	4.0

Table 7b: Amino acid composition of buckwheat products after receiving (mean±S.D.) in g kg^{-1} DW

AA	Crunchy products cocoa	%CV	Flour	%CV	Wholemeal flour	%CV	Pasta	%CV
Cys	1.8 ± 0.13	7.0	4.3 ± 0.04	1.0	4.5 ± 0.07	2.0	2.2 ± 0.19	8.0
Glu	6.7 ± 0.67	10.0	16.5 ± 1.07	6.0	19.1 ± 1.99	10.0	8.3 ± 0.80	10.0
Asp	4.2 ± 0.43	10.0	9.1 ± 0.88	10.0	11.1 ± 0.90	8.0	5.4 ± 0.60	11.0
Tyr	1.4 ± 0.09	6.0	3.0 ± 0.03	1.0	4.1 ± 0.37	9.0	1.8 ± 0.20	11.0
Ser	2.0 ± 0.18	9.0	4.6 ± 0.34	7.0	5.7 ± 0.71	12.0	2.5 ± 0.27	11.0
Pro	2.0 ± 0.10	5.0	4.0 ± 0.25	6.0	5.0 ± 0.38	8.0	2.2 ± 0.17	8.0
Gly	2.8 ± 0.32	11.0	6.2 ± 0.25	4.0	8.2 ± 0.77	9.0	4.0 ± 0.38	10.0
Ala	2.2 ± 0.18	8.0	4.5 ± 0.26	6.0	5.5 ± 0.56	10.0	2.7 ± 0.27	10.0
Val	2.5 ± 0.16	6.0	5.5 ± 0.38	7.0	6.5 ± 0.65	10.0	3.1 ± 0.32	10.0
Leu	3.2 ± 0.11	3.0	6.7 ± 0.59	9.0	7.3 ± 0.71	10.0	3.4 ± 0.32	9.0
Ile	1.9 ± 0.02	1.0	4.1 ± 0.50	12.0	4.5 ± 0.31	7.0	2.0 ± 0.18	9.0
Thr	1.8 ± 0.15	8.0	3.8 ± 0.32	8.0	4.8 ± 0.62	13.0	2.3 ± 0.22	10.0
Met	1.4 ± 0.17	12.0	2.9 ± 0.08	3.0	5.6 ± 0.13	2.0	2.3 ± 0.15	7.0
Lys	2.2 ± 0.18	8.0	6.2 ± 0.33	5.0	7.6 ± 0.70	9.0	3.1 ± 0.06	2.0
Phe	2.4 ± 0.24	10.0	4.9 ± 0.35	7.0	6.1 ± 0.30	5.0	2.7 ± 0.22	8.0
His	1.2 ± 0.03	3.0	2.8 ± 0.23	8.0	3.2 ± 0.37	12.0	1.7 ± 0.07	4.0
Arg	4.2 ± 0.59	14.0	11.9 ± 0.50	4.0	14.0 ± 1.48	11.0	5.0 ± 0.35	7.0

Table 8a: Amino acid composition of buckwheat products after best before date (mean±S.D.) in g kg^{-1} DW

AA	Peels	%CV	Groats	%CV	Broken groats	%CV	Crunchy products natural	%CV
Cys	0.5 ± 0.01	2.2	4.1 ± 0.00	0.0	3.1 ± 0.01	0.2	1.4 ± 0.08	4.6
Glu	2.0 ± 0.02	0.9	18.7 ± 0.69	3.7	12.5 ± 0.35	2.8	9.0 ± 0.20	2.2
Asp	2.0 ± 0.02	0.9	11.8 ± 0.48	4.1	7.9 ± 0.20	2.5	6.5 ± 0.45	6.9
Tyr	0.6 ± 0.01	2.3	3.0 ± 0.02	0.6	2.2 ± 0.01	0.6	1.6 ± 0.05	3.2
Ser	1.2 ± 0.03	2.4	5.4 ± 0.24	4.4	3.7 ± 0.07	1.9	2.8 ± 0.09	3.4
Pro	1.1 ± 0.00	0.4	4.0 ± 0.15	3.7	2.8 ± 0.12	4.4	2.4 ± 0.05	2.0
Gly	1.5 ± 0.03	2.0	6.7 ± 0.22	3.3	4.6 ± 0.02	0.4	3.6 ± 0.05	1.3
Ala	1.1 ± 0.04	3.3	5.2 ± 0.10	1.8	3.6 ± 0.13	3.6	3.0 ± 0.05	1.6
Val	1.2 ± 0.04	3.6	5.8 ± 0.17	2.9	4.0 ± 0.04	1.0	3.2 ± 0.03	0.9
Leu	1.6 ± 0.03	1.9	7.4 ± 0.28	3.8	5.2 ± 0.02	0.5	4.2 ± 0.10	2.4
Ile	1.0 ± 0.02	1.8	4.5 ± 0.14	3.2	3.1 ± 0.06	2.0	2.6 ± 0.06	2.3
Thr	1.1 ± 0.00	0.0	4.4 ± 0.13	2.9	3.1 ± 0.02	0.7	2.4 ± 0.06	2.7
Met	0.6 ± 0.02	3.9	3.0 ± 0.07	2.4	2.1 ± 0.00	0.2	1.4 ± 0.02	1.2
Lys	1.2 ± 0.01	0.9	7.0 ± 0.25	3.5	4.8 ± 0.00	0.0	3.5 ± 0.09	2.6
Phe	1.1 ± 0.00	0.4	5.4 ± 0.04	0.7	3.9 ± 0.13	3.4	3.1 ± 0.09	2.9
His	0.7 ± 0.04	5.7	2.7 ± 0.10	3.8	1.8 ± 0.09	5.0	1.4 ± 0.02	1.8
Arg	1.1 ± 0.03	2.9	13.0 ± 0.12	0.9	8.7 ± 0.31	3.6	5.9 ± 0.09	1.5

Table 8b: Amino acid composition of buckwheat products after best before date (mean±S.D.) in g kg^{-1} DW

AA	Crunchy products cocoa	%CV	Flour	%CV	Wholemeal flour	%CV	Pasta	%CV
Cys	1.8 ± 0.05	2.9	3.9 ± 0.24	6.3	4.4 ± 0.19	4.3	2.3 ± 0.00	0.2
Glu	8.6 ± 0.17	2.0	14.6 ± 0.12	0.8	20.1 ± 0.50	2.5	10.1 ± 0.17	1.7
Asp	5.8 ± 0.20	3.5	9.9 ± 0.22	2.2	13.5 ± 0.29	2.1	6.9 ± 0.05	0.8
Tyr	1.5 ± 0.07	5.0	2.5 ± 0.05	2.0	3.4 ± 0.04	1.1	1.5 ± 0.03	1.9
Ser	2.7 ± 0.07	2.6	4.2 ± 0.06	1.5	6.3 ± 0.15	2.4	3.2 ± 0.05	1.5
Pro	2.0 ± 0.06	3.1	3.0 ± 0.12	3.9	4.7 ± 0.21	4.5	2.4 ± 0.01	0.6
Gly	3.4 ± 0.07	2.1	6.0 ± 0.08	1.3	7.9 ± 0.20	2.6	3.9 ± 0.11	2.9
Ala	2.9 ± 0.04	1.3	4.1 ± 0.02	0.5	6.1 ± 0.03	0.5	3.2 ± 0.07	2.0
Val	3.1 ± 0.10	3.3	5.0 ± 0.16	3.2	6.6 ± 0.10	1.6	3.5 ± 0.02	0.5
Leu	4.0 ± 0.19	4.8	5.9 ± 0.19	3.3	8.5 ± 0.21	2.5	4.7 ± 0.12	2.5
Ile	2.4 ± 0.10	4.0	3.9 ± 0.19	5.0	5.2 ± 0.12	2.4	2.8 ± 0.08	2.7
Thr	2.4 ± 0.08	3.4	3.4 ± 0.14	4.1	5.2 ± 0.13	2.5	2.7 ± 0.08	2.9
Met	1.7 ± 0.05	2.9	3.1 ± 0.07	2.3	2.9 ± 0.13	4.4	1.9 ± 0.02	1.1
Lys	3.1 ± 0.12	3.8	5.8 ± 0.38	6.5	8.1 ± 0.22	2.7	4.2 ± 0.08	2.0
Phe	3.3 ± 0.10	3.1	4.6 ± 0.30	6.5	6.2 ± 0.20	3.3	3.3 ± 0.18	5.6
His	1.3 ± 0.04	3.3	2.6 ± 0.14	5.3	3.2 ± 0.09	2.8	1.6 ± 0.05	3.1
Arg	5.3 ± 0.16	3.1	10.6 ± 0.58	5.5	14.1 ± 0.35	2.5	6.5 ± 0.04	0.6

Table 9a: Amino acid composition of legumes after receiving (mean±S.D.) in g kg^{-1} DW

AA	*G. max*	%CV	*P. sativum*	%CV	*Ph. vulgaris*	%CV	*L. esculenta*	%CV
Cys	6.6 ± 0.55	8.0	3.6 ± 0.02	1.0	2.8 ± 0.02	1.0	2.6 ± 0.09	3.0
Glu	51.9 ± 4.11	8.0	22.0 ± 2.68	12.0	23.1 ± 0.52	2.0	24.7 ± 1.07	4.0
Asp	33.5 ± 1.22	4.0	16.0 ± 1.66	10.0	20.1 ± 0.12	1.0	17.9 ± 0.15	1.0
Tyr	10.1 ± 0.42	4.0	4.3 ± 0.42	10.0	5.3 ± 0.22	4.0	4.5 ± 0.25	6.0
Ser	14.1 ± 0.81	6.0	6.1 ± 0.71	12.0	9.1 ± 0.18	2.0	7.4 ± 0.16	2.0
Pro	16.7 ± 1.79	11.0	5.8 ± 0.76	13.0	6.6 ± 0.49	7.0	6.9 ± 0.05	1.0
Gly	12.6 ± 0.96	8.0	6.3 ± 0.65	10.0	6.9 ± 0.16	2.0	6.7 ± 0.22	3.0
Ala	12.7 ± 0.94	7.0	6.1 ± 0.68	11.0	7.0 ± 0.16	2.0	6.9 ± 0.23	3.0
Val	15.5 ± 1.47	10.0	7.2 ± 0.73	10.0	9.6 ± 0.25	3.0	8.6 ± 0.02	0.0
Leu	23.1 ± 1.29	6.0	10.4 ± 0.79	8.0	13.9 ± 0.65	5.0	12.2 ± 0.40	3.0
Ile	14.7 ± 1.12	8.0	6.4 ± 0.50	8.0	8.3 ± 0.29	3.0	7.5 ± 0.13	2.0
Thr	11.1 ± 0.72	6.0	5.1 ± 0.57	11.0	7.4 ± 0.02	0.0	5.7 ± 0.08	1.0
Met	28.6 ± 2.18	8.0	8.2 ± 1.16	14.0	6.3 ± 0.51	8.0	4.1 ± 0.13	3.0
Lys	18.9 ± 1.82	10.0	10.4 ± 1.01	10.0	12.0 ± 0.96	8.0	11.6 ± 0.88	8.0
Phe	16.0 ± 1.05	7.0	7.5 ± 0.68	9.0	10.3 ± 0.75	7.0	9.0 ± 0.32	4.0
His	7.9 ± 0.51	6.0	4.0 ± 0.41	10.0	5.2 ± 0.26	5.0	4.7 ± 0.11	2.0
Arg	27.4 ± 2.29	8.0	12.5 ± 0.94	7.0	13.4 ± 0.56	4.0	14.9 ± 0.66	4.0

Table 9b: Amino acid composition of legumes after best before date (mean±S.D.) in g kg^{-1} DW

AA	*G. max*	%CV	*P. sativum*	%CV	*Ph. vulgaris*	%CV	*L.esculenta*	%CV
Cys	6.5 ± 0.06	1.0	3.6 ± 0.02	0.4	3.1 ± 0.01	0.4	2.9 ± 0.11	3.7
Glu	60.9 ± 1.10	1.8	27.1 ± 0.41	1.5	29.9 ± 0.47	1.6	31.3 ± 0.09	0.3
Asp	41.3 ± 1.09	2.6	20.6 ± 0.22	1.1	26.7 ± 0.15	0.6	23.7 ± 0.53	2.2
Tyr	11.2 ± 0.37	3.3	4.6 ± 0.23	5.1	6.3 ± 0.12	1.8	5.7 ± 0.20	3.4
Ser	19.2 ± 0.42	2.2	8.0 ± 0.09	1.2	13.7 ± 0.02	0.2	11.1 ± 0.06	0.6
Pro	17.5 ± 0.30	1.7	6.7 ± 0.35	5.2	7.9 ± 0.39	5.0	7.5 ± 0.02	0.3
Gly	14.2 ± 0.19	1.3	7.4 ± 0.07	1.0	8.5 ± 0.04	0.5	8.0 ± 0.05	0.6
Ala	14.9 ± 0.53	3.6	7.4 ± 0.38	5.1	8.9 ± 0.44	4.9	8.6 ± 0.38	4.5
Val	13.3 ± 0.32	2.4	8.3 ± 0.04	0.5	9.0 ± 0.37	4.1	8.0 ± 0.14	1.8
Leu	25.8 ± 0.32	1.2	12.4 ± 0.02	0.2	16.4 ± 0.09	0.6	14.6 ± 0.12	0.8
Ile	12.9 ± 0.30	2.4	7.6 ± 0.07	0.9	7.9 ± 0.37	4.7	7.1 ± 0.10	1.4
Thr	13.7 ± 0.09	0.7	6.6 ± 0.03	0.4	10.2 ± 0.25	2.5	7.9 ± 0.00	0.0
Met	6.6 ± 0.04	0.7	3.0 ± 0.14	4.9	4.2 ± 0.08	1.9	2.9 ± 0.06	2.1
Lys	22.1 ± 0.38	1.7	13.2 ± 0.01	0.1	14.8 ± 0.09	0.6	14.3 ± 0.18	1.2
Phe	18.0 ± 0.30	1.7	8.5 ± 0.07	0.9	12.5 ± 0.03	0.3	10.3 ± 0.01	0.1
His	8.7 ± 0.08	0.9	4.1 ± 0.01	0.2	5.7 ± 0.03	0.6	4.9 ± 0.02	0.4
Arg	31.2 ± 0.80	2.6	15.8 ± 0.19	1.2	17.3 ± 0.22	1.3	17.9 ± 0.28	1.6

The evaluation of total essential amino acids (EAA) content (Table 10) in individual samples was also performed. Total content of EAA was changing during storage. In the most of examined samples it increased, only in *G. max* and flour it decreased. Groats, broken groats and both flours contained the highest amount of essential amino acids from all buckwheat products. All legume samples contained more than 50 g kg^{-1} of EAA in both samplings.

Table 10: Content of essential amino acids (g kg^{-1})

	∑EAA	
	Receiving	**Best before date**
Peels	6.3	7.7
Groats	32.9	37.4
Broken groats	24.9	26.1
Pasta	18.8	23.0
Crunchy products natural	16.8	20.4
Crunchy products cocoa	15.4	19.9
Flour	34.1	31.7
Wholemeal flour	42.3	42.7
G. max	127.8	112.3
P. sativum	55.1	59.5
Ph. vulgaris	67.7	75.0
L. esculenta	58.9	65.1

As stated by Edwardson (1996) buckwheat is one of the best sources of high quality, easily digestible protein in the plant kingdom. It has a balanced amino acid profile and high level of essential amino acids. It can be used a nutraceutical. Buckwheat extruded products are of a high nutritional quality when compared with products from cereals [101].

Protein quality of studied samples was evaluated by the essential amino acid index (EAAI). Calculated values are presented in Table 11. 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 [83].

Table 11: Essential amino acid index (EAAI, in %)

	EAAI	
	Receiving	**Best before date**
Peels	1.5	1.8
Groats	7.8	8.9
Broken groats	6.0	6.2
Pasta	4.5	5.4
Crunchy products natural	3.9	4.7
Crunchy products cocoa	3.8	4.6
Flour	8.2	7.5
Wholemeal flour	9.9	10.1
G. max	19.9	25.0
P. sativum	12.3	13.2
Ph. vulgaris	15.0	16.2
L. esculenta	12.9	14.0

4.2.6. Starch content

Content of starch was observed in buckwheat products. The working procedure is described in section 3.2.6. The starch content (Table 12) differs from one sample to another. Its content in studied products was in the range of 50 - 80% in dry matter, with the exception of peels, where the starch amount was only about 3.6%. The content of starch during storage was changing. In some products, it decreased, in groats, broken groats and crunchy products, it increased. Edwardson (1996) reported that starch as the major carbohydrate in buckwheat comprises from 50 to 67% of the seed [101]. Steadman *et al*. (2001) presented the content of starch in whole groats as 54.5% [70]. The starch in buckwheat seed is concentrated in endosperm.

Table 12: Average content of starch (mean±S.E.) in %

	Starch	
	Receiving	**Best before date**
Peels	3.6 ± 0.18	1.1 ± 0.00
Whole seed	53.3 ± 0.36	47.8 ± 0.00
Groats	61.7 ± 0.00	78.0 ± 0.18
Broken groats	69.0 ± 0.35	72.2 ± 0.00
Crunchy products natural	77.8 ± 0.18	82.3 ± 0.18
Crunchy products cocoa	72.9 ± 0.18	76.0 ± 0.00
Flour	66.8 ± 0.18	65.1 ± 0.00
Wholemeal flour	53.5 ± 0.36	49.6 ± 0.18
Pasta	75.6 ± 0.36	72.6 ± 0.18

4.2.7. Fibre content

The determination was performed according to the method mentioned in section 3.2.7. Table 13 shows the amount of fibre in particular samples. 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. During the storage experiment, the content of fibre diminished in all samples. Peels after receiving of samples contained more than 65% of fibre; after best before date the fibre content lowered to 40%. Bonafaccia *et al.* (2003) reported the fibre content in flour from common buckwheat as 6.5% [98]. Results from the experiment are in concordance with this study. Dalgetty *et al.* (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 [102]. 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.

Table 13: Average content of fibre (mean±S.E.) in %

	Fibre					
	Receiving			Best before date		
G. max	13.3	±	0.14	4.3	±	0.25
P. sativum	15.3	±	0.21	2.6	±	0.16
Ph. vulgaris	11.1	±	0.72	2.0	±	0.18
L. esculenta	5.6	±	0.29	1.3	±	0.08
Peels	65.9	±	1.17	40.4	±	1.04
Whole seed	14.8	±	1.05	8.4	±	0.12
Wholemeal flour	6.7	±	0.19	1.6	±	0.27
Groats	ND			ND		
Broken groats	ND			ND		
Crunchy products natural	ND			ND		
Crunchy products cocoa	ND			ND		
Flour	ND			ND		
Pasta	ND			ND		

4.2.8. Minerals

Minerals were determined using the method mentioned in section 3.2.8. The majority (Na, K, Mg, Ca), trace (Fe, Zn, Cr) and toxic elements (Pb, Cd) were determined only at the beginning of the experiment, not during the storage period.

In Tables 14a and 14b, mineral composition of examined buckwheat products is presented. Wholemeal flour is a very rich source of Ca, Fe and Zn. The content of these elements is 1171.8, 45.9 and 35.4 mg/kg of dry matter, respectively. Peels are also good source of Ca (999 mg/kg). The lowest content of Ca was determined in crunchy products cocoa, 87.9 mg/kg, maybe because of the processing. On the other hand, the highest content of toxic Pb was found in broken groats, more than 1 mg/kg. Both flours are rich in Mg; they contain more than 2000 mg/kg of this element. Ikeda *et al.* (2006) dealt in their study with minerals in buckwheat flour. They presented values of Fe, Zn, Ca and Mg contents as 2.9, 2.5, 12.4 and 375 mg/100 g, respectively [103]. After the conversion of units and subsequent comparison with values obtained in the experiment (Table 14b) it can be concluded that in the experiment higher contents of Zn, Fe and Ca, 32.6, 30.1 and 267 mg/kg, respectively were found; only the content of Mg is lower, 2000 mg/kg. Wijngaard and Arendt (2006) reported content of Fe as 3.03 mg/100 g and Zn as 2.92 mg/100 g in buckwheat groats [42]. These values for groats are close to those in Table 14a. To conclude, buckwheat flours are rich sources of many minerals.

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, resp. in lentil and 2.3 and 3.2 mg/100 g, respectively in peas [40]. Table 15 presents content of minerals in legume samples. Values for peas and lentil are higher than those reported by Iqbal *et al*. (2006).

From Table 15, 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 14a: Content of minerals in buckwheat products (mean±S.D.) in 1000 g of DW

		Peels	Whole seed	Groats	Broken groats	Crunchy products natural
Pb	µg	428.0 ± 4.27	510.0 ± 5.10	222.0 ± 2.15	1049.0 ± 10.47	194.0 ± 1.94
Cd	µg	44.0 ± 0.40	78.0 ± 0.77	73.0 ± 0.72	53.0 ± 0.52	67.0 ± 0.67
Cr	µg	64.0 ± 0.64	49.0 ± 0.49	69.0 ± 0.69	477.0 ± 4.77	109.0 ± 1.09
Zn	mg	5.6 ± 0.06	17.6 ± 0.18	27.9 ± 0.28	16.7 ± 0.17	12.0 ± 0.12
Cu	mg	4.7 ± 0.05	7.3 ± 0.07	6.7 ± 0.07	5.0 ± 0.05	4.8 ± 0.05
Na	mg	8.6 ± 0.09	6.8 ± 0.07	5.6 ± 0.06	1.6 ± 0.02	10.9 ± 0.11
Fe	mg	16.5 ± 0.17	24.3 ± 0.24	28.7 ± 0.29	16.9 ± 0.17	11.7 ± 0.12
Ca	mg	999.1 ± 5.00	533.2 ± 2.67	148.3 ± 0.74	113.6 ± 0.57	246.5 ± 1.23
Mg	g	1.1 ± 0.05	1.7 ± 0.01	2.2 ± 0.01	1.4 ± 0.01	0.9 ± 0.00
K	g	5.8 ± 0.03	4.8 ± 0.02	4.8 ± 0.02	3.2 ± 0.02	2.0 ± 0.01

Table 14b: Content of minerals in buckwheat products (mean±S.D.) in 1000 g of DW

		Crunchy products cocoa	Flour	Wholemeal flour	Pasta
Pb	µg	422.0 ± 4.22	412.0 ± 4.12	831.0 ± 8.31	384.0 ± 3.84
Cd	µg	44.0 ± 0.40	108.0 ± 1.06	130.0 ± 1.30	54.0 ± 0.05
Cr	µg	111.0 ± 1.11	144.0 ± 1.42	149.0 ± 1.49	113.0 ± 1.13
Zn	mg	17.6 ± 0.17	32.6 ± 0.32	35.4 ± 0.17	10.1 ± 0.12
Cu	mg	5.0 ± 0.05	7.8 ± 0.08	11.6 ± 0.12	5.7 ± 0.01
Na	mg	15.9 ± 0.15	2.1 ± 0.02	5.3 ± 0.05	5.9 ± 0.06
Fe	mg	20.1 ± 0.20	30.1 ± 0.30	45.9 ± 0.45	15.3 ± 0.15
Ca	mg	87.9 ± 0.87	266.6 ± 1.33	1171.8 ± 5.85	122.8 ± 1.22
Mg	g	0.9 ± 0.01	2.2 ± 0.01	2.4 ± 0.01	1.0 ± 0.01
K	g	2.7 ± 0.01	4.6 ± 0.02	6.1 ± 0.03	2.5 ± 0.01

Table 15: Content of minerals in legumes (mean±S.D.) in 1000 g of DW

		G. max	***P. sativum***	***Ph. vulgaris***	***L. esculenta***
Pb	µg	422.0 ± 4.22	146.0 ± 1.46	447.0 ± 4.47	166.0 ± 1.64
Cd	µg	78.0 ± 0.78	27.0 ± 0.27	30.0 ± 0.30	21.0 ± 0.21
Cr	µg	347.0 ± 3.46	405.0 ± 4.05	365.0 ± 3.64	286.0 ± 2.85
Zn	mg	40.7 ± 0.40	23.8 ± 0.23	32.1 ± 0.32	28.2 ± 0.28
Cu	mg	12.9 ± 0.12	4.4 ± 0.04	7.5 ± 0.07	7.1 ± 0.07
Na	mg	3.7 ± 0.04	22.4 ± .022	3.0 ± 0.03	8.8 ± 0.08
Fe	mg	70.2 ± 0.70	40.6 ± 0.40	76.9 ± 0.77	78.3 ± 0.78
Ca	mg	1807.3 ± 9.04	688.4 ± 3.44	1718.3 ± 8.59	695.5 ± 3.48
Mg	g	2.1 ± 0.02	1.2 ± 0.01	1.6 ± 0.02	1.1 ± 0.01
K	g	17.3 ± 0.17	9.8 ± 0.10	14.8 ± 0.15	9.4 ± 0.09

4.2.9. Rutin concentration

Determination of rutin concentration in buckwheat products was performed according to the method mentioned in section 3.2.9.

As can be seen in Figures 7 and 8, the highest concentration of rutin in both samplings was found in wholemeal flour, almost 703 μg g^{-1} after receiving and about 638 μg g^{-1} after best before date. On the other hand, the lowest concentration of rutin was determined in crunchy products and pasta. Kreft *et al*. (2006) dealt with rutin in buckwheat and they presented the value of rutin concentration in buckwheat groats, dark and light flour as 0.2, 0.2 and 0.1 mg/g, respectively [55]. The value of rutin concentration in light flour is close to value obtained in the experiment. Other values, when compared with the experiment, are a little bit different. The concentration of rutin in wholemeal flour is more then three times higher than the value reported by Kreft *et al*. (2006). Also Oomah *et al*. (1996) presented the level of rutin in buckwheat groats as 0.2 mg/g. Rutin level in buckwheat is dependent on growth location and cultivar of the plant. In addition to rutin antioxidant capacity, it can also help with treatment of chronic venous insufficiency [104,105].

The rutin concentration during storage at room temperature grew almost in all samples, only in crunchy products, wholemeal flour and pasta, its concentration decreased; in crunchy products natural it decreased almost three times.

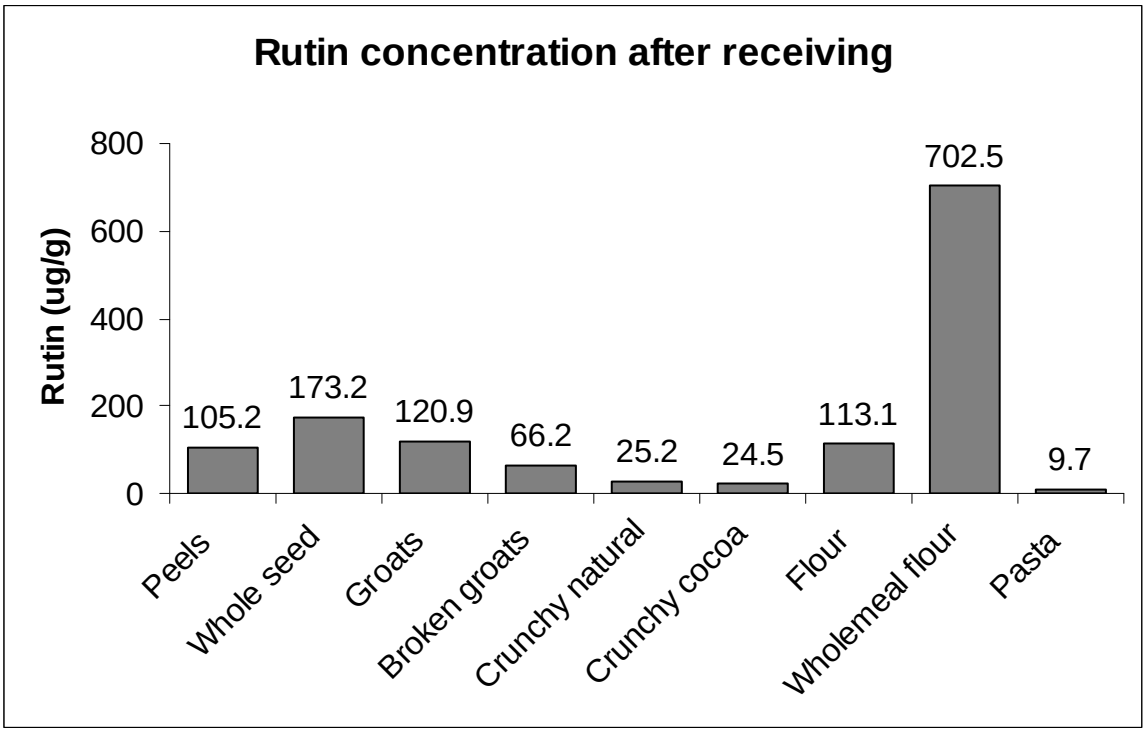

Figure 7: Concentration of rutin in μg g^{-1} DW after receiving samples

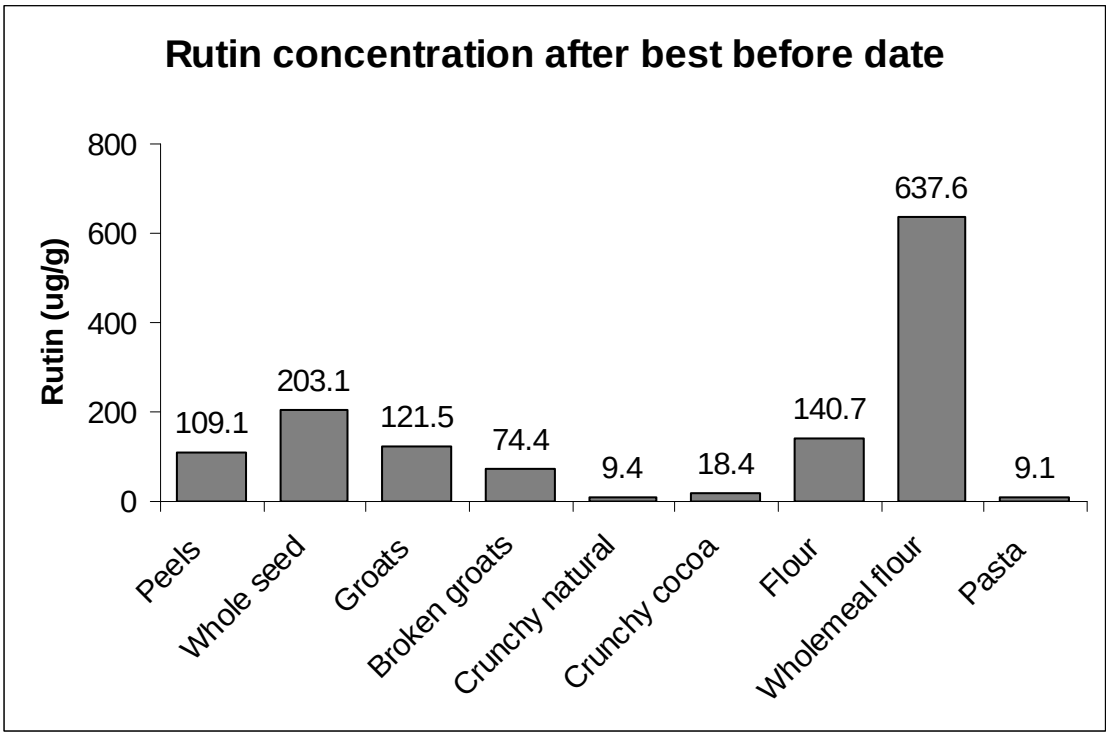

Figure 8: Concentration of rutin in μg g^{-1} DW after best before date

4.2.10. Phytic acid content

Phytic acid content in tested samples was determined by the modified Holt´s method, described in section 3.2.10. During the storage experiment, content of phytic acid in particular samples has declined.

Table 16 shows that the amount of phytate in dry matter of soybeans was about 2.0 g/100 g after receiving and 1.9 g/100 g after best before date. These values are slightly higher than those reported by Reddy *et al.* (1982) who presented a range of 1.0-1.5% of phytate content in dry matter of soybeans and 1.2% of phytate in dry matter of peas [106]. Values for peas are also lower than those from the experiment (Table 16). Hídvégi and Lásztity (2002) assigned the content of phytate in soybeans in the range of 1.2-1.8g/100 g and 0.7-1.2 g/100 g in peas [107]. These values are also lower than those presented in Table 16. For common beans, Hídvégi and Lásztity (2002) stated the range of phytate content as 0.6-1.7 g/100 g [107]. Data for common beans from this experiment does not suit to this extent. Amount of 2.0 and 1.8 g/100 g is higher.

The highest amount of phytate was found in common beans, soybeans, broken groats and wholemeal flour, about 2 g/100 g before storage. On the other hand, the lowest content of phytate was observed in buckwheat pasta, less than 1 g/100 g. 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 [108]. *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 quantification of phytate in *F. esculentum* groats was 1.9 g per 100 g of dry matter before storage and 1.5 g per 100 g of dry matter after best before date. The value after best before date is close to the average one reported by Egli *et al.* (2002) which was 1.4 g per 100 g of the sample [109].

Marounek *et al.* (2000) ascertained that phytates are problematic compounds in the environment. In animals with simple stomach phytates are not digested and go to excrements. Then, they are degraded by microorganisms [5].

Content of phytate in particular samples obtained during this experiment can differ from that obtained in previous studies because of many factors, e.g. climatic conditions, location, different varieties, reagents from different producers, etc.

Table 16: Content of phytic acid (mean±S.E.) in g 100 g^{-1}

	Receiving	**Best before date**
Peels	1.1 ± 0.01	1.0 ± 0.01
Groats	1.9 ± 0.01	1.5 ± 0.00
Broken groats	2.0 ± 0.01	1.3 ± 0.01
Flour	1.7 ± 0.00	1.6 ± 0.01
Wholemeal flour	2.0 ± 0.00	1.5 ± 0.00
Pasta	0.9 ± 0.00	0.9 ± 0.00
G. max	2.0 ± 0.01	1.9 ± 0.01
P. sativum	1.7 ± 0.00	1.5 ± 0.00
Ph. vulgaris	2.0 ± 0.00	1.8 ± 0.00
L. esculenta	1.7 ± 0.01	1.5 ± 0.00

4.2.11. Digestibility

In vitro digestibility was determined using incubator and enzymes pepsin and the combination of pepsin and pancreatin. Working procedure is mentioned in section 3.2.11. Coefficients of digestibility for particular samples are presented in Table 17. For better comparison of enzymes effects, graphical illustrations were created (Fig.9,10,11).

As can be seen from Figure 9, the highest coefficient of crude protein digestibility 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.

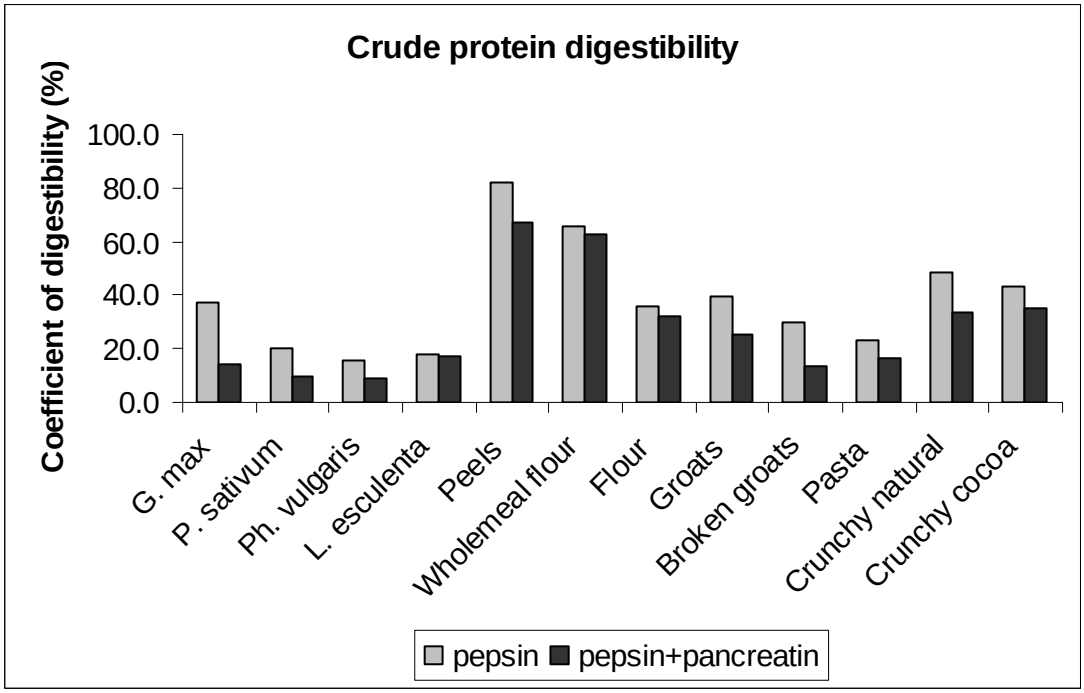

Figure 9: Digestibility of crude protein

Table 17: Coefficients of digestibility (%)

	Crude protein		**Fibre**		**Phytic acid**	
	Pepsin	*Pepsin + pancreatin*	*Pepsin*	*Pepsin + pancreatin*	*Pepsin*	*Pepsin + pancreatin*
G. max	37.3	14.4	18.4	18.6	46.3	39.3
P. sativum	20.3	10.0	3.3	9.6	41.3	50.1
Ph. vulgaris	15.6	8.7	ND	23.4	39.0	37.5
L. esculenta	18.0	16.9	9.5	15.4	45.0	50.5
Peels	81.8	67.5	55.8	64.4	89.3	86.4
Wholemeal flour	66.0	62.7	22.3	18.0	44.7	47.9
Flour	35.7	32.1	-	-	56.6	44.9
Groats	39.2	25.6	-	-	49.7	43.2
Broken groats	29.8	13.8	-	-	45.7	42.7
Pasta	22.9	16.1	-	-	-	-
Crunchy products natural	48.6	33.9	-	-	-	-
Crunchy products cocoa	43.1	35.0	-	-	-	-

In Figure 10, 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.

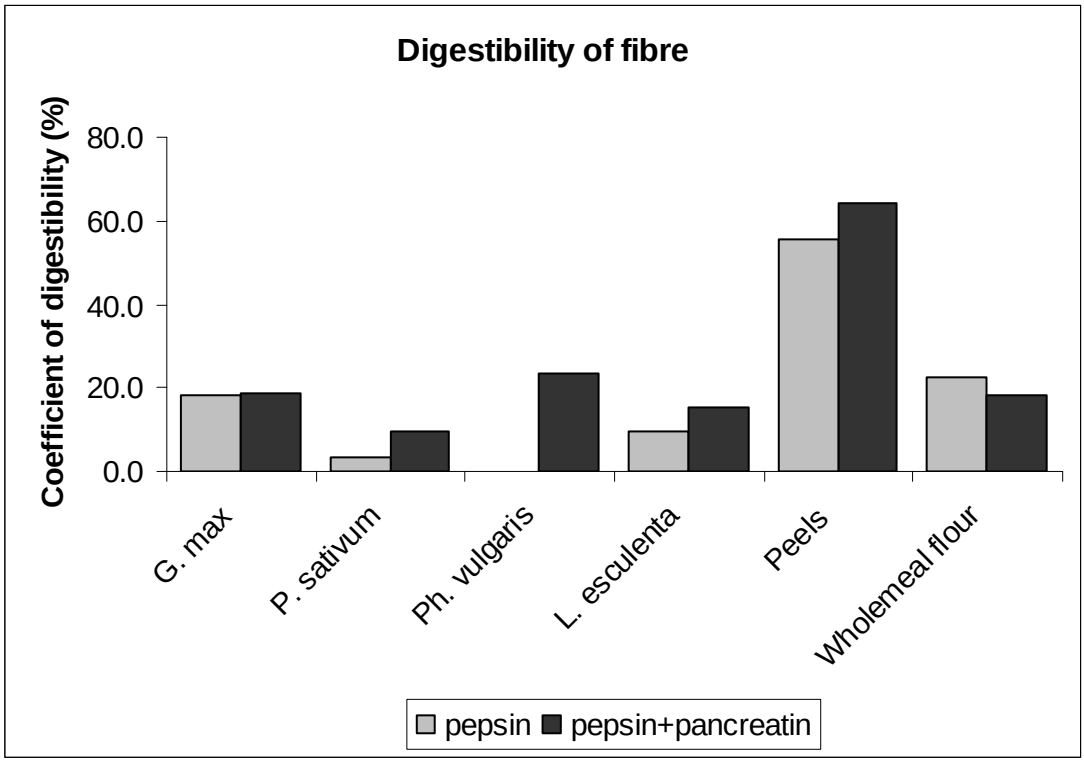

Figure 10: Digestibility of fibre

And finally, coefficients of digestibility for phytic acid (Fig.11) were calculated. These values were really interesting. 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.

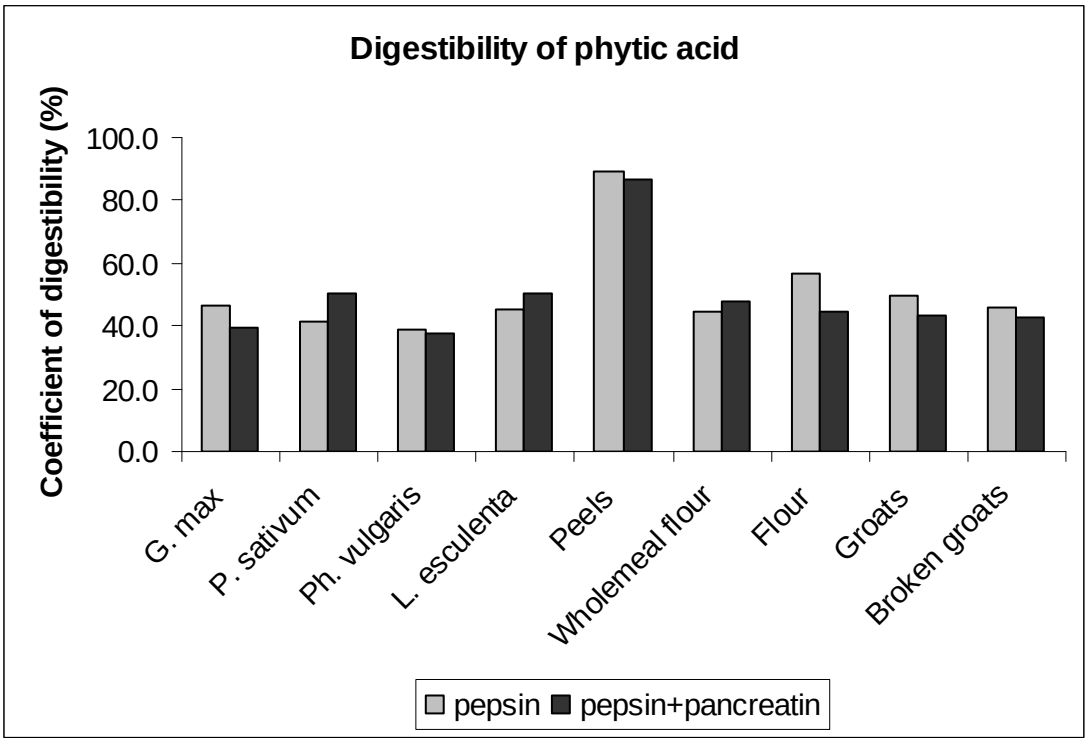

Figure 11: Digestibility of phytic acid

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 [110].

Digestibility can be influenced by many factors. Mainly digestibility of crude protein may be affected by the concentration of phytic acid. Phytates bind proteins and form indigestible complexes.

CONTRIBUTION TO THE SCIENCE AND PRACTICE

Issues addressed in the Doctoral thesis should contribute to the awareness of the nutritional value of examined samples. Important characteristics, mainly in buckwheat products of which composition has not yet been studied were observed and should be used especially for people with celiac disease as a source of information.

Contribution to the science

- More accurate information on nutrient composition of commonly consumed legumes and buckwheat products were obtained.
- Determination of phytic acid content in legumes and buckwheat products was performed.
- Determination of crude protein, fibre and phytic acid digestibility which comprises the nutritional value of foods was carried out.
- Results from the Doctoral thesis were published in international scientific journals and presented at scientific conferences.
- Cooperation with other scientific workplaces was established.

Contribution to the practice

- Results will be sent to Mr. Šmajstrla from Pohankový mlýn, s.r.o. Frenštát pod Radhoštěm for his needs.
- The gained knowledge should contribute to better public awareness (especially for people with celiac disease) on the nutritional value of buckwheat products available in stores in the Czech Republic.
- High protein content in legumes makes them sources of proteins especially in developing countries.

CONCLUSIONS

In the experimental part of the Doctoral thesis, the basic chemical composition of legumes and buckwheat products during the one-year storage experiment was determined.

The main emphasis 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. They form hardly usable complexes with minerals (e.g. Fe, Ca) and proteins. These complexes are insoluble in the physiological pH of the intestine and bound compounds are not absorbed. Phytic acid may also influence the digestibility of crude proteins, lipids and starch.

None of samples contained more than 11% of moisture before storage. During the storage experiment, a gradual reduction in moisture content, due to evaporation of water from each sample, was observed; only in soybeans a slight increase in moisture content was observed. Ash content slightly increased during storage; only in lentil a slight decrease of ash content was observed.

Content of total fat and crude protein was descending in most samples during the storage experiment; only in some of them a slight increase was observed. Content of fat in soybeans was about 17%, in other legumes it ranged from 1.5 to 2% of fat in dry matter. Soybeans are sometimes classified as oilseeds. Buckwheat products are considered to be low fat products. All studied legumes were rich in crude protein; the content ranged from 18% in *P. sativum* to almost 38% in *G. max*. These results confirmed that 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 and buckwheat products contained all seventeen amino acids. During storage amounts of amino acids was changing. All buckwheat products were rich in Glu, Asp and Arg; in legumes the greatest content of Cys, Glu, Asp, Leu, Lys and Arg was determined. Results from the experiment showed that legumes contained more than 50 g kg^{-1} of essential amino acids (EAA); on the other hand, the lowest content of EAA was discovered in peels which are not used for direct consumption but usually for making tea.

Starch and rutin contents were determined only in buckwheat products. Starch is mainly concentrated in the endosperm of buckwheat seed. Examined buckwheat products contained more than 50% of starch in dry matter except peels. The greatest rutin concentration was found in wholemeal flour in both samplings (almost 703 μg/g after receiving and 638 μg/g after the best before date). On the other hand, the lowest concentration of rutin was found in pasta. To conclude, rutin concentration during storage grew almost in all samples; in crunchy products natural a great decrease was observed, the content of rutin decreased almost three times.

Fibre content was determined only in legumes, peels, whole seeds and wholemeal flour. In other buckwheat products fibre was not detected. The richest source of fibre is peels; they contained more than 65% of fibre before the storage experiment. The lowest amount of fibre was found in *L. esculenta*. During the storage experiment, the content of fibre diminished.

Minerals were determined in all samples only at the beginning of the experiment. Wholemeal flour is rich in Ca, Fe, Mg and Zn; also peels are good source of calcium. Both flour contained more than 2 g/kg of magnesium. The experiment proved that legumes are rich in Mg and Ca.

The quantification of phytate in studied samples was higher than 1 g/100 g in all samples except pasta which contained lower amount of this compound. The highest content of phytic acid was discovered in *Ph. vulgaris*, *G. max*, groats, broken groats and wholemeal flour.

In the experiment, *in vitro* digestibility of crude protein, fibre and phytic acid was performed. The highest coefficients of crude protein digestibility were obtained when using pepsin. On the other hand, when the combination of pepsin and pancreatin was used, higher fibre digestibility coefficients were obtained. When digestibility of phytic acid was determined and only pepsin was used, higher coefficients of digestibility for *G. max*, *Ph. vulgaris*, peels, flour, groats and broken groats were found out. While when the combination of pepsin and pancreatin was used, higher phytic acid digestibility coefficients for *P. sativum*, *L. esculenta* and wholemeal flour were observed.

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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