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Studies concerning the phosphorus bioavailability improvement of some cereals used in nourishment

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Abstract

Possibilities to increase the phosphorus bioavailability and reduce the phytic acid content by soaking, germination and processing of germinated cereals seeds into extracts form, were investigated for some cereals used in nourishment. The modifications of soluble phosphorus content were discussed in relation with the changes that occurs in the phytic acid content. The phosphorus amount was spectrophotometrically estimated using the ammonium molybdate method. The phytic acid was indirectly estimated using a spectrophotometric method based on precipitation of phytate as ferric phytate. The phytic acid amount was calculated on the basis of iron and phosphorus content of ferric phytate. Cereals seeds germination is accompanied by extensive change in the soluble phosphorus content. The biggest solubilization of the phosphorus appears in the case of rye germination and the smallest for maize seeds. The phytic acid content for all seeds tested was reduced by germination and by processing in the extracts form, but the extent of reduction differed with cereals species. In the case of extracts from germinated cereals, 62-85% of the phosphorus content was found in the soluble form. The decrease of phytic acid content during investigated processes was found strongly correlated with the increase of soluble phosphorus amount.

Keywords: cereals, soluble phosphorus, phytic acid, soaking, germination, extracts of germinated cereals

Introduction

This study had as a starting point the nutritive recommendations of recent years which have led to increasing consumption of whole grain and food rich in ballast substances. Whole grains have been associated with a reduction of many chronic diseases such as heart disease, cancer, and diabetes mellitus. But, the cereals contain of some antinutrient components, such as phytic acid and its salts with Ca^{2+} and Mg^{2+} or K^{+} and Mg^{2+}, forms very stable, hardly soluble and not disposable for absorption at gastrointestinal level tract [1]. Phytic acid is a major storage form of phosphorus and the source of inositol in plant seeds. Approximately 75% of a mature seed's total phosphorus is found as a single compound called phytic acid, formally referred to as myo-inositol 1,2,3,4,5,6-hexakis dihydrogenphosphate. The biochemical behavior and the nutritional value of phytic acid are governed by the strong chelating ability of the molecule [2, 3]. Phytic acid forms complexes with proteins, starch and lipids decreasing their digestibility. It inhibits the action of some enzymes as amylase, acid phosphatases and trypsin, having an adverse influence upon the digestive process [2, 4]. In humans, phytic acid is chelating agent, which binds nutritionally important mono- and divalent minerals (i. e., calcium, magnesium, cobalt, manganese, zinc, copper, and iron) to form complex phytate [2-5]. Taking into account the nutritional involvement of phytic acid, its release from the complexes forms is necessary. For decreasing the phytic acid content, in literature [6-9] are suggested of some methods such as: boiling, fermentation, extrusion, ions exchange, gamma rays irradiation, ultra filtration, dialyze, microwave treatments etc. By most of these methods achieve a significant reduction of phytic acid content but are destroyed and certain nutrients. A mild process, which doesn't influence upon other components, is represented by soaking and germination of cereals seeds [6, 7, 10]. On the basis of these considerations, the aim of this work was the study of changes that occur in phytic acid and soluble phosphorus content by soaking and germination of some cereals cultivated in Romania and by processing of germinated cereals into extracts form. Konietzny and Greiner [7] noticed that the phytate level during soaking and germination of cereals seeds decreases with increasing of the phytase activity. The phytases (EC 3.1.3.8 for 3-phytase and EC 3.1.3.26 for 6-phytase) hydrolyses the phytic acid to myo-inositol and phosphoric acid in a stepwise manner, forming myo-inositol phosphate intermediates [2, 6, 7, 10]. Phytic acid degradation under the phytase action continues during the processing of germinated cereals seeds into extracts form.

Materials and methods

Samples

The samples consisted of wheat seeds (*Triticum Aestivum* – Lovrin 34), barley (*Hordeum vulgare L.* – Miraj), rye (*Secale cereale L. – Orizont*) and maize (Zea May – the Florencia hybrid). The cereals seeds were taken from the 2004 harvest year, from the western part of Romania (Didactic Experiment Station of Banat's University of Agricultural Sciences and Veterinary Medicine Timisoara).

Preparation of Samples

The cereals seeds were soaking for 40 hours (alternating periods of immersion in water and keeping without water under aeration), then germinated for 6 days using the pilot micromalting installation; the green malt was dried at low temperatures (below 60°C), until the humidity of the product declined to 6%. Extracts from germinated cereals were processed by mashing the mixture of finely ground germinated cereals seeds with water 1:3 (w:w) pursuant with a pre-established diagram (for the best extraction, the mash is held for fixed times at a series of specified temperatures, such as: 45°C, 55°C, 63°C, 70°C, 75°C), then filtering and evaporating the resulting liquid under vacuum (T=55°C) to give a stable extract of about 76% solids. By means of this procedure we obtained four types of extracts: from germinated wheat, rye, maize and barley.

Total and soluble phosphorus content analysis

Inorganic phosphorus content was determined colorimetrically following nitric/perchloric acid digestion, using the ammonium molybdate method, which is based on the formation of a phosphomolibddic acid. For reduction of phosphomolybdic acid it was used ascorbic acid as described by Nahapetian and Bassiri [11]. Inorganic phosphate concentration was determined from a standard curve using KH_2PO_4.

The soluble phosphorus compounds were extracted by stirring the probe with diluted HCl. The resulted supernatant of centrifugation was processed, in order to remove phytic acid, with Carez I and II reagents into ultrasonic bath (Transsonic T460), followed by the centrifugation of mixture. The soluble phosphorus compounds from supernatant were spectrophotometrically estimated using Spectrophotometer Specord 205 by Analytic Jena

[10]. Phosphorus content was determined using a standard curve and was expressed as mg P/g dry substance.

Phytic acid analysis

The extraction of phytate phosphorus was carried out using a modified procedure of Nahapetian and Bassiri [11]. Phytate phosphorus was determined by precipitating the phytate as ferric phytate [2, 10].

The phytic acid content was calculated by two ways: on the basis of iron and phytate phosphorus amount from ferric phytate. The results obtained through by two ways were comparatively presented. All values for phytic acid were calculated on the assumption that four ferric atoms combine with one molecule of phytic acid ($P_6C_6H_{18}O_{24}$), resulting ferric phytate ($Fe_4P_6C_6H_6O_{24}$) [2, 10]. Phytic acid content, expressed as mg/g d.s was calculated as follows:

1. on the base of phosphorus content from ferric phytate: Phytic acid (mg/g d.s.) = 3.55 x Phytic phosphorus (mg/g d.s.), where 3.55 represents the ratio between phytic acid molecular mass, $M(P_6C_6H_{18}O_{24})$=660g and phytic phosphorus molecular mass, $M(P_6)$=186g.
2. on the base of iron content from ferric phytate: Phytic acid (mg/g d.s.) =2.94 x Phytic iron (mg/g d.s.), where 2.94 represents the ratio between phytic acid molecular mass, $M(P_6C_6H_{18}O_{24})$=660g and phytic iron molecular mass, $M(Fe_4)$=224g.

Results and discussion

The effect of soaking process on the phosphorus bioavailability versus the seeds humidity is shown in the Table 1. The soluble phosphorus amount it was determined after each period of immersion in water.

Soaking of wheat						
Humidity (%)	12.71	31.07	40.17	43.31	45.06	
Soluble phosphorus (mg/g d.s.)	0.72	0.77	0.91	1.18	1.23	
Soaking of barley						
Humidity (%)	12.37	30.21	39.05	42.59	44.07	
Soluble phosphorus (mg/g d.s.)	0.82	0.91	1.01	1.10	1.18	
Soaking of rye						
Humidity (%)	12.94	30.78	41.26	44.09	46.84	
Soluble phosphorus (mg/g d.s.)	0.91	0.99	1.23	1.40	1.48	
Soaking of maize						
Humidity (%)	13.41	21.32	28.58	34.79	37.91	39.63
Soluble phosphorus (mg/g d.s.)	0.74	0.74	0.78	0.82	0.91	0.93

Table 1. The influence of cereals seeds soaking on the soluble phosphorus amount

In cereals seeds in native state, the soluble phosphorus content represents about 17-25% from total phosphorus. After soaking, soluble phosphorus content of investigated cereals seeds was increased. The highest accumulation of soluble phosphorus was induced by rye seeds soaking (62.63% of the initial value) and the smallest in the case of maize seeds soaking (25.68% of the initial value).

In the Table 2 are presented the total and soluble phosphorus amounts from seeds in native state and after each of the three processes: soaking, germination and processing in the malt extracts form.

In native state				
Phosphorus (mg/g d.s.)	Wheat	Maize	Rye	Barley
Total Phosphorus	4,03	4.26	3.78	4.06
Soluble phosphorus	0.7	0.74	0.91	1.02
After soaking				
Phosphorus (mg/g d.s.)	Soaked wheat	Soaked maize	Soaked rye	Soaked barley
Total Phosphorus	3.99	4.21	3.76	3.98
Soluble phosphorus	1.23	0.93	1.48	1.18
After germination				
Phosphorus (mg/g d.s.)	Germinated wheat	Germinated maize	Germinated rye	Germinated barley
Total Phosphorus	3.96	4.16	3.72	3.94
Soluble phosphorus	1.95	1.31	2.09	1.74
After processing in the extract form				
Phosphorus (mg/g d.s.)	Extract of germinated wheat	Extract of germinated maize	Extract of germinated rye	Extract of germinated barley
Total Phosphorus	3,61	2,54	3,3	2,87
Soluble phosphorus	3,07	1,6	2,71	2,33

Table 2. The impact of cereals processing on the soluble phosphorus amount

We can observe that cereals germination is accompanied by extensive change in the soluble phosphorus content. After germination, the soluble phosphorus content increases from two-tree time, depending on the cereals species. The largest phosphorus release through germination was registered in the case of rye seeds (from 0.91 mg/g d.s to 2.09 mg/g d.s) and the smallest one in the case of maize seeds (from 0.74 mg/g d.s to 1.31 mg/g d.s). It was noticed that, in the extracts of germinated cereals seeds, most of phosphorus contents found in soluble form. In the case of extract from germinated rye, wheat and barley approximately 80-85% of total phosphorus content was in soluble form. Only 62% from the phosphorus amount found in extract of germinated maize was in the soluble form. We explain these results on the basis of endogenous phytase activity synthesized in the germination time, which continues to act during processing of the germinated cereals in the extracts form. The biggest phosphorus bioavailability through germination was registered in the case of rye, and the smallest one in the case of maize, in accord with own phytase activity.

In the Table 3 are presented the results obtained in the case of phytic acid determination by two ways and the results have been comparatively showed.

In native state				
Phytic acid (mg/g d.s.)	Wheat	Maize	Rye	Barley
phytic acid content*	11.10	11.02	9.5	10.58
phytic acid content**	11.59	12.04	9.99	11.05
After soaking				
Phytic acid (mg/g d.s.)	Soaked wheat	Soaked maize	Soaked rye	Soaked barley

phytic acid content *	8.88	10.52	7.45	9.05
phytic acid content**	9.22	10.89	7.93	9.37
After germination				
Phytic acid (mg/g d.s.)	Germinated wheat	Germinated maize	Germinated rye	Germinated barley
phytic acid content *	6.47	8.79	5.15	6.91
phytic acid content**	7.07	9.23	5.5	7.19
After processing in the extract form				
Phytic acid (mg/g d.s.)	Extract of germinated wheat	Extract of germinated maize	Extract of germinated rye	Extract of germinated barley
phytic acid content *	1.48	2.92	1.83	1.65
phytic acid content**	1.56	3.21	2.12	1.94

* the way of phytic phosphorus analysis from ferric phytate;
** the way of iron analysis from ferric phytate.

Table 3. The impact of cereals processing on the phytic acid amount

Through soaking and germination of cereals seeds the phytate becomes unstable due to the endogenous phytase enzyme activity. It was registered a rapid degradation of phytic acid from all investigated cereals seeds by soaking, germination and by processing the germinated cereals in the extract. The magnitude of this reduction depends on the cereal species.

The degradation of phytate during of investigated processes is generally attributed to endogenous phytase activity or the formation of insoluble complexes between phytate and other components [7]. It was reported by some authors that in cereals in native state, the phytase activity is influenced by cultivar, climatic conditions and year. The biggest phytase activity it was registered for rye seeds and the lower value of this activity was showed for maize seeds [1, 7, 12].

Our results are in accord with these data: in the seeds in native state were registered the lower phytic acid amount and the biggest soluble phosphorus content for rye seeds. Contrary, the highest value of phytic acid and the lower value of soluble phosphorus amount were registered in maize seeds.

Many authors [3, 5, 6] mentioned that in the germination time phytase enzymatic activity was increased in order: rye>wheat>barley> maize. The changes in phytate phosphorus levels were influenced by the cereals type. During the soaking and germination, the phytic acid hydrolysis devolves more intense for rye; we notice that in the case of maize seeds germination, the hydrolysis devolves slowly. It can be said that for all the types of cereals, the phytic acid content decrease by soaking and germination, but the extent of reduction is different, depending on the cereals species. In the first moment, during the soaking (40 hours), we notice that the decreasing rate of phytic acid for barley, wheat and rye was emphasized, than in the beside germination time of these seeds. By soaking of wheat, rye and barley seeds, phytic acid amount was decreased with aproximate 14-21%, while in the germination time its hydrolysis devolved more slowly. After six days of germination, phytic acid content was decreased with approximate 45% of the initial value for rye, with 40% for wheat and with 35% for barley. During the maize soaking, the phytic acid amount was not significantly affected. By soaking, it was slightly decomposed (5%) and by germination until 20%, from the initial value. The loss values of phytic acid amount from extracts can be explained due the phytase action in the mashing time. As a result, the content of phytic acid decreased, while the released soluble phosphorus is accumulated in the mixture. Still, there is in the extracts, a small quantity of phytic acid, situation accountable by the passing in extract, during the filtration, of a small quantity of husks in which the phytic acid hydrolysis is incomplete. The phytic acid values obtained on the basis of phytic phosphorus are lower than those based on phytic iron.

Data from the Table 4 show that the Fe/P weight rations are higher than the theoretical ratio of 1.20 (assuming 4Fe/6P in ferric phytate).

Analyzed sample	Phytic phosphorus (mg/ g d.s.)	Phytic iron (mg/g d.s.)	Ratio Fe/P
Wheat	3.1256	3.9421	1.26
Maize	3.1051	4.0864	1.32
Rye	2.6748	3.3904	1.27
Barley	2.9814	3.7494	1.26
Soaked wheat	2.5015	3.1360	1.25
Soaked maize	2.9633	3.7041	1.25
Soaked rye	2.0986	2.6973	1.29
Soaked barley	2.5493	3.1871	1.25
Germinated wheat	1.8217	2.4005	1.32
Germinated maize	2.4761	3.1395	1.27
Germinated rye	1.4511	1.8663	1.29
Germinated barley	1.9451	2.4398	1.25
Extract of germinated wheat	0.4163	0.5308	1.28
Extract of germinated maize	0.8224	1.0898	1.33
Extract of germinated rye	0.5150	0.7187	1.40
Extract of germinated barley	0.4657	0.6584	1.41

Table 4. The values of ratio Fe/P in the case of phytic acid determination on the basis of phosphorus and ferric iron and from ferric phytate

This is true for measurements on purified phytic acid. For our case, is possible the occlusion of iron in ferric phytate, or the formation of complexes, which might explain the high Fe/P values. Nevertheless, the differences between phytic acid values, based on the determination of phosphate, comparatively with those based on iron, do not exceed 15%.

In Fig. 1 it is comparatively presented the phytic phosphorus, soluble phosphorus and estherified phosphorus, for germinated and not germinated cereals. The estherified phosphorus was calculated as difference between total and soluble phosphorus. These findings show a strong correlation between increasing of soluble phosphorus amount and decreasing of phytic acid amount.

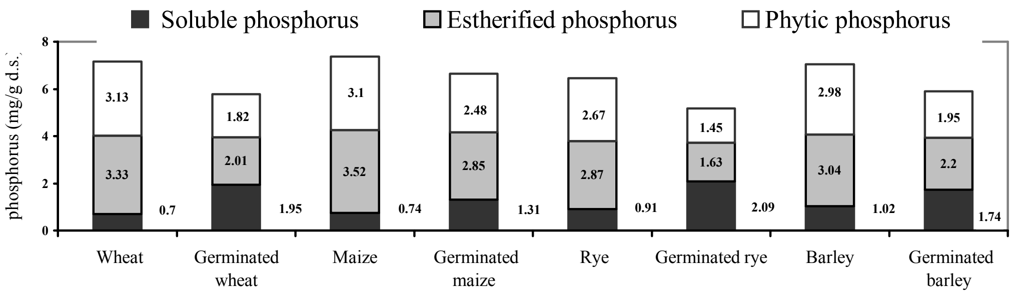

Figure 1. Relation between phytic phosphorus–soluble phosphorus–estherified phosphorus from cereals, before and after germination

The decreasing of phytic acid content from cereals seeds were influenced by the processing methods and by cereals species. Therefore, these cereals had shown a distinct phytase activity in the germination time. For all analyzed species, the value of the estherified phosphorus was more increased than the phytic phosphorus amount. We may say that the phytic acid and its salts represent the main constituents of estherified phosphorus. For wheat, barley and rye this difference is approximate 0.2 mg/g d.s, consisting in the inferior esters of myo-inositol phosphate and other compounds with phosphorus. In the maize case, this difference was situated in the range 0.37-0.42 mg/g d.s. This fact can be explained by the presence of some amounts of other organics compounds with phosphorus, such as: phospholipids, glycerolphosphates, sugar phosphates etc**.** These data are important, concerning the evaluation of the nutritional and health impact of the products obtained from cereals, seeing that they could be used as food supplements, to improve the nutritive value of the human diet.

Conclusions

In conclusion, results from these experiments indicated that during the soaking and germination processes, the soluble phosphorus amount was significantly increased. In opposition, we noticed a drastic decrease of phytic acid content from cereals seeds. The changes that were occurred in the phytic acid and soluble phosphorus content of tested cereals seeds were influenced by the processing methods and by cereals species. The major accumulation of soluble phosphorus is induced by rye soaking (62.63%) and the smallest, during the maize seeds (25.68% given of initial value). The results of this study show that the greatest reduction in phytate phosphorus was seen in germinated rye seeds, while the smallest decrease was found for maize seeds. In the extracts from germinated cereals, the soluble phosphorus represents 62-85% from total phoshorus amount. Phytate degradation during the investigated processes is generally attributed to endogenous phytase activity, or to the formation of insoluble phytate complexes. This study also shows that the phytic acid is the main form of estherified phosphorus from cereals seeds and it decomposition through soaking and germination has been correlated with the accumulation of soluble phosphorus.

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