Integral Wheat Flour Based Biscuits as Sources of Phosphorus in Everyday Nutrition

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Summary

Eight experimental integral wheat flour based biscuits were prepared and investigated for total and bioavailable phosphorus content. Results were compared to the values obtained for classic white wheat flour based biscuits in order to asses the impact of implantation of bran, different integral raw materials and fibers on the total phosphorus content and its availability. Since a study was conducted in the view of current trends of the excessive intake of this element in most of the developed countries, we expressed results obtained for total phosphorus content as percentages of allocated RDA values. Total phosphorus was determined by an official AOAC method (AOAC 2001) and its bioavailability by an *in vitro* enzymatic method (Schwedt et al. 1998). Total phosphorus content of investigated samples ranged from 1.093 g kg^{-1} (biscuit based on type 500 wheat flour) to 2.987 g kg^{-1} (biscuit enriched with integral wheat flour and amaranth). Phosphorus availability was the highest in biscuit based on type 500 wheat flour, as expected (86.1 %), and the lowest in the sample enriched with amaranth flour (53.0 %), due to a very high phytic acid content. Considering revealed values of total phosphorus content and its bioavailability, we concluded that the richest source of this important macroelement was the sample enriched with soy flour providing 1.671 g kg^{-1} of available phosphorus.

Key words

dietary phosphorus, phosphorus bioavailability, biscuits

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Introduction

Phosphorus represents the second most abundant mineral in the organism and is present mainly as constituent of bones (85 %) and muscles (14 %). It is used to maintain acid-base balance; it is involved in energy production and is necessary for other vital cellular functions.

The recommended intake levels for phosphorus are set based on life stage groups. For children Recommended Dietary Allowance (RDA) is set to 300 – 800 mg per day depending on the age; for youth (age 11 – 24) 1200 mg per day indicating the higher need for phosphorus during the adolescent growth spurt, and for adults (age > 25) 800 mg per day (DRIs, 1999). It should also be noted that there are no additional requirements for phosphorus during pregnancy or lactation.

Phosphorus is found widely distributed in foodstuffs and generally speaking, alimentary sources of phosphorus can be divided in three groups. It is found in raw and unprocessed foods containing phosphorus as cellular and protein constituent; it is also added to foods during food processing and it is contained in dietary supplements. The most important sources of phosphorus in the first group are milk and milk products, meat and fish, and grain products, each contributing 20 – 30 % of the daily intake of phosphorus for most age and sex groups (Uribarri and Calvo 2003). However, the contribution of food additives to dietary phosphorus intake is also increasing since highly processed foods are more and more represented in everyday nutrition. Phosphate additives are mostly used as flavor stabilizers, especially in popular cola beverages and as such can significantly contribute to inadequate phosphorus intake. For example, it has been reported that children consuming large amounts of soft drinks developed hypocalcemic tetany (Mazariegos-Ramos et al. 1995).

Since phosphorus is so plentiful in foodstuff and usually 60 – 70 % of dietary phosphorus is absorbed, deficiency does not appear in healthy population. If it occurs it is usually not due to inadequate dietary intake, but is rather the result of a high dose of antacid use, poorly managed diabetes, alcoholism, and some other forms of malnutrition.

On the other hand, excess phosphorus intake is very often in most developed countries. It has been estimated that over 50 % of all adults in United States ingest over 1500 mg per day of phosphorus, nearly double its RDA (Calvo 1996). The study conducted in Poland (Gronowska – Senger and Kotanska 2004) showed that intake of phosphorus was high in all investigated households and ranged between 34 – 96 % above RDA limit. In Japan, daily phosphorus intake increased from 1243 mg per day in 1960 to 1421 mg per day in 1995, due to increased consumption of milk, meat and chicken eggs. According to this study, the main sources of dietary phosphorus in 1995 were cereals which participated in total daily phosphorus intake with 24.4 % (Takeda et al. 2002).

It is also important to emphasize that the total amount of consumed phosphorus is probably less important than the molar ratio of dietary calcium to dietary phosphorus. Namely, if consumption and absorption of phosphorus is substantially grater than the consumption and absorption of calcium, then secondary hyperparathyroidism and decline in bone density may result. Therefore, a high intake of phosphorus increases the requirement for dietary calcium by impairing the homeostatic mechanism that allows the body to adapt low calcium intake (Sax 2001).

The consequences of long-term inadequate phosphate control are especially dangerous for patients with renal diseases where consequences of inadequate phosphate control include hyperparathyroidism, metabolic bone disease calcifying uremic arteriophaty, and cardiovascular calcification (Kuhlman 2006). It is also an important risk factor for developing osteoporosis due to its effects on bone mineralization (Omi et al. 2001; Basabe Tuero et al. 2004; Koshihara et al. 2005).

As stated before, cereals represent one of the most important sources of dietary phosphorus in everyday nutrition. A new generation of cereal based products includes integral products containing a high content of bran. Those products are generally richer in minerals, vitamins, dietary fibers, antioxidants, and numerous other functional and health protective components. Therefore, a group of such products was investigated in the framework of this study.

The goal of our research was to determine how the supplementation of white wheat flour based biscuit with whole grain wheat flour, dietary fibers, and other integral raw materials affects the total phosphorus content of the final product.

The most important determinant of how much phosphorus is absorbed from a diet is the total phosphorus content as well as phosphorus bioavailability that is affected by presence of its natural binders in the foodstuff (Uribarri and Calvo 2003). One of the most important natural binders in grains is phytic acid representing the major form of phosphorus in seeds, and accumulating in other plant tissues as well, such as pollen, roots, tubers, and turions. Its most important function is the storage and retrieval of phosphorus, minerals, and inositol during development and germination. It can represent from one to several percent of a typical seeds dry weight, and about 75% of seeds total phosphorus (Raboy 2003). It is the most important limiting factor of phosphorus availability in wheat and wheat-based products.

Our goal was to examine if it remaines so when other types of raw materials (carob, amaranth dietary fibers or soy) containing numerous other antinutritive compounds were implanted to standard recipe. Therefore the bioavailability of phosphorus was also determined in investigated samples by an *in vitro* enzymatic method.

Material and methods

In the frameworks of this research, nine experimental biscuits were prepared. Our reference sample (sample 1) was prepared using white wheat flour (type 500). In samples 2 and 3 white wheat flour was replaced with different amounts of integral wheat flour (65 % and 100 % respectively). All other samples were made of a mixture of white and integral wheat flour but each sample was additionally enriched with one of the following raw materials: wheat dietary fibers, oat dietary fibers, apple dietary fibers, full–fat soy flour, amaranth, and carob, that were added to the recipe on account of white wheat flour. Differences in composition of investigated samples are presented in Table 1.

After preparation, the dough was rolled to 0.7 cm, biscuits were formed and baked at 180 °C for approximately 15 min. Biscuits were ground to the particle size < 1 mm and stored in plastic containers at 4 °C for further analysis.

Total phosphorus was determined by AOAC spectrophotometric method (AOAC 2001). Samples were wet digested in MLD 2000 microwave unit by the addition of nitric acid (95 %) and hydrogen peroxide (30 %). Digested sample was diluted by the addition of deionized water. For spectrophotometric determination 1 – 5 mL of the digested sample was taken (depending on total phosphorus content) and mixed with 1 mL of 5 % ammonium molybdate, 1 mL 5% hydroquinone and 1 mL 20 % sodium sulfite solution. The mixtures were diluted with deionized water to 50 mL and kept at room temperature for 30 min for complete color development. Absorbance was determined at 650 nm.

Phosphorus bioavailability was determined using an *in vitro* enzymatic method (Schwedt et al. 1998). Samples were incubated with pepsin (Merck – EC 3.4.23.1) disolved in hydrochloric acid solution (pH=2) at 37 °C for three hours in order to mimic gastric digestion of the sample. Intestinal digestion was further simulated by adjusting the pH of gastric digest to 7.5 with sodium bicarbonate and adding pancreatin (Sigma P1750), bile salts (Sigma B8756), and amylase (Fluka 10070) to the sample. Incubation was conducted at 37 °C for the next two hours. Samples were then vacuum filtered and indigestible residue was wet digested in microwave unit and examined for phosphorus content as described before. The amount of phosphorus available for absorption was calculated from the difference between total and undigestible phosphorus.

Table 1. Differences in the compositions of investigated biscuits in relation to reference sample (sample 1)

Sample number	Raw materials added to the basic recipe
2	T 1700 (65%)*
3	T 1700 (100%)
4	T 1700 (65%) + soy flour (25%)
5	T 1700 (65%) + carob (25%)
6	T 1700 (65%) + amaranth (25%)
7	T 1700 (65%) + wheat fibers (17%)
8	T 1700 (65%) + oat fibers (17%)
9	T 1700 (65%) + apple fibers (17%)

*numbers in the brackets show the percentage of added raw materials considering the total amount of the flour in the recipe. The rest to 100% is the amount of type 500 wheat flour in the recipe.

Results and discussion

Obtained results show that implementation of different integral materials to white wheat flour based biscuit significantly contributes to total phosphorus content of the final product. Results are shown in Table 2.

The amount of total phosphorus in investigated samples ranged from 1.092 g kg^{-1} (biscuit based on type 500 wheat flour) to 2.987 g kg^{-1} (biscuits enriched with integral wheat flour and amaranth). It was particularly high in samples enriched with a mixture of integral wheat- and soy flour and also in biscuit made totally of integral wheat flour. Comparing the results obtained for the sample made of mixture of white and integral wheat flour (sample 2) to the samples additionally enriched with dietary fibers (samples 7, 8, 9) it is clear that the addition of dietary fibers decreased total phosphorus content. The same comparison shows that the addition of carob (sample 5) has no effect on total phosphorus content of the biscuit. The contribution of each raw material or mixtures of materials added to the reference sample to the total phosphorus amount in the final product is shown in Figure 1.

The bioavailability of phosphorus is presented in Table 2. Presented data show that it has been significantly decreased by implantation of all used integral raw materials to standard recipe compared to the reference sample (sample 1).

Availability of phosphorus in investigated samples varied from 86.1 % in the reference sample to 53.0 % in sample enriched with the mixture of integral wheat flour and amaranth. Significant decrease of phosphorus availability was also noted in samples 3 and 4 (additions of whole grain flour and its mixture with soy flour), and a lesser reduction of bioavailability was noted in samples 5, 7, 8 and 9 (samples enriched with a mixture of integral wheat flour, with carob or one of three used types of dietary fibers).

Table 2. Total, undigestible and bioavailable phosphorus in investigated samples

Sample number	Phosphorus fraction	g kg^{-1} original sample (average± stdev)	g kg^{-1} dry matter (average± stdev)	availability (%)
1	total P	1.092±0.005	1.133±0.005	86.091
	undigestible P	0.152±0.023	0.158±0.238	
	available P*	0.941	0.976	
2	total P	2.106±0.010	2.185±0.108	64.152
	undigestible P	0.755±0.043	0.783±0.045	
	available P	1.351	1.401	
3	total P	2.629±0.016	2.735±0.016	57.576
	undigestible P	1.115±0.013	1.160±0.133	
	available P	1.514	1.575	
4	total P	2.741±0.0158	2.896±0.017	57.709
	undigestible P	1.159±0	1.225±0	
	available P	1.582	1.671	
5	total P	2.099±0.021	2.186±0.022	68.672
	undigestible P	0.658±0.028	0.685±0.029	
	available P	1.441	1.501	
6	total P	2.987±0.011	3.119±0.011	53.012
	undigestible P	1.403±0.373	1.466±0.039	
	available P	1.584	1.653	
7	total P	1.925±0.180	2.049±0.019	64.615
	undigestible P	0.681±0.312	0.725±0.033	
	available P	1.244	1.324	
8	total P	1.955±0.021	2.081±0.022	66.532
	undigestible P	0.654±0.327	0.697±0.035	
	available P	1.301	1.385	
9	total P	1.900±0.094	2.015±0.100	66.069
	undigestible P	0.645±0.307	0.684±0.037	
	available P	1.255	1.331	

*Amount of available phosphorus was calculated from the difference between total and undigestible phosphorus content

The percentage of decrease of phosphorus availability in enriched samples compared to reference biscuit is shown in Figure 1.

Generally, the decrease in mineral availability in foodstuff is usually the consequence of higher content of natural chelators. As mentioned before, the most important limiting factor for phosphorus availability is phytic acid, and since it is mostly found in the aleuronic layer of wheat grain, implantation of bran to the reference sample caused significant decrease of bioavailability of investigated mineral. Replacement of white flour with 65 % (sample 2) and 100 % of integral flour (sample 3) resulted in 25.5 % and 33.1 % decrease in phosphorus availability respectively. A high decrease was also noted when white wheat flour in the recipe was substituted with a mixture of integral wheat flour and phytic acid rich raw materials such as soy flour and amaranth (samples 4 and 6). On the other hand when carob or either one of used dietary fibers were mixed with whole grain flour and used for enrichment of the reference sample, the effect on phosphorus availability was lesser (samples 7, 8, 9) or the same (sample 5) as

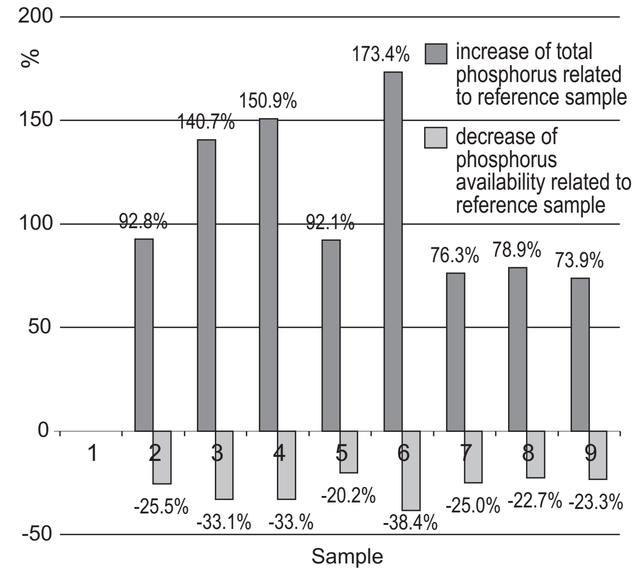

Figure 1. Changes of total phosphorus content and its availability in investigated samples related to reference sample (sample 1)

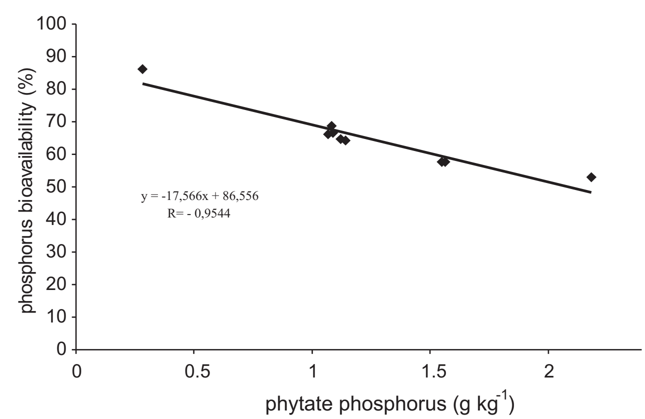

Figure 2. Correlation between the amount of phytate phosphorus and phosphorus bioavailability in investigated samples

when only integral wheat flour was added to the reference sample (sample 2). That effect was consistent with our expectations since phytic acid free fibers were used for the preparation of biscuits and our previous investigations showed that carob flour contained very low amounts of phytic acid (Vitali et al. 2007)

In order to confirm our belief that phytic acid is the main factor determining phosphorus availability in integral biscuits even when raw materials other than wheat are used for their preparation, we compared previously determined amounts of phytate phosphorus (Vitali et al. 2007) in investigated samples with phosphorus bioavailability determined in the framework of this investigation.

As expected, very high negative correlation was obtained as shown in Figure 2.

Our results show that despite the significant differences in phosphorus bioavailability among investigated samples, total phosphorus content could be considered as a good indicator of sample's value as the source of bioavailable forms of this element. For example, significantly higher phosphorus availability in white wheat flour classic biscuit (86.1 %) compared to other investigated samples could not compensate for the lack of total phosphorus content of the reference sample. Therefore it is considered to be the poorest source of phosphorus in nutrition providing only 0.976 g kg^{-1} of available phosphorus. Samples with the highest total of phosphorus content (samples 4 and 6) also proved to be the best sources of its available fraction, despite significantly lower bioavailability, containing 1.671 g kg^{-1} and 1.653 g kg^{-1} of available phosphorus, respectively.

Conclusion

Results of our investigation show that cereal based confectionery products can also be considered as significant sources of phosphorus in everyday nutrition, especially when enriched with different integral raw materials. Namely, obtained results show that 100 g of such biscuit can cover from 13.65 % up to 37.3 % of RDI set for an adult person (800 mg per day).

Implementation of integral raw materials to reference biscuit resulted in a decrease of phosphorus availability but even more significant an increase in total phosphorus content in all investigated samples. Therefore we can conclude that modifications that we made in the reference recipe resulted in a creation of samples that provide higher amount of bioavailable phosphorus in everyday nutrition despite the fact that their relative phosphorus availability was decreased.

Results of our work also proved that a decrease of phosphorus availability in modified samples was mostly due to the increased amounts of phytic acid that can be considered as the main limiting factor of phosphorus bioavailability, not only in pure wheat-based products but also when other raw materials such as amaranth, soy, carob and different sources of dietary fibers are incorporated to the recipe.

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