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Bioaccessibility of Ca, Mg, Mn and Cu from whole grain tea-biscuits: Impact of proteins, phytic acid and polyphenols

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Abstract

Levels of some essential minerals (Ca, Mg, Cu and Mn) were determined in ten different types of experimentally prepared hard biscuits. In relation to the wheat flour-based reference sample, other investigated samples were enriched with different ratios of integral raw materials of different origin or various dietary fibers in view of improving their functionality and nutritive quality. The goal of the research was to evaluate enriched biscuits as additional sources of calcium, magnesium, copper and manganese in nutrition and to investigate if the modifications of wheat flour based biscuit composition significantly change the amounts of total and bioaccessible minerals in the final product. Since our results indicated significant changes of mineral bioaccessibility among the samples, obtained results were correlated to the content of proteins, phytic acid and polyphenols for the sake of assessing their impact as limiting factors of mineral bioaccessibility in these types of foods.

Keywords: Bioaccessibility; Mineral; Biscuit; Phytic acid; Protein; Polyphenols

1. Introduction

In the past few decades there has been a graduate increase in production and consumption of novel cereal based products that can be categorized as functional foods designed to provide additional amounts of nutritive and health protecting substances to daily diet. In these terms, newly designed nutritionally improved biscuits can be recognized as foods exerting a beneficial effect on health and/or reducing the risk of developing chronic disease beyond their basic nutritional functions.

Improving nutritional quality of cereal based foods is often achieved by combining the cereals with some other raw materials such as legumes or pseudocereals. In that way it is possible to enhance protein content and quality as well as the content of some vitamins and essential minerals in the final product.

Due to their high content of nondigestible carbohydrates and as rich sources of dietary fibers that promote several beneficial effects (laxation, lowering blood cholesterol levels, preventing some types of cancer, diabetes, hart disease and obesity) (Charalampopoulos, Wang, Pandiella, & Webb, 2002), cereals or cereal constituents are often used in developing functional foods, mostly as fermentable substrates for growth of probiotic microorganisms or as prebiotics.

Dietary fibers obtained from different sources vary considerably in their chemical composition, insoluble/soluble dietary fiber ratio, particle size and physicochemical characteristics so consequentially they show different physiological effects as well (Figuerola Hurtado, Estevez, Chiffelle & Asenjo, 2005). Those variations in their chemical composition also affect their ability to bind minerals during intestinal digestion of foods. Namely, one of the most important properties of dietary fiber is the cation exchange. Therefore poor mineral utilization from certain types of fiber rich foods is probably due to the binding of minerals and electrolytes on fiber source. Binding efficiency depends mostly

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on fiber source, i.e. on the number of free carboxyl groups on sugar residues and the uronic content of polysaccharides (Olivares, Martinez, Lopez, & Ros, 2001). Additionally, polysaccharides in cereals are associated with many other substances, mostly with proteins, polyphenols and phytate which can modify mineral binding by dietary fiber. For example in *in vitro* mineral binding study by Idouraine (Idouraine, Khan, & Weber, 1996), it has been found that wheat bran bounds significantly more zinc, calcium and magnesium compared to rice bran and oat fiber while oat fiber bound significantly more copper compared to other investigated fiber sources. On the other hand, some indigestible polysaccharides, such as inulin and oligofructose, have often been reported as potent enhancers of mineral bioavailability in foods of plant origin. The improved mineral absorption in rats in the presence of inulin or oligofructose (Levrat, Remesy, & Demigne, 1991; Ohta et al., 1994) is probably associated with decreased pH of ileal, cecal and colonic contents, hypertrophy of cecal walls and increased concentrations of volatile fatty – and bile acids in cecal contents. Studies with humans partially confirmed those findings proving the beneficial effects of inulin and oligofructose on calcium absorption (Coudray et al., 1997; Van den Heuvel, Muys, Van Dokkum, & Schaafsma 1999).

In the view of above mentioned findings, the aim of our study was to examine the possibilities of improving Ca, Mg, Mn and Cu content and bioaccessibility from hard biscuit, by modifying its basic composition, based on white wheat flour, with various whole-grain raw materials and dietary fibers of different origin. Since enrichment of the basic recepture led to significant changes regarding the content of proteins, phytic acid and polyphenols, we also investigated whether those changes could be correlated with observed differences in mineral bioavailability among investigated samples.

2. Materials and methods

2.1. Sample preparation

Sample preparation has already been described elsewhere in details (Vitali, Vedrina-Dragojević, Šebečić, & Vujić, 2007). Shortly, tea biscuit recepture based on the use of the mixture of type T500 and type T1700 wheat flour was modified by addition of various dietary fibers or full grain raw materials of different origin. Major differences in the composition of investigated biscuits are presented in Table 1. As shown in the table, sample 1 contains only white wheat flour (flour type T500), while in sample 2 a ratio of white flour was substituted with integral wheat flour (flour type T 1700). Sample no 3 was entirely made of integral wheat flour. Those samples were prepared in order to evaluate the effect of the amount of wheat bran in biscuit on the content and bioaccessibility of investigated minerals.

Table 1
Differences in composition of ten investigated experimental biscuits

Composition of biscuits[a]

Sample	Basic raw materials		Additional raw materials	
	White wheat flour %	Whole grain wheat flour %	Integral raw material (%)	Pure fiber (%)
1	100	–	–	–
2	35	65	–	–
3	–	100	–	–
4	10	65	Soy flour (25)	–
5	10	65	Amaranth flour (25)	–
6	10	65	Carob flour (25)	–
7	18	65	–	Inulin (17)
8	18	65	–	Wheat fiber (17)
9	18	65	–	Oat fiber (17)
10	18	65	–	Apple fiber (17)

[a] All samples were prepared using same amounts of fat, sugar and powder milk. Differences refer only to different amounts and sorts of flours and fibers used in preparations by addition on account of white wheat flour.

All other samples (samples no 4–no 10) were additionally enriched with different ratios of other raw materials or dietary fibers on account of white wheat flour, while the share of integral wheat flour remained the same as in sample no 2 (i.e. 65% of the dough's dry weight). Therefore, in the rest of the text, sample 2 is referred as the reference sample.

For the purpose of obtaining as reliable data as possible, three series of biscuits were prepared, each on different experimental day using different batches of utilized raw materials made by the same producer. Each series of biscuit was investigates separately (in duplicates or triplicates, depending on the type of analysis) and results of three series of samples were averaged and presented in this work.

2.2. Determination of total mineral content

Investigated macroelements (Ca and Mg) and trace elements (Mn and Cu) were determined by Inductively Coupled Plasma Atomic Emission Spectrometry on a Trace Scan Thermo (Thermo Jarrell Ash Corporation, Franklin, USA) at 184.0, 279.0, 257.6 and 324.7 nm, respectively using standard technique (Jarrell Ash Corporation, 1995). Detection limits were 0.03 $\mu g\,L^{-1}$ (Ca, Mg), 0.10 $\mu g\,L^{-1}$ (Mn) and 0.50 $\mu g\,L^{-1}$ (Cu).

Prior to spectrometric analysis, samples were wet ashed using microwave digestion procedure. A 500 mg of sample was weight directly to digestion vessel. A 65% HNO_3 (v/v) (5 mL) and 30% H_2O_2 (v/v) (2 mL) were added to the samples, vessels were covered, placed into the rotator body of microwave oven Milestone MLS 1200 Mega Oven (Milestone, Bergamo, Italy) and digestion program was recalled. The digestion conditions were: 1 min at 250 W (smooth oxidation of organic matter); 1 min at 0 W (proceeding of reaction without addition of energy to avoid run-away temperatures and overpressures); 5 min at 250 W (termination of the soft oxidation of the organic compounds); 5 min at 400 W and 5 min at 600 W (final termination of oxidation processes by applying higher power). After cooling, digested samples were transferred to volumetric flasks and diluted to 50.0 mL using deionized water and finally transferred to 50 mL polyethylene flasks.

Microwave digestion procedure has been selected for sample preparation since it is free from contamination risk and is not time consuming. Due to relatively small amounts of samples used for digestion, excessive pressure during digestion procedure, the main restriction of microwave digestion, was avoided (Doner & Ege, 2004). All glassware used for mineral determination was washed with detergent and water, and soaked in 10% nitric acid (v/v) solution for 24 h. The solution was discarded and the soaking was repeated one more time for the next 24 h. After that, all glassware was rinsed several times with deionized water and dried.

2.3. Determination of *in vitro* mineral bioaccessibility

The amount of bioaccessible minerals was determined according to *in vitro* enzymatic method (Schwedt, Tawali, & Koch, 1998). The simulation of gastric digestion, during which samples were treated with pepsin (Merck – EC 3.4.23.1) in 0.02 M HCl/NaCl mixture (pH 2), was conducted in the rocking water bath, at 37 °C for 3 h. Prior to the simulation of intestinal digestion step, pH of the samples was adjusted to 7.5 using 6% $NaHCO_3$ and after addition of bile salt (Sigma B8756), pancreatin (Sigma P1750) and amylase (Fluka 10070) to digestion solutions, simulation of intestinal digestion was continued for another 2 h. Samples were vacuum filtered and residues were wet ashed using the same procedure described for total mineral determination. Afterwards, samples were diluted to 50 mL with deionized water and the content of insoluble Ca, Mg, Cu and Mn was determined by ICP-AES as described previously. Experiments were conducted in triplicates and bioaccessibility of investigated minerals was calculated as shown below:

$$\text{Mineral bioaccessibility}(\%) = \frac{(\text{total mineral} - \text{insoluble mineral}) \times 100}{\text{total mineral}}$$

2.4. Determination of protein, phytate, polyphenol and moisture content

Proteins were determined according to Kjeldahl procedure (AOAC, 2000) using semiautomatic Büchi system. Investigations were carried out in duplicates and conversion factor 6.25 was used for recalculating the content of organically bound nitrogen to protein content. Moisture was determined using the air oven method (AOAC, 1990) and investigations were carried out in duplicates.

Phytic acid content (Vitali, Vedrina-Dragojević, Šebečić, & Vujić, 2007) was determined spectrophotometrically (Haug & Lantzsch, 1983). Polyphenol content (Šebečić, Vedrina, Vitali, Hečimović, & Dragičević, 2007) was determined in defatted samples using Folin Ciocalteu spectrophotometric method (Gao, Wang, Oomah, & Mazza, 2002).

2.5. Statistical analysis

One-way ANOVA was used to calculate significance of possible differences among series of investigated biscuits regarding their total-, insoluble mineral and protein content. Data obtained for each series were subjected to statistical analytical process using mean ± standard deviation of parallel determinations. Equations, correlation coefficients and significance of the correlation of mineral bioaccessibility with the content of proteins, polyphenols, and phytic acid were calculated using linear regression. Statistica Version 6 was used for conducting all mentioned analysis.

3. Results and discussion

Enrichment of referent recepture (sample 2) with selected raw materials or dietary fibers resulted in significant differences among enriched biscuits regarding their protein, phytate and polyphenolic content (Table 2).

Obtained values were consistent with the results of our preliminary investigations, dealing with raw materials used in biscuit production (Table 3).

In the view of estimating the possibilities of increasing protein content and quality of wheat based confectionary product by substituting the ratio of white wheat flour with the mixture of other raw materials and with the aim to evaluate their impact on mineral solubility, protein content of enriched biscuits was compared to the values obtained in the reference sample.

As presented in Fig. 1, increase of protein content was achieved by addition of whole grain raw materials to refer-

Table 2
Content of phytic acid, polyphenols and proteins in investigated biscuits (means ± SD)

Sample	Phytic acid[a]	Polyphenols[b]	Proteins
	g/100 g dry matter		
1	0.15 ± 0.01	0.11 ± 0.02	8.7 ± 0.1
2	0.57 ± 0.04	0.14 ± 0.02	9.0 ± 0.1
3	0.78 ± 0.01	0.17 ± 0.01	9.3 ± 0.1
4	0.77 ± 0.02	0.23 ± 0.01	13.8 ± 0.2
5	1.08 ± 0.09	0.14 ± 0.01	9.9 ± 0.1
6	0.53 ± 0.02	0.58 ± 0.06	9.1 ± 0.2
7	0.53 ± 0.05	0.16 ± 0.02	8.4 ± 0.1
8	0.56 ± 0.02	0.09 ± 0.01	8.5 ± 0.1
9	0.53 ± 0.03	0.10 ± 0.02	8.6 ± 0.1
10	0.53 ± 0.01	0.23 ± 0.01	8.6 ± 0.1

[a] Vitali et al. (2007).

[b] Šebečić et al. (2007).

Table 3
Protein, polyphenol and phytic acid content of raw materials used in biscuit production (means ± SD)

Raw materials	Proteins	Polyphenols	Phytic acid[a]
	g/100 g dry matter		
Wheat flour T500	12.1 ± 0.0	0.10 ± 0.00	0.29 ± 0.08
Wheat flour T1700	13.6 ± 0.0	0.16 ± 0.00	0.71 ± 0.06
Full fat soy flour	48.5 ± 0.7	0.51 ± 0.03	1.04 ± 0.11
Amaranth	15.9 ± 0.1	0.16 ± 0.01	2.25 ± 0.25
Carob	6.1 ± 0.0	4.50 ± 0.22	0.05 ± 0.01
Inulin	ND[b]	0.01 ± 0.00	ND
Wheat fiber	0.2 ± 0.0	ND	ND
Oat fiber	0.1 ± 0.0	ND	ND
Apple fiber	0.3 ± 0.0	1.09 ± 0.01	ND

[a] Vitali et al. (2007).

[b] Content was below the limit of detection of applied method.

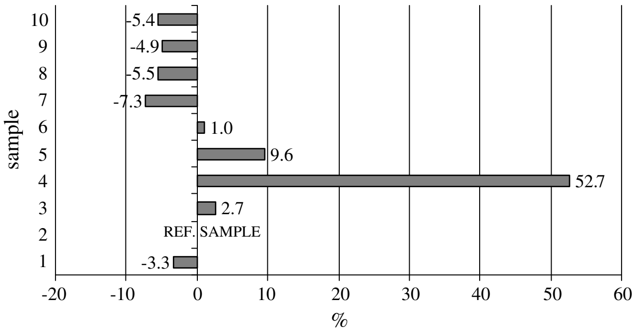

Fig. 1. Changes of protein content of investigated biscuits in relation to the reference sample.

ence recepture, especially by addition of soy flour (52.7%). Significant increase was also observed in the sample containing amaranth (9.6%), while addition of T1700 wheat flour and carob resulted in slighter change of protein content (2.7% and 1%, respectively). Addition of purified dietary fibers to reference sample as well as excluding bran from biscuit preparation (sample 1) significantly reduced protein content.

Applied modifications of reference sample (sample 2) also resulted in significantly changed mineral composition of modified biscuits. Obtained amounts of total and bioaccessible minerals are shown in Table 4. Regarding the total mineral content, the most abundant mineral was Ca, ranging from 40.4 mg/100 g in white wheat flour based biscuit (sample 1), to 104.8 mg/100 g in the sample enriched with carob. Significantly increased amounts of Ca, as compared to reference sample, were also found in samples containing soy flour and amaranth (90.1 and 83.4 mg/100 g dry matter, respectively). Mg levels ranged from 20.4 mg/100 g (sample based on T500 wheat flour) to 101.9 mg/100 g (sample enriched with amaranth). Significant increase of Mg content, as compared to reference sample, was also found in the biscuit containing soy flour (sample 4) and the integral wheat flour based biscuit (sample 3) (84.9 and 68.0 mg/100 g respectively). Addition of carob slightly increased total Mg, while addition of purified fibers to reference sample did not significantly change its content. Obtained levels of Mn and Cu were lower, as expected, ranging from 0.34 mg/100 g to 2.79 mg/100 g and from 0.09 mg/100 g to 0.26 mg/100 g respectively. The lowest amounts of investigated elements were found in the sample containing oat fiber (the lowest Cu content) and in the reference sample (the lowest Mn content), while the richest sources of Mn and Cu were integral wheat flour based biscuit (sample 3) and biscuit containing soy- flour (sample 4).

Generally, our results indicate that addition of integral raw materials to referent sample results in more considerable increase of mineral content compared to addition of pure dietary fibers. When comparing mineral content of such samples (samples 7–10) to the reference biscuit, small differences are observed. Addition of dietary fiber contributed to total Ca content with approximately 20%, increased Mg content for 10% and did not significantly affect Mn and Cu levels.

Obviously, the better approach for improving nutritive quality of these types of products is the use of whole grain flours resulting in significant increase of mineral content, improving protein content and increasing the amount of

Table 4
Amounts of total and bioaccessible Ca, Mg, Mn and Cu in investigated samples (mg/100 g dry sample)

Sample	Ca	Mg	Mn	Cu	Ca	Mg	Mn	Cu
	Total minerals[a]				Bioaccessible minerals[a]			
1	40.4 ± 0.5	20.4 ± 1.5	0.34 ± 0.02	0.11 ± 0.01	21.4 ± 0.6	15.9 ± 1.3	0.19 ± 0.01	0.06 ± 0.01
2	53.6 ± 2.8	50.9 ± 1.6	1.98 ± 0.05	0.15 ± 0.02	24.1 ± 1.6	36.7 ± 2.3	0.96 ± 0.06	0.09 ± 0.02
3	56.0 ± 1.3	68.0 ± 5.3	2.79 ± 0.27	0.18 ± 0.01	24.3 ± 1.9	50.2 ± 7.4	1.16 ± 0.17	0.12 ± 0.01
4	90.1 ± 3.5	84.9 ± 1.0	2.32 ± 0.06	0.26 ± 0.02	30.9 ± 3.0	58.9 ± 1.7	1.14 ± 0.09	0.17 ± 0.01
5	83.4 ± 3.6	101.9 ± 2.0	2.56 ± 0.23	0.21 ± 0.02	28.7 ± 3.6	68.6 ± 1.2	0.83 ± 0.09	0.14 ± 0.02
6	104.8 ± 6.6	59.1 ± 3.5	2.11 ± 0.07	0.25 ± 0.01	23.6 ± 4.5	38.5 ± 5.9	0.40 ± 0.07	0.12 ± 0.01
7	54.8 ± 3.1	51.7 ± 0.4	2.00 ± 0.02	0.13 ± 0.00	28.4 ± 0.6	39.0 ± 1.3	1.06 ± 0.06	0.08 ± 0.01
8	66.9 ± 1.7	55.7 ± 2.0	2.05 ± 0.03	0.14 ± 0.01	29.1 ± 0.6	39.9 ± 0.8	1.06 ± 0.11	0.08 ± 0.02
9	65.9 ± 1.8	54.5 ± 2.9	2.02 ± 0.08	0.09 ± 0.01	30.6 ± 2.2	39.4 ± 3.6	0.95 ± 0.11	0.05 ± 0.01
10	63.9 ± 1.7	52.8 ± 3.3	1.97 ± 0.09	0.19 ± 0.02	24.0 ± 2.2	35.3 ± 4.4	0.81 ± 0.05	0.12 ± 0.02

[a] Data are presented as averages of three investigated series of biscuits ± SD.

dietary fibers due to naturally present fibers in used raw materials.

However, due to the presence of natural chelating agents in whole grain flours used for biscuit preparation, total content of certain mineral does not necessarily reflect the actual potential of foodstuff as the source of that element since those compounds have been known to impair mineral bioaccessibility. Therefore, it is necessary to gain a better insight to the amounts of soluble mineral fraction that is available for absorption in small intestine (Table 4).

When reviewing the results in terms of the amounts of bioaccessible mineral fraction (total mineral – insoluble mineral), the most abundant mineral in investigated samples was Mg, with concentrations ranging from 15.9 mg/100 g (sample 1) to 68.6 mg/100 g (sample enriched with amaranth). Very good sources of available Mg were also biscuit with soy flour (sample 4) and integral wheat flour based biscuit (sample 3). In the case of Ca, quite uniformed results were obtained, ranging from 21.4 mg/100 g (sample 1) to 30.9 mg/100 g (sample with soy flour). This is the good example of the importance of determining the amounts of soluble mineral fractions because in some cases, like this one, total mineral content does not reflect the amount of mineral that is available for intestinal absorption. For example, the sample with carob flour contained significantly higher calcium content compared to all other investigated samples (104.8 mg/100 g) but in spite of that provided almost the lowest amount of bioaccessible calcium (23.6 mg/100 g).

The amounts of bioaccessible Mn and Cu ranged from 0.19 to 1.16 mg/100 g and from 0.05 to 0.17 mg/100 g, respectively and generally correlated well to the total amounts of Mn and Cu in investigated samples.

When observing obtained results in the terms of bioavailability i.e. the percentage of bioaccessible mineral in relation to mineral's total content, as shown in Fig. 2, it is obvious that in most of cases bioavailability of investigated minerals is decreased in relation to reference sample (sample 2)

Low bioaccessibility of minerals in some samples is probably due to the high content of antinutritive compounds known to impair mineral availability. In addition to phytate and polyphenols, proteins of plant origin have also been reported to decrease mineral availability. Therefore, we expressed obtained results as the percentages of elements available for absorption in relation to the total amount of particular mineral (Fig. 2) and correlated them with biscuiś content of proteins, phytic acid and polyphenols (Table 2). Obtained correlation coefficients are presented in Table 5.

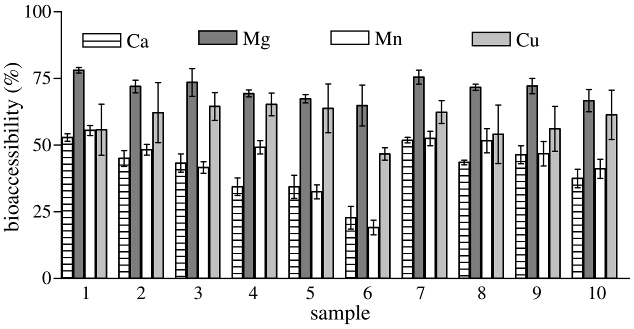

Fig. 2. Bioaccessibility of Ca, Mg, Mn and Cu in investigated samples.

As shown, impact of protein content on mineral availability was negligible in our study. The result is not surprising because all investigated samples, except for the sample that contained soy flour (sample 4) were similar regarding protein content and origin. Namely effects of proteins on mineral availability are usually noticeable when comparing foodstuff that differ significantly regarding protein origin (animal or vegetable), content and quality (amino acid composition) (Camara, Amaro, Barbera, & Clemente, 2005; Perez-Llamas, Larque, Marin, & Zamora, 2001). Soy protein has been cited to improve Mn bioavailability (Kannan, 2003), but that positive effect was not clear in our investigation. Namely, addition of soy flour to the recepture resulted in increase of Mn solubility from 48.3 to 49.1% what was not statistically significant.

Ca and Mg bioaccessibility negatively correlated with polyphenol and phytate content, while Mn bioaccessibility negatively correlated with phytic acid content. Cu availability did not seem to be impaired by the presence of any of investigated limiting factors, what is consistent with available literature data (Turnlund, King, Gong, Keyes, & Michael, 1985).

Ca accessibility ranged from 22.8 to 55.2% (samples 6 and 1, respectively). Again, impact of phytate was best visible in samples containing only wheat flour (samples 1–3) while enrichment with other raw materials increased the effect of other antinutritive compounds. The most visible effect was the one of the polyphenols, since the lowest Ca accessibility was found in samples rich in those compounds (samples 4, 5, 6 and 10).

Therefore, low bioaccessibility of Ca in sample enriched with carob or apple fiber is in relation to the high amount of polyphenols (0.58 g/100 g and 0.23 g/100 g, respectively) which is significantly higher compared to the samples containing wheat fiber, oat fiber or in relation to the reference sample (0.09 g/100 g, 0.10 g/100 g and 0.14 g/100 g, respectively) all showing higher calcium bioavailability.

Among investigated elements, Mg was characterized with the highest bioavailability ranging from 64.9% in sample containing carob (sample 6) to 78.1% in the reference

Table 5
Correlation coefficients obtained by correlating mineral bioaccessibility with phytate, polyphenolic and protein content

	Ca	Mg	Mn	Cu
Phytate	−0.553[a]	−0.512[a]	−0.385	0.412
Polyphenols	−0.659[a]	−0.633[a]	−0.788[a]	−0.442
Proteins	−0.357	−0.279	0.072	0.276

[a] Correlation is significant at the level $p > 0.05$.

sample (sample 1). Rather low Mg bioaccessibility was also found in samples containing amaranth and soy flour (samples 5 and 4, respectively). Those biscuits also contain the highest amounts of phytic acid (1.08 g/100 g and 0.77 g/100 g, respectively), so this decrease of magnesium solubility can be attributed to the restricting effect of dietary phytate that had already been confirmed even in *in vivo* studies (Pallauf, Pietsch, & Rimbach, 1998; Rimbach & Pallauf, 1999). Low Mg availability in the sample containing carob can be explained by already mentioned significantly higher polyphenol content in that sample.

Bioavailability of Cu was rather uniformed, ranging from 46.7% (sample 6) to 69.1% (sample 10). Although the lowest Cu solubility was found in the sample with the highest polyphenolic content (sample 6), other samples rich in phenolic compounds (samples 4 and 10) were not characterized with particularly low copper accessibilities (65.3% and 61.4%, respectively).

Mn bioaccessibility varied significantly among investigated samples ranging from 19.1% to 59.9% (samples 6 and 8, respectively) and significant correlation was determined with polyphenol content. The correlation with phytic acid was significant only in those samples that contained exclusively wheat flour (samples 1–3), while addition of other raw materials decreased the importance of phytate as major mineral chelating compound.

Although the impact of dietary fibers on mineral availability has often been cited in the literature, many times it has been stated that it is hard to distinguish the impact of dietary fibers themselves from the impact of fiber associated compounds such as phenolic compounds and/or phytic acid (Rosander, Sandberg, & Sandstrom, 1992). Since dietary fibers used in this investigation were phytic acid free and additionally, inulin, oat and wheat fibers contained negligible amounts of polyphenols (Table 3), it was possible to asses and compare the impact of dietary fibers of different origin on the bioaccessibility of Ca, Mg, Mn and Cu.

When assessing the impact of added fibers (in samples 7—10), the comparisons had to be made with the reference sample (sample 2) due to the same nutritive composition of compared samples (except for the amount of added pure dietary fibers).

Positive effect of inulin on Ca solubility that was already mentioned in the introduction was also confirmed in our investigation. Namely addition of inulin significantly increased Ca bioaccessibility from 44.0% to 53.9%. However, bioavailability of other minerals was not influenced by addition of inulin (it was slightly increased compared to sample 2 but those differences were statistically insignificant). Addition of wheat and oat fibers did not affect Ca solubility while it was decreased by addition of apple fibers, probably due to high content of associated polyphenols. Our results are consistent with the data published by Claye, Idouraine, and Weber (1998)), where apple fiber were found to have higher affinity for Ca compared to oat fiber. In the case of Mg all used dietary fibers showed similar, insignificant effect on its accessibility. Addition of inulin, wheat- or oat fiber resulted in changing Mg bioaccessibility from 72 to 75.5, 71.7 and 72.2%, respectively. Again, Mg accessibility was mostly affected by addition of apple fibers (66.7%). Obtained data are consistent with the work of Claye et al. (1998), where in vitro mineral binding capacity of five fiber sources for Mg was investigated. Their results also indicate apple fiber showed higher binding capacity compared to wheat fiber. Similar findings were also found in the work of Idouraine, Hassani, Claye, and Weber (1995) where binding capacity of various fiber sources for magnesium, zinc and copper was investigated.

In the case of Mn, bioaccessibility of the mineral was also mostly impaired by addition of apple fiber, while addition of oat- or wheat fiber didn't significantly affect its accessibility.

In the case of Cu, it can be concluded that addition of either one of used fibers showed negligible effect on its bioaccessibility, which is consistent with literature data stating that the most important factors that inhibit copper absorption are sugars (O'Dell, 1993), animal proteins, S-amino acids (Brown & Strain, 1990) and histidine (Harvey, Hunsaker and Allen, 1981) rather than dietary fibers.

4. Conclusion

This investigation showed that enrichment of wheat flour based biscuit (reference sample) with natural raw materials or/and dietary fibers can significantly improve its mineral composition. We have found that 100 g of enriched biscuit can cover up to 8.8% of Recommended Dietary Intake (RDI) for Ca, 29.1% of RDI for Mg, 26.1% of RDI for Cu and as much as 55.8% of RDI for Mn. Additionally, most of suggested modifications of reference biscuit's recepture resulted in significant improvement of biscuit's protein content and increased amounts of phytic acid and polyphenols – the bearers of biscuit's antioxidative capacity. This investigation indicates two possible ways of improving biscuits as sources of essential minerals in daily nutrition – increasing the total mineral content of the biscuit (by using whole grain raw materials in their production) which also results in increased amounts of bioaccessible mineral fraction, or by incorporating the enhancers of mineral solubility (such as inulin) to biscuit's recepture. Obtained results also indicate that phytic acid, and especially polyphenols, limit the solubility of investigated minerals, which results in significant decrease of their bioaccessibility, except in the case of Cu, while the effect of proteins on mineral bioaccessibility is negligible in the case of all investigated minerals.

Taking into account all obtained data (the amounts of total and bioaccessible minerals, improvement of protein content of the biscuit, the content of health protecting compounds – phytic acid and polyphenols) it could be recommended that for enrichment of standard receptures in these types of products it is better to use whole grain flours of different origin possibly in combination with inulin, rather than purified dietary fibers.

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