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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.

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### 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

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)

<sup>a</sup> 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<sub>3</sub> (v/v) (5 mL) and 30% H<sub>2</sub>O<sub>2</sub> (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

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