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Impact of modifying tea–biscuit composition on phytate levels and iron content and availability

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Abstract

The effect of modifying the standard recipe of wheat flour based tea–biscuit on phytate levels, iron content and in vitro availability was investigated. Standard recipe was enriched by addition of dietary fibers and integral raw materials. The average phytic acid content of investigated biscuits ranged from 0.138 to 1.084 g/100 g dry matter of biscuit, depending on biscuit composition. Phytic acid levels were also determined in dough in order to determine the influence of technological process on phytic acid content. Iron content of investigated samples ranged from 0.655 to 4.222 mg/100 g biscuit, and iron availability varied from 26% up to 56%. Data analysis showed that changes in sample composition resulted in significant changes in phytic acid, total and available iron content related to standard sample.

Keywords: Phytic acid; Total iron; Available iron; Biscuit

1. Introduction

Since the importance of cereals in human nutrition has been recognized in past few decades, the production of all kinds of whole grain cereal based products has been growing constantly ever since. Those products include the new generation of enriched biscuits which are, therefore, no longer considered just as high energy foods but also as sources of numerous nutritive and health protecting substances. New processing methods are being developed, and new unconventional raw materials (legumes and pseudocereals) are being introduced to standard recipes in order to achieve high nutritive quality and functionality of the final product. Cereal based foods are very good basis for development of functional foods due to high content of fibers and other associated components (phytic acid, polyphenols, etc.) known for their protective and prebiotic properties.

Unfortunately, these components also represent the main limitation in biological utilization of cereals and cereal based products and are often being defined as antinutritive factors decreasing bioavailability of starches, proteins and especially minerals. On the other hand, some substances present in such foods act as absorption promotors, counteracting the impact of antinutritives, and therefore, balancing their content through the modifications of recipes, better bioavailability of minerals could perhaps be achieved.

The aim of this study was to examine the possibilities of creating a healthy nutritive biscuit that could also be regarded as a valuable source of non-heme iron in diet by supplementing the standard tea–biscuit recipe with isolated dietary fibers and some integral raw materials. Iron was selected as target mineral of our study because iron-deficiency anemia is considered to be the most common nutritional deficiency affecting approximately 30% of the world population (Swanson, 2003). Iron-fortification of food has been proposed as a strategy to reduce the high prevalence of iron-deficiency. Poor consumer acceptance, unacceptable taste, and discoloration of the iron-fortified foods have been frequently listed as causes of unsuccessful iron-fortification programs (Bowell-Benjamin & Guinard, 2003). In order to avoid such problems we tried to increase

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the content of native iron and examine its bioavailability in the final product.

Different in vivo and in vitro approaches have been used for assessment of iron bioavailability resulting in large variability of obtained results. In vivo investigations usually include rat bioassays or human clinical studies, that serve as the reference point against which other methods for assessing Fe bioavailability are compared (Forbes et al., 1989). For assessment of Fe bioavailability by an in vitro method soluble and/or dialysible Fe fraction is usually determined. Comparison between dialysis and solubility methods showed significant differences in obtained results with considerable higher values obtained for soluble iron content (Camara, Amaro, Barbera, & Clemente, 2005). Recently, cell culture has become an extensively used in vitro method for assessing human iron bioavailability and investigations show consistency of results with human studies (Au & Reddy, 2000).

2. Materials and methods

2.1. Preparation of biscuits

For assessment of total and soluble iron content three series of 10 different types of biscuits were prepared in order to obtain as reliable data as possible. Standard tea–biscuit was prepared using the mixture of whole grain and white wheat flour while experimental samples were enriched with either one of four used types of fibers (wheat fibre (type WF 600-300), oat fiber (type HF 600), apple fiber (type AF 400-30) and inulin (Vitacel, Germany)) or by one of four different sorts of raw materials (whole grain wheat flour, full fat soy flour, amaranth and carob). In order to estimate the effect of complete elimination of bran on iron content and bioavailability of the final product, sample based on T-500 wheat flour only was prepared as well.

Doughs were rolled to 0.7 cm, formed and baked at 180 °C for 15 min. Biscuit samples were then ground to the particle size <1 mm and stored in dry containers at +4 °C for further analysis. Dough samples, to be used for phytic acid content determination, were freezed and stored at −10 °C. Since literature data indicate that significant changes of iron content and availability can occur in this type of foodstuff after more than 30 days of storage (Jood, Yadav, Gupta, & Khetarpaul, 2001), all investigations were conducted in each series of biscuits within that period. That is why the next series of biscuits was prepared only after all analysis of the previous one were finished.

Besides increasing the total iron content, the addition of fibers to standard recipe was supposed to improve functional properties of the biscuit, that is their prebiotic potential. Sufficient fiber intake is also very important for maintaining numerous physiological functions and it could be regarded as protective in relation to different disorders such as coloretical cancer, obesity, coronary disease, etc. (Hill, 1998; Jenkins, Marchie, Augustin, Ros, & Kendall, 2004; Li, Wang, Kaneko, Qin, & Sato, 2004).

Good prebiotic properties are usually attributed to inulin, plant derived carbohydrate with benefits of soluble dietary fiber. Inulin is composed of β(1–2) fructans with a chain length between 2 and 60, and it is present in many plants; asparagus, onion, artichoke and chicory roots (Lopez et al., 2000). Acting as prebiotic, inulin enhances gastrointestinal and immune system, by its stimulation of bifidobacterial growth in the intestine (Lopez-Molina et al., 2005).

Apple fibers are characterized by a well balanced proportion between soluble and insoluble fraction (Gorinstein et al., 2001), and they also show better quality than other dietary fibres due to the presence of associated bioactive compounds (Fernandez-Gines, Fernandez-Lopez, Sayas-Barbera, & Perez-Alvarez, 2003). They are also a very good source of pectin, proven to show antiinflammatory effect in the bowel and inhibiting the incidence of hepatic metastasis (Tazawa et al., 1999).

Dietary fibers from cereals are more frequently used than those from fruits although they show lower soluble fiber content, lower water and oil holding capacity and colonic fermentability (Figuerola, Hurtado, Estevez, Chiffelle, & Asenjo, 2005). The main reason for their use in our investigation was the high iron content of wheat and oat fibers obtained during our preliminary investigations (Table 3). Literature data show that wheat fiber can be considered as a very good laxative, placing wheat fiber among the most effective fibers for increasing fecal bulk (Vuksan et al., 1999). Preventing the constipation, they act protective considering the development of coloretical cancer.

Besides due to high iron content, oat fibers were chosen for biscuit enrichment due to a considerable content of β-glucan, proven to be an active hypolipidemic component. Clinical studies confirmed cholesterol-lowering effects of moderate amounts of oat fibers incorporated to diet. A significant dose response due to β-glucan concentration in the oat extract was observed in total cholesterol levels (Behall, Scholfield, & Hallfrisch, 1997).

In order to assess binding properties of used fibers and to determine their influence on iron content and in vitro availability in relation to their source and chemical properties and thereby avoiding the impact of phytate, phytic acid – free fibers were used for fortification of investigated samples.

Whole grain raw materials used for iron-fortification of standard tea–biscuit recipe were chosen based on their nutritional qualities, accessibility and flavour in order to improve its nutritive value in general, increasing at the same time total iron content of the biscuit. Wheat is known for low concentrations of some essential aminoacids, so addition of soy flour characterized by high quality proteins was the best way for improving protein quality and aminoacid content of the biscuit. In addition to its nutritive value soy protein also shows antiobese, sugar-lowering and cholesterol-lowering effect (Takamatsu, Tachibana, Matsumoto, & Abe, 2004). Amaranth was chosen due to its unique protein composition with regard to quality and quantity. Protein content of amaranth ranges from 12% to 18%, depending on sort, climate and agrotechnical conditions and proteins are characterized by higher lysine content compared to wheat. Ratio 30:70 of amaranth and wheat flour was chosen for preparation of amaranth supplemented experimental biscuit since it has been reported that with this ratio optimal protein quality is achieved (protein quality rises from 32 to 52 – based on casein) (Bressani, 1989). Carob is sold in the USA and other Western countries in the health food stores as a substitute for cocoa. It is mainly characterized by high sugar, low fat and high tannin and crude fiber content (Yousif & Alghzawi, 2000) but the complete absence of caffeine and theobromine (Craig & Nguyen, 1984), so in general, it could be considered as a natural sweetener and a natural healthy food.

Differences in composition of 10 investigated experimental biscuits are listed in Table 1.

2.2. Estimation of total and in vitro available iron

In order to determine total iron levels of investigated biscuits, homogenized samples were wet ashed using HNO_3 and H_2O_2 in Microwave digestion unit MLD 2000, and diluted afterwards with deionized water. Microwave digestion procedure has been selected for sample preparation since it is free from contamination risk and is not time consuming. Small amounts of sample were needed for our investigations (500 mg) so excessive pressure during the digestion, the main restriction of microwave digestion (Doner & Ege, 2004), was avoided.

Iron content was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at 238.204 nm. All measurements were carried out in duplicates.

Availability of iron in investigated experimental tea–biscuits was determined according to enzymatic method of Schwedt, Tawali, and Koch (1998). To simulate gastric digestion, samples were treated with pepsin (Merck – EC 3.4.23.1) in 0.02 M HCl/NaCl mixture (pH 2) at 37 °C for 3 h. After adjusting pH to 7.5 with conc. $NaHCO_3$ and adding bile salt (Sigma B8756), pankreatin (Sigma P1750) and amylase (Fluka 10070), simulation of intestinal digestion was continued for another 2 h. Samples were vacuum filtered and residues were wet ashed using HNO_3 and H_2O_2. Insoluble iron content was determined by ICP-AES. Experiments were conducted in triplicates.

2.3. Determination of phytic acid

Phytic acid was determined spectrophotometrically by the Haug and Lantzch method (Haug & Lantzsch, 1983). Ground samples were extracted with 0.2 M HCl for 90 min in shaking water bath at 25 °C. After extraction the solutions were centrifuged at 3000 rpm for 10 min and clear supernatants were used for phytic acid determination. Four milliliters of ferric solution (0.02% $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 0.2 M HCl) was added to 2 ml of supernatant (containing 3–30 μg/ml phytate phosphorus) and the solutions were mixed and heated in boiling water bath for 30 min. After cooling, 8 ml of 2.2′ biprydyl solution (10 g of 2.2′ biprydyl (Fluka EC No. 2066744) dissolved in 10 ml of thioglycollic acid in distilled water and made up to 1000 ml) was added to the solution, mixed, and absorption was measured at 519 nm. Calibration curve was made under the same conditions using sodium salt of phytic acid (Sigma P-8810). The measurements were performed in duplicates.

2.4. Statistical analysis

Since the study design contained three series of biscuits, one-way ANOVA was used to find out wether there were any significant differences between them. Data obtained for each sample within one series were subjected to statistical analytical process using mean ± standard deviation of three determinations. Statistica Version 6 was used for conducting the above mentioned analysis.

Table 1
Differences in composition of 10 investigated experimental biscuits

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

3. Results and discussion

3.1. Total and available iron levels in investigated biscuits

In order to evaluate experimental biscuits as a potential sources of non-heme iron in nutrition, total and available iron levels were determined. Means of total and available iron content of three investigated series of biscuits are presented in Fig. 1. It is obvious that all modifications of standard recepture resulted in increased total iron levels except in the T-500 wheat flour based biscuit (sample 1) that contained the lowest total iron levels among the investigated biscuits. The most significant iron enrichment of the standard sample (sample 2) was obtained by use of amaranth (sample 6), wheat fiber (sample 8) and oat fiber (sample 9).

Content of bioavailable iron varied significantly among investigated biscuits, most of them being decreased compared to standard sample. Iron availability of integral biscuits (samples 2–9), estimated based on soluble iron content, ranged from 26% (sample 10) to 56% (sample 4) with average availability around 39% what is consistent with data obtained by Jood et al. (2001), who investigated similar type of whole wheat flour based biscuit and found that 35.1% of total iron in the biscuit was soluble at pH 7.5.

Increase of bioavailability of iron in relation to standard sample (sample 2) was only achieved by excluding bran from standard sample (sample 1) and addition of soy flour (sample 4) and inulin (sample 7) to standard recepture.

3.2. Phytic acid content of investigated biscuits

Phytic acid levels in investigated biscuits ranged from 0.138 g/100 g dry matter in white wheat flour based biscuit (sample 1) to 1.084 g/100 g dry matter in the sample prepared by addition of amaranth (sample 6) (Table 2). Levels of phytic acid in examined biscuits were consistent with the data obtained by investigations of raw materials used for biscuit preparation (Table 3). Among added raw materials, carob was the only one that had slightly decreased phytic acid content related to standard sample what was expected since phytic acid is found in highest amount as a part of

Table 2
Phytic acid content in investigated biscuits

Sample number	Phytic acid (g/100 g dry matter)[a]		
	Series 1	Series 2	Series 3
1	0.138 ± 0.005	0.141 ± 0.001	0.161 ± 0.007
2	0.560 ± 0.043	0.640 ± 0.014	0.574 ± 0.014
3	0.767 ± 0.010	0.764 ± 0.013	0.784 ± 0.001
4	0.759 ± 0.019	0.800 ± 0.001	0.778 ± 0.023
5	0.531 ± 0.004	0.564 ± 0.013	0.531 ± 0.010
6	1.069 ± 0.001	0.925 ± 0.010	1.084 ± 0.004
7	0.576 ± 0.001	0.550 ± 0.014	0.478 ± 0.029
8	0.549 ± 0.014	0.561 ± 0.012	0.579 ± 0.027
9	0.533 ± 0.025	0.582 ± 0.008	0.535 ± 0.006
10	0.523 ± 0.003	0.538 ± 0.001	0.536 ± 0.014

[a] Mean ± SD.

Table 3
Total iron and phytic acid content of raw materials and fibers used for fortification of standard biscuit

Sample	Fe (mg/100 g)[a]	Phytic acid (g/100 g)[a]
T 500 wheat flour	1.30 ± 0.10	0.29 ± 0.08
T 1700 wheat flour	4.25 ± 0.15	0.71 ± 0.06
Full fat soy flour	6.81 ± 0.09	1.04 ± 0.11
Amaranth	9.85 ± 0.20	2.25 ± 0.25
Carob	1.73 ± 0.11	0.05 ± 0.01
Wheat fiber	21.81 ± 0.33	ND[b]
Oat fiber	15.25 ± 0.16	ND
Apple fiber	6.11 ± 0.20	ND
Inulin	ND	ND

[a] Means ± SD of triplicate analysis.
[b] Content was below the limit of detection of applied method.

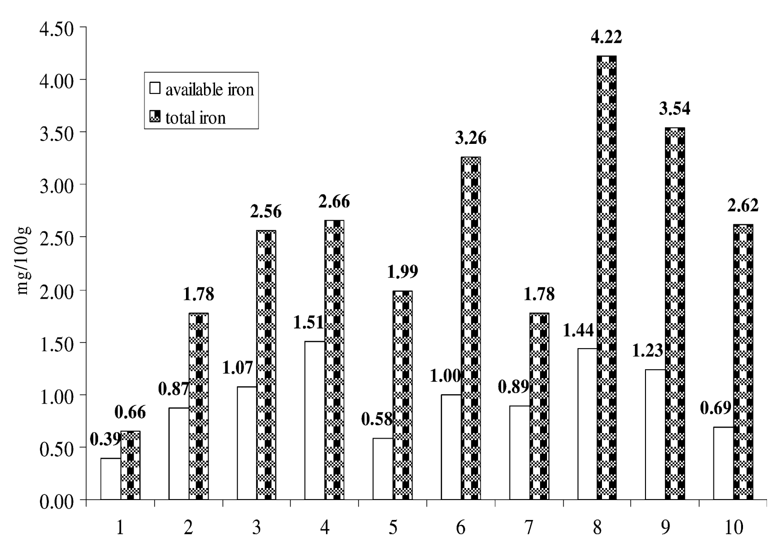

Fig. 1. Means of total and available iron content of investigated biscuits.

plant seed were it can represent from 1.0 to several percent of typical seeds dry weight, while its content in fruits, as carob, is much lower, almost negligible. The significant difference in determined phytate levels (73.7%) between standard sample (sample 2) based on the mixture of white and integral wheat flour and sample based on white wheat flour (sample 1) can be explained by the fact that in the small grains (barley, wheat, rice) it has been estimated that more than 80% of the whole grain's phytic acid is sequestered in the alurone layer. Addition of pure fibers had no significant effect (sample 8) or slightly decreased phytic acid concentration (samples 7, 9, 10), related to standard sample, since phytic acid free fibers were used in the investigation. Enrichment of standard sample with full fat soy flour and whole grain wheat flour also significantly increased phytic acid concentrations from 0.567 g/100 g in standard sample to 0.768 and 0.779 g/100 g, respectively. Statistical analysis showed that there were no significant differences in phytic acid concentrations between different series of investigated biscuit.

By determining phytic acid content in dough that was deep-frozen immediately after the preparation, we wanted to assess the impact of baking procedure on phytic acid degradation. Mean values obtained after analysing three series of biscuits showed a little higher phytic acid concentrations in dough compared to baked cookies with phytate degradation ranging from 1.05% (sample 8) to 11.35% (sample 5). Performing statistical analysis showed that those differences were not statistically significant ($p > 0.01$) so we concluded that baking had no considerable influence on phytic acid degradation. The results are consistent with literature data stating that decrease of phytate levels in whole grain based sample is possible through different technological procedures during sample preparation (soaking, germination or fermentation) (Haraldsson et al., 2005) or through activation of intestinal phytase, while very little phytate is hydrolized by baking (McKenzie-Parnell & Davies, 1986).

In order to evaluate the signification of phytic acid impact on decrease of mineral availability we established the correlation between phytic acid content and iron availability in investigated samples. Correlating the concentrations of phytic acid to the iron availability in investigated samples (Fig. 2) we obtained negative correlation coefficient ($r = -0.7576$) and concluded that in our samples too phytic acid negatively influences iron availability but that due to implementation of different whole raw materials to the basic recepture promotor/antinutrient ratio changes compared to pure wheat based product, and therefore, phytic acid can not be considered as the only factor for predicting iron availability.

3.3. Effect of standard recipe fortification on iron content and availability

Influence of introducing different integral raw materials to standard recipe on iron content and availability is presented in Fig. 3a. It is obvious that addition of all raw materials had positive effect on total iron content and that the most significant increase was achieved by addition of amaranth (3.26 mg Fe/100 g of biscuit) Addition of soy and whole wheat flour showed similar effect (2.66 and 2.56 mg Fe/100 g of biscuit, respectively) and the least significant increase was acquired by addition of carob (1.99 mg Fe/100 g of biscuit) Those results were consistent with our expectations based on total iron concentrations determined in used raw materials (Table 3).

As far as impact on iron availability is concerned, only addition of soy flour increased the percentage of available iron related to standard sample. Addition of other raw materials decreased bioavailability of iron and especially addition of carob and amaranth (40.4% and 36.8%, respectively). Influences of addition of whole wheat flour and amaranth were expected considering their phytic acid levels and the fact that phytic acid is often stated to have the main negative impact on availability of iron and availability of other minerals in general (Haug & Lantzsch, 1983). Results obtained for samples with carob added did not correlate with measured phytic acid concentrations, as mentioned

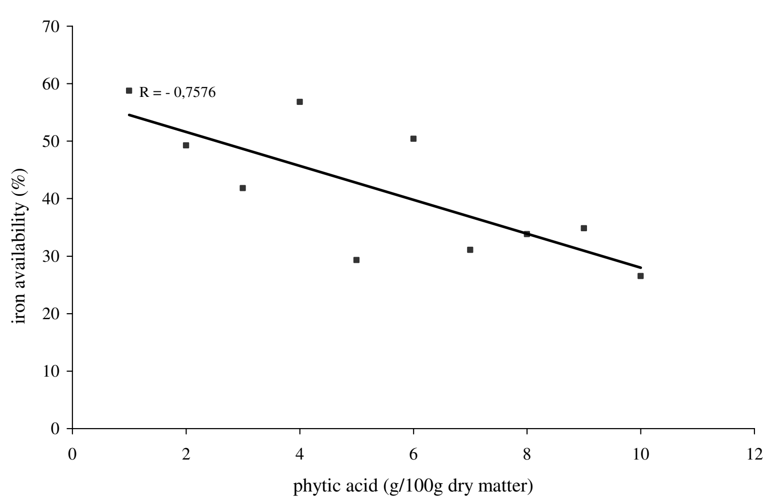

Fig. 2. Phytic acid–iron availability correlation.

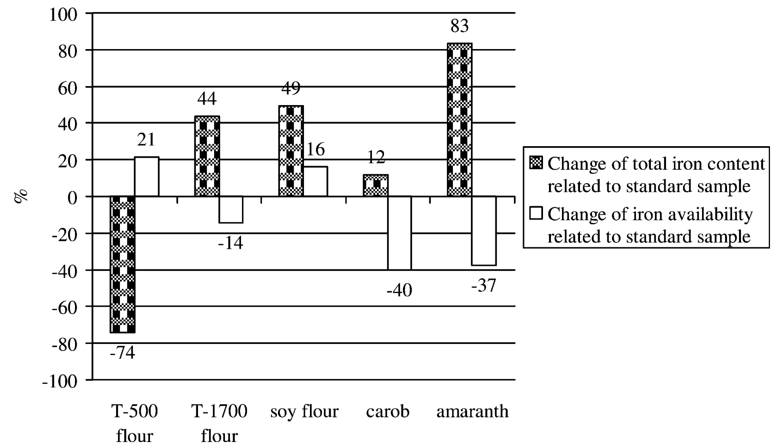

Fig. 3a. Influence of fortification of standard sample with different integral raw materials on iron content and availability (all data were obtained based on investigations of three series of biscuits).

before, so it was concluded that there were some other natural chelating compounds present in carob that also diminish iron solubility. Since it has been stated in literature that phenolic compounds (phenolic acids, flavonoids, tannins) can show such effects (Cook, Reddy, & Hurrel, 1995; Garcia Lopez, Erdman, & Sherman, 1990) and that carob flour contains significantly higher total phenol content compared to other investigated raw materials (Yousif & Alghzawi, 2000) we concluded that phenolic compounds are the main limiting factors considering iron availability in carob flour. Positive effect of soy flour on iron availability was also surprising, considering its high phytate content and negative impact of 7 S protein fraction of soybean (conglydine) that also depresses iron absorption (Lynch, Dassenko, Cook, Juillerat, & Hurrel, 1994). This inconsistency could perhaps be explained by significantly higher cystein and hystidine levels in soy flour compared to other used raw materials, since those aminoacids have often been reported as important enhancers of iron availability (Cummings, Edmond, & Magee, 2004).

By addition of wheat, oat and apple fiber to standard sample similar effects were obtained – increased total iron levels and decreased iron bioavailability (Fig. 3b). Addition of wheat fiber had the most significant influence on increasing total iron levels (4.22 mg/100 g of biscuit) what was consistent with our investigations of fibers used for biscuit preparation (Table 3) showing that fiber extracted from wheat bran contained the greatest amount of endogenous iron related to other used fiber sources. According to these results, wheat fiber could be considered as useful raw material for iron-fortification of foodstuff. Apple fibers were found to be the poorest source of additional iron among these three types of fibers (2.62 mg Fe/100 g of biscuit), at the same time showing the most adverse effect related to bioavailability of iron. In vitro studies showed that the ability of fiber to impair mineral availability depends on the type of fiber investigated and amount of fiber used in the diet, and that the results also vary because of the differences in the experimental conditions and length of study (Iduraine, Khan, & Weber, 1996). Mechanisms of those effects

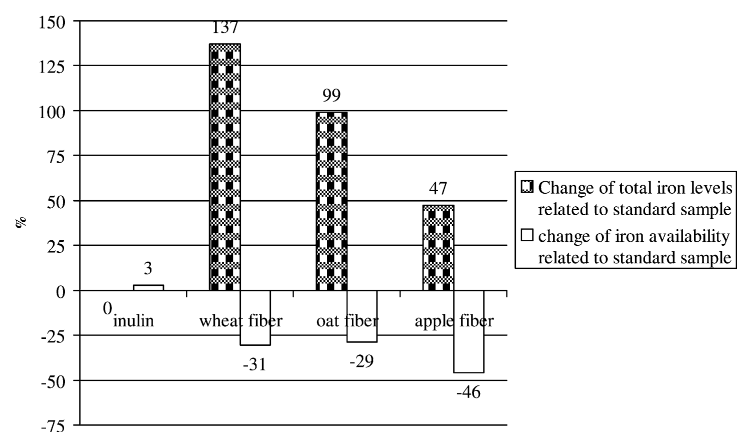

Fig. 3b. Influence of fortification of standard sample with different fibers on iron content and availability (all data were obtained based on investigations of three series of biscuits).

have not yet been fully understood but the main way of fibers affecting mineral bioavailability are by binding, diluting and trapping minerals within dietary fiber particles (Idouraine, Hassani, Claye, & Weber, 1995). Therefore, the differences that fibers showed in decreasing iron availability could be explained by their different binding properties partially due to different amount of associated polyphenols. Namely, polyphenols are considered to be fiber constituents (Bravo, Abia, Goni, & Saura – Calixto, 1995) that show different adverse effects relating iron availability through different mechanisms (catecholic complexation, polymerization reactions) (Watzke, 1998) so different impacts on iron availability could be explained by different polyphenol content.

Addition of inulin showed no significant effects regarding iron content (0.90 mg Fe/100 g of biscuit) and bioavailability indicating differences regarding chemical properties related to other three types of fibers used. It has been reported that inulin enhances mineral/iron availability counteracting the effects of different absorption inhibitors (Gerger, 1999; Lopez et al., 2000) through different mechanisms. The improved absorption of minerals in the presence of inulin is the result of decreased pH of ileal, cecal and colonic contents, hypertrophy of cecal walls and increased concentrations of volatile fatty acids and bile acids in cecal content (Gerger, 1999). However, those effects are only expressed in vivo, so they could not have been noted in our investigation. Based on obtained data we concluded that inulin does not contain any significant levels of endogenous iron nor it shows iron binding capacity due to its specific chemical composition. Because of those characteristics it can not be used as the source of additional iron in the nutrition but since it shows the potential for increasing mineral availability and because of its numerous health promoting properties it can be considered as very desirable supplement for the whole grain cereal based products.

4. Conclusions

Based on available iron content, biscuits enriched with soy flour, wheat fiber and oat fiber were found to be the most valuable sources of non-heme iron among investigated samples. Recommended dietary allowances for iron are 8 mg/dne for men and 18 mg/dne for women (DRI, 2001) meaning that 100 g of supplemented biscuits can cover approximately 10–24% of recommended dietary allowance (RDA) of iron for women and 22–53% of RDA for men so that investigated integral biscuits can be regarded as an important additional sources of non-heme iron in nutrition.

Finally, we can conclude that it is possible to create integral biscuit with both improved nutritive characteristics and satisfactory iron content and availability only through careful combining of used raw materials. Since procedures like phytate degradation, dehusking, or addition of iron salts are avoided, complete nutritive and health protecting potential of used raw materials is preserved in the final product.

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