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EVALUATION OF THE CONTENT AND THE POTENTIAL BIOAVAILABILITY OF IRON FROM FORTIFIED WITH IRON AND NON-FORTIFIED FOOD PRODUCTS

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Background. Fortified food products contain usually higher amounts of certain nutrients. However, the information about the nutritional quality of such products is limited. The objective of this study was to determine the content and the release of iron from fortified and non-fortified food products available on the Polish market.
Material and methods. A group of 29 fortified with Fe and non-fortified food products, such as cereal products (16) and confectionaries (13), were purchased from local market between October and November 2009. The content of Fe in these products, as well as the amount of Fe released in enzymatic digestion *in vitro* was determined by the flame atomic absorption spectrometry method.
Results. It was found that most of the fortified with Fe food products had significantly higher amount and the potential bioavailability of this element in comparison with the non-fortified analogues, however the content of Fe determined analytically not always matched the values declared on the label.
Conclusions. Products fortified with Fe appear to be better sources of potentially bioavailable Fe in comparison with the non-fortified analogues.

Key words: content, release, iron, fortified products, non-fortified products

INTRODUCTION

Dietary minerals are essentials components of human diet due to their role in metabolism. They should be provided in adequate quantities as our body is not able to synthesize them. Both mineral excess and deficiency bring about serious health problems, including diseases connected with a specific role of an element and its biological function. Generally, balanced nutrition ensures adequate intake of macro- and micro-

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elements, however, results of nutritional studies conducted in various European countries and world-wide showed that some minerals intakes (Ca, Mg, Fe, Zn, Se and I) are often inadequate and do not meet the recommendations in some populations [Frontela et al. 2009, Hemalatha et al. 2009].

The most common nutritional disorder affecting at least one third of world's population is iron deficiency. The populations at risk of iron deficiency are considered adult women, especially during pregnancy, individuals on restricted diets (eg. vegans), children, adolescents and elderly [Best et al. 2010, Perez-Llamas et al. 2010]. Although there is no clear relationship between dietary iron intake and iron status, isotope studies have identified multiple dietary factors that influence iron absorption, such as ascorbic acid, animal tissue, phytates and polyphenols [Heath et al. 2002]. The consequence of inadequate iron uptake, due to its low intake or poor bioavailability is iron-deficiency anaemia [Ford et al. 2010]. Maternal iron deficiency anaemia (IDA) has significant negative effects on the foetal outcome, such as poor intrauterine growth and increased risk of preterm births and low birth weight rates [Akhter et al. 2010]. For this reason, early detection and effective management of anaemia in pregnancy is very important both for maternal and foetal health, and prevention of undernutrition in childhood, and further life [Kalaivani 2009].

One of the strategies applied to combat dietary mineral inadequacies is food fortification that is considered as a relatively low cost, high efficient, and safer than diet supplementation approach in prevention of nutritional deficiencies [Fairweather-Tait et al. 2002, Grajeta 2006].

Fortified food products usually contain higher amounts of certain nutrients. They are widely advertised and consumed in an increasing manner worldwide, which makes consumers to think that they are of better quality than the non-fortified ones. However, the information about the nutritional quality, the composition and bioavailability of various forms of minerals from fortified and non-fortified food products is limited. In our previous works the effect of technological processing (fermentation and extrusion) on the potential bioavailability of minerals (Fe, Zn and Cu) *in vitro* from lupine grain preparations was described [Suliburska et al. 2009, Krejpcio et al. 2009].

The objective of this study was to determine the content and the potential bioavailability of iron (based on the amount released during the enzymatic digestion *in vitro*) from selected fortified with Fe and non-fortified food products available on the Polish market.

MATERIAL AND METHODS

The materials studied were 29 selected fortified with Fe and non-fortified food products: cereal based foods (16 items) and confectionary products (13 items; Table 1).

All the food products were purchased from the local market (the city of Poznan, October-December 2009). Depending on the package volume, its content was mixed and homogenized in an electric grinder. Finely ground food product was transferred to plastic bags and stored frozen (–20°C) until analyzed. Enzymatic digestion *in vitro* was performed according to the method developed by Skibniewska et al. [2002].

Samples (approx. 2 g) of finely ground food product were weighed in conical beakers and treated with deionised water (20 ml) and shaked for 10 min. In order to create

Table 1. Characteristics of the analysed food products

Products fortified with Fe	Fortificant	Declared mineral content mg/100 g	Products non-fortified with Fe
Cereal products			
Corn flakes	Fe-reduced	Fe: 3.6	Corn flakes
Honey flakes *Honey Chrups*	$CaCO_3$ Fe-reduced	Ca: 120 Fe: 2.1	Honey flakes *Kółeczka miodowe*
Flakes *Shelly Chrups*	$CaCO_3$ Fe-reduced	Ca: 120 Fe: 5.1	Chocolate flakes *Muszelki czekoladowe*
Balls *Nesquik*	$CaCO_3$ Fe-reduced	Ca: 230 Fe: 3.6	Chocolate flakes *Kuleczki czekoladowe*
Muesli *Fitness Fruits*	$CaCO_3$ Fe-reduced	Ca: 360 Fe: 8.4	Muesli spring dream
Flakes *Cookie Crisp*	$CaCO_3$ Fe-reduced	Ca: 230 Fe: 3.6	Multi grain muesli
Flakes *Dotty Chrups*	$CaCO_3$ Fe-reduced	Ca: 120 Fe: 3.5	Oat flakes
			Oat bran
			Bran with currant flavour
Confectionary products			
Cookies *Miśkopty*	$CaCO_3$ Fe-reduced	Ca: 320 Fe: 6	Cookies *Hippolitki*
Cookies *Go! musli z owocami*	Fe-reduced	Fe: 2.9	Cookies *Digestive*
Cookies *Go! Kakao*	Fe-reduced	Fe: 3.5	Cookies *Krakuski zbożowe*
Cookies *Go! 4 zboża + mleko*	Fe-reduced	Fe: 2.9	Cookies *Złotokłose*
			Biscuits *Owsiaki*
			Apple muesli bar
			Strawberry bar *Minute truskawka*
			Apple and cinnamon bar *Minute jabłko + cynamon*
			Bar *Cini minis*

suitable conditions for pepsin action, pH was brought to 2 using 0.1M HCl aqueous solution (Suprapure, Merck), than pepsin solution (0.5 ml/100 ml) was added to the homogenate. Subsequently samples were placed in a thermostat shaker (37°C) for 2 hours. During the incubation process, pH was assured or corrected by addition of 6M HCl aqueous solution, when necessary. After 2 hours, digested samples were treated with 6% $NaHCO_3$ aqueous solution (Extrapure, Merck) to bring pH to 6.8-7.0, and subjected to pancreatin solution (10 ml/40 ml of homogenate), and placed in a thermostatic shaker (37°C) for 4 hours.

Afterwards, digested samples were centrifuged for 10 min (3.800 r/min), and clear solution was quantitatively transferred to qwartz cruicibles, and treated with a mixture of concentrated nitric (65% w/w) and perchloric (70% w/w) acids (10 ml:5 ml; Suprapure, Merck). Crucibles with samples were placed in a thermostatic block and heated until complete mineralization.

In order to determine the total content of Fe in native products, food samples (2 g) were ashed in a muffle furnace at 450°C until complete mineralization.

The content of Fe in digested and native food products was determined by the flame atomic absorption spectrometry method (AAS-3, Zeiss spectrometer). The accuracy of the method was assured by simultaneous determination of Fe in the reference material (*Virginia Tabacco Leaves, CTA-VTL-2*), which reached 102% of the average target value.

Based on the obtained results, the degree of Fe release (total bioavailability of Fe), expressed as the amount of Fe (mg) liberated during the enzymatic digestion *in vitro* from 100 g of product (TBV), as well as relative potential bioavailability of Fe (RBV) (expressed as percentage of Fe released vs. total content of Fe x 100%) was calculated.

The statistical analysis was carried out employing the STATISTICA 7.0 software and using the Student's t-test at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The content of Fe in fortified with Fe and non-fortified food products

The content of Fe in fortified and non-fortified food products are presented in Table 2 and 3. As can be seen from Table 2, four out of eleven fortified products had lower content of Fe, while six had higher content of Fe than declared on their label. The greatest difference between the determined and declared Fe content (2-fold) was found in muesli *Fitness Fruits*. Discrepancies between declared and analytically determined content of Fe in fortified with Fe food products were also reported by other authors [Jantarska et al. 2007, Ratkovska et al. 2007].

Table 2. The content of Fe in fortified food products ($\bar{x}$ ±SD)

Product	The content of Fe, mg/100 g	
	determined	declared
1	2	2
Cereal products		
Corn flakes	1.51 ±0.03	3.6
Honey flakes *Honey Chrups*	1.73 ±0.02	2.1
Flakes *Shelly Chrups*	5.79 ±0.09	5.1
Balls *Nesquik*	7.29 ±0.08	3.6
Muesli *Fitness Fruits*	4.27 ±0.54	8.4
Flakes *Cookie Crisp*	5.45 ±0.03	3.6
Flakes *Dotty Chrups*	2.68 ±0.02	3.5

Table 2 – cont.

1	2	2
Confectionary products		
Cookies *Miśkopty*	10.68 ±1.4	6
Cookies *Go! musli z owocami*	4.81 ±0.01	2.9
Cookies *Go! Kakao*	4.22 ±0.3	3.5
Cookies *Go! 4 zboża + mleko*	5.22 ±0.1	2.9

Table 3. The content of Fe in non-fortified with Fe food products ($\overline{x}$ ±SD)

Product	The content of Fe, mg/100 g
Cereal products	
Corn flakes	1.32 ±0.09
Honey flakes *Kółeczka miodowe*	2.34 ±0.11
Chocolate flakes *Muszelki czekoladowe*	4.84 ±0.21
Chocolate flakes *Kuleczki czekoladowe*	3.05 ±0.01
Muesli spring dream	2.83 ±0.07
Multi grain muesli	3.24 ±0.05
Oat flakes	3.48 ±0.12
Oat bran	3.30 ±0.15
Bran with black currant flavour	5.65 ±0.04
Confectionary products	
Cookies *Hippolitki*	0.72 ±0.01
Cookies *Digestive*	1.03 ±0.04
Cookies *Krakuski zbożowe*	1.79 ±0.03
Cookies *Złotokłose*	2.29 ±0.06
Biscuits *Owsiaki*	2.04 ±0.04
Apple muesli bar	1.78 ±0.05
Strawberry bar *Minute truskawka*	1.46 ±0.05
Apple and cinnamon bar *Minute jabłko + cynamon*	1.11 ±0.03
Bar *Cini minis*	6.92 ±0.06

The highest Fe content was measured in fortified cookies *Miśkopty* (10.68 ±1.40 mg Fe/100 g), while the lowest, as expected, in the non-fortified ones, cookies *Hippolitki* (0.72 ±0.01 mg/100 g). In the group of non-fortified foods relatively high content of Fe was determined in *bran with black currant flavour*, since cereal bran is usually rich in minerals. Similar values were reported by Paczkowska and Kunachowicz [2006] who found higher content of Fe in wheat bran in comparison with other cereal food products.

The content of Fe in *Corn flakes* analysed in this study was slightly higher from the value reported by Kosse et al. [2001]. The content of Fe in honey-flakes *Kółeczka miodowe* (2.34 ±0.11 mg/100 g) was lower than that reported by Frontela et al. [2009] (above 8 mg/100 g).

Differences in the cereal products Fe contents found in this study can be explained by variability of concentration of this element in cereals, dependent on the plant variety, fertilizers used, soil and climatic conditions, as well as technological processing applied during production. Many authors reported that technological processing can significantly affect mineral contents of final food products [Hemalatha et al. 2007, 2009, Paczkowska and Kunachowicz 2006, Sebastia et al. 2001, Skibniewska et al. 2002].

Bioavailability of Fe from fortified with Fe and non-fortified food products

The total bioavailability of Fe (TBV) expressed as the amount of this element released in mg Fe/100 g, as well the relative bioavailability of Fe (RBV), defined as percentage of the total content of Fe released during enzymatic digestion *in vitro* are presented in Tables 4 and 5. The highest RBV values was obtained for cookies *Go! musli z owocami* (49.97 ±0.02%) and cookies *Go! 4 zboża + mleko* (49.76 ±2.56%), while the lowest for flakes *Shelly Chrups* (14.11 ±1.35%) and honey flakes *Honey Chrups* (15.50 ±11.36; Table 4).

Significantly lower RBV values for Fe were reported by Garcia-Casal et al. [2003], however they applied different method and model *in vivo* (human subjects). In such case, Fe bioavailability also depends on various factors connected with individual Fe status and interactions with other nutrients and non-nutrients. Besides, these authors reported that Fe uptake in human subjects increased from 4% to 25% after addition of vitamin C. Concerning the non-fortified with Fe food products, the highest RBV of Fe

Table 4. Bioavailability of Fe from fortified food products ($\overline{x}$ ±SD)

Product	Total bioavailability of Fe, mg/100 g	Relative bioavailability of Fe, %
Cereal products		
Corn flakes	0.56 ±0.02	36.79 ±2.15
Honey flakes *Honey Chrups*	0.27 ±0.20	15.50 ±11.36
Flakes *Shelly Chrups*	0.82 ±0.07	14.11 ±1.35
Balls *Nesquik*	1.40 ±0.01	19.23 ±0.19
Muesli *Fitness Fruits*	1.76 ±0.04	41.43 ±4.31
Flakes *Cookie Crisp*	2.06 ±0.23	37.79 ±4.38
Flakes *Dotty Chrups*	0.64 ±0.15	23.99 ±5.79
Confectionary products		
Cookies *Miśkopty*	3.97 ±0.24	37.71 ±7.32
Cookies *Go! musli z owocami*	2.40 ±0.01	49.97 ±0.02
Cookies *Go! Kakao*	0.79 ±0.14	18.76 ±2.19
Cookies *Go! 4 zboża + mleko*	2.60 ±0.09	49.76 ±2.56

Table 5. Bioavailability of Fe from non-fortified with Fe food products ($\overline{x}$ ±SD)

Product	Total bioavailability of Fe mg/100 g	Relative bioavailability of Fe, %
Cereal products		
Corn flakes	0.38 ±0.03	28.78 ±4.37
Honey flakes *Kółeczka miodowe*	0.88 ±0.00	37.50 ±1.59
Chocolate flakes *Muszelki czekoladowe*	0.89 ±0.00	18.36 ±0.78
Chocolate flakes *Kuleczki czekoladowe*	0.28 ±0.12	9.04 ±4.03
Muesli spring dream	1.05 ±0.05	36.92 ±0.73
Multi grain muesli	0.80 ±0.05	24.72 ±1.19
Oat flakes	0.37 ±0.06	10.79 ±2.23
Oat bran	1.06 ±0.08	32.12 ±3.21
Bran with black currant flavour	1.29 ±0.00	22.86 ±0.17
Confectionary products		
Cookies *Hippolitki*	0.50 ±0.08	69.24 ±9.60
Cookies *Digestive*	0.76 ±0.10	74.11 ±11.86
Cookies *Krakuski zbożowe*	1.24 ±0.06	69.61 ±1.78
Cookies *Złotokłose*	0.83 ±0.27	36.62 ±2.83
Biscuits *Owsiaki*	0.74 ±0.07	36.36 ±4.39
Apple muesli bar	1.16 ±0.27	65.31 ±17.20
Strawberry bar *Minute truskawka*	0.19 ±0.05	12.76 ±2.86
Apple and cinnamon bar *Minute jabłko + cynamon*	0.34 ±0.10	30.31 ±9.67
Bar *Cini minis*	3.30 ±0.25	47.65 ±4.01

value was found for cookies *Digestive* (74.11 ±11.86%), while the lowest in chocolate balls *Kuleczki czekoladowe* (9.04 ±4.03%; Table 5). Relatively low RBV values were determined for *oat flakes* (11%) that can result from high content of fiber, phytate and other chelating compounds present in the cereal matrix. Similarly, decreased RBV of Fe values for foods rich in fiber were reported by Skibniewska et al. [2002, 2005]. On the other hand, fibre-rich food products usually contain higher concentration of minerals that, to some extent, can compensate for their lower bioavailability.

Concerning the TBV of Fe values, the highest values were determined for products fortified with Fe such as cookies *Miśkopty* (3.97 ±0.24 mg/100 g) and cookies *Go! 4 zboża + mleko* (2.60 ±0.09 mg/100 g), while the lowest for the non-fortified ones, such as strawberry bar *Minute truskawka* (0.19 ±0.05 mg/100 g).

Comparison of the content and bioavailability of Fe from fortified with Fe and non-fortified food products

Food fortification with minerals and other nutrients has become trendy over last years, and there are more and more of such products on international and national markets. Therefore it is necessary to control the efficacy of the fortification process, the quality of final products, as well as to address the question of bioavailability of a mineral from such enriched products. For this reason, in this study the content of Fe and its potential bioavailability (total and relative) for seven pairs of food products, fortified with Fe and non-fortified equivalents was determined (Tables 6 and 7).

Table 6. Food products compared in respect to the content and bioavailability of Fe

Product	Fortified with Fe	Non-fortified with Fe
Corn flakes	Corn flakes, Nestle	Corn flakes, Carrefour
Honey flakes	Honey Chrups, Otmuchów	*Kółeczka miodowe*, Lubella
Chocolate flakes	Shelly Chrups, Otmuchów	*Muszelki czekoladowe*, Lubella
Flakes *Chocolate balls*	Balls *Nesquik*, Nestle	*Kuleczki czekoladowe* Mr. Breakfast
Cereal muesli	Muesli *Fitness Fruits*, Nestle	*Muesli spring dream*, Lubella
Baby cookies	Cookies *Miśkopty*, Nestle	Cookies *Hippolitki* Hipp
Cereal cookies	Cookies *Go! musli z owocami* LU	Cookies *Digestive* LU

Table 7. The content and bioavailability of Fe from selected pairs of fortified with Fe and non-fortified food products

Content of Fe, mg/100 g					
Product	fortified with Fe		non-fortified with Fe		p
1	2		3		4
Corn flakes	1.51 ±0.03	a	1.32 ±0.09	a	NI
Honey flakes	1.73 ±0.02	a	2.34 ±0.11	b	< 0.05
Chocolate flakes	5.79 ±0.09	b	4.84 ±0.21	a	< 0.05
Flakes *Chocolate balls*	7.29 ±0.08	b	3.05 ±0.01	a	< 0.05
Cereal muesli	4.27 ±0.54	a	2.83 ±0.07	a	NI
Baby cookies	10.68 ±1.45	b	0.72 ±0.01	a	< 0.01
Cereal cookies	5.22 ±0.09	b	1.03 ±0.04	a	< 0.01
Relative bioavailability of Fe, %					
Corn flakes	36.79 ±2.15	a	28.78 ±4.38	a	NI
Honey flakes	15.49 ±11.35	a	37.50 ±1.59	b	< 0.01
Chocolate flakes	14.11 ±1.35	a	18.36 ±0.78	a	NI
Flakes *Chocolate balls*	19.23 ±0.19	b	9.04 ±4.03	a	< 0.01

Table 7 – cont.

1	2		3		4
Cereal muesli	41.43 ±4.31	a	36.92 ±0.73	a	NI
Baby cookies	37.71 ±7.32	a	69.24 ±9.60	b	< 0.01
Cereal cookies	49.97 ±0.02	a	74.11 ±11.87	b	< 0.01
Total bioavailability of Fe, mg/100 g					
Corn flakes	0.56 ±0.02	b	0.38 ±0.03	a	< 0.01
Honey flakes	0.27 ±0.20	a	0.88 ±0.00	b	< 0.01
Chocolate flakes	0.82 ±0.07	a	0.89 ±0.00	a	NI
Flakes *Chocolate balls*	1.40 ±0.00	b	0.28 ±0.12	a	< 0.05
Cereal muesli	1.76 ±0.04	b	1.05 ±0.05	a	< 0.05
Baby cookies	3.97 ±0.24	b	0.50 ±0.08	a	< 0.01
Cereal cookies	2.40 ±0.01	b	0.76 ±0.09	a	< 0.01

a, b – values in rows marked with different letters are statistically different at $p < 0.05$.

As can be seen in Table 7, most of food products fortified with Fe, as can be expected, contained significantly higher amounts of Fe in comparison with the non-fortified analogues. However, two out of seven food products fortified with Fe contained comparable amounts of Fe with the non-fortified ones, and one out of seven food products non-fortified with Fe contained markedly higher amount of Fe than the fortified analogue that can result from poor efficacy of the mixing process (uneven distribution of fortificant particles in the product matrix).

The highest discrepancy in the content of Fe between the fortified with Fe and non-fortified food products was found in the fortified with Fe baby cookies (10.68 ±1.45 mg/100 g vs. 0.72 ±0.01 mg/100 g).

The RBV of Fe values obtained for three of the non- fortified with Fe food products were significantly higher in comparison with the fortified ones (honey flakes, baby cookies and cereal cookies). Only in the fortified flakes *chocolate balls* the RBV of Fe value was markedly higher than in the non-fortified product.

From nutritional point of view, the most important seems the TBV value, expressed in mg of Fe released from 100 g of a product. It was found that most of food products fortified with Fe, as can be expected, not only contained significantly higher amounts of this nutrient, but also featured higher the TBV of Fe values in comparison with their non-fortified analogues. Such trend was observed for most (five out of seven) pairs of the compared food products. The exception to this rule was found for the fortified with Fe products, such as honey flakes (0.27 ±0.20 mg/100 g vs. 0.88 ±0.00 mg/100 g) and chocolate flakes (0.82 ±0.07 mg/100 g vs. 0.89 ±0.00 mg/100 g) that featured lower the TBV of Fe values than the fortified with Fe analogues.

CONCLUSIONS

1. Most of the analysed food products fortified with Fe had significantly higher contents and total and relative bioavailability of this element in comparison with the non-fortified analogues.
2. The analytically determined content of Fe in the fortified with Fe food products not always matched with the values declared on the label.
3. The fortified with Fe food products appear to be better sources of potentially bioavailable Fe in comparison with the non-fortified analogues.

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OCENA ZAWARTOŚCI ORAZ STOPNIA UWALNIANIA ŻELAZA Z WYBRANYCH PRODUKTÓW WZBOGACONYCH I NIEWZBOGACONYCH W TEN SKŁADNIK MINERALNY

Wstęp. Produkty wzbogacane zawierają zwykle większą ilość składników odżywczych. Niewiele jest jednak informacji dotyczących jakości odżywczej tego rodzaju żywności. Celem pracy było określenie zawartości żelaza oraz jego potencjalnej biodostępności z produktów wzbogaconych i niewzbogaconych w żelazo dostępnych na rynku polskim.
Materiał i metody. Materiałem do badań było 29 produktów spożywczych różnych producentów, dostępnych w handlu detalicznym. Zakupiono je w okresie od października do grudnia 2009 roku. Badaniami objęto dwie grupy wzbogacone i niewzbogacone w żelazo: przetwory zbożowe (16 produktów) oraz wyroby cukiernicze (13 produktów). Próbki poddano trawieniu enzymatycznemu w warunkach *in vitro*. Zawartość żelaza w preparatach oraz supernatancie po trawieniu określono metodą AAS. Analizę statystyczną wykonano z wykorzystaniem programu komputerowego Statistica 7.0.
Wyniki. Stwierdzono, że znacząca większość badanych produktów wzbogacanych w żelazo charakteryzowała się istotnie większą zawartością i uwalnianiem tego pierwiastka, w porównaniu z ich odpowiednikami niewzbogaconymi. Oznaczona analitycznie zawartość żelaza nie zawsze była zgodna z ilością podaną na etykiecie.
Wnioski. Produkty wzbogacone w żelazo wydają się być lepszym źródłem potencjalnie dostępnego żelaza niż produkty niewzbogacone w ten składnik.

Słowa kluczowe: zawartość, uwalnianie, żelazo, produkty wzbogacone, produkty niewzbogacone

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