EFFICIENCY OF IRON IN FORTIFIED BREAD

Ciobanu C., Deseatnicov O.

Technical University of Moldova, Chisinau, Republic of Moldova,

Deseatnicov Olga: olga22@hotbox.ru

Abstract: The present work was designed to compare the effects of different kinds of bread fermentation on iron bioavailability. Bread making procedure was executed by yeast bread fermentation and by lactic acid fermentation methods. In both cases bread was fortified with iron salts. An optimal result in fortification of bakery products with iron is achieved with use of lactic acid fermentation method. Lactic acid fermentation method provides a higher degree of phytate solubility and a higher bioavailability of non-hem iron.

Key words: iron, UNICEF, bread.

Introduction

Iron deficiency anaemia remains an enormous global problem. Up to half of the children and women of childbearing age in developing countries and about 10% in developed countries are estimated to be iron deficient (Algarin *et al.*, 2003). Iron deficiency leads to impair physical work performance, cognitive impairment, and adverse pregnancy outcomes. Increasing evidence shows that iron deficiency anaemia in infants and toddlers can lead to irreversible developmental delays (Hertrampf, 2002).

The study of the food consumption and nutritional statute of Moldavian people, realised with the support of UNICEF in spring-autumn 1998, showed that the products used in the nutrition of women aged 18 – 45 years old contained only 28-53% of the iron daily amount (Hertrampf, 2002). The consequence of this deficiency is the prevalence of iron deficiency anaemia in 47% of children aged 6-12 months and in 28% of children aged 1-5 years old, showed by the same study.

Three intervention strategies are available to prevent iron deficiency and, therefore, iron deficiency anaemia. These are supplementation, dietary diversification, and both targeted and untargeted food fortifications UNICEF (2002). Food fortification programs are cost effective means for reducing the prevalence of iron deficiency (Hurrell, 1999). The effectiveness of a food fortification program depends on the consistent and uniform addition of iron compounds to appropriate food vehicles, such as flour, which are widely consumed by the target population (Nalubola, 2000).

Mineral intake and bioavailability are critical factors for meeting mineral nutritional needs. Whole grain bread contains high levels of potassium, magnesium, iron and zinc, but the presence of phytic acid compromises mineral and trace element absorption in humans. However, during bread making the content of phytic acid decreases due to the action of phytases in the dough. The activity of baker's yeast seems to have no significant effect on these conditions.

Nevertheless, if very little phytate is hydrolyzed in unleavened whole meal breads including breads containing bicarbonate of soda, phytic acid hydrolysis occurs during all stages of yeast bread making. Reduction of phytic acid content in different bread types varies between 13% and 100%, with the lowest decrease being in unleavened breads. Phytic acid content of rye bread may be, under optimal conditions, reduced to near-zero values. The substantial decrease of phytic acid in whole wheat

products can improve mineral availability in humans. Because consumption of whole grain breads is increasing, a whole wheat bread with low phytic acid levels and increased mineral bioavailability would be beneficial and attractive in improving mineral status and, hence, preventive nutrition.

Quality control of fortification iron and fortified food requires a simple and reliable method to determine potentially bioavailable iron. Bioavailability refers to the amount of iron that is absorbed from the diet for normal use in metabolic processes and functions, and is influenced by both dietary factors and host-related factors. As a biological concept, bioavailability should be determined by measurements *in vivo*, but human *in vivo* studies, however, are time-consuming and very expensive. Comparatively, *in vitro* methods are simple, rapid and inexpensive. The effectiveness of several methods for predicting bioavailability of several iron sources in human subjects has been investigated comparatively in an inter-laboratory study (Luten *et al.*, 1996).

The objective of this study was to investigate the potential iron bioavailability in iron fortified white bread prepared by traditional method (mono- and double phase) as well as by development of lactic acid fermentation procedure, with further evaluation of soluble of the amount of soluble and absorbable iron. The correlation between phytate solubilisation and Fe-dializability has been evaluated.

Materials and methods

Materials

Distilled, de-ionised water was used throughout the experiments. All glassware was washed with detergent, rinsed with water, soaked overnight in 20% HNO_3, rinsed again and dried. All chemicals were of analytical grade.

Preparation of test bread: For the bread preparation was used high quality flour, as ferrous additive was taken the ferrous sulfate. As bread making procedures were used traditional methods-mono and biphasic (starter leaven fermentation) and the lactic acid fermentation performed in two stages: the simple mixture of ingredients and further lactic fermentation (I lactic acid method A-L I) (Lopez et al., 2001), and the dough scalding method (II lactic acid method A-L II). The lactic acid spontaneous fermentation procedure was performed using different durations of fermentation (1, 2, 3 days). Also there was tested starter leaven addition in different concentrations (25-75% of the traditional dose). After cooking the iron fortified bread was dried and homogenised. Ten grams were soaked in 100 ml double-distilled water.

Pepsin digestion mixture: 16 grams of pepsin were suspended in 100 ml of 0.1 N HCl.

Pepsin digestion: the *p*H was adjusted to 2.0 by adding 6 N HCl. Freshly prepared pepsin suspension (1.6 ml) was added and the mixture incubated in a shaking water bath at 37°C for 2 h using 100 rev/min as stirring speed. During the pepsin digestion, the 25-g digest aliquots were placed in ice-water bath to cool and to stop the digestion procedure, and centrifuged at 8000 rpm for 30 min to obtain soluble fractions.

Reagents and Chemical analysis

In a 1-l volumetric flask, 50 g hidroxylamine monohydrochloride was first dissolved in water. Concentrated HCl (100 ml) and 100 g trichloroacetic acid were

added and the solution was brought to volume with water. Bathophephenanthroline disulfonic acid (BPDS; 300 mg) was dissolved in 10-15 ml of water in a 1-l volumetric flask. The solution was brought to volume with 3 M sodium acetate. The solution was stored in an amber coloured glass bottle to minimise light catalysed deterioration. Iron standards were perorated, in duplicate, by diluting a stock iron solution (1000 μg Fe/ml in 1% HCl) with iron extracting solution to achieve the following concentrations of iron: 0, 1, 2, 3, 4, 5, 8 and 10 μg/ml. A 1-ml aliquot of the iron standard or the sample filtrate was mixed with 3 ml of the chromogen solution. After standing at room temperature for 15 min, absorbency was measured spectrophotometrically ar 535 nm (Kosse *et al.*, 2002).

A standard curve was constructed with the iron standards using linear regression and the food sample readings were plugged into the regression equation to determine their iron concentrations. A filtrate blank was also prepared for each food sample by adding 3 ml of 3 M sodium acetate without the chromogen reagent (BPDS) to 1 ml of the food sample filtrate. The reading obtained from the blank was then subtracted from the reading obtained from the corresponding sample filtrate containing the chromogen reagent.

Phytic acid content in the extract was determined colorimetrically (Haug and Lentzsch, 1983).

Estimation of absorbable iron

Absorbable iron was estimated with the model of Miller *et al*. (1981). After statistical comparison of the standard deviations, a multiple range test using the Student-Newman-Keuls method was performed to compare the means.

Results and discussion

Some dietary constituents interfere with non-hem iron solubility by forming insoluble macromolecules resulting in poor iron availability. Phytic acid content of several cereals has adversely influenced protein and starch digestibility and the availability of essential minerals (Steven, 2004).

This study has evaluated the influence of the process of bread making on iron bioavailability and the degree of enzymolysis of wheat phytates. It was shown that the amount of soluble iron varies considerably in function of the method used for bread making. The absorbable iron and soluble phytate contents in bread during the gastric and trypsic digestion are present in Table 1.

It is considered that in majority of cases the content of dialyzable iron at the end of gastro-intestinal digestion cycle in vitro (2h gastric digestion, at pH = 2.0 in the presence of pepsin and 2h of intestinal digestion, at *p*H = 8.0, in the presence of trypsin) is considerably higher than in case of bread made by traditional method. The duration of fermentation and added starter leaven considerably influence iron bioavailability.

It is well known that phytate enzymolisis process is directly connected with the conditions of bread baking process. A special influence has the pH of dough. According to the literature data an optimal *p*H for phytate enzymolisis is 5.5.

During dough fermentation process the accumulation of lactic, acetic, butyric, propionic, formic, succinic acids takes place. Accumulation of lactic and acetic acids in dough is the result of fermentation of heterofermentative lactic bacteria. Bacteria introduced into the dough mainly with flour plays an important role in accumulation of

lactic acid in the dough. In order to achieve faster the desired acidity, a mixture of water and flour is prepared and left for spontaneous fermentation. In the situation of lack of starter leaven I lactic acid method of fermentation takes place (A-L I) (Lopez *et al.*, 2001).

Table 1. The influence of technological parameters and method of bread making on in vitro iron bioavailability in non-fortified products

Method of bread making	Duration of ferm. (day)	Starter leaven %	$Fe_{soluble}$, mg% (± 0.05)			$Fe_{solub.}$ % (± 0.5)	$Phytate_{soluble}$, mg% (± 0.5)			Solub. Phyt. % (± 0.5)
			Gastr.	Tryps.	Total		Gastr.	Tryps.	Total	
Trad.monof.	-	100	0.23	-	0.23	9.2	30.8	53.1	83.9	14.5
Trad.bifaz	-	100	0.21	-	0.21	8.0	35.6	56.7	92.3	15.9
A-L I	1	-	0.29	0.25	0.54	21.6	121.9	83.7	205.6	35.4
A-L I	1	25	0.32	0.24	0.56	22.4	129.6	81.5	211.1	35.9
A-L I	1	50	0.26	0.08	0.34	13.6	123.5	78.6	202.1	34.4
A-L I	1	75	0.22	0.24	0.46	18.4	125.2	89.5	214.7	36.5
A-L I	2	-	0.19	0.32	0.41	16.4	112.6	87.5	300.1	51.0
A-L I	2	25	0.31	0.20	0.51	20.4	197.9	99.7	297.6	50.6
A-L I	2	50	0.29	0.28	0.57	22.8	111.4	75.3	186.7	31.7
A-L I	2	75	0.18	0.12	0.30	12.0	129.3	85.6	214.9	36.6
A-L I	3	-	0.42	0.18	0.60	24.0	204.1	22.6	226.7	39.2
A-L I	3	25	0.52	0.12	0.64	25.6	97.5	87.6	185.1	31.5
A-L I	3	50	0.29	0.18	0.47	18.9	98.7	112.7	211.4	35.9
A-L I	3	75	0.29	0.24	0.53	21.2	217.4	29.1	246.6	42.7
A-L II	1	-	0.28	0.23	0.51	20.2	114.4	15.0	129.4	22.4
A-L II	1	25	0.30	0.36	0.66	26.4	131.5	75.8	207.3	35.3
A-L II	1	50	0.35	0.23	0.58	23.2	182.5	47.6	230.1	39.8
A-L II	1	75	0.34	0.25	0.59	23.6	122.6	46.2	168.8	24.2
A-L II	2	-	0.27	0.12	0.39	15.6	108.7	75.8	184.5	31.4
A-L II	2	25	0.19	0.13	0.32	12.8	121.5	73.8	197.3	33.5
A-L II	2	50	0.22	0.15	0.37	14.8	119.6	109.7	229.3	38.9
A-L II	2	75	0.26	0.22	0.48	15.2	165.1	32.8	197.9	34.3
A-L II	3	-	0.14	0.17	0.31	12.2	95.0	112.7	208.3	35.4
A-L II	3	50	0.29	0.16	0.45	12.4	122.9	43.5	166.4	28.8
A-L II	3	75	0.22	0.26	0.48	15.2	82.7	136.5	219.2	37.3

* The total amount of phytates in the product - 578 mg of phytic acid /100g of product; $Fe_{total\ bread}$ = 2.5 mg%

Lactic acid has influence on swelling of proteins and gelling of starch. In the presence of lactic acid partial peptisation of proteins happens. Peptised proteins increase osmotic pressure in exterior of protein globule what determines water diffusion from globule into exterior, in such a way the globule becomes more compact. Peptised gluten gets superior rheological properties; respectively the dough and bread appear to be of higher quality. Also lactic acid prevents bread from development of bread disease.

Acetic and lactic acids, alcohol, carbon dioxide and small amounts of nitrogen are formed in the result of lactic acid fermentation. To speed up the fermentation and

replication of bacteria processes, method of scalding - the II lactic acid method is used (A-L II). It also allows starch gelatination. In the result starch is exposed to amylolytic hydrolysis in such a manner accelerating lactic acid fermentation too (Fretzdorff and Brümmer, 1992).

At the result of performed analysis it was established that phytate enzymolysis goes on more intensive in case of samples produced by the I lactic acid method (50% of hydrolysed phytates in comparison with 35-39% in case of the II type of lactic acid fermentation). But in both cases products made by lactic acid method have a high degree of phytates enzymolysis by contrast to the products made by traditional methods. Potential iron bioavailability, characterised by fraction of soluble iron that is capable to penetrate gastrointestinal barrier, is considered greater: 12.2 – 26.4% in case of I and II A-L methods in comparison with 8.1 – 9.2 when traditional methods with bakery starter leaven are used.

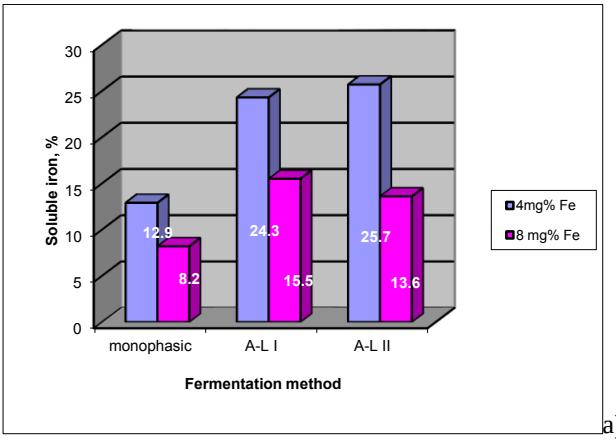
Further bread fortified with non-hem iron with the use of acid-lactic fermentation technology was prepared. The obtained data are presented in table 2.

It was determined that in case of traditional bakery methods (mono- and biphasic), iron solubility degree in bread fortified with non-hem iron is 8.6 – 9.2%, and the quantity of soluble iron after 4h of in vitro digestion reaches 0.6 – 0.9 mg of Fe for 100 mg of the product. The most of added compound is unaffected by dietary components which may either enhance or inhibit the availability of non-hem iron.

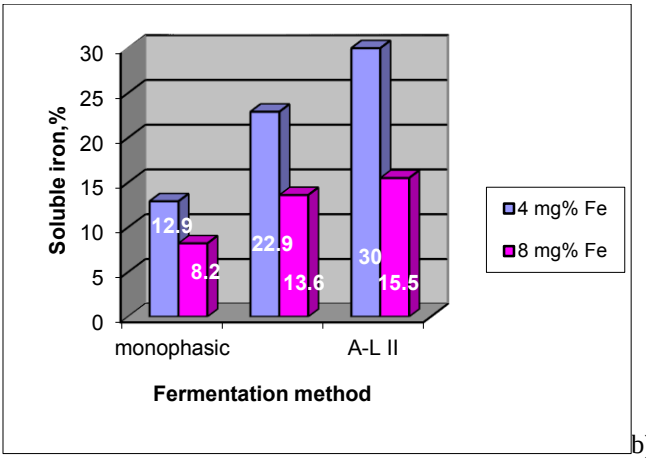
In bakery products made by I lactic acid method the content of soluble iron at the end of 4h of gastro-intestinal digestion in vitro achieves 1.5 – 1.7 mg%. This fact has a positive correlation with the increase of the degree of phytates enzymolysis that practically redoubles.

The most impact on the bioavailability has the portion of the supplement. Thus, the study of influence of supplement concentration on iron bioavailability in bread produced by traditional method (monophasic) shows that the optimal portion of supplement is 4 mg%, what means that total iron amount in bread will be 6.5 mg%.

The same effect was manifested in the case of iron fortified bakery products made by I and II lactic acid method (figures 1a and 1b).



a)



b)

Figure1. The influence of the method of production on iron bioavailability: a) with 50% of starter leaven; b) with 75% of starter leaven

Hereby in all three used methods of baking iron bioavailability (supplement – 4 mg%) is higher than with the use of supplement more than (8 mg%). Comparison of the results obtained by I lactic acid method and II lactic acid method shows that in the last case iron bioavailability is a little higher, especially with the supplement of 4 mg % Fe. This fact is evidently connected with higher level of phytate solubilisation.

Table 2. The influence of preparation technology on in vitro iron bioavailability and phytates enzymolysis in fortified bakery products

Method of bread making	**Duration of ferm. (day)**	**Starter leaven %**	**Fe mg% fortif.**	**$Fe_{soluble}$, mg% (± 0.05)**			**$Fe_{solub.}$ % (± 0.5)**	**$Phytate_{soluble}$, mg% (± 0.5)**			**Solub. Phyt. % (±0.5)**
				Gastr.	**Tryps**	**Total**		**Gastr.**	**Tryps.**	**Total**	
Monofazic	-	100	4	0.5	0.1	0.6	9.2	38.4	36.2	74.6	12.9
Bifazic	-	100	8	0.8	0.1	0.9	8.6	44.2	32.2	76.4	13.2
A-L I	2	50	4	1.2	0.5	1.7	26.2	89.6	43.5	133.1	23.0
A-L I	2	50	8	1.1	0.6	1.7	16.2	78.9	46.9	125.8	21.8
A-L I	2	75	4	0.9	0.6	1.5	23.1	93.6	41.5	135.1	23.4
A-L I	2	75	8	1.1	0.5	1.6	15.2	87.2	37.5	124.7	21.6
A-L II	2	50	4	1.3	0.5	1.8	27.7	137.6	67.6	205.2	35.5
A-L II	2	50	8	1.4	0.4	1.8	17.1	89.2	42.7	131.9	22.8
A-L II	2	75	4	1.8	0.3	2.1	32.3	165.5	51.0	216.8	37.5
A-L II	2	75	8	1.6	0.6	2.2	20.9	81.9	39.7	121.6	21.0

*Compound – Ferrous sulfate

Hereby in all three used methods of baking iron bioavailability (supplement – 4 mg%) is higher than with the use of supplement more than (8 mg%). Comparison of the results obtained by I lactic acid method and II lactic acid method shows that in the last case iron bioavailability is a little higher, especially with the supplement of 4 mg % Fe. This fact is evidently connected with higher level of phytate solubilisation.

Conclusions

An optimal result in fortification of bakery products with iron is achieved under the following conditions:

- Use of lactic acid fermentation method;
- Quantity of iron supplement of 4 mg %;
- Additive of 50% of starter leaven in case of I lactic acid method or 75% of starter leaven in case of II lactic acid method;
- II lactic acid method provides a higher degree of phytate solubility and a higher bioavailability of non-hem iron.

References

1. **Algarin, C., Peirano, P. et. au.** (2003): Iron deficiency anemia in infancy: long-lasting effects on auditoryand visual system functioning. Pediatr. Res., **53:217-223**.
2. **Fretzdorff, B. and Brümmer, J.M.** (1992): Reduction of phytic acid during bread making of whole-wheat breads, Cereal Chem. **69**: 266.
3. **Halberg, L, Brune, M. and Rossander, L.** (1989): Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate**,** Am. J. Clin. Nutr., **49**:140-144.
4. **Haug, W. and Lentzsch, H.G.** (1983): Sensitive method for the rapid determination of phytate in cereals and cereal products, J. Sci. Food Agric., **34**: 1423-1426.
5. **Hertrampf, E.** (2002): Iron Fortification in Americans, Nutrition Reviews, **60** (7), S22-S25.
6. **Hurrell, R.F.** (1999): Mineral fortification of food. England, Leatherhead Food Research Association.
7. **Kosse, J.S., et au** (2002): A rapid method for iron determination in fortified foods, Food Chemistry **75**: 371-376.
8. **Lopez, H.W., et al.** (2001) Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium, J. Agric. Food Chem. **49**: 2657.
9. **Luten, J., et al.** (1996): Interlaboratory trail on the determination of the in vitro iron dialysability from food, J. Sci Food Agric., **72**: 415-424.
10. **Miller, D.D., Schricker, B.R. and Rasmussen, R.R.** (1981): An in vitro estimation of iron availability from meals. Am. J. Clin. Nutr. **34:** 2248-2256.
11. **Nalubola, R.N.** (2000): Wheat flour fortification with iron. Arlington, VA, The MOST Project http:/ www.mostproject.org.pubs.htm.
12. **Steven, A.A.** (2004): New approaches to iron fortification: role of bioavailability studies, Am. J. Clin. Nutr., **80**: 1104-1105.
13. UNICEF (2002): Raportul UNICEF Alimentaţia şi nutriţia umană în Republica Moldova.