FORTIFICATION OF BAKERY PRODUCTS WITH IRON AND IRON ABSORPTION ENHANCERS

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Abstract

The ascorbic acid or the vitamin C is able to increase the absorption of iron from the fortified foods. This effect is due to the property of the vitamin C to reduce the capacity of the chelating action, the importance of the action of the ascorbic acid depends on the added quantity, of the iron concentration, and of the quantity of the inhibitors present in the product. In this paper were carried out researches concerning the efficiency of iron fortifications of bakery products with addition of iron enhancers, and the optimization of the fabrication procedure through mathematic models.
***Keywords**: iron, fortification, hip-rose, bioavailability, mathematic models*

Introduction

The prevention of nutritional deficiencies represent a indispensable method for the prophylaxis of various pathologies, which are require enormous expences for hospitalization, drugs and multiples investigations. Iron deficiency is a major health problem in the world today (M. J. Salgueiro, 2002), it affects over 2 mlrd of people. Iron anaemia is the severe form of iron deficiency. It can result in low resistance to infection, impaired psychomotor development, and cognitive function at children, poor academic performance, as well as fatigue and poor physical/work endurance, the etymology of this in most of the cases proceeds from dietetic errors concerning the nutrients amount and iron deficit.

The study of the food consumption and nutritional status of Moldavian people, realised with the support of UNICEF in spring-autumn 2000, showed that diets of women aged 18 – 45 years old contained only 23-53% of the daily amount of iron (UNICEF, 2000). 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.

Personal researches, concerning the total iron amount and in vitro iron bioavailability from the daily ration of institutionalized children in R. of

Moldova indicate the average daily intake of 9,66±0.09 mg Fe/day, which represent only 53,7% from the reccomended daily intake. The amount of soluble iron was 1,8±0,07 mg Fe/zi, that is only 18,6% of the total iron.

According to FAO/WHO estimations, the average iron intake in the R. of Moldova doesn't reach 100% of nutritional requirements for any population category (UNICEF, 2002). It is obvious that only considerate correction could rectify these disorders.

In 2002 WHO declared iron deficiency as being one of the most important risks for the human health, which can be prevented. The vitamin C is an important enhancer of dietary iron absorption, which decreases the inhibitory effect of phytates and tanins (Darnton-Hill I, 1999). Multiples researches indicate, that hemic and non hemic iron bioavailability could be considerably enhanced by the presence of vitamin C (Cook JD, 1996). This effect is due to the capacity of ascorbic acid to reduce the activity of chelating agent of iron. The vitamin C represents also a reducer, able to mantain iron in reduced form (Fe^{2+}). The ability of ascorbic acid to reduce iron and to prevet the formation of poor soluble ferric compounds represent an important enhancing mecanism, espetially for the non hemic iron.

In light of the above, a practical interest represents the examination of the influence of the vitamin C from a natural product as the hip-rose, by its incorporation in iron enriched rolls, intended to curative diet of institutionalized children.

Experimental

For the researches of the present study there have been prepared rolls enriched with iron and hip-rose addition. For the preparation of bakery products was used wheat flour of superior quality (STAS-26574-85). As additive was used the ferrous sulphate – $(NH_4)_2\,Fe\,(SO_4)_2 \cdot 7H_2O$ (Mohr salt), which represents the most stable iron bivalent iron compound, with a high purity degree (impurities concentration does not exceed 0,01%). The hip-rose was dried until a constant mass, was crushed in a fine powder and was bolted by a sieve to exclude all no homogeneity of the product. In order to protect the ascorbic acid from the destruction during drying, this one was realized at a fixed temperature of 80° C in the installation of convective drying.

The enriched rolls have been prepared by the monophase method (all ingredients are mixed at the same time), the biphasic method I (iron and hip-rose added to the leaven) and the biphasic method II (iron and hip-rose added to the dough after the fermentation). The bread samples were dried until a constant mass, crumbled and portioned (by 10,0±0,2g).

Relative iron availability was estimated using the in vitro method which involves a two stage digestion at 37±1°C and continuous shaking (pepsin at pH=2 for 2 hours and pancreatin-bile at pH=7,5 for 2 hours) of a 10g bread sample (Miller D.D., 1981). The dialysate was analysed for bathophenanthroline disulphate reactive iron by spectrophotometric method which was considered as the amount of available iron in bread (Jennifer S. Kosse, 2002).

Results and Discussion

Researches concerning potential bioavailability of iron in bakery products fortified with iron and hip-rose addition

Iron incorporated in a complex system like the foods, confronts various problems, like oxidation and precipitation. The bioavailability of Fe (heme and non-heme) is directly correlated with the presence in the ration of the enhancers, such as the vitamin C (ascorbic acid).

It was noted, that the hip-rose addition has an outstanding influence on the physicochemical and organoleptic characteristics of the rolls. In the case of the product obtained by the direct method (monophase), the specific volume of the rolls increase significantly, comparing to the witness, also the organoleptic appreciation established by the experts (tab.1).

Table 1: Influence of hip-rose on the physicochemical and organoleptic characteristics of the product

N d/o	Hip-rose content, mg-%	monophase method			biphasic method I			biphasic method II		
		V spec. cm 3/g	pH	organoleptic appreciation	V spec cm 3/g	pH	organoleptic appreciation	V spec , cm 3/g	pH	organoleptic appreciation
1.	-	2.680	5.10	8/10	3.005	5.32	7.5/10	3.005	5.32	7.5/10
2.	0.5	3.250	5.02	8.7/10	2.833	5.23	7.0/10	3.000	5.09	8.0/10
3.	1.0	3.865	4.97	8.2/10	3.012	5.17	6.5/10	3.090	5.02	7.3/10
4.	1.5	3.015	4.50	7/10	2.847	5.13	6.0/10	3.060	4.92	6/10

* Biphasic method I- hip-rose incorporated at the leaven stage

Biphasic method II- hip-rose incorporated at the dough stage

The best results have been obtained for the rate of hip-rose 0.5 and 1.0%. At the same time, the pH of the crumb of rolls decreases not considerably (with 0.08-0.13 units), while the addition of 1.5% hip-rose leads to a decrease of pH on 0.6 units of pH. For the rolls prepared by the diphase method I and II, a less outstanding influence on specific volume and acidity was noted.

The incorporation of hip-rose at the leaven stage led to the no significant variation of specific volume (1.0 mg-% hip-rose), and has the considerable reduction of specific volume for the addition of 1.5 mg-%, variation of pH represents 0.15-0.19 units of pH.

The rolls prepared by the diphase method II (hip-rose added at the dough stage) have values close to specific volume. The pH varies not significantly, while organoleptic appreciation is higher (0.5 mg-% hip-rose) or close (1.0 mg-% hip-rose) to the value obtained by the witness.

Thus, the addition of a small quantity of hip-rose (0.5-1.0 mg-%) can positively influence the organoleptic and physicochemical properties of the rolls, prepared by the monophase and biphasic II method. A higher quantity of dry hip-rose leads to a decrease of the organoleptic properties, probably due to the high acidity and grayish coloring of the rolls.

After drying, the rolls were crushed and the content of total Fe and dialyzable Fe during *in vitro* gastro-intestinal digestion was determined. The results for the rolls prepared by the biphasic method are shown in table 2. It was established that in the case of witness (rolls not enriched with iron), also in the case of enriched rolls without addition of hip-rose the bioavailability of iron is very low, only 8.9% of total iron is in soluble form after 4 hours of gastro-intestinal digestion, which represents 0.2 mg per 100 g of product. The hip-rose addition enhances iron solubility degree even in unfortified samples. Thus, addition of 1.0-1.5% of hip-rose ensures up to 0.3-0.35 mg Fe/100 g product of soluble iron in unfortified products.

The increase of iron solubility is higher in the case of bakery products enriched with iron and hip-rose, and it correlates directly with the amount of the hip-rose addition. It isn't noticed any essential difference between the hip-rose addition stage.

The most important enhancing effect is seen in case of 1.5% of hi-rose addition, so at the end of 4 hours of digestion the amount of soluble iron is estimated at 36-39%. But organoleptic appreciation in this case is lower compared to the witness and the biphase method. The enhancing effect of 0.5-1.0% of hip-rose is also very important and provides an increase of soluble iron up to 30-35%.

Table 2: Potential iron bioavailability in iron fortified bakery products with hip-rose addition (biphasic method)

$C_{hip-rose}$, %	Hip-rose aadministration stage	C_{Fe} additive, mgFe/100g flour	Total iron amount, mg Fe/100g product	Soluble iron amount, mg Fe/100g product				% soluble iron (final)
				Gastric stage (pepsin, pH=2)		Intestinal stage (tripsin, pH=8,2)		
				1 h	2 h	3 h	4 h	
-	-	-	1.63 ± 0.12	0.05 ± 0.01	0.11 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	8.9
		4	3.89 ± 0.16	0.23 ± 0.02	0.42 ± 0.02	0.69 ± 0.03	0.83 ± 0.09	21.4
		8	8.96 ± 0.14	0.28 ± 0.03	0.86 ± 0.02	1.28 ± 0.06	1.68 ± 0.07	18.8
0,5	leaven	-	1.59 ± 0.07	0.07 ± 0.01	0.13 ± 0.06	0.19 ± 0.06	0.22 ± 0.06	13.9
		4	4.38 ± 0.15	0.27 ± 0.04	0.89 ± 0.07	1.12 ± 0.09	1.29 ± 0.09	29.6
		8	8.96 ± 0.23	0.34 ± 0.11	1.07 ± 0.13	1.47 ± 0.16	2.54 ± 0.17	28.4
	dough	-	1.56 ± 0.11	0.06 ± 0.07	0.14 ± 0.07	0.18 ± 0.09	0.23 ± 0.10	14.7
		4	4.87 ± 0.17	0.25 ± 0.05	0.91 ± 0.07	1.23 ± 0.11	1.55 ± 0.15	31.9
		8	8.67 ± 0.26	0.41 ± 0.07	1.04 ± 0.09	1.94 ± 0.16	2.54 ± 0.19	29.3
1,0	leaven	-	1.58 ± 0.11	0.07 ± 0.01	0.12 ± 0.02	0.19 ± 0.07	0.29 ± 0.09	18.7
		4	4.19 ± 0.19	0.27 ± 0.01	0.84 ± 0.08	1.07 ± 0.11	1.34 ± 0.12	32.1
		8	9.04 ± 0.16	0.66 ± 0.03	1.36 ± 0.13	2.09 ± 0.21	2.96 ± 0.23	32.7
	dough	-	1.46 ± 0.18	0.06 ± 0.02	0.15 ± 0.05	0.20 ± 0.07	0.29 ± 0.07	19.7
		4	4.37 ± 0.21	0.32 ± 0.07	0.92 ± 0.06	1.27 ± 0.13	1.52 ± 0.17	34.7
		8	8.59 ± 0.17	0.50 ± 0.11	1.71 ± 0.12	2.40 ± 0.19	2.90 ± 0.21	33.8
1,5	leaven	-	1.61 ± 0.12	0.11 ± 0.03	0.19 ± 0.09	0.27 ± 0.09	0.35 ± 0.11	21.7
		4	4.27 ± 0.27	0.38 ± 0.05	0.78 ± 0.12	1.16 ± 0.12	1.68 ± 0.15	39.3
		8	9.08 ± 0.19	0.63 ± 0.04	2.04 ± 0.17	2.90 ± 0.21	3.40 ± 0.24	37.5
	dough	-	1.54 ± 0.19	0.09 ± 0.05	0.17 ± 0.04	0.25 ± 0.07	0.34 ± 0.07	22.3
		4	4.71 ± 0.18	0.33 ± 0.11	0.97 ± 0.11	1.56 ± 0.14	1.82 ± 0.22	38.7
		8	8.34 ± 0.26	0.56 ± 0.14	2.12 ± 0.13	2.89 ± 0.18	3.02 ± 0.19	36.2

The amount of iron additive does not have a considerable influence on the amount of soluble iron, though the maximum percentage in all analyzed samples is noticed for 4 mg Fe/100 g flour of additive. Evidently, the amount of soluble iron is higher in samples with 8 mg Fe/100 g flour of additive.

The results of the analysed samples prepared by the monophasic method are shown in table 3.

It is established, as in case of rolls prepared by the biphasic method, that the addition of hop-rose provide an increase in the amount of soluble iron even for in the unfortified samples. Evidently, in iron fortified samples this effect is noteworthy. Thus, in case of 0.5% of hip-rose addition the amount of soluble iron increase 1.5-fold in rolls prepared by the biphasic method, and 2-fold in case of monophasic samples. The addition of 1.0% of hip-rose provide a 1.7-fold increase in biphasic samples and 2.3-fold in monophasic samples, and in case of 1.5% of hip-rose addition, the amount of soluble iron increase 1.9-fold in biphasic samples and 2.5-fold in monophasic samples.

Table 3: Potential iron bioavailability in iron fortified bakery products with hip-rose addition (monophasic method)

$C_{hip\text{-}rose}$, %	C_{Fe} additive, mgFe/10 g flour	Total iron amoun, mg Fe/100g product	Soluble iron amount, mg Fe/100g product				% Soluble iron (final)
			Gastric stage (pepsin, pH=2)		Intestinal stage (tripsin, pH=8,2)		
			1 h	2 h	3 h	4 h	
-	-	1.43 ± 0.11	0.05 ± 0.01	0.07 ± 0.02	0.10 ± 0.02	0.12 ± 0.04	8.6
	4	4.19 ± 0.14	0.13 ± 0.03	0.39 ± 0.03	0.49 ± 0.06	0.60 ± 0.08	14.4
	8	8.82 ± 0.12	0.21 ± 0.03	0.76 ± 0.07	1.02 ± 0.07	1.22 ± 0.09	13.8
0,5	-	1.36 ± 0.05	0.05 ± 0.01	0.10 ± 0.05	0.14 ± 0.07	0.16 ± 0.07	11.7
	4	4.96 ± 0.14	0.29 ± 0.05	0.81 ± 0.04	1.11 ± 0.08	1.37 ± 0.08	27.7
	8	9.07 ± 0.22	0.41 ± 0.07	1.04 ± 0.09	1.94 ± 0.16	2.54 ± 0.19	26.3
1.0	-	1.56 ± 0.12	0.07 ± 0.01	0.13 ± 0.02	0.18 ± 0.02	0.20 ± 0.09	12.6
	4	4.97 ± 0.18	0.37 ± 0.05	0.88 ± 0.06	1.33 ± 0.15	1.68 ± 0.18	33.9
	8	8.52 ± 0.17	0.45 ± 0.05	1.11 ± 0.09	2.09 ± 0.17	2.71 ± 0.19	31.8
1.5	-	1.64 ± 0.17	0.07 ± 0.03	0.16 ± 0.05	0.24 ± 0.07	0.27 ± 0.09	16.3
	4	4.81 ± 0.18	0.36 ± 0.11	0.91 ± 0.08	1.37 ± 0.15	1.77 ± 0.17	36.7
	8	8.94 ± 0.23	0.61 ± 0.16	1.92 ± 0.11	2.73 ± 0.19	3.08 ± 0.19	34.5

The enhancing effect is due primarly to the ascorbic acid presence in hip-rose, which in case of 0.5% hip-rose addition is ≈20 mg AA/100 g flour; at 1.0% hip-rose addition it makes up ≈40 mg AA/100 g flour; and for the addition of 1.5% of hip-rose the ascorbic acid amount represent ≈60 mg AA/100 g flour. It is obvious that AA is extremly vulnerable to the thermic treatment, so that after baking its amount decrease considerable, and does not exceed 25-33% of initial values. After drying, grinding and *in vitro* digestion, AA amount is reduced to 10-12% of initial value. But the iron solubilization effect is exposed through all fermentation period, which contributes to a higher amount of final soluble iron amount in case of fortified samples compared to unfortified.

Thus, it was established that the addition of dried hip-rose in iron fortified bakery products could improve appreciably iron solubility in gastro-intestinal conditions, which represents the main condition for non hemic iron assimilation from food products.

Optimization of the fabrication procedure of fortified bakery products through mathematic models

With a view to optimization of the fabrication procedure of bakery products, fortified with iron and iron enhancers (dried hip-rose addition), it was performed an experimental research concerning the influence of the hip-rose addition, of the the bread making procedure and the additive amount on the soluble iron amount (in the conditions of *in vitro* gastro-intestinal digestion). Experimental results were used in mathematic models, which allowed to establish the influence of each analyzed factor on parameters wich determine the efficiency of fortification (Balan V. ,2004).

For the three bread making procedures, the analytic expressions for the iron amount represent polynominals of third degree given the hip-rose concentration, which vary in intervals 0-1.5%. The coefficients were calculated by the processing of experimental results by the mathematical regression method and interpretation of the obtained results by means of the applied system.

For the optimization of the fabrication procedure of fortified bakery products it is necessary to consider the total of factor with major influence on iron potential bioavailability in product. The mathematic models were elaborate for the three bread making procedures, then the obtained spatial models were compared.

Initial experimental data served for drawing up the matrix of the discret values of the independent variables (x) and the vector of the discret values of the function $[Fe]_{solubil} = f([M],[Fe]_{total})$. Further were calculated the regression coefficients (C) and was established the analytic expression of the dependence of the amount of soluble iron on the hip-rose content and the total iron amount. In the analytic expression were considered the interaction termes of the two independent variables, of the 2 and 3 degree.

Monophasic method

$$C(Fe_{solubil}) = -2.22678 + 0.45790[M] - 0.93882[M]^2 + 0.47111[M]^3 + 0.00939[Fe_{total}]^3 + \ldots$$
$$-0.207196[Fe_{total}]^2 + \ldots + 1.381996[Fe_{total}] + 0.22103[M]\cdot[Fe_{total}] - 0.14342[M]^2\cdot[Fe_{total}] +$$
$$0.0149[Fe_{total}]\cdot[M]^2$$

Biphasic method (addition of hip-rose at leaven stage)

$$C(Fe_{solubil}) = -1.65925 - 0.173707[M] - 2.92132 \cdot [M]^2 + 1.74667 \cdot [M]^3 + 0.01160 [Fe_{total}]^3 + \ldots$$
$$- 0.22761 \cdot [Fe_{total}]^2 + \ldots + 1.30345 [Fe_{total}] + 0.80073 [M] \cdot [Fe_{total}] - 0.29842 [M]^2 \cdot [Fe_{total}] -$$
$$0.0190 [Fe_{total}] \cdot [M]$$

Biphasic method (addition of hip-rose at dough stage)

$$C(Fe_{solubil}) = -1.51549 - 4.04522 \cdot [M] + 4.06500 \cdot [M]^2 - 1.295556 \cdot [M]^3 + 0.012297 \cdot [Fe_{total}]^3 + \ldots$$
$$- 0.22883 \cdot [Fe_{total}]^2 + \ldots + 1.41875 \cdot [Fe_{total}] + 0.83835 \cdot [M] \cdot [Fe_{total}] - 0.27000 \cdot [M]^2 \cdot [Fe_{total}] -$$
$$03415 \cdot [Fe_{total}] \cdot [M]$$

On the basis of analytic expressions were drawn up the functions of spatial representation.

In figure 1 are represented the graphic functions, which allow to compare the soluble iron amount in bakery products fortified with iron and hip-rose addition, fabricated by three bread making procedures. The exalination of this graphic functions indicates, that in all three cases the maximum of soluble iron is obtained at hip-rose concentrations included in 0.3-1.0% at the concentration of total iron amount at 7.5-12.5 mg Fe/100 g flour.

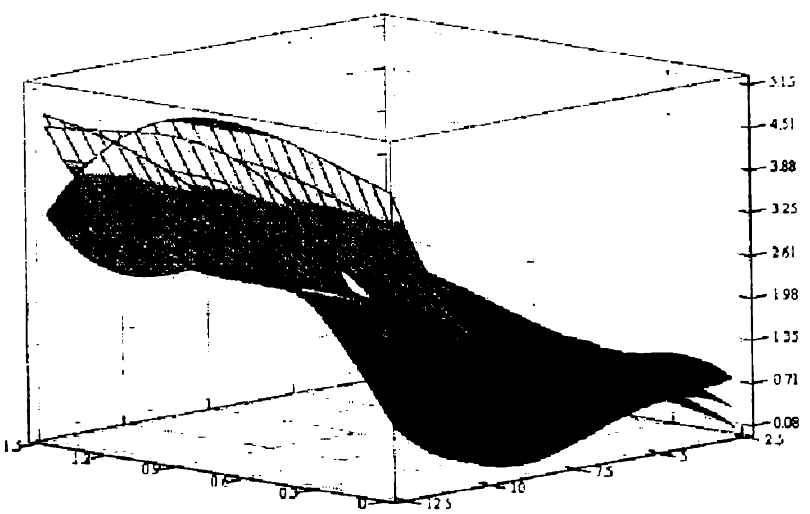

Fig. 1: Comparing of the three spatial representations of the soluble iron amount (Z) depending on the hip-rose concentration (X) and total iron amount (Y) for each bread making procedure

Considering the fact that in fortification are used smaller doses of iron, there can be taken the total iron concentrations 5 - 6.5 mg Fe/100 g flour, were the soluble iron amount has been identified very high in all analysed procedures.

For each examined case, generally are observed the same natures of a law (analytic expressions). For the bakery products prepared by the biphasic method (method 2 and 3), the maximum is identified in the interval of hip-rose concentration 0.8-1.3%, being more marked for the maximum concentration of total iron. Because in this interval of hip-rose concentration is registered a high amount of soluble iron, it is obvious, that between this two parameters exists a direct relation.

Conclusion

1. The influence of the hip-rose addition on the physicochemical and organoleptic characteristics of the enriched rolls, prepared by the three methods of bread making, was highlighted. The addition of hip-rose carries out the increase of the specific volume of the enriched bread (0,5-1,0 mg % hip-rose) and positively influences the result of the organoleptic test in the case of the rolls prepared by the monophase method and biphasic (hip-rose added to the dough stage). The value of the pH of the crumb of the rolls decreases not significantly (0,5-1.0 mg % hip-rose), while for the higher amounts (1,5 mg % hip-rose) the acidity increases considerably (on 0,5-0,6 units of pH).

2. The influence of the rate of hip-rose on the potential bioavailability of Fe during 4 hours of *in vitro* digestion was examined. It was established that the higher the rate of hip-rose is, the amount of soluble iron increase. The addition of 0,5 % of hip-rose (biphasic method) and of 1,5 % (monophase method) increases the amount of the soluble Fe from 12 -18 % (witness) until 48- 50 %.

3. The kinetics of solubilization of Fe during *in vitro* digestion of the iron enriched rolls with addition of hip-rose was examined. It was established that for the samples prepared by the three methods of bread making, the solubilization of the Fe in samples without addition of hip-rose is much slower, than in presence of hip-rose. The hip-rose expresses quite outstanding promoter effect on iron bioavailability in fortified bakery products.

4. The addition of small doses of hip-rose (0,5- 1,0%) in the receipt of the iron enriched rolls (4-10 mg % Fe), prepared by the biphasic method (hip-rose being mixed at the same time with the ferrous additive), could improve the potential bioavailability of Fe considerably and will improve the organoleptic indices of the rolls. A similar effect, but less outstanding, could be obtained for the rolls prepared by the monophase method of bread manufacture.

5. On the basis of the experimental researches and the MATCAD program was done the optimization of the fabrication procedure for bakery products, fortified with iron and hip-rose addition. It was established, that the most important contribution on the soluble iron content has the total iron content, followed by the hip-rose content and the interacion factor of these two independent variables. The maximum of soluble iron accumulation is registered in the interval of total iron concentration 7.5-12.5 mg Fe/100 g flour, and hip-rose concentrations comprised between 0.3-0.9%. It was also remarked a maximum less outlined at the average total iron concentrations (5–6.5 mg Fe/100 g flour) and hip-rose concentration 0.3 – 1.0%. Considering this facts, for the fortification of bakery products with iron and hip-rose addition, can be used additive concentrations 5-6.5 mg Fe/100 g flour.

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