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ScienceDirect
Food Chemistry 102 (2007) 309–316
Food Chemistry
www.elsevier.com/locate/foodchem

Sensory characteristics and iron dialyzability of gluten-free bread fortified with iron

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Received 2 December 2005; received in revised form 8 May 2006; accepted 17 May 2006

Abstract

The objectives of the present study were (a) to produce gluten-free bread, fortified with iron (GFB-Fe), using selected iron compounds (ferric pyrophosphate, ferric pyrophosphate with emulsifiers, NaFeEDTA, electrolytic iron, ferrous gluconate, ferrous lactate and ferrous sulphate) (b) to test sensory characteristics of the GFB-Fe (feel-mouth texture, crumb colour, aroma and taste) (c) to compare iron dialyzability of various iron compounds in GFB-Fe. The most acceptable products were those fortified with ferric pyrophosphate with emulsifiers and ferric pyrophosphate. Ferrous dialyzable iron (ferrous iron with molecular weight lower than 8000 Da, an index for prediction of iron bioavailability) was measured under simulated gastrointestinal conditions. Ferrous dialyzable iron in GFB-Fe fortified with ferric pyrophosphate with emulsifiers, NaFeEDTA, ferrous bis-glycinate, ferrous gluconate or ferrous sulphate was higher than that in GFB-Fe fortified with electrolytic iron, ferrous lactate or ferric pyrophosphate ($P < 0.05$). These results are promising for the development of GFB-Fe products in the future.

Keywords: Iron bioavailability; Dialyzability; Gluten-free bread; Fortification; Sensory; Celiac disease

1. Introduction

Coeliac disease is a genetically based autoimmune enteropathy caused by a permanent sensitivity to gluten (Hamer, 2005; Hill et al., 2005). In susceptible individuals the ingestion of gluten induces an immunologically toxic reaction that results in damage to the mucosal surface of the small intestine (Howard et al., 2002). This interferes with the absorption of nutrients, including iron (Doganci & Bozkurt, 2004; Mody, Brown, & Wechsler, 2003). Thus, the importance of coeliac disease as a possible cause of iron deficiency anaemia is increasingly being recognized (Hershko, Lahad, & Kereth, 2005). It is estimated that coeliac disease may account for 3–5% of the prevalence of iron deficiency anaemia (Grisolano et al., 2004; Howard et al., 2002). Upon diagnosis, the coeliac disease patient is directed to a gluten-free diet for life. The gluten-free diet excludes the intake of storage proteins found in wheat, rye, barley and of hybrids of these grains, such as kamut and triticale. This diet prevents morbidity and reduces the incidence of the associated gastrointestinal malignancy (Storsrud, Hulthen, & Lenner, 2003), but it is difficult to adhere to (Kupper, 2005). Moreover, the gluten-free products are often low in micronutrients therefore adding to the risk of deficiencies (Thompson, Dennis, Higgins, Lee, & Sharrett, 2005). It has been shown that among common deficiencies associated with a gluten-free diet is iron deficiency (Kapur, Patwari, Narayan, & Anand, 2003). Fortified or enriched gluten free products are rare, but it has

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doi:10.1016/j.foodchem.2006.05.022

been suggested that development of such products would improve the quality of the diet (Kapur et al., 2003; Kupper, 2005).

Fortification is an effective approach to increase dietary iron intake, provided that certain conditions apply (Hurrell, 2002). Therefore, iron fortification of selected foods is supported by various scientific and governmental bodies, as well as the industry. Among foods frequently fortified with iron are cereal products and rice. It is therefore within adopted practice to fortify gluten free products.

Successful fortification requires an iron compound that is adequately absorbed and does not affect the sensory properties of the products. Ferrous sulphate is the most popular source of iron for the fortification of various foods however other iron forms may exhibit higher bioavailability than ferrous sulphate and may present alternative choices (Lynch & Stoltzfus, 2003).

The objectives of this study were: (a) to produce a gluten-free bread fortified with iron (GFB-Fe) using selected iron compounds (electrolytic iron, NaFeEDTA, ferric pyrophosphate, ferric pyrophosphate with emulsifiers, ferrous bis-glycinate, ferrous gluconate, ferrous lactate, ferrous sulphate) (b) to test the sensory characteristics of the iron-fortified product (c) to compare the *in vitro* dialyzability of various iron compounds added in the GFB-Fe.

2. Materials and methods

2.1. Materials

The following ingredients were used for the production of gluten-free bread: gluten-free flour (Glutenfreies "St. Georgsmehl", Kunstmuehle, Leobersdorf, Austria, consisting of rice flour, maize starch, potato starch and locust bean gum), amaranth flour (amaranthus cruentus, genotype "amar", Life Power Posch Innovative Produkte, St. Poelten, Austria), albumen (egg white powder, type low whip, Enthoven – Bouwhuis Eiproducten B.V., Raalte, Germany), vegetable fat powder (REVEL * BEP, Loders Croklaan, The Netherlands), enzyme of α-amylase with additional transglutaminase and hemicullolase activity (VERON CLX AB Enzymes, Darmstadt, Germany), moist yeast (L'hirondelle, S.I.Lesaffre, France), salt (iodised sea salt, Kallas, Greece) and emulsifier-DATEM (Diacetyl-tartaric esters of mono- and diglycerides, Danisco, Copenhagen, Denmark).

The iron compounds tested for the formulation of GFB-Fe were ferric pyrophosphate with emulsifiers (24% iron according to the manufacturer, SunActive F-P80®, Taiyokagaku. Vokkaichi, Japan), ferrous bis-glycinate (20.1% iron according to the manufacturer, Ferrochel®, Albion Laboratories Inc., Clearfield, UT, USA), ferrous gluconate (12.5% iron according to the manufacturer, Gluconal Fe-G-USP®, Avebe, Veendam, The Netherlands), ferric pyrophosphate (25% iron according to the manufacturer, Dr. Paul Lohmann, Emmerthal, Germany) ferrous lactate (24% iron according to the manufacturer, Ferrous Lactate®, Jost Chemical, Namur, Belgium), elemental iron (Haganas, Hoganas, Sweden), NaFeEDTA (Ferazone®, AkzoNobel) functional chemicals, and ferrous sulphate (Merck, Darmstadt, Germany). Ferrous bis-glycinate, ferrous gluconate, ferric pyrophosphate, ferrous lactate, elemental iron and NaFeEDTA were donated by the respective producing companies.

The materials used in the *in vitro* digestion experiment were as follows. Pepsin was a porcine pepsin preparation, suspended in 0.1 M HCl at 4 g/100 mL in 0.1 M HCl. Pancreatin/bile mixture was a porcine pancreatin (0.2 g) and a bile extract (1.2 g) suspended in 100 mL 0.1 M $NaHCO_3$. PIPES buffer pH 9.1, 0.15 M PIPES (piperazine-*N*,*N*′-bis[2-ethane-sulfonic acid] disodium salt), was adjusted to pH 6.3 using concentrated HCl. HEPES buffer, 0.3 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid sodium salt) was used without pH adjustment. Protein precipitant solution (reducing) was 100 g trichloroacetic acid, 50 g hydroxylamine monohydrochloride and 100 mL concentrated HCl per 1 L of water. Protein precipitant solution (non-reducing) was prepared as the reducing solution except that the hydroxylamine monohydrochloride was not added. Ferrozine chromogen solution (5 mg/mL) was of ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4 triazine, disodium salt). Spectrapore® I dialysis tubing with a molecular weight cut-off of 6000–8000 (Spectrum Laboratories, Rancho Dominguez, CA, USA) was cut into 25 cm lengths and soaked in water for at least 1 h prior to use and stored in 0.15 M PIPES buffer pH 6.1, until use.

All chemicals were purchased from Sigma–Aldrich (Munich, Germany). Double distilled, deionised water was used throughout the experiments. All glassware was washed, soaked overnight in 1 N HCl and rinsed with distilled deionised water.

2.2. Experimental protocols

Gluten-free flour (111 g) and amaranth flour (74 g) were mixed and gradually added to the iron compound (amount calculated for each compound to provide 0.296 g of Fe). Subsequently the iron and flour mixture was mixed with egg white powder (9.25 g), vegetable fat powder (7.4 g), salt (3.7 g), emulsifier (0.925 g) and α-amylase (0.0925 g) in a Hobart mixer (Hobart N50, Hobart Co., Troy, OH, USA) for 1 min. Moist yeast (5.55 g) was dispersed in water (148 g) and added to the dry mixture. The mixing process continued for 3 more min. The dough was then hand-moulded, divided into 60 g pieces, put into rectangular greased cake pans (60 mm × 100 mm) and stored in polyethylene bags in a freezer at −18 °C (Ariston, ECH 145EU, Ancona, Italy). For each baking formula 6 bread loaves were prepared. The rational behind the choice of developing a frozen product instead of a fresh one was that the target group for GFB is small; therefore the GF baked products may not be marketed fresh but frozen. After storage for 10 days, the dough was defrosted under ambient conditions. Dough

temperature was checked by a temperature probe (Testostor® 171, Testo GmbH & Co., Lenzkirch, Germany) inserted at the midpoint of the dough. The samples were considered fully defrosted, when the temperature was 25–30 °C. Subsequently, the dough was fermented for 10 min at 27 °C in a preheated oven (Heraeus RT360, NTM, Hanuau, Germany), hand-stirred with a spoon and fermented for 20 min more. Samples were rested for proofing for 55 min at 27 °C and baked in an air oven (Memmert Model U, Winsconsin Oven Co., East Troy, WI, USA) for 30 min at 185 °C. The average air velocity in the oven was 1.1 m/s. The relative humidity in the oven was adjusted at 80% by placing a pot filled with water at the bottom of the oven. After baking, the breads were removed from the pans, stored for 24 h at ambient conditions and were used for further testing. Bread slices, 1 cm thick, were scanned and images were taken.

A randomized design was used for the order of baking different breads, but samples of the same composition used were from a single baking process. Baking parameters were carefully controlled to minimize variability due to oven to baking: Samples of the same weight and volume were put carefully at similar positions in each baking process, the temperature profile inside the crumb was followed by a thermocouple and the conditions in the oven chamber were checked for temperature, air velocity and humidity. During the training sessions of sensory analysis, samples of the same composition but of different baking were offered to the same panellist. No significant differences were detected between the different baking processes.

2.3. Sensory analysis

Sensory analysis was performed by a panel trained in different sessions as described in Mandala and Daouaher (2005). Twenty volunteers (ages 18–35, both sexes) were recruited from the student community, screened for their perception of colour, aroma, taste and texture and trained. The training (15 h divided into four sessions) aimed to familiarize the panellists with the methodology and terminology of the sensory analysis and to improve their ability to detect and describe qualitative and quantitative sensory aspects of a GFB-Fe in precise and reproducible evaluations. Panellists were given a coded reference samples several times during training and pre-evaluation procedure. Eleven trained panellists were selected. The unfortified sample (GFB) and all GFB-Fe were tested with the exception of GFB-Fe fortified with $FeSO_4$. This sample was not tested because the $FeSO_4$ used was a chemical not certified for human consumption. Samples were presented once in slices (1 cm thick) on plastic dishes coded with three-digit random numbers and served in a randomized order. All slices given were from the same location within the bread which was the geometrical centre of the loaf. Samples were served at room temperature (22 ± 2 °C) and analyses were performed under normal lighting conditions. The assessors sat in a round table and after tasting a sample, they discussed and pointed out its undesirable or desirable properties (e.g. soft texture, sharp aroma) and described the key parameters selected in the final sensory evaluation. The attributes tested were aroma, crumb colour, taste and mouth-feel texture (chewiness and crumb firmness). For the evaluation of texture the following definitions were given to the panellists: Firmness (hardness): force necessary to bite through a piece of the centre of the slice with the front teeth, chewiness: number of chews required before the product is swallowed (Szczesniak, 1963, 1998; Szczesniak, Brand, & Friedman, 1963). The attribute intensities were rated on continuous, unstructured graphical intensity scales. Panellists were then asked to score the samples on a 10-cm line scale with no divisions, the left side of the scale corresponding to the lowest intensity (value 0) and the right side to the highest intensity (value 10) of the attribute. For aroma and taste the degree of balanced flavour was rated (0 not balanced, 10 perfectly balanced aroma or taste). No balanced flavour included aroma and taste characteristics such as sour, sharp aroma, burnt flavour, yeast taste, "metallic", bitter, mouldy taste. These characteristics were pointed out by the assessors in the training sessions. The point marked on the line was measured with a ruler and the mean score for each sample was calculated. After evaluating each property separately, an average score was calculated using the following significance factors for each attribute: 9 for taste, 5 for texture, 4 for colour and 3 for aroma. These factors were selected according to the instructions in DLG (1995) for bread attributes and opinions of panelists. This average score represented the overall quality score of each product.

2.4. In vitro digestion

The digestion process is described in detail by Kapsokefalou and Miller (1991). This *in vitro* model simulates the gastrointestinal digestion by subjecting samples to incubation for 4.5 h at 37 °C, at different pHs, in the presence of peptic enzymes and by fractionating digests through the aid of a dialysis membrane. Briefly, samples of 20 mL, pH adjusted to 2.8 with concentrated HCl, were transferred in 120 mL screw cap vials and placed in a shaking water bath maintained at 37 °C. The samples were incubated for 2 h in the presence of 1 mL pepsin suspension added to each sample. At the end of this incubation, the pH of the samples was adjusted gradually from 2.8 to 6 with the aid of a dialysis sac, filled with 20 mL of PIPES buffer, pH 6.3. The dialysis sac was immersed into the incubating samples. After 30 min, 5 mL of a pancreatin–bile salt mixture was added to the samples and the incubation continued for another 2 h. At the end of this incubation period, the dialysis sac was removed. The dialysate, consisting of soluble compounds of low molecular weight, was collected. The contents of the dialysis bag were saved for measurements of the iron concentration.

2.5. Iron analysis

Ferrous and total (ferrous + ferric) iron concentrations in the dialysates were measured using a modification of the ferrozine method proposed by Reddy, Chidambaram, Foneca, and Bates (1986). Briefly, for total iron determination reducing protein precipitant solution (0.5 mL) was added to 1 mL aliquot of each dialysate. For ferrous iron determination, non-reducing protein precipitant solution (0.5 mL) was added to 1 mL aliquot of each dialysate. The samples were held overnight at room temperature. Subsequently they were centrifuged at 5000*g* for 10 min. Aliquots of the supernatants (0.5 mL in duplicate) were transferred to separate tubes. Ferrozine solution (0.25 mL) and HEPES buffer (1.0 mL) were added to each tube. Absorbance was measured at 562 nm immediately after chromogen addition for the ferrous iron determination or 1 h after addition for the total iron determination. Sample iron concentrations were calculated from the absorbance readings using a regression equation derived from data generated from standards of $FeCl_3$ in the presence of protein precipitant solution.

Dialyzable ferrous iron and dialyzable total iron were expressed as percentages of the calculated total amount of iron in the treatment at the beginning of the digestion.

2.6. Statistical analysis

The Statgraphics Statistical Graphics System, Version 2.1 (Statgraphics, Rockville, MD, USA) was used for statistical analysis of the results of sensory evaluation. Panel mean scores for each sensory data were calculated and Fisher's LSD was used to determine significant differences between samples. A *P*-value of <0.05 was considered significant.

For the dialyzable ferrous and total iron determination, each individual sample was run in duplicate and each experiment was repeated three times. Differences among samples containing the selected iron compounds were tested with LSD test when ANOVA was significant. Means were concluded to be significantly different at 95% confidence interval, after testing for normality (Zar, 1999). Analysis of data was carried out with the program Statistica, version 5.1 (StatSoft, Tulsa, OK, USA).

3. Results

Iron fortification of GFB affected all sensory characteristics tested ($P < 0.05$) except firmness ($P > 0.05$) (Table 1). In most cases, differences were observed between the unfortified and the fortified products as well as among GFB-Fe formulated with different iron compounds. Ferric pyrophosphate with emulsifiers was the iron compound that produced the most acceptable GFB-Fe product, while ferrous lactate was the least acceptable product.

In general, in most GFB-Fe the colour of crumbs was darker than that of GFB. In some cases very low scores were obtained, indicating an undesirable dark crumb colour. Scores for crumb colour assigned to GFB-Fe fortified with ferric pyrophosphate with emulsifiers or with ferric pyrophosphate or with electrolytic iron were higher than those assigned to the other GFB-Fe ($P < 0.05$) and similar to the scores of GFB ($P > 0.05$). The score of GFB-Fe fortified with NaFeEDTA was lower than that of GFB-Fe fortified with ferric pyrophosphate ($P < 0.05$). Scores assigned to GFB-Fe fortified with ferrous bis-glycinate or ferrous L-lactate or ferrous gluconate were lower ($P < 0.05$) than those assigned to all other samples. In GFB-Fe fortified with ferrous L-lactate, green spots were noticed in a bread slice justifying the low score assigned to this product.

All GFB and GFB-Fe were characterized by a sharp aroma. Aroma scores of GFB-Fe fortified with ferric pyrophosphate with emulsifiers or with NaFeEDTA were higher than those fortified with ferrous L-lactate ($P < 0.05$). Differences in aroma scores were not significant among all other GFB-Fe and GFB samples ($P > 0.05$).

Low to moderate scores for taste were obtained for GFB and GFB-Fe, typical for this kind of products. The panel concluded that the GFB-Fe fortified with ferric pyrophosphate with emulsifiers was the most acceptable one and that it had significantly better taste than GFB. GFB or GFB-Fe fortified with ferrous bis-glycinate or with ferric pyrophosphate or with NaFeEDTA or with ferrous gluconate had taste scores similar to GFB ($P < 0.05$). GFB-Fe fortified with ferrous L-lactate or with electrolytic iron received the lowest scores. GFB-Fe fortified with ferrous L-lactate had the worst taste ($P < 0.05$) from all other samples except GFB-Fe fortified with electrolytic iron.

Scores for mouth-feel texture (chewiness and crumb firmness) of all GFB and GFB-Fe were not different ($P > 0.05$). However they were higher for GFB-Fe fortified with ferric pyrophosphate with emulsifiers, or with electrolytic iron or with NaFeEDTA. Chewiness score for GFB-Fe fortified with ferric pyrophosphate with emulsifiers was higher from that of unfortified GFB ($P < 0.05$).

The calculated average quality score was higher than 5, corresponding to a medium/satisfactory quality product, for GFB-Fe fortified with ferric pyrophosphate with emulsifiers or with ferrous bis-glycinate or with ferric pyrophosphate or with NaFeEDTA. Among these products, GFB-Fe fortified with ferric pyrophosphate or with ferric pyrophosphate with emulsifiers had the highest score, therefore were the most promising ones. On the contrary, GFB-Fe fortified with ferrous L-lactate received a score lower than 3, corresponding to an unacceptable quality with no desirable characteristics.

Variability in scores obtained could be explained by the fact that the panellists used were not strongly motivated tasting such products. This means, that they were not celiac disease suffers, who are more aware of such products. Many of them found that both aroma and flavour of samples were sharp, a fact that could influence their judgement for the other properties as well.

Table 1
Sensory attributes tested in GFB and GFB-Fe fortified with selected iron compounds by a panel of 11 trained volunteers[a]

Attributes tested	GFB	GFB-Fe fortified with selected iron compounds						
		Ferric pyrophosphate with emulsifiers	NaFeEDTA	Ferrous bis-glycinate	Electrolytic iron	Ferrous lactate	Ferric pyrophosphate	Ferrous gluconate
Aroma	4.7 ± 1.8 ab	6.4 ± 2.3 b	6.1 ± 2.4 b	5.1 ± 2.6 ab	5.8 ± 2.8 ab	3.6 ± 2.9 a	5.1 ± 2.5 ab	5.4 ± 3.5 ab
Chewiness	4.1 ± 3.1 a	6.5 ± 3.0 b	5.9 ± 1.5 ab	4.3 ± 2.7 ab	5.7 ± 2.3 ab	4.5 ± 3.0 ab	5.1 ± 2.3 ab	5.3 ± 2.6 ab
Firmness	6.2 ± 1.8 a	4.9 ± 3.1 a	5.2 ± 2.8 a	5.8 ± 1.8 a	5.4 ± 2.3 a	5.0 ± 1.5 a	5.1 ± 2.1 a	5.1 ± 1.9 a
Crumb colour	7.0 ± 1.8 bc	7.1 ± 1.8 bc	6.0 ± 2.1 b	2.2 ± 0.7 a	6.7 ± 1.6 bc	1.1 ± 0.6 a	7.9 ± 1.2 c	1.3 ± 0.9 a
Taste	4.3 ± 1.9 bc	6.3 ± 2.2 d	4.7 ± 2.1 bcd	5.0 ± 1.7 bcd	3.4 ± 2.1 ab	2.2 ± 2.5 a	5.3 ± 1.9 cd	5.9 ± 2.9 cd
Overall quality	5.1	6.3	5.3	5.0	4.9	2.9	5.7	4.8

[a] Means ± standard deviation for 11 measurements. Values with different letters are significantly different: $P < 0.05$.

Some representative images are presented in Fig. 2. Crumb structure is different from that of a white bread containing gluten. Air pore coalescence seems to take place resulting in large pores of uneven shape. This was especially evident in unfortified samples. GFB and GFB-Fe present some differences accordingly to their grain characteristics. Larger pores were found in case of unfortified GFB (data not shown). According to an image analysis programme, samples fortified with ferric pyrophosphate with emulsifiers or with ferrous lactate (Fig. 2B and C) present a smaller average pore size than unfortified GFB samples. Additionally, samples of GFB-Fe fortified with ferrous lactate (Fig. 2C) had a greater number of smaller pores than GFB-Fe fortified with ferric pyrophosphate with emulsifiers, but both had similar average air pore area (data not shown).

A comparison of the concentration of ferrous dialyzable and total dialyzable iron in gluten-free bread fortified with different iron compounds is presented in Fig. 1.

Ferrous dialyzable iron in GFB-Fe products fortified with ferric pyrophosphate with emulsifiers, NaFeEDTA, ferrous bis-glycinate, ferrous gluconate or ferrous sulphate was higher than that in GFB-Fe fortified with electrolytic iron, ferrous lactate or ferric pyrophosphate ($P < 0.05$).

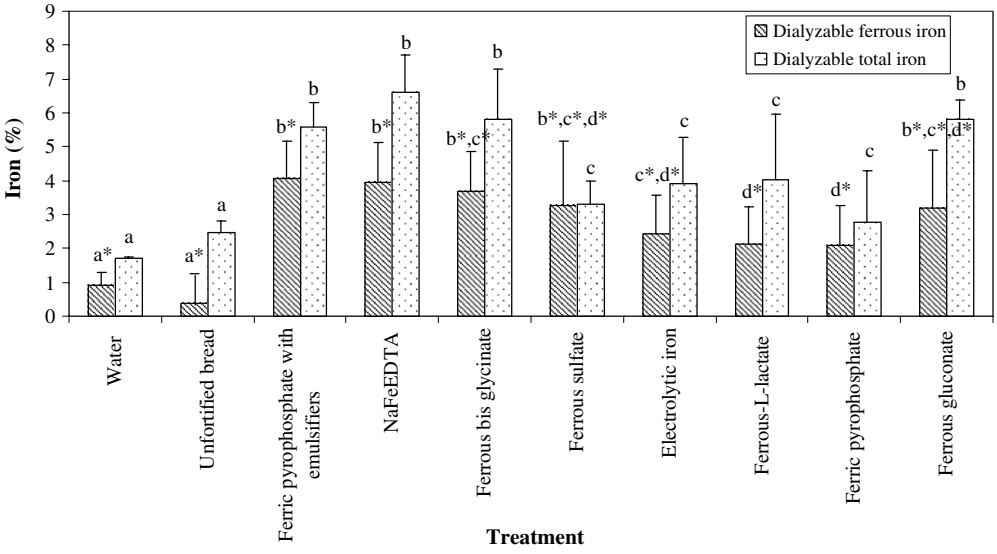

Fig. 1. Dialyzable (ferrous and total) iron formed after *in vitro* digestion in samples of gluten-free bread fortified with various iron forms. Results are expressed as percentage of iron before incubation. Means ± standard deviation for four experiments. Values with different letters are significantly different: $P < 0.05$. The letters with an asterisk refer to comparisons on dialyzable ferrous iron while letters without an asterisk refer to comparisons on total dialyzable iron.

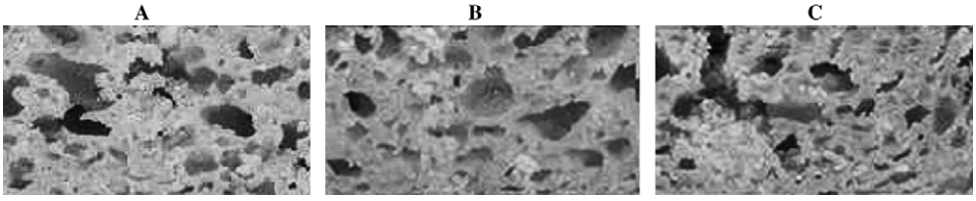

Fig. 2. Photographs of crumb structure in gluten-free breads (GFBs). (A) unfortified GFB (B) GFB-Fe fortified with ferric pyrophosphate with emulsifiers (C) GFB-Fe fortified with ferrous lactate.

Similar results were obtained for total dialyzable iron (Fig. 1).

4. Discussion

The first finding emerging from the present study was that the iron compounds that were used for the formulation of GFB-Fe products affected the sensory characteristics of GFB in a different manner. Some iron compounds had a positive effect while others had a negative effect on the sensory properties of GFB (Table 1). Ferric pyrophosphate with emulsifiers produced the most acceptable product.

Iron has been known to affect the sensory properties of a fortified food, particularly taste and colour, which is a critical characteristic for crumb appearance (Bovell-Benjamin & Guinard, 2003; Hurrell et al., 2004). Iron fortification, however, is suggested as a means to increase iron intake (Centers for Disease Control & Prevention, 1998). Therefore, it is adopted as a strategy for the control of iron deficiency worldwide, but only after a careful evaluation of the effect of iron compounds on the sensory properties of a food (Bovell-Benjamin & Guinard, 2003). As a result of this integrated approach, iron fortification of staple foods such as wheat bread or rice has been applied successfully in national food fortification programs (Dary, 2002; Hurrell, 2002). Ferrous sulphate and elemental iron have been suggested for the fortification of white wheat bread (Walter, Pizarro, Abrams, & Boy, 2004). Other iron compounds such as iron gluconate (Salgueiro et al., 2005) or NaFeEDTA (Hurrell et al., 2004; Kloots, Op den Kamp, & Abrahamse, 2004) have been studied as well. The fortification of GFB, however presents further challenges. In general the taste of a GFB is inferior to that of white wheat bread, therefore the moderate scores obtained were typical for GFB and were considered acceptable for such products (Berti, Riso, Monti, & Porrini, 2004). Moreover, the GFB-Fe developed for this study was a frozen product. In these frozen formulations the iron compound remained in the aqueous environment of the dough for longer time than it would in a fresh white wheat bread product. In this experiment the fortified products in the form of dough remained at −18 °C for 10 days before they were baked and tested. Differences in reactivity of the iron compounds in the physicochemical environment of the dough or of baked bread, may explain the observed differences in scores assigned to the different formulations. However, it is difficult to explain that some GFB-Fe samples were assigned better scores than GFB. In the case of ferric pyrophosphate with emulsifiers the scores were significantly higher than those of GFB in most attributes tested. This suggests that iron fortification by this compound improves some sensory characteristics of GFB. A plausible explanation may be that the emulsifier used in this formula led to a good moisture distribution in the crumb and a desirable aerated structure. It was observed that the crumb of this product had medium size air pores of good uniformity. Furthermore its grain characteristics (pore size and distribution) had the greatest similarities to those of unfortified sample. These observations suggest that further investigation of the physicochemical characteristics of the GFB-Fe is needed as it may reveal important effects of iron compounds on the properties of the baked products. Furthermore it may explain differences noted by the trained panellists.

The second finding of the present study refers to the ferrous and total iron dialyzability measured in the GFB-Fe under conditions of *in vitro* digestion. The evaluation of the various iron compounds included measurements of ferrous dialyzable and total (ferrous and ferric) dialyzable iron. These indices for the prediction of iron bioavailability have been reported in the literature. Total dialyzable iron was initially proposed in the *in vitro* model employed herein (Forbes et al., 1989; Hazell & Johnson, 1987; Miller & Berner, 1989; Schricker, Miller, Rasmussen, & Van Campen, 1981; Whittaker, Spivey Fox, & Forbes, 1989). However, ferrous dialyzable iron has been evaluated as a better index to total dialyzable iron because it exhibits better correlation with results on iron uptake by cells from various food environments (Glahn & Van Campen, 1997) and with data on iron absorption by humans (Kapsokefalou & Miller, 1991). Although solely a predictor and not a true measurement of iron bioavailability, formation of ferrous dialyzable iron provides information on bioavailability because it depicts the affinity and interaction of iron with various dietary factors and their digestion products under conditions that mimic digestion. Solubility of ferrous or total iron is a less reliable index of iron bioavailability. Nevertheless it provides information on the chemical transformations of iron that may occur during digestion. Herein, conclusions on the prediction of iron bioavailability are, therefore, based on results on ferrous dialyzable iron. Total dialyzable iron is reported as well, to provide information on the chemical behavior of the iron fortificants in these samples digested *in vitro*.

There have been no previous studies on the bioavailability of iron in fortified gluten free bread. On the contrary, the bioavailability of various iron compounds have been studied systematically in water or in many different food environments (Brise & Hallberg, 1962; Hurrell, 2002). The fortification of cereal bread has been extensively studied mainly because of the central role of fortified cereal products in many national fortification programs (Hurrell et al., 2004). Numerous studies have been conducted to optimise the conditions of fortification, including the selection of the most economically feasible, most organoleptically and technologically acceptable and most bioavailable compound (Mannar & Gallego, 2002). Ferrous sulphate and electrolytic iron are most common iron sources used in the fortification of cereal food staples (Hurrell et al., 2004). However, the ingredients of GFB are different than those of cereal bread. In this study, GFB contained rice flour, maize starch, potato starch, locust bean gum, egg albumin, vegetable fat and emulsifiers. Therefore suggestions/guidelines for cereal bread may not

apply for GFB. For this reason, a series of iron compounds was tested instead of the two that are proposed for the fortification of cereal bread (i.e. ferrous sulphate or electrolytic iron, Hurrell et al., 2004). Results of this study suggest that other iron compounds may be more appropriate for use in GFB, when iron is the only mineral fortificant. In addition to our finding on the sensory properties of GFB-Fe, bioavailability predicted by this *in vitro* model of ferric pyrophosphate with emulsifiers, NaFeEDTA and ferrous bis-glycinate, ferrous gluconate, ferrous lactate was higher than that of ferrous sulphate or electrolytic iron (Fig. 1). There are numerous studies on the absorption of these iron compounds (Lysionek et al., 2001; Olivares et al., 1997; Sakaguchi, Rao, Nakata, Nanbu, & Juneja, 2004; Trinidad et al., 2002). Among the iron compounds tested, ferric pyrophosphate with emulsifiers and NaFeEDTA had the highest formation of ferrous and total dialyzable iron. The absorption of these compounds from various foods has been studied by others. For example, ferric pyrophosphate with emulsifiers, added in an infant cereal and a yoghurt drink, was as well absorbed as ferrous sulphate in adults (Fidler et al., 2004). This compound is of particular interest because it provided a GFB-Fe with good sensory characteristics. There are various reports on the absorption of NaFeEDTA suggesting that this is a promising iron compound particularly in food that are rich in inhibitors of iron bioavailability (Bothwell & MacPhail, 2005; Hurrell, Reddy, Burri, & Cook, 2000; Hurrell et al., 2004; Thuy et al., 2003; Trinidad et al., 2002). However, various aspects of the physiology of iron that is delivered through NaFeEDTA have been under investigation (Yeung, Zhu, Glahn, & Miller, 2005).

It is not possible to conclude on the absorption of added iron to GFB on the basis of iron dialyzability reported herein. Although studies on human subjects may be required, these data are encouraging for further investigations targeting to the development of GFB-Fe with high iron bioavailability.

5. Conclusions

A series of iron compounds was tested for the production of gluten free bread fortified with iron. Ferric pyrophosphate with emulsifiers was the iron compound that produced the most acceptable iron fortified gluten free bread. The predicted bioavailability of this compound, based on the ferrous iron dialyzability, was similar or higher than other iron compounds tested. Overall, the attempt of producing iron-fortified gluten free bread was successful; the bread produced had satisfactory sensory and nutritional characteristics. This encourages further research for developing such products and for testing them in humans, particularly gluten sensitive individuals.

6. Uncited reference

Miller, Schricker, Rasmussen, and Van Campen (1981).

Acknowledgements

The authors thank Prof. E. Berghofer and Dr. R. Schoenlechter in BOKU (University of Natural Resources and Applied Life Sciences, Department of Food Science and Technology, Vienna, Austria) for their contribution in the development of the final recipe of GFB and for their help in providing some ingredients used in GFB recipe. The authors also thank the producers of iron compounds, mentioned in Section 2 for donating samples.

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