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Determination of visual detection thresholds of selected iron fortificants and formulation of iron-fortified pocket-type flat bread

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Wheaten pocket-type flat breads were baked from refined flours fortified to contain iron from ferrous sulphate ($FeSO_4$), hydrogen-reduced elemental iron (Fe) or sodium iron ethylenediaminetetraacetic acid (NaFeEDTA). Individual and group visual detection thresholds were determined by the 3-alternative forced choice (3-AFC) test according to the American Society of Testing and Materials (ASTM) method E-1432. The group visual detection thresholds of $FeSO_4$, NaFeEDTA and Fe in bread were established in samples baked from the corresponding wheat flours fortified with 69.46, 236.82 and 304.97 mg iron/kg flour, respectively. Sensory testing showed that iron-fortified pocket-type flat breads were similar ($P < 0.01$) to regular bread when baked from flours formulated to contain iron at levels lower by 25% than the group visual detection thresholds of Fe and $FeSO_4$ and two 25% increments lower than the threshold of NaFeEDTA. These findings indicate that iron-fortified pocket-type flat breads, which are sensorially similar to regular bread, baked from flours that contained 52.1, 133.22 and 228.73 mg iron/kg flour as $FeSO_4$, NaFeEDTA and Fe, would provide the segment of the population at the highest risk of iron-deficiency anaemia, specifically women of childbearing age, with 91%, 207% and 346% of their recommended daily intakes for iron, respectively.

1. Introduction

Iron deficiency is the most common nutritional deficiency affecting nearly 20% of the world's population with the groups mostly at risk being young children and women of reproductive age (Martínez-Navarrete et al., 2002). Chronic iron deficiency leads to iron-deficiency anaemia (IDA). An estimated 200 million people in the Eastern Mediterranean might be suffering from anaemia with iron deficiency constituting one of the factors responsible for this high incidence of anaemia (WHO/UNICEF/MI, 2003). Data on the incidence of IDA in Eastern Mediterranean countries are sketchy or, at best, difficult to locate. However, the prevalence of IDA in the Eastern Mediterranean region has been reported to range between 15% and 74% in preschool children, 18–41% among women of childbearing age and 14–45% among pregnant women (Muwakkit et al., 2008; WHO, 2004).

Insufficient iron intake and low iron bioavailability are the main contributors to the high incidence of iron deficiency (Hansen et al., 2005). In developing countries, however, the major causative factor is low iron absorption (Hurrell, 1997) because diets are largely based on cereals and legumes which contain high levels of phytate, a potent inhibitor of non-haem iron absorption (Hernández et al., 2003). Intervention strategies to reduce the prevalence of iron deficiency include supplementation, dietary diversification, and food fortification with fortification being regarded as the most cost-effective long-term approach (Hurrell, 1997).

The potential efficiency of dietary fortification is determined by the iron status of the target group, total iron intake, selection of the food vehicle, bioavailability of the iron, and the balance of inhibitors and enhancers of iron absorption in the diet (Tetens et al., 2005). The most important consideration in any successful food fortification program, however, is the combination of the iron fortificant and the food vehicle. The fortified food must be acceptable to and consumed by the target population, must provide iron in a stable and highly bioavailable form and the chosen fortificant must not adversely affect the organoleptic qualities and shelf-life of the food vehicle (Martínez-Navarrete et al., 2002). The vehicle used in fortification programs is determined by the locally available foods and the dietary patterns of the target populations. To this end, a number of vehicles have been investigated including rice, milk,

Abbreviations: 3-AFC, 3-alternative forced choice; CV, coefficient of variation; Fe, elemental iron; $FeSO_4$, ferrous sulphate; IDA, iron-deficiency anaemia; NaFeEDTA, sodium iron ethylenediaminetetraacetic acid.

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salt, sugar, soy sauce, fish sauce and fruit juices with wheat and maize flours being the most used in iron-fortification programs (Huma et al., 2007; Sadighi et al., 2008; Tazhibayev et al., 2008; WHO/FAO, 2006). Wheaten pocket-type flat bread, known as Pita bread in Europe and North America, is the main dietary staple of the people in the Eastern Mediterranean region. This two-layered flat bread is regularly consumed with meals and also serves as a carrier for other foods consumed during the meal. The bread can be filled with food, as practiced in Europe and North America, topped with food and rolled to a cylinder-like sandwich or cut into pieces and shaped like scoops to carry food into the mouth. The relatively low cost and widespread consumption of wheat flour bread (Arredondo et al., 2006) render pocket-type flat bread a particularly appealing vehicle for iron-fortification programs in the region.

A number of iron compounds could potentially be used as food fortificants with ferrous sulphate ($FeSO_4$) and hydrogen-reduced elemental iron (Fe) being the most used in the fortification of maize and wheat flours (Huma et al., 2007; WHO/FAO, 2006). Sodium iron ethylenediaminetetraacetic acid (NaFeEDTA) is also being increasingly used in fortification of wheat flour due to its high bioavailability from phytate-containing food matrices (Hurrell, 2002; WHO/FAO, 2006). Iron fortificants are known to impart colour tints, ranging from grey to green, as well as textural changes and off-flavours to bread (Alam et al., 2007; Le et al., 2007; Morales et al., 2008; Rehman et al., 2006; Richins et al., 2008; Van Stuijvenberg et al., 2008). Since iron-fortified foods are usually rejected by consumers due to unacceptable discoloration and flavour and textural changes (Bovell-Benjamin and Guinard, 2003), the levels at which iron fortificants could be added to foods without imparting unacceptable sensory changes need to be established for any successful iron-fortification program (WHO/FAO, 2006).

In common with other breads, colour and other sensory changes are expected to take place in pocket-type flat bread upon fortification with Fe, $FeSO_4$ or NaFeEDTA. To safeguard against sensory changes in the bread, the iron fortificants must be added at levels below their detection threshold (Ziadeh et al., 2005). Lim and Lawless (2006) determined the taste detection thresholds of $FeSO_4$, ferrous chloride and ferrous gluconate in deionised water. However, so far, no studies have reported on the sensory thresholds of the different iron fortificants used in wheat flour breads. Colour is the first perceived attribute of foods and is very often used by consumers as a cue about flavour, quality and safety of foods (Emerton, 2008). Accordingly, the iron fortificants should be added below their visual detection thresholds before testing whether such levels will induce other sensory changes in the fortified bread. Differences in the sensory properties of food products may be determined by a small group of trained subjects under laboratory settings, although different values might result under actual consumption conditions by the public depending on the proportion and sensitivity of discriminators in the population. Furthermore, the iron-fortified bread should, ideally, exhibit same/similar sensory characteristics as the regular bread to minimize the possibility of its rejection by the target population. To this end, testing for similarity, which utilizes a relatively large panel in conjunction with specifying a proportion of discriminators in order to satisfy statistical hypothesis testing, has the potential for identifying products that are judged as exhibiting the "same" characteristics (Bi, 2005; Meilgaard et al., 2007). Consequently, to minimize the possibility of rejection of the iron-fortified bread by the consumers due to changes in sensory characteristics, it is imperative to determine the levels, at the threshold and/or lower-than-threshold values, at which the fortified bread would be judged as similar to its regular counterpart. The objectives of this study were to (1) determine the visual detection thresholds of $FeSO_4$, Fe and NaFeEDTA in pocket-type, wheaten flat bread and (2) establish the maximum levels at which these fortificants can be added to the flour to yield iron-fortified pocket-type flat breads with similar sensory properties to their regular counterpart.

2. Materials and methods

2.1. Materials

Food-grade $FeSO_4$, hydrogen-reduced Fe (Nutrafine™ RS; 325 mesh, particle size <45 μm), and NaFeEDTA (Ferrazone® FX) were obtained from Spectrum Laboratory Inc. (Gardena, CA, U.S.A.), Höganäs AB (Höganäs, Sweden) and Azko Nobel (Amersfoort, The Netherlands), respectively. Wheat flour of 78% extraction rate was obtained from Societe Industrielle du Levant S.A.L. (Beirut, Lebanon). Dry yeast (Vahiné; Monteux, France), salt and sugar were obtained from the local market.

2.2. Chemical analysis

Iron fortificants and bread samples were ashed and their iron contents were determined by flame atomic absorption spectrophotometry (SOLAAR Atomic Absorption Spectrophotometer, Thermo Labsystems, MA, U.S.A.) as described by Abebe et al. (2007). The accuracy of iron determination was assessed through analysis of a standard reference material (SRM) – wheat flour (SRM-1567a) – obtained from the National Institute of Standards and Technology (Gaithersburg, MD, U.S.A.) with average replicate analysis being 14.63 ± 1.2 μg/g (C.V. 8.5%) compared to the reference's certified value of 14.1 ± 0.5 μg/g.

2.3. Flour fortification and bread preparation

Threshold testing entails evaluation of a series of samples with consistent increase or decrease in concentration of a particular additive. The iron fortificants were added to regular flour to produce the highest concentration level; and lower concentration levels were obtained by diluting sub-samples, taken from the concentrated batch, with regular flour. In total, six batches of flour were fortified for each iron source, with iron concentrations ranging from 40 to 90 mg iron/kg flour for $FeSO_4$, and 90 to 500 mg iron/kg flour for Fe and NaFeEDTA. The American Society of Testing and Materials (ASTM) method used for threshold determination typically requires six or seven concentration levels or steps with each differing from the previous one by a factor between 2 and 4 (ASTM E-1432, 1997). Preliminary experiments indicated that dilution factors between 2 and 4 yielded excessively large differences between successive concentrations and were not conducive to accurate determination of the threshold values. Accordingly, because a factor between 2 and 4 could not be used, flours were fortified to contain iron from $FeSO_4$, Fe and NaFeEDTA at six ascending levels differing by logarithmic steps. For $FeSO_4$, the levels chosen were 40, 50, 60, 70, 80 and 90 mg iron/kg flour. Hydrogen-reduced Fe was added to produce levels of 90, 100, 200, 300, 400 and 500 mg iron/kg flour. NaFeEDTA was added to yield flours containing 90, 100, 200, 300, 400 and 500 mg iron/kg flour. The concentrated flour sample and the fortified samples prepared by dilution were sifted three times, using a common household flour sifter, in order to ensure uniform distribution of the iron.

Bread samples were prepared as described by Toufeili et al. (1999). Doughs contained flour (100 parts), sugar (2.4 parts), salt (1 part), yeast (0.8 parts) and deionised water (50 parts). The ingredients were mixed in a dough mixer (DITO SAMA, Model BM 20S, France) at low speed for 7 min until a smooth continuous dough was obtained. Doughs were then fermented at 40 °C for

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25 min, divided into balls weighing 75 g ± 3 g each and the dough balls were proofed at 40 °C for 25 min. The balls were flattened into sheets 1.7 mm thick, proofed at 40 °C for 15 min and baked at 500 °C to optimum crust colour (55 s to 1 min). The bread loaves were allowed to cool to room temperature, placed in polyethylene bags to prevent moisture loss, and stored at −20 °C until used. Bread loaves were prepared in batches with either 1000 or 2000 g flour. Under these conditions of dough preparation and baking, 20 bread loaves (mass 60.8 ± 1.55 g; diameter 19.4 ± 1.31 cm) were obtained from 1000 g of flour.

2.4. Determination of visual detection thresholds

Individual and group visual detection thresholds were determined by the 3-alternative forced choice (3-AFC) test according to ASTM method E-1432 (ASTM, 1997). Fourteen subjects (4 males and 10 females; 22–28 years of age) were trained for 2 one-hour sessions before serving on the panel. During training, the panellists were shown bread samples prepared from flours fortified with high concentrations of the iron fortificants, namely 300, 800 and 600 mg iron/kg flour as $FeSO_4$, Fe and NaFeEDTA, respectively, to note the colour tints imparted by the iron fortificants and were familiarised with the logistics of the 3-AFC test. Two of the panellists were dropped due to lack of motivation and data were obtained with the 12 panellists (3 males and 9 females; 22–28 years of age) who participated in the whole study. Bread loaves were taken out of the freezer, 2 h prior to testing, thawed and placed in 20 × 30 cm polyethylene bags which were then sealed and coded with 3-digit random numbers. At each level of the iron fortificant, the panellists were presented with three entire flat bread samples (two blanks and one containing the iron fortificant/stimulus) and asked to identify the sample containing the stimulus. The procedure was repeated for the 6 levels of each fortificant. The order of presentation based on left-to-right presentation of the blanks and fortified samples was counterbalanced to ensure that each of the possible sample combinations was presented an equal number of times. Sequential presentation of the 6 iron level sets was also randomized among panellists who received sample combinations chosen according to the laws of chance. This was done in order to account for bias which may be caused by the order of presentation (Meilgaard et al., 2007). The whole procedure was replicated 6 times and, thus, each panellist evaluated a total of 36 3-AFC presentations (one 3-AFC combination at each of the six concentrations and 6 replications) for each iron compound. At each fortificant level, the panellists were presented with bread loaves prepared from a single batch of flour in each replicate determination. Each panellist evaluated the bread samples with the 6 levels of one of the fortificants in a session. A total of 6 sessions were carried out on successive days for each iron compound. Testing was done on 18 days with one session being conducted in a day.

The data was analysed by logistic non-linear regression to determine the individual thresholds (Meilgaard et al., 2007). The model used was:

$$P = \frac{\frac{1}{3} + e^{B(T - \text{Log } X)}}{1 + e^{B(T - \text{Log } X)}} \quad (1)$$

where P = Proportion of correct identifications at each concentration in the six replicate determinations, B = Slope of the logistic curve, X = Concentration (mg/kg) of stimulus ($FeSO_4$, Fe and NaFeEDTA) in flour, and T = Threshold value in Log (mg/kg).

The parameters B and T were estimated by fitting the proportional correct identifications, in the six replications of the ascending forced choice tests to Equation (1) using the sequential quadratic programming option, with a maximum of 30 iterations, of the Non-linear Regression module of SPSS software (SPSS®16.0 for Windows, SPSS Inc., Chicago, U.S.A.). The group mean threshold was calculated as the geometric mean of the individual thresholds. The logistic curves of the individual panellists for the three iron compounds, corresponding to Equation (1), were plotted using Origin 6.0 data analysis and graphing software (OriginLab Corporation, MA, U.S.A.).

2.5. Overall difference and similarity testing

In statistical hypothesis testing, the conclusions drawn upon accepting/rejecting the null hypothesis are subject to the so-called Type I and Type II errors. The probabilities of making Type I and Type II errors are denoted by the α-risk and β-risk, respectively. In difference testing, the experiment is designed to have a low α-risk to reduce the probability of Type I error. In similarity testing, the experiment is designed to reduce the β-risk because the ramifications that result from making Type II error are more serious as it leads to the conclusion that the products are similar when, in fact, they are not. In the context of the present work, making a Type II error would lead to the conclusion that the iron-fortified bread will be judged, by the population, as being similar to the regular bread when in fact it is not. To reduce the probability of wrongly concluding that two products are similar sensorially, the null hypothesis is tested at a low level for the Type II error (β-risk), a moderate-to-large level for the Type I error (α-risk) and specifying a proportion of discriminators in the population. For the conditions of similarity testing to be satisfied a proportionately higher number of panellists, as compared to difference testing, is required (Lawless and Heymann, 1998; Meilgaard et al., 2007). To reduce the number of tests required to establish similarity, difference testing was first carried out to establish the levels at which no detectable differences between fortified and unfortified samples were observed by the panellists. Fortificant levels for difference testing were determined based on preliminary visual threshold testing. Similarity testing was then conducted on breads that contained iron at the levels at which no perceptible differences existed, and at lower levels if need be, until similarity was established between the iron fortified and regular breads.

2.5.1. Difference testing

Difference testing was carried out to determine whether a perceptible overall difference (i.e. difference in colour, odour, texture and/or taste) existed between the iron-fortified flat breads at the determined visual threshold level for each iron compound and regular non-fortified flat bread. Twenty-one panellists (7 males and 14 females; 20–57 years of age) evaluated bread samples made from flours fortified with $FeSO_4$, Fe and NaFeEDTA corresponding to the respective group detection thresholds (i.e. 69.46 mg iron/kg for $FeSO_4$, 304.97 mg iron/kg for Fe and 236.82 mg iron/kg for NaFeEDTA). When differences were detected between the regular bread and the iron-fortified counterparts, the concentrations corresponding to the threshold values were lowered in steps differing by 25% and the resulting fortified breads were tested for differences from non-fortified bread until levels at which no perceptible differences were reached. $FeSO_4$ was tested at a concentration of 69.46 mg iron/kg flour, Fe was tested at 304.97 mg iron/kg flour and NaFeEDTA at 236.82 and 177.62 mg iron/kg flour.

2.5.2. Similarity testing

Bread samples that contained iron at levels shown not to result in differences from the regular bread, were subjected to similarity testing in order to establish the levels at which iron-fortified breads are judged as being sufficiently similar to non-fortified bread to ensure that iron fortification would remain undetected by consumers. When similarity was not established, the levels

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determined not to cause differences from the control were lowered by increments of 25% and the resulting iron-fortified breads were subjected to similarity testing until the iron-fortified breads were judged as being similar to the regular bread. Sixty-six panellists (18 males and 48 females; 18–58 years of age) participated in this phase of the study. $FeSO_4$ was tested at concentrations of 69.46 and 52.1 mg iron/kg flour, Fe was tested at 304.97 and 228.73 mg iron/kg flour and NaFeEDTA at 177.62 and 133.22 mg iron/kg flour.

The triangle test was used for both difference and similarity testing. An hour prior to each evaluation session, bread samples were taken out of the freezer, thawed, cut into 2×5 cm rectangular strips and placed in 10×10 cm polyethylene bags that were then sealed and coded with 3-digit random numbers. Panellists were presented with three samples with half of the panellists receiving two regular bread samples and one iron-fortified sample while the other half received two iron-fortified samples and one regular bread sample. The panellists were asked to look at, smell and chew the breads and indicate the odd sample. Sample order was counterbalanced and randomized over sessions and subjects. Mineral water was provided for rinsing the palate between sample evaluations in order to avoid any carry-over effects. Sensory testing was carried out in partitioned booths equipped with daylight and paper ballots were used in all the sessions. Ten sessions, 4 for difference testing and 6 for similarity testing, were conducted with one session being held in a day.

The panellists for the threshold determinations, the difference and similarity tests were recruited from the Faculty of Agricultural and Food Sciences at the American University of Beirut and were not compensated for serving on the panel. The use of human subjects in the study was approved by the institutional review board of the American University of Beirut.

Difference testing was determined at an α-level of 5%. Similarity testing was conducted at an α-level of 0.2 (probability of concluding that a perceptible difference exists when it does not), a β-level of 0.01 (probability of concluding that no perceptible difference exists when it does) and a *Pd* of 0.3 (proportion of the population able to detect differences between samples). The significance of the test was ascertained by reference to tables of correct responses required to establish significance in the triangle test in difference and similarity testing (Meilgaard et al., 2007).

3. Results and discussion

Representative logistic curves fitted to an individual panellist's data and individual and group visual detection thresholds of bread samples baked from wheat flour fortified with $FeSO_4$, Fe or NaFeEDTA are shown in Fig. 1 and Table 1, respectively.

Breads baked from $FeSO_4$-fortified flours had the lowest visual group detection threshold at 69.46 mg iron/kg flour. The group detection threshold of breads baked from Fe-fortified flours was the highest at 304.97 mg iron/kg flour followed by the threshold of breads baked from NaFeEDTA-fortified flours (236.82 mg iron/kg flour) and the group mean threshold for breads made with $FeSO_4$-fortified flours (69.46 mg iron/kg flour) (Table 1). At high levels of fortification, $FeSO_4$ imparted a greenish tint, Fe a grey-like tint and NaFeEDTA a yellowish colour to the breads. A markedly narrower range of individual thresholds was observed for breads prepared from $FeSO_4$-fortified flours than the ranges obtained for breads baked from flours with added NaFeEDTA and Fe. This suggests that the greenish tint imparted to the bread by $FeSO_4$ is more easily discerned than the tint imparted to the bread by the other two iron compounds, thus limiting the maximal quantity of this iron source that can be used for fortification purposes. The higher group mean threshold values of breads baked from flours with added NaFeEDTA and Fe make them the preferred iron sources to consider when

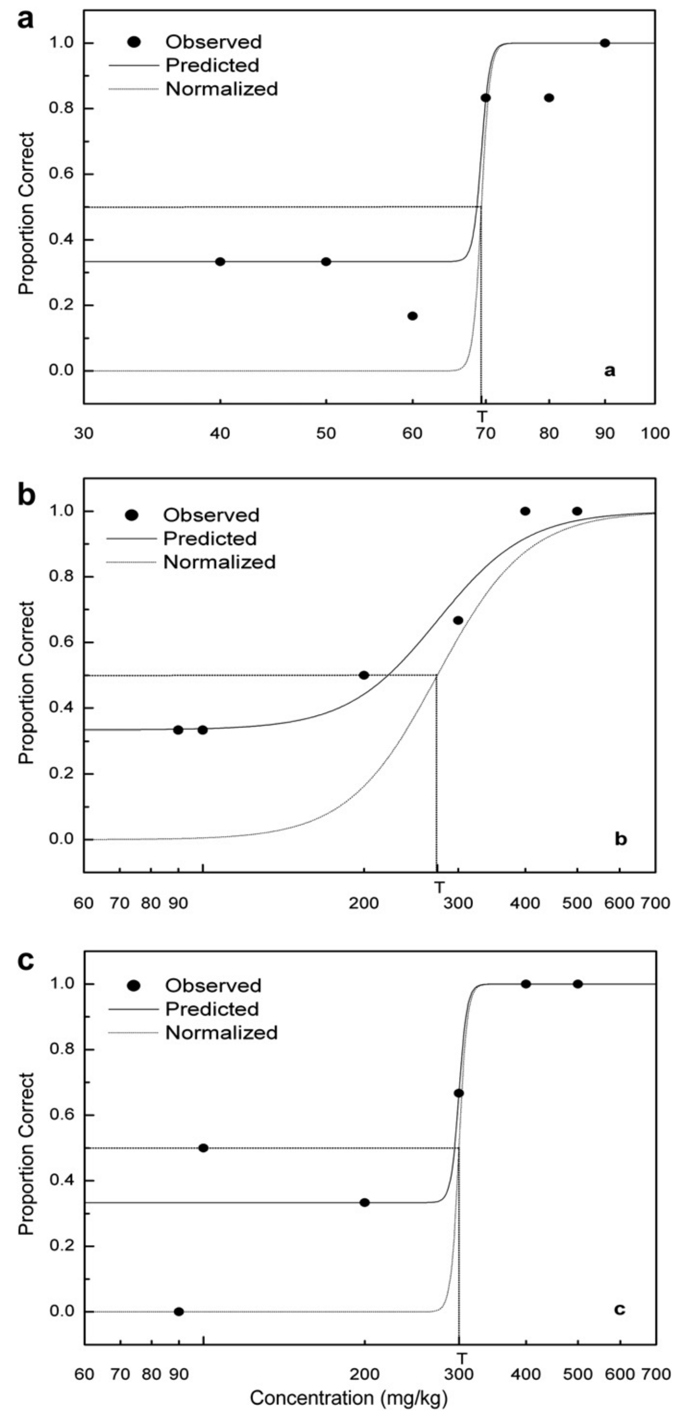

Fig. 1. Proportion of correct judgments by panellist number 9 at different concentrations of ferrous sulphate (a), elemental iron (b) and ferric sodium EDTA (c) in wheat flour pocket-type flat bread. Each point (●) is the proportion correct of six judgments in 3-Alternative Forced Choice (AFC) tests with one sample containing the indicated concentration of iron and the other samples having no added iron. The threshold value (T), 69.34 (a), 274.16 (b) and 299.92 (c), is the concentration that corresponds to 50% correct identifications on the normalized curves.

colour changes induced by fortificants to flat bread are of concern, with elemental iron constituting the better choice of the two. These findings are consistent with those reported by Richins et al. (2008) who showed that fortifying corn-flour tortillas with 40 mg iron/kg flour as $FeSO_4$ had a greater impact on colour and appearance of the samples compared to tortillas fortified with an equal amount of elemental iron. A dull-greyish colour has been reported for breads

Table 1
Individual and group visual detection thresholds for ferrous sulphate, hydrogen-reduced elemental iron and sodium iron EDTA.

Panelist number	Individual threshold (mg iron/kg flour)[a]		
	Ferrous sulphate ($FeSO_4$)	Elemental iron (Fe)	Sodium iron EDTA (NaFeEDTA)
1	61.66	207.49	94.41
2	69.18	291.74	399.95
3	80.91	300.61	172.19
4	80.91	191.87	374.97
5	70.96	545.76	172.58
6	67.92	180.30	339.63
7	55.34	318.42	313.33
8	57.68	337.29	334.97
9	69.34	274.16	299.92
10	69.98	434.51	372.39
11	82.79	490.91	187.07
12	73.11	299.92	99.31
Group mean threshold (mg iron/kg flour)	69.46	304.97	236.82
Standard log deviation	0.056	0.152	0.224

[a] Determined by logistic regression of percentage correct identifications in six replicate presentations of six stimulant-containing bread samples as per the ASTM method E-1432.

prepared from high-extraction wheat flour fortified with NaFeEDTA at the rate of 10 mg/kg flour while Fe caused no changes in bread colour when added at a level of 35 mg/kg flour (Van Stuijvenberg et al., 2008). The results of the present work lend further support to the suggestion that elemental iron is the iron fortificant of choice when the objective is to minimize colour changes in iron-fortified staples.

In breads prepared from flours with iron at the obtained group threshold values, both $FeSO_4$- and Fe-fortified breads were perceived as being not different ($P > 0.05$) from non-fortified control breads while NaFeEDTA-fortified bread differed significantly ($P < 0.05$) from the regular bread. This finding indicates that NaFeEDTA causes changes in the sensory properties of bread beyond visual effects when added at the level of its visual detection threshold of 236.82 mg iron/kg flour. Consequently, the level of flour fortification with NaFeEDTA was further lowered by 25% at which level the iron-fortified bread was judged as being not significantly different ($P > 0.05$) from the control.

In similarity testing, triangle tests indicated that iron-fortified bread samples were similar ($P < 0.01$) sensorially to their non-fortified counterparts when baked from flours fortified with Fe, $FeSO_4$ and NaFeEDTA at levels 25% lower than those established not to cause differences. More specifically, iron-fortified breads were found to have similar organoleptic properties to their non-iron fortified counterparts when baked from flours that contained 52.1, 133.22 and 228.73 mg iron/kg flour as $FeSO_4$, NaFeEDTA and Fe, respectively. These levels represent the maximum iron concentrations which can be added to flour intended for the preparation of pocket-type flat bread without affecting the sensory qualities of the baked product. The calculated 99% CI indicate that pocket-type flat bread can be baked from flour fortified with 52.1 mg/kg flour of iron in the form of $FeSO_4$, 133.22 mg/kg flour of iron in the form of NaFeEDTA and 228.73 of iron in the form of Fe with 99% confidence that no more than 10%, 25% and 23% of the population will be able to detect differences between the iron-fortified breads and the regular bread, respectively. Fe again proved to be the superior fortificant from a sensory standpoint, with the capacity of being added to wheat flour at levels 4.4 and 1.72 times higher than $FeSO_4$ and NaFeEDTA, respectively, without imparting any sensory changes to the bread. Elemental iron's low potential to cause adverse organoleptic changes, attributed to its water-insoluble character, coupled with its relatively low cost are largely responsible for its use in world-wide cereal fortification programs.

At the aforementioned levels of flour fortification, pocket-type flat bread will provide 5.07 ± 1.03, 19.3 ± 0.47 and 11.54 ± 0.31 mg iron/100 g bread when prepared from $FeSO_4$-, Fe- and NaFeEDTA-fortified wheat flours, respectively. The per capita consumption of bread in the Eastern Mediterranean has been estimated at 323 g/day (WHO, 2003). At this level of consumption, fortified bread would provide 16.38, 62.34 and 37.27 mg iron/day when baked from flours fortified with $FeSO_4$, Fe and NaFeEDTA, respectively. With recommended intakes of 18 mg iron/day for women 19–50 years of age (Food and Nutrition Board, 2000), breads fortified with $FeSO_4$, Fe and NaFeEDTA would provide 91%, 346% and 207% of the recommended daily intakes for iron to women in the Eastern Mediterranean, respectively.

Notwithstanding the overriding priority not to cause sensory changes in the product, the fortificant must exhibit high bioavailability from the food matrix and be of reasonable cost. *In vivo* and *in vitro* studies suggest that Fe is at best 65% as available as $FeSO_4$ and that iron from NaFeEDTA is 2–4 times more available than iron in the form of $FeSO_4$ from wheat based staple foods (Hurrell et al., 2000; Sun et al., 2007; Walter et al., 2004). However, the cost of elemental iron is lower than those of $FeSO_4$ and NaFeEDTA (Hurrell, 2002). The low cost of Fe, the absence of sensory changes when added at a level 1.72 times higher than NaFeEDTA, and the potential inhibitory effect of Fe on mould growth during storage of flour (Akhtar et al., 2008) might offset the relatively low bioavailability of Fe and render it an attractive candidate for iron-fortification programs of pocket-type flat breads.

4. Conclusions

Visual detection thresholds for $FeSO_4$, NaFeEDTA, and Fe were determined to be 69.46, 236.82 and 304.97 mg iron/kg flour, respectively, in pocket-type flat breads prepared from iron-fortified flours. Similarity testing showed that breads baked from flours fortified at levels of 52.1, 133.22 and 228.73 mg iron/kg flour as $FeSO_4$, NaFeEDTA and Fe, respectively, were judged as being similar to regular bread ($P < 0.01$). These results suggest that hydrogen-reduced elemental iron is the compound of choice for wheat flour fortification. The advantages of using hydrogen-reduced elemental iron in fortification programs include its low impact on the organoleptic properties of pocket-type flat bread, its low cost and its potential for extending the microbiological stability and shelf-life of whole wheat flour. Studies on iron bioavailability of hydrogen-reduced elemental iron in pocket-type flat bread are warranted.

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