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Effect of Iron Source on Color and Appearance of Micronutrient-Fortified Corn Flour Tortillas

A. T. Richins, K. E. Burton, H. F. Pahulu, L. Jefferies, and M. L. Dunn

ABSTRACT

Iron deficiency anemia is a widespread occurrence. Consequently, iron is commonly added in cereal fortification programs. However, many iron sources cause undesirable sensory changes, especially color changes, in the food being fortified. This study evaluated the effect of different iron sources on CIE $L^*a^*b^*$ color values and sensory color perception in fortified corn tortillas. Corn masa flour was fortified with micronutrient premix containing vitamins, zinc, and one of eight iron compounds. Iron sources included ferrous fumarate (F), ferrous sulfate (S), ferric orthophosphate (OP), ferrous lactate (L), ferrous gluconate (G), ferric pyrophosphate (PP), sodium iron (III)-EDTA, and A-131 electrolytic iron (E), with addition levels adjusted based on bioavailability. Control (Ct) samples were prepared with all micronutrients except iron. All iron-fortified tortillas had lower $L^*$ values and were significantly darker than control tortillas. Based on instrumental color values and Mexican regulatory recommendations, five treatments were selected for further testing. A difference-from-control sensory test was conducted comparing PP, E, OP, F, and S with Ct tortillas. Sensory rankings were Ct $>$ E $>$ PP $>$ OP $>$ F $>$ S. A-131 electrolytic iron is recommended for fortification of corn tortillas due to minimal effect on color and significantly lower cost than other iron sources evaluated.

Iron deficiency has been classified as the most prevalent micronutrient deficiency in the world (Dary et al. 2002; Looker et al. 2002). Inadequate iron intake leads to anemia in young children, adolescents, and women. And it leads to significant irreversible developmental delays and cognitive and motor deficits in infants, children, and adolescents (Looker et al. 2002). Because of the widespread nature of iron deficiency, iron is considered a basic component in most food-fortification programs (Lynch 2005). Iron fortification of wheat flour is compulsory in Mexico (Secretaria de Salud 1996). However, a large percentage of the Mexican population consumes only minimal amounts of wheat-flour products. This consumption pattern is especially true among the portions of the population more nutritionally at risk, where the corn tortillas constitute 60–90% of cereal product intake (Villalpando 2004). Consequently, a 2005 regulatory proposal from the Mexican Comisión Federal para la Protección Contra Riesgos Sanitarios (COFEPRIS) included corn flour and nixtamalized corn flour as additional vehicles for mandatory fortification (Secretaria de Salud 2005). The initial proposal specified that iron be derived from ferrous sulfate or ferrous fumarate. Due to significant concern from industry regarding the potential negative impact on color and stability associated with these more reactive iron sources, the proposal was modified to allow the use of other iron sources, as long as addition rates are adjusted to deliver a bioavailable amount of iron equivalent to that provided by ferrous sulfate or ferrous fumarate. The micronutrient levels in the proposed regulations are shown in Table I.

Hurrell (2002) reported that iron is the most problematic mineral to add to foods. Many of the compounds used as iron fortificants cause unacceptable color and flavor changes in the foods that are being fortified. For a fortification program to be successful, it is important that the combination of the fortificant and the vehicle are acceptable to the target population (Bovell-Benjamin and Guinard 2003). This requirement largely relates to sensory properties of the fortified food but also includes economic viability and efficacy or bioavailability.

A variety of iron forms with widely varying bioavailabilities and sensory effects have been evaluated in fortified foods. Moretti et al. (2005) evaluated a number of different iron sources as fortificants in extruded rice grains. They found that the only iron source that did not cause significant color changes in the finished product was ferrie pyrophosphate, whereas ferrous sulfate (NaFe EDTA) and electrolytic iron all had negative effects on color. The reduced iron, ferric ammonium citrate, ferrous sulfate, ferrous chloride, and ferrous gluconate had no significant effect on color in Arabic bread when added at Fe levels $\leq 50$ mg/lb (110 mg/kg) (Mohammad and Hallab 1973). Ferrous sulfate did significantly affect the color of flat bread (Alam et al. 2007). Unfortified maize porridge had a brighter yellow color than porridge fortified with ferrous sulfate, ferrous bisglycinate, ferrous trisglycinate, or iron-EDTA (Bovell-Benjamin et al. 1999). Rosado et al. (2005) examined the effect of micronutrient addition to corn flour tortillas and found that addition of iron in the form of elemental reduced iron did not cause tortilla color changes. However, Burton et al. (2008) found that fortification of nixtamal corn tortillas with a micronutrient premix containing ferrous fumarate caused tortillas to be darker than unfortified control tortillas according to CIE light-dark ($L^*$) values. Preliminary studies in tortilla mills in Mexico indicated that millers had adverse reactions to observable color differences in fortified tortillas when using ferrous fumarate as a fortificant (Dunn et al. 2007).

The objective of this research was to evaluate the effects of different iron sources on the color and appearance of fortified corn tortillas when added at levels designed to yield approximately equivalent absorption based on bioavailability levels indicated in the literature.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Min Level (mg/kg)$^b$</th>
<th>Recommended Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid</td>
<td>2</td>
<td>Folic acid</td>
</tr>
<tr>
<td>Iron</td>
<td>40</td>
<td>Sulfate or fumarate$^c$</td>
</tr>
<tr>
<td>Thiamin</td>
<td>5</td>
<td>Thiamin mononitrate</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>3</td>
<td>Riboflavin</td>
</tr>
<tr>
<td>Niacin</td>
<td>35</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Zinc</td>
<td>40</td>
<td>Zinc oxide$^c$</td>
</tr>
</tbody>
</table>

$^a$ Norma Oficial Mexicana PROY-NOM-000-SSA1-2005.

$^b$ Minimum level of addition in flour.

$^c$ It is permissible to use other sources of iron and zinc as long as the amount added delivers a bioavailable amount equivalent to the recommended sources.

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MATERIALS AND METHODS

Materials
Corn tortillas were made from unfortified, instant corn masa flour (CMF) (Quaker, Chicago). Supplemental vitamins and zinc were added through a micronutrient premix consisting of: zinc oxide, riboflavin USP, thiamin mononitrate, folic acid, and nicotinic acid at levels indicated in Table I. Premix was provided by DSM Nutritional Products México, El Salto, Jalisco, México. Ferrous fumarate (F), ferrous sulfate (S), ferric orthophosphate (OP), ferrous lactate (L), ferrous gluconate (G), and sodium iron (III)-EDTA (EDTA) were also provided by DSM. Other iron sources evaluated included ferric pyrophosphate (PP) (Paul Lohmann, Emmerthal, Germany) and A-131 electrolytic iron (E) (Research Products Co., Salina, KS).

Tortilla Preparation
Tortillas for initial colorimetric evaluation were prepared in a laboratory by adding the micronutrient premix and an individual iron source to 220 g of CMF in the amounts listed in Table II. Iron source addition was adjusted to provide an approximately equivalent absorbable dose of iron (40 mg/kg of flour), based on estimates of bioavailability available in the literature (Hurrell 1999; Hurrell et al 2002; Walter et al 2003; Bothwell and MacPhail 2004; Hurrell et al 2006). A control treatment was also prepared by adding the micronutrient premix without iron. The dry ingredients were mixed for 5 min using a bench-top planetary mixer with a dough-hook attachment. The dry ingredients were then added through a micronutrient premix consisting of: zinc oxide, riboflavin USP, thiamin mononitrate, folic acid, and nicotinic acid at levels indicated in Table I. Premix was provided by DSM Nutritional Products México, El Salto, Jalisco, México. Ferrous fumarate (F), ferrous sulfate (S), ferric orthophosphate (OP), ferrous lactate (L), ferrous gluconate (G), and sodium iron (III)-EDTA (EDTA) were also provided by DSM. Other iron sources evaluated included ferric pyrophosphate (PP) (Paul Lohmann, Emmerthal, Germany) and A-131 electrolytic iron (E) (Research Products Co., Salina, KS).

Iron Source | Amount (mg/kg) |
---|---|
Control (none) | 0 |
Ferrous sulfate | 133.33 |
Ferrous fumarate | 121.21 |
Ferric orthophosphate | 336.84 |
Ferric orthophosphate | 501.25 |
Ferric orthophosphate | 140.35 |
A-131 electrolytic iron | 82.47 |
Ferrous lactate | 198.61 |
Ferrous gluconate | 374.53 |
NaFe EDTA | 130.65 |

*a Approximate addition levels required to deliver bioavailable amount equivalent to 40 mg of iron/kg of corn flour.
* Based on specification sheets or Hurrell (1999).
* Hurrell (1999). Where ranges were given, midpoint in the range was used.
booths under fluorescent lighting. Panelists were instructed to hold each coded sample next to the control, evaluating the overall color and shade of each coded sample compared with the control, and then rate how different each sample was from the control, ignoring any scorch marks created by the tortilla oven. They were also informed that at least one of the coded samples could be the same as the control. Panelists marked the difference from control on a 0 to 9 scale, with 0 being “no difference” or same as control and 9 being “very different” or furthest from control. The same panelists evaluated batch 1 and batch 2 samples as separate events on the same day with different blinding codes. Data were collected using Compusense five v.4.6 (Guelph, ON, Canada) software and then exported to SAS for statistical analysis. The study was approved by the University Institutional Review Board for Human Subjects and panelists provided informed consent. Panelists received monetary compensation for their time.

Data Analyses

Data were analyzed with statistical software (SAS Institute, Cary, NC) using a mixed model analysis of variance (Proc Mixed) and the Tukey-Kramer procedure to determine significant differences among means. Significance level was 0.05.

RESULTS AND DISCUSSION

Color

Results of instrumental color readings for both laboratory and tortilleria produced tortillas are shown in Table III. All iron-fortified tortillas were significantly darker than control tortillas, regardless of iron source. L* color values for tortilleria-produced tortillas closely matched those of laboratory tortillas and showed a similar trend in rankings, with PP, E, and OP most closely matching the control. The a* color values for laboratory and tortilleria tortillas were –1.30 to 0.77 and –0.16 to 1.80, respectively. Although significant differences were found among treatments, these differences may not be of practical significance because all values fell in such a narrow range. There did not appear to be any consistent pattern in the a* values due to fortification with iron. The b* color values followed a trend that was quite similar to L* values, with E, OP, and PP scoring closest to the control. However, for b* color values, EDTA treatments were more yellow than the control, whereas all other fortificants caused the tortillas to be less yellow.

The instrumental color effect of NaFeEDTA in this study contrasts with results found in iron-fortified maize porridge (Bovell-Benjamin et al 1999). In this research, unfortified control samples were compared with samples fortified with ferrous bisglycinate, ferrous trisglycinate, ferrous sulfate, and iron-EDTA using sensory descriptive analysis. The control porridge had a brighter yellow color than mostfortified samples. The dulles-colored samples were those fortified with ferrous sulfate and iron-EDTA. Although the effect of ferrous sulfate in porridge is consistent with the results reported in this study, EDTA seemed to have the opposite effect and caused tortillas to be much more yellow than the control. However, for L*, iron-EDTA tortillas were significantly darker than control tortillas. This darkening effect may have caused them to appear duller during sensory analysis.

Sensory Panel

Results of the tortilla sensory panel are shown in Table IV. The difference-from-control scores for all iron treatments were significantly greater than differences from the “blind” control. E and PP were not significantly different and were most similar in color to the control. Sulfate and fumarate treatments, which were specifically recommended in the proposed 2005 Mexican regulation, were the most different from the control, with fumarate being the best of the two. These results are consistent with those reported by Moretti et al (2005), who found PP to have the least effect on sensory scores in extruded rice grains compared with EDTA and S. However, they also reported that rice fortified with E received lower visual and colorimetric scores than PP. The data presented here indicates that PP and E were not significantly different in the sensory test, despite PP having an L* value significantly closer to the control. Possibly the greater inherent coloration of the tortilla compared with rice resulted in less visible change when using E as a fortificant.

L* and b* color values for laboratory tortillas were strongly correlated with sensory scores (r² = 0.82 and 0.78, respectively). It appears that instrumental L* and b* color may be good predictors of discernible sensory differences in corn tortillas. As we described previously, a* values varied widely among treatments and thus were not strongly correlated with sensory appearance.

DISCUSSION

Based on instrumental color values and sensory appearance scores, E and PP appear to be the best candidates for iron fortification of corn masa tortillas. However, cost and bioavailability

### Table III

<table>
<thead>
<tr>
<th>Iron Source</th>
<th>L* (lab)</th>
<th>L* (tort)</th>
<th>a* (lab)</th>
<th>a* (tort)</th>
<th>b* (lab)</th>
<th>b* (tort)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (none)</td>
<td>71.25a</td>
<td>71.51a</td>
<td>0.42b</td>
<td>0.99c</td>
<td>27.39b</td>
<td>20.85a</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>60.69f</td>
<td>60.12d</td>
<td>–1.30f</td>
<td>–0.16e</td>
<td>19.45c</td>
<td>16.20d</td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>65.78c</td>
<td>65.12c</td>
<td>0.01cd</td>
<td>0.41d</td>
<td>22.78d</td>
<td>17.48c</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>69.18b</td>
<td>67.05b</td>
<td>0.47b</td>
<td>1.36b</td>
<td>25.79c</td>
<td>20.47ab</td>
</tr>
<tr>
<td>Ferric orthophosphate</td>
<td>67.13d</td>
<td>65.76c</td>
<td>0.77a</td>
<td>1.31b</td>
<td>25.80c</td>
<td>19.87b</td>
</tr>
<tr>
<td>A-131 electrolytic iron</td>
<td>68.11c</td>
<td>66.99b</td>
<td>–0.16d</td>
<td>1.80a</td>
<td>24.92c</td>
<td>20.32ab</td>
</tr>
<tr>
<td>Ferrous lactate</td>
<td>58.06h</td>
<td>–</td>
<td>–1.26f</td>
<td>–</td>
<td>19.86e</td>
<td>–</td>
</tr>
<tr>
<td>Ferrous gluconate</td>
<td>59.24g</td>
<td>–</td>
<td>–0.87e</td>
<td>–</td>
<td>19.35e</td>
<td>–</td>
</tr>
<tr>
<td>NaFe EDTA</td>
<td>66.16e</td>
<td>–</td>
<td>0.28bc</td>
<td>–</td>
<td>42.76a</td>
<td>–</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>0.218</td>
<td>0.262</td>
<td>0.063</td>
<td>0.051</td>
<td>0.242</td>
<td>0.155</td>
</tr>
</tbody>
</table>

* Laboratory (lab) and commercial tortilla (tort). Values followed by the same letter within columns are not significantly different (P > 0.05). CIE scale L*: 0 = black, 100 = white; a*: negative values indicate green, positive values indicate red; b*: negative values indicate blue, positive values indicate yellow.

### Table IV

<table>
<thead>
<tr>
<th>Iron Source</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (blind)</td>
<td>1.3a</td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>4.0d</td>
</tr>
<tr>
<td>Ferric orthophosphate</td>
<td>3.0c</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>6.3e</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>2.4b</td>
</tr>
<tr>
<td>A-131 electrolytic iron</td>
<td>2.2b</td>
</tr>
</tbody>
</table>

* Scores indicate difference from control on a point scale of 0 (no difference or same as control) to 9 (very different or furthest from control). Values followed by the same letter are not significantly different (P > 0.05). Standard error of the mean = 0.193.
must also be taken into consideration when choosing an ideal iron source (Whittaker 1998). Electrolytic iron has a significantly lower cost than the other iron sources but has a lower bioavailability than sulfate or fumarate (Hurrell 1999). The bioavailability of electrolytic iron reported in the literature varies greatly. One study found that maize porridge fortified with E did not decrease the prevalence of iron-deficiency anemia in children and did not improve any of the iron-status indicators evaluated (Andang'o et al 2007). Swain et al (2003) reported that A-131 electrolytic iron had 54% of the bioavailability of ferrous sulfate based on rat studies. However, more recent research has shown E to be effective in improving iron-status in humans. Hoppe et al (2006) found that A-131 electrolytic iron had 65% absorption in Swedish subjects consuming fortified wheat rolls, compared with ferrous sulfate. In a study comparing elemental iron powders using in vitro solubilities and human efficacy trials, electrolytic iron had 51–73% effective dissolution depending on the source. The most soluble electrolytic source was A-131, which resulted in 77% relative bioavailability compared with ferrous sulfate in Thai women consuming fortified wheat-based snacks (Zimmerman et al 2005; Lynch and Bothwell 2007). Moretti et al (2006) stated that relative bioavailabilities vary widely with food matrix and iron status. Thus, it may be difficult to compare studies of different fortified foods among different populations. However, these findings indicate that electrolytic iron can be effectively used as an iron fortificant. Addition of A-131 electrolytic iron at double the intended addition rate (the rate used in this study) has been specifically recommended for fortification of cereal food staples when ferrous sulfate and ferrous fumarate cause unacceptable changes in the color of fortified foods (SUSTAIN 2001; Hurrell et al 2002).

Iron-EDTA has received attention because it has reportedly high bioavailability, especially in cereal-based foods (Hurrell et al 2000). This was shown specifically in corn-masa tortillas, where NaFeEDTA had higher bioavailability than ferrous fumarate, ferrous bisglycinate, ferrous sulfate, and reduced iron (Davidsson et al 2002; Walter et al 2003). This compound also has little effect on the sensory qualities of some foods such as instant noodles (Kongkachuichai 2007). However, in our study, NaFe EDTA significantly affected tortilla color even when added at less than half the iron dosage of iron sulfate. This result is reinforced by the study of Duarte-Vazquez et al (2004), who reported that NaFe EDTA had a greater effect on corn tortilla color than reduced iron, ferrous sulfate, and ferrous fumarate (Duarte-Vazquez et al 2004). In addition to its negative effect on tortilla color, NaFe EDTA is also more expensive, with a cost more than double that of ferrous sulfate (personal communication).

Although electrolytic iron may have reduced bioavailability compared with iron-EDTA, it has the least effect on tortilla appearance. Additionally, its lower cost makes it economically feasible to incorporate the iron at a higher level to account for the lower bioavailability. Despite the importance of other factors, one of the initial hurdles to overcome in any fortification program is consumer acceptance stemming from sensory effects and cost (Salgueiro et al 2002). On this basis, it appears that electrolytic iron may be the most suitable fortificant when incorporated at the higher recommended levels.

CONCLUSIONS

All iron fortificants used in this experiment significantly affected both the instrumental and sensory color of fortified tortillas made from corn flour. Of these compounds, ferric pyrophosphate and A-131 electrolytic iron caused the least amount of change, even when added at double the iron dosage level of ferrous sulfate. A-131 electrolytic iron has a significantly lower cost than most other iron sources. Furthermore, a sensory test using 100 Mexican consumers indicated that there were no significant differences in acceptability of color, appearance, aroma, texture, or flavor in side-by-side comparisons of tortillas fortified with electrolytic iron at levels used in this study and unfortified control samples (Dunn et al 2007). Tortillas fortified with electrolytic iron could be a viable option to reduce the prevalence of iron deficiency in the Mexican population.

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LITERATURE CITED


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