Effect of Micronutrient Fortification on Nutritional and Other Properties of Nixtamal Tortillas

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Effect of Micronutrient Fortification on Nutritional and Other Properties of Nixtamal Tortillas

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ABSTRACT

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Micronutrient fortification of traditional tortillas made from nixtamal, coupled with fortification of CMF, could improve the nutritional status of a significant percentage of the Mexican population. The effect of micronutrient fortification on nixtamal tortillas has been evaluated in several previous studies. Figueroa et al (2003) incorporated vitamins and minerals into nixtamalized corn tortillas before the milling stage and evaluated the effect of fortification on masa and tortillas. However, nutrient increases from fortification were relatively small compared with the very high premix addition rates indicated in their study. Tovar and Larios-Saldaña (2005) investigated iron and zinc addition during nixtamalization. The minerals were mixed with the lime and added during the cooking step, which is not ideal. After cooking and steeping, the aqueous phase is drained and the nixtamal is typically washed several times with fresh water before milling, which results in significant losses of added micronutrients. Actual increases in iron and zinc were 5–10 times lower than expected theoretical increases. Consumer sensory data, an important aspect of overall acceptability, was not reported in either of these studies.

Rosado et al (2005) measured vitamin and mineral losses during conversion of fortified corn masa flour into tortillas. They found that added riboflavin and thiamin incurred losses of 37 and 45%, respectively. Mineral losses reported were not significant. A thorough assessment of the effects of micronutrient fortification on nutritional, sensory, and other properties of commercially produced nixtamal tortillas is needed. The objectives of this research were to evaluate these properties in commercially prepared masa and tortillas prepared from micronutrient fortified nixtamal.

MATERIALS AND METHODS

Materials

A micronutrient premix in dry powder form was provided by DSM Nutritional Products Mexico S.A. de C.V. (Mexico, DF, Mexico). Premix composition was based on vitamin and mineral levels proposed by COFEPRIS for addition to corn flour (G. Arroyo, personal communication), with adjustments made to account for the higher moisture content of nixtamal (Table I). The premix included a maltodextrin carrier that was incorporated to allow dosing of 1 g of premix/kg of nixtamal, as well as 0.1% silicon dioxide as a flow agent. Ferrous fumarate (7.46% w/w in premix), zinc oxide (2.91%), nicotinamide (2.02%), thiamin mononitrate (0.36%), riboflavin (0.17%), and folic acid (0.13%) were used as micronutrient sources.

Corn was commercial whole-kernel, white dent corn (Honeyville Food Products, Rancho Cucamonga, CA), with a thousand kernel weight average of 319 g. Lime was Food Chemicals Codex grade calcium hydroxide (Mississippi Lime, St. Genevieve, MO).
Commercial Nixtamalization and Sample Collection

Nixtamal and tortillas for this study were prepared at a commercial tortilleria in Taylorsville, UT, using the tortilleria’s standard manufacturing practices. Unfortified nixtamal was prepared from two different lots of dried corn on separate days. Dried corn, water, and lime in a 1:3:0.01 (w/w) ratio were combined in an open tank and heated gently for \( \approx 1 \) hr. The mixture was manually stirred periodically during cooking. The corn was then steeped for 16–20 hr without application of additional heat. After steeping, the cooked corn (nixtamal) was transferred to a holding tank where it was washed and cooled with running tap water for 5 min. The liquid was then drained from the nixtamal.

On each of two days, a portion of the nixtamal was split into two 32-kg batches of unfortified nixtamal, which were used to produce two treatments: 1) control (no premix) or 2) micronutrient premix addition. Unfortified control (Ct) nixtamal was prepared by mixing 32 kg of nixtamal in the tortilleria’s paddle-blender for 2 min. Micronutrient fortified (MF) nixtamal was prepared in the same way with addition of micronutrient premix (32 g) distributed evenly throughout the mixer. Mixing time was based on previous homogeneity testing using iron as a marker.

After mixing in the paddle blender, each batch of nixtamal was conveyed to the mill hopper and milled into masa dough using traditional volcanic mill stones. During milling, additional water was added by the mill operator as needed to provide an appropriate texture of masa for tortilla making and to reduce masa temperature. This is standard practice in the nixtamal tortilla industry (Rooney and Serna-Saldívar 2003). Masa was manually kneaded as it came out of the mill to form a cohesive mass and to ensure masa homogeneity. The resulting mass was transferred to a tortilla-forming machine (maquinas tortilladoras, Celorio, Naucalpan, México), which consisted of a mixer, extruder, and former in one unit. Formed tortillas were baked for 35–40 sec in a triple-pass, gas-fired oven heated to 240°C. While in the oven, tortillas were inverted twice.

Masa and tortilla samples (250 g) were collected at the beginning, middle, and end of each treatment run. Baked tortillas were stacked and allowed to sit at ambient temperature for 10–15 min before packaging in plastic sample bags and storing on ice. Tortillas were transported to the laboratory on ice and placed in a –20.0°C freezer for 48 hr before shipping frozen by overnight courier to Medallion Laboratories (Minneapolis, MN) for micronutrient analysis. Micronutrient analyses were typically completed within 12 days of sample collection. Masa samples were collected as uncooked masa disks immediately upon exiting the tortilla former and were treated in the same fashion as the tortillas. Masa and tortilla samples for texture and color analysis were transported back to the laboratory on ice and evaluated the same day they were produced. Tortillas were allowed to equilibrate to room temperature before reading.

Masa Evaluation

Hardness and adhesiveness. Masa hardness and adhesiveness were determined with a TA-XT2 texture analyzer (Texture Technologies, Scarsdale, NY) using methods provided by the manufacturer for tortilla doughs. Eighteen masa samples, three each from beginning, middle, and end of run on each of two days were analyzed for each treatment. To measure hardness, a 1.00 ± 0.05 g masa sample was weighed and molded into a disk 1.8–1.9 cm in diameter and 0.3–0.4 cm in height. The sample of masa was placed on the texture analyzer platform. A TA 4 38.1-mm acrylic cylinder was positioned at 20.00 mm above the platform. The cylinder compressed the masa disk within 1.00 mm of the platform. The test speed was 3.0 mm/sec and the post-test speed was 10.0 mm/sec. Hardness was defined as the maximum force required to flatten the masa sample.

For adhesiveness, a 2.00 ± 0.05 g masa sample was weighed and molded into a disk 1.8–1.9 cm in diameter and 0.6–0.7 cm in height. A TA 4 38.1-mm acrylic cylinder started descent at 55.00 mm above the platform. The cylinder applied a constant force of 150 g on the masa sample for 10 sec. The test speed and the post-test speed were the same as for hardness. Adhesiveness was defined as the maximum force needed to pull the cylinder away from the masa sample.

pH. Masa pH was measured directly, without addition of water, using a pH meter (Orion perpHect LogR, model 320, Thermo Electron Corporation, Round Rock, TX) calibrated with pH 7 and 10 buffer standards.

Baked Tortilla Evaluation

Texture. Rollability and stretchability tests were used to evaluate the texture of the baked tortillas. For rollability testing, a commonly used method of Arámbula et al (1999) was followed. A method of Mao et al (2002) was followed to measure tortilla stretchability. In this study, the distance the TA probe traveled was changed from 60 to 20 mm. As with masa, 18 tortilla samples (three each from beginning, middle, and end of run on each of two days) were analyzed for each treatment.

Color. Tortilla color was determined with a spectrophotometer (Hunter Color Flex, Hunter Associates Laboratory, Reston, VA). An 18 × 18 cm glass plate was placed on the colorimeter’s measuring port, followed by two stacked tortillas, double-baked side down. The tortillas were held in place using another glass plate. Each set of two tortillas was read three times, moving the tortillas from left to right in a straight line (Waliszewski et al 2004). Average CIE \( L^* \) (light-dark), \( a^* \) (green-red), and \( b^* \) (blue-yellow) colors were reported for each sample. Eighteen sets of tortilla samples (three each from beginning, middle, and end of run on each of two days) were analyzed for each treatment.

Sensory testing. A central-location consumer test took place at two Utah markets that cater to the local Latino community. A total of 51 expatriate Mexican (n = 47) and Central American (n = 4) consumers who regularly consume corn tortillas were recruited to evaluate sensory properties of the tortillas. Consumer panels consisted of 25 males and 26 females, predominantly between 19 and 40 years of age, in the lower to lower-middle socio-economic class. Panels were conducted outdoors on separate days for each location between the late morning and early afternoon hours.

Overall liking, color, aroma, flavor, and texture of the tortillas were rated by panelists using a 9-point hedonic scale, with 1 = “dislike extremely”, 5 = “neither like nor dislike”, and 9 = “like extremely.” Tortillas were held refrigerated (4.0°C) for two to three days before the consumer test. Before serving, the tortillas were reheated on a gas-fired, cast iron griddle for 20–25 sec on each side at 180°C. The reheating method is a modification of a standard method from the Instituto Nacional de Nutrición Salvador Zubirán (INNSZ) (National Institute of Nutrition) in Mexico.

Tortillas from each treatment were presented using a randomized complete block design. Samples prepared on different days were represented equally. Questions were delivered and responses recorded on a paper ballot written in Spanish. The samples were

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Fortification of Corn Masa Flour (mg/kg)</th>
<th>Fortification of Nixtamal (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>40.0</td>
<td>23.11</td>
</tr>
<tr>
<td>Zinc</td>
<td>40.0</td>
<td>23.11</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2.0</td>
<td>1.16</td>
</tr>
<tr>
<td>Niacin</td>
<td>35.0</td>
<td>20.22</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>3.0</td>
<td>1.73</td>
</tr>
<tr>
<td>Thiamin</td>
<td>5.0</td>
<td>2.89</td>
</tr>
</tbody>
</table>

\(^a\) Arroyo (2004).

\(^b\) Conversion based on estimated average of 90% solids content in flour and 52% solids content in nixtamal.
presented in sequential monadic format on paper plates labeled with 3-digit blinding codes. Panelists were instructed to use an unsalted cracker and filtered water to cleanse the palate between samples. Data was later entered into software (Compusense 5, Guelph, ON, Canada) 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.

**Evaluative Nutritional Analysis**

Dry corn, nixtamal, masa, and tortilla samples were packaged in plastic sample bags and shipped frozen to an external analytical laboratory for nutritional analysis. Iron, zinc, folic acid, niacin, riboflavin, and thiamin were analyzed in each of two composite samples from each day’s production. Subsets of tortillas were re-heated on a griddle at 180°C for 20 sec on each side to mimic consumer reheating; these were also sent for nutritional analysis. The nutritional profile of the tortillas after reheating indicates the extent of nutritional change resulting from in-home use. It also indicates the actual nutritional benefit for the consumer. Iron and zinc were not reported in masa or reheated tortillas because it was not expected that mineral content for these samples would differ from results in tortillas as calculated on a dry basis. Moisture was also measured in each sample to calculate nutrient values on a dry basis, which allowed for direct comparison of results between samples from different stages of the process.

The external analytical laboratory used AOAC methods (AOAC 2000) for mineral and vitamin analysis. Iron and zinc were determined according to 990.08.32. Samples for folic acid analysis were prepared according to 960.46 and measured using 992.05. Samples for riboflavin analysis were prepared according to 960.46 and measured according to 940.33. Thiamin was measured with 942.23. Samples for niacin analysis were prepared according to 960.46 and analyzed using 944.13. Moisture was tested by 925.09 and 926.08.

### Statistical Analyses

Differences between Ct and MF treatment means for masa properties and tortilla texture, color, and sensory attributes were assessed using the dependent t-test. Differences in vitamin and mineral content during the nixtamalization process and among Ct, MF, and MF reheated tortillas were analyzed using analysis of variance (ANOVA); comparison of means was performed according to Tukey-Kramer using the Statistical Analysis System v.9.1 (SAS Institute, Cary, NC). A significance value of $P = 0.05$ was used to distinguish significant differences among treatments.

### RESULTS AND DISCUSSION

#### Masa and Tortilla Chemical, Physical, and Sensory Properties

There was no significant difference in masa hardness, adhesiveness, or pH between the Ct and MF treatments (Table II). Premix addition also had no significant effect on tortilla rollability (Table III). However, tortilla stretchability significantly decreased with the addition of micronutrient premix. Premix is composed of vitamins, minerals, a maltodextrin carrier, and a small amount of silicon dioxide as a flow agent. The maltodextrin makes up >80% of the premix but <0.08% of the masa. It is unlikely that this level of carrier would significantly affect tortilla texture. Mohammad and Hallab (1973) did find adverse effects on wheat dough and bread quality upon addition of >0.011% iron in the form of sulfate, gluconate, or trichloride. It is possible that iron, or one of the other micronutrients present in the premix, adversely affected stretchability properties of the fortified tortillas, though the exact mechanism is unclear.

Significant differences in the Hunter CIE $L^*$, $a^*$, and $b^*$ color values were found between fortified and unfortified tortillas (Table III). The addition of micronutrient premix made the tortillas slightly darker and more blue-green in color. It is widely known that xanthophyll and anthoxanthin pigments in corn are affected by pH (Paulsen et al. 2003). Miller in certain regions adds additional lime to their tortillas to produce a more yellow tortilla, whereas millers in other areas wash the nixtamal more extensively to produce whiter tortillas. Tortillas produced at the mill in this study were quite alkaline and therefore had a somewhat yellow appearance. A component elimination study with individual premix fortificants revealed that the darkening of the tortilla upon enrichment was almost entirely caused by ferrous fumarate. This compound is very dark brown in appearance and reacts with phenolic and sulfur-containing compounds in the corn to produce an even darker appearance (Hurrell 1985). The darkening effect of ferrous fumarate has also been observed in nixtamalized corn flour. Dary (2002) reported that off-colors in tortillas were observed for levels of fumarate addition >30 mg of Fe/kg. The significant differences in CIE values in this study are not unexpected, given the COFEPRIS recommendation of 40 mg of Fe/kg in the form of fumarate or sulfate. The blue-green shift could possibly result from the iron as well, though riboflavin, which is yellow-green in solution, could also have contributed. These objective differences in CIE color are important, but of greater practical concern is the effect of this change on consumer acceptance.

Despite the apparent color difference in tortillas, consumer sensory test results indicated no significant differences in hedonic scores between Ct and MF treatments for any of the attributes tested (Table IV). MF tortillas were found to be comparable to Ct tortillas in overall acceptability and other sensory attributes. Of particular interest is the fact that the instrumental color difference did not significantly affect consumer acceptance of tortillas when presented in sequential monadic fashion. It is possible that a side-by-side evaluation might produce different results. However, the

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### TABLE II

<table>
<thead>
<tr>
<th>Treatmentb</th>
<th>Hardness (g)</th>
<th>Adhesiveness (g)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct</td>
<td>12.297a</td>
<td>80.5a</td>
<td>8.9a</td>
</tr>
<tr>
<td>MF</td>
<td>13.878a</td>
<td>93.1a</td>
<td>9.0a</td>
</tr>
<tr>
<td>SEc</td>
<td>1.298</td>
<td>6.53</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### TABLE III

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stretchability (kg)</th>
<th>Rollability</th>
<th>CIE $L^*$</th>
<th>CIE $a^*$</th>
<th>CIE $b^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct</td>
<td>0.34a</td>
<td>1.0a</td>
<td>69.5a</td>
<td>0.36a</td>
<td>22.6a</td>
</tr>
<tr>
<td>MF</td>
<td>0.25b</td>
<td>1.1a</td>
<td>67.5b</td>
<td>0.02b</td>
<td>20.4b</td>
</tr>
<tr>
<td>SEc</td>
<td>0.03</td>
<td>0.1</td>
<td>0.42</td>
<td>0.09</td>
<td>0.22</td>
</tr>
</tbody>
</table>

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$^a$ Values followed by the same letter within columns are not significantly different ($P > 0.05$).

$^b$ Treatments include control (Ct) and micronutrient fortified (MF) samples.

$^c$ SE, standard error of the mean.
sequential monadic testing is more in keeping with typical consumer purchase practices.

**Effect of Fortification on Nutritional Properties**

Alkaline nixtamalization of corn dramatically affects the pH of the masa and resulting tortillas and can significantly alter the nutritional properties of these products because many vitamins are affected by changes in pH. Ball (2006) reports that thiamin and riboflavin are stable in acidic media but become increasingly unstable at alkaline pH, especially at elevated temperature. Folic acid, on the other hand, is quite stable in the range of pH 5–12, but unstable below pH 5 (Ball 2006). Although minerals are elemental in nature and do not degrade, reduced bioavailability of minerals such as iron can occur at alkaline pH (Hurrell 1999).

Due to differing consumer preferences and increasing use of acidic preservatives by certain manufacturers, commercial tortillas now cover a wide pH range from moderately acidic (pH 5.5) to alkaline (pH 8.5–9) (Ortiz and Carrillo 1997). The tortillas produced at the mill participating in this study are in the higher alkaline range and it was expected that micronutrient stability would be affected accordingly.

Vitamin levels in dry corn, as well as Ct and MF nixtamal, masa, and tortillas are reported in Table V. Process-related vitamin losses in Ct and MF products can be determined by comparing vitamin levels in intermediate and finished products to the initial levels in dried corn. Total loss of vitamins in processed fortified products can be determined by comparing MF tortilla micronutrient levels to the theoretical levels calculated based on actual premix addition to MF nixtamal.

Folic acid in native corn decreased by 24% (w/w) during the tortilla manufacturing process, with most of the degradation occurring during baking in the oven. Folic acid in MF tortillas showed a 33% (w/w) loss during progression from the point of premix addition through baking, as compared with the theoretical value. This represented a statistically significant decline, comparable to the loss in the control product. Of particular interest is the fact that much of the degradation took place during mixing with the nixtamal, indicating that the warm, moist, alkaline environment had a destabilizing effect on this vitamin. Figueroa et al (2003) observed a somewhat larger loss of 46% (w/w) in added folic acid through the masa/tortilla making process. The extent of the loss in the tortilla process is surprising given the fact that both Ball (2006) and Gregory (1996) have generalized that folic acid is quite resistant to pH and thermal degradation.

The most labile vitamin in the present study proved to be thiamin, which was almost completely degraded during nixtamalization. Total loss of native thiamin following tortilla baking was 88% (w/w). The dramatic loss of thiamin is supported by the observation that thiamin is extremely labile in alkaline conditions such as those occurring during nixtamalization (Gregory 1996). Thiamin loss in MF tortillas was 91% (w/w), as compared to theoretical. Consequently, no significant increase in thiamin resulted from fortification during this study. As indicated by samples of fortified nixtamal collected directly after mixing, most of the thiamin (81% w/w) was lost in the initial mixing step, as noted previously with folic acid. Bressani et al (1958) also found significant losses of thiamin when evaluating nixtamalized corn tortillas prepared in a household setting. During nixtamalization and baking, losses of native thiamin reached 62% (w/w) in their study. Figueroa et al (2003) showed only a 36% (w/w) loss in added thiamin during processing. Rosado et al (2005) showed similar thiamin losses (35% w/w) to Figueroa et al (2003) during conversion of fortified masa flour into tortillas.

Niacin is one of the few micronutrients that show favorable effects from nixtamalization. During the traditional corn tortilla manufacturing process, niacin is reportedly released from complex derivatives called niacytin, resulting in improved measurement and bioavailability (Heathcote et al 1952; Gregory 1996). Niacin, which is considered extremely stable in most types of cooking matrices and preparation conditions (Gregory 1996; Ball 2006), showed excellent stability in both control and fortified products in this study, with no losses being observed. Figueroa et al (2003)
also reported good stability of niacin during tortilla manufacturing, with a directional (8% w/w) net increase in niacin through the process. Bressani et al. (1958), however, showed significant losses of native niacin (32% w/w) in corn during preparation of nixtamal tortillas in a household setting.

Native riboflavin showed a loss of 50% (w/w) in converting dried corn to control tortilla. This loss was not surprising considering riboflavin degrades in alkaline conditions despite good retention during heat processing (Gregory 1996). Riboflavin in MF tortillas showed a loss of 24% (w/w) during processing, as compared with theoretical addition levels. In contrast, during the study by Figueroa et al. (2003), riboflavin decreased 80% during processing. Rosado et al. (2005) measured process losses during conversion of fortified masa flour into tortillas. They found that riboflavin decreased by 37% (w/w), which is comparable to total losses reported in this study. Bressani et al. (1958) reported 42% (w/w) loss of riboflavin during nixtamalization and baking in the home.

Despite losses in added vitamins during processing, fortification of nixtamal did result in micronutrient increases in the finished tortillas. Table V shows that there was a significant increase in folic acid, niacin, and riboflavin in the MF tortillas compared with the Ct tortillas. However, as noted previously, there was not a significant increase in thiamin. The vitamin content in the MF reheated tortillas is also shown in Table V. Only small changes in nutrient levels occurred during reheating, indicating that it is not a significant cause of nutrient loss.

Because they are elements, iron and zinc would not be expected to degrade due to thermal or chemical processing. However, losses due to leaching during steeping and washing could certainly be possible. Table V shows that iron and zinc levels in native corn were not affected by nixtamalization or other steps in the tortilla manufacturing process. The content of these minerals in fortified tortillas was more than double the levels in control samples. Rosado et al. (2005) measured mineral levels during baking of fortified masa flour into tortillas and found no significant decreases from theoretical. Figueroa et al. (2003) found >50% loss of iron during conversion of corn to nixtamal tortillas. It is not clear if their reported loss in iron was actually due to processing, or possibly caused by a problem with the analysis.

In Table VI, the vitamin and mineral contents of Ct and MF tortillas are compared with the official recommended nutrient intakes listed in the current Mexican food labeling regulations, Norma Oficial Mexicana NOM-086-SSA1-1994 (Online at www.cofepris.gob.mx/mj/documentos/nom3.htm). The average consumers in Mexico may eat up to 10 tortillas per day (unpublished data), therefore, consumers could receive a significant percentage of the recommended intake of these nutrients from fortified tortillas.

Overall, tortilla fortification resulted in significant increases in all of the vitamins and minerals except thiamin, showing that added folic acid, niacin, riboflavin, iron, and zinc had acceptable stability through masa/tortilla manufacturing. Effective fortification with thiamin may require excess addition or encapsulation to withstand the moist, alkaline environment.

Because fortification of nixtamal tortillas could significantly reduce micronutrient deficiencies in target populations, and be a great benefit to those who are beyond the scope of current cereal fortification programs, additional studies in commercial mills in Mexico are being undertaken to evaluate this technology in an actual production setting.

CONCLUSIONS

Even though significant differences were found in instrumental tortilla texture and color between control and fortified treatments, these differences were not substantial enough to affect hedonic ratings during consumer testing.

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