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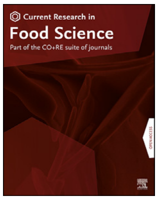

Research Paper

Effect of maize processing methods on the retention of minerals, phytic acid and amino acids when using high kernel-zinc maize

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ABSTRACT

High kernel-zinc maize varieties are available to consumers in several countries in Latin America to contribute to increase the zinc intake of their populations. Minerals, phytic acid and amino acids retention were measured after processing six maize varieties including three high kernel-zinc, one quality protein maize and two conventional maize. Grain for each variety was processed into *tortillas*, *arepas* and *mazamorra*, common maize dishes in the region. To evaluate the effect of processing kernel-zinc maize varieties on zinc retention, varieties were grouped in zinc biofortified maize (ZBM) and non-ZBM. Iron, zinc, phytic acid, tryptophan and lysine concentrations in non-processed maize were 17.1–19.1 μg/g DW, 23.9–33.0 μg/g DW, 9.9–10.0 mg/g DW, 0.06–0.08% and 0.27–0.37%, respectively. In *tortillas*, the iron, zinc, phytic acid and lysine content did not change ($p < 0.05$) compared to raw grain, while tryptophan decreased by 32%. True retention of iron in *arepas* and *mazamorra* was 43.9 and 60.0%, for zinc 36.8 and 41.3%, and for phytic acid 19.3 and 25.1%. *Tortillas* had higher zinc retention than *arepas* and *mazamorra* due to use of whole grain in the nixtamalization process. Therefore, to contribute to higher zinc intake, nixtamalized tortilla prepared with biofortified zinc maize is recommended. Additionally, promotion of whole grain flour to prepare arepas should be explored to enhance the intake of minerals that are usually confined to aleurone layers and germ.

1. Introduction

Maize is the most consumed cereal in Mexico and several countries in Central and South America. It is also a staple crop in Sub-Saharan Africa. In Mexico and Guatemala, the daily per capita consumption of maize is 290 g, while in Venezuela and Colombia is approximate 100 g (Ranum et al., 2014; Govaerts et al., 2019; Ekpa et al., 2019).

In Latin America, maize dishes include beverages, snacks, and flatbreads. In Mexico and Central America, more than 600 food products and dishes are derived from maize and about 300 of them use the ancient alkaline cooking process called nixtamalization, which consists of boiling and steeping whole maize grains in an alkaline solution. *Tortillas* are the most consumed product prepared from nixtamalized dough and today tortillas are found worldwide (Ekpa et al., 2018; Escalante-Aburto et al., 2020). The most common maize product in Venezuela, Colombia and Panama is a flatbread called *arepa*, a thick round dough generally prepared with pre-cooked decorticated and degermed maize kernels although in some regions of Colombia is made of whole grain previously treated with ash or lime (Ranum et al., 2014; Espinoza et al., 2018; Guzzon et al., 2021). Another popular form of maize consumption in Colombia, Argentina and Peru is *mazamorra*. In Colombia, it is boiled decorticated and degermed maize normally served semi-liquid in combination with milk (Nuss and Tanumihardjo, 2010; Guzzon et al., 2021).

Biofortification, a breeding strategy to develop staple crops with higher micronutrient content while ensuring good agronomical performance it has been led for the last 20 years by Harvest Plus working with several stakeholders (Bouis and Saltzman, 2017). The first considered biofortified maize, however, was developed in 1970's, Quality Protein Maize (QPM), maize with higher content of lysine and tryptophan, two essential amino acids (Atlin et al., 2011). Most recently, high kernel-zinc

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Table 1
Biofortified and control varieties used. Group non-ZBM: non-biofortified maize varieties (A-B-C), and group ZBM: high kernel-zinc biofortified maize varieties (D-E-F).

Group	Code	Variety	Type of maize	
Non-ZBM	A	S06TLWQHGAB02-B	No zinc	QPM
	B	S11TLWNHGAB06-B	No zinc	No QPM
	C	Commercial check	No zinc	No QPM
ZBM	D	S13LTWQHZNHGAB01	Zinc	QPM
	E	S13LTWQHZNHGAB02	Zinc	QPM
	F	S13LTWQHZNHGAB03-B	Zinc	QPM

maize was developed and made available to farmers in Colombia, Guatemala, Honduras and Nicaragua (Listman et al., 2019). Zinc (Zn) is an essential micronutrient which deficiency results in a compromised immune system. In order to consider a maize variety high in zinc, HarvestPlus recommended a target increase in the kernel of more than 12 μg/g (total Zn concentration >30 μg/g) compared to non-biofortified maize (Listman et al., 2019). Bauman (1975) and Chakraborti et al. (2011) reported a significant positive correlation between QPM and Zn content in maize kernels. Currently, most of the commercialized high Zn maize varieties and hybrids are both high Zn and QPM.

The Zn content in maize kernels varies due to the genetic background and the environmental conditions where the crop is grown (Hindu et al., 2018). Post-harvest management and cooking processes can affect the vitamin, mineral and amino acid composition in the final product as compared to raw kernels (Taleon et al., 2017). Since the majority of zinc is located in the maize embryo and pericarp (Cheah et al., 2020) processes that remove large quantities of these two grain structures could reduce the zinc content in the final food product, even below to 10 ppm (Bevis and Hestrin, 2020). Processes where not much embryo or pericarp is removed yields high zinc retention (Bressani et al., 2004). Zinc bioavailability is affected by phytic acid which binds zinc to produce zinc-phytate (Miller et al., 2007). Phytate is also located largely in the embryo and pericarp (Raboy et al., 2000). High zinc maize could improve zinc deficiency in populations only if the zinc is retained in similar or higher quantities compared to conventional maize. Up to date no information is available on the zinc and phytic acid retention in biofortified maize.

In this work, we used high kernel-zinc maize varieties to elucidate the effect of processing and cooking methods (nixtamalized *tortillas, arepas* and *mazamorra)* on Zn retention and to assess the implications for Zn bioavailability.

2. Materials and methods

2.1. Maize germplasm

Five white maize breeding experimental varieties (hybrids) were used: three high kernel-zinc biofortified varieties (ZBM) and two non-biofortified varieties (non-ZBM). The non-ZBM varieties were: one QPM variety and one conventional maize variety. One commercial non-ZBM hybrid was used as a control (Table 1).

2.2. Grain physical traits

Subsamples (100 g) were used for physical characterization, including kernel size (length, width and thickness), thousand kernel weight (TKW), percentage of vitreous (VE) and floury endosperm (FE), and kernel hardness (flotation index and hectoliter weight), according to methodologies described by Palacios-Rojas (2018).

2.3. Food products preparation processes

All food products were prepared in duplicate and sampled at every different stage in the processes.

2.3.1. Arepa and mazamorra preparation

Whole maize (10 kg) was threshed during 5 min (5 hp engine at 500 rpm) in a dry-degermination machine, used for pilot laboratory studies, to mechanically remove the pericarp, tip cap and most of the germ (maize bran). Maize bran was estimated based on a preliminary test and according to maize weight after degermination and visual verification (16.0–20.5% of the kernel weight).

For *arepas*, 200 g of threshed maize was washed and mixed with 2500 mL of ultrapure water (18.0 MΩ) (MilliQ® Merck-Millipore, Germany) and 2 g of sodium bicarbonate (baking soda). Maize was cooked in a pressure cooker for 90 min, cooled down without the cooking water, and then ground using a disk mill (Corona grain mill grinder, Landers & Co, Colombia). Butter (25 g) and salt (2.5 g) were added to the dough. Cakes weighing 50 g (1 cm thick) were formed by hand and cooked in a Teflon-coated pan, preheated at 150 °C, for 8 min per side (two times). For *mazamorra*, the same quantities of threshed maize, ultrapure water and baking soda were used, but the grain was cooked in a pressure cooker for 120 min. The cooked maize was cooled down in the same water where it was cooked.

2.3.2. Nixtamalization and tortilla preparation

Whole maize kernels (250 g) were cooked at 85 °C with 750 mL of ultrapure water and 2.5 g of calcium hydroxide ($Ca(OH)_2$, 1% w/w) for 50 min. The nixtamalized grain (*nixtamal*) was soaked at room temperature for 16 h. The cooking liquid (*nejayote*) was drained and the nixtamalized samples were washed with ultrapure water twice to remove excess of $Ca(OH)_2$ and the released pericarp of the *nixtamal*. The washed *nixtamal* was weighed and then ground using a disk mill (Corona grain mill grinder, Landers & Co, Colombia). Ultrapure water was added to make the dough smooth and malleable. Balls (25 g) were formed, pressed flat in a tortilla press, and cooked on a Teflon-coated pan preheated at 270 °C for 45 s per side (three times).

2.4. Chemical analysis

Samples of raw kernels, threshed grains, nixtamalized grain and dough, cooked grain and dough, *tortillas*, *arepas* and *mazamorra* were lyophilized and ground using a Retsch Mixer Mill MM 400 fitted with zirconium grinding jars and balls (Retsch GmbH & Co KG, Germany). Samples were stored at 4 °C until further analysis.

2.4.1. Determination of minerals and phytic acid (PA)

Iron (Fe), Zn and Ca concentrations were determined in duplicate using inductively-coupled plasma-optical emission spectroscopy (ICP-OES) (7500cx; Agilent Technologies, USA) after a closed-tube nitric acid/hydrogen peroxide digestion method (Wheal et al., 2011). Aluminum was analyzed as an indicator of potential sample contamination (Palacios-Rojas, 2018). PA content was determined following extraction in 1.25% H_2SO_4 and subsequent analysis by Dionex liquid chromatography using 200 mM NaOH and deionized water as the eluents (Dipti et al., 2017).

2.4.2. Determination of molar ratio of PA:minerals

Molar ratios between PA and Fe and Zn in food were calculated to estimate the bioavailability of Fe and Zn contained in the final food products (Norhaizan & Nor Faizadatul Ain, 2009). The molar ratios were obtained after dividing the mole of PA by the mole of minerals, which were calculated by dividing their weight by atomic weight. As described by Gibson et al. (2010), values of PA:Fe > 1 and PA:Zn > 15 were used as indicators of the low bioavailability (<15% absorption) of such minerals.

2.4.3. Determination of lysine, tryptophan and protein content

Protein and lysine were measured as described elsewhere (Palacios-Rojas, 2018). For tryptophan determination the colorimetric method described by Nurit et al. (2009) was used.

Table 2
Physical grain characteristics of six non-ZBM and ZBM varieties.

Code	Variety	100-Kernel Weight (g)	Flotation Index (%)	Pedicel (%)	Pericarp (%)	Germ (%)	Floury Endosperm (%)	Vitreous Endosperm (%)	VE/FE Ratio[a]
A	S06TLWQHGAB02-B	29.57 ± 0.97 ab	11.0 ± 2.8a	4.8 ± 1.0a	5.0 ± 0.1 ab	12.2 ± 0.1a	18.5 ± 2.5a	59.6 ± 1.3d	3.3 ± 0.5d
B	S11TLWNHGAB06-B	31.46 ± 0.44a	3.5 ± 0.7b	4.2 ± 0.2a	5.5 ± 0.5a	10.4 ± 0.8a	9.5 ± 2.0c	70.4 ± 3.4 ab	7.7 ± 2.0abc
C	Commercial check	31.76 ± 0.79a	4.0 ± 0.0 ab	4.1 ± 0.1a	4.1 ± 0.2c	9.1 ± 0.6b	6.5 ± 1.0c	76.2 ± 1.3a	11.9 ± 2.0a
D	S13LTWQHZNHGAB01	25.33 ± 0.19c	5.0 ± 2.8 ab	5.2 ± 1.0a	4.6 ± 0.1abc	12.7 ± 0.1a	8.7 ± 0.2c	68.8 ± 1.0bc	7.9 ± 0.1abc
E	S13LTWQHZNHGAB02	26.49 ± 0.30c	1.0 ± 0.0b	4.0 ± 0.1a	4.5 ± 0.2bc	12.4 ± 0.1a	9.6 ± 0.1bc	69.5 ± 0.3b	7.2 ± 0.1bcd
F	S13LTWQHZNHGAB03-B	27.87 ± 0.23bc	5.5 ± 2.1 ab	5.3 ± 0.7a	4.7 ± 0.2abc	12.6 ± 1.1a	14.6 ± 0.6 ab	62.8 ± 1.2cd	4.3 ± 0.3cd

Differences between means were considered significant at $p < 0.05$.
[a] Ratio of vitreous to floury endosperm.

2.5. *Nutrients true retention calculation*

True retention measures the proportion of a nutrient remaining in processed food compared to the amount of that nutrient originally present in a given weight of the food before processing (Taleon et al., 2017).

Percentage of true retention (%TR) of Fe, Zn and PA was calculated using the equation *%TR* = (Nutrient content/g of processed food * g of food after processing/Nutrient content/g of raw food * g of food before processing) * 100.

2.6. *Contribution to the daily intake of Zn*

The potential contribution of foods made from high kernel-zinc maize to the daily intake of Zn and Estimated Average Requirement (EAR) was calculated based on the average amount of maize product consumed (100 g for children aged 4–6 years and 250 g for women), and the typical processing methods used in Latin American countries with high maize consumption (Ranum et al., 2014). An EAR for Zn of 1390 μg/day for children aged 4–6 years and 2969 μg/day for women of childbearing age were used as a reference, and a 30% bioavailability was considered to calculate the total absorbed zinc, following the recommendations of the European Food Safety Authority (EFSA, 2014).

2.7. *Statistical analysis*

To evaluate the effect of processing kernel-zinc maize varieties on zinc retention, varieties were grouped as ZBM and non-ZBM. Fe, Zn, Ca and PA content, %TR was expressed as mean ± standard deviation of 3 varieties (repetitions). The effect of processing type and maize type was evaluated using a two-way analysis of variance model. Analysis of variance was done using the mixed procedure in SAS 9.4 (SAS Institute, Cary, NC). Means separation for processing type was calculated using Tukey's method. Differences between means were considered significant at $p < 0.05$.

3. Results and discussion

3.1. *Physical and chemical characterization of maize kernels*

Physical properties of the maize kernels were analyzed (Table 2). Although significant differences are observed for most of the physical parameters measured, in general, all evaluated varieties are classified within similar types of kernels, very hard ones with low percentage flotation index (0–12%) and low percentage of floury endosperm. The commercial check had similar hardness but with higher ratio of vitreous/floury endosperm (VE/FE) compared to the other varieties analyzed (Table 2). The higher the VE/FE ratio, the higher the flour extraction rate expected during degerming/threshing, and therefore this type of maize is commonly used in processing of *arepas* (Ranum et al., 2014; Gwirtz and Garcia-Casal, 2014). The variety B as well as the commercial check had the lower percentages of germ (10.4% and 9.1%, respectively) and the higher kernel weight (31.5% and 31.8%, respectively) compared to the ZBM group and the QPM maize genotype. The differences in grain hardness, kernel size, weight and percentage of germ caused final threshed products with different percentages of bran removal (16.1–20.4%). Cheah, Kopittke, Scheckel, Noerpel, and Bell (2020), showed that large amount of Zn accumulated in the germ compared to other areas of the kernels; thus, differences in the percentages of germ could also explain the differences in Zn content. Average Fe content in non-ZBM and ZBM grain (whole kernel) was 19.07 μg/g (ranged 16.61–23.02 μg/g) and 17.10 μg/g (ranged 16.18–17.80 μg/g), respectively; while, Zn content in the non-ZBM group was 23.91 μg/g (19.71–26.07 μg/g) and that of the ZBM group was 33.02 μg/g (32.55–33.67 μg/g) (Table 3 and Suppl. Table 2). The average Zn increment in the ZBM compared to the non-ZBM group was +9.1 μg/g. Fe and Zn levels were within values reported for maize (8.19–25.65 μg/g for Fe and 17.11–43.69 μg/g for Zn) from a diverse panel of 923 inbred lines (Hindu et al., 2018; Ortiz-Monasterio et al., 2007).

Calcium content of whole kernel ranged 32.60 and 91.79 mg/g and PA content was 8.61–11.36 mg/g, similar to values reported for maize

Table 3
Mineral and phytic acid (PA) content of two groups of maize (non-ZBM and ZBM) and molar ratio of PA:minerals in raw kernels and processed products.

Group/Varieties	Product	Fe (μg/g DW)	Zn (μg/g DW)	Ca (μg/g DW)	PA (mg/g DW)	Ratio PA:Fe	Ratio PA:Zn
Non-ZBM (A + B + C)	Raw kernel	19.07 ± 3.59a-c	23.91 ± 3.81b	59.60 ± 28.44b	10.04 ± 1.04b	46.05 ± 10.36a	42.10 ± 4.33a
	Tortillas	22.25 ± 4.96a	25.72 ± 4.31b	1560 ± 384a	11.48 ± 1.14a	44.98 ± 8.22a	44.84 ± 5.44a
	Arepas	13.38 ± 5.42de	10.98 ± 6.00e	86.09 ± 9.32b	2.12 ± 1.21d	12.77 ± 3.92d	18.91 ± 1.94cd
	Mazamorra	9.82 ± 4.54e	13.63 ± 7.47de	43.18 ± 13.81b	3.03 ± 1.68d	24.57 ± 6.73bc	21.95 ± 2.47c
ZBM (D + E + F)	Raw kernel	17.10 ± 0.95b-d	33.02 ± 1.39a	56.61 ± 5.40b	9.92 ± 1.55b	49.01 ± 6.59a	29.77 ± 4.55b
	Tortillas	20.99 ± 1.99 ab	34.82 ± 0.85a	1445 ± 239a	11.10 ± 0.51 ab	45.08 ± 4.75a	31.58 ± 1.40b
	Arepas	15.20 ± 3.06cd	18.56 ± 2.72cd	88.85 ± 9.09b	3.06 ± 0.57d	17.72 ± 4.94cd	16.85 ± 4.76d
	Mazamorra	12.94 ± 1.58de	22.68 ± 2.86bc	41.66 ± 4.94b	4.43 ± 0.78c	28.99 ± 4.02b	19.30 ± 1.92cd

Data are means ± SD of duplicated analyses. Different letters indicate a statistically significant difference ($p < 0.05$) between products.

Table 4
Protein, lysine and tryptophan content of two groups of maize (non-QPM and QPM maize) and true retention (%TR) after processing as *tortillas*, *arepas* and *mazamorra*.

Group/Varieties	Final product	Protein (%)	Tryptophan (%)	Lysine (%)	Protein (%TR)	Tryptophan (%TR)	Lysine (%TR)
Non-QPM (B + C)	Raw kernel	9.57 ± 0.25b	0.055 ± 0.002b	0.269 ± 0.006c	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Tortillas	11.06 ± 0.38a	0.038 ± 0.006c	0.306 ± 0.019b	104.18 ± 6.06a	62.27 ± 10.38b	102.65 ± 4.05a
	Arepas	10.62 ± 0.22a	0.013 ± 0.002e	0.159 ± 0.028e	77.89 ± 4.49b	16.67 ± 3.80c	41.66 ± 7.82b
	Mazamorra	8.34 ± 0.56d	0.012 ± 0.003e	0.164 ± 0.033e	62.90 ± 7.09c	15.91 ± 2.33c	43.18 ± 1.42b
QPM (A + D + E + F)	Raw kernel	9.09 ± 0.56bc	0.076 ± 0.009a	0.367 ± 0.024a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	Tortillas	10.50 ± 0.36a	0.050 ± 0.005b	0.383 ± 0.020a	101.94 ± 6.75a	66.29 ± 13.30b	102.73 ± 14.68a
	Arepas	8.58 ± 0.79cd	0.021 ± 0.002d	0.237 ± 0.014d	69.18 ± 9.00c	23.44 ± 4.27c	52.63 ± 7.18b
	Mazamorra	8.60 ± 0.60cd	0.020 ± 0.003d	0.241 ± 0.024d	62.72 ± 6.96c	19.69 ± 4.44c	48.86 ± 10.96b

Data are means ± SD of duplicated analyses. Different letters indicate a statistically significant difference ($p < 0.05$) between products.

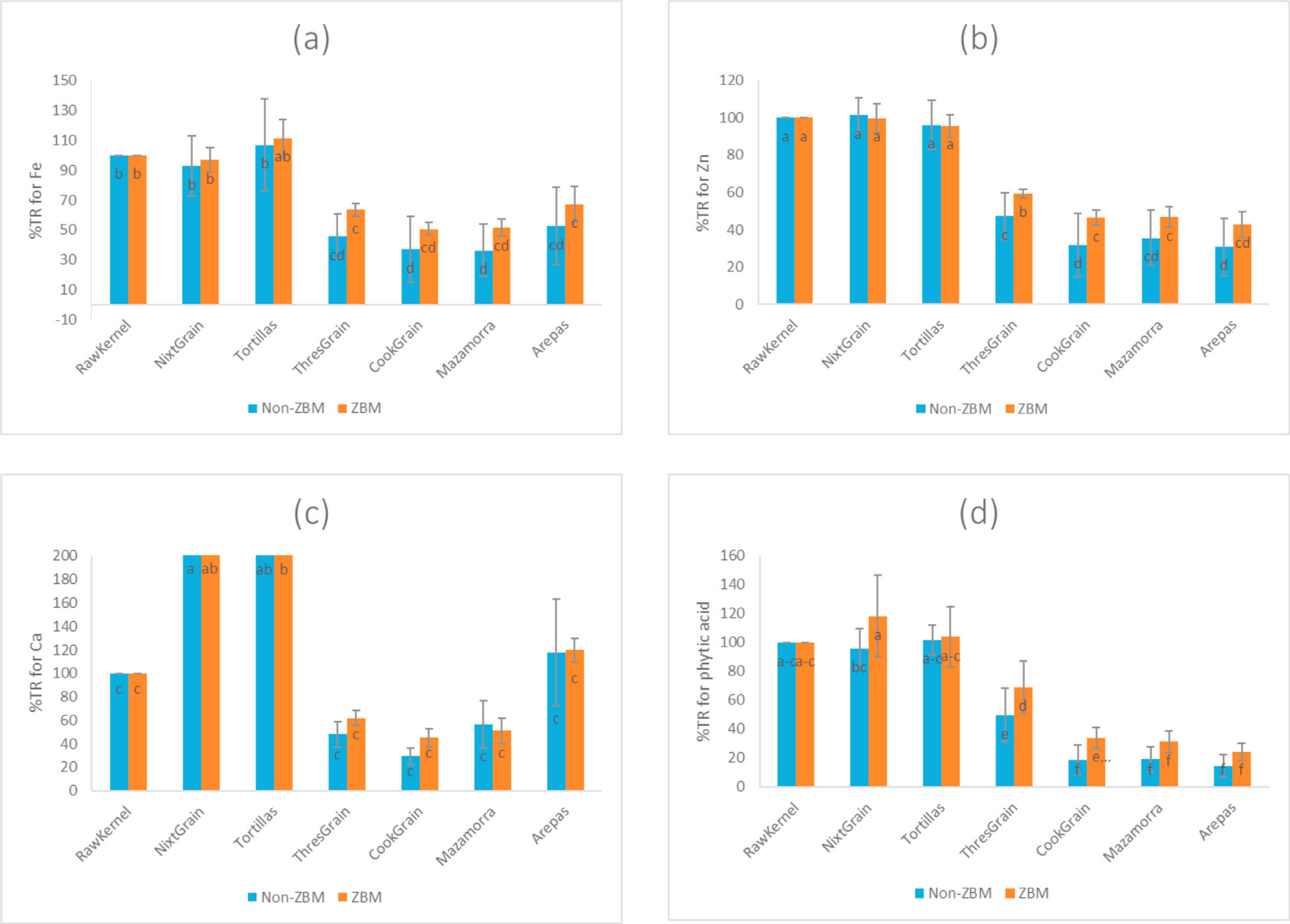

Fig. 1. True retention of iron (a), zinc (b), calcium (c) and phytic acid (d) in raw kernels, intermediate and final products elaborated with two groups of maize (non-ZBM and ZBM). Note y-scale is cut in Figure 2c to better show details of each product.

grain (Bressani et al., 2004). There was no significant difference between the non-ZBM and the ZBM groups for either of these two compounds (Table 3). All samples had similar protein content (8.6–9.7%). Maize is normally considered QPM when more than 0.07% of tryptophan and 0.35% of lysine is present in the whole kernel (Nurit et al., 2009). Grain from samples A, D, E and F had the expected high content of tryptophan (0.065–0.081%) and lysine (0.33–0.38%), while samples B and C had 0.053–0.056% and 0.27% of tryptophan and lysine, respectively (Table 4).

3.2. Fe, Zn, Ca and PA content in arepas and mazamorra

Fe and Zn content in the threshed maize from the non-ZBM group was 10.29 μg/g (8.10–12.77 μg/g) and 14.24 μg/g (7.77–18.40 μg/g), respectively, whereas for the ZBM group it was 13.19 μg/g (12.97–13.55 μg/g) and 23.88 μg/g (23.27–24.29 μg/g), respectively (Suppl. Table 1). Individual data on the mineral content of each maize variety after threshing is shown in the Suppl. Table 2. After degermination, Fe TR was 45.8% and Zn TR was 47.2% for the non-ZBM group, whereas in ZBM varieties it was 63.4% and 59.4%, respectively. Significant decreases in TR found in the cooking of threshed maize kernels compared to raw whole kernels, corresponding to 37.1% TR for Fe and 31.9% TR for Zn for the non-ZBM group, and 50.6% TR for Fe and 46.4% TR for Zn for the ZBM group (Fig. 1 a-b).

Fe and Zn content in *arepas* from the non-ZBM group was 13.39 μg/g (ranged 7.21–16.87 μg/g) and 10.98 μg/g (3.66–15.31 μg/g), respectively, whereas for the ZBM group it was 15.20 μg/g (11.53–17.21 μg/g) and 18.56 μg/g (16.64–21.96 μg/g), respectively (Table 3). Fe TR was

52.7% and Zn TR was 30.9% for the non-ZBM group, whereas in ZBM varieties it was 67.3% and 42.8%, respectively (Fig. 1 a-b). On the other hand, Fe and Zn content in *mazamorra* was 9.82 μg/g (4.19–13.97 μg/g) and 13.63 μg/g (4.09–19.43 μg/g) for non-ZBM varieties and 12.94 μg/g (11.93–14.78 μg/g) and 22.68 μg/g (20.27–25.83 μg/g) for the ZBM group, respectively (Table 3); the non-ZBM group had 36.3% TR for Fe and 35.7% TR for Zn, while the ZBM group had 51.6% TR for Fe and 46.9% TR for Zn, which was not significantly different from *arepas* (Fig. 1 a-b). Traditionally, *arepas* and *mazamorra* are generally made by dehulling and degerming whole kernels using a mechanical thresher. In such processes, the separation of maize constituents (bran, germen and some endosperm) varies, but could range from 25 to 30% depending on the grain morphology, type of thresher used and consumer preferences, affecting the vitamin and mineral content of the finished product (Cheah et al., 2020; Bressani et al., 2002). Threshing is done to ensure sufficient shelf life of the product by excluding rancidity development factors (fatty acids) which can cause off-odor or off-taste and poor industrial performance. Since threshing efficiency depends on grain characteristics, it is important to ensure that the grain used has adequate physicochemical characteristics to prevent excessive dry matter losses during threshing and consequently higher micronutrient losses.

The calcium content in *arepas* and *mazamorra* produced from non-ZBM varieties was 86.09 μg/g (77.09–91.06 μg/g) and 43.18 μg/g (29.14–53.28 μg/g), respectively; whereas that of ZBM varieties, Ca content was 88.85 μg/g (81.57–99.97 μg/g) in *arepas* and 41.66 μg/g (36.89–45.43 μg/g) in *mazamorra*, which was not significantly different from raw threshed grain of non-ZBM and ZBM varieties (Table 3). Calcium content did not change, as previously reported by Bressani et al.(2004), when cooking whole maize kernels in water. Noteworthy, the TR was higher than 100% due to the use of butter in the *arepas* making process, (Fig. 1 c).

PA is a powerful chelator of divalent cations such as Ca^{2+}, Fe^{2+} and Zn^{2+}, limiting their bioavailability due to the formation of insoluble complexes. However, PA is also important antioxidant with potential to reduce lipid peroxidation (Vashishth et al., 2017). PA content of the threshed maize was 2.90 and 9.24 mg/g, whereas that of cooked threshed grain was 0.88–5.19 mg/g. PA content of the threshed and cooked grain of non-ZBM varieties (2.90–8.81 mg/g and 0.88–4.29 mg/g, respectively) was significantly different from that of the ZBM varieties (6.52–9.24 mg/g and 4.67–5.25 mg/g) (Suppl. Table 1). The losses of PA in *arepas* and *mazamorra* were large due to the removal of most of the germ and pericarp which contain a large amount of it (Cheah et al., 2020). PA content in *arepas* made from non-ZBM group did not show significant differences compared to the ones made with ZBM, ranging from 0.67 to 3.59 mg/g. Similarly, no difference in PA content was found between *mazamorra*, made with non-ZBM maize and ZBM (0.89–4.93 mg/g) (Table 3).

The large losses of PA in non-ZBM threshed maize and cooked grain represented TR of 49.9% and 18.7% respectively, whereas for ZBM intermediate products it was 69.0% and 33.6%, respectively. No significant differences in TR of PA between the non-ZBM and ZBM groups in *arepas* (14.5% and 24.1%, respectively) and *mazamorra* (19.1% and 31.2%, respectively) was found (Fig. 1 d). PA retention was significantly correlated to the retention of Zn ($R^2 = 0.84$) and Fe ($R^2 = 0.60$). Fractionation steps have shown to decrease not only chelating compounds such as PA and fiber, but also minerals including Fe and Zn. This is because both, chelating agents and minerals, are mainly in the germ and aleurone layer (Greffeuille et al., 2011; Cheah et al., 2020; Gannon and Tanumihardjo, 2014). For threshed maize intermediate and final products, large losses of PA and to a lesser extent Fe and Zn were observed due to the physical losses of germ and pericarp.

3.3. Fe, Zn, Ca and PA content in lime-cooked tortillas

Iron content did not change during nixtamalization for ZBM and non-ZBM varieties. Fe content in the *nixtamal* was 17.84 μg/g (16.80–18.89 μg/g) for the non-ZBM group and 16.98 μg/g (15.91–18.42 μg/g) for the ZBM group, whereas in *tortillas* it was 19.88–24.92 μg/g for the non-ZBM group and 22.25 μg/g (20.06–21.85 μg/g) for the ZBM group (Suppl. Table 1). Likewise, zinc content did not change when maize was nixtamalized. The Zn content in *nixtamal* of non-ZBM varieties was 25.22 μg/g (19.44–28.25 μg/g), while in *tortillas* it was 25.72 μg/g (20.39–29.09 μg/g); for the ZBM group, Zn content was 33.67 μg/g (33.11–34.79 μg/g) in *nixtamal* and 34.82 μg/g (34.66–35.05 μg/g) in *tortillas* (Suppl. Table 1). There was no significant difference in %TR between the ZBM and non-ZBM groups for Fe as well as Zn (Fig. 1 a-b). %TR for Fe and Zn in *nixtamal* in the non-ZBM was 92.9% and 101.4%, whereas for the ZBM it was 96.9% and 99.7%, respectively. %TR for Fe and Zn in *tortillas* of non-ZBM was 106.9% and 96.1%, whereas for the ZBM it was 111.4% and 95.6%, respectively (Fig. 1 a-b). In general, for the evaluated ZBM and non-ZBM groups, Fe and Zn were highly retained during the nixtamalization process, and more than 90% of their content was found in the *tortillas* (Escalante-Aburto et al., 2020). The high retention of these minerals could be due to the low dry matter loss in *tortillas*, since most of the zinc is located in the endosperm and germ, and only part of the pericarp was removed during the nixtamalization process. It is well documented that mineral retention when preparing *tortillas* varies according to the type and concentration of Ca $(OH)_2$ used, the cooking time and the steeping time (Bressani et al., 2004; Bressani et al., 2002; Mariscal et al., 2015). No significant losses of Zn or Fe were observed in *tortillas*, most likely because during nixtamalization, only the outermost pericarp layer was removed, which is mostly composed of polysaccharides/hemicelluloses, whereas the aleurone and germ were not removed. Although intrinsic minerals can be reduced during processing, iron contamination of maize products has been found during postharvest treatments, such as harvesting, milling, cooking, under uncontrolled conditions (Greffeuille et al., 2011). Thus, the mineral content in *tortillas* could also come from the stone grinder. Similarly, no differences were observed in %TR of minerals in QPM vs non-QPM varieties. This is in agreement with previous results reported by Serna-Saldivar et al. (1991), who compared QPM and normal endosperm maize processed into nixtamalized *tortillas*. For these set of samples, the proportion of variability in %TR due to genotype was similar to the proportion due to processing, suggesting that processing is as important as the type of grain when it comes to Zn retention in the final product and therefore the Zn intake of consumers.

Ca content in *nixtamal* ranged from 1215 to 2350 μg/g which represented an increase of 16–72 times the original content. Ca content in *nixtamal* and *tortillas* for the non-ZBM group was 1750 μg/g (1435–2350 μg/g) and 1560 μg/g (1325–2015 μg/g), respectively, whereas the Ca content for the ZBM group was 1560 μg/g (1215–1895 μg/g) in *nixtamal* and 1445 μg/g (1305–1690 μg/g) in *tortillas* (Suppl. Table 1), which indicates an expected and well documented significant increase in Ca due to the process of cooking and steeping raw whole grain with $Ca(OH)_2$. (Escalante-Aburto et al., 2020). In fact, nixtamalization has been recognized as a way to increase the Ca supply to consumers, even when PA content is elevated (>1500 mg/180 g of *tortilla*). Rosado, Diaz, Rosas, Griffit, and Garcia (2005) found that the percentage of Ca absorption was high in a group of Mexican women who consumed nixtamalized *tortillas*. Ca uptake by the kernels during nixtamalization has found to be dependent on Ca concentration, steeping time, changes in the pericarp and the diffusive mechanisms. Additionally, differences in Ca uptake by dent or flint kernels, have been reported. *Tortillas* prepared with soft kernels cooked at higher temperatures and longer steeping times will retain more Ca (Bressani et al., 2004; Bressani et al., 2002; Mariscal et al., 2015; Samil and Sait, 2016). The six varieties used in this study showed the same trend in Ca retention in the *tortilla* (Fig. 1 c) and as reported previously, slightly more Ca was observed in the *nixtamal* compared to the *tortillas* (Escalante-Aburto et al., 2020).

PA content of the *nixtamal* for non-ZBM (8.93–10.94 mg/g) was not significantly different than for ZBM varieties (10.07–13.14 mg/g). PA content of *tortillas* for non-ZBM (10.29–12.67 mg/g) were similar than

Table 5
Contribution of maize to zinc EAR% for children 4–6 years old and women of childbearing age.

Population	Process	Maize type	Zn in maize (μg/g)	Zn process retention (%)	Zn in processed maize (μg/g)	Zn daily intake (mg)[a]	Zn absorbed (μg)[b]	Contribution to EAR (%)[c]
Children	Not processed	ZBM	37	100	37.0	3.70	925	80
		non-ZBM	25	100	25.0	2.50	625	54
	Tortillas (limited bran removal)	ZBM	37	96	35.4	3.54	884	76
		non-ZBM	25	96	24.0	2.40	601	52
	Mazamorra (extensive bran removal)	ZBM	37	47	17.3	1.73	434	37
		non-ZBM	25	36	8.9	0.89	223	19
	Arepas (extensive bran removal)	ZBM	37	43	15.8	1.58	396	34
		non-ZBM	25	31	7.7	0.77	193	17
Women	Not processed	ZBM	37	100	37.0	9.25	2313	93
		non-ZBM	25	100	25.0	6.25	1563	63
	Tortillas (limited bran removal)	ZBM	37	96	35.4	8.84	2211	89
		non-ZBM	25	96	24.0	6.01	1502	61
	Mazamorra (extensive bran removal)	ZBM	37	47	17.3	4.34	1084	44
		non-ZBM	25	36	8.9	2.23	557	23
	Arepas (extensive bran removal)	ZBM	37	43	15.8	3.96	990	40
		non-ZBM	25	31	7.7	1.93	482	19

[a] Maize intake = 100 g for children 4–6 years and 250 g for women.
[b] Bioavailability = 30% (EFSA, 2014).
[c] Physiological daily average requirement for zinc = 1390 μg for children 4–6 years and 2969 μg for women of childbearing age (EFSA, 2014).

ZBM varieties (10.70–11.45 mg/g) (Suppl. Table 1). %TR of PA in *nixtamal* and *tortilla* for non-ZBM group was 95.9% and 101.9%, respectively. For ZBM varieties, %TR of PA in *nixtamal* and *tortilla* was 118.3% and 103.9%, respectively (Fig. 1 d). Since the dry matter losses were low and most of the germ remained in the dough, the PA content was as expected (Greffeuille et al., 2011). Between 0 and 35% PA reductions have also been reported; however, in this study, losses of PA in *tortillas* were not found. Such differences in PA loss are dependent on the maize genotype and PA levels in the raw kernels, percentage of $Ca(OH)_2$, cooking and steeping time, as well as the intensity at which the *nixtamal* is washed, prior to milling (Bressani et al., 2004; Bressani et al., 2002). Research on the effect of nixtamalization on PA content is not extensive, but previous studies have shown a decrease in PA of 0.25–35% (Gomez-Aldapa, Martínez-Rustos, Figueroa-Cárdenas, Orodrica-Falomir and González-Hernández, 1996; Urizar and Bressani, 1997). Bressani, Turcios, Colmenares de Ruiz, and Palacios de Palomo (2004) showed higher PA loss when using 1% lime and 75 min of cooking. A reduction of about 18% in PA content in whole grain and germ was reported but losses were higher in the endosperm (46%). Greffeuille et al. (2011), reported PA losses of up to 14% in the first steps of *ogi* preparation, which are like the nixtamalization steps, but without using an alkaline solution. In the present study, nixtamalization was done using 1% of Ca $(OH)_2$, 50 min of cooking and a relatively long steeping time (16 h); however, no significant losses of PA were observed, presumably due to the relatively hard kernels, which limited the effect of lime during cooking and steeping.

3.4. True retention of protein, lysine and tryptophan in final products

The protein content in *tortillas*, *arepas* and *mazamorra* in non-QPM varieties was 11.1%, 10.5–10.8% and 7.9–8.8%, corresponding to TR of 104.2%, 77.9% and 62.9%, respectively. Protein content in the same foods made from the QPM group was 10.0–10.8%, 8.0–9.8% and 8.0–9.3%, corresponding to TR of 101.9%, 69.2% and 62.7% for *tortillas*, *arepas* and *mazamorra*, respectively (Table 4). Tryptophan content in *tortillas* ranged between 0.033–0.042% and 0.045–0.053% when using non-QPM and QPM grains, respectively, while in *arepas* and *mazamorra* it was 0.011–0.015% in non-QPM and 0.017–0.023% in QPM. Lysine content in *tortillas* was 0.29–0.32%, while in *arepas* and *mazamorra*, it was 0.14–0.18% for both when using non-QPM varieties; in the QPM group, lysine content was 0.37–0.41% for *tortillas*, 0.22–0.25% for *arepas* and 0.21–0.27% for *mazamorra*. %TR for tryptophan and lysine among processed products was not significantly different between non-QPM and QPM groups (Table 4). These results agree with previous reports where in average more than 90% and 80% TR has been found for lysine and tryptophan, respectively, in nixtamalized *tortillas* (Gomez-Aldapa, Martínez-Rustos, Figueroa-Cárdenas, Orodrica-Falomir and González-Hernández, 1996; Vasquez-Carrillo et al., 2014; Bressani et al., 1990).

3.5. Mineral bioavailability in final products

In whole grain the PA:Fe molar ratio was 46.1 (33.6–56.0) for the non-ZBM group and 49.0 (45.1–55.5) for ZBM, without significant differences between groups. The PA:Zn ratio in non-ZBM (38.4–46.1) was significantly higher than for ZBM (26.0–34.6) (Table 3). For non-ZBM *tortillas*, PA:Fe and PA:Zn molar ratios were 45.0 (41.4–48.9) and 44.8 (41.0–50.4), whereas for ZBM *tortillas*, the molar ratio were 45.1 (43.1–48.5) and 31.6 (30.5–32.7), respectively. In general, molar ratios in tortillas were similar to the ratios found in raw maize grain probably because no major Fe, Zn and PA losses were found after processing maize into *tortillas* (Table 3). The PA:Fe molar ratio for *arepas* produced with non-ZBM and ZBM was 12.8 (8.9–14.9) and 17.7 (12.6–22.7), respectively, whereas the PA:Zn molar ratio was 18.9 (17.5–21.2) and 16.9 (11.2–21.3), respectively. The PA:Fe molar ratio for *mazamorra* produced with non-ZBM and ZBM was 24.6 (17.8–32.4) and 29.0 (25.9–32.8), respectively, while the PA:Zn molar ratio was 22.0 (19.7–24.7) and 19.3 (18.1–20.9), respectively. Ratios were not different between non-ZBM and ZBM (Table 3). Theoretical estimations of Zn bioavailability based on PA:Zn molar ratios (EFSA, 2014) suggest that their absorption might be 25–45% with lower absorption at high PA:Zn ratios. Hambidge et al. (2017) reported Zn absorption of 22–34% when using maize as the main component of a diet with relatively high PA, and 22–40% when using maize in a diet with lower PA. High Ca levels could exacerbate the bounding of Zn to PA during processing and make Zn less available.

Additionally, large amounts of Ca in nixtamalized products may reduce Fe bioavailability (Bressani et al., 2004), and therefore the bioavailability of Zn and Fe in products made with nixtamalized maize could be low (Gibson et al., 2010; Suri and Tanumihardjo, 2016). However, for Mexican and several Central American populations, the high intake of Ca through nixtamalized products is recognized and well documented (Rosado et al., 2005). Although PA content in *mazamorra* and *arepas* was lower than in *tortillas*, PA:Zn and PA:Fe ratios were also higher than 15, suggesting low mineral bioavailability. Furthermore, the lower PA:Zn ratio found in tortillas made with ZBM could result in better Zn absorption compared to tortillas made with non-ZBM. In vitro studies with caco-2 cells or absorption studies will be helpful to further validate these results.

3.6. Impact of high kernel-zinc maize on Zn intake

The contribution of ZBM to Zn EAR would be up to 76% for children aged 4–6 years and 89% for women of child-bearing age when consumed as minimally processed products such as *tortilla* and if ZBM is the only type of maize consumed (Table 5). The contribution to daily Zn intake could be lower and directly related to the proportion of ZBM consumed relative to the total amount of maize consumed. Furthermore, the contribution of ZBM would be only 34–44% if consumed as *arepa* or *mazamorra* because the process removes the germ and pericarp of the grain, that contain most of the Zn (Table 5). However, the Zn bioavailability in processed *arepas* and *mazamorra* could be higher compared to *tortillas* due to the lower PA concentration in threshed grain used to make these products. Besides, although the most common type of arepa is prepared with decorticated kernels, recent healthier trends may create a consumer demand for whole grain products over highly processed ones (Willet et al., 2019; Palacios-Rojas et al., 2020). In summary, the potential contribution of zinc to the EAR for children 4–6 years old and women of child-bearing age was between 18 and 29% higher in all products made with ZBM than non-ZBM (Table 5).

4. Conclusions

In the present study, *tortillas* had a higher Fe, Zn and PA retention than *arepas* and *mazamorra*. QPM products had higher lysine and tryptophan content compared to products prepared with non-QPM. In countries where *arepas* and *mazamorra* are consumed, the promotion of whole grain flour should be explored to enhance the intake of minerals that are usually confined to aleurone layers and germ. Zn biofortified maize could contribute to increase the Zn intake of populations where maize is an essential part of the diet.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2021.03.007.Multim

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