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Original Research

Optimization of the Effects of Nixtamalization on the Nutritional and Anti-Nutritional Contents of Quality Protein Maize Flour

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Abstract

Maize is the world's most significant cereal grain, which provides nourishment for both human beings and the living animals. For that reason, the utilization of maize in developing countries calls for investigations of processing methods to evaluate and enhance nutritional compositions. This study aimed to evaluate the effect of nixtamalization on the nutrient and anti-nutrient contents of quality protein maize (QPM). A response surface experimental design was used at three levels of each factor: cooking time (20, 40, and 60 minutes), steeping time (8, 12, and 16 hours), and lime concentration (1.0, 1.5, and 2.0%). The results showed that all the factors significantly ($p<0.05$) affected the nutrient and anti-nutrient composition of the nixtamalized QPM (NQPM). Numerical optimization showed that higher nutrient and lower anti-nutrient contents were obtained at cooking time of 20 minutes, steeping time of 8 hours, and lime concentration of 1.0%. The mean values of moisture, protein, fat, fiber, ash, carbohydrate, energy, tannin, phytate, and oxalate composition of the NQPM flour were 8.9%, 10.9%, 5.4%, 1.7%, 1.9%, 71.0%, 376.8 kcal/100g, 2.0 mg/100g, 6.5 mg/100g and 1.2 mg/100g, respectively. The nutritional value of NQPM flour was improved at the optimum condition.

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INTRODUCTION

Maize (*Zea mays*) is one of the most important crops in the world for feed, food, and industrial applications (Revilla *et al.,* 2022). After wheat, maize is the second-most significant cereal crop in the world, followed by rice. In terms of research and adaptability, maize has garnered significantly more attention even though it is one of the most recent entries to the list of food crops in Africa (Anteneh & Asrat, 2020). As a result, maize has overtaken other crops as the most important contributor to modern agriculture and food security in sub-Saharan Africa (Anteneh & Asrat, 2020). The crop supplies a significant amount of nutrients that motivate the food industries to produce many maize-based food items (Escalante-aburto *et al*., 2019). In Africa, each country has different processing methods, food products, and ways of eating maize and maize-based foods (Mensah *et al*., 2013). Countries have one or more dishes unique to their culture in terms of maize utilization for food. For instance, in Central and Latin America, maize is consumed in the form of tortillas (Yetneberk *et al*., 2000).

Maize is also one of the most important and strategic grains that play an important role in the food security and livelihood of Ethiopian farmers (Balemi *et al*., 2020). About 302,054,260.58 quintals (88.36%) of the grain production came from cereals. The grain output was dominated by maize, teff, wheat, and sorghum, accounting for 30.88% (105,570,935.92 quintals), 16.12% (55,099,615.14 quintals), 16.91% (57,801,305.96 quintals), and 13.22% (45,173,502.18 quintals), respectively (CSA, 2020). The per capita consumption of maize in the country is about 60 kg per year (Demeke, 2018). The traditional maize processing techniques may affect the nutritional contents and their availabilities. For instance, during the traditional food preparations the pericarp germs are sieved out as unwanted material, which results in the loss of many nutrients present in the maize kernel structures (Ekpa *et al*., 2018). Maize is a

highly productive cereal crop in Ethiopia; therefore, there is the need to introduce a variety of maize-based foods with higher nutritional value through appropriate processing techniques.

Nixtamalization is one of the processes that enhance the nutritional quality of maize and helps to develop some maize-based food types that might be convenient for utilization (Inyang *et al.*, 2019). The nixtamalization process has some limitations on the industrial scale: inefficient heat transfer, excessive water waste, and polluting effluents due to the industrial's nejayote alkalinity (Mendoza-Madrigal *et al*., 2017). Some of the factors that increase the loss of dry matter are grain's physical characteristics, such as mechanical damage, low density, thinner pericarp, and soft hardness. And some of the nixtamalization conditions like long cooking time with higher temperature followed by long steeping time, higher steeping temperature, and higher lime concentration (Escalante-Aburto *et al*., 2019).

During the process, the addition of calcium and water into the maize grains causes significant physicochemical effects on pericarp, germ, and endosperm, which contributes to good nutritional and sensory properties. Traditional processing methods, such as fermentation, germination, and roasting, are inexpensive, straightforward, and practiced for many years and established different eating habits (Ejigui *et al*., 2007). However, nixtamalization is vital due to its use both as a traditional and industrial method for processing maize. In addition, nixtamalization facilitates the pericarp removal, partial gelatinization of starches, and solubilisation of protein. Furthermore, the use of lime in the processing of maize grains increases calcium content of the product (Morales & Zepeda, 2017). Thus, the use of nixtamalization to process quality protein maize (QPM) may help to enhance availability of nutrients and protein quality, minimize loss of nutrients, and reduce anti-nutrients. Therefore, this study was aimed to optimize the effect of nixtamalization process on the proximate composition and anti-nutritional factors of QPM flour.

MATERIALS AND METHODS

Study Site

This study was conducted at Jimma University, College of Agriculture and Veterinary Medicine, Department of Postharvest management laboratory, and Animal nutrition laboratory.

Experimental materials

The QPM for this experiment was obtained from Melkassa Agricultural Research Center (MARC). The registered name of the QPM variety is Melkassa 6Q (QPM Melkassa 6Q). The QPM maize kernels 15kg were taken from MARC to Jimma University, College of Agriculture and Veterinary Medicine, Department of Postharvest Management, Postharvest laboratory.

Raw material preparation of QPM

The quality protein maize QPM grain was manually cleaned and foreign materials, immature grains, and damaged seeds were removed. Then, cleaned grains were stored in plastic bags and transported to Jimma University, College of Agriculture and Veterinary Medicine, Postharvest management laboratory. The QPM intended for this experiment was selected for good kernel weight. Food-grade $Ca(OH)_2$ (lime concentration) used for this study was taken from a postharvest management laboratory store, 95% purity $Ca(OH)_2$ (supplier postharvest management laboratory store, 95% purity).

Raw materials

A QPM grain of 15kg was obtained from Melkassa Agricultural Research Center (MARC). The QPM was selected for its good kernel weight. The maize grain was manually cleaned and foreign materials, immature grains, and damaged seeds were removed. Then, cleaned grains were stored in plastic bags and transported to Jimma University, Postharvest laboratory. Food-grade $Ca(OH)_2$ of 95% purity used for this study was obtained from postharvest laboratory store.

Experimental design

The design expert software (Design Expert version 13.0.5.0 64-bit) was used to determine treatment combinations using response surface method (RSM). The central composite design (CCD) approach with three numerical factors (cooking time, steeping time, and concentration of lime) was used to determine the best combination of the nixtamalization process to produce nixtamalized QPM flour. Then, 20 experimental runs in three blocks were generated. The response variables chosen were proximate composition and anti-nutrient composition of nixtamalized QPM flour.

Nixtamalization process

The overall process of nixtamalization procedure is described on Figure 1. For each sample treatments, 500 g of maize kernels were cooked in 2000 mL of distilled water for different lime ($Ca(OH)_2$) concentrations (1.0%, 1.5%, and 2.0%). Then, the maize kernels were cooked at 90 °C for different cooking times (20, 40, and 60 minutes). After cooking, the maize was steeped for different steeping times (8, 12, and 16 hours). The nejayote (cooking liquor) was drained off; the cooked maize kernels were washed twice in running tap water by stirring the grains in the washing water. Then, the sample was dried at 55 °C for 10 hours and finally milled into flour. The control sample was prepared by milling maize kernels without cooking and addition of lime. The nixtamalized QPM flour and the raw flour (control) were packed in a polyethylene bag and stored at ambient temperature till laboratory analysis conducted at Jimma University, College of Agriculture and Veterinary Medicine, Department of Food Science and Postharvest Management Technology, Postharvest Laboratory.

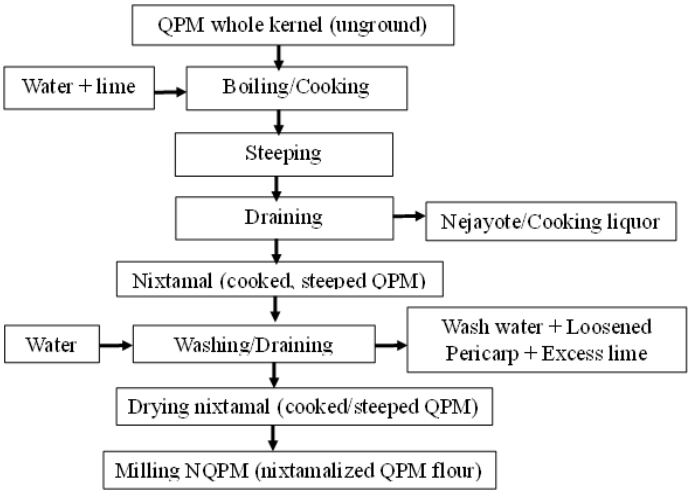

Figure 1: The processing procedure for traditional nixtamalization process for the production of nixtamalized maize flour

Experimental analysis

Determination of proximate composition

The proximate compositions (dry matter basis) of the nixtamalized QPM flours were determined following the Association of Official Analytical Chemists (AOAC) methods: moisture (925.05.), crude protein (979.09), crude fiber (920.169), crude fat (920.39) and ash (941.12) (AOAC, 2000). The total carbohydrate content of the samples was determined by difference using equation (1).The energy value (Kcal) of each sample was determined according to the already established Atwater factor method, as described in equation (2) (Lewu *et al.*, 2010).

$$\%\ \text{Carbohydrate content} = 100\% - (\%\ \text{moisture} + \%\ \text{protein} + \%\ \text{Fat} + \%\ \text{Fiber} + \%\ \text{Ash}) \quad (1)$$

$$\text{E.V} = (9 \times \text{Crude Fat}\ \%) + (4 \times \text{Crude Protein}\ \%) + (4 \times \text{Carbohydrate}\ \%) \quad (2)$$

Anti-nutritional factors

Phytate

Determination of phytate content was done as described by Vaintraub & Lapteva (1988). About 0.5 g of each sample was extracted with 10 mL of 2.4% HCl in a mechanical shaker (*HY-2(A), Speed Adjusting Multi-purpose Vibrator, China*) for 1 hour at room temperature. The extract was centrifuged at 3000 rpm for 30 minutes. The clear supernatant was used for phytate content determination. Then, 1 mL of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicylic acid in water) was added to 3 mL of the supernatant solution. A series of standard solutions with different concentrations (0 – 150 µg/mL) of phytic acid (analytical grade sodium phytate) were prepared in 0.2N HCl and absorbance was measured. A 3 mL of the standard was added into 15 mL of centrifuge tubes with 3 mL of water used as a blank. Next, 1 mL of the wade reagent was added to each test tube, and the solution was mixed on a Vortex mixer for 5 seconds. The mixture was centrifuged for 10 minutes, and the absorbance of the solutions (both samples and standards) was measured at 500 nm using UV-VIS spectrophotometer (*Beckman DU-64- spectrophotometer, USA*). A standard curve was derived from absorbance versus concentration of sodium phytate standard (Y = -0.006X+1.250, R^2 = 0.992). The phytate content was calculated by using the following formula (3) and the result was reported in mg/100g.

$$Phytate\ \left(\frac{mg}{100g}\right) = \frac{[(AbS - AbB) - Intercept] * 10}{Slope * W * 3} \quad (3)$$

Where: W = Weight of sample in gram
AbS = Sample absorbance
AbB = Blank absorbance
10 = Conversion factor from µg/mL to mg/100g
3 = Volume of aliquot

Oxalate

Oxalate content was determined according to the official method (AOAC, 2000). In brief, 1 g of each sample was mixed with 190 mL of distilled water and 10 mL of 3 M HCl before being digested at 100 °C for 1 h. The digested sample was filtered, cooled, and diluted to a volume of 125 mL. Four drops of methyl red indicator were added to 125 mL of the filtrate, which had been transferred to a beaker. Concentrated NH_4OH was used to titrate the solution until the test solution's salmon pink color changed to a faint yellow hue (pH 4 to 4.5). Then, each portion was heated to 90 °C, cooled, and filtered to remove ferrous ion precipitate. Each piece contained 75 mL of solution. With steady stirring, 10 mL of a 5% $CaCl_2$ solution was added after the filtrate had been heated to 90 °C once more. The filtrate was heated, cooled, and then allowed to stand overnight in a refrigerator (5 °C). Sigma laboratory centrifuges, 2- 16 KC, Germany, were used to centrifuge the solution for 5 minutes at 2500 rpm. The precipitate was completely dissolved in 10 mL of a 20% (v/v) H2SO4 solution after the supernatant was decanted. After 1 g of the sample was digested, a total of 150 mL of filtrate was produced. A faint pink color that remained for 30 seconds was achieved by titrating 75 mL aliquots of the filtrate with 0.01 M KMnO4 standard solution after heated almost to boiling. Finally, the oxalate concentration in each sample was calculated using equation (4).

$$\text{Oxalate}\ \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{T\ x\ Vme\ x\ DF\ x\ 10S}{ME\ x\ MF} \quad (4)$$

where T is the titre of $KMnO_4$ (mL), V_{me} is the volume-mass equivalent (i.e. 1 cm^3 of 0.1 N $KMnO_4$ solution is equivalent to 0.006303 g anhydrous oxalic acid), DF is the dilution factor, ME is the molar equivalent of $KMnO_4$ in oxalate, and MF is the mass of flour used.

Tannin

Tannin content was determined as described by Maxson and Rooney (1972). One gram of the sample was weighed and mixed with 10 mL of 1% HCl solution in methanol in a screw cap test tube. Then the tube was shaken for 24 hr at room temperature on a mechanical shaker (*HY-2(A), Speed Adjusting Multi-purpose Vibrator, China*). The solution was centrifuged at 3000 rpm for 30 minutes. Then, 1 mL of supernatant was transferred to another test tube and mixed with 5 mL of vanillin-HCl reagent (prepared by combining a volume of 8% concentrated HCl and 4% vanillin in methanol). D-catechin standard was used for condensed tannin determination. A stock solution was prepared by dissolving 40 mg of D-catechin in 100 mL of 1% HCl solution in methanol. Series of standard solutions of different concentrations (0 – 60 mg /100 mL) were prepared from the stock solution. The volume of each test tube was adjusted to 1 mL with 1% HCl in methanol, and 5 mL of vanillin-HCl reagent was added to each test tube. After 20 minutes, the absorbance of sample and standard solutions were

measured at 500nm using UV-VIS spectrophotometer (*Beckman DU-64- spectrophotometer, USA*). A calibration curve (R^2 =0.993) was derived from absorbance versus concentration of catechin standard (Y = 0.0114x+0.0526, R^2 = 0.9926). The final results were calculated from the slope and intercept of the calibration curve using the following equation (5) and reported in mg/100g.

$$Tannin\ \left(\frac{mg}{100g}\right) = \frac{[(AbS-AbB)-Intercept]*10}{Slope*W*d} \text{---------- (5)}$$

Where: AbS = Sample absorbance
AbB = Blank absorbance
W = Weight of sample in gram
d = Density of solution (0.791g/ml)
10 = Conversion factor from µg/mL to mg/100g

Numerical Optimization

Numerical optimization was carried out for the process variables in nixtamalization of the QPM at the desired goals assigned for all the parameters by maximizing values of protein, fat, total ash, and energy. Conversely, minimizing the cooking time, steeping time, and the anti-nutritional factors (tannin, phytate, and oxalate). The total carbohydrate, moisture content, crude fiber, and lime concentration were set in the range.

Statistical analysis

The data were analyzed and modeled using Design Expert (version 13.0.5.0 64-bit) to generate second-degree polynomial models with response surface effects. ANOVA was used to identify the significant terms in the models for each response and accepted at a 0.05 level of probability (*P < 0.05*). The Design expert statistical software package was also used for the optimization of the nutritional and anti-nutritional contents of QPM flour.

RESULTS AND DISCUSSION

Proximate compositions

Moisture content

The finding indicated that steeping time and the interaction effect of steeping time and lime concentration significantly (*p≤0.01*) affected moisture content (MC) of the nixtamalized QPM flour (Table 1). The MC of the nixtamalized QPM ranged from 7.6% to 10.2%, while the MC of the raw QPM was 9.3% (Table 2). The highest moisture content was found in nixtamalized maize flour, but all samples fell below the level set by Mexican Official Norm NOM-147-SSA1-1996 (Normas Oficiales Mexicanas, 1999) for this type of product. This indicates that the flour developed can be considered secure dry products with a long shelf life (Rodrguez-Miranda et al., 2011). This value is within the recommended range (<13%) for safe storage of maize grains as reported by Prieto *et al*. (2021). The highest moisture content recorded was 10.2% at a cooking time of 20 and 60 minutes, steeping time of 16 hours and lime concentrations of 1% and 2%. Whereas, the lowest MC was 7.6% recorded at 40 minutes cooking time, 12 hours steeping time and 2.34% lime concentration (Table 2). This result indicates that a longer cooking time and steeping time with lower lime concentration might result in higher MC. But, shorter cooking time and steeping time, and higher lime concentration may result in lower MC. Similar was reported by Kadir *et al*. (2019), in which the moisture content was highest in the treatments cooked for 60 minutes with 0.5% of lime; while the moisture content was lowest at 30 minutes of cooking time with lime concentration of 1.5%. Similar finding by Upreti *et al*. (2006) was also reported that the higher the calcium content, the smaller is the amount of water that the flour might trap. Similarly, the decrease in the MC of nixtamalized corn when treated with higher lime concentration was also reported by Kadir *et al*. (2019). The result shows that the MC increases as steeping time increases with a slight decrease in lime concentration at constant cooking time.

3.1.2. Crude protein content

Both cooking time and steeping time significantly (*p≤0.05*) affected the crude protein content of the nixtamalized QPM flour (Table 1). The crude protein content of the nixtamalized QPM flour samples ranged from 9.7–11.5%; which was lower compared to the protein content of the raw control maize grains 12.9% (Table 2). The value found in this work was in the range 6.7-11.6% reported by Almeida-Dominguez *et al*. (1996) for common maize. Similarly, Méndez-Montealvo *et al*. (2005) reported protein content of 7.6–11.1% in QPM genotypes. However, the average result of this study is higher compared to the protein contents of yellow QPM (9.5%) and white QPM (10.4%) (Bressani, 1990). The interaction effect of cooking time, steeping time and lime concentration showed a significant (*p≤0.05*) effect on crude protein content (Table 1). Similar decrease in the protein content of the QPM by nixtamalization process compared to the control was also reported in other works (Farinde *et al*., 2021; Maureen *et al.*, 2020). These sources reported the reduction of protein content in the nixtamalized maize compared to the raw. The decrease might be attributed to partial separation of the germ or germ losses from the kernel and other protein losses during the nixtamalization process (Maureen *et al.*, 2020). This study indicated that protein content of nixtamalized QPM flour can be preserved by the reduction of steeping time and cooking time.

Table 1: Regression coefficients of a quadratic polynomial model, R^2, and lack of fit for proximate composition parameters of nixtamalized QPM flour

Source	MC	CP	CF	FC	Ash	CHO	Energy
Model	5.43**	2.48**	3.39*	9.14**	2.23*	16.23*	542.26**
Intercept	8.89	10.53	5.38	2.09	2.00	71.32	374.98
A	0.085	-0.17**	0.22*	-0.22*	-0.096	0.18	1.77
B	0.48**	-0.27**	-0.14	0.65**	0.049	-0.77**	-5.72**
C	-0.19	-9.674E-004	-0.050	-0.11	0.30**	0.042	0.027
A^2	0.096	-0.049	0.018	-0.054	-0.027	-	0.15
B^2	0.10	-0.012	-0.13	-0.15	0.14	-	-0.90
C^2	-0.12	0.21**	-0.12	-0.22*	-0.065	-	1.15
AB	0.17	-0.16*	-0.025	-0.090	0.21**	-0.078	-0.76
AC	-0.15	0.046	-0.17	0.078	0.022	0.17	-1.12
BC	0.32*	-0.13	0.47**	0.42**	-0.12	-0.96**	-0.64
R^2	0.84	0.89	0.81	0.92	0.87	0.64	0.86
lack of fit	*0.2033*	0.2004	0.5782	0.3807	0.8264	0.0561	0.7257

*A= Cooking time, B= Steeping time, C=Lime concentration, MC=Moisture Content CP=Crude Protein, CF =Crude Fat, FC= Fiber Content, CHO=Carbohydrate, *Significant at P≤ 0.05 level, **Significant at P≤ 0.01.*

Crude fat content

The result showed a significant effect of cooking time ($p \leq 0.05$) and interaction of steeping time and lime concentration ($P \leq 0.01$) on the crude fat content of QPM flour (Table 1). The crude fat content of nixtamalized QPM flour in this study ranged from 4.2–6.0%, while the fat content of raw QPM was 5.6% (Table 2). The highest fat content (6.0%) was observed at 60 minutes cooking time, 8 hours steeping time, and 1% lime concentration, whereas the lowest (4.2%) at 20 minutes cooking time, 16 hours steeping time and 1% lime concentration. The result showed that the nixtamalization process slightly decreased the fat content of QPM. Similar decrease from 6.1 to 5.0 in the fat content of nixtamalized QPM was reported by Pflugfelder *et al.* (1988). This effect could be due to removal of the pericarp and germ tissues during the cooking and washing of the nixtamal that decreases the fat content (Gómez *et al*., 1991). The decrease in fat content might also be attributed to the high temperature and metal ions (Ca^{2+} in this case) promoting the oxidation and decomposition of fat (Charley and Weaver, 1998). Although the crude fat content of the flour is lower, it is advantageous in extending shelf life of the product (Olaoye *et al*., 2007). On the other hand, the fat content was higher when the steeping time and lime concentration were lower. The overall effect shows that the cooking time is the main factor, and the interaction of steeping time and lime concentration is a significant factor affecting the fat content of nixtamalized QPM flour.

Crude fiber content

The result of this study showed that the cooking time, steeping time, and interaction of steeping time and lime concentration significantly ($p \leq 0.05$) affected the crude fiber content of nixtamalized QPM flour (Table 1). The crude fiber content of the nixtamalized QPM flour ranged in 0.2–3.3% (Table 2). The average crude fiber content of the nixtamalized QPM was higher compared to the raw QPM flour (1.6%). Similar crude fiber levels (3.3%) in the nixtamalized maize flour, for classic nixtamalized maize flour (2.7), and traditionally nixtamalized maize flour (2.5%) were reported (Sunico *et al*., 2021). The finding shows that the percentage of fiber content increased with increasing steeping time and lime concentration. Similar an increase in the crude fiber content was reported by Sunico *et al.* (2021), in which the fiber content of QPM increased with steeping time. The increase may be due to the interaction of ions (Ca^{2+} and OH^-) in the cooking medium with the components of the grain, which produces some indigestible products enhancing the crude fiber (Ocheme *et al*., 2010). This can be an advantage to prevent constipation, as crude fibers contribute to facilitating the evacuation of the bowels from the human intestine.

Table 2: Proximate compositions (%) of nixtamalized QPM flour

A	B	C	MC	CP	CF	CFC	Ash	CHO	E (kcal/100g)
20	8	1	9.49±0.04	11.1±0.02	5.85±0.02	1.61±0.02	1.68±0.02	70.27±0.04	378.13±0.15
40	12	1.5	8.65±0.01	10.73±0.04	5.89±0.01	1.79±0.04	1.47±0.06	71.47±0.05	381.81±0.13
20	16	2	10.16±0.01	10.76±0.05	5.86±0.02	2.81±0.03	1.95±0.04	68.41±0.03	369.62±0.10
40	12	1.5	9.23±0.04	10.47±0.03	5.27±0.03	2.08±0.02	1.91±0.05	71.04±0.05	373.47±0.35
60	16	1	10.24±0.02	10.28±0.04	5.05±0.02	1.41±0.03	1.73±0.02	71.29±0.06	371.73±0.10
60	8	2	8.51±0.01	11.46±0.02	5.15±0.04	0.53±0.04	1.77±0.04	72.51±0.03	382.51±0.20
40	12	1.5	8.94±0.04	10.54±0.02	5.48±0.05	1.76±0.03	2.41±0.03	70.87±0.04	374.96±0.16
60	8	1	8.81±0.03	10.51±0.05	5.99±0.07	0.88±0.04	1.37±0.03	72.44±0.04	385.71±0.31
20	8	2	8.13±0.01	10.88±0.04	4.83±0.08	0.24±0.05	2.89±0.04	73.07±0.05	383.11±0.13
60	16	2	9.53±0.02	9.71±0.02	5.23±0.03	2.03±0.04	2.56±0.03	70.94±0.09	369.67±0.11
20	16	1	8.58±0.01	10.53±0.05	4.16±0.02	1.79±0.03	2.11±0.02	72.83±0.02	370.88±0.15
40	12	1.5	9.05±0.02	10.46±0.05	5.15±0.05	2.28±0.04	2.26±0.03	70.1±0.06	371.39±0.12
40	12	1.5	8.75±0.05	10.58±0.01	5.07±0.04	2.2±0.04	2.19±0.04	71.21±0.02	372.79±0.12
6.36	12	1.5	8.62±0.05	10.75±0.04	4.53±0.04	2.83±0.06	2.05±0.02	71.22±0.03	368.65±0.11
40	12	2.34	7.61±0.02	11.07±0.01	4.62±0.02	1.51±0.07	2.44±0.04	72.75±0.04	376.86±0.10
73.64	12	1.5	8.88±0.05	10.15±0.05	5.9±0.02	1.97±0.08	1.98±0.05	71.15±0.05	378.3±0.15
40	12	1.5	8.72±0.01	10.41±0.04	5.45±0.03	2.41±0.06	1.78±0.03	71.22±0.03	375.57±0.18
40	5.27	1.5	7.89±0.03	10.85±0.06	4.91±0.02	0.89±0.05	2.49±0.05	73±0.04	379.59±0.13
40	12	0.66	8.66±0.01	11.31±0.03	5.04±0.01	2.33±0.04	1.38±0.04	71.28±0.05	375.72±0.15
40	18.73	1.5	9.64±0.03	10.26±0.04	4.67±0.05	3.34±0.05	2.51±0.08	69.58±0.04	361.39±0.13
Raw QPM (control)			9.28±0.07	12.88±0.04	5.58±0.03	1.57±0.06	1.22±0.06	69.47±0.05	379.62±0.14

A=Cooking time (min), B=Steeping time (hrs), C=Amount of lime (%), MC= Moisture Content, CP = Crude Protein, CF = Crude Fat, CFC = Crude fiber content, CHO = Crude carbohydrate, E = Energy, Std Dev=Standard Deviation

Ash content

The finding showed that there is a significant effect of the lime concentration (*p ≤ 0.01)* and the interaction of cooking time and steeping time (*p ≤ 0.01)* on ash content of the nixtamalized QPM flour (Table 1). There is an increase in the ash content of the nixtamalized QPM (1.4–2.9%) compared to the raw QPM flour (1.2%) (Table 2). Since ash content is the reflection of mineral composition, the increase could be due to the addition of lime during nixtamalization that increases calcium (Ca^{2+}) content (Salazar *et al*., 2014; Serna-Saldivar *et al*., 1991). The results in this study agrees with the ash content of traditionally nixtamalized QPM flour (2.3%) (Sunico *et al*., 2021). The highest value of ash content was found at cooking time of 20 minutes and steeping time of 8 hours. The finding indicated that the ash content increased by reducing cooking time and steeping time, which agrees with the report of Salazar *et al*. (2014). Therefore, the consumption of QPM cooked with lime is beneficial in terms of mineral content.

Carbohydrate content

The result of this study showed that steeping time and interaction of steeping time with lime concentration significantly (*P ≤ 0.01)* affected carbohydrate content of the nixtamalized QPM flour (Table 1). The total carbohydrate content of the nixtamalized QPM flour was ranged in 68.4–73.1% (Table 2). There was an increase in average carbohydrate content of the nixtamalized QPM flour compared to the control (69.5%). The values of carbohydrate content in this study were slightly lower than the result reported by Kadir *et al*. (2019), which ranged in 74.4–75.4%. Short steeping time preserved more carbohydrate, while long steeping time may lead to the loss of carbohydrate. According to Ocheme *et al*. (2010), carbohydrate contents are affected by the other proximate compositions such as protein and fat contents. On the other hand, several variables influence the cooking or processing of carbohydrates that might cause either positive or negative impact on the nutritional or quality attributes (Frias *et al*., 2000). Generally, the result indicated that the level of carbohydrate in nixtamalized QPM flour increased with an increase in the lime concentration and reduction in steeping time.

Total energy

The result showed that steeping time significantly (*P ≤ 0.01)* affected energy content of the nixtamalized QPM flour (Table 1).

The energy values of the nixtamalized QPM flour samples were ranged from 361.4 kcal/100g to 385.7 kcal/100g (Table 2). These values agrees with that reported by Reyes-Moreno *et al*. (2013) in commercial nixtamalized maize flour MASECA^{TM} (374.98 kcal/100g). Similar energy content of 382.87 kcal/100g in nixtamalized maize was reported by Jiménez *et al*. (2020). On the other hand, the energy content of nixtamalized QPM flour in this study was higher than the energy value reported in nixtamalized maize (364.75 kcal/100g) *(Sunico et al*., 2021). But lower than the energy content found in optimized nixtamalized QPM (417.4 kcal/100g) (Milan-Carrillo *et al.*, 2004). The energy content of the nixtamalized QPM flour was higher when the cooking time and steeping time are at lower range. The highest energy value (385.71 kcal/100g) was recorded at cooking time of 60 minutes, steeping time of 8 hours and lime concentration of 1%. While the lowest (361.39 kcal/100g) was found at 40 minutes cooking time, 18.73 hours steeping time and 1.5% lime concentration (Table 2). The result indicated that the energy value of nixtamalized QPM flour increased by rducing steeping time and lime concentration.

Anti-nutritional factors

Phytate content

The result showed that the phytate content of the nixtamalized QPM was significantly affected by lime concentration ($p \leq 0.01$), interaction of cooking time and lime concentration ($p \leq 0.01$), and interaction of steeping time and lime concentration ($p \leq 0.01$) (Table 3). Phytate content of the samples were found in the range 0.61 mg/100g – 9.86 mg/100g, which is lower than phytate content of the raw QPM flour (15.9mg/100g) (Table 4). Similar in the reduction of phytate content of maize grains on the nixtamalization were reported by *Sunico et al*. (2021). The reduction can also be due to degradation of phytic acid by phytase enzyme, which were reported to be activated in alkaline solution during the soaking of maize kernels *(Ocheme et al*., 2010). Similarly, Escalante-aburto *et al*. (2019) reported the loss of phytate content in the samples cooked with higher lime concentration. The reduction of phytic acid level might enhance calcium, iron, and zinc bioavailability (Bressani *et al*., 2004). The highest phytate content was recorded at the cooking time of 20 minutes, steeping time of 16 hours and lime concentration of 1%; While the lowest was recorded at the cooking time of 40 minutes, steeping time of 12 hours, and lime concentration of 2.34% (Table 4). The finding showed that the phytate content of the nixtamalized QPM decreases as the lime concentration, and cooking time increase

Table 3: Regression coefficients of a quadratic polynomial, R^2 and lack of fit for anti-nutritional parameters of nixtamalized QPM flour

Source	Tannin	Phytate	Oxalate
Model	7.91**	375.41**	0.42*
Intercept	2.80	3.60	1.17
A	0.25*	0.36	0.015
B	0.099	0.11	-8.958E-003
C	9.645E-003	-3.63**	0.085*
A^2	-0.27*	-0.17	5.055E-003
B^2	-0.070	2.32**	-0.085
C^2	-0.40**	1.80**	-0.059
AB	0.65**	-0.10	-0.13**
AC	-0.22	1.53**	0.039
BC	-0.094	-2.68**	0.051
R^2	0.87	0.98	0.80
lack of fit	0.7066	0.0891	0.3652

*A= Cooking time, B= Steeping time, C=Lime concentration ($Ca(OH)_2$) *Significant at P≤0.05 level, **Significant at P≤0.01 level.*

Oxalate content

The result showed significant ($p \leq 0.05$) linear term of lime concentration and strong interaction effect of cooking time and steeping time ($p \leq 0.01$) on the oxalate content of nixtamalized QPM (Table 3). Oxalate content of the nixtamalized QPM was found in the range 0.6mg/100g–1.3mg/100g, while oxalate content of 1.3mg/100g was observed in the raw maize grains (Table 4). The finding showed a decrease in oxalate content of the nixtamalized QPM compared to the control. The reduction might be attributed to solubility of the oxalate in water during the cooking process (Sotelo *et al*., 2010). The highest oxalate level (1.3mg/100g) was recorded at cooking time of 20 minutes, steeping time of 16 hours, and lime concentration of 2%; while the lowest (0.6mg/100g) was recorded at the cooking time, steeping time, and lime concentration of 60 minutes, 16 hours, and 1%, respectively (Table 4). The oxalate content of the nixtamalized QPM decreased as cooking time increased and lime concentration reduced. The oxalate is considered as undesirable compound in

foods because it is not suitable for human health since it limit calcium bioavailability and also lead to the formation of kidney stone; so that reduction of oxalate content is advantageous (Sotelo *et al*., 2010).

Table 4: Anti-nutrient composition of nixtamalized quality protein maize flour (mg/100g)

A	B	C	Phytate	Oxalate	Tannin
20	8	1	6.80±0.04	0.76±0.04	3.14±0.05
40	12	1.5	1.66±0.04	1.01±0.03	3.89±0.03
20	16	2	0.98±0.06	1.26±0.04	1.81±0.05
40	12	1.5	2.56±0.03	1.24±0.03	3.71±0.04
60	16	1	9.18±0.05	0.63±0.03	4.63±0.03
60	8	2	6.53±0.08	1.22±0.04	2.38±0.08
40	12	1.5	2.08±0.04	1.2±0.03	3.53±0.05
60	8	1	4.21±0.02	1.08±0.04	2.96±0.06
20	8	2	2.88±0.05	0.98±0.05	3.88±0.04
60	16	2	2.17±0.03	1.21±0.04	3.77±0.05
20	16	1	9.89±0.04	1.07±0.05	1.73±0.06
40	12	1.5	2.10±0.06	1.19±0.06	4.71±0.05
40	12	1.5	2.21±0.04	1.23±0.02	2.40±0.05
6.36	12	1.5	1.04±0.03	1.17±0.04	1.38±0.04
40	12	2.34	0.61±0.04	1.04±0.03	2.73±0.03
73.64	12	1.5	2.03±0.04	1.25±0.05	2.08±0.04
40	12	1.5	2.27±0.05	1.18±0.06	3.00±0.04
40	5.27	1.5	6.47±0.03	1.03±0.05	1.79±0.05
40	12	0.66	9.86±0.07	1.02±0.06	1.00±0.05
40	18.73	1.5	5.98±0.06	0.88±0.07	3.05±0.04
Raw QPM (control)			15.9±0.08	1.26±0.05	5.59±0.05

A=Cooking time (min), B=Steeping time (hrs), C=Lime concentration (%), Std Dev=Standard Deviation

Tannin content

The tannin content of the nixtamalized QPM flour was found in the range 1.00 mg/100g–4.71 mg/100g, as compared to the raw maize grains (5.6mg/100g) (Table 4). Evaluation of the results showed that tannin content of the nixtamalized QPM was significantly affected by cooking time ($p \leq 0.05$) and interaction of cooking time and steeping time ($P \leq 0.01$) (Table 3). The study showed that nixtamalization has reduced the tannin content of the QPM flour. The reduction might be due to removal of tannin along with pericarp by thermo-alkali treatment, since tannin is known to be mainly concentrated in the pericarp (Gaytán-martínez *et al*., 2017). The average decrease is slightly higher during long steeping time with lower cooking time, demonstrating that long steeping time has greater effect on the tannin content. Similar reduction in the tannin content for millet grains soaked in lime compared to the millet grains cooked in tap water was reported (Boniface & Gladys, 2011). The finding is also consistent with the observations of Price & Butler (1977), in which the long-term moistening of grains with lime solution effectively inactivated and detoxified bird-resistant sorghum tannins. The result indicates major reduction of tannin content can be attributed to the long steeping time than the cooking time.

Numerical optimization

The numerical optimization results for preparing nixtamalized QPM flour at the targeted values for each processing parameters gave cooking time of 20 minutes, steeping time of 8 hours, and lime concentration of 1% (Table 5). At these levels of the independent parameters nixtamalization of QPM resulted in a flour with higher nutritional value and lower anti-nutrient composition. The result showed a reasonably higher degree of desirability score of 0.66 (Table 5). Therefore, the specified nixtamalization parameters considered in this study for preparation of QPM flour at the optimum values determined can be used to maximize the nutritional values and minimize anti-nutrients.

Table 5: Response optimization for nixtamalization process, proximate and anti-nutritional value of nixtamalized QPM flour

Name	Unit	Lower Limit	Upper Limit	The optimum value of the NQPMF
Cooking time	Minutes	20	60	20
Steeping time	Hours	8	16	8
Lime concentration	%	1	2	1
Moisture	%	7.61	10.24	8.92
Protein	%	9.71	11.46	10.88
Fat	%	4.16	5.99	5.39
Fiber	%	0.24	3.34	1.74
Ash	%	1.37	2.89	1.92
Carbohydrate	%	68.41	73.07	71.0
Energy	kcal/100g	361.39	385.71	376.79
Tannin	mg/100g)	0.79	3.73	2.03
Phytate	mg/100g)	0.92	14.8	9.46
Oxalate	mg/100g)	0.63	1.26	0.90
Desirability	-	-	-	0.66

NQPMF=nixtamalized quality protein maize flour

CONCLUSIONS

The results of the study showed that there is a significant ($P \leq 0.05$) difference in the proximate and anti-nutritional contents of nixtamalized QPM flour compared to the raw maize grains. Moisture, protein, crude fat, fiber, ash, carbohydrate, and energy contents of the flour were significantly ($P \leq 0.05$) affected by cooking time, steeping time, and lime concentration. The numerical optimization indicated that better nutrient contents and lower anti-nutrients were obtained at 20 minutes cooking time, 8 hours steeping time, and 1% lime concentration. The finding might have a useful implication for preparation of maize-based food varieties through the nixtamalization processing.

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Conflict of Interest

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

This study does not involve any human or animal testing.

REFERENCES

Almeida-Dominguez, H. D., Cepeda, M., & Rooney, L. W. (1996). Properties of commercial nixtamalized corn flours. *Cereal Foods World*, *41*(7), 624–630.

Anteneh, A., & Asrat, D. (2020). Wheat production and marketing in Ethiopia: Review study. *Cogent Food and Agriculture*, *6*(1). https://doi.org/10.1080/23311932.2020.1778893

AOAC International. (2005). *Official methods of analysis of AOAC International* (18th ed.). AOAC International.

Balemi, T., Kebede, M., Golla, B., Tufa, T., & Chala, G. (2020). Phenological and grain yield response of hybrid maize varieties , released for differing agro-ecologies , to growing temperatures and planting dates in Ethiopia. *African Journal of Agricultural Research*, 16(12), 1730–1739. https://doi.org/10.5897/AJAR2020.15103

Boniface, O. O., & Gladys, M. E. (2011). Effect of Alkaline Soaking and Cooking on the Proximate , Functional and Some Anti-Nutritional Properties of Sorghum Flour. 14 (3), 210–216.

Bressani, R. (1990). Chemistry, Technology, and Nutritive Value of Maize Tortillas. *Food Reviews International*, *6*(2), 225–264.

Bressani, R., Turcios, J. C., Colmenares De Ruiz, A. S., & De Palomo, P. P. (2004). Effect of Processing Conditions on Phytic Acid, Calcium, Iron, and Zinc Contents of Lime-Cooked Maize. *Journal of Agricultural and Food Chemistry*, *52*(5), 1157–1162. https://doi.org/10.1021/jf030636k

Central Statistical Agency (CSA). (2020). *The Federa Democratic Republic of Ethiopia Report on Area and Production of Majr Crops*. *I*.ed amnu

Demeke, K. H. (2018). Nutritional Quality Evaluation of Seven Maize Varieties Grown in Ethiopia. *3*(2), 45–48. https://doi.org/10.11648/j.bmb.20180302.11

Ejigui, J., Savoie, L., Marin, J., & Desrosiers, T. (2007). Improvement of the nutritional quality of a traditional complementary porridge made of fermented yellow maize (Zea mays): Effect of maize – legume combinations and traditional processing methods. 28(1), 23–34.

Ekpa, O., Palacios-rojas, N., Kruseman, G., Fogliano, V., & Linnemann, A. R. (2018). Sub-Saharan African maize-based foods : Technological perspectives to increase the food and nutrition security impacts of maize breeding programmes. *Global Food Security*, *17*(March), 48–56. https://doi.org/10.1016/j.gfs.2018.03.007

Escalante-aburto, A., Mariscal-moreno, R. M., Santiago-, D., & Ponce-garcía, N. (2019). An Update of Different Nixtamalization Technologies , and Its Effects on Chemical Composition and Nutritional Value of Corn Tortillas: An Update of Different Nixtamalization Technologies. *Food Reviews International*, *0*(0), 1–43. https://doi.org/10.1080/87559129.2019.1649693

Farinde, E. O., Dauda, T. O., & Obatolu, V. A. (2021). Heteroscedasticity analysis of nixtamalization effect on nutrients, digestibility, and functional properties of quality protein maize and indigenous local maize flour. *Journal of Food Processing and Preservation*, 45(4), 1–11. https://doi.org/10.1111/jfpp.15303

Frias, J., Vidal-Valverde, C., Sotomayor, C., Diaz-Pollan, C., & Urbano, G. (2000). Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. *European Food Research and Technology*, 210(5), 340–345. https://doi.org/10.1007/s002170050560

Gaytán-martínez, M., Cabrera-ramírez, Á. H., Morales-Sanchez, E., Ramírez-jiménez, A. K., Cruz-ramírez, J., Campos-vega, R., Velazquez, G., Loarca-Piña, G., & Mendoza, S. (2017). Effect of nixtamalization process on the content and composition of phenolic compounds and antioxidant activity of two sorghums varieties. *Journal of Cereal Science Please*. https://doi.org/10.1016/j.jcs.2017.06.014

Gómez, M. H., Waniska, R. D., & Rooney, L. W. (1991). Starch Characterization of Nixtamalized Corn Flour. *Cereal Chemistry*, 68 (6), 578–582.

Inyang, U. E., Akindolu, B. E., & Elijah, A. I. (2019). Nutrient Composition , Amino Acid Profile and Anti- nutritional Factors of Nixtamalized Maize Flour Supplemented with Sprouted Soybean Flour. *European Journal of Nutrition & Food Safety*, 9(1), 41–51. https://doi.org/10.9734/EJNFS/2019/46150

Jiménez, B. C., Toledo, M. E. O., Mier, L. G., Bravo, R. M., Alberto, C., Gutiérrez, G., Curiel, F., Rodriguez-garcia, M. E., Contreras, B., Toledo, M. E. O., Mier, L. G., Bravo, R. M., Alberto, C., Gutiérrez, G., Ayala, F. C., & Rodriguez-Garcia, M. E. (2020). Physicochemical study of nixtamalized corn masa and tortillas fortified with " chapulin " (grasshopper , Sphenarium purpurascens) flour. *CyTA - Journal of Food*, 18(1), 527–534. https://doi.org/10.1080/19476337.2020.1794980

Kadir, S., Ahmad, L., & Bait, Y. (2019). Proximate and calcium analysis of nixtamalized corn grits as a raw material of Gorontalo traditional meal , Indonesia. 11(1). https://doi.org/10.13057/nusbiosci/n1101xx

Lewu, M. N., Adebola, P. O., & Afolayan, A. J. (2010). Comparative assessment of the nutritional value of commercially available cocoyam and potato tubers in South Africa. *Journal of Food Quality*, *33*(4), 461–476. https://doi.org/10.1111/j.1745-4557.2010.00325.x

Maureen, N., Kaaya, A. N., Kauffman, J., Narrod, C., & Atukwase, A. (2020). *Enhancing Nutritional Benefits and Reducing Mycotoxin Contamination of Maize through Nixtamalization*. https://doi.org/10.3923/jbs.2020.153.162

Maxson, E. D., & Rooney, L. W. (1972). Two Methods of Tannin Analysis for Sorghum Bicolor (L.) Moench Grain. *Crop Scince*, *12*, 253–254.

Méndez-Montealvo, G., Solorza-Feria, J., Valle, M. V. del, Gómez-Montiel, N., Paredes-López, O., & Bello-Pérez, L. A. (2005). Chemical Composition and Calorimetric Characterization of Hybrids and Varieties of Maize Cultivated In México. *Publicado Como ARTÍCULO En Agrociencia*, *39*(3), 267–274.

Mendoza-Madrigal, A.G. Dur´an-P´aramo, E. Valencia del Toro, G. Chanona-P´erez, J.J. Celeste O. Mart´ınez-Ram´ırez, I. Arzate-V´azquez A. V. (2017). Viability Kinetics of Free and Immobilized Bifidobacterium Bifidum in Presence of Food Samples Under Gastrointestinal in Vitro Conditions. *Revista Mexicana de Ingeniería Química VIABILITY*, *16*(1), 159–168.

Mensah, J., Aidoo, R., & Teye, A. (2013). Analysis of Street Food Consumption Across Various Income Groups in the Kumasi Metropolis of Ghana. *International Review of Management and Business Research*, *2*(4), 951–961.

Milan-Carrillo, J., Gutierrez-Dorado, R., Cuevas-Rodriguez, E. O., Garzon-Tiznado, J. A., & Reyes-Moreno, C. (2004). Nixtamalized Flour From Quality Protein Maize (Zea mays L). Optimization of Alkaline Processing. *Plant Foods for Human Nutrition*, *59*, 35–44.

Morales, J. C., & Zepeda, R. A. G. (2017). Effect of Different Corn Processing Techniques in the Nutritional Composition of Nixtamalized Corn Tortillas. *Journal of Nutrition & Food Sciences*. 7(2). https://doi.org/10.4172/2155-9600.1000580

Ocheme, O. B., Oludamilola, O. O., & Gladys, M. E. (2010). Effect of Lime Soaking and Cooking (Nixtamalization) on the Proximate , Functional and Some Anti-nutritional Properties of Millet Flour. *AU J.T.*, 14(2), 131–138.

Official Mexican Standards (1999). NOM-147-SSA1-1996. Goods and services. Cereals and their products. Cereal flours, semolina, or semolina. Cereal-based foods with edible seeds, flour, semolina, and semolina or their mixtures. Bakery products. Provisions and sanitary and nutritional specifications. General Directorate of Standards. (in Spanish)

Olaoye, O. A., Onilude, A. A., & Oladoye, C. O. (2007). Breadfruit flour in biscuit making : effects on product quality. *African*

Journal of Food Science, 020–023.

Pflugfelder, R. L., Rooney, L. W., & Waniska, R. D. (1988). Dry matter losses in commercial corn masa production. *Cereal Chemistry*, *65*(2), 127–132.

Price, M. L., & Butler, L. G. (1977). Rapid Visual Estimation and Spectrophotometric Determination of Tannin Content of Sorghum Grain. *Journal of Agriculture and Food Chemistry,* 25(6), 1268–1273.

Prieto, S., Ricker-Gilbert, J., & Bauchet, O. (2021). Incomplete Information and Product Quality in Rural Markets : Evidence from an Experimental Auction for Maize in Senegal. *Economic Development and Cultural Change*, 1351–1377.

Revilla, P., Alves, M. L., Andelković, V., Balconi, C., Dinis, I., Mendes-Moreira, P., Redaelli, R., Ruiz de Galarreta, J. I., Vaz Patto, M. C., Žilić, S., & Malvar, R. A. (2022). Traditional Foods From Maize (Zea mays L.) in Europe. *Frontiers in Nutrition*, *8*(January). https://doi.org/10.3389/fnut.2021.683399

Reyes-Moreno, C., Ayala-Rodríguez, A. E., Milán-Carrillo, J., Mora-Rochín, S., López-Valenzuela, J. A., Valdez-Ortiz, A., Paredes-López, O., & Gutiérrez-Dorado, R. (2013). Production of nixtamalized flour and tortillas from amarantin transgenic maize lime-cooked in a thermoplastic extruder. *Journal of Cereal Science*, *58*(3), 465–471. https://doi.org/10.1016/j.jcs.2013.09.008

Rodríguez-miranda, J., Ruiz-lópez, I. I., Herman-lara, E., Martínez-sánchez, C. E., & Delgado-licon, E. (2011). LWT - Food Science and Technology Development of extruded snacks using taro (Colocasia esculenta) and nixtamalized maize (Zea mays) fl our blends. *LWT - Food Science and Technology*, *44*(3), 673–680. https://doi.org/10.1016/j.lwt.2010.06.036

Salazar, R., Arámbula-villa, G., Luna-bárcenas, G., Figueroa-cárdenas, J. D., & Azuara, E. (2014). LWT - Food Science and Technology Effect of added calcium hydroxide during corn nixtamalization on acrylamide content in tortilla chips. *LWT - Food Science and Technology*, *56*(1), 87–92. https://doi.org/10.1016/j.lwt.2013.10.046

Serna-Saldivar, S. O., Rooney, L. W., & Greene, L. W. (1991). Effect of Lime Treatment on the Availability of Calcium in Diets of Tortillas and Beans: Rat Growth and Balance Studies. *Ceral Chemistry*, *68*(6), 565–570.

Sotelo, A., González-osnaya, L., Sánchez-chinchillas, A., & Trejo, A. (2010). Role of oxate , phytate , tannins and cooking on iron bioavailability from foods commonly consumed in Mexico. *International Journal of Food Sciences and Nutrition*, *61*(February), 29–39. https://doi.org/10.3109/09637480903213649

Sunico, D. J. A., Rodriguez, F. M., Tuaño, A. P. P., Mopera, L. E., Atienza, L. M., & Juanico, C. B. (2021). Physicochemical and Nutritional Properties of Nixtamalized Quality Protein Maize Flour and its Potential as Substitute in Philippine Salt Bread. *Chiang Mai University Journal of Natural Sciences*, *20*(2), 1–15. https://doi.org/10.12982/CMUJNS.2021.035

Upreti, P., Mckay, L. L., & Metzger, L. E. (2006). Influence of Calcium and Phosphorus , Lactose , and Salt-to-Moisture Ratio on Cheddar Cheese Quality : Changes in Residual Sugars and Water-Soluble Organic Acids During Ripening. *Journal of Dairy Science*, *89*(2), 429–443. https://doi.org/10.3168/jds.S0022-0302(06)72107-5

Vaintraub, I. A., & Lapteva, N. A. (1988). Calorimetric Determination of Phytate in Unpurified Extracts of Seeds and the Products of Their Processing. *Analytical Biochemistry*, *175*, 227–230.

Yetneberk, S., Teamir, M., & Teklewold, A. (2000). *Maize Based Food Products in Ethiopia: A Review of Traditional Practices and Research Outputs*. 1–16.