ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

Proximate Properties, Functional Components, and Vitamin and Mineral Content of Nixtamalized Lagkitan Corn (*Zea mays* L.)

Ann C Cayetano, Liezl M Atienza* and Clarissa B Juanico

Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, Philippines

***Corresponding Author**: Liezl M Atienza, Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, Philippines.

Received: February 15, 2019 ; **Published:** March 27, 2019

Abstract

Nixtamalization, the process of alkaline treatment, had been widely used across the globe and its positive effects had been the focus of several studies. However, little attention had been given in testing the effect of this process on Philippine corn variety and in comparing the effect of three nixtamalization processes, namely classical, ecological, and traditional, on different parameters. Therefore, this study aims to identify the most effective nixtamalization process in increasing functional properties and nutrient content of corn. This study utilized the Lagkitan corn variety (Zea mays L.) and it was found out that there are significant differences in terms of proximate and functional properties and mineral content between nixtamalized and non-nixtamalized treatments. Moreover, it had been observed that among the three processes, the traditional nixtamalization process yielded the highest significant amounts of antioxidants and nutrients.

Keywords: *Nixtamalization; Corn; Proximate Analysis; Classical; Ecological; Traditional*

Abbreviations

TNP: Traditional Nixtamalization Process; ENP: Ecological Nixtamalization Process; CNP: Classical Nixtamalization Process; NN: Non-nixtamalized

Introduction

Rice is the staple food of Filipinos. According to the Food and Agriculture Organization of the United Nations as cited by Exconde [1], a Filipino consumes an average of 119 kg of rice per year in 2013. Moreover, domestic consumption of milled rice is continuously increasing from 2,515,000 metric tons in 1960 to 13,400,000 metric tons in 2018 [2]. Meanwhile, total palay output reaches about 12.688 million metric tons (MMT) at an average milling recovery rate (MRR) of 65.4% [3], which is insufficient to supply the country for the year. Palay production in April to June 2018 had also been reported to decline by 1.44%, from 4.15 MMT last year down to 4.09 MMT [4]. The Philippine Statistics Authority attributed these decrements to several factors such as decreasing harvest area, advance harvesting due to hot weather condition, less planting due to insufficient rainfall, change in cropping due to changing weather pattern, and closure and rehabilitations of some National Irrigation Administration canals.

The insufficient rice supply and the upsurging demand submits the Philippines to increase rice importation despite being the 8th largest producer of rice in the world. According to the report of Arcalas [3], Philippine rice import increased by 250,000 MT this year, with a total of 750,000 MT. In addition, rice prices had increased 24 times from January to June 2018 despite the importation [5]. This situation highlights the need for rice substitutes, which can be locally grown, has low cost production, easily maintained, and culturally acceptable. Among the alternatives, white corn grits are relatively cheaper and nutritious.

According to de los Santos and Lansigan [6], corn is the second most important crop and staple food of Filipinos. Together with rice, they take up two-thirds of the country's farm land. Filipinos consume an average of 11 kg of corn [7,8] and produces about 7.9 MMT in a year but is expected to increase to 8.1 MMT [9]. Corn production needs same amounts of fertilizers as with rice. However, despite the same amounts of agricultural inputs, corn produces higher yields in metric tons. Corn production is also relatively easier since corn can grow in different areas and climate prolifically since they are efficient in photosynthesis [10]. These factors may contribute to the lower price of corn as compared with rice, with Php 14.56/kg and Php 23.04/kg, respectively [11], and to the higher increase in the demand for corn more than the demand for rice and wheat globally [12].

In terms of nutritional content, white corn grits provide higher amounts of calorie, protein, and fats than rice based on the Food Composition tables [13]. However, one of the issues in using corn as main staple is its deficiency in certain key nutrients such as niacin and some essential amino acids including lysine and tryptophan [14]. Naturally, white corns are rich in niacin; however, these nutrients are protein-bound thus limiting its bioavailability. Lack of this vitamin can lead to niacin deficiency which can develop to diseases like pellagra.

With these identified concerns with regards to consumption of corn as staple, several processes are developed to utilize the protein-bound nutrients. One of the most popular is nixtamalization, a technique commonly used in Mexico and Central America [15]. Nixtamalization is a cooking process utilizing alkaline reagents, which has three methods: traditional, classical, and ecological. Several studies had shown that this process helps increase the bioavailability of protein-bound nutrients in corn. The effect of the three different methods on the nutritional and functional composition of different corn varieties were also observed. However, none of the literature tested these methods to Philippine corn varieties such as "Lagkitan".

Lagkitan is a white glutinous, hybrid corn variety with excellent eating quality, higher yield, and is resistant to downy mildew. White corn is also commonly used to make a dish called 'binatog' which also uses an alkaline treatment. The said variety is therefore suitable for the study and since the nutrient content and functional properties of nixtamalized Lagkitan is lacking, the study aims to answer these knowledge gaps.

The upsurging demand of Filipinos for rice supply calls for a substitute staple, of which corn remains to be the most appropriate as it is the second most consumed, culturally-acceptable, and affordable. In fact, rice-corn grits (a mixture of rice and corn-grits) is now currently considered as a healthier and more acceptable alternative to rice. However, it is widely known that corn consumption subjects the consumers to diseases related to micronutrient deficiencies such as pellagra, particularly if consumed as majority of the diet. Therefore, it is important to determine the process that will render corn nutritionally suitable for consumption to maximize the potential of this crop. The present study used three different nixtamalization processes to determine the effects of these treatments to the functional components and nutritional content of Lagkitan.

Nixtamalization offers a solution to utilize corn as staple without the risk of suffering from micronutrient deficiency-related diseases. The objective of this study was to assess the effects of different nixtamalization methods on the nutrient contents of maize products by individual micronutrients (including vitamins and minerals) and some other important phytochemicals and antioxidants. The results of this study may help in developing practical and economical processes for corn consumption with improved nutritional characteristics.

The results of this study can also help in addressing the problem on rice shortage and motivating corn farmers, maximizing the potential of corn in providing essential micronutrients and contributing to the existing set of interventions in addressing these problems in the country since niacin has a role in diabetes and cancer prevention.

Materials and Methods

Raw corn samples were procured from the Institute of Plant Breeding in Los Baños, Laguna. These samples were planted since July 5, 2018 and were matured when harvested. It was ensured that the samples used in the study were free of any extensive defects or damages. The study used the corn variety Lagkitan and included four treatments: one non-nixtamalized (control) and three nixtamalized corn samples using three different methods of nixtamalization. The scientific name of the corn variety was verified at the Museum of Natural History, University of the Philippines Los Baños. Data collection procedures were done at the Bioassay Laboratory in the Institute of Human

Nutrition and Food, UPLB except for crude fiber which was analyzed in the Animal Science Laboratory in the same campus. All analyses were done in triplicates and were subjected to statistical analyses.

Nixtamalization

In this study, nixtamalization was done in three different procedures as described in the study of Mariscal-Moreno., *et al* [16]. These include: (1) traditional with lime (TNP), (2) classical with ashes (CNP), and (3) ecological with calcium salts (ENP).

Traditional Nixtamalization (TNP)

For 23 - 30 minutes, 1 kg of corn was cooked in two liters of water with 1.0% (w/w) calcium hydroxide at 90 - 94°C. The cooked kernels were steeped for 16 hours at room temperature, then the cooking liquor or nejayote was separated from the nixtamal or the nixtamalized corn kernels. The nixtamal was rinsed and ground in a grinder to obtain fresh masa. The masa was put in an oven dryer at 45°C for 16 hours to obtain dehydrated flour. The flour was grinded using a pulverizer.

Classical nixtamalization (CNP)

In this process, one kilogram of corn was cooked in four liters of water with 1% wood ashes at 94°C for 1 hour and was steeped for 16 hours at room temperature. The nejayote was separated from the nixtamal and the nixtamal was rinsed and ground in a grinder to obtain fresh masa. The masa was put in an oven dryer at 45°C for 16 hours to obtain dehydrated flour. The flour was grinded using a pulverizer.

Ecological nixtamalization (ENP)

For 30 minutes, 1 kg of corn was cooked in two liters of water an 1% (w/w) of either calcium propionate or calcium carbonate. The cooked kernels were steeped for 16 hours at room temperature, then the nejayote was separated from the nixtamal or the nixtamalized corn kernels. The nixtamal was rinsed and ground in a grinder to obtain fresh masa. The masa was put in an oven dryer at 45°C for 16 hours to obtain dehydrated flour. The flour was grinded using a pulverizer.

Proximate analysis

Proximate analysis included measuring the moisture, crude fat, crude protein, total ash, crude fiber, and nitrogen content of the three treatments. Protocols in doing the proximate analyses were based from Association of Official Analytical Chemists (1980) as cited by Rodriguez and Hurtada [17].

Analysis of moisture content

In this analysis, 10 grams of nixtamalized samples were cut into smaller pieces. This was done in triplicate and were placed in a moisture dish. The samples were put in an oven dryer at 45°C overnight. The samples were then be removed from the oven and were placed in a desiccator. Samples were brought to room temperature. Percent moisture content was calculated using the following equation:

% Moisture = (weight of water/weight of sample) * 100

where: weight of water = weight of sample before drying - weight of sample after drying

Analysis of crude fat

Small sheets of filter paper were folded into filter paper thimbles, sealing off one end by stapler. One gram of dried ground sample was placed, and the exact weight of the paper thimble and the combined weight of the thimble and the sample were measured. The other end of the thimble was also stapled, and the thimble was placed on a Soxhlet fat extractor. Petroleum ether was put in a boiling flask and the samples were refluxed for 8 hours. The samples were removed from the extractor and were placed in an oven at 45°C for at least one hour. The samples were equilibrated in a desiccator. The exact weight of the thimble and the defatted samples were then weighed. Percent fat was calculated using the following equation:

% Crude fat = (weight of fat/weight of sample) * 100

where:

Weight of fat = (weight of thimble + sample before refluxing) - (weight of thimble + sample after drying)

Weight of sample = (weight of sample + thimble) - (weight of thimble)

Analysis of nitrogen

This procedure utilized the Kjeldahl method wherein 50 mg of dried, defatted powdered sample was placed into a 30 ml digestion flask and added with 0.20g selenium catalyst mixture and 2.0 ml concentrated sulfuric acid. The resulting solution was digested in a microdigestor until the solution was clear and was cooled to room temperature. The solution was transferred into a test tube and was diluted to 10 ml volume with distilled water. 0.01 ml of aliquot was procured and was placed in a test tube, added with 1.5 ml working buffer, 0.40 ml salicylate reagent and 0.20 ml hypochlorite solution. The solution was mixed every addition of reagent. The solution was left to stand at room temperature for 30 minutes for color development and was diluted to final volume of 10 ml with distilled water and mixed well. Absorbance was read at 660 nm. A blank and a standard curve was done using 100 ugN/ml ammonium sulfate. The slope was plotted, and the nitrogen content of the sample was calculated. Nitrogen was converted to protein by multiplying a factor of 6.25.

The following formula was used in calculating the nitrogen content of the samples:

% Nitrogen = (weight of nitrogen/weight of sample) x 100

Analysis of total ash

100 mg of defatted, ground samples were placed in a 30 mL porcelain crucible. The samples were placed in a furnace and were ignited overnight at 650°C. After cooling the samples to a lower temperature, they were transferred in a desiccator and were equilibrated at room temperature. Exact weights were measured, and percent total ash was computed as follows:

% Total Ash = (weight of ash/weight of sample) * 100

Where: Weight of ash = (weight of crucible + sample after ignition) - (weight of crucible)

Analysis of crude fiber

100 mg of dry defatted samples were placed in a 100 ml Bercillus beaker and were added with 50 ml 2.5% sulfuric acid. The samples were placed in a reflux apparatus and were refluxed for 30 minutes. The samples were then washed with hot water until the washing is neutral with litmus paper and 50 ml of 2.5% sodium hydroxide were then added. Samples were refluxed for another 30 minutes and were placed in an oven dryer at 105°C overnight. The samples were allowed to cool in a desiccator and the exact weights were measured. The samples were then ignited in a furnace and the exact weight were measured again.

Percent crude fiber was computed using the following formula:

% Crude fiber = (weight of fiber/weight of sample) * 100

Where: Weight of fiber = (weight of crucible + sample after drying) - (weight of crucible + sample after ignition)

Functional components analysis

Analysis of functional components included measurement of anthocyanidin content, antioxidant activity, beta-carotene, total phenols, vitamin C, and tryptophan. Anthocyanidin and total phenolic content were determined by using a slightly modified protocol of Ranganna [18] and Ainsworth and Gillespie [19], respectively. Antioxidant capacity was measured following the protocol of Shimada., *et al* [20] while tryptophan was analyzed using the method of Opienska-Blauth., *et al* [21]. Vitamin C and beta-carotene analyses were adopted from Ishiwu Charles., *et al* [22]. Protocols were followed and slightly modified as described by Rodriguez and Hurtada [17].

Analysis of anthocyanidins

Anthocyanidins are food pigments like anthocyanins, without the sugar. To measure this, ten ml of methanol solution was added to one gram of sample and was macerated in a mortar and pestle. The mixture was filtered using a Whatman coarse filter paper and 0.20 ml of methanolic extract was acquired. One ml of 1% vanillin in methanol, 1 ml of 9.0N hydrochloric acid, and 1.80 ml of distilled water was mixed to the extract. The solution was let to stand for 20 minutes and absorbance was measured at 520 nm.

A standard curve was prepared by plotting the absorbance against the concentration of the standard. Concentration of anthocyanidin as catechin was calculated from the standard curve. Percent anthocyanidins was then computed as follows:

% anthocyanidins = (weight of anthocyanidins/weight of sample) * 100

Analysis of antioxidant capacity

Five ml of extract was prepared by placing 100 mg samples in a test tube, added with 5 ml of 50% methanol solution. The solution was mixed intermittently for 10 minutes in a vortex mixer and was filtered in a clean test tube with cover. The extract was stored in a refrigerator until use.

To get the antioxidant capacity, 1 ml of aliquot from the prepared extract was added with 4 ml distilled water and 1 ml of freshly prepared 1 Mm dinitrophenyl picryl hydrazyl radical (DPPH) methanolic solution. The solutions were left to stand for 30 minutes and the absorbance was measured at 517 nm. The lower the absorbance at 517 nm represents the higher DPPH scavenging activity. Antioxidant capacity was then computed using the following formula:

% DPPH scavenging activity = [1 - (test sample absorbance/DPPH absorbance)] * 100

Analysis of beta-carotene

The beta-carotene contents of the samples were determined by weighing 1g sample into a beaker and macerating it with 10 ml mixture of acetone and n-hexane (1:1). The mixture was filtered and the filtrate was added with 10 ml of 50% ammonium sulfate solution, was vigorously shake and were allowed to settle. The upper layer was collected and the absorbance was read at 450 nm against hexane as blank. The following formula was used in determining beta-carotene content:

Beta-carotene (mg/100 ml) = (mean absorbance x dilution)/slope

Where: Slope = 1.249, calculated from the standard curve of beta-carotene against absorbance

Analysis of vitamin C

One gram of dried sample was weighed and macerated with 30 ml of 0.4% oxalic acid solution and was filtered. One ml of filtrate was pipetted into a test tube and 0.2 ml of 0.01% methylene blue solution was added. One ml of acetate buffer pH 4.2 was added into the solution and was diluted to 5 ml using distilled water. Absorbance of the solution was read using Spectrophotometer at 450 nm. The amount of Vitamin C was calculated using the following equation:

Vitamin C (mg/100 ml) = (Mean absorbance * dilution factor)/slope

Where: Slope = 0.0693, calculated from the standard curve of vitamin C against absorbance

Analysis of tryptophan

Three ml papain solution was added to a 100 mg dried, defatted, powder sample and was mixed in a vortex mixer. The sample was incubated at 65°C in an incubator and after 16 hours, it was allowed to cool to room temperature. The solution was centrifuged at 3000 rpm for 5 minutes and hydrolysate was collected. One ml aliquot was placed in another test tube and 4 ml of reagent C (combination of 27 mg iron trichloride hexahydrate dissolved in 50 ul distilled water and diluted to volume with glacial acetic acid; and diluted 83.3 ml concentrated sulfuric acid to 100 ml distilled water) was added. The solution was incubated at 65°C for 15 minutes and was allowed to

cool to room temperature. Absorbance was measured at 545 nm and papain was used as blank to zero the instrument. A standard curve was prepared, and tryptophan was computed using the formula:

% tryptophan = (weight of tryptophan/weight of sample) * 100

Where: weight of sample = (100 mg/3) * 1 ml = 33.3 mg

Analysis of Total Phenols

Fifty milligrams of dried, powdered sample were added with 5.0 ml absolute methanol and was shaken for 30 minutes in a vortex mixer. The mixture was then centrifuged at 3000 rpm for 5 minutes and 0.2 ml of supernate was collected. Distilled water (2.8 ml), sodium carbonate Na_2Co_3 (1.0 ml), and Folin-Ciocalteus Phenol reagent (0.2 ml) were added individually. The mixture was mixed thoroughly and was put in a boiling water bath for 15 minutes. The mixture was cooled to room temperature and the absorbance was read at 710 nm. A standard curve was prepared and the total phenolics content was calculated using the formula:

% phenols = (weight of phenols/weight of sample) * 100

Mineral analysis

Mineral analysis was composed of measuring the calcium, iron, and zinc content of the three treatments. Protocols of calcium and iron analyses were based from AOAC (1980) while zinc determination was conducted by following the protocols described by Platte and Marcy (1959) and Valdman., *et al* (2007) as cited by Rodriguez and Hurtada [17].

Analysis of calcium

In this method, samples were prepared by making a Solution A, which was used for the succeeding analyses. One gram of dried sample was placed in a 500 ml borosilicate beaker and in a cool muffle furnace. The temperature was increased to 500 to 550°C until the ash is white or nearly so, for 4 - 5 hours. The samples were then removed from the muffle and were allowed to cool and evaporate to dryness with 3 ml of 5N nitric acid. The samples were placed in a cool furnace and were heated to 400°C for about 15 minutes. The samples were removed from the furnace and were allowed to cool and moisten with little distilled water. Three ml of concentrated HCl was added and was allowed to evaporate to dryness in the steam plate. The samples were allowed to bake for one hour to dehydrate the silica and after removing from the hot plate, 5 ml of 2N nitric acid was added and was stirred with rubber policeman to dissolve the residue of salts. The beaker was rinsed with hot distilled water and was allowed to cool and mixed well. Silica was allowed to settle before taking aliquots for analysis.

In a 125 ml Erlenmeyer flask, 5 ml of solution A was added with distilled water to bring to a volume of 50 ml. The pH was adjusted to 12 with about 2 ml KOH. 50 mg murexide indicator was then added and was titrated with EDTA to a violet endpoint. Calcium content was computed using the formula:

% Calcium = (ml EDTA) * 0.050

Analysis of iron

This analysis required the preparation of an ash solution from 100 mg sample which was ignited at 550°C overnight until ash is white. Then, 1.0 ml of concentrated hydrochloric acid was added and was gently mixed using a stirring rod. A 4.0 ml volume of distilled water was also added, and the solution was heated on a hot plate at 100°C, allowed to evaporate to about 2.0 ml. A 2.0 ml volume of distilled water was again added and heated to 90°C. The solution was filtered and the filtrate was diluted to 20.0 ml volume with distilled water.

A 0.1 ml aliquot of the ash solution was added with 0.01 g ascorbic acid crystals and 5 ml sodium acetate trihydrate with pH 5.4 buffer solution. The solution was mixed and 1 ml of 2' 2' bipyridine solution was added and the solution was diluted to 25 ml. The solution was allowed to stand for one hour and the absorbance was read at 520 nm. A blank was run using the same procedure and reagents and a standard curve was prepared. Iron content of the sample was computed using the following equation:

% iron = (weight of iron/weight of sample) * 100

Analysis of zinc content

For zinc analysis, the sample was prepared using a 25g fresh sample which was charred and ignited at a temperature not exceeding 500°C. The ash was dissolved in minimum volume of 50% HCl and 20 ml distilled water was added and evaporated to near dryness. 20 ml of 0.1N HCl was then added and was heated for another 5 minutes. The filtrate was transferred into a 100 ml volumetric flask and was diluted to volume with 0.1 N HCl.

One ml of aliquot was pipetted into a 5 ml test tube and was added with 0.5 ml buffer solution. The solution was mixed well and added with zincon solution drop by drop until the red color is one drop in excess, was mixed well and was diluted to 5 ml using distilled water. The absorbance was measured at 620 nm and a standard curve was prepared using zinc sulfate. The following formula was used in determining the zinc content of the samples:

% Zinc = (weight of zinc/ weight of sample) x 100

Data processing and analysis

The data that were gathered were analyzed using MS Excel and PhStat. Analysis of Variance (ANOVA) and Tukey Kramer analysis (p = 0.005) were used to determine if the treatments have significant differences.

Results and Discussion

Corn has been one of the most widely consumed staples in the country. Several techniques had been developed to maximize its nutritional content and one of these is the nixtamalization process. Several studies had established that nixtamalization increases the functional properties of corn; however, data is lacking comparing the three methods. This study, therefore, aimed to close that research gap. The corn variety Lagkitan was subjected to three different methods, namely: classical (CNP), ecological (ENP), and traditional (TNP), each of which utilizes different alkaline reagents. Non-nixtamalized corn (NN) was also analyzed and results were compared with those of nixtamalized corn. The results of the proximate, functional properties, and mineral content analyses of the three treatments are presented below.

Proximate analysis

Results of the proximate analyses are presented below including the moisture content, crude fat, crude fiber, total ash, and nitrogen and protein content.

Component	Classical (CNP)	Ecological (ENP)	Traditional (TNP)	Non-nixtamalized (NN/control)	p-value
Moisture (%)	44.00 ± 1.00^{abc}	47.00 ± 3.00^{ade}	48.33 ± 12.33^{ce}	41.67 ± 1.33^{bd}	0.0183
Crude fat (%)	4.06 ± 0.02^{a}	3.40 ± 0.02^{b}	4.16 ± 0.02^{a}	3.66 ± 0.02^{b}	0.0005
Crude fiber[+] (%)	1.92 ± 0.018	1.85 ± 0.03	1.96 ± 0.05	1.99 ± 0.009	0.7128
Total ash[+] (%)	1.90 ± 0.37	1.37 ± 0.1	2.37 ± 0.14	1.70 ± 0.010	0.0814
Nitrogen (%)	0.0002 ± 0.0^{a}	0.0002 ± 0.0^{a}	0.0004 ± 0.0	0.0003 ± 0.0	0.0001
Protein (%)	0.0010 ± 0.0^{a}	0.0010 ± 0.0^{a}	0.0027 ± 0.0	0.0018 ± 0.0	0.0001

Table 1: *Results of proximate analysis of non-nixtamalized and nixtamalized corn subjected to different nixtamalization processes. Values are reported as mean ± variance; means followed by the same superscript within the same row are not significantly different from one another at 0.05 level of significance.*

[+]: There is no significant difference between all treatments.

Table 1 shows that TNP had the highest amount of moisture content among all treatments and was the only treatment that yielded a significant increase in the moisture content as compared to control. This finding agrees with the results of Pappa., *et al* [23], in which a higher increase in the moisture level was observed in TNP than CNP. However, this observation contradicts the results of Morales and

Zepeda [24] which had observed a 5% decrease in the moisture content in the nixtamalized dough. The increase in moisture content in TNP may be due to the effect of calcium hydroxide on the corn granules. In the study of Contreras-Jimenez., *et al* [25], it had been shown that calcium hydroxide affects the diffusion of water into the starch granules by observing that corn grits steeped in water without calcium hydroxide showed lower water absorption than those with calcium hydroxide. It had been explained that grinding increases the surface area of corn, which facilitates greater water diffusion and increases the access of reagents into the interior of the starch granules. This explanation agrees with the results of Ruiz-Gutierrez., *et al* [26] which also showed that alkaline-treated samples facilitate the diffusion of water throughout the whole grain.

However, these results contradict the explanation that pH is also a factor. According to Rodriguez., *et al.* [27], increase in pH charges the amylose and amylopectin glucose chains making them interact with calcium, therefore inhibiting water absorption in the starch granules. The calcium content of calcium hydroxide should inhibit water absorption; however, the opposite was observed. Based on the observations of Gutierrez., *et al.* [28], water absorption during thermo-alkaline treatment is limited by the presence of the pericarp. Since most of the pericarp had been removed due to softening of the cell walls, there is an increased surface area for water absorption, therefore observing an increase in the moisture content.

For crude fat, it was observed that both CNP and TNP values are significantly different with NN. However, the crude fat content of these two treatments are not significantly different with each other. There is a limited literature showing the effect of the nixtamalization on crude fat. In the study of Morales and Zepeda [24], the crude fat content of tortillas made from nixtamalized dough and nixtamalized flour was compared and they found out that nixtamalized dough has 1% higher crude fat than nixtamalized flour. It had been explained that the decrease can be attributed to about 30% germ losses during the nixtamalization process and 17% during rinsing. On the other hand, the increment in the fat content of nixtamalized corns observed in the present study may be due to the softening and dissolution of the content of the cell walls. Moreover, according to Arambula-Villa., *et al.* [29], crude fat measures not only the true fats but also fat-like substances such as alcohols, waxes, steroids, pigments, and aldehydes, among others. Since nixtamalization makes these substances more available, crude fat content will also increase. It is therefore recommended to perform subsequent or validation tests such as fatty acid determination.

On the other hand, it was found that there is no significant difference in the amounts of crude fiber and total ash between nixtamalized and non-nixtamalized samples. It was further observed that there is a lower crude fiber content among nixtamalized treatments that NN. These results agree with the results of Mariscal-Moreno., *et al.* [16], in which they observed that lower crude fiber in nixtamalized tortillas were due to the loss of pericarp and external layers throughout the process of nixtamalization. Moreover, higher amounts of ash in CNP and TNP treatments were observed in the present study. This observation agrees with Owusu-Kwarteng [30] who observed that nixtamalization improved crude protein and ash contents of millet dough samples. On the other hand, the observed decrease in ash content in the ENP treatment agrees with the results of Boniface and Mikailu [31]. This observation can be attributed to the addition of wood ash in the CNP treatment and the loss of some components during the nixtamalization process.

For protein and nitrogen, all of them have values that are significantly different with the control. Moreover, there is no significant difference between CNP and ENP, while TNP yielded the highest protein and nitrogen amounts among the three. In the present study, CNP and ENP was found to have lower protein content than NN. There is a limited evidence on the effect of different alkaline treatments on protein content; however, Pappa., *et al.* [16] showed that nixtamalized tortillas have lower protein quality than that of raw corn. This observation may be due to the effect of alkaline treatments on proteins, giving rise to peptides which are not biologically available and is detrimental to protein quality. Moreover, Chu., *et al.* (1976) as cited by Pappa., *et al.* [16] showed the possibility that alkaline ions may have an effect on the formation of chemical compounds and induce physical and functional characteristics in corn. Also, the denaturation of proteins caused by heat also disrupts its structure which may also explain the observed phenomenon.

Functional properties

Table 2 reflects that all treatments and control have high significant differences between each other in all analyzed functional properties.

Component	Classical (CNP)	Ecological (ENP)	Traditional (TNP)	Non-nixtamalized (NN/control)	p-value
Antioxidant Activity (DPPH)	92.561 ± 0.18^{ab}	93.34 ± 0.18^{ac}	93.23 ± 0.15^{bc}	87.64 ± 0.35	0.0001
Anthocyanidin (%)	0.22 ± 0.0009^{ab}	0.27 ± 0.0015^{ac}	0.17 ± 0.0002^{b}	0.33 ± 0.0004^{c}	0.0005
B-carotene (%)	0.29 ± 0.0018^{a}	0.36 ± 0.0011^{ab}	0.73 ± 0.0096	0.44 ± 0.0006^{b}	0.0001
Vitamin C (%)	311.21 ± 96.48	77.44 ± 0.69	370.85 ± 27.07	508.90 ± 25.68	0.0001
Total Phenols (%)	0.29 ± 0.0008^{ab}	0.32 ± 0.0057^{ac}	0.51 ± 0.0001	0.29 ± 0.0017^{bc}	0.0009
Tryptophan (%)	0.0043 ± 0.0^{ab}	0.0044 ± 0.0^{ac}	0.0042 ± 0.0^{bc}	0.0067 ± 0.0	0.0001

Table 2: *Results of analysis of functional properties of non-nixtamalized and nixtamalized corn subjected to different nixtamalization processes.*

Values are reported as mean ± variance; means followed by the same superscript within the same row are not significantly different from one another at 0.05 level of significance.

For antioxidant activity (DPPH) and tryptophan, all treatments were significantly different from NN but there was no significant difference between the three treatments. This agrees with the results of Bello-Perez., *et al.* [32] who observed that tortillas prepared with calcium salts had higher antioxidant capacity than traditional tortillas treated with ferric reducing antioxidant. However, this contradicts the observation of De La Parra., *et al.* [33] wherein nixtamalization had reduced the antioxidant activity by 75%. On the other hand, only the TNP had a significant difference in total phenolic content compared to NN and between treatments.

The observed decreased in tryptophan levels among nixtamalized samples contradicts the very limited studies on the effect of nixtamalization on tryptophan. According to Wacher [15], tryptophan and niacin levels had increased in nixtamalization. Corn is naturally abundant in niacin; however, according to Bressani., *et al* (1958) and Kodicek., *et al* (1956) as cited by Suri and Tanumihardjo [34], about 30% was lost during the process but the remaining niacin becomes more bioavailable.

On the other hand, significant difference on anthocyanidin content was only found between non-nixtamalized corn and CNP and TNP, with ENP having an insignificant difference with other treatments. Since data is lacking on the effect of nixtamalization on anthocyanidin, anthocyanin is used to explain the observation since anthocyanidin is the sugar-free analogues to anthocyanins. The observed decrease in the anthocyanidin content agrees with the results of Escalante-Aburto., *et al.* [35], which observed that up to 100% anthocyanins in blue corn may be due to leaching into the lime solution due to cell wall damage. This loss is aggravated by additional processing steps during production.

For beta-carotene, only the ENP has no significant difference with NN. Moreover, only the TNP has yielded a significantly different value between the three treatments. The observed decrease in the amounts of beta-carotene among CNP and ENP treatments agrees with the results of Gutierrez-Uribe., *et al* [36]. They found out that carotenoids are released from the kernels during nixtamalization. De La Parra., *et al.* [33] had also observed that there is a decreased in beta-carotene by 38% and beta-cryptoxanthin by (84%) during nixtamalization. Moreover, it was observed that the vitamin C content of all treatments are significantly different from NN and from each other. These phenomena are also attributed to the cell wall softening, inducing loss of essential components. Also, loss of vitamin C can be attributed to the increased temperature during nixtamalization since vitamin C is highly volatile.

Mineral Content

Table 3 shows that there is a significant difference between all treatments in terms of iron, calcium, and zinc contents.

Component	Classical (CNP)	Ecological (ENP)	Traditional (TNP)	Non-nixtamalized (NN/control)	p-value
% Iron	0.012 ± 0.0^{ab}	0.012 ± 0.0^{ac}	0.032 ± 0.0	0.016 ± 0.0^{bc}	0.0001
% Calcium	0.018 ± 0.0^{a}	0.018 ± 0.0^{a}	0.028 ± 0.0	0.008 ± 0.0	0.0001
% Zinc	0.003 ± 0.0^{a}	0.004 ± 0.0^{b}	0.002 ± 0.0	0.004 ± 0.0^{ab}	0.0001

Table 3: *Results of analysis of functional properties of non-nixtamalized and nixtamalized corn subjected to different nixtamalization processes.*

Values are reported as mean ± variance; means followed by the same superscript within the same row are not significantly different from one another at 0.05 level of significance.

For iron, only TNP had significant increase in iron content compared to NN while all nixtamalized treatments had significant increase in calcium content as compared to NN. The observed increased iron content in the present study agrees with the results of Morales and Zepeda [24] with 0.5% increase in iron content in nixtamalized corn tortillas. Nixtamalized corn had been tested by Rong and Kang-Ning [37] to piglets and they found out that it can improve the iron status and growth performance of piglets suffering from IDA.

On the other hand, a decrease in zinc content was observed in TNP while CNP and ENP had no significant difference in zinc content with that of control. According to Bressani., *et al.* [38], the holding time of nixtamal affects zinc content of nixtamalized corn, losing about 11% of zinc from the initial content. This may affect the zinc content of the samples since several preparations were done to get an aliquot. The observed increase in both zinc and iron may be due to the decrement in the phytic acid content by 20% which increases the bioavailability of iron and zinc. However, Bressani., *et al.* [38] had observed otherwise. They found out that the steeping procedure during nixtamalization decreases iron levels due to an increase in calcium content which inhibits iron bioavailability.

Moreover, there was a significant increase in calcium content of all nixtamalized corns compared to non-nixtamalized, with TNP yielding the highest amount. This observation agrees with the results of Bressani., *et al.* [39], observing an increase in the calcium content of nixtamalized corn. This can be attributed to the natural calcium content of the alkaline reagents and the increased calcium concomitant due to increased alkalinity.

Conclusion

Corn nixtamalization has been widely studied in the MesoAmerican countries. In the Philippines, corn is one of the main staples but is usually associated with micronutrient deficiencies, especially niacin. Studies have shown that nixtamalization increases the bioavailability of several nutrients including vitamin B3. Therefore, in this study, three different methods of nixtamalization was utilized to determine their effect on the proximate and functional properties and mineral content.

Results showed that there was a significant difference on the moisture, crude fat, nitrogen, and protein content of the three treatments while there was no significant difference on the crude fiber and total ash contents. Generally, only moisture content had increased among all treatment and only the TNP yielded a significant increase in nitrogen and protein content as compared to NN. Also, TNP yielded the highest amounts of all tested proximate components among the three treatments. However, due to limited evidences on the effect of alkaline treatment on proximate properties, particularly protein and crude fat content, it is recommended to conduct research and validation studies using more advanced protocols and methodologies.

Moreover, the study reflects that all treatments and control have high significant differences between each other in all analyzed functional properties. Generally, antioxidant activity and total phenols had increased in nixtamalized corn while beta-carotene had increased only in TNP. On the other hand, the amounts of anthocyanidin, vitamin C, and tryptophan had decreased. Overall, TNP has the highest beta-carotene, vitamin C, and total phenolic contents among the treatments.

It had also been shown that there was a significant difference between all treatments in terms of iron, calcium, and zinc contents. Only TNP had significant difference compared to the other two treatments and to NN. Moreover, only TNP had significant increase in iron content compared to NN while all nixtamalized treatments have significant increase in calcium content as compared to NN.

Based on the results, it can be concluded that nixtamalization has a significant positive effect on the proximate and functional properties and mineral content of corn, especially using the traditional process.

Acknowledgment

The authors would like to acknowledge the Philippine Rice Research Institute-UP Los Baños and Prof. Arvin Paul Tuaño for providing some reagents used in the study and in giving assistance on data analysis, and Mrs. Ellen Penezuela for aiding during the data collection process.

Bibliography

1. Exconde B. "Rice Production and Consumption Trends in the Philippines from 2000 to 2015". *Yuchengco Center* (2016).
2. Index Mundi. "Philippines Milled Rice Domestic Consumption by Year". Index Mundi (2018).
3. Arcalas JY. "Philippines importing 250,000 MT rice to pull down rising prices". Business Mirror (2018).
4. Philippine Statistics Authority. "Rice and Corn Situation and Outlook, July 2018 Round". Philippine Statistics Authority (2018).
5. Villanueva Rhodina. "Rice import dependence compromising Philippine food security". Philstar Global (2018).
6. de los Santos WL., et al. "Linking Corn Production, Climate Information and Farm-Level Decision-Making: A Case Study in Isabela, Philippines". In: Sivakumar M.V.K., Hansen J. (eds) Climate Prediction and Agriculture. Springer, Berlin, Heidelberg (2007): 157-164.
7. Philippine Statistics Authority. "Consumption of Selected Agricultural Commodities in the Philippines: Volume 2 - By Barangay Classification". Quezon City, Philippines (2017).
8. de los Santos WL., et al. "Corn industry in the Philippines [2002]". Food and Agriculture Organization of the United Nations (2002).
9. Gomez, EJ. "PH corn output seen rising in 2019-2020 market year". The Manila Times (2019).
10. Haspel T. "In defense of corn, the world's most important food crop". The Washington Post (2015).
11. Philippine Statistics Authority. "Updates on Palay, Rice, and Corn Prices: Second Week of September 2018". Philippine Statistics Authority (2018).
12. Chen Xun Yao. "Why corn is taking over demand growth from wheat and rice". *Market Realist* 24 (2013).
13. Department of Science and Technology, Food and Nutrition Research Institute. "The Philippines Food Composition Tables" (1997).
14. Mendez-Alborez A., *et al*. "Impact of different alkaline-heating processes on technological and nutritional properties of maize tortillas". *Journal of Food and Nutrition Research* 53.1 (2014): 60-70.
15. Wacher C. "Nixtamalization, a Mesoamerican technology to process maize at small-scale with great potential for improving the nutritional quality of maize based foods". *Paper presented at the 2nd International Workshop: Food-based approaches for a healthy nutrition, Mexico* (2003).
16. Mariscal-Moreno RM., *et al*. "The effect of different nixtamalisation processes on some physicochemical properties, nutritional composition, and glycemic index". *Journal of Cereal Science* 65 (2015): 140-146.
17. Rodriguez FM and Hurtada W. "Analytical Procedures in Food Research". Los Baños, Laguna, Philippines (2010).
18. Ranganna S. "Handbook of Analysis and Quality Control for Fruit and Vegetable Products". 2nd edition. New Delhi: Tata McGraw-Hill Publishing Company Ltd (1986).
19. Ainsworth Elizabeth A and Kelly M Gillespie. "Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent". *Nature Protocols* 2.4 (2007): 875.
20. Shimada K., et al. "Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion". *Journal of Agricultural and Food Chemistry* 40.6 (1992): 945-948.
21. Opienska-Blauth J., et al. "A new, rapid method of determining tryptophan". *Analytical Biochemistry* 6.1 (1963): 69-76.
22. Ishiwu CN., *et al*. "Effect of thermal processing on lycopene, beta-carotene, and vitamin C content of tomato". *Journal of Food and Nutrition Sciences* 2.3 (2014): 87-92.

23. Pappa MR., *et al*. "Effect of Lime and Wood Ash on the Nixtamalization of Maize and Tortilla Chemical and Nutritional Characteristics". *Plant Foods for Human Nutrition* 65.2 (2010): 130-135.

24. Morales JC and Zepeda RAG. "Effect of Different Corn Processing Techniques in the Nutritional Composition of Nixtamalized Corn Tortillas". *Journal of Nutrition and Food Sciences* 7.2 (2017): 1-7.

25. Contreras-Jimenez B., *et al*. "Effect of steeping time and calcium hydroxide concentration on the water absorption and pasting profile of corn grits". *Journal of Food Engineering* 122 (2014) 72-77.

26. Ruiz-Gutiérrez., *et al*. "Changes in mass transfer, thermal and physicochemical properties during nixtamalization of corn with and without agitation at different temperatures". *Journal of Food Engineering* 98.1 (2010): 76-83.

27. Rodríguez ME., *et al.* "Influence of the structural changes during alkaline cooking on the thermal, rheological and dielectric properties of maize tortillas". *Cereal Chemistry* 73.5 (1996): 593-600.

28. Gutiérrez, E., *et al*. "Study of Calcium Ion Diffusion in Nixtamalized Quality Protein Maize as a Function of Cooking Temperature". *Cereal Chemistry* 84 (2007): 186-194.

29. Arambula-Villa G., *et al*. "Thermal properties of maize masa and tortillas with different components from maize grains, and additives". *Journal of Food Engineering* 80.1 (2007): 55-60.

30. Owusu-Kwarteng James and Fortune Akabanda. "Applicability of nixtamalization in the processing of millet-based maasa, a fermented food in Ghana". *Journal of Food Research* 2.1 (2013): 59.

31. Boniface Ocheme Ocheme and Mikailu Esther Gladys. "Effect of alkaline soaking and cooking on the proximate, functional and some anti-nutritional properties of sorghum flour". *AUJT* 14.3 (2011): 210-216.

32. Bello-Perez LA., *et al*. "Effect of nixtamalization with calcium carbonate on the indigestible carbohydrate content and starch digestibility of corn tortilla". *Journal of Cereal Science* 60.2 (2014): 421-425.

33. de la Parra Columba., et al. "Effect of processing on the phytochemical profiles and antioxidant activity of corn for production of masa, tortillas, and tortilla chips". *Journal of Agricultural and Food Chemistry* 55.10 (2007): 4177-4183.

34. Suri Devika J and Sherry A Tanumihardjo. "Effects of different processing methods on the micronutrient and phytochemical contents of maize: From A to Z". *Comprehensive Reviews in Food Science and Food Safety* 15.5 (2016): 912-926.

35. Escalante-Aburto, A., et al. "Effect of extrusion processing parameters on anthocyanin content and physicochemical properties of nixtamalized blue corn expanded extrudates". *CyTA-Journal of Food* 11.1 (2013): 29-37.

36. Gutiérrez-Uribe Janet A., et al. "Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize kernels processed into masa for tortillas". *Journal of Cereal Science* 52.3 (2010): 410-416.

37. Rong Li and Kang-Ning W. "Nixtamalization Effects on the Contents of Phytic Acid in the Varieties of Maize and the Bioavailability of Iron in Nixtamalized Maize to Young Pigs". *Pakistan Journal of Nutrition* 8.6 (2009): 905-909.

38. Bressani R., *et al*. " Effect of processing conditions on phytic acid, calcium, iron, and zinc contents of lime-cooked maize". *Journal of Agricultural and Food Chemistry* 52.5 (2004): 1157-1162.

39. Bressani R., *et al*. "Nixtamalization effects on the contents of phytic acid, calcium, iron and zinc in the whole grain, endosperm and germ of maize". *Food Science and Technology International* 8.2 (2002): 81-86.

40. Argun MS and Dogan IS. "Effects of Varying Nixtamalization Conditions on the Calcium Absorption and Pasting Properties of Dent and Flint Corn Flours". *Journal of Food Process Engineering* (2016).

41. Cooper A. "Effects of nixtamalization on maize processing in Malawi". *Journal of Undergraduate Research* (2018).

42. Cuevas-Martinez D., *et al*. "Nutrition and Texture Evaluation of Maize-White Common Bean Nixtamalized Tortillas". *Mexico* 35.11 (2010): 828-832.

43. DA BPO. "UPLB study identifies corn varieties resistant to Fusarium ear rot. Laguna, Philippines". *Philippines* (2015).

44. De Girolamo A., *et al*. "Effect of alkaline cooking of maize on the content of fumonisins B1 and B2 and their hydrolysed forms". *Food Chemistry* 192 (2016) 1083-1089.

45. García-Rosas M., *et al*. "Resistant starch content and structural changes in maize (Zea mays) tortillas during storage". *Starch/Stärke* 61.7 (2009): 414-421.

46. Gerpacio R V., *et al*. "Maize in the Philippines: Production Systems, Constraints, and Research Priorities". *Mexico* (2004).

47. Gutiérrez, E., *et al*. "Study of Calcium Ion Diffusion in Nixtamalized Quality Protein Maize as a Function of Cooking Temperature". *Cereal Chemistry* 84 (2007): 186-194.

48. Gutiérrez-Cortez E., *et al*. "Effect of processing conditions on the production of nixtamalized corn flours by the traditional method". *Journal of Food* 11 (2013): 46-53.

49. Guzman AQ., *et al*. "Changes on the structure, consistency, physicochemical and viscoelastic properties of corn (Zea mays sp.) under different nixtamalization conditions". *Carbohydrate Polymers* 78.4 (2009): 908-916.

50. K E Burton., *et al*. "Effect of Micronutrient Fortification on Nutritional and Other Properties of Nixtamal Tortillas". *Cereal Chemistry* 85.1 (2008): 7-75.

51. Khampas S., *et al*. "Variability in phytochemicals and antioxidant activity in corn at immaturity and physiological maturity stages". *International Food Research Journal* 20.6 (2013): 3149-3157.

52. Martínez HE., *et al*. "Studies and biological assays in maize tortillas made from fresh masa prepared by extrusion and nixtamalization processes". *Journal of Food Science* 67 (2002): 1196-1199.

53. Mendoza C., *et al*. "Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas". *The American Journal of Clinical Nutrition* 68.5 (1998): 1123-1127.

54. Mora-Escobedo R., *et al*. "Changes in select nutrients and microstructure of white starch quality maize and common maize during tortilla preparation and storage". *Food Science and Technology International* 10.2 (2004): 79-87.

55. MPRO_admin. "Promoting rice and white corn combination as a staple for Filipinos". University of the Philippines (2018).

56. Palencia E., *et al*. "Total Fumonisins Are Reduced in Tortillas Using the Traditional Nixtamalization Method of Mayan Communities". *American Society for Nutritional Sciences* 133.10 (2003): 3200-3203.

57. Peña-Reyes RA., *et al*. "Effect of nixtamalization processing temperature on maize hydration and the textural properties of masa and tortillas". *Journal of Food Processing and Preservation* (2016).

58. Ranum P., *et al*. "Global maize production, utilization, and consumption". *Annals of the New York Academy of Sciences* 1312 (2014): 105-112.

59. Robles-Ramirez MDC., *et al*. "Physiological effects of products obtained byn ixtamalization and extrusion of quality protein maize". *International Biotechnology Color Journal* 1.1 (2011): 20-25.

60. Rodriguez-Martinez NA., *et al*. "Effect of Malting and Nixtamalization Processes on the Physicochemical Properties of Instant Extruded Corn Flour and Tortilla Quality". *Plant Foods for Human Nutrition* 70.3 (2015): 275-280.

61. Salazar R., *et al*. "Effect of added calcium hydroxide during corn nixtamalization on acrylamide content in tortilla chips". *LWT - Food Science and Technology* 56.1 (2014): 87-92.

62. Sefa-Dedeh S., *et al*. "Effect of nixtamalization on the chemical and functional properties of maize". *Food Chemistry* 86.3 (2004): 317-324.

63. Slavin JL., *et al*. "Grain processing and nutrition". *Critical Reviews in Food Science and Nutrition* 40.4 (2000): 309-326.

64. Treviño-Mejia D., *et al*. "Fortification of Commercial Nixtamalized Maize (Zea Mays L.) With Common Bean (Phaseolus Vulgaris L.) Increased the Nutritional and Nutraceutical Content of Tortillas Without Modifying Sensory Properties". *Journal of Food Quality* 39.6 (2016): 569-579.

65. Urizar AH and Bressani R. "The effect of lime cooking of corn on phytic acid, calcium, total and ionizable iron content". *Archivos latinoamericanos de nutricion* 47.3 (1997): 217-223.

Volume 14 Issue 4 April 2019