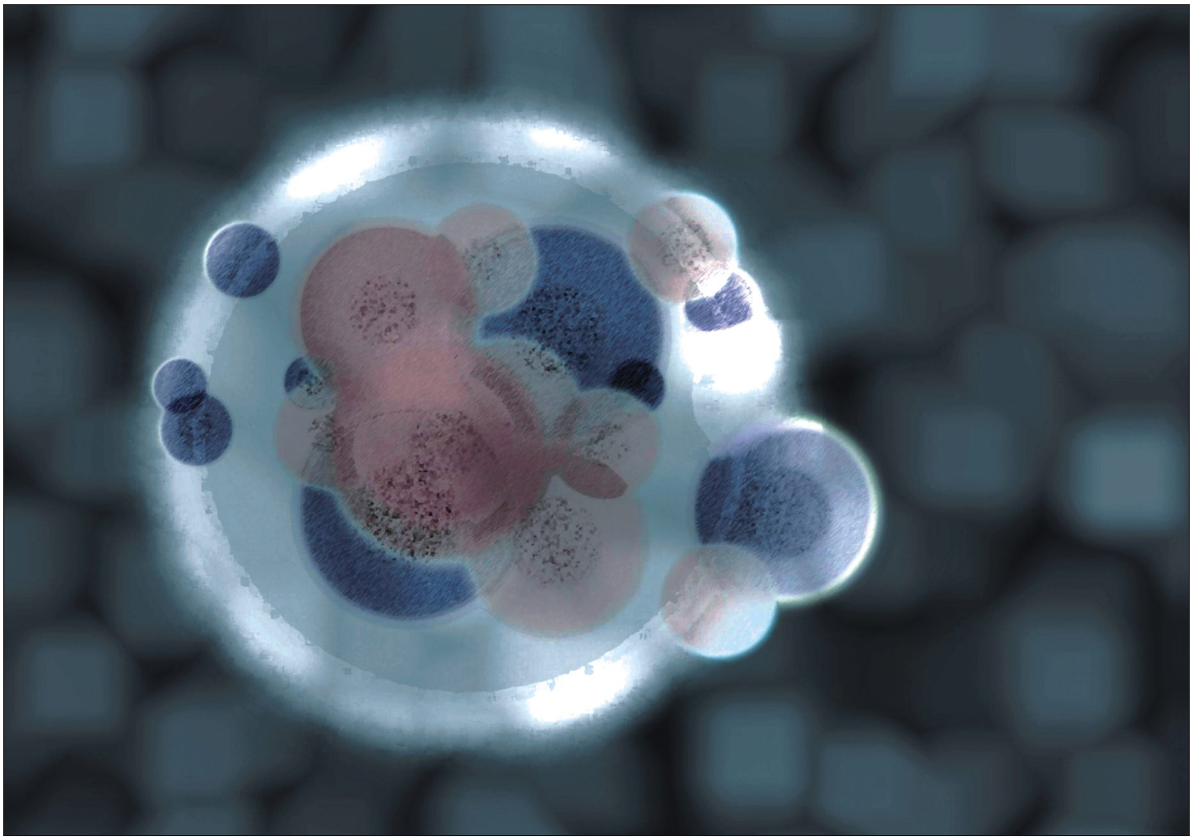

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Research Article
Enhancing Nutritional Benefits and Reducing Mycotoxin Contamination of Maize through Nixtamalization

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Abstract

Background and Objective: Maize is majorly consumed in Uganda but has the low nutrient quality and high mycotoxin contamination. This study established the effect of nixtamalization on mycotoxin content, nutritional composition, physicochemical and sensory properties of Ugandan maize. **Materials and Methods:** The samples were nixtamalized before milling by soaking maize grains in a solution of; (a) 1% slaked lime ($(Ca(OH)_2$) and (b) 1% traditional liquid ash. The maize grains from each solution were washed in clean water and dried at 60°C to constant weight and milled into flour. The milled samples were analyzed for aflatoxins, fumonisins, nutrient content and digestibility. The pasting properties and sensory acceptability of the nixtamalized maize flour products (posho and porridge) were also studied. **Results:** Nixtamalization significantly ($p<0.05$) reduced aflatoxin (up to 90%) and fumonisins (up to 80%) but increased the ash and niacin content. However, the process slightly reduced fat, sugars, protein and dietary fiber. The flour prepared from lime-nixtamalized maize had the lowest peak viscosity (711cP), followed by ash-nixtamalized flour (1377cP), while the flour from non-nixtamalized maize had the highest peak viscosity of 1465cP. Overall, there was no significant difference among the sensory properties for porridge and posho prepared from nixtamal and non-nixtamal maize flour. **Conclusion:** The study findings suggest that nixtamalization is a promising and affordable processing technology for reducing mycotoxin levels in maize and enhances the nutrient profile of the maize products without compromising consumer acceptability in Uganda.

Key words: Nixtamalization, mycotoxins, nutrient profile, sensory acceptability, affordability

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize (*Zea mays* L.) is an important staple food crop in Uganda and has emerged as a cash crop for smallholder farmers. Maize has, therefore, been prioritized by the Government of Uganda for continuous improvement through the national agricultural research system, resulting in the release of several improved varieties according to Ajambo *et al.*[1]. The production system is dominated by peasant farmers, with 75% of the country's output grown on plots of land that are between 0.2-0.5 hectares as reported by Daly[2]. Overall, Uganda is Africa's third-largest exporter of maize and second-leading exporter of maize flour[2]. To underline the importance of this crop, the Government of Uganda has prioritized maize as one of the 10 agricultural crops within the Rural Development and National Zoning Strategy as reported by MAAIF and ACF[3,4]. This position maize to play an increasingly significant role in the poverty alleviation agenda of the country[1,3,5]. However, the production and utilization of maize have several constraints including among others, contamination with mycotoxins especially aflatoxins[6,7,8] and fumonisins[9,10] and nutritional limitations, especially the quantity and quality of essential amino acids and niacin as mentioned by Carmen[11]. Unless it is prepared by specific techniques, Carmen[11] believes that the nutritional value of maize is marginal and any human population that depends on it as a major staple would suffer some degree of malnutrition and pellagra.

In Uganda, several strategies have been put in place to prevent mycotoxin contamination in maize during production, postharvest handling and storage[7,9]. However, according to Kaaya *et al.*[12] these strategies have not been successful in reducing the mycotoxin contamination to acceptable levels. There is thus a need to identify appropriate and affordable technologies that can decontaminate maize from mycotoxins[12,7]. One of the technologies used to decontaminate maize from mycotoxins but also improve nutritional value is nixtamalization[13,14,15]. Nixtamalization treatment of maize using slated lime facilitates pericarp removal, reduces mycotoxin levels, enhances water uptake, increases gelatinization of starch granules, and improves nutritional value through increased availability of niacin[16,17,18]. This research, therefore, aimed at establishing an appropriate nixtamalization method for maize. The objective of the study was to establish the effect of nixtamalization on mycotoxin (aflatoxin and fumonisin) content, nutritional composition, and physicochemical and sensory properties of Ugandan maize.

MATERIALS AND METHODS

Sampling maize: Maize samples were purchased during December 2018-March 2019, from farmers in each of the four major maize growing districts of Uganda (Mubende, Kiryandongo, Gulu, Iganga) located in four agro-ecological zones where aflatoxin and fumonisin contamination of maize has been previously reported[7,9] and from two maize collection centers (Nateete and Kisenyi) in Kampala Capital City Authority where the quality of maize was visually bad. The samples were stored at -18°C until further analyses.

Maize nixtamalization: The nixtamalization process involved (a) soaking maize grains in 1% slaked lime ($Ca(OH)_2$) solution; (b) soaking maize grains in a 1 % traditional liquid ash solution (Fig. 1) as per the relevant literature[19,20,21]. The maize grains were washed in each solution three times and dried at 60°C, for 16 hrs to constant weight and ground into flour. Part of the flour was analyzed for aflatoxin and fumonisin content and the other was saved for nutrient and physico-chemical analyses as well as preparation of porridge and posho (paste) for sensory evaluation.

Determination of aflatoxin and fumonisin content: Total aflatoxin (parts per billion) and fumonisin content (parts per million) of nixtamal and non-nixtamal (control) maize were determined and quantified using the VICAM[22] Fluorometer method following the manufacturer's method (VICAM L. P, USA).

Proximate analysis: The flours from nixtamal and non-nixtamal maize were analyzed for crude fiber, ash, crude fat, and crude protein content and digestibility using the standardized methods recommended by AOAC[22].

The total crude fiber was calculated as the sum of the insoluble and soluble dietary fiber determined in the samples. Ash content was calculated in percentage dry basis using the original sample weight and dry matter coefficient. Crude fat was determined on a dry basis as grams (g) of fat in the flour sample while crude protein was determined from the obtained supernatant after cooling.

Determination of protein digestibility: Protein digestibility (%) in nixtamal and non-nixtamal maize flour was determined using the porcine pepsin method as adapted by Gomez *et al.*[23]. The protein content of the sample was determined with a Nitrogen (N)-analyzer (Elementar Americas; Inc., Mt. Laurel, NJ) before and after digestion.

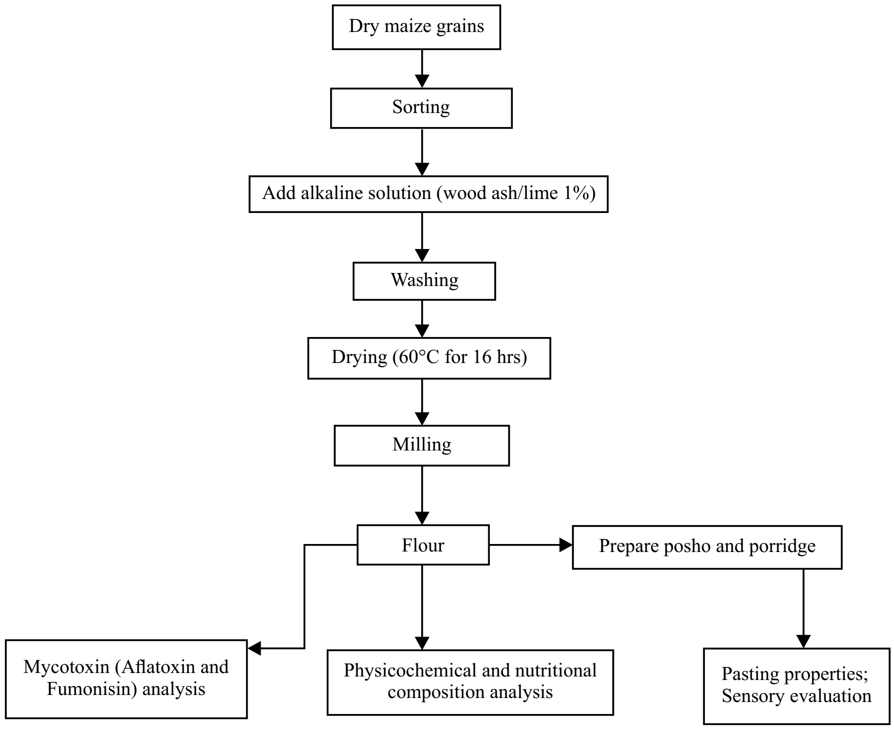

Fig. 1: Summary of the processes followed during nixtamalization

Determination of starch content: Starch content was determined according to the method described by Nielsen[24] by reference to a standard curve.

Determination of Niacin: Niacin was extracted from the flour by alkaline treatment and quantified using High-Performance Liquid Chromatography (HPLC) with fluorimetric detection after post-column derivatization with UV irradiation according to Lawrence[25].

Determination of pasting properties: Starch pasting properties were determined using a Rapid Visco Analyser (RVA-4500, Perten Instruments, Australia) equipped with thermocline for Windows Software, using Standard profile 1 as described by Fresnellia *et al.*[26]. Peak viscosity (PV), trough/hold viscosity (HV), breakdown (BD), final viscosity (FV), setback (SB), pasting temperature (PT) and peak time were recorded from the RVA curve.

Sensory evaluation of porridge and Posho: Sensory evaluation was conducted on the porridge and posho prepared from both the nixtamal and non-nixtamal maize flour. Porridge was prepared according to the village household technology by adding separately 100 g of flour in 200 mL of cold water. The resulting paste was added to 500 mL of boiling water and cooked for 15 min with constant stirring. The prepared porridge was kept in coded thermos vacuum flasks to keep it hot till sensory evaluation tests by panelists. Posho (maize paste) was also prepared according to village household technology by adding water to a heavy-bottomed saucepan and brought to a boil. Approximately 500 g of flour (from either nixtamal or control maize) was added to boiling water little at a time. A flat wooden spoon was used to stir the mixture to form a porridge-like consistency. More maize flour was added a little at a time while pressing to the sides of the saucepan to remove any lumps. The procedure continued for 10 min to allow the paste to become more firm, thus forming the Posho. The Posho was then transferred to a flat plate and cut into small pieces and allowed to cool before presenting it to the panelists to undertake the sensory evaluation.

Sensory evaluation of the porridge and Posho was evaluated by a panel of 32 consumers who regularly consume these products following a method described by Mbela *et al.*[27]. The sensory attributes that were evaluated included; color, taste, aroma and overall acceptability. These attributes were selected because they are prone to the changes associated with chemical reactions that take place in the product during storage. The panelists rated the sensory attributes of the porridges and Posho on a nine-point hedonic scale described by Wichchukita and O'Mahony[28] where 9 = like extremely and 1 = dislike extremely.

RESULTS AND DISCUSSION

Effect of nixtamalization on aflatoxin contamination of maize: Results in Fig. 2 show that the nixtamalization process using both slaked lime and traditional wood ash significantly ($p<0.05$) reduced the amount of aflatoxin content in all the maize samples. In maize samples from some districts, the reduction in aflatoxin was up to more than 90%. According to another study[29] traditional nixtamalization process for maize reduced aflatoxin levels by 90% while ecological nixtamalization process reduced the toxins by 92%. Research to understand the role of slaked lime, $Ca(OH)_2$ indicated that, in addition to aiding pericarp removal, lime incorporation is responsible for the cross-linking of starch molecules via formation of a calcium bridge with negatively charged amylose molecules[18,30,31]. The high pH of the alkaline processing that promotes ionization of starch hydroxyl groups is also responsible for hydrolysis of the parent aflatoxin B1 (FB1) to hydrolyzed aflatoxin B1 (HFB1)[16].

In order to compare the effect of the two nixtamalization methods on aflatoxin decontamination of maize, the aflatoxin results of the maize samples from different districts were combined (Table 1). Nixtamalization significantly reduced aflatoxin levels up to about 46%; however, there was no significant difference ($p>0.05$) between aflatoxin content of the maize treated with lime and that treated with ash (Table 1).

Effect of nixtamalization on fumonisin content in maize: Results in Table 2 indicate that nixtamalization significantly reduced total fumonisin contamination in maize. The results in Table 2 indicate that fumonisin levels decreased by more than 45 and 80% due to treatment with lime and ash respectively. Fumonisins are very water-soluble mycotoxins, which can thus leach into the liquid fraction during cooking and steeping procedures[13,20]. Furthermore, an alkaline treatment can result in hydrolysis of the O-acyl bonds of fumonisins, leading to the formation of hydrolyzed fumonisins[20]. Sara and Carsten[20] established that upon steeping of maize kernels and maize meal in 0.1 M $Ca(OH)_2$ (at room temperature, under continuous stirring), a reduction in FB1 concentrations and an accumulation of fully hydrolyzed FB1 (HFB1; also referred to as aminopentol) occurred. Palencia *et al.*[13] reported that cooking corn with lime and allowing it to steep overnight, reduced fumonisin content by 18.5% while Sara and Carsten[20] established that nixtamalization of maize by Mayan communities in South America reduced fumonisin levels by 78-89%. Atukwase *et al.*[9,10], reported that fumonisin levels of maize from three agro ecological zones in Uganda ranged from 0.27-10 ppm with a mean of 4.93 ppm, while current study results of the control samples (non-nixtamal maize) indicate

Table 1: Effect of different nixtamalization methods on aflatoxin content of maize

Treatment	Mean aflatoxin levels (ppb)
Control	11.588 ± 1.222^b
Lime (1%)	6.996 ± 0.662^a
Ash (%)	6.266 ± 0.058^a

Means with different superscripts in a column are significantly different ($p<0.05$)

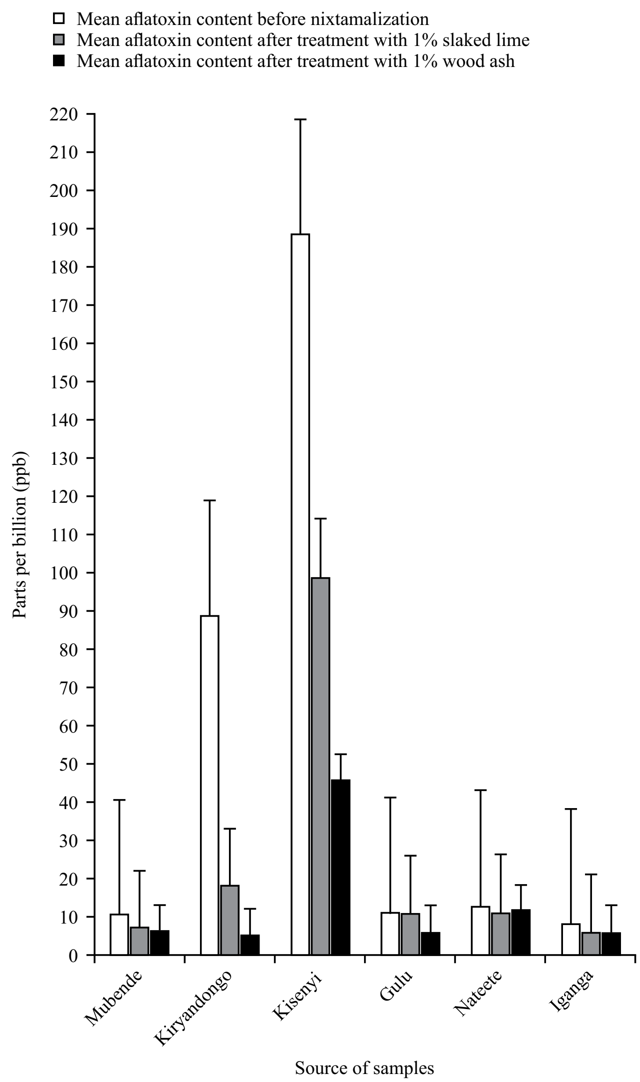

Fig. 2: Effect of Nixtamalization on mean aflatoxin content of maize from different parts of Uganda

that mean fumonisin contamination of maize was 5.75 ppm implying that these mycotoxins are still a problem in Uganda thus requiring effective control and management.

Effect of nixtamalization on crude fat, sugars and crude fibre: The results of the effect of nixtamalization on crude fat, sugars and crude fibre are presented in Fig 3. In the current study, results show that there was a decrease in dietary fibre (12.82-10.042%). However, the decrease was greater in maize nixtamalized with wood ash as compared to that nixtamalized with lime. The nixtamalization process helps to eliminate the pericarp, therefore, insoluble dietary fibre decreases from raw to nixtamalized maize[16,11]. However, the relatively high levels of dietary fibre that remains in the maize are of nutritional significance[11]. Nixtamalization process involving both slaked lime and wood ash solutions significantly ($p<0.05$) reduced crude fat, total sugars and crude fibre amounts in maize. Bressani[18] studied chemical changes during rural tortilla production in Guatemala using nixtamalization and reported that dietary fibre of nixtamal dough (9.3-9.6%) was lower than that of raw maize (12.2-12.8%).

In the current study, nixtamalization also significantly reduced the amount of total sugar content in maize from 28.8-22.2% which possibly was due to retrodagation and high pH of 7.1 and 7.2[32]. According to Mora-Rochin *et al.*[31] to maize products with a low caloric content and a high resistant starch value help to maintain a healthy intestine. Furthermore, the decrease in crude fat content (3.5%) was more significant in maize nixtamalized with wood ash as compared to lime-nixtamalized maize whose reduction was 4% (Fig. 3). The crude fat content of maize decreases as the kernel is nixtamalized; this results from the loss of the seed-coat, the tip cap, the aleuronic layer and possibly part of the germ[32,33].

Effect of nixtamalization on ash content: Nixtamalization slightly increased the ash content although the differences were not statistically significant (Table 3). An increase in ash content of nixtamal maize has been reported by several other researchers[11,16,17,33] due to the ash and lime used in the process. An increase in wood ash would imply enhancement of certain important minerals which could be lacking or inadequate in maize thus making it very important in addressing challenges of micronutrient malnutrition in Uganda. According Ohno[34] wood ash contains micro-nutrients such as; iron (Fe), copper (Cu), zinc (Zn). Calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P). Thus the use of calcium hydroxide improves the calcium-to-phosphorus ratio in nixtamal, which possibly favours the utilization of the calcium ion by the animals and human beings as believed by sefa-dedeh *et al.*[21].

Effect of nixtamalization on protein content and digestibility: There was a slight reduction in protein content of nixtamalized maize (Fig. 4). Similar results were obtained by Sefa-dedeh *et al.*[21] and Bressani[18] who reported that for the uncooked maize samples (steeped without cooking), the

Table 2: Effect of nixtamalization on fumonisin content of maize

Treatment	Mean fumonisin levels (ppm)
Control	5.75±0.012[a]
Lime (%)	3.15±0.014[b]
Ash (%)	1.12±0.011[c]

Means with different superscripts in a column are significantly different ($p<0.05$)

Table 3: Effect of nixtamalization on ash content of maize

Treatment	Ash (%)
Control	1.35±0.02[a]
Lime	1.47±0.03[a]
Ash	1.42±0.05[a]

Means with same superscripts in a column are not significantly different ($p>0.05$)

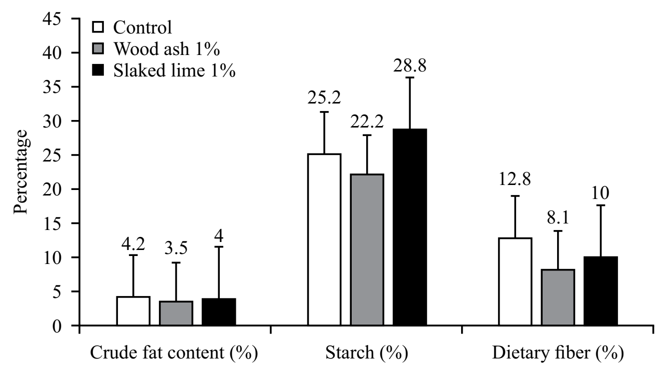

Fig. 3: Effect of nixtamalization on crude fat, starch and dietary fiber content of maize

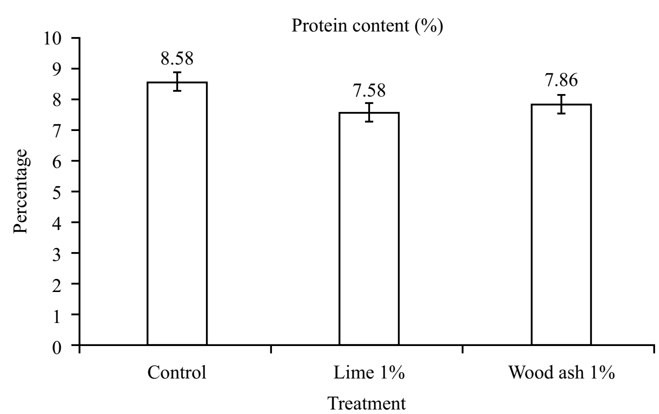

Fig. 4: Effect of nixtamalization on the protein content of maize

protein content generally decreased with increasing lime concentration although the decrease was not significant (p>0.05). Soaking maize in an alkaline causes changes in proteins through processes such as cross-linking, hydrophobic interaction, racemization, degradation, denaturation or the formation of lysinoalanine; these processes may as well affect protein content[16,35,36]. In addition, some researchers have reported that even though chemical losses in some nutrients take place upon lime cooking of maize, the protein quality is better in tortillas than in raw maize[16,35,36].

Results of the protein digestibility of maize are presented in Fig. 5. Nixtamalization caused a decrease in the protein digestibility of maize from 66-44.44%. According to Gutiérrez-Dorado[37] nixtamalization process decreased *in vitro* protein digestibility from 79.05-75.42%, a reduction less than what was established in the current study. Soaking maize in an alkaline solution causes changes in proteins through processes such as cross-linking, hydrophobic interaction, racemization, degradation, denaturation or the formation of lysinoalanine, and, these processes lead to decrease in protein digestibility[16,36,37]. This suggests that a decrease in protein digestibility occurs when the raw maize grain is nixtamalized[16,31]. According to Carlos *et al.*[38], changes in protein structure through the formation of secondary crosslinks or iso-peptide bonds may reduce digestibility by blocking the active sites of enzyme attack or by inducing the formation of compounds that inhibit digestive enzymes (inhibition of amino peptidase by an advanced Maillard derivative of lysine). Although protein digestibility reduced, nixtamalization has been reported to improve availability of particular essential amino acids in cooked tortillas thus improving overall protein quality[38]. Therefore, subsequent studies could establish the effect of nixtamalization on essential amino acids in maize products (porridge and posho) rather than on protein digestibility.

Effect of nixtamalization on niacin content: The nixtamalization process caused a significant increase in the niacin content available in maize as presented in Fig. 6. Maize nixtamalized with ash had the highest level of niacin (93 μ kg^{-1}) followed by lime with 89 μ kg^{-1} while the control had the least content of 66 μ kg^{-1}. This is a highly significant increase with the ash treated maize having a 41% increase, and the lime treated maize having a 34.8% increase in niacin over the control samples. The increase in the niacin content of nixtamal maize has been reported by other researchers[16,11]. According to Bressani[18], Lime treatment results in a release of the bound niacin. After lime-cooking, the endosperm contributed around 68% of the total niacin, and the germ around 5.5%. Of the total, 26% was found in the cooking water. They also found that the percentage of niacin extracted in water from the raw grain was 68.5% of the total; and from lime-cooked maize 76%. Since maize consumption in Uganda and all of Sub-Saharan Africa is quite high and is increasing[2,39,40], findings indicate that nixtamalization has the potential to decrease pellagra, an endemic disease caused by a lack of the vitamin niacin (vitamin B_3) in the body[11].

Effect of nixtamalization on the sensory properties of porridge and posho: Results of the sensory evaluation of Posho and porridge prepared from nixtamal and non-nixtamal maize are presented in Table 4 and 5 respectively. There was no significant difference (p>0.05) between porridge made from maize nixtamalized with ash, lime and the control in terms of general appearance, colour, aroma, thickness, taste, mouth feel and overall acceptability (Table 4). As observed for porridge, all sensory attributes tested by panelists did not

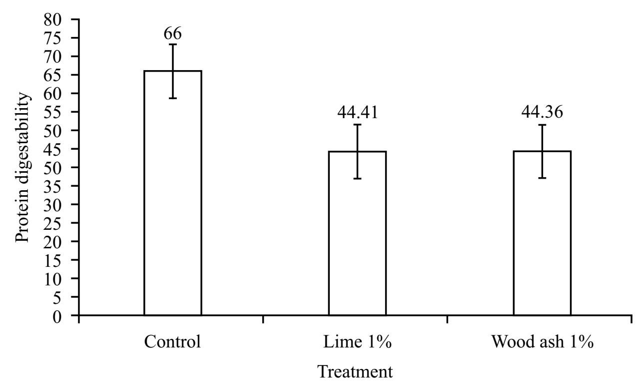

Fig. 5: Effect of nixtamalization on the protein digestibility of maize

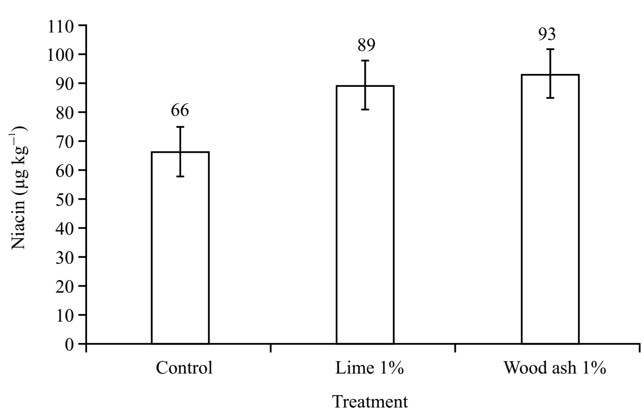

Fig. 6: Effect of nixtamalization on niacin content of maize

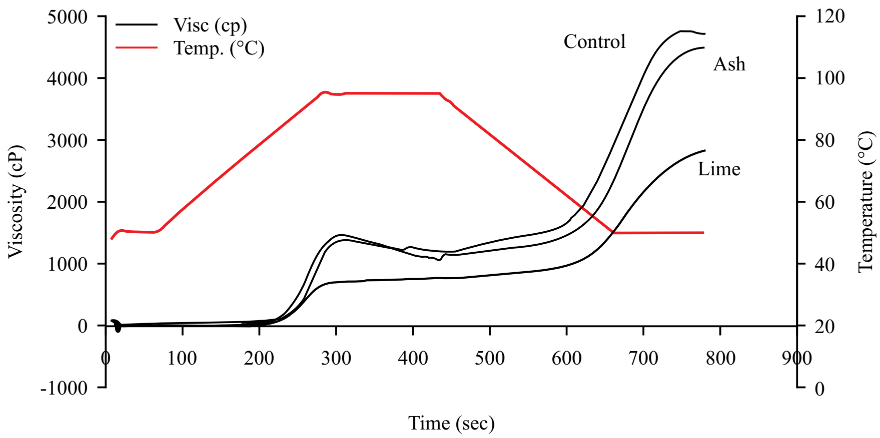

Fig. 7: Viscosity plots for lime, ash and control treatments

Table 4: Effect of nixtamalization on the sensory properties of Posho

	Treatment		
Attribute	Lime	Ash	Control
General appearance	6.1±1.80[a]	6.3±1.90[a]	6.1±2.00[a]
Colour	6.0±1.72[a]	6.1±2.10[a]	5.6±2.03[a]
Aroma/Flavour	5.8±1.68[a]	6.2±1.63[a]	5.5±1.59[a]
Texture	5.9±1.54[a]	6.0±1.69[a]	5.9±1.76[a]
Taste	5.1±1.98[a]	5.8±1.91[a]	5.1±1.89[a]
Mouth feel	5.1±1.86[a]	5.8±1.81[a]	5.3±2.07[a]
After taste	5.2±1.89[a]	5.4±2.05[a]	5.4±1.90[a]
General acceptability	5.6±1.86[a]	6.2±1.74[a]	5.6±1.62[a]

Data are Means±standard deviation (n = 32), Mean values in the same row with same superscript letters are not significantly different (p>0.05), Anchors for the hedonic scale used were: 9: Like extremely, 8: Like very much, 7: Like moderately, 6: Like slightly, 5: Neither like nor dislike, 4: Dislike slightly, 3: Dislike moderately, 2: Dislike very much, 1: Dislike extremely

Table 5: Effect of nixtamalization on the sensory properties of maize porridge

	Treatment		
Attribute	Lime	Ash	Control
General appearance	6.7±1.73[a]	6.8±1.36[a]	7.4±1.34[a]
Colour	6.8±1.31[a]	6.6±1.61[a]	7.2±1.50[a]
Aroma/Flavour	6.8±1.72[a]	6.3±1.32[a]	6.8±1.66[a]
Thickness	6.7±1.45[ab]	6.0±1.60[b]	7.1±1.21[a]
Taste	7.1±1.55[a]	7.0±1.27[a]	7.3±1.18[a]
Mouth feel	6.6±1.76[a]	6.8±1.41[a]	7.1±1.41[a]
After taste	6.2±1.64[a]	6.4±1.44[ab]	7.0±1.39[b]
General acceptability	6.7±1.515[a]	6.8±1.06[a]	7.3±1.62[a]

Data are Means±standard deviation (n = 32), Mean values in the same row with different superscript letters are significantly different (p<0.05), Anchors for the hedonic scale used were: 9: Like extremely, 8: Like very much, 7: Like moderately, 6: Like slightly, 5: Neither like nor dislike, 4: Dislike slightly, 3: Dislike moderately, 2: Dislike very much, 1: Dislike extremely

differ significantly. All the posho prepared from both nixtamal and non-nixtamal maize was generally acceptable.

Majority of the panelists mentioned that porridge nixtamalized with ash and lime had a slightly bitter and flat taste respectively. Since Ugandans are used to consuming porridge from unfermented maize, it was anticipated that nixtamalization could negatively affect porridge sensory properties hence acceptability. However, nixtamalization did not affect the overall taste and acceptability (Table 4 and 5). Therefore, this implies that use of lime and ash could be used to nixtamalize maize in order to enhance the additional benefits from porridge. Furthermore, Ugandans usually consume posho prepared from unfermented maize flour. Thus, nixtamalization using slaked lime and wood ash can be a good process for preparing flour prior to mingling Posho out of it.

Effect of nixtamalization on pasting properties of maize flour: Results show that the pasting properties differed among treatments, with flour prepared from maize nixtamalized using lime having the lowest peak viscosity, followed by flour prepared from maize nixtamalized using wood ash, while the flour from the control (non-nixtamalized) had the highest peak viscosity (Fig. 7). However, flour prepared from maize nixtamalized with ash had almost the same viscosity trend with that prepared from the control. The pasting characteristics of starch are used to obtain information about its functional behaviour during heating and cooling periods, which is common during the processing of starchy products (like porridge and Posho) or in those foods where starch is added as an ingredient[41]. According to Garcia-Diaz *et al.*[32] it is apparent that calcium hydroxide affects the maize starch supra-molecular structure along two ways: (1) calcium ions are linked to starch chains to form cross-linked structures that enhance water retention and viscoelasticity and (2) hydroxyl ions break down ramified amylopectin molecules, improving both calcium-ion diffusion and viscoelasticity of the treated maize starch gels. The binding of calcium to starch chains and the ionic strength by the remnant calcium and carbonate ions modifies the affinity of the starch gel to water[32,38]. In turn, this effect should be reflected in the water affinity of the nixtamalized starch chains[32]. There is extensive wettability of the starch molecules, which might be attributed to breakage of starch chains[38,42]. Furthermore, according to Fresnellia et al[26] pasting properties were more affected by the moisture content and more influenced by starch type and quantity rather than protein presence of the grains. This could explain the observed pasting properties of maize treated with lime during the current study.

CONCLUSION

Based on the findings of this study, the nixtamalization process improved maize quality by reducing aflatoxin and fumonisin contamination and increasing niacin (B3) content. Consumers who represented Ugandans during sensory evaluation found both the Posho and porridge forms acceptable under experimental conditions. Since the nixtamal maize had better-pasting properties, this may imply a cost-effective strategy for consumers to use the product. Specifically, the fact that ash was equally or even more effective in reducing aflatoxins and fumonisins than slaked lime, is quite important in the practical application of the method.

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SIGNIFICANCE STATEMENT

The results of this study have broad public health implications, as ash is readily available to the poor in Uganda and Africa in general. These findings further indicate that if the practice of nixtamalization is scaled up, then there could be a significant improvement in body immunity and reduction in child malnutrition due to reduced aflatoxins, fumonisins and pellagra.

This study discovered that nixtamalization method using wood ash improves Ugandan maize quality and safety that can be beneficial for improvement of body immunity and reduction in malnutrition due to reduced aflatoxins, fumonisins and pellagra. This study will help the researchers to uncover the critical areas of cost-effective means of enhancing health benefits of Ugandan maize that many researchers were not able to explore. Thus a new theory on use wood ash which is affordable to Ugandans may be arrived at.

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