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Effect of Alkaline Steeping on the Nutritional, Antinutritional and Functional Properties of Malted Millet *(Pennisetum glaucum)* Flour

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ABSTRACT
Pearl millet seeds were subjected to two alkaline steeping methods (lime solution and unripe plantain peel ash solution) for duration of 24, 48 and 72 h followed by 24 h sprouting, oven drying, milling and sieving to get fine flour. Flour of unsteeped seeds served as control. Standard methods were used to determine the proximate, mineral, antinutrients and functional properties. 72 h steeping in plantain ash solution significantly ($p<0.05$) increased the crude protein and crude lipid of the flours while crude fibre and ash content were increased by 72 h lime steeping. Both steeping solutions significantly ($p>0.05$) reduced carbohydrate and energy contents of the flours. Higher concentrations of calcium, potassium and zinc were observed at 72 h ash steeping as a result of significant ($p<0.05$) reduction in tannin content with gradual reduction in cyanide and phytate as steeping duration advanced. Ash steeping produced millet flours of low bulk density, good water and oil absorption capacity, emulsification, gelation temperature, wettability and water solubility which therefore have the most suitable potential for the production of complementary foods.
Keywords: Lime, Millet, Ash, Nutrients, Functional properties, Minerals

INTRODUCTION
Pearl millet *(Pennisetum glaucum)* grains are small seeded annual coarse cereals grown throughout the world (Jaybhayei *et al.,* 2014). It serves as an important source of dietary proteins, carbohydrates, vitamins, fibre and minerals which forms a vital component of the food security in the developing world (Abdalla *et al*., 1998). It is also one of the most widely grown and drought resistant crops among cereals because of its ability to establish and thrive in harsh conditions of low rainfall, high temperatures and poor soil fertility (Devi *et al*., 2011; Adekunle, 2012; Issoufou *et al.*, 2013; Kindiki *et al*., 2015). They are nutritionally comparable or even superior to staple cereals such as rice and wheat (Sehgal and Kawatra, 2003; Yang *et al*., 2012). Millet serves as a major food component and beverages in many African and Asian countries, such as bread, fermented cereal based porridge (“Akamu” or “Ogi”), indigenous fermented beverage, such as “kunu-zaki” and snack foods specifically among the non-affluent segment in their respective societies (Zakari *et al*., 2010; Amadou *et al.*, 2011; Chandrasekara *et al*., 2012; Oluwajoba *et al*., 2013; Abdullahi*et al.*, 2015).
Millet flour is an important food ingredient and its food and industrial uses would increase if appropriate applications were developed. One way of increasing uses of raw materials is to promote functional modifications. As with other cereals certain nutritional inhibitors, such as phytic acid and tannins are associated with pearl millet (Ali *et al*., 2009). These factors affect the nutritional value of the grain by inhibiting protein and starch digestibility and mineral bioavailability (Abdel-Rahaman *et al*., 2007; Nour *et al*., 2015). However, different food processing technologies have been developed over the centuries and are adopted to alleviate micronutrient deficiencies (Afify *et al*., 2012). These methods make the final product more attractive in flavour, appearance, taste, consistency etc. Besides these aspects of consumer preferences, several of the methods aim at making the food safe, wholesome and increase its shelf life.

Malting is a traditional process used in West Africa to prepare traditional beers, nonalcoholic beverages, porridges for children and other range of food products. It is a process which involves steeping, sprouting and drying, aims to change grains into malt with high enzymes and vitamins contents (Jaybhayei et *al*., 2014). Steeping is the process of soaking the grains in water or other liquid for the purpose of softening, cleansing and extraction of some constituents. During steeping, water is absorbed by the seed and thus causing the entire seed to swell up for the sprouting to occur. These led to decrease in tannins and total phenols content whereby vitamins content increases significantly (Abdullahi *et al.,* 2015). Notwithstanding, some minerals are lost and this phenomenon could be attributed to solubilisation and leaching during steeping. Therefore, the objective of this work was to investigate the effect of alkaline ($Ca(OH)_2$ (lime) and plantain peel ash) steeping and period on functional properties of malted millet flour.

MATERIALS AND METHODS

Sample collection: Pearl millet grains were purchased from Itam Market, Uyo, Akwa Ibom State, Nigeria. Fully matured unripe plantain was also purchased from the same market. Chemical used were of analar grade.

Preparation of ash: Plantain was properly washed with distilled water to remove dirt and blot dried. The skin was then peeled off manually using a knife. Plantain skin was then pre-ashed in an electric stove to burn off the organic content present. Pre-ashed sample were then transferred into a muffle furnace, initially set at 550 ^{o}C until properly ashed. Ash was then transferred into a plastic container with lid and stored until when needed.

Sample treatment: The method of Ocheme and Mikailu (2011) was adopted for the grain steeping with slight modification. Millet grains were cleaned to remove dirt, stones, damaged and discoloured seeds. This was done through hand picking, winnowing, and washing with distilled water. Millet was then drained by transferring to a sieving bowl. Grains were divided into three lots and each weighing 200 g. The first lot was unsteeped sample which served as control. The second and third lots were steeped in 100 ml 2% $Ca(OH)_2$ solution and 100 ml 2% ash solution, respectively. Steeping was carried out for 24, 48 and 72 h, steeped water was decanted and the seeds were placed on a moist muslin cloth for 24 h for sprouting to occur. Sprouted grains were kilned in a conventional air oven (model P.P. 22 US, Genlab, England) at 60 ^{o}C for 90 minutes to inactivate all enzymes and dry the grains. Dried grains were then cleaned of rootlets, milled, sieved using a 2 mm mesh screen and packaged in a high density polyethylene bag and stored at 4^{o}C for analysis.

Sample Analyses

Determination of proximate composition and total energy content: The flour samples were analyzed for their moisture, ash, crude protein, crude lipid and crude fibre contents according to the method described by AOAC (2005). Carbohydrate was determined by difference and energy content was determined by using the Atwater factor (carbohydrate and protein values were each multiplied by 4 kcal/g, whereas fat values were each multiplied by 9 kcal/g).

Determination of mineral composition: Selected mineral (Calcium, Magnesium, Zinc, Potassium and Phosphorus) were determined using the method of AOAC (2005). For the determination of each mineral, standards were prepared from the stock solution and were used to calibrate the equipment in concentration mode. The flour sample (2.0 g) was put in a crucible and placed into a muffle furnace, ashed at 550°C for 4 h and then cooled. The ash was washed into a 100 ml volumetric flask with 1% HNO_3, made up to the mark and filtered through a Whatman No. 1 filter paper. The filtered digest was then transferred into the Atomic Absorption Spectrophotometer (AAS) auto sampler vial and used directly for the determination calcium, magnesium and zinc by the atomic absorption spectrophotometer (UNICAM, Model 939, UK). Potassium was determined using the standard flame emission photometer while phosphorus was analysed using vanado-molybdate method.

Determination of selected antinutrients content: The hydrogen cyanide and tannin contents of the flours were determined by the method described by AOAC (2005). Modified method of Ijarotimi and Oluwalana (2013) was used for the determination of phytate.

Determination of functional properties: The bulk density, water absorption capacity, oil absorption capacity and wettability of the flour samples were determined by method of Onwuka (2005). Emulsification capacity was determined using the method of Kaushal *et al*. (2012), gelation temperature was determined according to the method of Narayana and Narasinga (1982) and solubility was determined following the method of Subramanian *et al*. (1994).

Statistical analysis

Data obtained were subjected to Analysis of Variance (ANOVA) using SPSS statistical software version 21.0. Means of triplicate data found to be significantly ($p<0.05$) different were separated using Duncan New Multiple Range Test.

RESULT AND DISCUSSION

Effect of alkaline steeping on the malted millet flour nutrients

The proximate composition and energy content of the malted millet flour samples are shown in Table 1. There were significant ($p<0.05$) increase in moisture, ash, crude protein, crude lipid and crude fibre contents and decrease in carbohydrate and energy contents were observed as the steeping duration progressed in both alkaline solutions. Moisture content ranged from 8.04-9.87% and 9.12-11.26% for lime and plantain ash steeping, respectively. These values were higher than the control (unsteeped millet flour) while the flour produced from millet steeped in ash solution for 72 h and sprouted for 24 h had the highest moisture content (11.26%). Lower value recorded for the control (6.81%) compared well with the value reported for fermentation of millet (Onweluzo and Nwabugwu, 2009) and this might be due to its low dry matter content (Adebowale and Maliki, 2011). Moisture content is an important parameter in flour and also an index of storage stability of the flour and its products. Flour with moisture content less than 13% are more stable from moisture-dependent deterioration (Shahzadi *et al*., 2005). Flour samples produced from millet steeped in lime solution observed to have higher ash content increased from 2.42-3.53% while flour gotten from plantain ash had lower values ranged from 0.96-1.25% for 24 h and 72 h, respectively. Plantain ash steeping increase the protein content of the flour samples from 11.20% (24 h)-11.82% (72 h) and had higher values compared with control (9.45%) and lime steeping which ranged between 9.10 (24 h) and 10.50% (72 h). The highest crude lipid content (5.48%) was found in the control sample followed by the 72 h ash steeping with value (5.27%) whereas 24 h lime steeping gave the lowest value (4.53%). The fibre content of the lime steeping samples were higher than the control and ash steeping samples. The highest (2.45%) and lowest value (0.74%) were 72 h lime steeping and 24 h ash steeping, respectively. Lime steeping gave the highest values which increased from 1.71-2.45% for 24 h and 72 h, respectively. The carbohydrate and energy content of all the steeped flour samples decreased as the steeping duration progressed. The carbohydrate content ranged from 68.68-76.10% with control sample had the highest value (76.10%). For steeping flour, 74 h lime steeping had the highest (74.20%) while 24 h lime steeping had the lowest value (68.68%). The control had higher energy value (391.52%) while among the steeping samples, 24 h ash steeping had the highest value (381.12 kcal/100g). Table 2 shows the minerals content of unsteeped and alkaline steeping millet flours. Millet flour samples were observed to contain fairly good amount of calcium and magnesium. A significant ($p<0.05$) increase in calcium, magnesium and potassium were observed in the flour samples while lower concentration and reduction in values were observed in phosphorus and zinc as the steeping duration progressed. Steeping in ash solution significantly ($p<0.05$) increased the concentration of calcium from 62.15 mg/100g (24 h) to 63.46 mg/100g (72 h). Lime steeping increased from 54.28 mg/100g (24 h) to 54.63 mg/100g (48 h) and later decreased to 53.02 mg/100g at 72 h while the control had the least value (49.36 mg/100g). Similar

Table 1: Proximate composition and energy content of alkaline steeped and unsteeped malted millet flours

	Lime steeping duration with 24 h sprouting			Ash steeping duration with 24 h sprouting			
	24 h	48 h	72 h	24 h	48 h	72 h	Control
Moisture (%)	8.04^{e}±0.01	9.14^{d}±0.03	9.87^{c}±0.01	9.12^{d}±0.02	10.05^{b}±0.01	11.26^{a}±0.02	6.81^{g}±0.02
Ash (%)	2.42^{c}±0.03	3.34^{b}±0.02	3.53^{a}±0.02	0.96^{g}±0.02	1.03^{f}±0.02	1.25^{d}±0.02	1.20^{e}±0.02
Crude protein(%)	9.10^{g}±0.02	9.80^{e}±0.02	10.50^{d}±0.05	11.20^{c}±0.02	11.50^{b}±0.05	11.82^{a}±0.02	9.45^{f}±0.05
Crude lipid(%)	4.53^{e}±0.03	4.91^{d}±0.02	4.97^{c}±0.01	4.88^{d}±0.02	4.96^{d}±0.03	5.27^{b}±0.02	5.48^{a}±0.01
Crude fibre(%)	1.71^{c}±0.01	2.33^{b}±0.02	2.45^{a}±0.01	0.74^{g}±0.03	0.79^{f}±0.01	0.83^{e}±0.02	0.96^{d}±0.03
Carbohydrate(%)	74.20^{b}±0.02	70.48^{e}±0.02	68.68^{f}±0.01	73.10^{c}±0.02	71.67^{d}±0.02	69.57^{d}±0.05	76.10^{a}±0.01
Energy(kcal/100g)	373.97^{e}±0.35	365.31^{f}±0.47	361.45^{g}±0.41	381.12^{c}±0.38	377.32^{d}±0.55	372.99^{b}±0.24	391.52^{b}±0.47

Values are means±SD, values with the same superscript in the same row do not differ significantly (p>0.05).

Table 2: Mineral composition of alkaline steeped and unsteeped malted millet flours (mg/100g)

	Lime steeping duration with 24 h sprouting			Ash steeping duration with 24 h sprouting			
	24 h	48 h	72 h	24 h	48 h	72 h	Control
Calcium	54.28^{e}±0.02	54.63^{d}±0.03	53.02^{f}±0.02	62.15^{c}±0.02	63.10^{b}±0.03	63.46^{a}±0.03	49.36^{g}±0.03
Magnesium	27.32^{c}±0.01	28.49^{b}±0.01	28.53^{a}±0.02	15.79^{f}±0.01	16.33^{e}±0.02	16.52^{d}±0.02	15.14^{g}±0.01
Phosphorus	0.26^{f}±0.02	1.70^{a}±0.02	1.12^{b}±0.02	1.10^{b}±0.02	0.91^{c}±0.01	0.80^{d}±0.01	0.65^{e}±0.01
Potassium	11.31^{e}±0.01	11.37^{d}±0.01	11.24^{f}±0.02	24.87^{c}±0.01	36.12^{b}±0.02	38.66^{a}±0.01	1.84^{g}±0.02
Zinc	4.82^{c}±0.02	4.54^{d}±0.02	4.08^{e}±0.02	5.32^{a}±0.02	5.17^{b}±0.01	4.83^{c}±0.02	2.63^{f}±0.03

Values are means±SD, values with the same superscript in the same row do not differ significantly (p>0.05).

calcium value had been reported for untreated pearl millet (Abdel-Rahaman *et al*., 2005). Lime steeping increased the concentration of magnesium in the flour samples from 27.32 mg/100g (24 h) to 28.53 mg/100g (72 h) while the control gave a lowest value (15.14 mg/100g). Phosphorus concentration in the lime steeped flour samples increased from 0.26 mg/100g (24 h) to 1.70 mg/100g (48 h) and later decreased at 72 h to 1.12 mg/100g. Gradual reduction was observed in ash steeping from 1.10 to 0.80 mg/100g while the control had 0.65 mg/100g. Alkaline steeping greatly increased the concentration of potassium in the flour samples while the control had the least value (1.84 mg/100g). The ash steeping increased from 24.87 mg/100g (24 h) to 38.66 mg/100g (72 h) while lime steeping increased from 11.31 mg/100g (24 h) to 11.37 mg/100g (48 h) and then decreased at 72 h (11.24 mg/100g). These increase values are as a result of higher level of potassium in plantain peel ash solution as this support the findings of Olabanji *et al*. (2012) and Israel and Akpan (2016). Zinc concentrations of all the flour samples were higher than the control (2.63 mg/100g) and decreased significantly as the steeping duration progressed. For the steeping samples, higher value (5.32 mg/100g) were observed in ash steeping at 24 h while 72 h lime steeping gave the least value (4.08 mg/100g).

Effect of alkaline steeping on the malted millet flour antinutrients

Table 3 shows the results of the antinutritional content of unsteeped and steeped malted millet flours. Cyanide content ranged from 0.01-0.05 mg/100g with flour produced from ash steeping having the highest value and flour from lime steeping having the lowest value. 24 and 48 h lime steeping flour samples were not significantly (p>0.05) different from the control.

Table 3: Antinutrient contents of alkaline steeped and unsteeped malted millet flours (mg/100g)

	Lime steeping duration with 24 h sprouting			Ash steeping duration with 24 h sprouting			
	24 h	48 h	72 h	24 h	48 h	72 h	Control
Cyanide	0.02^{d}±0.02	0.02^{d}±0.01	0.01^{e}±0.01	0.03^{c}±0.03	0.04^{ab}±0.02	0.05^{b}±0.02	0.02^{a}±0.02
Tannin	0.17^{b}±0.02	0.17^{b}±0.01	0.12^{c}±0.02	0.02^{c}±0.02	0.02^{c}±0.01	0.02^{c}±0.01	0.19^{a}±0.01
Phytate	6.93^{a}±0.02	6.05^{b}±0.01	3.99^{c}±0.01	3.93^{c}±0.02	3.24^{d}±0.02	2.83^{e}±0.02	2.32^{f}±0.01

Values are means±SD, values with the same superscript in the same row do not differ significantly (p>0.05).

Alkaline steeping significantly (p<0.05) increased the tannin content of the ash steeped flours. This increases could be at the point of sprouting due to increase inherent enzyme activities in the sprouting seeds. These enzymes were inactive in the dry seeds and during de-vegetation cyanide contents were reduced (Mbaeyi, and Onweluzo, 2010). There was no significant different in all the ash steeping durations and also 24 h was not significantly (p>0.05) different from 48 h lime steeping. Increase in phytate content of the flour samples at the first 24 h of steeping was observed in both solutions. Control had the lowest value (2.32 mg/100g) followed by ash steeping samples which decreased from 3.93-2.83 mg/100g and highest value (6.93 mg/100g) was recorded for 24 h lime steeping. Results obtained were lower than the reported values by Eltayeb *et al.* 2007.

Effect of alkaline steeping and duration on the malted millet flour functional properties: The functional properties of unsteeped and steeped malted flour samples are presented in Table 4.

Table 4: Functional properties of alkaline steeped and unsteeped malted millet flours

	Lime steeping duration with 24 h sprouting			Ash steeping duration with 24 h sprouting			
	24 h	48 h	72 h	24 h	48 h	72 h	Control
Bulk density (g/cm3)	0.76^{a}±0.02	0.77^{a} ±0.02	0.74^{a} ±0.01	0.75^{a} ±0.01	0.76^{a} ±0.01	0.77^{a} ±0.01	0.76^{a} ±0.03
Water absorption (g/g)	2.40^{abc}±0.03	2.60^{a}±0.03	2.40^{abc}±0.10	2.50^{ab}±0.05	2.20^{abc}±0.02	2.00^{c}±0.50	2.10^{bc}±0.02
Oil absorption (g/g)	2.50^{a}±0.05	2.50^{a}±0.02	1.70^{d}±0.05	1.60^{e}±0.02	2.00^{b}±0.12	1.90^{c}±0.05	2.00^{b}±0.02
Emulsification	4.90^{d}±0.02	5.00^{c}±0.01	5.10^{b}±0.03	4.80^{e}±0.03	4.90^{d}±0.02	5.20^{a}±0,02	3.50^{f}±0.01
Gelation temp. (°C)	83.30^{f}±0.05	94.60^{c}±0.02	95.30^{a}±0.05	80.00^{g}±0.02	91.50^{d}±0.02	94.70^{b}±0.02	85.60^{e}±0.05
Wettability (g/g)	2.50^{b}±0.02	1.32^{f}±0.02	1.16^{g}±0.02	2.15^{c}±0.02	2.00^{d}±0.02	1.84^{e}±0.04	3.20^{a}±0.02
Water solubility (g/g)	0.52^{bc}±0.01	0.53^{b}±0.02	0.57^{a}±0.02	0.51^{bc}±0.01	0.54^{b}±0.02	0.58^{a}±0.02	0.49^{c}±0.01

Values are means±SD, values with the same superscript in the same row do not differ significantly (p>0.05).

The bulk density of the flour samples ranged from 0.74-0.77 g/cm^3. Bulk density of the steeped flour samples were not significantly (p>0.05) different from the control. The low bulk density reported for all the samples in this study showed that millet flour could be an advantage in the preparation of complementary foods, because high bulk density limits the caloric and nutrient intake per feed of a child (Peter-Ikechukwu *et al*., 2016). Water and oil absorption capacity ranged from 2.00-2.60 g/cm^3 and 1.60-2.50 g/cm^3, respectively. Significant (p<0.05) reductions in water and oil absorption capacity were observed for flour produced from ash steeping while increase value was noticed in 48 h lime steeping. This increase might have been as a result of the production of compounds having good water holding capacity such as soluble sugars and this depends on the water bounding capacities of food components. The values were higher than those obtained from sorghum flour (Ocheme *et al*., 2015). Emulsification, gelation temperature and water solubility of flour samples increased significantly (p<0.05) as the steeping duration progressed. Control had the highest wettability value (3.20 g/g) while alkaline steeping gradually reduced the values of all the flour samples. Lime steeping decreased from 2.50-1.16 g/g and ash steeping from 2.15-1.84 g/g. The results are in conformity with the finding of Onweluzo and Nwabugwu (2009) who reported decreased in tannin and cyanide level of fermented millet flour and pigeon pea flour.

CONCLUSION

The result obtained have shown that the steeping in lime and plantain peel ash solution prior to 24 h sprouting of pearl millet at different steeping duration resulted to a significant desirable variation in proximate, mineral, antinutrients and functional properties. Ash steeping increased the crude protein and crude lipid while crude fibre and ash increased by lime steeping. Carbohydrate and energy contents were reduced by both steeping solutions. Lime steeping increased magnesium and phosphorus while ash steeping improved and increased calcium, potassium and zinc bioavailability in the millet flours by gradual reduction in tannin content and lower level of cyanide and phytate contents. Both alkaline steeping solution produce millet flour of low bulk density, good water and oil absorption capacity, good gelation and water solubility. The findings therefore suggest that good quality, highly nutritious and acceptable complementary foods can be produced from low cost and underutilized millet flour obtained especially from ash steeping.

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