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Original Research Article.

Effect of Some Preservatives on Nutritional Quality and Sensory Characteristics of Pickled African Walnut (*Tetracarpidium conophorum*)

ABSTRACT

Aim: To determine the effect of citric and lactic acid used as preservatives on the nutritional quality and sensory characteristics of African walnut pickle during storage.

Place and Duration of Study: This study was carried out between July-September, 2017 at Food and Industrial Microbiology Laboratory, University of Port Harcourt, Port Harcourt Nigeria.

Methodology: Mature raw African walnut was cooked for 1 hr and pickled using brine solution. 1 % (v/v), 3 % (v/v) and 5 % (v/v) citric acid were separately added to brine solution containing pickled African walnut inside three glass jars, and sterilized. Similarly, 1 % (v/v), 3 % (v/v) and 5 % (v/v) lactic acid was added to already sterilized pickled African walnut mixed with brine solution inside three glass jars. Pickled African walnut without preservative is the control. The entire setup was stored for 6 Wks at room temperature (28±2 ^{o}C). At 1 Wk interval, pH of the samples was monitored. Proximate composition and antinutrients in freshly cooked African walnut and pickle from each glass jar after 6 Wks storage were determined using standard methods and sensory characteristics by 9 point Hedonic scale.

Results: The level of antinutrients in freshly cooked African walnut is higher than that of pickled African walnut. In terms of nutrients, protein which ranged between 24.12-25.20 % is the least affected by the treatments. Flavonoid which ranged between 0-0.89 mg is the lowest antinutrient in the samples. All the parameters evaluated showed significant differences ($P < 0.05$) among samples. During storage, pH of freshly cooked African walnut steadily increased from 5.6-7.0 but that of pickled African walnut which ranged between1.8 - 4.0 decreased. The pickles met U.S. Code of Federal Regulations (21 CFR Part 114) which stipulate that pH of food products must be 4.6 and below. Sensory evaluation revealed that pickled African walnut without preservative and the pickle preserved with 5 % lactic acid is the least and most preferred, respectively.

Conclusion: Addition of citric and lactic acid in pickled African walnut reduced its pH and antinutrients; slightly affected its nutritional composition as well as improved its sensory characteristics.

Keywords: Pickled, Preservatives, Sensory, African walnut, Nutritional

1.0 INTRODUCTION

Walnut (*Tetracarpidium conophorum*) is a small flowering plant which produces a popular nut. It is commonly known as African walnut because of its African origin [1, 2]. African walnut grows in parts of Nigeria and Cameroon. It belongs to the family Eupohorbiacea [3]. In Southern Nigeria where it grows abundantly, the Igbos, Yorubas and Edos know it by the name "*ukpa*", "*asala*", "*okhue* or *okwe*", respectively. The Efiks and Ibibios call African walnut "*ekporo*" in their native language [3, 4].

Tetracarpidium conophorum is a dry nut enclosed between two semi-spherical hard shell attached to each other. The fruit has the shape of a brain [5, 6]. The African walnut is enclosed in a pod which accommodates one, two or three shelled nuts. The colour of walnut shell is either black or brown. After the shell is cracked, the whitish nut which has thin layer between two halves becomes visible [1].

African black walnut can be cooked and eaten, roasted or deep fried with the shell. It can also be dried and the roasted seed ground like melon seeds and used as soup thickener [7, 8]. African black walnut is a source of income for many rural dwellers [8]. The seeds, leaves and roots of African walnut have application in herbal

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medicine because it contains bioactive compounds such as oxalates, phytate, tannins, saponins and alkaloids [4]. After eating walnuts, a bitter aftertaste is felt while drinking water. This could be attributed to alkaloids present in the walnuts [3, 9]. Studies have shown that African walnuts are rich in protein, carbohydrate, vitamins, minerals, fats and oil [1]. Ros [10] listed a lot of health benefits that could be derived from consumption of nuts. Poly-unsaturated fat in the seeds could reduce body cholesterol while African walnut extracts rich in dietary omega-3-fatty is helpful in preventing depression and dementia in humans [11, 12].

Pickling is a method of preserving food in an edible antimicrobial liquid such as natural salt, vinegar (acetic acid) or oil. It can be broadly categorized as chemical and fermentation pickling [13]. Food subjected to pickling is known as pickle. It is heat-treated in order to achieve long-term preservation. Spices and sugar are usually added to improve the flavour of pickles. Sometimes, preservatives such as lactic acid, benzoate and sorbate are also added to enhance microbial safety of pickle [14, 15].

Cooked African walnuts have a short shelf life which is between 1-2 days [16]. Therefore, [17] subjected African walnut to different preservation methods. To the best of our knowledge, there has not been any reported study on pickled African walnut. Therefore, this study is aimed at determining the effect of lactic and citric acid used as preservative on the proximate composition, physicochemical property, antinutritional factors and sensory characteristics of pickled African Walnut.

2.0 Materials and Methods

Freshly matured raw African black walnuts shown in Plate 1 were purchased using sterile plastic bags from Eke Akpara market in Aba, Abia State and were immediately transported to Food and Industrial Microbiology Laboratory, University of Port Harcourt for analysis.

Plate 1 Fresh raw African walnut

2.1.1 Preparation of raw African walnut

The method described by [9, 18] with slight modification was adopted in preparing raw African walnut. Freshly matured raw African black walnut was dehusked, washed with distilled water and then cooked for 1 hr followed by sieving and shelling.

2.1.2 Preparation of pickling brine solution

The method described by Sultana *et al.,* [19] with slight modification was adopted in preparation of pickling brine solution. Five gram (5 g) NaCl mixed with 100 ml distilled water was separately poured into seven clean glass jars.

2.1.3 Preparation of pickle

Pickling process described by Ibrahim et al. [20] with slight modification was adopted. One hundred gram (100 g) of cooked African walnut was separately transferred into three labeled glass jars containing 100 ml brine solution followed by addition of 1 % (v/v), 3 % (v/v), and 5 % (v/v) citric acid. The glass jars were covered with a lid followed by gentle shaking with two hands and then sterilized at 121 ^{o}C for 15 min. Similarly, 100 g of cooked African walnut was separately poured into another set of three labeled glass jars containing 100 ml brine

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solution. Gently the three glass jars was shaken and then sterilized (121 °C for 15 min.) followed by addition of 1 % (v/v), 3 % (v/v) and 5 % (v/v) lactic acid. The glass jar containing 100 g cooked African walnut and 100 ml brine solution which has been sterilized without addition of lactic or citric acid is the control. Each of the glass jars containing pickled African walnut was stored at room temperature (28±2 °C) for a period of 6 Weeks.

2.2 Proximate analysis

AOAC [21] method was used to determine the protein, fat, moisture, crude fibre and ash content of freshly cooked African walnut as well as pickled African walnut preserved with citric and lactic acid after 6 Weeks storage period. Carbohydrate content of the samples was determined by difference method.

2.2.1 Determination of Protein Content (Kjeldahl method)

Two grams (2 g) sample was weighed into a boiling flask and 2.5 ml concentrated H_2SO_4 was added to the flask followed by two tablets of kjeldahl tablet. The flask was set on a heating mantle in a fume cupboard to boil until a clear solution was observed which marks complete digestion. The content of the flask was transferred into another 500 ml boiling flask followed by addition of 75 ml of 50 % NaOH and 300 ml of distilled water. Thereafter, the content of the flask was set to boil using quick fit apparatus with a condenser connected to a conical flask containing 50 ml of 2 % boric acid and 10 drops of methyl red. The distillate (Ammonia gas-NH3) was collected into the conical flask until 15 ml mark was attained. The distillate was titrated against 0.5 ml HCl until a sharp change in colour from pale yellow to deep orange or slight pink was observed which marks the end point. Percentage (%) nitrogen is calculated using the formula:

$$\% \text{ Nitrogen} = \frac{14.0\,(Vs - Vb)\ x\ M\ of\ acid\ x\ 100}{Wt\ of\ Sample\ \ x\ 100}$$

Where:

V_s = volume of acid used
V_b = volume of blank (acid)

Therefore, % protein = % N x 6.25 (Where 6.25 is the conversion factor of protein)

2.2.2 Determination of fat content (Soxhlet fixation method)

The continuous solvent extraction method was adopted. Approximately 2.5 g of fine ground sample was weighed into a filter paper and then transferred into a thimble. The thimble was guarded with a cotton wool. A dry boiling flask containing 3-4 anti-bump granules was weighed and 200 ml petroleum ether was poured into the flask. The sample in the thimble was put inside the Soxhlet apparatus and then mounted on the boiling flask. The assembled Soxhlet apparatus was allowed to reflux for 1-2 hours on a thermal heater. As petroleum ether was boiling, the solvent refluxes into the thimble and extract the fat which flows through the thimble down to the boiling flask. The solvent was recovered and the flask free of solvent was dried in an air oven (vacuum oven) at 60 °C for 30 minutes after which it was allowed to cool inside a dessicator before it was reweighed. The fat content of the sample was calculated using the formula below.

$$\% \text{ Fat} = \frac{W2 - W1}{Wt\ of\ sample}\ X\ \frac{100}{1}$$

Where:

W_2 = Weight of flask + anti-bump granules + sample after extraction
W_1 = Weight of flask + anti-bump granules + sample before extraction

2.2.3 Determination of carbohydrate content

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The carbohydrate content of the samples was estimated by the difference method which is expressed as nitrogen free extract using the relationship below.

$$\% \text{ Carbohydrate} = 100 - \% (A + B + C + D + E)$$

Where: A, B, C, D and E represent percentage protein, fat, ash, crude fibre and moisture content, respectively.

2.2.4 Determination of moisture content

The moisture content of the sample was determined by weighing 10 g sample into a clean, dry pre-weighed Petri dish and then placed inside oven to dry at a constant temperature of 105 ^{o} C for 3 hours. On completion of drying, the sample was transferred to a dessicator to cool for 30 minutes after which the Petri dish and its content was reweighed and the moisture content was determined by comparing the weight lost.

$$\% \text{ Moisture content} = \frac{W3 - W1}{W2 - W1} \text{ X } \frac{100}{1}$$

Where:

W_1 = Weight of dish
W_2 = Weight of dish with sample before drying
W_3 = Weight of dish and sample after drying

2.2.5 Determination of crude fibre

Two grams (2 g) of the sample defatted with petroleum ether was weighed into a mounted 500 ml boiling flask followed by pouring 200 ml of 1.25 % H_2SO_4 into the flask and boiling chips added to the set up. The mixture was allowed to boil under reflux for 30 min after which the solution was filtered through 0.5µ porous filter paper and boiled water was used to wash off H_2S0_4. The residue was transferred to a boiling flask and allowed to boil for another 30 min. with 700 ml NaOH. Thereafter, the mixture was filtered using a pre-weighed Whatman filter paper. The residue obtained was washed with boiled distilled water in order to wash off NaOH. The next step was to carefully put the filter paper containing the residue inside oven and allow it to dry until a constant weight was achieved. The filter paper containing the residue was reweighed. The fibre content was calculated using the formula:

$$\% \text{ Crude fibre} = \frac{W2 - W1}{Wt\ of\ sample} \text{ X } \frac{100}{1}$$

Where:

W_1 = Weight of filter paper before drying;
W_2 = Weight of filter paper and sample after drying

2.2.6 Determination of ash content (Dry ash method using furnace)

Accurately, 3 g of the sample was weighed into a pre-weighed porcelain crucible. The crucible was put inside pre-heated furnace using crucible thong. The sample was ashed at 600 ^{o}C for 4 hours. Thereafter, the sample was carefully removed from the muffle furnace and kept inside a dessicator for 30 min in order to cool. The ashed sample inside the crucible was reweighed and ash content was calculated using the formula.

$$\% \text{ Ash content} = \frac{W2 - W3}{W2 - W1} \text{ X } \frac{100}{1}$$

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Where:

W_1 = Weight of empty crucible

W_2 = Weight of crucible plus sample before ashing

W_3 = Weight of crucible plus ashed sample

2.3 Antinutritional factors

The quantity of antinutrional factors namely tannin, flavonoids, alkaloids, saponin and terpenoid present in freshly cooked African walnut and pickled African walnut separately preserved with 1 % (v/v), 3 % (v/v), 5 % (v/v) citric acid as well as 1 % (v/v), 3 % (v/v), 5 % (v/v) lactic acid for 6 Weeks was determined using AOAC (2005) methods.

2.3.1 Determination of tannin (Folin-Denis method)

Accurately, 1.0 g of the sample was weighed into a conical flask and 10 ml distilled water was added. The solution was placed inside a mechanical shaker for 2-5 minutes after which it was allowed to stand at room temperature (28±2 ^{0}C) for 30 min. The mixture was filtered using a filter paper and the residue was washed with distilled water to bring the extract volume to 10 ml. One mililitre (1 ml) of the extract and 1 ml 0.05 % tannic acid were dispersed into separate test tubes after which 0.2 ml Folin Denis reagent was added to each of the test tube followed by 1 ml sodium tricarbonate (Na_2CO_3) solution. The content of each of the test tubes was made up to 10 ml mark using distilled water and then incubated at room temperature (28±2 ^{0}C) for 90 min. The absorbance of the solution in the test tubes was measured at 760 nm using a spectrophotometer. Tannin content was calculated using the formula.

$$\% \text{ Tannin} = \frac{Au}{As} \times \frac{100}{W} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

Where:

Au = Absorbance of test samples

As = Absorbance of standard tannic solution

C = Concentration of standard tannic solution in mg/ml

W = Weight of sample analyzed

V_f = Total extract volume

V_a = Volume of extract analyzed

D = Dilution factor where applicable

2.2.2 Determination of flavonoid (Harbone method)

Two grams (2 g) of powdered sample was accurately weighed and transferred into a test tube followed by addition of 50 ml of 0.1M HCl. The solution was boiled for 30 min and then allowed to cool before it was filtered using What man filter paper No. 10. A few drops of ethyl acetate extract which contained the flavonoids were recovered while the aqueous layer was discarded. A pre-weighed Whatman filter paper No. 40 was used to filter the second extract and the residue was then placed in an oven to dry at 60 ^{o}C after which the weight was measured. The quantity of flavonoids was determined using the formula.

$$\% \text{ Flavonoid} = \frac{W2-W1}{Wt\ of\ sample} \times \frac{100}{1}$$

Where:

W_1 = Weight of empty filter paper

W_2 = Weight of paper + flavonoids i.e. extract (residue)

2.2.3 Determination of alkaloid (Alkaline precipitation gravimetric method)

Two grams (2 g) dry sample was weighed and soaked in 20 ml of 10 % ethanol acetic acid. The mixture was allowed to stand for 4 hr at room temperature (28±2 ^{0}C) after which it was filtered using Whatman filter paper No. 40. The filtrate was concentrated by evaporation over a steam to ¼ of its original volume. To the precipitate

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of the alkaloids, concentrated ammonia solution was added in drops to the extract until it was in excess. Thereafter, the resulting alkaloid precipitate was washed with 1 % ammonium solution and dried in the oven at 60 °C for 30 min., cooled in a dessicator and weighed. The weight of the alkaloid was determined by difference and expressed as

$$\% \text{ Alkaloid} = \frac{W2-W1}{Wt\ of\ Sample} \times \frac{100}{1}$$

Where: W_1 = Weight of filter paper
W_2 = Weight of paper + alkaloid precipitate

2.2.4 Determination of saponin (Double extract gravimetric method)

Two grams (2 g) of powdered sample was accurately weighed and mixed with 50 ml of 20 % aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 30 min at 55 °C and then passed throuhgh a filter paper. The residue was extracted with 50 ml of 20 % ethanol. The volume of extracts were reduced to about 40 ml at 90 °C and then transferred to a separating funnel followed by addition of 40 ml diethyl ether and shaken vigorously. Separation was by partition during which ether layer was discarded and the aqueous layer collected. Re-extraction partition was repeated until the aqueous layer becomes clear. Saponin was extracted with 10 ml normal butanol and the combined extracts were washed with 5 % aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. The saponin content was determined using the formula.

$$\% \text{ Saponin} = \frac{W2 - W1}{Wt\ of\ sample} \times \frac{100}{1}$$

Where:
W_1 = Weight of empty evaporation dish
W_2 = Weight of dish + Saponin extract

2.2.1.1 Sensory evaluation of freshly cooked and African walnut pickle

Ten untrained sensory panelists which comprise staff and graduate students in the Department of Microbiology, University of Port Harcourt were given freshly cooked African walnut and pickled African walnut containing citric and lactic acid as preservatives after 6 Weeks shelf life study to evaluate their sensory characteristics using the method described by [17, 22] with slight modifications. The sensory panelists were instructed to taste the coded samples without rinsing their mouth and also do same after rinsing their mouth with potable water. Consequently, they were asked to rate the coded samples based on aroma, taste, mouth feel, colour and overall acceptability using a 9-point hedonic scale which starts from (9) liked extremely to (1) dislike extremely.

2.4 Statistical analysis

The data obtained were subjected to statistical analysis using one-way ANOVA with the help of statistical package for social sciences IBM statistical version 22.0 (SPSS.22) by Java Technologies, United States. Statistical differences were calculated using p-value < 0.05 while the graphical presentation were produced using Microsoft Excel 2013.

3.0 Results and Discussion

African walnuts like other nuts are rich in nutrients. However, they also contain some quantity of antinutrients. Different processing and preservation methods could influence the level of nutrients and antinutrients present in

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African walnut as well as its sensory characteristics. Plate 2 shows the photograph of pickled African walnut preserved with different concentrations of citric and lactic acid.

Plate 2 The developed pickled African walnut

3.1 Proximate composition

The result obtained from this study revealed that freshly cooked African walnut contains 30.03 % moisture, 24. 31 % protein, 25.63 % fat, 15.38 % carbohydrate, 1.15 % fibre and 3.50 % ash. Okonkwo and Ozoude [23] as well as [24] reported similar results from their respective studies. Hosain *et al.* [25] and [26] in their separate studies stated that processing of food affects its nutritional composition.

Table 1 shows that freshly cooked African walnut had a lower moisture content compared with pickled African walnut separately preserved with lactic and citric acid for 6 Weeks. Increase in moisture content of pickled African walnut during the period of storage is an indication that the shelf life of the product was reducing with time. According to [27], the moisture content of raw African walnut is 4.30 %. Based on the results obtained from this study, boiling of raw African walnut might have contributed enormously to increase the moisture content of pickled African walnut above other nuts such as hazelnuts and pine nuts. Generally, products that have high moisture content usually have short shelf life [28].

Ash content is often considered as an index of minerals present in a biological mass. According to [27], ash content of raw African walnut is 7.0 %. Meanwhile, the ash content of freshly cooked African walnut (sample FCAW) is 3.50 %. The difference in ash content could be as a result of leaching of some minerals while African walnut was undergoing hydrodermal processing. Arinola and Adesina [29] reported that 3.32 % ash content of raw African walnut reduced to 2.77 % and 2.16 % in African walnut boiled with the shell and that without the shell, respectively. The ash content of pickled African walnut samples preserved with either citric or lactic acid ranged between 2.88 - 3.22 %.

The protein content (25.20 %) of pickled African walnut preserved with 5 % NaCl + 1 % citric acid is significantly different from that of other pickled samples including the freshly cooked African walnut which ranged between 24.12-24.31 %. In terms of proximate composition, this study have shown that protein content of African walnut is least affected by the processing and preservation methods they were subjected to. Similar studies by [29] reported that protein content of boiled African walnut together with the shell and the one without the shell is 24.13 % and 22. 47 %, respectively. The protein content of freshly cooked and that of pickled African walnut separately preserved with citric and lactic acid is comparable with other nuts such as almonds, pistachios and peanuts [28]. Protein is an important structural component of body tissues and muscle. It also helps in production of hormones, enzymes and haemoglobin. One interesting thing about human consumption of high quantity of plant protein is because it reduces the risk of overweight, obesity and certain chronic diseases unlike animal protein [30]. However, [31] cautioned against high dependent on plant protein for human body needs because it is deficient in many essential amino acids.

Table 1 show that fat content of freshly cooked African walnut is lower than that of pickled African walnut samples after 6 Weeks of storage. The fat content of freshly cooked African walnut is 25.63 % but that of pickled African walnut preserved with either lactic acid or citric acid ranged between 28.30 - 29.24 %. This result trend is in agreement with a related study carried out by [29]. However, the fat content reported by [29] is

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higher than the results obtained from this study. The differences in fat content could be as a result of African walnut variety and geographical location where it was planted.

Considering the proximate composition of both freshly cooked and pickled African walnut samples, the numerical value for crude fibre content of each of the samples is lower than that of crude protein, fat, fibre, moisture and carbohydrate content. The crude fibre content of the samples evaluated in this study range between 0.9 - 1.21 %. This should be of concern because regular intake of high dietary fibre has the potential to reduce serum cholesterol level, risk of coronary heart diseases, rate at which glucose is released into blood stream, improve digestibility, assist bowel movement as well as prevent development of bowel and breast cancer [29, 32].

Udedi et al. [32] reported that total carbohydrate content of cooked African walnut is 15.90 %. This is in agreement with the result obtained from this study. However, there was significant reduction in carbohydrate content of pickled African walnut samples which ranged between 10.08 % -10.49 %. In a related study that involved determination of the effect of pickling on carbohydrate content of five selected fruits, [33] reported that pickling reduced the carbohydrate content of the fruits. The reduction in carbohydrate content could be as a result of activities of microorganisms which metabolized carbohydrate as source of nutrients and energy. A similar result reported by [34] was attributed to Maillard reaction which involves non-enzymatic browning of carbohydrates (reducing sugars).

Test parameter	FCAW	PAWFF	PAWFE	PAWFD	PAWFC	PAWFB	PAWFA
Moisture	30.03±0.82^{a}	33.33±0.16^{d}	33.13±0.13^{d}	33.12±0.06^{d}	32.43±0.10^{c}	33.21±0.05^{d}	31.55±0.06^{b}
Ash	3.50±0.42^{c}	2.88±0.09^{a}	3.08±0.05^{ab}	3.08±0.07^{ab}	3.22±0.08^{bc}	3.22±0.05^{bc}	2.94±0.05^{ab}
Crude fat	25.63±0.46^{a}	28.30±0.14^{b}	28.40±0.07^{b}	29.20±0.04^{c}	29.11±0.10^{c}	28.45±0.06^{b}	29.24±0.05^{c}
Crude protein	24.31±0.25^{a}	24.24±0.11^{a}	24.20±0.05^{a}	24.12±0.08^{a}	24.15±0.11^{a}	24.26±0.06^{a}	25.20±0.07^{b}
Crude Fibre	1.15±0.08^{bc}	1.13±0.05^{bc}	1.12±0.03^{bc}	0.9±0.04^{a}	1.10±0.06^{b}	1.21±0.05^{c}	1.07±0.06^{b}
Carbohydrate	15.38±0.08^{c}	10.49±0.32^{b}	10.39±0.19^{b}	10.08±0.05^{a}	10.25±0.06^{ab}	10.09±0.05^{a}	10.08±0.05^{a}

Table 1 Proximate composition (%) of freshly cooked and pickled African walnut samples

Mean Standard Error (MSE), using Least Significant Difference (LSD); p-value < 0.05. The alphabets indicate the points of relatedness of the data tested. The Figures with same alphabets suggest that both data is related. The difference in the data suggests points of statistical significance. PAWFA represent 100 g of African walnut + 5 % NaCl + 1 % citric acid; PAWFB represent 100 g of African walnut + 5 % NaCl + 3 % citric acid; PAWFC represent 100 g of African walnut + 5 % NaCl + 5 % citric acid; PAWFD represent 100 g of African walnut + 5 % NaCl + 1 % Lactic acid; PAWFE represent 100 g of African walnut + 5% NaCl + 3% Lactic acid; PAWFF represent 100 g of African walnut + 5 % NaCl + 5 % Lactic acid; FCAW represent freshly cooked African walnut.

3.2 Antinutritional factors

Generally, antinutrients are linked with reduction in nutrient utilization, and or food intake [35]. It is important to emphasize that antinutrients have numerous potential health benefits as well as adverse effects [36]. This study revealed that per 100 g freshly cooked African walnut, its antinutritional factors are: tannin (1.89mg), saponin (8.10mg), alkaloid (3.19mg) and flavonoid (0.92mg). The level of antinutrients present in freshly cooked African walnut is in agreement with the results reported by [17] from a similar study.

Results presented in Table 2 shows that pickled African walnut samples had significantly lower (P> 0.05) tannin, flavonoids, saponin and alkaloid content compared with that of freshly cooked African walnut. This result suggests that pickling process decreased the quantity of antinutritional factors present in pickled African

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walnut samples. Reduction in antinutritional factors by processing methods such as moist heat treatment (boiling) was reported by [17]. According to [24], processing methods such as toasting, roasting and cooking is capable of reducing antinutritional factors present in food. This observation is in agreement with [37] which stated that soaking food materials in water and salt solutions that contains additives or not encourages loss in antinutritional factors especially in legume seeds. Other physical processing methods that could also reduce quantity of antinutrients are autoclaving/pressure cooking/steaming, blanching, ordinary cooking, extrusion, roasting and the use of processing chemicals [37].

Studies have shown that flavonoids possess antioxidative property and free radical scavenging property. According to [38], flavonoids could prevent coronary heart disease and lower the risk of cancer development. Considering the antinutrients in freshly cooked as well as pickled African walnut evaluated in this study, flavonoid content has the lowest numerical values which range between 0 - 0.89 %. Table 2 shows that flavonoids content of freshly cooked African walnut is higher than that of pickled African walnut samples. Reduction in flavonoids content could be as a result of pickling of the samples which were stored for a period of 6 Weeks.

Contrary to the flavonoids content in the African walnut samples, results presented in Table 2 clearly shows that saponin content of freshly cooked and pickled African walnut samples is higher than other antinutrients evaluated in this study. This result trend is in agreement with the findings of [39]. Several studies have linked saponin content in food materials to numerous health benefits [16].

Alkaloids present in plants have therapeutic uses because it possesses antimicrobial and antibacterial properties. It can also be used as an analgesic [38]. Results from this study shows that alkaloids is present in pickled African walnut preserved with lactic acid but was not detected in pickled African walnut preserved with citric acid except the sample preserved with 1 % citric acid which has 0.93 mg of alkaloids. It could be that citric acid had more significant effect in reducing the level of alkaloids in pickled African walnuts than lactic acid after 6 Weeks of storage.

Table 2 shows that there is no significant difference in pickled African walnut preserved with citric acid unlike that which was preserved with lactic acid in terms of tannin content. Protein bioavailability could be reduced as a result of insoluble complexes formed by tannins and protein. The result obtained from this study is in agreement with a similar study by [33]. Their study revealed that there was reduction in tannin content of five selected fruits that were subjected to pickling. The presence of tannin in African walnut supports its use in herbal medicine as well as healing haemorrhoids, frost bite and varicose ulcers [1].

Table 2 Antinutrient composition (mg) of freshly cooked and pickled African walnut samples

Parameter	FCAW	PAWFF	PAWFE	PAWFD	PAWFC	PAWFB	PAWFA
Tannin	1.89±0.04^{c}	1.06±0.05^{a}	1.25±0.05^{b}	1.25±0.07^{b}	1.85±0.03^{c}	1.83±0.04^{c}	1.88±0.04^{c}
Saponin	8.11±0.04^{e}	8.08±0.04^{e}	6.84±0.04^{d}	2.16±0.02^{c}	0	0	0.85±0.04^{b}
Alkaloids	3.21±0.04^{d}	2.12±0.03^{c}	3.17±0.03^{d}	0.90±0.04^{b}	0	0	0.93±0.03^{b}

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Flavonoids	0.89±0.04[c]	0.85±0.02[bc]	0.84±0.03[b]	0.85±0.01[bc]	0.85±0.02[bc]	0.87±0.03[bc]	0

Mean Standard Error (MSE), using Least Significant Difference (LSD); p-value < 0.05. The alphabets indicate the points of relatedness of the data tested. Values with same alphabets across the row are not significantly different but with different alphabets are significantly different. PAWFA represent 100 g of African walnut + 5 % NaCl + 1 % citric acid; PAWFB represent 100 g of African walnut + 5 % NaCl + 3 % citric acid; PAWFC represent 100 g of African walnut + 5 % NaCl + 5 % citric acid; PAWFD represent 100 g of African walnut + 5 % NaCl + 1 % lactic acid; PAWFE represent 100 g of African walnut + 5 % NaCl + 3 % lactic acid; PAWFF represent 100 g of African walnut + 5 % NaCl + 5 % lactic acid; FCAW represent freshly cooked African walnut.

3.3 pH

Figure 1 shows that pH of freshly cooked African walnut steadily increased from 5.6 – 7.0 during the period of storage. The results obtained from this study further indicate that pH of pickled African walnut without any organic acid added is higher than that of pickled African walnut preserved with different concentrations of citric and lactic acids. Lower pH of pickled African walnut could be as a result of organic acids added to the pickling brine. Usually, acidic food has an equilibrium pH of 4.6 or below [40]. Decrease in pH during storage of pickled African walnut was reported in a similar study carried out by [41, 42, 43]. Ibrahim *et al,* [20] stated that highly acidic foods with a pH below 4.6 are safe for consumption because most pathogens cannot survive on food materials that have very low pH. According to United States Code of Federal Regulations (21 CFR Part 114), the pH of food products should be maintained at or below 4.6 using acid or acid ingredients especially acetic acid (vinegar). To further prevent growth of pathogenic microorganisms in such products, heat treatment must be part of the process [44]. Based on the above food regulatory standard, pickled African walnut preserved with lactic or citric acid could be considered safe for human consumption.

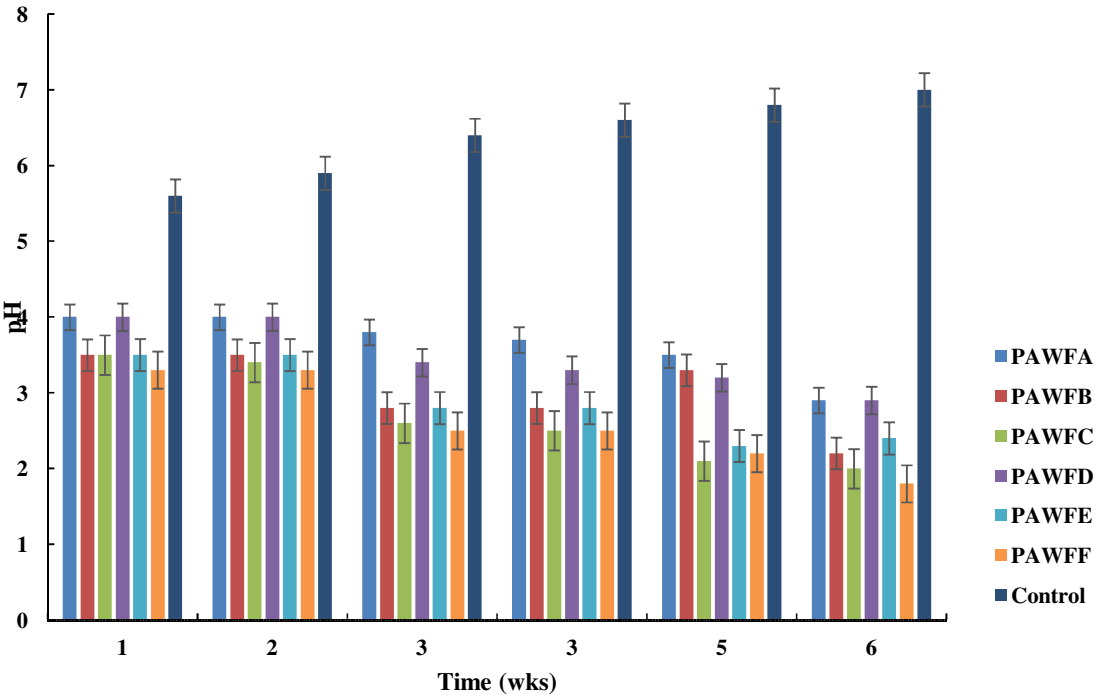

Fig.1 pH of pickled African walnut preparations stored for six weeks

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PAWFA represent 100 g of African walnut + 5 % NaCl + 1 % citric acid; PAWFB represent 100 g of African walnut + 5 % NaCl + 3 % citric acid; PAWFC represent 100 g of African walnut + 5 % NaCl + 5 % citric acid; PAWFD represent 100 g of African walnut + 5 % NaCl + 1 % lactic acid; PAWFE represent 100 g of African walnut + 5% NaCl + 3% lactic acid; PAWFF represent 100 g of African walnut + 5 % NaCl + 5 % lactic acid; Control represent pickled African walnut without preservative.

3.4 Sensory evaluation

Sensory evaluation of pickled African walnut preserved with lactic and citric acid revealed that the most and least preferred sample is the one preserved with 5 % lactic acid and 1 % citric acid, respectively. Table 3 further revealed that taste of the pickles preserved with lactic acid after the samples were rinsed with potable water were preferable than similar samples tasted without being rinsed with potable water. However, it was the reverse in the case of African walnut pickle preserved with citric acid. This study further revealed that African walnut pickle preserved with lactic acid was assigned higher sensory score than the ones preserved with citric acid. Previous studies have shown that lactic acid and its salts are capable of improving sensory characteristics of 'naan' [45]. In a related study, [41] reported that sensory characteristics of seas date (*Phownix dactylifera* L.) pickle that underwent pickling process using brine mixed with organic acid recorded higher score in sensory evaluation than seas pickles prepared without addition of organic acid. Therefore, addition of organic acid to African walnut pickle contributed in the improvement of its sensory characteristics. However, the control was very much disliked based on sensory evaluation score. Absence of organic acid in the control might have favoured growth of spoilage microorganisms which contributed in poor sensory attributes of the product.

Table 3: Sensory evaluation of freshly cooked and African walnut pickles

Sample Code	Taste (R)	Taste (NR)	Aroma	Mouth feel	Colour	Overall acceptability
PAWFF	8.1±0.88^{e}	8.0±0.67^{f}	8.2±0.63^{e}	8.1±0.88^{e}	8.9±0.32^{e}	9.0±0.0^{f}
PAWFE	7.2±1.14^{d}	7.1±1.14^{e}	8.2±0.79^{e}	7.4±0.97^{e}	9.0±0.0^{e}	8.2±0.92^{e}
PAWFD	7.1±0.99^{d}	6.3±0.95^{e}	7.0±1.15^{d}	5.4±1.07^{d}	7.2±1.03^{d}	7.3±1.06^{d}
PAWFC	4.3±0.95^{c}	5.3±1.42^{d}	5.6±0.97^{c}	4.3±0.82^{c}	5.4±0.97^{c}	5.0±0.94^{c}
PAWFB	3.1±0.99^{b}	4.1±0.88^{c}	4.1±0.99^{b}	3.3±0.82^{b}	5.0±0.82^{c}	4.1±0.88^{b}
PAWFA	2.1±0.99^{a}	2.2±1.14^{b}	1.4±0.52^{a}	2.3±0.67^{a}	3.1±1.10^{b}	2.1±0.88^{a}
Control	1.3±0.48^{a}	1.1±0.44^{a}	1.4±0.52^{a}	2.1±0.74^{a}	1.2±0.42^{a}	2.4±0.70^{a}

R- Rinsed; NR-Not rinsed; Mean Standard Error (MSE), using Least Significant Difference (LSD); p-value < 0.05. The alphabets indicate the points of relatedness of the data tested. Values with same alphabets across the row are not significantly different but with different alphabets are significantly different. PAWFA represent 100 g of African walnut + 5 % NaCl + 1 % citric acid; PAWFB represent 100 g of African walnut + 5 % NaCl + 3 % citric acid; PAWFC represent 100 g of African walnut + 5 % NaCl + 5 % citric acid; PAWFD represent 100 g of African walnut + 5 % NaCl + 1 % lactic acid; PAWFE represent 100 g of African walnut + 5 % NaCl + 3 % lactic acid; PAWFF represent 100 g of African walnut + 5 % NaCl + 5 % lactic acid; Control represent freshly cooked African walnut.

Liking Score		**Panelist Hedonic Rating**
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	-	DislikeVery Much

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1 - Dislike Extremely

Conclusion

The preservatives added to pickled African walnuts reduced the pH of the samples during storage. Therefore, the product met the requirement of U.S. Code of Federal Regulations (21 CFR Part 114). Interestingly, the level of antinutrients in the pickled samples with added preservatives is lower than that of freshly cooked African walnut. However, addition of citric acid as a preservative demonstrated more effectiveness than lactic acid in reducing level of antinutrients in the pickled samples. The moisture and fat content of freshly cooked African walnuts is lower than that of pickled samples but it was the reverse in terms of ash, crude fibre and carbohydrate content. Remarkably, protein content of pickled African walnut was least affected by pickling process and separate addition of two organic acids used in this study as preservatives. Based on sensory evaluation of the pickled samples, that which was preserved with 5 % lactic acid was the most preferred product. Importantly, potable water used to rinse pickled African walnut preserved with lactic acid gives it a preferable taste than similar samples that were not rinsed with potable water. On the contrary, the taste of pickled African walnut preserved with citric acid without rinsing the pickle with potable water gives it a better taste than similar samples rinsed with potable water. Based on the results obtained from this study, pickled African walnut preserved with lactic and citric acid is safe, nutritious and well acceptable to consumers.

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