European Journal of Nutrition & Food Safety

9(1): 72-83, 2019; Article no.EJNFS.2019.015
ISSN: 2347-5641

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

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IA and NNO designed and supervised the study. Author NM performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author VCE managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2019/45065

Original Research Article

Received 19 September 2018
Accepted 27 November 2018
Published 01 February 2019

ABSTRACT

This study was carried out between July-September, 2017 at Food and Industrial Microbiology Laboratory, University of Port Harcourt, Port Harcourt Nigeria. 1 %, 3 % and 5 % citric acid were separately added to brine solution containing pickled African walnut inside three glass jars, and sterilised. Similarly, 1 %, 3 % and 5 % lactic acid was added to already sterilised pickled African walnut mixed with brine solution. Pickled African walnut without preservative was the control sample. The entire setup was stored for 6 Wks at room temperature (28±2°C). At 1 Wk interval, pH of the samples was monitored. Proximate composition and antinutrients in freshly cooked African walnut (FCAW) and pickle from each glass jar were determined using standard methods after 6 Wks storage period and sensory characteristics by 9 point Hedonic scale. The antinutrients, fibre, ash and carbohydrate content of FCAW were higher than that of pickled samples after 6 weeks storage period. The protein content of the pickles ranged between 24.12-25.20 %, ash 2.88-3.22 %, moisture 31.55-33.33 %, fat 28.30-29.24 %, fibre 0.90-1.21 % and carbohydrate 10.08-10.49 %. All the parameters evaluated showed significant differences (P = .05) among the samples. Very strong correlation exist between the proximate composition of the pickles preserved with same concentration of citric and lactic acid but level of antinutrients and sensory evaluation scores of the pickles exhibited weaker correlations with few exceptions. During storage, pH of FCAW steadily increased from 5.6-7.0 but that of pickled samples which ranged between1.8-4.0 decreased. The pickles met U.S. Code of Federal Regulations (21 CFR Part 114) which stipulate that acetic acid added to food products must maintain its pH at 4.6 or below. Sensory evaluation revealed that

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pickled African walnut preserved with 5 % lactic acid was most preferred. Pickled African walnut preserved with citric and lactic acid is well acceptable to consumers. The preservatives slightly affected its nutritional composition, reduced its pH and level of antinutrients.

Keywords: Pickled; preservatives; sensory; African walnut; nutritional.

1. INTRODUCTION

Walnut (*Tetracarpidium conophorum*) is a small flowering plant which produces a popular nut 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].

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 [5,6]. African black walnut is a source of income for many rural dwellers [6]. The seeds, leaves and roots of African walnut have application in herbal 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 [3,7]. The nutritional composition of African walnuts has been determined by several researchers [1,8,9]. Studies by Igara et al. [10] reported that the nuts contain 9.64 mg/100 g vitamin A, 0.04 mg/100 g vitamin B_1, 0.08 mg/100 g vitamin B_2, 0.02 mg/100 g vitamin B_3, 6.98 mg/100 g vitamin C and 96.42 mg/100 g vitamin E. According to Ros [11], nuts which include African walnut could be described as nutrient dense foods associated with health benefits. 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 [12,13].

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 categorised as chemical and fermentation pickling [14]. Any food subjected to pickling process is known as a pickle. It is heat-treated in order to achieve long-term preservation. Sometimes, preservatives such as lactic acid, benzoate and sorbate are also added to enhance microbial safety of pickle. Spices and sugar are usually added to improve the flavour of pickles [15,16].

Cooked African walnuts have a short shelf life which is between 1-2 days [17]. Therefore, Ekwe and Ihemeje [18] 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. MATERIALS AND METHODS

Fresh matured raw African black walnuts 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.

2.1 Preparation of Raw African Walnut

The method described by Arinola and Adesina [7] and Inyang et al. [19] 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 h followed by sieving and shelling.

2.2 Preparation of Pickling Brine Solution

The method described by Sultana et al. [20] 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.

2.3 Preparation of Pickle

Pickling process described by Ibrahim et al. [21] with slight modification was adopted. One hundred gram (100 g) of cooked African walnut which lasted for 1 h was separately transferred into three labeled glass jars containing 100 ml brine solution followed by addition of 1 % (w/v), 3 % (w/v), and 5 % (w/v) citric acid. The glass jars were covered with a lid followed by gentle shaking with two hands and then sterilised 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 solution. Gently, the three glass jars were shaken and then sterilised (121°C for 15 min.) followed by addition of 1 % (v/v), 3 % (v/v) and 5 % (v/v) lactic acid separately into the glass jars. The glass jar containing 100 g cooked African walnut and 100 ml brine solution which had been sterilised 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.

2.4 Proximate Analysis

AOAC 2005 method [22] 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.

2.5 Antinutritional Factors

The quantity of antinutritional factors namely tannin, flavonoids, alkaloids, saponin and terpenoid present in freshly cooked African walnut and pickled African walnut separately preserved with 1 % (w/v), 3 % (w/v), 5 % (w/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.

2.6 Sensory Evaluation of Freshly Cooked and African Walnut Pickle

Ten untrained sensory panelists were selected. Five of the panelist ages between 35-50 years were staff and the remaining panelist ages between 25-35 years were graduate students in the Department of Microbiology, University of Port Harcourt. The panelists were given pickled African walnut samples containing citric and lactic acid as preservatives after 6 Weeks shelf life study to evaluate their sensory characteristics using the method described by Ekwe and Ihemeje [18] and Odu et al. [23] with slight modifications. The sensory panelists were instructed to taste each of the coded samples without rinsing it with potable water and also do same after rinsing it with potable water. Also, each of the panelists was instructed to rinse their mouth with potable water before tasting every sample. 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.

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	-	Dislike Very Much
1	-	Dislike Extremely

2.7 Statistical Analysis

The data obtained were subjected to statistical analysis using One-Way ANOVA and partial correlation with the help of statistical package for social sciences IBM statistical version 22.0 (SPSS.22) by Java Technologies, United States. A probability value at $p < .05$ was considered as being statistically different using Duncan multiple range test.

3. RESULTS AND DISCUSSION

The proximate composition and antinutrients present before and after freshly cooked African walnut samples were subjected to pickling process that involved separate addition of different concentrations (1 %, 3 % and 5 %) of citric and (1 %, 3 % and 5 %) lactic acid which were stored for 6 Weeks at room temperature is discussed in the following subsections. Similarly, pH of the formulations monitored within the storage period is also discussed. Finally, result from sensory evaluation of the pickles stored for 6 Weeks is discussed.

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 [24] as well as Nwosu et al. [25] reported similar results from their respective studies. Hosain et al. [26] and Canan [27] in their separate studies stated that processing of food affects its nutritional composition. This is evidenced by changes in proximate composition of pickled African walnut samples when compared with that of freshly cooked African walnut.

Table 1 shows that freshly cooked African walnut had a lower moisture content compared with pickled African walnut samples separately preserved by lactic and citric acid for 6 Weeks. According to Apeh et al. [28], the moisture content of raw and cooked walnut seeds is 40.11 % and 46.52%, respectively. It could be that cooking process increased the moisture content of raw African walnut above other nuts such as hazelnuts and pine nuts. This study has shown that pickled African walnut samples after 6 Wks storage had higher moisture content which range between 31.55-33.33 % when compared with 30.03 % moisture content of freshly cooked African walnut. Generally, edible products that have high moisture content usually have short shelf life [29].

Ash content is often considered as an index of minerals present in a biological mass. Therefore, 7.0 % ash content of raw African walnut is an indication that it contains reasonable quantity of minerals [30]. Meanwhile, 3.50 % ash content of freshly cooked African walnut indicates lower quantity of minerals than the raw sample. The difference in ash content could be as a result of leaching of some minerals such as sodium, iron, manganese, zinc and phosphorus while African walnut was undergoing hydrodermal processing [31]. Arinola and Adesina [32] 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. In this study, the ash content of freshly cooked African walnut is higher than that of pickled African walnut samples preserved with either citric or lactic acid for 6 Wks which ranged between 2.88-3.22 %. This could also be as a result of leaching of some minerals during storage of the pickles.

The protein content (25.20 %) of pickled African walnut preserved with 1 % citric acid is slightly higher than that of other pickled samples including the freshly cooked African walnut which ranged between 24.12-24.31 %. Despite the slight differences in protein content, it can be generalised that pickling process which involved addition of organic acid did not cause remarkable changes in the crude protein content of pickled African walnut samples after 6 Wks of storage. Arinola and Adesina [32] 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 [31]. 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 [33]. However, Krajcovicova-Kudlackova et al. [34] cautioned against high dependence 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 Arinola and Adesina [32]. However, the fat content reported by Arinola and Adesina [32] was 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 [32,35].

Udedi et al. [35] 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 stored for 6 Wks 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, Saranya and Rajani [36] 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 metabolised carbohydrate as source of nutrients and energy. A similar result reported by Djikeng et al. [37] was attributed to Maillard reaction which involves non-enzymatic browning of carbohydrates (reducing sugars).

3.2 Correlating Proximate Composition of the Pickles Preserved with Same Concentration of Lactic and Citric Acid

A partial correlation to determine the relationship between proximate composition of the pickles preserved with same concentration of citric acid and lactic acid while controlling for sample (FCAW) revealed that there was a very strong, positive partial correlation, which was statistically significant, between pickled African walnut preserved with 5 % lactic acid and that which was preserved with 5 % citric acid because $r(15) = 0.977$, $p = 0.000$. Similarly, partial correlation between pickled African walnut preserved with 3 % lactic acid and that which was preserved with 3 % citric acid showed that $r(15) = 0.999$, $p = 0.000$ whereas that of pickled African walnut preserved with 1 % lactic acid and that which was preserved with 1 % citric acid showed that $r(15) = 0.957$, $p = 0.000$.

However, zero-order correlations showed that there was a statistically significant, very strong, and positive correlation between proximate composition of the pickle preserved with 5 % lactic acid and that which was preserved with 5 % citric acid because ($r(16) = 0.999$, $p < 0.000$) whereas that between the pickles preserved with 1 % citric acid and the one preserved with 1 % lactic acid showed that ($r(16) = 0.998$, $p < 0.000$). Meanwhile, it was a perfect, statistically significant, positive correlation between the proximate composition of the pickles preserved with 3 % lactic acid and that which was preserved with 3 % lactic acid because ($r(16) = 1.000$, $p < 0.000$). Therefore, the control sample (FCAW) had very low influence in controlling for the relationship between the proximate composition of the pickles preserved with 1 % citric and 1 % lactic acid; 3 % citric and 3 % lactic acid; 5 % citric and 5 % lactic acid.

3.3 Antinutritional Factors

Generally, antinutrients are linked with reduction in nutrient utilisation, and or food intake [38]. It is important to emphasise that antinutrients have numerous potential health benefits as well as adverse effects [31]. This study revealed that per 100 g freshly cooked African walnut, its antinutritional factors are: tannin (1.89 mg), saponin (8.10 mg), alkaloid (3.19 mg) and flavonoid (0.92 mg). The level of antinutrients present in freshly cooked African walnut is in agreement with the results reported by Ekwe and Ihemeje [18] 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 walnut samples. Reduction in antinutritional factors by processing methods such as moist heat treatment (boiling) was reported by Ekwe and Ihemeje [18]. According to Nwosu et al. [25], processing methods such as toasting, roasting and cooking is capable of reducing antinutritional factors present in food. This observation is in agreement with Khokhar and Apenten [39] 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 [39].

Studies have shown that flavonoids possess antioxidative and free radical scavenging property. According to Igara et al. [10], flavonoids could prevent coronary heart disease and lower the risk of cancer development. Considering the

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

Test parameter	FCAW	PAWFF	PAWFE	PAWFD	PAWFC	PAWFB	PAWFA
Moisture	30.03±0.94^{a}	33.33±0.25^{d}	33.13±0.18^{d}	33.12±0.09^{d}	32.43±0.15^{c}	33.21±0.05^{d}	31.55±0.07^{b}
Ash	3.50±0.46^{b}	2.88±0.07^{a}	3.08±0.05^{a}	3.08±0.06^{a}	3.22±0.05^{ab}	3.22±0.05^{ab}	2.94±0.02^{a}
Crude fat	25.63±0.49^{a}	28.30±0.20^{b}	28.40±0.07^{b}	29.20±0.02^{c}	29.11±0.10^{c}	28.45±0.09^{b}	29.24±0.05^{c}
Crude protein	24.31±0.30^{a}	24.24±0.12^{a}	24.20±0.07^{a}	24.12±0.02^{a}	24.15±0.11^{a}	24.26±0.04^{a}	25.20±0.08^{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}

Values are mean of triplicate samples. Values with the same superscript along the row are not significantly ($p < 0.05$) 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.

Table 2. Antinutrient composition (mg/100 g) 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}	ND	ND	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}	ND	ND	0.93±0.03^{b}
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}	ND

Values are mean of triplicate samples. Values with the same superscript along the row are not significantly ($p < 0.05$) different. ND = Not detected; 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.

antinutrients in freshly cooked as well as pickled African walnut formulations evaluated in this study, flavonoid content had the lowest numerical values which did not exceed 0.89 %. Table 2 shows that flavonoids content of freshly cooked African walnut is higher than that of pickled African walnut formulations. Reduction in flavonoids content could be as a result of pickling of African walnut and addition of citric or lactic acids to the samples.

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 formulations is higher than other antinutrients evaluated in this study except the pickles preserved with citric acid. This result trend is similar with the findings of Nwaoguikpe et al. [40]. According to Ayeni and Nuhu [17], saponin content in food has numerous health benefits.

Alkaloids present in plants have therapeutic uses because it possesses antimicrobial and antibacterial properties. It can also be used as an analgesic [10]. 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 Saranya and Rajani [36]. Their study revealed that there was reduction in tannin content of five selected fruits that were subjected to pickling process. The presence of tannin in African walnut supports its use in herbal medicine as well as healing haemorrhoids, frost bite and varicose ulcers [1].

3.4 Correlating Level of Antinutrients in the Pickles Preserved with Same Concentration of Lactic and Citric Acid

A partial correlation to determine the relationship between the level of antinutrients in pickled African walnut preserved with same concentration of citric and lactic acid while controlling for sample (FCAW) revealed that $r(9) = 0.059$, $p = 0.864$ was a weak, positive partial correlation that was not statistically significant between the level of antinutrients in pickled African walnut preserved with 5 % lactic acid and that which was preserved with 5 % citric acid. Similarly, that of pickled African walnut preserved with 1 % lactic acid and that which was preserved with 1 % citric acid showed that $r(9) = 0.368$, $p = 0.265$ was a weaker, positive partial correlation that was not statistically significant. On the contrary, partial correlation showed that between the level of antinutrients in pickles preserved with 3 % lactic acid and that which was preserved with 3 % citric acid, there was a very strong, negative partial correlation that was statistically significant because $r(9) = -0.981$, $p = 0.000$.

However, zero-order correlations showed that there was a statistically significant, strong negative correlation between level of antinutrients in pickled African walnut preserved with 5 % lactic acid and that which was preserved with 5 % citric acid because ($r(10) = -0.609$, $p < 0.036$). Therefore, the control sample (FCAW) had a very high influence in controlling the relationship between the level of antinutrients in the pickles preserved with 5 % lactic and 5 % citric acid. Zero-order correlation between pickled African walnut preserved with 3 % lactic acid and that which was preserved with 3 % citric acid showed that ($r(10) = -0.719$, $p < 0.008$) was a strong, negative correlation that was statistically significant. Similarly, zero-order correlation between pickled African walnut preserved with 1 % lactic acid and that which was preserved with 1 % citric acid showed that ($r(10) = 0.217$, $p < 0.498$) was a very weak, positive correlation that was not statistically significant. Therefore, the control sample (FCAW) had a moderate influence in controlling the relationship between the level of antinutrients in the pickles preserved with 1 % lactic and 1 % citric acid; 3 % lactic acid and 3 % citric acid.

3.5 pH

Fig. 1 shows the pH of pickled African walnut formulations monitored for 6 weeks. During the storage period, pH of the control sample (African walnut pickled without addition of citric or lactic acid) steadily increased from 5.6–7.0. The pH of control sample is higher than that of pickled African walnut formulations (pH 4.0-1.8) stored

for 6 Weeks previously subjected to a pickling process that involved addition of citric or lactic acids. Low pH of pickled African walnut preserved with citric or lactic acids could be as a result of the organic acids added to the pickling brine. Usually, acidic food has an equilibrium pH of 4.6 or below [41]. Decrease in pH during storage of pickled African walnut was reported in a similar study carried out by El-Shehawy and El-Kady [42], Khaskheli et al. [43] and Erdoğan et al. [44]. According to Ibrahim et al. [21], 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 [45]. Microbiological Guidelines for Ready-to-Eat Food [46] stipulate that the population of *Bacillus cereus* is satisfactory if it is less than 10^3 cfu/g and that of *Staphylococcus aureus* below 20 cfu/g in the product. Specific spoilage microorganisms between 10^5 - 10^8 cfu/ml in food products could be tolerated for human consumption based on Public Health Laboratory Service Guideline (PHLSG) [47] and Rho and Schaffner [48]. Total aerobic colony which must be less than 10^6 cfu/g in ready-to-eat foods is acceptable by Fylde Borough Council referencing manual of PHLSG [47]. Considering these standards, the pickled African walnut preserved with lactic or citric acid could be considered safe for human consumption [8].

3.6 Sensory Evaluation

Table 3 shows the result of sensory evaluation of pickled African walnut formulations preserved with citric or lactic acid for a period of 6 weeks. Among the pickles preserved with organic acids (citric and lactic acids), the sensory panelists reported that the most and least preferred sample is the one preserved with 5 % lactic acid and 1 % citric acid, respectively. However, the pickle without any organic acid added to it had the lowest score for all the sensory attributes as compared with pickled samples preserved with organic acids. This study revealed that pickled African walnut preserved with lactic acid have a preferable taste when the pickles were rinsed

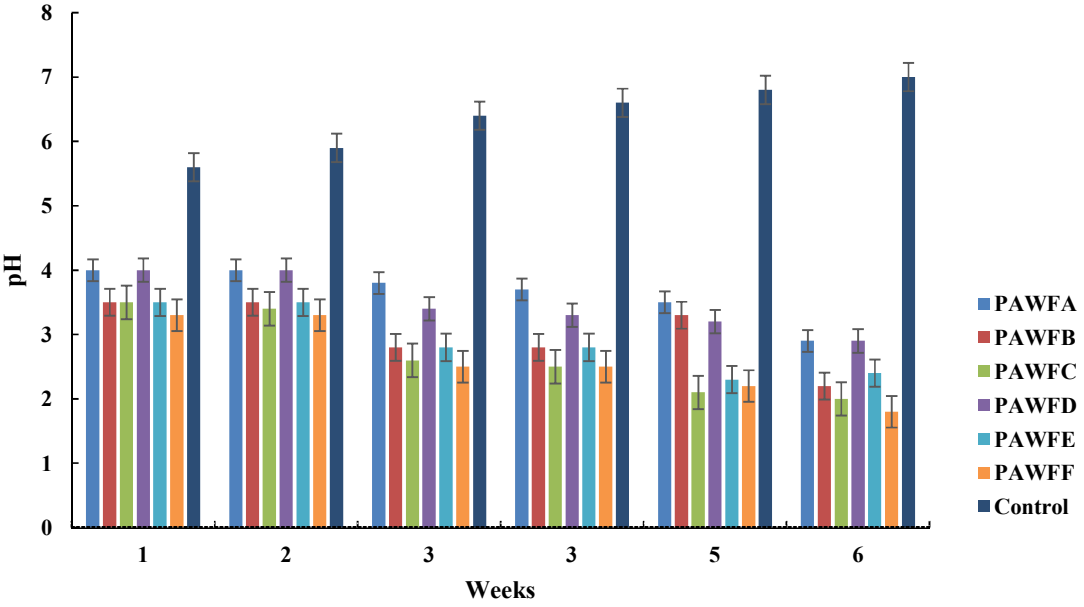

Fig. 1. pH of pickled African walnut preparations stored for six weeks

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 100 g of African walnut + 5 % NaCl

with potable water than similar samples that were not rinsed with potable water. However, it was the reverse in the case of pickled African walnut preserved with citric acid. Table 3 shows that all the sensory characteristics of African walnut pickles preserved with lactic acid were assigned higher sensory scores 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' [49]. In a related study, El-Shehawy and El-Kady [42] reported that sensory characteristics of seas date (*Phownix dactylifera* L.) pickled with brine mixed with organic acid had a higher sensory score than seas pickles prepared without addition of organic acid. Therefore, addition of the organic acid to African walnut pickle might have contributed in the improvement of its sensory characteristics. However, the control sample (pickled African walnut without addition of organic acid) was very much disliked by the panelists based on sensory evaluation score assigned to the product after 6 weeks storage period. Absence of organic acid in the control sample might have favoured growth of spoilage microorganisms which contributed in poor sensory attributes of the product.

3.7 Correlating Sensory Evaluation Scores of the Pickles Preserved with Same Concentration of Lactic and Citric Acid

A partial correlation to determine the relationship between the sensory evaluation scores assigned to pickled African walnut preserved with same concentration of citric and lactic acid while controlling for sample without preservative revealed that there was a very weak, positive partial correlation which was not statistically significant between pickled African walnut preserved with 5 % lactic acid and that which was preserved with 5 % citric acid because $r(57) = 0.295$, $p = 0.023$. Similarly, it was $r(57) = 0.456$, $p = 0.008$ between pickled African walnut preserved with 3 % lactic acid and that which was preserved with 3 % citric acid. Considering partial correlation of sensory evaluation scores assigned to pickles preserved with 1 % lactic acid and that which was preserved with 1 % citric acid while controlling for sample without preservatives, $r(57) = -0.006$, $p = 0.963$ showed that it was a very weak, negative partial correlation that was not statistically significant.

However, zero-order correlation showed that ($r(58) = 0.277$, $p < 0.032$) was a statistically not significant, very weak positive correlation between the sensory evaluation scores assigned to pickled African walnut preserved with 5 % lactic acid and that which was preserved with 5 % citric acid. Similarly, zero-order correlation also showed that sensory evaluation scores assigned to pickled African walnut preserved with 3 % lactic acid and that which was preserved with 3 % citric acid was ($r(58) = 0.458$, $p < 0.000$). Therefore, the control sample had a very low influence in controlling for the relationship between the pickles preserved with 5 % lactic and 5 % citric acid; 3 % citric and 3 % lactic acid. On the contrary, zero-order correlation between the pickles preserved with 1 % lactic acid and that which was preserved with 1 % citric acid based on sensory evaluation scores

Table 3. Sensory evaluation of African walnut pickles preserved with lactic and citric acid after 6 weeks storage period

Sample code	Taste (R)	Taste (NR)	Aroma	Mouth feel	Colour	Overall acceptability
PAWFF	8.1±0.88^{e}	8.0±0.47^{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±0.79^{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±0.32^{a}	1.4±0.52^{a}	2.1±0.74^{a}	1.2±0.42^{a}	2.0±0.70^{a}

Values are mean of triplicate samples. Values with the same superscript down the column are not significantly ($p < .05$) different. R - Rinsed sample; NR - Not rinsed sample; 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 sample represent 100 g of African walnut + 5 % NaCl

assigned to the pickles was ($r(58) = 0.007$, $p < 0.960$). Therefore, the control sample had a very strong influence in controlling for the relationship between the pickles preserved with 1 % lactic acid and that which was preserved with 1 % citric acid.

4. CONCLUSION

Citric and lactic acids added during pickling of African walnuts reduced the pH of the stored samples. Consequently, pH of the pickles were within the range stipulated by U.S. Code of Federal Regulations (21 CFR Part 114) except the control sample. Citric acid had more effect than lactic acid in reducing the level of antinutrients in the pickled African walnut samples. However, the level of antinutrients in freshly cooked African walnut (FCAW) was higher than what was obtainable in the pickles preserved with organic acids (citric or lactic acids) for 6 Wks. Proximate composition of the samples revealed that moisture and fat content of FCAW was lower than that of pickled samples preserved with organic acids but it was the reverse in terms of ash, crude fibre and carbohydrate content. Interestingly, protein content was least affected by the pickling process and addition of organic acids. Pickled African walnut preserved with 5 % lactic acid was the most preferred formulation based on sensory evaluation. Furthermore, the pickles preserved with lactic acid had a preferable taste when it was rinsed with potable water than similar samples that were not rinsed. This is contrary to what was observed in pickles preserved with citric acid. Based on the results obtained from this study, pickled African walnut preserved with lactic or citric acid is safe, nutritious and well acceptable to consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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