Microbiology Research Journal International

25(4): 1-9, 2018; Article no.MRJI.41024
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Effect of Processing on Microbial Load, Physicochemical Properties and Nutritional Composition of *Plukenetia conophora* (Africa Walnut)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOI and TRO designed the study. Authors TRO and OSF performed the statistical analysis. Authors AOI and TRO wrote the protocol and wrote the first draft of the manuscript. Authors AOI, TRO and OSF managed the analyses of the study. Authors AOI, TRO and OSF managed the literature searches. Authors AOI, TRO and OSF read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/41024
Editor(s):
(1) Dr. Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.
Reviewers:
(1) Ojo Omolara Comfort, University of Lagos, Nigeria.
(2) Supreena Srisaikham, Burapha University-Sa Kaeo, Thailand.
Complete Peer review History: http://www.sciencedomain.org/review-history/27275

Original Research Article

Received 14 January 2018
Accepted 21 April 2018
Published 17 November 2018

ABSTRACT

This research was carried out to determine the effect(s) of different processing methods on the nutritional composition of *Plukenetia conophora* (Africa walnut) seeds. The dried walnut seeds were sorted, cleaned, cracked and dehulled by abrasion to get the cotyledons. The raw sample and processed (boiled and fermented) seeds were analyzed for microbial load, physicochemical properties (pH, total titratable acidity and moisture contents), proximate composition, vitamin contents and antioxidant level. The boiled sample had the least microbial load (7.55 Cfu/ml) followed by the raw sample 8.50 (Cfu/ml). There was a progressive increase in the microbial load with an increase in fermentation time from 8.18 Cfu/ml to 8.40 Cfu/g in FW24 and FW120, respectively. The raw sample had least pH and moisture content of 5.78 and 9.50%, respectively. Boiling and increase in fermentation time led to significant increase in pH and moisture content of the samples.

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The raw sample had the highest TTA (2.88N) while there was a significant decrease in (Total Titerable Acidity) TTA with an increase in fermentation time. The raw sample had the least protein (26.78%), fat (30.11%) and ash (4.43%) contents while the fermented sample had the highest percentage of protein (29.01%), fat (32.67%) and ash (6.23%). The antioxidant properties of the fermented samples, i.e. total phenolics (17.67 mg/g) and DPPH (24.57 mg/g) were significantly higher than the boiled and raw samples. The fermented sample had highest vitamin A (33.71 mg/g), vitamin B1 (0.16 mg/g) and B2 (01.2 mg/g) vitamin C (22.67 mg/g). The vitamin B2 (0.02 mg/g) and vitamin E (23.91 mg/g) of the boiled sample were significantly higher than the fermented and raw sample. From the study, it was observed that walnut is better consumed fermented for its important roles in health and nutrition than boiling and raw consumption.

Keywords: Plukenetia conophora; processing; microbial load; antioxidants; vitamins.

1. INTRODUCTION

African walnuts (*Plukenetia conophora*) is a member of the Euphorbiaceae family. It is a climber found in the forest region of India and Western Africa. It is predominantly found in the Southwest States of Nigeria [1]. Conophora plants are cultivated principally for the nuts which are usually cooked and consumed as snacks [2]. The plant is mostly maintained by cultural preferences and traditional practices which made its potential value to be underestimated, undervalued and underexploited.

The stems of *Plukenetia conophora* is usually 3–15 m (9.8–49.2 ft) long, though can be up to 30 m (98 ft) long. The seed is thin-shelled and about 25 mm (0.98 in) long. It is contained in a pod which may be a one-shelled nut (single) or more. Traditionally, it was believed that walnut is better cultivated with the one-shelled nut (single) for a high yield. The walnut shells could be black (when the fruit is getting rotten) or brown (when the fruit is yet fresh or freshly plucked) from the plant. The nut is whitish upon cracking from the shell. The nut has a thin layer in between two halves (when a nut is divided into two equal parts) of nut [3]. Each seed is about 2.5 cm in diameter. Small-scale farmers constitute the highest producers of walnut [4].

Approximately an ounce of walnuts daily over a period of 2-3 months can help reduce the metabolic syndrome-related problem. The addition of walnut to diets decreased abdominal adiposity [5]. The root is used in Nigerian traditional medicine for the treatment of cancer and also used in the treatment of asthma and hypertension. In Nigeria the leaf extract when taken combat malaria and prolonged hiccups. In men, the seeds increase sperm counts while the leaf juice improves fertility in women. The fresh uncooked nuts when consumed acts as anti-poison against snake bite. Walnut could be boiled or roasted for consumption. In some parts of Nigeria, walnut is blended/ ground on mill stone after it is boiled to cook soup. It serves as an alternative source to thicken the soup when the melon is scarce or economically unaffordable. Therefore, there is need to study the effect of various processing methods on the nutritional quality of walnut in other to identify the best form by which walnut could be consumed for nutrition and its health potential.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Africa walnut seeds (*Plukenetia conophora)* was purchased from Atakumosa modern market in Ilesa West Local Government Ilesa, Osun state. The seeds were authenticated by a taxonomist in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti State Nigeria.

2.2 Sample Preparation

The dehulled walnut seeds were sorted to remove grit, dirt and decomposing ones. These were divided into three groups and were kept in sterile polythene bags ready for laboratory further analysis.

2.3 Raw Sample

The seeds were dehulled by abrasion. These were then cleaned and separated from grits, oven dried at 60°C for 96 h, pulverized using blade homogenization and poured into a sterile container having screw cap for analysis.

2.4 Boiled Sample

The unshelled walnut seeds were firstly dehulled by abrasion. The seeds were washed using

distilled water and cooked for three h in boiling water. The boiled seeds were then oven dried at 60°C for 96 h, pulverized using blade homogenization and poured into a sterile container having screw cap for analysis.

2.5 Fermented Sample

The unshelled walnut seeds were dehulled by abrasion. The seeds were washed with distilled water, cooked for four h in boiling water and allowed to cool to about 30°C. The cooled walnut seeds were wrapped in aluminium foil and incubated at 35°C for 120 h. The fermented seeds were then oven dried at 60°C for 96 h, pulverized using blade homogenization and poured into a sterile container having screw cap for analysis.

2.6 Microbial Analysis

The total viable counts of the samples were analyzed daily by the method of Olutiola et al. which include isolation of microorganism from the sample, determination of total viable counts (microbial load), direct and microscopic observation and biochemical identification of the isolates.

2.7 Determination of pH

The pH was determined according to the method of AOAC 2005. Each sample (5 g) was weighed into a sterile mortar and mashed with a clean pestle, and 50 ml of distilled water was added. It was mixed thoroughly to form a slurry and filtered with Whatman No. 14 filter paper. A standard buffer solution (pH 6.0) was prepared, and this was used to standardize the pH meter (Checker, produced by Hanna instruments, model no-16607). The electrode of the digital pH meter was dipped in the slurry. The pH readings were recorded.

2.8 Determination of Total Titratable Acidity (TTA)

The amount of lactic acid in the fermenting mass was determined as described by Omodara and Aderibigbe (2013). Twenty millilitres (20 ml) of the filtrate obtained from 5 g seed dissolved in 20 ml distilled water was titrated against 0.1 M NaOH using phenolphthalein indicator. The titre value was then used to calculate the titratable acidity as percentage lactic acid using $M_1V_1 = M_2V_2$.

2.9 Moisture Determination

This was determined according to AOAC 2005 by weighing a clean and well-labelled Petri dish that has been oven dried (W1), 5 g of the sample was added to the dish and this was reweighed (W2). The dish and its content were transferred to the oven at 105°C for about 24 h. After which it was transferred into a desiccator and cooled for about one hour and weighed again. This was repeated severally to get a constant weight (W3).

% Moisture= [(W2 -W3)100]/ (W2-W1)

3. PROXIMATE ANALYSIS

The proximate compositions of the raw, boiled and fermented samples were determined using standard procedures of AOAC (2005). The parameters determined were protein, ash, crude fibre, fat and carbohydrate.

3.1 Ash Composition

2 g of the sample is weighed into a pre-heated crucible. The crucible is placed into muffle furnace at 400-600°C for four h until whitish-grey ash is obtained. The crucible is then placed in the desiccator and weighed:

% Ash = (wt. of crucible +ash – wt. of crucible)/ Wt. of sample

3.2 Crude Fibre Determination

2 g sample was put into 200 mL of 1.25% of H_2SO_4 and boiled for 30 min. The solution and content then poured into butcher funnel equipped with a muslin cloth and secured with elastic band. This was allowed to filter, and the residue washed with hot water to free it from acid. The residue was then put into 200 mL boiling 1.25% NaOH and boiled for 30 min, then filtered. It was then washed twice with alcohol; the material obtained was washed thrice with petroleum ester. The residue obtained was put in a clean, dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. Then difference of weight (i.e. loss in ignition) is recorded as crucible fibre and expressed in percentage of the original weight.

Percentage crude fibre = [(W1 - W2)/ Wt)] X 100

Where:

W 1 = Weight of sample before incineration

W 2 = Weight of sample after incineration
W 3 = Weight of original sample.

3.3 Determination of Fat Content

The sample, 2 g was loosely wrapped with a filter paper and put into the thimble which is fitted to a clean round-bottomed flask, which has been cleaned, dried and weighed. The flask contained 120 mL of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for five h. The heating was then stopped, and the thimbles with the spent samples kept and later weighed. The difference in weight was received as the mass of fat and is expressed in percentage of the sample. The percentage oil content is calculated;

Percentage Fat = [(W2-W1) / W3] x 100

Where

W1 = Weight of the empty extraction flask
W2= Weight of the flask and oil extracted
W3 = Weight of the sample

3.4 Determination of Crude Concentration of Protein

The micro kjeldahl method described by AOAC (2005) was used. 2 g, each of the samples was mixed with ten mL of concentrated H_2SO_4 in a heating tube. One tablet of selenium catalyst was added to the tube and mixture heated inside a fume cupboard. The digest was transferred into a 100 mL volumetric flask and made up with distilled water. Ten mL portion of the digest was mixed with equal volume of 45% NaOH solution and poured into a kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 4% boric acid solution containing three drops of zua age indicator. A total of 50 mL distillate was collected and titrate as well. The sample was duplicated and the average value was taken. The nitrogen content was calculated and multiplied by 6.25 to obtain the crude protein content.

Percentage Nitrogen=[(100 x N x14 x Vf) T]/ 100 x Va

Where:

W = Weight of the ample
N = Normality of the titrate (0.1 N)
vf = Total volume of the digest = 100 mL
T = Titre value
va = Aliquot volume distilled.

3.5 Carbohydrate Content Determination

The nitrogen-free method described by AOAC 2005 was used. The carbohydrate is calculated as weight by the difference between 100 and the summation of other proximate parameters as Nitrogen Free Extract (NFE).

Percentage carbohydrate (NFE) = 100 – (M + P +F 1 + F2)

Where

M = moisture; P = protein; F 1 = fat; A = ash; F 2 = fibre.

3.6 Determination of vitamin A

The sample (1 g) was weighed and macerated with 20 mL of petroleum ether. It was evaporated to dryness, and 0.2 mL of chloroform acetic anhydride was added, and two mL of TCA chloroform were added and the absorbance measured at 620 nm. Then the concentration of vitamin A was extrapolated from the standard curve.

3.7 Determination of Vitamin B1 (Thiamine)

The samples (5 g) are homogenized with 50 mL of ethanolic sodium hydroxide solution. This was filtered into a 100 mL flask. The filtrate (10 mL) was pipetted into a beaker and colour developed by the addition of 10 mL potassium dichromate. The absorbance is read at 360 nm. A blank sample was also prepared and read at the same wavelength. The values are extrapolated from a standard curve [6].

3.8 Determination of Riboflavin (Vitamin B2)

Each of the samples (5 g) was extracted with 100 mL of 50 % ethanol solution shaken for one h. This was filtered into a 100 mL of 30% hydrogen peroxide (H_2O_2) and allowed to stand over a hot water bath for 30 mins. 2 mL of 40% sodium sulphate added to make up the 50 mL mark and absorbance read at 510 nm in a spectrophotometer [6].

3.9 Determination of Niacin (Vitamin B3)

The sample (5 g) was blended and 100 mL of distilled water added to dissolve all nicotinic acid or niacin present. The solution (5 mL) was drawn

into 100 mL volumetric flask and makeup to mark with distilled water. 10-50 ppm of Niacin stock solution was prepared. The absorbance of diluted stock solution and sample extract were measured at a wavelength of 385 nm on a spectrophotometer. Different concentrations of the standard stock solutions were read on the spectrophotometer for absorbance at the specified wavelength to obtain the Gradient factor. Amount of niacin in the sample was calculated using the formula:

Mg/100 g niacin= Absorbance x dilution factor x Gradient factor stock solution / 10

3.10 Determination of Ascorbic Acid (Vitamin C)

Vitamin C content was determined according to the method of Baraket *et al*., (1973). Five grams (5 g) of the sample was weighed into an extraction tube and 100 mL of EDTA/TCA (2:1) extracting solution were mixed and the mixture was shaken for 30 min. This was transferred to a centrifuge tube and centrifuged at 3000 rpm for 20 min. It was transferred to a 100 mL volumetric flask and made up to 100 mL mark with the extracting solution. 20 mL of the extract was pipetted into the volumetric flask and 1 % starch indicator was added. These were titrated with 20 % $CuSO_4$ solution to get a dark end point [7].

4. DETERMINATION OF VITAMIN E

The sample (1 g) was weighed and macerated with 20 mL of ethanol. One mL of 0.2 % ferric chloride in ethanol was added, then one mL of 0.5 % α, α-dipyridyl was also added, it was diluted to 5 mL with distilled water and absorbance was measured at 520 nm. Then concentration of Vitamin E was extrapolated from the standard curve.

4.1 DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of methanolic, hexanoic, and aqueous extracts of the sample was determined according to the method reported by Brand-Williams et al. 1995. The stock solution of the radical, prepared by dissolving 24 mg DPPH in 100 ml methanol, was kept in a refrigerator until further use. The working solution of the radical was prepared by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 (± 0.02) at 517 nm. In a test tube, three mL DPPH working solution was mixed with 100 µl plant extract (1 mg/ml) or the standard solution. The absorbance was measured at 517 nm for 30 min. The percent antioxidant or radical scavenging activity was calculated using the following formula:

% Antioxidant activity = [(Ac-As)/ Ac] X 100

Where, Ac and As is the absorbance of control and sample, respectively. The control contained 100 µl methanol in place of the plant sample

4.2 Determinations of Antioxidant Activity

The antioxidant activity was determined using DPPH radical scavenging assay. To 0.2 ml of each extracted sample and the standard Trolox solutions, 3.8 ml of 0.1 mm DPPH solution was added to a test tube. The mixtures were shaken for 1 min and then left in the dark for 30 min after which the absorbance was read using spectrophotometer at 517 nm against the blank. The absorbance of negative control (A control) was taken after adding DPPH radical solution to 0.2 ml of the extraction solvent (distilled water).

% DPPH radical inhibitor = x 100

From the equation, the free radical scavenging (antioxidant) activity was expressed as the mean micromole of Trolox equivalent (µMTE/g).

4.3 Total Phenolics

The total phenolic content was measured using the Folin Ciocalteu reagent, [8]. An aliquot of the extract (100 µl) was mixed with 250 µl of Folin Ciocalteu's reagent and incubated at room temperature for 5 min. 1.5 mL of 20 % sodium bicarbonate was added to the mixture and incubated again at room temperature for two h. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The results were expressed regarding µg gallic acid equivalents (GAE)/ mg dry extract [9].

4.4 Data Analysis

Data from all the determination were analyzed using analysis of variance (ANOVA) using IBM/SPSS 20.0 Statistical package for windows. Different means were separated using the Least Significant Different (LSD) method, and a significant difference was accepted at $p<0.05$.

5. RESULTS AND DISCUSSION

Table 1 shows the microbial load of raw, boiled and fermented walnut seeds on nutrient agar (NA) and potato dextrose agar. The boiled sample had the least bacterial load (7.55 log Cfu/g), followed by the raw sample (7.59 log Cfu/g). There was a progressive increase in bacterial load due to increase in fermentation time. The load increased from 8.18 log Cfu/g to 8.40 Cfu/g in FW24 and FW120, respectively. The boiled sample also had the least fungal growth (5.80 Cfu/g) followed by the raw sample. There was also a progressive increase in fungal growth due to increase in fermentation time. The microbial count showed that bacteria were predominant in the sample than the fungi. The least number of microorganisms present in the boiled sample might be due to the effect of heat on the microorganism while the increase in microbial load due to increase in fermentation time might be due to hydrolysis and the release of the nutrient from their bound state into a form that is readily available for metabolic activities by the fermenting organisms. This is in conformation with the reports of Omodara and Aderibigbe 2013.

The physicochemical parameters are presented in Table 2. Processing has a significant effect on the pH, TTA and moisture content of the samples. The Boiled sample had the least pH (5.24), the raw sample (5.78) while the increase in fermentation time led to a significant increase in the pH of the samples. The least pH recorded in the boiled sample might be due to the effect of heat on the pH. The increase in pH with an increase in fermentation might be as a result break down of complex protein molecules with the release of ammonia [10]. The TTA of the raw walnut was observed to be higher than the boiled seed while there was a significant decrease in pH with an increase fermentation time. Processing led to significant increase in the moisture content of the sample.

Table 3 shows the proximate composition of the raw, boiled and fermented walnut seeds. The moisture content of the seeds increased after boiling which may be due to inhibition of water during boiling, but the moisture content of the seeds decreases the longer it stays in the fermentation process. The fermented seeds had the highest percentage of protein (29.01%), crude fibre (1.92%), ash (6.23%), and fat (32.67%) while the boiled sample had the highest percentage of carbohydrate. This is in agreement with the findings of Kuku et al. (2013) when working on cassava in Nigeria. Table 4 and Table 5 shows the antioxidants and vitamins level of the raw, boiled and fermented samples. Fermentation led to a significant increase in the total phenolics (17.67mg/g), DPPH (24.57) Vitamins A (33.7mg/g), B1 (0.16mg/g) B3 and C (22.67) in the sample while the boiled sample had the highest amount of Vitamin B2. The increase in the antioxidant and vitamin level when fermented revealed the chemo-preventive properties of *P. Conophora* (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways [11]. The highest level of DPPH in the fermented when compared with the boiled and raw sample indicates fermentation is the best processing method to project 2,2-diphenyl-1-picrylhydrazyl in walnut seed for scavenging free radicals. The vitamins concentration in raw, boiled and fermented samples of *Plukenetia conophora* showed that the most abundant vitamin in walnut seed was vitamin A while the lowest concentration recorded was vitamin B_2. It was also observed that boiling reduced vitamin C in *Placentia conophora* seed while fermentation significantly increased vitamin C in the seed. Walnut is better taken fermented for its use in herbal medicine for the treatment of skin condition including eczema, psoriasis, and pruritus [12]. The high vitamin content of the fermented seed can also make the fermented seed be used for the treatment of common cold and other diseases like prostate cancer [13]. Vitamin E in walnut seed supports its use in the Southern Nigeria ethnomedicine as a male fertility agent [14]. Heat processing generally increases fat-soluble vitamins the concentration of most of the water-soluble vitamins reduced with the application of heat. Similar results have been reported for vitamins A and D_3 in raw English walnut by Ernest et al. [15]. It is evident also from the result that walnut has anticancer activities. The *in-vitro*. radical scavenging ability of walnut was highest for DPPH when fermented so also for total phenolics. More to this, the antioxidant vitamins A and C increased with fermentation, but vitamin E only increased when boiled. The antioxidant properties of walnut will help lower the risk of chronic oxidative stress.

Table 1. The microbial load of raw, boiled and fermented walnut seed

Samples	Microbial load on Na (log_{10} CFU/ml)	Microbial load on PDA (log_{10} CFU/ml)
RW	8.50	6.22
BW	7.55	5.80
FW_{24}	8.18	5.65
FW_{48}	8.22	6.12
FW_{72}	8.29	6.43
FW_{96}	8.41	7.04
FW_{120}	8.40	7.23

Keys: RW-raw walnut, BW- boiled walnut, NA-Nutrient agar, PDA-Potato Dextrose Agar, TTA- Total titratable acidity, FW_{24}- fermented walnut at 24 hours, FW_{48}- fermented walnut at 48 hours, FW_{72}- fermented walnut at 72 hours, FW_{96}- fermented walnut seed at 96 hours, FW_{120}- fermented walnut at 120 hours

Table 2. Physicochemical properties of raw, boiled and fermented walnut seed

Samples	pH	TTA	Moisture content
RW	5.78^{e}±0.04	2.88^{a}±0.02	9.50^{f}±1.00
BW	5.24^{d}±0.05	2.21^{c}±0.02	36.97^{e}±0.02
FW_{24}	5.84^{d}±0.04	2.47^{b}±0.10	48.28^{d}±0.03
FW_{48}	6.12^{c}±0.22	2.16^{c}±0.03	48.36^{d}±0.10
FW_{72}	6.12^{c}±0.22	1.93^{d}±0.17	48.57^{c}±0.02
FW_{96}	6.32^{b}±0.10	1.89^{d}±.0.2	49.60^{b}±0.10
FW_{120}	6.59^{a}±0.09	0.999^{e}±0.01	50.78^{a}±0.02

Keys: RW-raw walnut, BW- boiled walnut, NA-Nutrient agar, PDA-Potato Dextrose Agar, TTA- Total titratable acidity, FW_{24}- fermented walnut at 24 hours, FW_{48}- fermented walnut at 48 hours, FW_{72}- fermented walnut at 72 hours, FW_{96}- fermented walnut seed at 96 hours, FW_{120}- fermented walnut at 120 hours

Table 3. Proximate composition of the raw, boiled and fermented walnut seed

Proximate (%)	Raw	Boiled	Fermented
Ash content	4.43^{c}±0.09	5.09^{b}±0.11	6.23^{a}±0.06
Moisture Content	9.50^{f}±1.00	36.97^{e}±0.02	48.28^{d}±0.03
Crude fiber	1.41^{b}±0.06	1.32^{c}±0.03	1.92^{a}±0.05
Fat content	30.11^{c}±0.04	31.37^{b}±0.28	32.67^{a}±0.07
Protein content	26.78^{c}±0.12	28.01^{b}±0.13	29.01^{a}±0.23
Carbohydrate	34.12^{c}±0.24	33.57^{ab}±0.19	33.54^{b}±0.37

Data are expressed in mean ±SD from triplicates experiments (n=3). Values having different superscript letters in a row differ significantly at P≤0.05

Table 4. Antioxidants properties of walnut seeds

	Raw	Boiled	Fermented
Total phenolics	13.76^{b}±0.08	9.98^{c}±0.02	17.67^{a}±0.15
DPPH scavenging Activities	23.12^{b}±0.03	19.33^{c}±0.08	24.57^{a}±0.11

Note: Data are expressed in mean ± SD from triplicate experiments (n=3). Values having different superscript letters in a row are differ significantly at p≤0.05

Table 5. Vitamin contents of walnut seeds

Vitamin A	27.25^{c}±0.04	33.42^{b}±0.07	33.71^{a}±0.14
Vitamin B1 (Thiamine)	0.11^{b}±0.02	0.07^{c}±0.02	0.16^{a}±0.02
Vitamin B2 (Riboflavin)	0.15^{a}±0.01	0.02^{a}±0.01	0.12^{b}±0.03
Vitamin B3 Niacin	1.14^{b}±0.29	0.42^{c}±0.03	1.2^{a}±0.02
Vitamin C	22.12^{b}±0.03	12.50^{c}±0.14	22.67^{a}±0.08
Vitamin E	19.17^{b}±0.05	23.91^{a}±0.03	15.42^{c}±0.06

Note: Data are expressed in mean ± SD from triplicate experiments (n=3). Values having different superscript letters in a row are differ significantly at p≤0.05

6. CONCLUSION

The seeds of *Plukenetia conophora* is better consumed when fermented and boiled for its health supportive potential evident in the nutritional composition. Walnut seed has high nutritional values which indicated it as a good source of the dietary element. Processing (fermentation and boiling) may be encouraged for maximum bioavailability of the nutrients in the seeds which when utilized properly could solve the problem of malnutrition and prevent the risk of some non-communicable diseases. Boiling is encouraged before ingestion to reduce the number of microorganisms entering the bowel from the seed. Heat processing increased the values of the fat-soluble vitamins while the values of most of the water-soluble vitamins reduced with the application of heat.

7. RECOMMENDATION

1. Consumers should be enlightened on the nutritional and health benefits of *Plukenetia conophora* consumption.
2. It should be cultivated in large quantity and be commercialized across the whole world for consumption.
3. Close attention should be paid to its preservation procedure to achieve round the year availability of this important nut, *Plukenetia conophora.*
4. Consumers should be enlightened that the best processing method for the consumption of walnut is by fermentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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