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Effect of boiling methods on the nutritional profile, phytochemicals, functional properties, lipid quality and consumer acceptability of African walnut (*Tetracarpidium conophorum*)

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Abstract

This study evaluated how various boiling methods affected the nutritional content, phytochemicals, functional properties, and lipid quality of African walnut *(Tetracarpidium conophorum)*. Fresh walnuts were boiled with or without shells at 100°C for 30 and 60 minutes. Boiling considerably decreased protein content while increasing crude fiber and ash content, indicating better mineral availability and shelf life. Amino acid analysis revealed that glutamic acid and aspartic acid were predominant, with important amino acids such as leucine and lysine suggesting high protein quality. Boiled samples showed more antioxidant activity and lower levels of phytates and tannins, which improved nutritional bioavailability. Lipid quality study revealed that boiling walnuts had higher oxidative stability but lower unsaturation. These findings suggest that adequate boiling procedures can improve the nutritional and functional features of African walnuts, indicating their potential usage as beneficial functional food ingredients.

Keywords: African walnut; Boiling method; Nutritional quality; Antioxidant properties; Functional properties

Introduction

African walnut (*Tetracarpidium conophorum*) is a prominent Central and Western African plant grown primarily for its nuts, which are typically consumed as snacks after being cooked and cracked. It is endemic to tropical western and central Africa, from Togo to Congo, Sierra Leone, Nigeria, and Cameroon (Chikezie, 2017). African walnut is an edible seed found in the plant Euphorbiaceae, specifically *Tetracarpidium conophorum. T. conophorum* is high in protein (29.14%), fat (54.14%), carbohydrate (4.17%), ash (3.32%), and a variety of vitamins and minerals such as magnesium, phosphorus, copper, and other micronutrients, making it a cholesterol-free alternative for heart-healthy eating, (Baiyeri and Olajide. 2022).

Walnuts have a rich and diversified nutritional profile, making them an important component of a health-promoting diet. Whole walnuts and walnut oil contain high levels of omega-3 polyunsaturated fatty acids (n-3 PUFAs), which have been linked to improved health (Øvrebø, 2019; Uzun *et al.* 2021). Walnut oil, in particular, is defined by its beneficial fatty acid content, which consists mostly of oleic acid, linoleic acid, and linolenic acid (Uzun *et al.* 2021; Demirag, 2019; Kafkas *et al.* 2020). Walnut oil's high polyunsaturated fatty acid concentration increases its nutritional worth and health potential, making it one of the most beneficial nut oils available.

African walnut has nutritional, therapeutic, and antioxidant characteristics due to bioactive polyphenols (Adetunji *et al.* 2021). They include phenolic compounds, which function as antioxidants, inhibiting LDL-lipoprotein oxidation, platelet aggregation,

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and red blood cell destruction. Walnuts have been shown in scientific studies to lower blood glucose and cholesterol levels while also lowering blood pressure (Lockyer *et al.* 2023). Consumption of walnuts has been linked to a variety of health advantages, including lower cancer mortality due to omega-3 fatty acids, antioxidants, and phytosterols. Other benefits include weight loss, better endocrine parameters in polycystic ovarian syndrome (PCOS), diabetic management, and increased cognitive capacities (Kalgaonkar *et al.* 2011). Alkaloids in the nuts produce a bitter taste, which is commonly noticed when drinking water shortly after eating the nuts.

Walnuts health-promoting properties are a result of their chemical profile, which includes ascorbic acid, flavonoids, quercetin, and caffeic acid (Goncaives *et al.* 2023). With a moisture level of around 35–40%, fresh walnuts are prone to germination and deterioration upon harvest, which results in low quality stability and a brief shelf life for walnut products. Several processing techniques, including fermentation, freezing, refrigeration, and thermal processing, have been used to overcome these hurdles. While thermal processing effectively reduces anti-nutritional components, it also poses a challenge by affecting walnuts' nutritional and functional properties. Similarly, thermal and mechanical processing methods have been developed to enhance walnuts' palatability, safety, and shelf life. Temperature and processing technique selection have a significant influence on walnuts' nutritional value. These barriers constrain the full-scale production of walnuts and the investigation of their intrinsic potential, which has a substantial effect on the market. Addressing these challenges becomes imperative for walnuts' sustainable development and industrial utilisation, particularly in regions like Nigeria, where storage facilities are lacking. Therefore, this study assessed the impact of boiling techniques affected the proximate, mineral content, anti-nutritional, functional, and lipid quality of African walnuts in order to extend their shelf life and investigate their potential for use in food formulations and as a source of antioxidants for the consumers

Materials and methods

Sample collection

Fresh African walnut seeds *(Tetracarpidium conophorum)* were obtained from a local market in Ogbomoso, Oyo State, Nigeria. The seeds were selected for uniform size and maturity, and transported to the laboratory in clean, breathable jute bags for processing and analysis, A total of 2.5 Kg of fresh walnut seeds used in this study.

Chemical and reagents

All of the chemicals and reagents utilized in this study were analytical grade.

Sample preparation

About 2.5 kg of fresh walnut seeds were weighed and sorted to remove impurities such as twigs, leaves, sand, and broken seeds. After thorough cleaning, the sorted seeds were divided into five equal portions of 500 grams each. The first portion, which served as the control, was not boiled. The walnuts were manually sliced, oven-dried at 60°C for 12 h, milled using an electric blender, packaged in zip-lock bags, and stored at room temperature (28 ± 2°C) until further use. The second portion was boiled with the shell at 100°C for 30 min. The water was drained using a strainer, after which the seeds were sliced, oven-dried at 60°C for 12 h, milled, packaged in zip-lock bags, and stored at room temperature. The third portion was first deshelled, then boiled at 100°C for 30 min. The seeds were drained, sliced, oven-dried at 60°C for 12 h, milled, packaged in zip-lock bags, and stored at room temperature. The same procedures were followed for the remaining two portions, which were boiled for 60 min.

Determination of nutritional composition

The nutritional composition of treated/non-treated walnut flour samples was determined using conventional procedures provided in the Association of Official Analytical Chemists' standard methodologies (AOAC, 2015). Moisture content was determined by drying the walnut flour samples in a hot air oven at 105°C for 3 hours; protein content was determined using the micro-Kjeldahl method with 6.25 as the conversion factor for total nitrogen to protein; fat content was determined using the Soxhlet extraction method with petroleum ether; and ash content was determined using a muffle furnace at 550°C until the ash turned into grey. Crude fibre determination was conducted by defatting the samples, followed by acid digestion and subsequent incineration in a muffle furnace at 600°C for 6 hours, as described by AOAC (2010) and carbohydrate content was determined by difference. The dry-ash methods of AOAC (2015) were used to analyze minerals using atomic absorption spectroscopy (PerkinElmer 410021, USA).

Determination of anti-nutritional factors

Tannin content

The tannin concentration of the walnut sample was done using Vanillin-HCL in methanol methods. For 15 hours, a one-gram sample of powdered flour was incubated at 30°C in

10 mL of acidified methanol (1 mL of concentrated HCl in 100 mL of methanol). After that, the sample was centrifuged for 15 minutes at 10,000 rpm. 1 mL of the supernatant was extracted and placed in a test tube. Equal amounts of vanillin solution (4 g/100 mL methanol) and acidified methanol (8 mL concentrated HCl in 100 mL methanol) were added to create the vanillin-HCl staining reagent. A tannic acid solution (1 g of tannic acid in 100 mL of acidified methanol) was employed as the standard (R2 = 0.9969). The extract and the standard were mixed with 5 mL of Vanillin-HCl reagent, and after 20 minutes of dark incubation, the absorbance was measured at 500 nm using a UV-V is Spectrophotometer against the Vanillin-HCl reagent as a blank. The tannin content was expressed as mg of tannic acid equivalent per 100 g of sample (Yadav *et al.* 2020), and the trypsin inhibitor was estimated using the spectrophotometric method described by Liu (2021).

Oxalate content

The oxalate content was ascertained using the volumetric analysis method, as AOAC (2015). Five (5) grams of the sample were put in a 250 ml beaker, and 200 ml of 10% acetic acid (CH_3COOH) in ethanol (C_2H_5OH). At 25°C, the mixture was covered and left to stand for four hours. Following the application of a paper filter, the filtrate was concentrated in a water bath until it had decreased to 25% of its initial volume. Until all of the precipitation was gone, concentrated NH_4OH was added dropwise. The precipitate was then gathered on weighed filter paper and cleaned with diluted NH_4OH after the mixture had been given time to settle. According to Udedi *et al.* (2014), the alkaloid precipitate was dried and weighed.

Determination of antioxidant properties

Ferric-reducing antioxidant power

By reducing ferric ions and measuring absorbance at 700 nm, the ferric-reducing antioxidant power (FRAP) was calculated as Zhang *et al.* (2020) reported. The method of Adeloye *et al.* (2021) was used to measure the hydroxyl radical scavenging activity. The reaction contained 1,10-phenanthroline, $FeSO_4$, and hydrogen peroxide, and the absorbance was measured at 536 nm.

DPPH radical scavenging activity

The extracts' capacity as antioxidants was assessed using both tests. A method developed by Sedej *et al.* (2011) was used to test the DPPH radical scavenging activity. The antioxidant capacity of the extracts was evaluated using a 90 mM DPPH solution made in 95% methanol. After adding the extracts at different concentrations (0.1, 0.5, 1.0, and 2.0 mg/mL) to the DPPH solution and shaking it vigorously, the mixture was kept in the dark for an hour. A spectrophotometer was used to measure absorbance at 517 nm; a lower absorbance implies a higher level of radical scavenging action.

Total phenolic content (TPC)

To determine the total phenolic content (TPC), the Folin–Ciocalteu technique was used. Depending on how soluble the extracts or fractions were in water, 500 µL of various concentrations were combined with 2.5 mL of Folin-Ciocalteu reagent (0.2 N). 2 mL of Na_2CO_3 solution (75 g/L) was added after 5 minutes, and the optical density was measured at 760 nm against a blank after 120 minutes of dark standing. According to the gallic acid calibration curve, the total phenolic contents were computed and reported as gallic acid equivalents (GAE) in mL/g of the sample (Adeyemo *et al.* 2022).

Total flavonoid content

A colorimetric approach, as outlined by Ayoub *et al.* (2019), was used to determine the total flavonoid content. First, $NaNO_2$ was added to the sample, then $AlCl_3$ and NaOH, and last, 2.5 mL of distilled water was added to get the final volume. Absorbance at 510 nm was measured during a short incubation period at room temperature. A catechin calibration curve was used to determine the total flavonoid content, which was then reported in microgrammes of catechin equivalents (CE) per gramme of extract.

Alkaloid content

A 250 ml beaker containing 5 g of the alkaloid was filled with 200 ml of 10% acetic acid (CH_3CO_2H) in ethanol (C_2H_5OH). After being covered, the mixture was let to stand at 25°C for four hours. The filtrate was then concentrated in a water bath until it had a quarter of its initial volume after being filtered using filter paper. Until all of the precipitation had evaporated, concentrated NH_4OH was applied dropwise. The precipitate was collected on weighted filter paper and cleaned with diluted NH_4OH after the mixture had been given time to settle. After drying, the alkaloid precipitate was weighed. Difference was used to determine the percentage alkaloid (Obdoni and Ochuko, 2001; Udedi *et al.* 2014).

Determination of fat quality

Acid value

The acid value was calculated using the method outlined by AFNOR (1981). 1 g of oil was added to a 250 mL beaker,

followed by 100 mL of 95^{0}C ethanol. Two drops of 1% phenolphthalein solution were added to the beaker contents, and the mixture was titrated with 0.1 N potassium hydroxide solution. This titration is also performed with the blank test, and the amount of KOH used is recorded. The acid index was calculated as mg KOH/g of oil.

Iodine value

According to the AOAC (2010), the Wijs technique was used to calculate the Iodine Value. A flask with 15 mL of carbon tetrachloride solution and 25 mL of Wijs' reagent was filled with 0.2 g of oil. Following a gentle shake and an hour in a dark box, 20 ml of a potassium iodide (10%) aqueous solution, 15 mL of distilled water, and five drops of 1% starch are added to the hermetically sealed container. After titrating the solution in the flask with 0.1 N sodium thiosulphate solution, the amount of sodium thiosulphate required to cause the solution to turn blue and vanish from the flask was recorded. Additionally, the blank test was used to make this titration. The II was expressed as g I2/100 g of sample.

Peroxide index

The Peroxide index was determined using the methods. After adding 9.8 mL of a chloroform-methanol combination (7:3 v/v) to a glass test tube with a capacity of 10 mL and 50 mg of oil sample, the liquid is vortexed for two to four seconds. 50 µL of a 30% aqueous solution of ammonium thiocyanate was then added, and the mixture was vortexed for two to four seconds. Next, 50 µL of an aqueous solution of iron chloride II was added. For two to four seconds, the mixture is vortexed after again. Using a spectrophotometer (Perkin-Elmer, Norwalk CT, USA), the absorbance of the reaction mixture is measured at 500 nm against a blank that contains all of the reagents except the oil after it has been incubated for five minutes at room temperature. Milliequivalents of O_2/kg of oil were used to represent the peroxide value.

Thiobarbituric acid number

Thiobarbituric acid number (TBA) was calculated using the technique. A 10 mL test tube was filled with 1 g of oil, followed by an aqueous solution of 0.1% trichloroacetic acid and a violent vortex. 1 mL each of a 0.375% thiobarbituric acid solution, 15% trichloroacetic acid solution, and 0.25 N hydrochloric acid solution were then added to this tube. The contents were then shaken further before being incubated for 30 minutes at 95°C in a water bath. Using a spectrophotometer (PerkinElmer, Norwalk CT, USA) against a white, the optical density of the aqueous phase was measured at 532 nm after the tubes were taken out of the bath and allowed to cool to room temperature. In ml of malondialdehyde (MDA) per kg of oil, the TBA was stated.

Yield value

Crude walnut oil fractions from three successive replicates were combined, and the yield was estimated as a percentage of the oil extracted from the walnut. The yield was determined using the following equation as published by Nazir *et al.* (2020).

Yields (%): (Extracted oil (g) / (Weight of sample collected (g)) ×100

Refractive index

The refractive index was measured using the standard AOCS procedures. The refractive index was measured at 30°C.

Determination of fatty acid profile

To assess the fatty acid profile of walnut oil, the extracted lipids were methylated to generate fatty acid methyl esters (FAMEs), which were then analyzed using gas chromatography-mass spectrometry (GC-MS) on the Shimadzu GC-2010 Plus model. According to Harynuk *et al.* (2006), 250 mg of extracted lipids were warmed in an oven at 70-80°C and saponified in a test tube with 1.5 mL of methanolic sodium hydroxide solution. The mixture was sonicated for 5 minutes before being treated with 2 mL of boron trifluoride (BF3) to catalyze methylation. Following that, 5 mL of saturated NaCl and 1 mL of iso-octane were added to the solution, which was agitated vigorously. A clear FAME solution was extracted from the aqueous phase after ten minutes, and 1 mL of the organic layer was pipetted into a vial for GC–MS examination.

The GC-MS apparatus was washed three times with hexane before to injection of the sample. A 1 µL aliquot of the FAME solution was injected into the GC-MS equipped with a CP-Sil 5CB capillary column and a preset oven temperature gradient from 60°C to 220°C at a rate of 10°C per minute. The carrier gas was helium at 12 kPa with a split ratio of 1:50, and the total flow rate was 11 mL/min. The obtained chromatogram allowed for the qualitative and quantitative identification of fatty acids in walnut oil, including saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), as detailed by Nazir *et al.* (2020).

Determination of *amino acid profile*

High-performance liquid chromatography (HPLC) was used to determine the amino acid profile of the African walnut flour, in accordance with the procedure outlined by Aderinola and Adeoye (2023). Performic acid and sodium metabisulfite were used to hydrolyse the sample, and it was then heated in hydrochloric acid. The hydrolysate was filtered, diluted, and partly dried after chilling. To get ready for dansylation, the leftover residue was frozen after being reconstituted in a sodium carbonate buffer. Following dansylation and an overnight incubation period, the sample was subjected to HPLC analysis, with findings represented as g/100 g of protein.

Statistical analysis

The experiment's data were analysed using ANOVA, and Duncan's multiple range test was used to identify significant differences ($p<0.05$) among the sample. Using SPSS version 25.0, Duncan's New Multiple Range (DNMR) Test was significant at a p-value < 0.05.

Results and discussion

The proximate effect of boiling on African walnut

Table I indicates the effects of boiling on the proximate composition of African walnut samples. The raw nut sample's proximate composition is similar to that reported by Arinola and Adesina (2014), confirming walnuts' high protein and fat content.

The protein composition of the sample differed significantly; boiling lowered the protein content of the nut from 35.52% for raw nuts to 32.47%, 34.05%, 28.83%, and 29.98% when cooked without shell for 30 minutes, with shell for 30 minutes, without shell for 60 minutes, and with shell for 60 minutes. As shown in the results, boiling with a shell for 30 minutes preserves the protein content more than boiling without a shell. The loss in protein content of African walnuts during boiling might be attributed to denaturation and/or leaching of some nitrogenous component during processing.

The crude fibre content of the African walnut samples increased significantly upon boiling, rising from 1.00% in the raw nut to 6.02%, 7.05%, 8.86%, and 9.69% for the 30-minute and 60-minute boiled samples without shell and with shell, respectively. While in some studies, it was observed that boiling can lead to a reduction in crude fiber due to leaching of soluble fiber into the cooking water. However, in this study of African walnut showed that boiling can increase the crude fiber content. This crude fibre increase could be attributed to the heat treatment promoting the breakdown of other compounds in the walnut matrix, potentially releasing bound fiber or altering the structure of existing fiber components in a way that increases the measured crude fiber content. The crude fibre in the diet has been shown to improve digestibility, decrease the release of glucose into the circulation, facilitate bowel movement, and prevent cancer.

The moisture content of the sample ranged between 2.84% and 3.53%. The maximum moisture level of 3.53% is found in boiling walnuts without shell after 60 minutes of cooking, while the lowest moisture content is found in boiled walnuts

Table I. Proximate composition of African walnut

Samples	Crude protein (%)	Crude fiber (%)	Moisture content (%)	Ash content (%)	Crude fat (%)	CHO (%)
A	32.47 ± 0.44^{c}	6.02 ± 0.03^{d}	2.84 ± 0.08^{c}	3.89 ± 0.07^{c}	50.98 ± 0.09^{a}	3.79 ± 0.48^{b}
B	34.05 ± 0.07^{b}	7.05 ± 0.02^{c}	2.97 ± 0.08^{c}	4.04 ± 0.04^{b}	50.25 ± 0.15^{d}	1.63 ± 0.30^{c}
C	28.83 ± 0.12^{c}	8.86 ± 0.11^{b}	3.53 ± 0.04^{a}	3.24 ± 0.04^{d}	51.12 ± 0.18^{a}	4.41 ± 0.43^{b}
D	29.98 ± 0.07^{d}	9.69 ± 0.13^{a}	3.11 ± 0.13^{b}	4.92 ± 0.07^{a}	48.59 ± 0.12^{c}	3.70 ± 0.35^{d}
E	35.52 ± 0.09^{a}	1.00 ± 0.00^{e}	3.09 ± 0.07^{b}	1.00 ± 0.04^{e}	51.21 ± 0.64^{a}	8.11 ± 0.67^{a}

The same column indicates that values with different superscripts differ significantly ($p<0.05$).
Sample A: boiled for 30mins without shell
Sample B: boiled for 30mins with shell
Sample C: boiled for 60min without shell
Sample D: boiled for 60min with shell
Sample E: Control

without shell after 30 minutes. There was no significant difference between samples A and B or D and E ($p<0.05$). These low values suggest that samples A and B have a longer shelf life because microbiological activity, chemical and metabolic processes are considerably slowed. The low moisture level is extremely beneficial since it reduces spoiling caused by the proliferation of these germs.

The boiled samples had higher ash contents (3.89%, 4.04%, 3.24%, and 4.92%) than the raw nuts (1.00%). Comparing the increased ash content to Neji and Agwupuye (2019), it is ridiculous. While sample E has the lowest value (1.00%), sample D has the highest value (4.92%). Sample D may contain a sufficient amount of mineral elements for maintaining bodily tissues and constructing a healthy body, according to this study.

The crude fat content was highest in sample E (51.21%) compared to samples B and D, while samples A (50.98%) and C (50.25%) showed minimal variation. This suggests that boiling had little to no effect on fat content in samples A and C, whereas a noticeable reduction was observed in samples B and D. These findings are consistent with the report by Okonkwo and Ozoude (2014), who observed a decrease in the ether extract of walnut from 15.84% to 11.54% following processing, indicating that thermal treatment may contribute to fat loss, potentially due to leaching or heat-induced degradation.

Raw and boiling walnut seeds have varying amounts of carbohydrates (1.63% to 8.11%). When compared to the Control (sample E), the amounts of carbohydrates in samples A, B, C, and D have dramatically dropped during the course of the treatments. Given that carbohydrates (reducing sugars) are also substrates of nonenzymatic browning, this may have something to do with the Maillard process (Tenyang *et al.* 2017). These findings supported the findings reported by Onyeike *et al.* (2015), which showed that processing reduced the walnut seed's total carbohydrate content.

Effect of boiling on mineral content in African walnut

African walnut samples' mineral content after boiling is shown in Table II. The macrominerals found in walnuts were found to be significant, with potassium, phosphorus, and sodium being the most prevalent. Compared to the preceding minerals, calcium and magnesium were also present, albeit in less amounts. These mineral elements have previously been shown to be important for human health. Enzymatic reactions, energy production, nerve impulse transmission, and several biological responses are among the bodily processes they are linked to Djikeng Tonfack *et al.* (2017). As compared to samples cooked with a shell, the calcium content of the sample boiled without a shell is much lower, according to the data. Samples C and D contained the least quantity of calcium, 47.06 and 53.09 mg/100g, respectively. This mineral is necessary for bone metabolism, together with phosphorus. Sample E had a sodium level of 88.42 mg/100g, whereas samples A, B, C, and D had concentrations of 56.88, 64.11, 44.89, and 67.09 mg/100g, respectively. This suggests that greater mineral leaching caused the salt composition of the shell-free boiling samples to decrease. Sample E had a magnesium content concentration of 70.98 mg/100g, whereas samples A, B, C, and D had concentrations of 49.02, 62.13, 36.44, and 46.09 mg/100g, respectively. This suggests a decrease in the magnesium content of the shell-free boiling samples, which may be explained by the mineral's dissolution in water during process-

Table II. Mineral composition of African walnut

Sample	Calcium (Ca)	Sodium (Na)	Magnesium (Mg)	Potassium (K)	Phosphorus (P)
A	64.78 ± 0.01^{c}	56.88 ± 0.01^{d}	49.02 ± 0.01^{c}	111.79 ± 0.01^{c}	124.22 ± 0.02^{d}
B	78.36 ± 0.01^{a}	64.11 ± 0.01^{c}	62.13 ± 0.01^{b}	145.31 ± 0.01^{b}	165.80 ± 0.01^{b}
C	47.06 ± 0.01^{e}	44.86 ± 0.02^{e}	36.44 ± 1.16^{e}	76.85 ± 0.01^{e}	143.88 ± 0.02^{e}
D	53.09 ± 0.01^{d}	67.09 ± 0.01^{b}	64.09 ± 0.10^{d}	85.73 ± 0.00^{d}	177.96 ± 0.01^{a}
E	77.43 ± 0.02^{b}	88.42 ± 0.01^{a}	70.98 ± 0.01^{a}	197.43 ± 0.04^{a}	86.71 ± 0.02^{e}

The same column indicates that values with different superscripts differ significantly ($p<0.05$).
Sample A: boiled for 30mins without shell
Sample B: boiled for 30mins with shell
Sample C: boiled for 60min without shell
Sample D: boiled for 60min with shell
Sample E: Control

ing by means of diffusion. However, its content may have increased in samples E and B due to the large destruction of the anti-nutritional in the nut that was complicated to the mineral (Makinde and Akinoso, 2013). It is also evident that, in comparison to the Control (sample E), the potassium content has considerably dropped during boiling. Given their clear connection to human hypertension, the mineral's presence in African walnuts is also advantageous. The plant may be utilised to prevent and manage high blood pressure because of this (James, 2000). All cooked samples of walnuts had higher phosphorus contents. It has a concentration range of 86.71 to 177.96 mg/100g.

Antioxidant

Table III presents the boiling samples' Ferric Reducing Antioxidant Properties (FRAP). The results indicated that compared to the boiled and unshelled samples, the boiled and shelled samples exhibited a substantial ($p<0.05$) decreasing property. Additionally, at the maximum concentration, the extract from shelled walnuts had a greater capacity to scavenge DPPH than the extract from unshelled walnuts.

Table III. Antioxidant properties of African walnut

Samples	FRAP	DDPH	Phenolic	Alkaloid	Flavonoid
A	102 ± 0.05^{b}	96.67 ± 0.51^{c}	155 ± 0.35^{d}	14.25 ± 0.11^{b}	38.52 ± 0.09^{d}
B	88.22 ± 0.06^{e}	87.79 ± 0.04^{d}	203 ± 0.41^{c}	13.82 ± 0.52^{b}	82.53 ± 0.29^{b}
C	98.92 ± 0.13^{c}	108.12 ± 0.98^{b}	138.76 ± 0.50^{e}	11.86 ± 0.07^{d}	47.16 ± 0.10^{c}
D	94.25 ± 0.06^{d}	77.00 ± 0.01^{e}	320.51 ± 0.96^{a}	11.08 ± 0.02^{d}	112.86 ± 0.32^{a}
E	124.08 ± 0.11^{a}	141.19 ± 0.57^{a}	288.22 ± 0.68^{b}	25.54 ± 0.36^{a}	20.66 ± 0.91^{e}

The same column indicates that values with different superscripts differ significantly ($p<0.05$).
Sample A: boiled for 30mins without shell
Sample B: boiled for 30mins with shell
Sample C: boiled for 60min without shell
Sample D: boiled for 60min with shell
Sample E: Control

Boiling walnuts without shells reduced the FRAP of unshelled walnut extract, according to the results of ferric reducing antioxidant capabilities of walnut seed extracts. The findings of this study are consistent with a previous study by Adefegha and Oboh (2011), which found that boiling green leafy vegetables increased their decreased qualities when compared to their raw state. However, the reduction in its capacity to convert Fe3+ to Fe2+ may be the result of prolonged boiling, particularly when the walnuts are unshelled. It has been suggested that dietary antioxidants, like those found in walnuts, may provide protective and preventive measures (Kanu *et al.* 2015) by halting oxidative stress by preventing free radical-induced damage to biomolecules like DNA, proteins, and lipids. This is because oxidative stress has long been linked to the pathogenicity of many degenerative diseases (Ademiluyi *et al.* 2015). Both shelled and unshelled walnut fruit extracts' capacity to scavenge free radicals (DPPH) suggests that walnuts are a great dietary source of antioxidants.

According to the total phenol and total flavonoid, the shelled walnut extracts (having a higher value) scavenged DPPH radicals more than the unshelled walnut extracts, considerably ($p<0.05$). This conclusion is consistent with other research showing that plant extracts' phenolic content is correlated with their ability to scavenge free radicals (DPPH) (Adefegha and Oboh, 2011; Ademiluyi *et al.* 2015). The relationship between the walnut extracts' capacity to scavenge free radicals and their percentages of flavonoids and phenols indicates that these bioactive substances are principally in charge of their antioxidant qualities.

The removal of the shell before cooking, however, may expose the antioxidant components of the unshelled walnut fruit—particularly phenolics—to higher heat degradation and a corresponding decrease in their ability to scavenge free radicals (DPPH). By producing the very reactive hydroxyl radical (OH), which may initiate and spread the lipid peroxidation chain reaction, iron can turn harmful when it combines with hydrogen peroxide through the Fenton reaction (Valko *et al.* 2005). Therefore, it is important to consider the possibility of removing excessive iron buildup from the body. Remarkably, the chelation of Fe2+ by both extracts (shelled and unshelled) was concentration-dependent. It is therefore possible that the antioxidant components of the unshelled walnut seed, particularly the phenolics, were subjected to

greater heat degradation when the shell was removed prior to cooking, which resulted in a considerable decrease in their capacity to chelate Fe2+ and scavenge OH. seen in extracts from unshelled walnuts.

The control sample E had more alkaloids than the boiling samples. Human toxicity and negative consequences are caused by high levels of alkaloids, particularly in physiological and neurological functions. Fowomola (2010) states that some alkaloids, such solanine, might lead to neurological diseases and gastrointestinal disturbances, particularly when consumed in excess of 200 mg/100 g of sample. Alkaloids pharmacological effects mediate significant physiological activities, including analgesia, blood pressure reduction, tumour cell destruction, and circulation and respiration stimulation (Makkar *et al.* 2007). However, dosages distinguish between toxicity. In terms of alkaloid toxicity, plant foods are harmless, particularly African walnuts, if they are properly cooked. Both the boiling and raw samples have low levels of cyanogenic glycosides.

Antinutritional content

Table IV shows the anti-nutritional component activity of the five African walnut samples. It is recognised that anti-nutritional substances decrease the body's ability to absorb nutrients; tannins create insoluble complexes with proteins that lower protein bioavailability, while phytates chelate mineral elements and render them metabolically inaccessible. The nut's phytate concentration was greatly decreased by boiling; this reduction may have resulted from phytate leaching into the cooking medium, heat breakdown, and the creation of insoluble complexes between phytate and other ingredients including protein and minerals. African walnuts' tannin content was similarly decreased by boiling them; this decrease may be caused by phenols leaking into the cooking medium as a result of a concentration gradient.

All polyphenolic chemicals, including tannins, are known to be soluble in water. Similar results on the decrease of phytate and tannin levels in vegetable cowpea (*Sesquipedalis*) seeds after boiling were reported by Udensi *et al.* (2007). Compared to boiling with a shell, boiling without one reduced both anti-nutritional components more.

Oxalate is a salt made from oxalic acid that binds to nutrients and prevents the body from using them. Oxalic acid with a number of other minerals, including calcium, magnesium, sodium, and potassium, create strong connections. Salt is the product of this chemical interaction. Consuming meals high in oxalate inhibits peptic digestion by reacting with proteins to produce complexes. Kidney stones can also result from it precipitating around soft tissues like the kidney. The findings in Table V demonstrate that boiling lowers the amount of oxalate in African walnut. This finding demonstrates how boiling, particularly without a shell, may greatly increase the nutrients' bioavailability in African walnuts.

Amino acid profiles of the African walnut flour

The findings show that the most prevalent amino acids in the samples are aspartic acid (8.89 g/100 g protein) and glutamic acid (10.44 g/100 g protein). Akinhanmi *et al.* (2008) and Ogungbenle and Omaejalile (2010) have reported that African walnuts are high in glutamic acid, which gives them their distinctive umami flavour when cooked. This discovery is in line with their findings. The citric acid cycle and energy generation depend heavily on aspartic acid, whereas glutamic acid is an essential neurotransmitter and intermediate in the metabolism of amino acids.

Table IV. Anti-nutritional analysis result

Sample	Tannin	Phytate	Oxalate
A	96.19 ± 0.55^{e}	0.55 ± 0.00^{b}	25.40 ± 0.44^{c}
B	172.32 ± 0.96^{c}	0.56 ± 0.00^{a}	52.12 ± 0.33^{b}
C	110.09 ± 0.50^{d}	0.43 ± 0.00^{d}	39.37 ± 1.06^{c}
D	180.61 ± 1.05^{b}	0.49 ± 0.00^{c}	33.31 ± 0.44^{d}
E	183.95 ± 0.52^{a}	0.57 ± 0.00^{a}	55.82 ± 0.87^{a}

The same column indicates that values with different superscripts differ significantly ($p<0.05$).
Sample A: boiled for 30mins without shell
Sample B: boiled for 30mins with shell
Sample C: boiled for 60min without shell
Sample D: boiled for 60min with shell
Sample E: Control

Table V. Amino acid composition of African walnuts boiled with their shells for 30 minutes

Essential Amino Acid	g/100g protein
Histidine	2.38
Isoleucine	3.61
Leucine	5.81
Lysine	5.09
Phenylalanine	4.58
Methionine	2.05
Threonine	3.83
Tryptophan	0.91
Valine	4.73
Non-Essential Amino Acid	g/100g protein
Alanine	5.10
Arginine	3.72
Aspartic acid	8.89
Cystine	1.38
Proline	3.65
Glutamic acid	10.44
Glycine	5.07
Serine	4.25
Tyrosine	2.70

Leucine (5.81 g/100 g protein) and lysine (5.09 g/100 g protein) are prevalent among the essential amino acids (EAAs). These amino acids are essential for the repair of tissues, growth, and the production of muscle proteins. The amounts of valine (4.73 g/100 g protein), phenylalanine (4.58 g/100 g protein), and threonine (3.83 g/100 g protein) are likewise much higher than the FAO/WHO-recommended values for protein consumption in the diet. African walnuts are positioned by these EAAs as a potentially beneficial addition to cereal-based diets, which are generally lacking in lysine and threonine.

Glutamic acid, alanine (5.10 g/100 g protein), and glycine (5.07 g/100 g protein) are the most common non-essential amino acids (NEAAs) in the samples. These amino acids are necessary for several metabolic functions, such as protein metabolism, detoxification, and immunological response. As reported in research on legumes and nuts (Adebisi *et al.* 2020; Kanu *et al.* 2009), partial degradation during processing may be the cause of the lower quantities of thermolabile amino acids like tryptophan (0.91 g/100g protein) and cystine (1.38 g/100g protein).

African walnuts amino acid digestibility is improved by boiling them because it lowers anti-nutritional elements such phytates and tannins (Oboh *et al.* 2010). This affirms the existing findings that the protein quality of the samples is supported by the overall amino acid profile. Further supporting African walnuts' potential as a dietary protein source is the fact that their amino acid composition is comparable to that of legumes like soybeans.

Lipid quality

TBA Value

Thiobarbituric acid value (TBA) measures the emission of secondary oxidation, mostly malonaldehyde, which can give oxidised oil an off-flavor (Tonfack-Djikeng *et al.* 2017). Table VI illustrates the impact of varying boiling periods on the TBA value of walnut oil. A significant difference ($p>0.05$) was observed between samples B and E's TBA values. However, as compared to samples B and E, the boiling samples showed a substantial increase ($p<0.05$) in this parameter. The greatest TBA value was shown by Sample C. This suggests that the rate of primary and secondary oxidation in this sample was substantially greater. This demonstrates that, in comparison to other methods, drying and boiling walnut oil for 60 minutes without a shell has substantially changed its quality. The production of malonaldehyde, a secondary oxidation product derived from the breakdown of hydroperoxide, is the cause of the rise in TBA value seen in the boiling samples (Womeni *et al.* 2016). It has previously been shown that boiling can speed up the pace at which edible seeds produce secondary oxidation products. According to Tenyang *et al.* (2017), the p-anisidine value of sesame oil rises considerably with increasing boiling temperatures and intervals.

Acid value

An essential indicator of food rancidity may be the increase in the acid value (AV) of oils. Triglyceride hydrolysis produces free fatty acids, which can be accelerated by oil-moisture interactions. The table VI shows how the acidity of walnut oil changes throughout processing. Between the following treated samples (A, B, C, and D) and the Control (sample E), a significant change was observed. However, as compared to the Control and the other processed samples, sample BAV significantly increased, while sample C AV significantly decreased. The rapid hydrolysis of sample B

Table VI. Lipid quality of the African walnut

Sample	TBA	Refractive Index	Yield	Acid value	Peroxide	Iodine
A	6.52 ± 0.03^{b}	1.48 ± 0.00^{d}	50.63 ± 0.22^{c}	14.40 ± 0.02^{d}	9.99 ± 0.08^{a}	109.74 ± 1.63^{a}
B	4.05 ± 0.00^{c}	1.48 ± 0.00^{a}	50.99 ± 0.01^{b}	31.39 ± 0.06^{b}	11.07 ± 0.12^{b}	100.04 ± 0.08^{b}
C	9.64 ± 0.21^{a}	1.48 ± 0.00^{b}	52.05 ± 0.06^{a}	13.40 ± 0.01^{e}	8.51 ± 0.10^{c}	92.28 ± 0.36^{c}
D	6.39 ± 0.02^{b}	1.48 ± 0.00^{c}	49.04 ± 0.03^{d}	29.10 ± 0.01^{c}	7.10 ± 0.04^{d}	83.06 ± 0.07^{d}
E	3.03 ± 0.02^{d}	1.48 ± 0.00^{d}	52.01 ± 0.05^{a}	70.62 ± 0.10^{a}	4.97 ± 0.01^{b}	101.03 ± 0.47^{b}

The same column indicates that values with different superscripts differ significantly ($p<0.05$).
Sample A: boiled for 30mins without shell
Sample B: boiled for 30mins with shell
Sample C: boiled for 60min without shell
Sample D: boiled for 60min with shell
Sample E: Control

triglycerides, which results in the buildup of free fatty acids, may be the cause of the significant spike in AV observed in that sample. The rapidly occurring transformation of the free fatty acid present into hydroperoxides may be the cause of sample C lower acid value when compared to sample E.

Peroxide value

The amount of primary oxidation products in oils, primarily hydroperoxides, is frequently assessed using the peroxide value (PV) (Shahidi and Wanasundara, 2008). The table VI shows the variations in PV of samples of walnut oil. In comparison to the Control (sample E), the peroxide values of all treated samples (samples A, B, C, and D) have increased noticeably. The maximum PV of 11.07, observed in sample B, was much higher than the acceptable peroxide value of oils and fats of 10meq O2/kg. Nevertheless, the PV of the other treated samples and the control sample was below the suggested value, indicating that these procedures preserved walnut oil better than sample B. According to Nyam *et al.* (2013) and Womeni *et al.* (2016), the buildup of hydroperoxides caused by free radicals attacking the unsaturated fatty acids in oil is probably the reason for the spike in peroxide value in the treated samples when compared to the Control. These findings concur with those of Tenyang *et al.* (2017), who showed that boiling sesame seeds increases their peroxide content.

Iodine value

The level of unsaturation of edible oils and fats is often determined by the iodine value (IV). According to Tynek *et al.* (2001), a decline in this parameter is typically ascribed to free radicals breaking the double bonds of polyunsaturated fatty acids. The table VI demonstrates how the IV of walnut oil samples changed throughout processing. In comparison to the Control (sample E), the treatments have significantly decreased and increased the iodine value. The most affected sample was the sample boiled with a shell for 60 minutes (sample D) because its IV decreased from 101.03 (IV of the Control) to 83.06. As previously stated, the significant reduction in iodine value observed in certain treated samples can be linked to the free radicals generated during processing breaking the double bonds of their unsaturated fatty acids. When sample D's reduction was more evident than in the other treated samples, it suggests a significant change in the unsaturation of its fatty acids. Particularly, the iodine amounts found in nearly all of the samples were within the recommended range of 120 to 155g I2/100g for walnut oil.

Refractive index

The refractive index, also known as an index of refractive, is a measurement of the rate at which a light beam blends when it moves through different materials (Oyedele and Oladipo,

Table VII. Identified Fatty acids of African walnut (Control, with or without shell 30 minutes, without or without shell 60 minutes)

S/N	Compound name	Abundance (%)	Molecular weight	Molecular formula	Common/Other names
Raw walnut (as control)					
1	Hexadecanoic acid, ethyl ester	1.33	284	C18H36O2	Palmitic acid ethyl ester
2	9,12-Octadecadienoic acid, methyl ester	0.59	294	C19H34O2	
3	9,12,15-Octadecatrienoic acid, methyl ester	1.01	292	C19H32O2	Linolenic acid methyl ester
4	9,12-Octadecadienoic acid, ethyl ester	19.15	308	C20H36O2	
5	9,12,15-Octadecatrienoic acid, ethyl ester	32.94	306	C20H34O2	Linolenic acid ethyl ester
6	Octadecanoic acid, ethyl ester	2.18	312	C20H40O2	Stearic acid, ethyl ester
7	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	2.56	568	C35H68O5	Dipalmitin
8	14-Methyl-8-hexadecyn-1-ol	23.55	252	C17H32O	
9	9,12,15-Octadecatrienoic acid,	10.87	278	C18H30O2	Linolenic acid
10	Octadecanoic acid, 2-hydroxy-1,3-propanediyl	3.10	624	C39H76O5	Glycerin 1,3-distearate
Walnut with shell and boiling for 30 minutes					
1	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	5.25	568	C35H68O5	Dipalmitin
2	9,12-Octadecadienoic acid -2-hydroxy-1-(hydroxymethyl) ethyl ester	1.18	354	C21H38O4	beta.-Monolinolein
3	14-Methyl-8-hexadecyn-1-ol	48.90	280	C18H32O2	cis,cis-Linoleic acid
4	9,12,15-Octadecatrienoic acid	30.92	278	C18H30O2	Linolenic acid
5	Octadecanoic acid, 2-hydroxy-1,3-propanediyl	11.18	624	C39H76O5	Glycerin 1,3-distearate
Walnut without shell and boiling for 30 minutes					
1	Ethyl Oleate	3.32	310	C20H38O2	Oleic acid, ethyl ester
2	9,12,15-Octadecatrienoic acid, ethyl ester	4.93	306	C20H34O2	Linolenic acid, ethyl ester
3	Hexadecanoic acid, ethyl ester	0.95	284	C18H36O2	Palmitic acid, ethyl ester
4	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	3.02	568	C35H68O5	Dipalmitin
5	9-Octadecenamide	1.00	281	C18H35NO	Oleic acid amide
6	9,12,15-Octadecatrienoic acid, methyl ester	1.42	292	C19H32O2	Linolenic acid, methyl ester
7	9,12-Octadecadienoyl chloride,	35.68	298	C18H31ClO	Linoleic acid chloride
8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	35.52	278	C18H30O2	Linolenic acid
9	Octadecanoic acid, 2-hydroxy-1,3-propanediyl	13.17	624	C39H76O5	Glycerin 1,3-distearate
Walnut with shell and boiling for 60 minutes					
1	Hexadecanoic acid, methyl ester	5.61	270	C17H34O2	Palmitic acid, methyl ester
2	14,17-Octadecadienoic acid, methyl ester	58.46	294	C19H34O2	Punicic acid
3	9,12,15-Octadecatrienoic acid, methyl ester	34.63	292	C19H32O2	Linolenic acid, methyl ester
4	Cis-11-Eicosenoic acid, methyl ester	1.31	324	C21H40O2	
Walnut without shell and boiling for 60 minutes					
1	3,13-Octadecadien-1-ol	2.88	266	C18H34O	
2	9,12,15-Octadecatrienoic acid, methyl ester	5.26	292	C19H32O2	Linolenic acid, methyl ester
3	Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	3.70	568	C35H68O5	Dipalmitin
4	9,12-Octadecadienoic acid	38.03	280	C18H32O2	cis,cis-Linoleic acid
5	9,12,15-Octadecatrienoic acid	35.94	278	C18H30O2	Linolenic acid
6	Octadecanoic acid, 2-hydroxy-1,3-propanediyl	11.49	624	C39H76O5	Glycerin 1,3-distearate

2014). Another way to define it is as a dimensionless number that characterises the way radiation, such as light, moves through a medium. This study used the refractometer as described by Ayo and Agu (2012) to calculate the refractive index. The refractive index of the raw and processed samples did not differ significantly, indicating that the African walnut oils' refractive index is unaffected by variations in temperature and time.

Total yield

Table VI presents the oil yield from raw and cooked African walnuts using various techniques (shelled and unshelled), and the results are significantly different ($p<0.05$). Although the oil output of the raw sample is greater than that of the cooked samples, sample E and sample C did not differ significantly. It would be cost-effective to produce African walnut oil for industrial or culinary uses, according to this. This finding suggests that the oil production in samples A, B, and D is decreased by thermal processing.

Fatty acid profile

Walnuts typically have a high oil content, however this might differ depending on the species, cultivar, and geographic area. Fatty acids abound in walnut oil, with linoleic acid, oleic acid, linolenic acid, palmitic acid, and stearic acid being the main fatty acids found in walnuts (Batun *et al.* 2017). Polyunsaturated Fatty Acids (PUFA) are also known to be present in significant amounts in walnut oil. Studies have indicated that consuming a high PUFA intake lowers blood pressure and LDL cholesterol, as well as total cholesterol, which lowers the risk of coronary heart disease in individuals (Iso *et al.* 2002; Batun *et al.* 2017). However, these health advantages, walnuts' high PUFA content renders them oxidisable, which shortens the product's shelf life (Jensen *et al.* 2003).

Tables VII-XI summarised the identified fatty acids as primarily Fatty Acid Methyl Esters (FAME) and a few as Fatty Acid Ethyl Esters (FAEE), along with their corresponding percentage abundance, molecular weight, and chemical formula.

Figures 1–5 show the chromatogram of the fatty acid profile of the walnut samples. The fatty acids observed in this study's results are consistent with those found by Batun *et al.* (2016) and Petrovi'c-Oggiano *et al.* (2020), who found that the main fatty acids in walnuts are oleic, linolenic, palmitic, and stearic acids. The control sample, on the other hand, contains a higher concentration of linolenic acid than stearic acid, although palmitic acid was found at a low concentration (1.33%). It is found that the main ingredient in all of the therapies is linolenic acid. All of the samples share it, and it remained highly abundant even after different heat treatments with and without shells. This indicates that the amount of linolenic acid in walnuts is mostly unaffected by heat treatment.

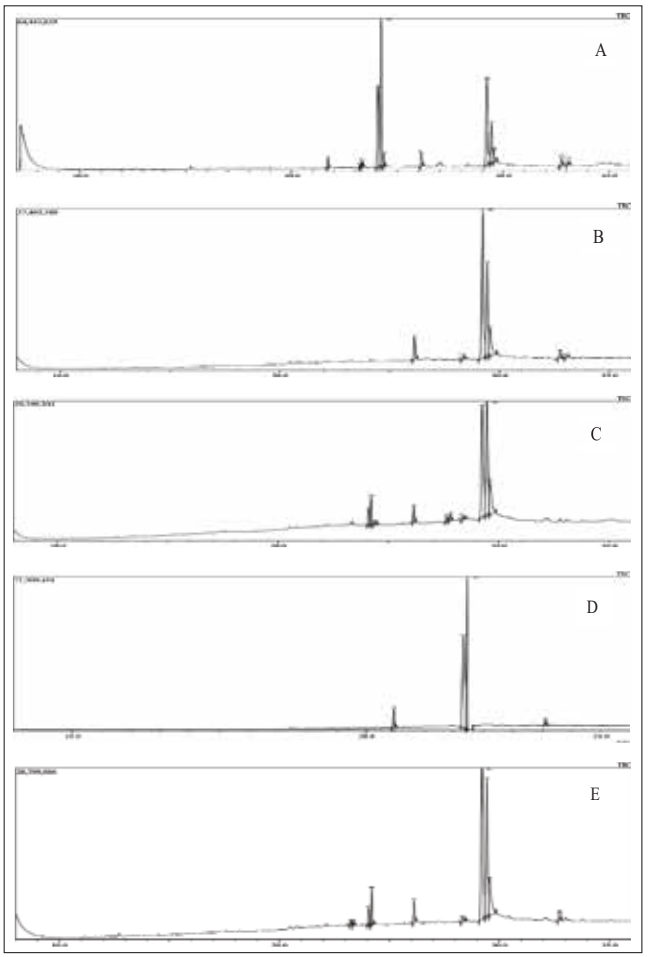

Fig. 1. Chromatogram of fatty acid profile of (A) control; (B) with shell 30 minutes; (C) without shell 30 minutes; (D) with shell 60 minutes; (E) without shell 60 minutes

Nonetheless, a recent study by Khaled *et al.* (2024) shown that repeatedly heating edible oils can reduce their fatty acid composition, physicochemical characteristics, and generally quality. Additionally, the results showed that, with the exception of the shell 60-minute treatment, dipalmitin—a diacylglycerol made up of two palmitic acid molecules esterified to glycerol as the backbone—was present in all treatments, albeit in varying amounts (2.56–5.25%).

The control sample (1.33%) had palmitic acid after 30 minutes without a shell (0.95%) and 60 minutes with a shell (5.65%); the samples without a shell 60 and with a shell 30 had insufficient amounts of the fatty acid. For 30 minutes, oleic acid was only detected without a shell at 3.32% abundance.

Only the sample with shell for 60 minutes has the largest amount of punicic acid (58.46%). Pomegranate seed oil is the primary source of this unsaturated fatty acid, which has been shown to offer possible health advantages like anti-inflammatory, anti-carcinogenic, and antidiabetic effects. It also includes conjugated linolenic acid (Koba and Yanagita, 2011).

Conclusion

The substantial nutritional potential of African walnuts is demonstrated by this study, which also shows that boiling improves the composition of the fruit. Increased crude fibre, greater protein levels, and improved nutritional advantages were all maintained by boiling with the shell. The amino acid analysis showed a rich profile, with important amino acids including leucine, lysine, and valine contributing to its excellent protein quality and glutamic acid and aspartic acid being the most prevalent. Especially for cultures with limited access to animal protein, our findings imply that adding African walnut meal to traditional foods, such stiff dough meals, can greatly increase protein consumption. High amounts of lysine and threonine fill important nutritional gaps in diets based on cereals, and non-essential amino acids support general structural and metabolic processes. Furthermore, the mineral results revealed that the cooked samples with the shell had much stronger antioxidant qualities and vital minerals, with the phosphorus level increasing after boiling. A further indication of increased nutrient bioavailability is the decrease in anti-nutritional agents. Overall, walnut proves to be a useful food item, supporting its inclusion in diets to increase nutritional diversity in environments with limited resources, address protein deficiencies and promote health.

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