COMPARATIVE STUDIES ON THE EFFECT OF BOILING AND FERMENTATION ON CALABASH GOURD MELON (*LAGENARIA SICERARIA*) SEEDS

ABSTRACT

The research was carried out to collect some useful information on *Lagenaria siceraria* (Calabash gourd melon) seeds. The dried melon seeds were sorted, cleaned, soaked in water for easy removal and dehulled by abrasion to get the cotyledons, which were divided into three parts. The first part was used directly for analysis, the second part was boiled while the third part was fermented and analysed. Boiled melon had the highest fat and protein content while the raw melon had the highest carbohydrate content, however there was no significant increase in the ash content of the samples. There was significant reduction in the antinutritional factors of boiled and fermented melon samples compared to the raw melon. Processing led to significant increase in the antioxidant activities and vitamin contents. The study observed that processing led to significant increase in the nutritional composition of the melon seeds and significant reduction in the antinutritional factors.

Keyword: *Lagenaria siceraria*; Processing- Fermentation and Boiling; proximate composition; Antioxidants; Antinutritional factors; Vitamins.

INTRODUCTION

Plant seeds are good source of food for animals, as well as humans, since they contain nutrients necessary for plant growth, including many healthy fats, such as Omega fats. In fact, the majority of foods consumed by human beings are seed-based foods, nevertheless, all seeds are not edible (Burnham and Johnson, 2004). Melon seeds are rich in fat and protein for some cucurbitaceous plants (squash, melon, gourd), which after being dried and ground are used as a major ingredient in West African cuisine (Massaquoi, 2011). Melon is a cucurbit crop that belongs to the cucurbitaceae family having fibrous and shallow root system. It is a tendril climber or crawling annual crop, mostly grown as subsidiary crop interplanted with early maize and yam in some savannah belt of Nigeria. Melons are major food crops with several varieties which serve as food sources (Mabalaha *et al*., 2007).

Cucurbitaceae family is commonly known as the gourd, melon or pumpkin family, is medium sized generally a climbing plants family, composing 118 genera and 825 species having wide distribution in the warmer regions of the world. The plants of cucurbitaceae family provide the major contribution for economically important domesticated species and many of these are earliest cultivated plants and are used for medical and nutritional values (Habib-ur-Rahaman, 2003). Among all plants of the cucurbitaceae family, *Lagenaria* species is the most popular (Minocha, 2015). *Lagenaria siceraria* is an annual climbing or crawling plant that is commonly cultivated in Nigeria. It is called calabash gourd or bottle gourd in English, members of this family consist of different varieties such as calabash, bottle gourd and calabash gourd and are widely used for ornamental purposes (Sani *et al*., 2013). The numerous variations in shapes of these fruits are as a result of cross pollination of flowers of different species; this yields a different fruit with shape and size different from that of parent flowers (Decker-Walter *et al*., 2004). This resulted into a dilemma on assigning scientific names to each variety; hence some of the varieties were called using local names of the geographical location of their origin. Its seeds are brown in colour, rectangular in shape and of variable size depending on the variety (Decker-Walter *et al*., 2004 & Tsieri *et al*., 2008).

The genus *Lagenaria* contains six species, probably all originally old world and mainly African (Teppner, 2004). Ibiok *et al*. (1991) showed that variation exist in morphology of leaves, fruits and seeds of the five different cultivars grown in southern Nigeria. In most local African communities, a number of land races, are cultivated for myriad of uses such as food and containers, corresponding to the characteristics of the fruit. As the morphological variation in *L. siceraria* is diverse and continuous, it is difficult to classify the land races into distant groups. However, they are generally distinguished by the size and shape of their fruits with common names (Heiser *et al*., 1979, Essien *et al*., 2013).

Lagenaria siceraria (previously known as *L. vulgaris* ser.) is indigenous to Africa and that it reached temperate and tropical areas in Asia and America.

It can be cultivated in all kinds of soil but thrives best in heavily manured loams. It requires warm humid climate or plenty of water when grown during dry weather. *Lagenaria siceraria* gourds are grown in most part of Nigeria examples include *L. siceraria* (African Wine kettle), otherwise known as "Akeregbe", *L. siceraria* (Basketball gourd) called "Igbaademu", and *L. siceraria* (Bushel Giant Gourd) known as "Igba-je" in Yoruba land (Ogundele *et al*., 2010).

Bottle gourd can be used for the treatment of mental health disorders due to its highest content of choline. The seeds of the bottle gourd, pumpkin and melons are encapsulated with innumerable phytochemicals, vitamins, minerals and amino acids along with essential fatty acids, omega fatty acids which are the major components of the communicating membranes of the brain (Rahman, 2003).

The fruits, leaves, oil, and seeds are edible and used by local people as folk medicines in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. The fruit pulp is used as an emetic, sedative, purgative, cooling, diuretic, antibilious, and pectoral. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness (Kirtikar *et al*., 2005, Rahman, 2003). In Curacao, a leaf decoction is taken for flatulence. A poultice of the crushed leaves has been applied to the head to treat headaches. Taken with *Achyranthes* spp., the seed is used to treat aching teeth and gums, boils, etc. Pulverized seed kernels are taken to expel intestinal worms (Pullaiah, 2006, Duke *et al*., 1985).

It has been observed that not much has been reported on the microbiological and biochemical effect of processing on *Lagenaria siceraria.* Therefore, this study will elucidate how processing such as boiling and fermentation affects its nutritional composition and health potential, thus enhancing the knowledge of suitable food processing for consumption of *Lagenaria siceraria* (calabash gourd melon known as 'Egusi Igba' in Yoruba land) seeds.

MATERIALS AND METHODS

Collection of Samples

The melon seeds (*Lagenaria siceraria*) were purchased from Oja Oba market in Ado- Ekiti and from Agbado main market, Agbado in Ekiti state. The seeds were authenticated at the plant science Department of Ekiti State University, Ado-Ekiti, Ekiti state, Nigeria.

Processing of Sample

The undehulled melon 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

Raw Sample: The seeds were dehulled by abrasion. These were then cleaned and separated from grits, oven dried at 60^0C for 96 h, pulverized using blade homogenization and poured into a sterile container covered with screw cap for analysis.

Boiled Sample: The unshelled melon seeds were firstly dehulled by abrasion. The seeds were washed using distilled water and cooked for 3 h in boiling water. The boiled seeds were then oven dried at 60^0C for 96 h, pulverized using blade homogenization and poured into a sterile container covered with screw cap for analysis.

Fermented Sample: The unshelled melon seeds were dehulled by abrasion. The seeds were washed with distilled water, cooked for 4 h in boiling water and allowed to cool to about 30°C. The cooled melon seeds were wrapped in aluminum foil and incubated at 35°C for 120 h. The fermented seeds were then oven dried at 60^0C for 96 h, pulverized using blade homogenization and poured into a sterile container covered with screw cap for analysis.

2.5.3 Determination of Moisture Content

Two Petri dishes were washed and dried in an oven, cooled in a desiccator and weighed. 2 g of the sample was added to the dishes labeled 1 and 2 and transferred to the oven set at 100°C and left for 24 h, the samples were cooled in the desiccator and reweighed.

% Moisture= X 100

W_1 = weight of sample

W_2 = weight of sample + dish before drying

W_3 = Weight of sample + dish after drying

2.6.1 Determination of Fat Content

Procedure:

60 g of pulverised sample was weighed into a filter paper, the wrapped filter paper was placed inside the inner part of the soxhlet extractor. The apparatus was then fitted to a round bottom flask, which contained 200 cm^3 of hexane solvent. It was then attached to a reflux condenser. The set-up was clamped and heated on water bath. After the extraction has been certified completed by the extracting solution being clear, the solvent was distilled off in the distillation set. The oil was then poured into a bottle and left for 5 days for the remaining solvent to evaporate. The oil was then weighed and the percentage oil content determined.

% Oil yield = x 100

2.6.2 Determination of Crude Fibre

The organic residue left after sequential extraction of sample with ether can be used to determine the crude fibre. The fat-free material is then transferred into a flask/beaker and 200 mL of pre-heated 1.25 % H_2SO_4 is added and the solution is gently boiled for about 30 min, maintaining constant volume of acid by the addition of hot water. The buckner flask funnel fitted with Whatman filter is pre-heated by pouring hot water into the funnel. The boiled acid sample mixture is then filtered hot through the funnel under sufficient suction. The residue is then washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200 mL of pre-heated 1.25 % Na_2SO_4 is added and boiled for another 30 min. Filter under suction and wash thoroughly with hot water and twice with ethanol. The residue is dried at 65°C for about 24 h and weighed. The residue is transferred into a crucible and placed in muffle furnace (400-600°C) and ash for 4 h, then cool in a desiccator and weigh.

% Crude fibre =   X 100

W_1 = weight of sample

W_2 = Dry weight of residue before ashing

W_3 = Weight of residue before ashing

2.6.3 Determination of Crude Protein

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16 % nitrogen. Crude protein is determined by Kjeldahl method**.** The method involves: Digestion, Distillation and Titration.

Digestion: weigh about 2 g of the sample into kjeldahl flask and add 25 mL of concentrated sulphuric acid, 0.5 g of $CuSO_4$, 5 g of Na_2SO_4 and a speck of selenium tablet. Apply heat in a fume cupboard slowly at first to prevent undue frothing, continue to digest for 45 min until the digesta become clear pale green. Leave until completely cool and rapidly add 100 mL of distilled water. Rinse the digestion flask 2-3 times and add the rinsing to the bulk.

Distillation: Markham distillation apparatus is used for distillation. Steam up the distillation apparatus and add about 10 mL of the digest into the apparatus via a funnel and allow it to boil.

Add 10 mL of sodium hydroxide from the measuring cylinder so that ammonia is not lost. Distil into 50 mL of 2 % boric acid containing screened methyl red indicator.

Titration: the alkaline ammonium borate formed is titrated directly with 0.1 N HCl. The titre value which is the volume of acid used is recorded. The volume of acid used is fitted into the formula which becomes

% N =   X 100

VA = volume of acid used w = weight of sample

% crude protein = % N x 6.25

2.6.4 Determination of Ash Content

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600°C in muffle furnace for 4 h. 2 g of the sample is weighed into a pre-heated crucible. The crucible is placed into muffle furnace at 400-600°C for 4 h or until whitish-grey ash is obtained. The crucible is then placed in the desiccator and weighed

% Ash =   X 100

2.6.5 Determination of Carbohydrate Content

This was determined by difference in percentage.

(% Carbohydrate = 100 - total weight of other nutritional factors)

2.7.1 Determination of Saponin

The saponin content of the sample was determined by double extraction gravimetric method (Harborne, 1973 and Uematsu, 2000). 5 g of the powdered sample was mixed with 50 ML of 20 % aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 min at 55°C; it was then filtered through Whatman filter paper (No.42). The residue was extracted with 50 mL of 20 % ethanol and both extract was poured together and the combined extract was reduced to about 40 mL at 90°C and transferred to a separating funnel where 40 mL of Diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60 mL of normal butanol. The combined extracts were washed with 5 % aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60^{o}C in the oven and reweighed after cooling in a desiccator. The process was repeated two more times to get an average saponin content was determined by difference and calculated as a percentage of the original sample thus;

% Saponin = X 100

W_1 = weight of evaporating dish

W_2 = weight of dish + sample

W_3 = weight of sample

2.7.2 Determination of Phytate

2 g of each sample was weighed into 250 mL conical flask 100 mL of 2 % HCl was added to soak each sample in the conical flask for 3 h. This was filtered through a double layer of hardened filter paper. 50 ml of each filtrate was placed in 0.50 mL conical flask and 107 mL

distilled water was added in each case to give proper acidity. 10 mL of 0.3 % Ammonium Thiocyanate (NH_4SCN) solution was added into each solution as indicated. This was titrated with the standard iron (III) chloride solution which contained 0.00195 g Iron per mL, the end point was slightly brownish-yellow which persisted for 5 min. The % phytic acid was calculated using the formula:

% Phytic Acid =

2.7.3 Determination of Flavonoid

The flavonoid content of the seeds was determined by the gravimetric method as was described by Harborne (1973). 5 g of the pulverized sample was placed into a conical flask and 50 mL of water and 2 mL HCL solution was added. The solution was allowed to boil for 30 min. The boiled mixture was allowed to cool before it was filtered through what-man filter paper (N0. 42). 10 mL of ethyl acetate extract which contained flavonoid was recorded while the aqueous layer was discarded. A pre-weighed what-man filter paper was used to filter the sample (ethyl-acetate layer), the residue was then placed in an oven to dry at 60^oC. It was cooled in a desiccator and weighed. The quantity of flavonoid was determined using the formula.

% Flavonoid = X 100

Where:

W_1 = Weight of empty filter paper

W_2 = Weight of filter paper and Flavonoid extract

W_3 = Weight of sample

2.7.4 Tannin determination

The tannin content of the seeds was determined using the Folin Dennis spectrophotometric method described by Pearson (1976). 2 g of the powdered sample was mixed with 50 mL of distilled water and shaken for 30 min in the shaker. The mixture was filtered and the filtrate used for the experiment. 5 mL of the filtrate was measured into 50mL volume flask and diluted with 3 mL of distilled water. Similarly, 5 mL of standard tanuric acid solution and 5 mL of distilled water was added separately. 1 mL of Folin-Dennis reagent was added to each of the flask followed by 2.5 mL of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90 min at room temperature. The absorbance of the developed colour was measured at 760 nm wave length with the reagent blank at zero. The process was repeated two more time to get an average. The tannin content was calculated as shown below.

% Tannin = x x x x D

Where,

W = Weight of sample analyzed

AU = Absorbance of the test sample

AS = Absorbance of the standard solution

C = Concentration of standard in mg/mL

VA = Volume of filtrates analyzed

D = Dilution factor where applicable

2.7.5 Determination of Cardiac gylcosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy *et al.* (1994). 1 g of the fine powder of plant was soaked in 10 mL of 70 % alcohol for 2 h, and then filtered. The extract obtained was then purified using lead acetate and

Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95 mL aqueous picric acid + 5 mL 10 % aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

2.7.6 Determination of inhibitor activity

Reagents:

1. Tris buffer (0.05 M, pH 8.2) containing $CaCl_2$ (Dissolve 6.05 g tris (hydroxymethylamine) methane and 2.94 g $CaCl_2{\cdot}H_2O$ in 900 mL water. Adjust pH to 8.2, and dilute volume to 1 litre with water).

2. Substrate solution (containing 40 mg benzoyl-DLarginine-p-nitroanalide (BAPA) hydrochloride in 100 mL reagent 1. Dissolve 40 mg BAPA in 1 mL dimethyl sulfoxide and dilute to 100 mL with reagent 1, prewarmed to 37°C).

3. Trypsin solution (containing 4 mg trypsin (2 ×crystallized, salt-free) in 200 mL 0.001M HCl.

4. Acetic acid solution containing 30 mL glacial acetic acid in 70 mL water).

Procedure:

Preparation of sample

1 g of powdered sample (defatted) was extracted with 50 ml NaOH/ g of sample for 3 h, with magnetic stirrer at low setting. Portions (0, 0.6, 1.0, 1.4, and 1.8 mL) of diluted suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 mL with water. 2 mL trypsin solution (reagent 3) was added to each test tube and placed in water bath at 37°C and mixed thoroughly.

5 mL substrate solution (reagent 2) previously warmed to 37°C was added and the reaction was stopped at exactly 10 min later by adding 1 mL acetic acid solution (reagent4) and then mixed.

The solution was filtered with Whatman no. 2 paper and the absorbance was measured at 410 nm.

For Blank preparation:

5 mL (reagent 2) was added to 2 mL sample extract, incubated at 37°C for 10 min, and 1 mL (reagent 4) added and followed by addition of 2 mL (reagent 3).

One trypsin unit is arbitrarily defined as increase of 0.01 absorbance unit at 410 nm per 10 mL of reaction mixture under conditions used herein. Trypsin inhibitor activity is expressed in terms of trypsin inhibitor units (TIU).

Calculations

Plot of TIU/mL versus volume of extract (mL) taken for analyses was prepared on regular graph paper and extrapolated to zero.

NOTE: TIU/g sample = extrapolated value ×dilution factor.

2.8.1.1 Determination of vitamin A

1 g of the sample 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 2 ml of TCA chloroform were added and the absorbance measured at 620 nm. Then concentration of vitamin A was extrapolated from the standard curve.

2.8.1.2 Determination of vitamin B_1 (thiamine)

5 g of samples are homogenized with 50 mL of ethanolic sodium hydroxide solution. This was filtered into a 100 mL flask. 10 mL of the filtrate was pipetted into a beaker and color 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 (Okwu, 2005).

2.8.1.3 Determination of riboflavin (Vitamin B_2)

Five grams (5 g) of each of the samples was extracted with 100 mL of 50 % ethanol solution shaken for 1 hr. This was filtered into a 100 ml of 30 % hydrogen peroxide (H_2O_2) and allowed to stand over hot water bath for 30 min. 2 mL of 40% sodium sulphate added to make up the 50 mL mark and absorbance read at 510 nm in a spectrophotometer (Okwu, 2005).

2.8.1.4 Determination of niacin (Vitamin B_3)

5 g of sample was blended and 100 mL of distilled water added to dissolve all nicotinic acid or niacin present. 5 mL of this solution was drawn into 100 mL volumetric flask and make up 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 sample was calculated using the formula:

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

2.8.1.5 Determination of ascorbic acid (Vitamin C)

Vitamin C content was determined according to the method of Barakat *et al*. (1973). 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 shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for 20 min. It was transferred into 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 (Baraket *et al*., 1973).

2.8.1.6 Determination of vitamin E

1 g of the sample was weighed and macerated with 20 mL of ethanol. 1 mL of 0.2 % ferric chloride in ethanol was added, then 1 mL of0.5 % 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.

2.8.2 Determination of Antioxidant Activity

The antioxidant activity was determined by means of 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 in a test tube. The mixtures were shaken for 1 min and then left in the dark

% 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).

2.8.2.1 DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of methanolic, hexanic, and aqueous extracts of sample was determined according to the method reported by Brad-Williams *et al.* (1995) with slight modification. 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 about0.98 (± 0.02) at 517 nm.

In a test tube, 3 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 a period of 30 min. The percent antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As)/ Ac] \times 100$$

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

2.8.2.2 Total phenolics

The total phenolic content was measured using the Folin Ciocalteu reagent McDonald *et al*. (2001). 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 2 h. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The results were expressed in terms of µg gallic acid equivalents (GAE)/ mg dry extract Soni N *et al.* (2014).

RESULTS

The proximate composition of samples is shown in Table 1. There was no statistical difference in the ash content of the sample, the values were 3.03±0.22 for RM, 3.00±0.22 for BM, 3.41±0.18 for FM. The crude fibre is statistically different with fermented melon having the highest value (4.33±0.54) and the raw melon had the lowest value (3.20±0.25). The fat content shows significant difference, the raw and boiled melon samples had the same highest value (30.43±0.09) compared to the fermented melon with 29.81±0.29. The Protein content of the boiled melon was significantly higher than the raw and fermented melon. The carbohydrate content of the raw melon was observed to be higher than others. The boiled melon had the lowest value (40.88±0.32). Table 2 & 3 shows the anti-nutritional (%) and the antioxidant activity (%) of the melon samples. The anti-nutritional factors determined were saponin, Trypsin inhibitor, flavonoid, phytate, cardiac glycoside and tannin.

TABLE 1: Proximate composition of raw, boiled and fermented melon seed

Proximates (%)	Raw	Boiled	Fermented
Moisture content	$6.00^{e}\pm0.87$	$34.96^{c}\pm0.09$	$41.67^{e}\pm0.26$
Ash content	$3.03^{a}\pm0.22$	$3.00^{a}\pm0.22$	$3.41^{a}\pm0.18$
Crude fibre	$3.20^{b}\pm0.25$	$3.72^{ab}\pm0.55$	$4.33^{a}\pm0.54$
Fat content	$30.43^{a}\pm0.09$	$30.43^{a}\pm0.09$	$29.81^{b}\pm0.29$
Protein content	$20.43^{b}\pm0.26$	$21.97^{a}\pm0.62$	$20.91^{b}\pm0.30$
Carbohydrate	$42.91^{a}\pm0.04$	$40.88^{c}\pm0.32$	$41.54^{b}\pm0.30$

Note: Data are expressed in mean ± SD from triplicate experiments (n=3). Values having different superscript letters in a row are differ significantly at $p\leq0.05$.

TABLE 2: The antinutritional factors of raw, boiled and fermented melon seed.

ANTINUTRIONALS	Raw	Boiled	Fermented
Saponin	$1.62^{a}\pm0.42$	$0.72^{b}\pm0.38$	$0.21^{b}\pm0.09$
Trypsin inhibitor	$1.48^{a}\pm0.46$	$0.11^{b}\pm0.15$	$0.20^{b}\pm0.01$
Flavonoid	$2.48^{a}\pm0.21$	$0.30^{b}\pm0.05$	$0.34^{b}\pm0.05$
Phytate	$0.93^{a}\pm0.53$	$0.10^{b}\pm0.01$	$0.12^{b}\pm0.08$
Cardiac Glycoside	$0.01^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
Tannin	$0.67^{a}\pm0.31$	ND	$0.01^{b}\pm0.01$

TABLE 3: The Antioxidant activity of raw, boiled and fermented melon seed.

Antioxidant	Raw	Boiled	Fermented
Total phenolics	$14.12^{b}\pm0.24$	$9.14^{c}\pm0.54$	$15.98^{a}\pm0.47$
DPPH scavenging activities	$25.58^{b}\pm0.49$	$21.78^{a}\pm0.64$	$26.67^{a}\pm0.40$

Note: Data are expressed in mean ± SD from triplicate experiments (n=3). Values having different superscript letters in a row are differ significantly at $p\leq0.05$.

TABLE 4: Antioxidant vitamins of raw, boiled and fermented melon seed.

Antioxidant vitamins	Raw	Boiled	Fermented
Vitamin A	$20.74^{c}\pm0.45$	$23.47^{b}\pm0.77$	$31.00^{a}\pm1.31$
Vitamin B_1	$0.12^{a}\pm0.06$	$0.04^{a}\pm0.01$	$0.20^{a}\pm0.14$
Vitamin B_2	$0.14^{a}\pm0.01$	$0.09^{b}\pm0.01$	$0.15^{a}\pm0.01$
Vitamin B_3	$1.11^{a}\pm0.02$	$0.61^{b}\pm0.09$	$1.14^{a}\pm0.01$
Vitamin C	$19.06^{b}\pm0.72$	$10.02^{c}\pm0.40$	$21.33^{a}\pm0.49$
Vitamin E	$19.74^{b}\pm0.39$	$24.47^{a}\pm0.64$	$15.09^{c}\pm0.40$

DISCUSSION

There was no significant difference in the fat content of the melon seed after boiling but decreased after fermentation in Table 1. This may be attributed to the breakdown of fat into free fatty acids, some of which might have been used in flavor and aroma generation. There was no observable statistical difference in the protein content of the raw and fermented melon but increased in boiled melon. The crude fibre was found to increase after fermentation. The high amount of fiber will prevent constipation, piles and flatulence. In the carbohydrate content, a statistical difference was observed; the raw melon seed had the highest carbohydrate content. This might probably be due to some undigested oligosaccharides which may be present in the melon seed but for their solubility characteristics leached away during boiling and fermentation process. This study revealed that boiling and fermentation have varying efficiencies in increasing the proximate composition of melon seed. This may simply indicate that the proximate composition of melon seed is processing dependent.

- Table 2 presented flavonoid, among other antinutrient to be highest in raw and fermented melon seed but penultimate highest in boiled melon seed. Flavonoids are highly bioactive and play a wide variety of different roles in the health of plants, animals, and human health. Flavonoids are best known for their antioxidant and anti-inflammatory health benefits as well as the support of the cardiovascular and nervous systems. Because they also help support detoxification of potentially tissue-damaging molecules, their intake has often, although not always, been associated with decreased risk of certain types of cancers, including lung and breast cancer (Chun et al., 2007). Tannin was not detected in the boiled melon seed probably because of thermal decomposition of Tannin compound during boiling. A significant difference ($p<0.05$) was observed in the saponin content, as it decreased after boiling and after fermentation. This is also similar to other anti-nutritionals quantified in the melon seeds. Saponins are phytochemicals, possessing detergent qualities that foam when mixed with water. Commercially, saponins appear in beverages and cosmetics as emulsifiers or sweeteners (Price et al., 1987). The unprocessed melon seed has higher content of anti-nutritional factors. There was decline in the values of anti-nutrients of boiled and fermented melon seeds compared to the raw melon seed. This may be attributed to leaching of anti-nutrient in water during boiling (Philips and Abbey, 1989). Fasoyiro *et al*. (2006) reported that boiling reduced anti nutrients in some legumes; also Ikechukwu *et al*. (2015) reported decrease in the antinutrient content of varieties of melon seed after boiling. Cardiac glycoside was totally lost after processing which indicated that though it was found in trace amount in raw sample but can never be a good nutraceutical against cardiac arrest since melon must undergo processing for human consumption.

The reduction observed in anti-nutritional factors after fermentation, indicated that fermentation of the melon seeds reduced the antinutrients present in the melon seed. Esenwah and Ikenebomeh (2008) reported decrease antinutrient content which may be due to enzymatic activities of fermenting microorganisms. Nwosu and Ojimelukwe (1993) also submitted that fermentation is the most effective processing technique that reduced anti-nutritional factors of fluted pumpkin seed. Both boiling and fermentation was found to have varying efficiencies in reducing the anti-nutritional content of the melon seed. It may be paraphrased that reduction of antinutrients in food are processing dependent.

From Table 3, there was significant increase ($p<0.05$) in the antioxidant activity of the melon seeds. This work showed that the various processed forms of the melon seed exhibited antioxidant properties (ability to inhibit oxidation). Fermented melon seed was observed to have higher content for antioxidant activity, while the boiled melon seed have lowest. Both the total phenolics and DPPH radical scavenging activity decreased after boiling the melon seed, indicating that boiling lowered the antioxidant activity for the melon seed. Yu-wei and Wang (2015) reported that cooking lowered the antioxidant activity in varieties of pulses (Soybeans, Azuki beans). Awoyinka *et al*. (2016) also indicated that processing such as malting decreased DPPH content in beans. Also, Baroga *et al.* (1985) reported that cooking and boiling reduced the amount of phenolics in legumes. It was observed that fermentation increased the DPPH radical scavenging activity and total phenolics in melon seed. Phenolic compounds are known to exhibit antioxidant properties and play important role in cancer prevention and treatment (Lacatusu *et al*., 2010). This result had shown that fermentation increases the antioxidant efficacy of the melon seed. Ileola and Omodara (2017), reported increase in antioxidant activity of fermented *Citrullus vulgaris.*

The vitamin levels in Table 4 for raw, boiled and fermented samples of *L. siceraria* showed that the most abundant vitamin in the plant food at all levels of processing was vitamin A (a fat soluble vitamin which is a very powerful antioxidant) with 31.00±1.31 mg/100 mg while the lowest concentration was obtained in vitamin B_1 with 0.04±0.01 mg/100mg. Vitamin A functions in various capacities as collagen breakdown, keratinization, mucopolysaccharide and glycoprotein synthesis, gene expression and tissue differentiation. The Vitamin content of the raw melon seeds increased after fermentation which concours with the work of Ileola and omodara (2017) that there was an increase in vitamin content of fermented *Citrullus vulgaris.* This work also revealed high Vitamin C content after fermentation. High intake of vitamin C reduces the wrinkles and dryness of skin (Minocha, 2015). Deficiency of vitamin C may cause failure to deposit intercellular cement substance. Vitamin E was observed to reduce after fermentation. Vitamin E, being the most powerful natural antioxidant observed is found to be involved in mopping free radicals and also prevent peroxidation on unsaturated lipids of membranes thereby helping the maintenance of cell membrane integrity and reduction in the risk of atherosclerotic disease (Arinathan *et al*.,2003; Arun *et al*.,2003).

There was significant difference ($p<0.05$) in the vitamin E content of the melon in which boiled melon seed had the highest content. There was no statistical difference between the raw melon and fermented melon in vitamins B_1, B_2 and B_3 content. This may be attributed to the fact that they were mostly found in trace amount and processing independent statistically.

4.2 CONCLUSION

The fermentation process was noticed to have increased the crude fibre, protein and carbohydrate contents while the boiling process led to an increase in the fat content and also protein content of the melon seed. The anti-nutritional components were reduced after boiling and fermentation. These processing methods have been shown to have varying efficiencies in improving the nutritional quality and antioxidant activity of melon seed.

4.2 RECOMMENDATION

Proper caution should be taken in processing the plant to retain or maximize the amount of nutrients that will be present. Processing such as fermentation should be employed for effective treatment before consumption.

Lagenaria siceraria has high antioxidant efficacy. Therefore, cultivation and exploitation of the seeds should be encouraged as an alternative source of food for the future.

There is need for further research into the toxicity and its pharmacological activities. Also, further study may be taken on commercialization and modern storage technique so that this food may be available at any time of the year.

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