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Comparative analysis of Proximate and Heavy metal composition of Ogiri Produced from Seeds of Climbing Melon (*Cucumeropsis*) and Fluted Pumpkin (*Telfairia occidendalist*)

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ABSTRACT

The Proximate and Heavy Metal Composition of "ogiri" produced from seeds of climbing melon (*Cucumeropsis*) and fluted pumpkin (*Telfairia occidentalist*) were studied. The raw samples were bought from Nkwo Market in Nnewi North Local Government Area of Anambra State, Nigeria. The samples were prepared using modified traditional method of processing "ogiri". The proximate and heavy metal composition of the processed samples of "ogiri" were analyzed using standard physicochemical methods. The percentage protein (4.80±0.27), carbohydrate (56.88±0.01), titrable acidity (0.60±0.01), fat (22.00±0.01) and volatile acidity (0.07±0.01) were higher in *Cucumeropsis* "ogiri" than those of *Telfairia* "ogiri", while the reverse is the case in the percentage moisture (20.65±0.01) and crude fibre (0.40±0.10) of *Cucumeropsis* "ogiri" and Telfairia "ogiri". The heavy metal contents were higher in *Cucumeropsis* "ogiri" than in *Telfairia "ogiri"*.
Keywords:Proximate, Heavy metal, Ogiri, Seeds, *Cucumeropsis* and *Telfairia occidendalist*

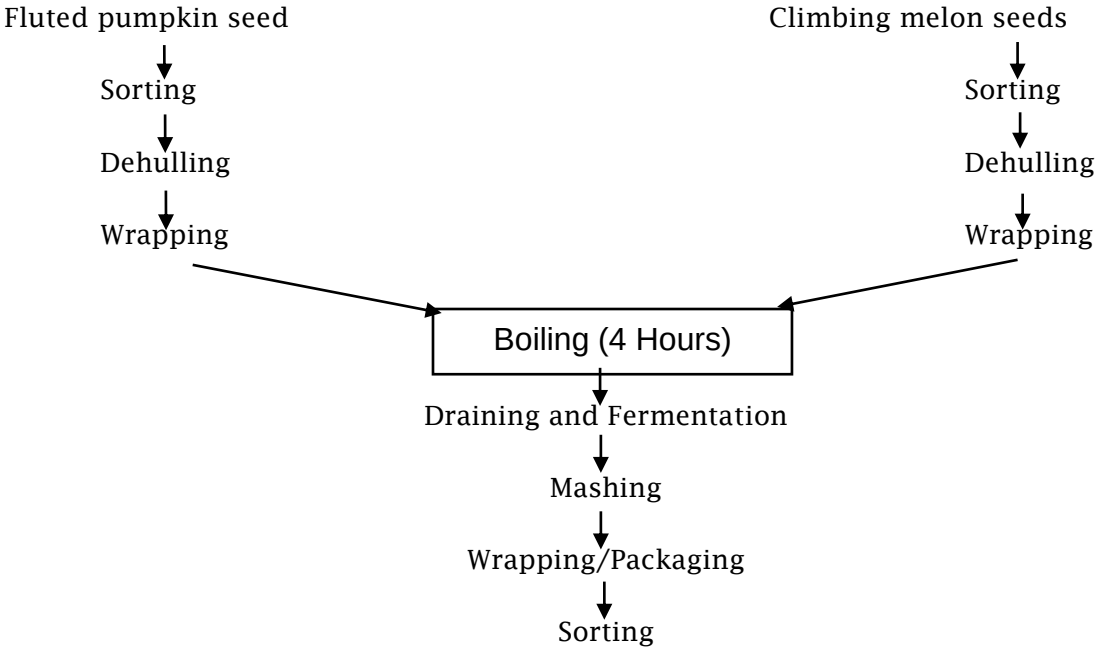
INTRODUCTION

Ogiri is a food condiment produced mainly from oil seeds especially creeping melon seeds (*Citrilus vulgaris*), climbing melon (Cucumeropsis), castor oil seeds (*Ricinus communis*) and fluted pumpkin seeds (*Telfairia occidentalis*) [1,2,3,4]. It is of great importance to South-East occupants of Nigeria especially the Ibos [5,6]. The production of "ogiri" from these seeds is based on fermentation by species of microorganisms which may be indigenous to the seeds or occur in their production environment [7,8,9]. The traditional preparation of "'ogiri" from these seeds is by method of uncontrolled fermentation [10] and this involves boiling of the raw seeds after which they are dehulled and boiled again to soften the seeds for fermentation [11]. Heavy metals are metallic elements with relative high density and are toxic even at low concentrations. They are stable and cannot be metabolized by the body [12]. It has been reported that bacteria in genus *Bacillus* were responsible for the fermentation of the fermented food products [13,14]. Through fermentation process, the anti nutritional factors in the oil seeds are reduced or eliminated in the products, flavourous compounds are developed and their characteristic ammonical taste enhances the taste of foods, beverages and drugs containing them [15]. Through proteolytic activities, the quality of fermented products are enhanced with such attributes as improved protein quality, texture as well as characteristic aroma and taste [16,17].The aim of this study is to compare the proximate and heavy metal compositions of "ogiri" produced from the seeds of *Felfairia* and *Cucumeropsis*.

MATERIALS AND METHODS

The raw materials, climbing melon seed and fluted pumpkin seeds were bought from Nkwo Nnewi in Nnewi North Local Government Area of Anambra State of Nigeria. The traditional method of processing "ogiri" by [12] was modified to become appropriate for the study as shown in Figure 1. The seeds were sorted and aseptically dehulled. Ten wraps of 250g each were boiled for 4 hours. The boiled seeds were drained and left to ferment at room

temperature. One wrap of each was mashed in a disinfected mortar and the resultant paste, the "ogiri" was finally wrapped in blached plaintain leaves (*Musa sapientum*) and stored prior to the proximate and heavy metal analysis.



Determination of Protein

Determination of protein was carried out by Kjeldahl's method and in NAFDAC Standard Operation (SOP) for protein determination. The analysis was carried out in three stages: Digestion, Distillation and Titration.

Digestion

Two grams of each sample was weighed out using electronic weighing balance (METLAR 200) and put in 250ml Kjeldahl digestion flask. Then 10g of copper sulphate and 3g of sodium sulphate were added together and 3g of the mixture was added to the sample. Two pieces of Kjeldahl tablet was also used as alternative to mixture of copper sulphate and sodium sulphate. One gram of anti-bumping granules (chips) was added as well as 20ml of concentrated H_2SO_4. The flask and its content was placed in Kjeldahl temperature regulated digester and digested for 3-4 hours and allowed to cool prior to distillation.

Distillation

Fifty milliliters of 10% sodium citrate solution was added to the digest in the flask. The10ml of 30% w/v of NaOH, two pieces of zinc pellets (granules) and 10ml of 2% boric acid were added in a 250ml conical flask. Also 1-2 drops of methyl red indicator solution was added to the mixture in the conical flask and placed under the mouth of the condenser. The distillate was then collected in the receiver flask and was used for titration.

Titration

Thirty milliliters of the distillate was put in a 250ml conical flask and titrated against 0.1M HCL to a purplish end point. The percentage nitrogen was calculated and then multiplied by a factor (6.25) to get the percentage protein.

$$\% \text{ Nitrogen} = \frac{\text{Titre} \times Ma \times 0.0140}{W} \times 100$$

where Ma = Molarity of acid used
w = weight of sample
Then % protein = % nitrogen x 6.25

Determination of Free Fatty Acid

This was carried out using Association of Official Analytical Chemist [5]. Five grams of each sample was put in a conical flask and heated at 60-70°C. Then 100ml of ethanol and 2ml phenolphthalein indicator were added to the heated sample and titrated against 0.25m NaOH solution to a faint purple colour that persisted for 20 minutes. Then the free fatty acid content was calculated as oleic acid and also as palmitic acid and expressed in mg/ml.

Determination of Total Ash

This was carried out by the method described in NAFDAC Standard Operation Procedure for determination of total as. For each sample, a clean dish (crucible) was weighed and the weight recorded as W_1. Then 5g of the sample was weighed directly into the dish and the weight (weight of the dish and sample) and recorded as W_2. The dish and the sample were charred over a flame in a fume cupboard until no smoke was given off. The crucible and the content were transferred into a muffle furnace (AAF 1100) at 550°C-600°C and left to ash. The fully ashed sample (black ash in colour) was cooled in a decicator and the weight recorded as W_3. The percentage ash was then calculated using the formula.

$$\% \text{ ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Determination of Moisture Content

This was done using electronic moisture analyzer – Sartorius moisture analyzer (MA45). One gram of each sample was weighed using the in-built balance of the moisture analyzer. The start/on button was pressed. The reading was taken when signal for completed test (steady temperature of 105°C was noted).

Determination of Crude Fibre

This was carried out by the method described in NAFDAC Standard Operation Procedure (SOP) for crude fibre determination. Two grams of the sample was put in a 250ml conical flask. Then 20ml petroleum ether and 20ml of 1.25% NaOH was added into the conical flask and boiled in a hot plate stirrer for 30 minutes. The residue was filtered out using Whatman's No. 1 filter paper. The weight of empty crucible was recorded as W_1. The filter paper and the residue was placed on the crucible dried in the furnace and the weight of the crucible together with that of residue was recorded as W_2. Then the loss of weight of samples after ashing was taken as crude fibre content.

$$\% \text{ crude fibre} = \frac{W_3 - W_6}{\text{Wt of samples}} \times 100$$

Where W^3 = weight of residue
W_6 = Wt of ash = $W_5 - W_4$
W_5 = Wt of crucible + ash
W_4 = Wt of empty crucible

Determination of Total Carbohydrate

Total carbohydrate was obtained by different using
Total carbohydrate =100 – (% moisture + ash + protein + fat)

Determination of Volatile Acidity

This was carried out by the method described by [9]. Five grams of each sample was dissolved in 100ml of distilled water and poured into round bottom flask and also 1g of anti-bumping chips added. Then 1L (1000ml) of water was poured into the steam developer (vapour developer) vessels. Ten milliliter of distilled water was poured in the receptor flask meant for collecting the distillate. Sixty milliliter of the distillate was poured into 250ml conical flask, heated to boil, 1ml of 1% alcoholic phenolphthalein indicator solution was added and filtred with 0.1M NaOH to a faint pink colour and the volume recorded as 'a' ml. Five milliliter of 25% H_2SO_4 and 0.1mle of 1% starch solution was also added and immediately titrated with 0.2M iodine solution in the burette to a blue colour lasting for 10 minutes and the volume recorded as 'b'ml. Then the volatile acid content was calculated as acetic acid in mg/l using the formula 0-12 (a-2b)

where 'a' = volume of NaOH used 'b' = volume of iodine used

Determination of Tritrable Acidity

The method of [4] was used. Two hundred milliliter of distilled water was poured into a 250ml conical flask and brought to boil. Then 2 drops of 1% aqueous phenolphthalein indicator was added to the boiling water in the conical flask and titrated against 0.1M NaOH to a faint pink colour and the volume recorded as V_1. Five milliliter of the sample suspension was added and titrated against 0.1M NaOH to a faint pink colour and the volume recorded as V_2. The titrable acidity was calculated and expressed as tartaric acid in g/100ml using the formula.

$$\text{Titrable Acidity g/100ml} = \frac{V_1 \times mV \times 75 \times 100}{V_2 \times 1000}$$

where V_1 = Volume of NaOH for neutralizing the water
V_2 = Volume of NaOH for neutralizing the sample
M = Molarity of NaOH
V = Volume of sample used
75 = Equivalent weight of tartaric acid

Determination of pH

The pH values of the samples were determined with pH meter (JENWAY 3510). The pH meter was standardized with buffers of pH 4 and pH 8 respectively and then with distilled water. The electrode was then inserted in each sample suspension and the readings were taken.

Determination of Cadmium

This was determined by the use of smart electrophotometer (Lamotte). Cadmium was selected from the menu button. The samples were extracted using soxhlet extraction apparatus (GT 301). Ten milliliters of the extracted sample were poured into a cuvette (tube). The tube was inserted into the spectrophotometer chamber and scanned as blank. The tube was removed from the spectrophotometer. The 1ml of buffered ammonium reagent (4021), 2 drops of 10% sodium citrate, 0.5ml of PAN indicator (4021) cap and 0.5ml stabilizing reagent (4022) cap were added into the test tube, mixed together and inserted into the spectrophotometer chamber. Scan sample was selected from the menu and the result recorded in ppm.

Determination of Lead

This was determined using smart spectrophotometer. The tube to be used was rinsed with test sample and filled with 10ml sample (extract) and scanned as blank. The tube was removed from the spectrophotometer. Then 5ml of ammonium chloride buffer (4022), 3 drops of 10% of sodium cyanide, 0.5m Pyridylazo resorcinol (PAR) indicator and 0.5ml of stabilizing reagent were also added to the tube, mixed and inserted into the chamber. The menu scan sample was selected and the result recorded in ppm. Thirty three copper Unit Dose Vials (UDV) was selected from the menu. The tube was rinsed with test sample water filled with 3ml of the test sample and scanned as blank. The tube was removed from the chamber. Then 3ml of sample was also added to a copper UDV (4314) and allowed to stand for two minutes. The tube was inverted severally to mix and scan sample menu was selected and the result recorded in ppm.

Determination of Iron

This was determined using smart spectrophotometer. The tube was rinsed with sample water and filled back with 10ml sample. The tube containing the sample was inserted into the chamber and scanned as blank. The tube was removed from the chamber and six (6) drops of Acid phenanthiolene indicator (2776) cap were added. The tube was inverted several times to mix the reagents and allowed to stand for five minutes for colour development. The tube was inserted into the chamber and the menu, scroll sample was chosen and the total iron content was read off and recorded in ppm.

Determination of Calcium and Magnesium

This was carried out by Unit Dose Vials (UDV) method using smart spectrophotometer. The menu thirteen (13) Calcium and Magnesium Hard – UDV was chosen. The vial was rinsed in sample water. Three (3) milliliter of the sample was added to the vial with syringe (1184). The vial was inserted into the chamber and the menu scan blank was chosen. The vial as removed from the chamber of the spectrophotometer. Then 3ml of sample of calcium hardness UDV vial (4309) and shaken vigorously for 10 seconds. The tube was inserted into the chamber and the menu, scan sample was chosen and the total hardness recorded. Concentration of calcium calculated using

$$Ca = \frac{\text{Total hardness} \times 2}{3}$$

while the magnesium content was calculated using

where Ca = concentration of Calcium

RESULTS AND DISCUSSION

The protein content is low in both Cucumeropsis "ogiri" and Telfairia "ogiri" (4.80±0.27% and 4.20±0.10% respectively). The low amounts of protein may be attributable to increase in the population of organisms during fermentation which resulted in increased utilization of the substrate by the organisms. This agrees with the findings of [11]. The pH is high in both *Cucumeropsis* "ogiri" and *Telfairia* "ogiri". The high pH is in line with the findings of [3]. The high pH could also be attributable to the ability of the fermenting organisms to degrade protein [8]. The low crude fibre and ash contents (Table 1) are in agreement with that of [12] for "ogiri" produced from melon seeds. The increase in the volatile acidity in both samples indicates similar increase in the production of free fatty acids by the lipolytic fermenting organisms and this agrees with the findings of [8]. High levels of heavy metals (Table 2) may be attributed to raw material sources because most of the substrates used in producing "ogiri" are produced in the affected areas and are distributed all over the country. Lead and Copper are used for making pesticides which is used for crop preservation and pest control. Also crops grown in Cadmium contaminated irrigation water is likely to be contaminated with Cadmium [9]. The presence of Lead and Cadmium poses a public health hazard to the consumers. The possible source could be from water around mining areas due to industrial effluent used in reconstitution, soil contaminated with these elements, poor mining and hygienic practices [7]. Moisture content is higher in the "ogiri" produced from Telfairia (73.79%±0.01) and that of *Cucumeropsis* (20.65%±0.01). The carbohydrate content is higher in Cucumeropsis "ogiri" that in Telfairia "ogiri" (Table 1).

Table 1: Proximate Composition of "Ogiri" Produced from *Cucumeropsis* and *Telfairia occidentalis* seeds.

	Cucumeropsis	*Telfairia*
Protein (%)	4.80±0.27	4.20±10
Fat (%)	22.00±1.00	5.00±1.00
Free Fatty Acid (mg/ml)	4.50±0.10	7.68±0.08
Ash (%)	0.15±0.01	1.60±0.10
Moisture Content (%)	20.65±0.01	73.79±0.01
Carbohydrate (%)	56.88±0.01	38.70±0.01
Titrable Acidity (g/100ml)	0.60±0.01	0.40±0.10
Volatile Acidity (g/100ml)	0.07±0.01	0.06±0.01
pH	7.50±0.17	7.10±0.10
Crude Fibre (%)	0.40±0.10	1,50±0.10

Table 2: Heavy metal composition of "Ogiri" from *Cucumeropsis* and *Telfairia* Seeds.

	Cucumeropsis	*Telfairia*
Calcium (ppm)	18.75±0.01	72.70±0.17
Cadmium (ppm)	0.40±0.22	0.10±0.02
Copper (ppm)	0.27±0.03	0.26±0.01
Lead (ppm)	0.20±0.10	0.10±0.01
Iron (ppm)	0.70±0.01	0.53±0.01
Magnesium (ppm)	6.25±0.10	14.30±0.17

CONCLUSION

Proximate and heavy metal contents of "ogiri" produced from *Cucumeropsis* and *Telfairia* seeds have been established. It has also been revealed that "ogiri" would contribute to protein and energy intake as well as nutrition to the consumers.

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