COMPARATIVE STUDY OF THE NUTRIENT AND ANTI-NUTRIENT COMPOSITION OF RAW AND FERMENTED MELON SEEDS OF THE CUCURBITACEAE FAMILY

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Abstract
Raw seeds of Citrullus Lanatus (Thumb), Citrullus Lanatus (Thunb), Lagenaria Siceraria and Cucumeropis Manni (Naud melon) were processed into flour and a portion of it was fermented. The proximate, mineral and anti-nutrient contents of both raw and fermented melon flour samples were analysed using standard methods and the effect of fermentation on the melon seed varieties used for Ogiri production was studied. Significant changes were observed in the nutrient compositions of the samples as results obtained showed that fermentation increased significantly ($p<0.05$) the moisture, crude fat, crude fibre and most importantly crude protein contents while carbohydrate value decreased significantly across the melon seeds analysed. An increase in the content of vital minerals such as zinc, iron and calcium was observed following fermentation with Calcium being the most abundant mineral among others analysed in both raw (0.38-0.59)mg/100g and fermented melons (1.76-2.24)mg/100g. Of the three anti-nutrients analysed, phytates and tannin were respectively the highest and lowest in both raw and fermented samples but they all were reduced significantly following fermentation. It was concluded that fermentation has a positive nutritive effect on the melon seeds analysed as it improved the nutrient and reduced the anti-nutritional compositions and these changes observed could have been facilitated by the enzymatic activities of the fermenting organisms

Key words: fermentation, Citrullus Lanatus (Thumb), Citrullus Lanatus (Thunb), Lagenaria Siceraria and Cucumeropis Manni.

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1. INTRODUCTION

In Nigeria, melons are major food crops with different varieties used as food sources (Mabalaha 2007); they have been widely reported to contain good amounts of crude protein ranging from 24.3 to 41.6% (Fokou et al 2004), carbohydrate of about 4.56-13.3% (David and Aderibigbe 2010) and a good level of amino acids.

The Cucurbitaceae family is considered the highest of plant families that provides edible products used as human food (Bisognin, 2002). A number of edible melons which can also be fermented into condiments have been identified in this family, some of which include:

Lagenaria siceraria (Molina), also known as Bottle gourd melon which has fruits used as containers and musical instruments, and seeds used as soup, soup thickner and source of highly nutritive edible oilseeds used for domestic purposes (Achu, Fokou, Tchiegang, Fotso, & Tchouanguep, 2005; Fokou, Achu, & Techounguel, 2004).

Cucumeropsis mannii, also known as Naud melon also belongs to the cucurbitaceae family (Sanjur et al. 2002) and it is widely cultivated in sub-saharan African countries especially in Nigeria where the seeds are processed into snack, roasted or fermented into condiments used in foods. The plant is also grown in Cameroon where it serves as an economical and valuable source of oilseeds (Fomekong et al 2008).

*Citrullus lanatus (*Thumb and Thunb) Mansf. also known as watermelon are species of the cucurbitaceae family cultivated annually in an open field particularly in Western Nigeria, for the food in the seed, usually inter-planted with maize, cassava, or other crops. (van der Vossen 2004). It usually produces large-sized fruit with great amount of seeds, however breeders have developed new varieties with smaller fruits

such as mini watermelons (Bomfim et al., 2013).

These melon seeds are processed into vegetable oils, blended to prepare soup or other delicacies or fermented into ogiri, a local condiment for seasoning foods. Ogiri is a product of fermentation usually produced through uncontrolled solid state fermentation and boiling for specific hours to soften selected legumes such as the melon seeds (Achi 2005). It is a less expensive condiment used in flavouring of stews and soups and it is usually produced by traditional fermentation of seeds like melon seeds, castor oils seeds. fluted pumpkin etc (Achi, 2013). Nutritional analyses of most fermented vegetable proteins of African origin have shown that these condiments are rich sources of protein, essential amino acids, vitamins and minerals which have been found to increase during the process of fermentation.

Fermentation is an age long food processing method generally reported to improve the nutritive value and safety of fermented foods; with other benefits such a food preservation, fortification, increase in diet variety, degradation of anti-nutritional factors, antioxidant and antimicrobial properties (Jimoh *et al*., 2012; Oyewole and Isah, 2012) etc. Fermented foods either of plant or animal origin are intricate part of human diet with lots of nutritional benefits. Fermented vegetable proteins are commonly used as seasonings in Africa especially by the rural dwellers. Some of the common fermented vegetable condiments in West Africa include *iru or dawadawa* from locust bean (*Parkia biglobosa*), *ogiri* from melon seeds (*Citrullus vulgaris*), *daddawa* from soybean (*Glycine max*), *soumbala* from soybean (*Glycine max*), *ugba* from African oil bean seed (*Pentaclethra macrophylla*) and *owoh* from cotton seeds (*Gossypium hirsutum*). (Nurudeen Ayoade et al 2019).

The usefulness of these melon seeds as sources of protein like other oilseeds and legumes in human foods, animal feeds and pharmaceutical purposes is limited due to paucity of information on the nutritional, antinutritional composition and the effect of fermentation on the nutrients and antinutrients present in these seeds.

Thus this research work is aimed at investigating the proximate, mineral and antinutrient composition of fermented and unfermented melon seeds of *Lagenaria siceraria, Cucumeropsis mannii, Citrullus lanatus (*Thumb) Mansf and *Citrullus lanatus (*Thunb) Mansf. Such information on these species of cucurbitaceae could make them useful as functional ingredients as well as sources of vegetable proteins.

2. MATERIALS AND METHODS

2.1. Sample materials

Fresh seeds of *C. Lanatus (Thumb), C. Lanatus (Thunb), L. Siceraria and C. Manni* were purchased from Bodija market in Ibadan, Oyo state and the seeds were authenticated at the Botany Department of the University of Ibadan.

The seeds were shelled, sorted and a portion was milled into flour using a blender and stored in an air-tight polyethylene bag. The un-milled portion was fermented as described below and both were analysed.

2.2. Fermentation of samples

The traditional method of ogiri production was used**.** Exactly 200g each of the dehulled seeds of *C. Lanatus (Thumb), C. Lanatus (Thunb), L. Siceraria and C. M*anni were boiled in a pot with distilled water (1:2) for 6hours to aid softening.

Intermittently, water was added to the pot to prevent burning. The seeds were afterwards drained and allowed to cool for 30 minutes after which the seeds were mashed and wrapped in plantain leaves (Musa spp).

After that, samples were put into a clean sack bag and allowed to ferment at room temperature for four days after which the analyses were carried out.

*Citrullus Lanatus (Thumb)*Mansf

*C*ucumeropis *M*anni (Naud melon)

Lagenaria Siceraria

Citrullus Lanatus (Thunb)(Bara)

Figure1: Pictures of the four melon varieties analysed

2.3. Determination of proximate composition

The crude protein, crude fat, crude fibre, moisture and ash contents of both fermented and unfermented melon flour were determined according to the method described by AOAC 2005 while the Carbohydrate content was calculated as the percentage difference of the other proximate parameters from 100 as shown below:

%carbohydrate = 100 – (%crude protein + %crude fat + %moisture + %crude fibre + %ash). AOAC 2005

2.4. Estimation of Energy Value

This was calculated according to the method used by Onyeike and oguike (2003) which involves multiplying the mean values of crude protein, crude fat and total carbohydrate by the factors of 4, 9, 4 respectively and then taking the sum of the products in kcal /100g.

2.5. Determination of mineral composition

The mineral content of the test sample was determined by the dry ash extraction method reported by Damaris et al (2015). 2.0g of samples was burnt to ashes in a muffle and the resulting ash was digested using 100ml of dilute hydrochloric acid and then diluted to 100ml in a volumetric flask using distilled water. The digest obtained was used for the mineral analyses. Zinc and iron content was determined using atomic absorption spectroscopy (AAS), Calcium content was determined by versanale EDTA complexiometric titration.

ANTINUTRIENT ANALYSIS

***Anti-nutrient determination*:** phytate, tannin andoxalate contents were determine according to the method described by AOAC.

***Phytate determination*:** Four grams of finely ground melon samples were soaked in 100 ml of 2% HCl for 3 hrs and then filtered. The 25 ml of the filtrate was placed in a 100 ml conical flask and 5 cm^3 of 0.03% of ammonium thiocynate solution (NH_4SCN) was added as indicator. To maintain proper acidity, 50ml of distilled water was added to the solution and this was titrated against 0.00566 g ml^{-1} of standard iron (iii) chloride solution that contained about 0.00195 g of iron per milligram until a brownish yellow colouration persisted for 5 minutes. The equivalent was obtained and from this, the percentage phytate content was calculated as:

% Phytic Acid =

$$\frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100 \times 3.55}{\text{Wt. of sample}}$$

Tannin determination: About 0.2 g of finely ground sample was weighed into a sample bottle. Ten milliliters of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2 h at 30°C. Each solution was then centrifuged and the supernatant stored in ice. A 0.2 ml of each solution was pipetted into the test tube and 0.8 mL of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg mL^{-1} of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folin Ciocaeteau reagent was added to both, the sample and the standard, followed by 2.5 ml of 20% Na_2CO_3. The solution was then vortexed and allowed to incubate for 40 min at room temperature after which its absorbance was read at 725 nm AJ- IC03 spectrophotometer against a reagent blank concentration of the same solution from a standard tannic acid curve that was prepared.

***Oxalate determination*:** One gram of the sample was weighed into 1000 ml conical flask. Then 0.75 M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for 1 h and then filtered using Whatman No. 1 filter paper. Twenty five milliliter of sample filtrate (extract) was collected and titrated hot (80-90°C) against 0.1 M $KMnO_4$ solution until a pink colour that persisted appeared.

Statistical analysis

All determinations were carried out in triplicate, and the errors were reported as standard deviation from the mean. Results were subjected to ANOVA and means separated by Duncan multiple range test using SPSS 17 computer program.

3. RESULTS AND DISCUSSION

***Proximate composition*:** The proximate compositions of raw and fermented melon samples;*C. Lanatus (Thumb), C. Lanatus (Thunb), L. Siceraria and C. M*anni, fermented samples are presented below. Table 1 shows the proximate compositions of the raw and fermented seeds of *Citrullus. Lanatus (Thumb), Citrullus Lanatus (Thunb), Lagenaria Siceraria and Cucumeropis Manni.*

The moisture content was found to range between 5.20% and 9.40% in the raw samples. This agrees with the work of Peter-Ikechukwu et al (2016) who reported a moisture content of about 5.29%-8.93% in some raw melon seeds of the curcubit family.

Table 1. Median lethal dose (LD 50) of crude and degummed *Citrullus lanatus* oil on albino mice

	Cirullus lanatus (thumb)		Citrullus lanatus(thunb)		*Lagenaria Siceraria*		*Cucumeropis manni*	
	Raw	Fermented	Raw	Fermented	Raw	Fermented	Raw	Fermented
% Moisture	5.20±0.74^a	9.37±0.20^b	7.99±0.19^c	12.82±0.25^d	9.40±0.86^e	12.10±0.66^f	5.79±0.18^g	13.17±0.36^h
% Ash	2.20±0.00^a	2.75±0.20^b	2.40±0.10^c	2.93±0.20^c	1.60±0.15^e	1.73±0.20^e	0.90±0.00^g	1.58±0.10^h
% C. Protein	27.75±0.30^a	29.10±0.15^b	27.95±0.31^c	28.94±0.15^c	27.40±0.25^e	27.74±0.13^e	23.98±0.33^g	24.81±0.13^g
% Crude fibre	17.92±0.04^a	18.61±0.05^a	12.06±0.01^c	14.04±0.00^d	18.76±0.15^e	19.53±0.12^e	13.33±0.07^g	14.73±0.01^g
% Fat	23.33±0.20^a	24.17±0.22^a	28.60±0.01^c	29.87±0.20^d	24.04±0.00^e	28.30±0.10^f	30.00±0.00^g	31.71±0.15^h
Carbohydrate	23.60±0.01^a	16.00±0.05^b	21.00±0.00^c	11.40±0.03^d	18.80±0.12^e	10.60±0.15^f	26.00±0.05^g	14.00±0.02^h
Energy value	415.37^a	397.93^b	453.20^c	430.19^d	401.16^e	408.06	469.92^g	440.63^h

An increase in moisture content was observed after fermentation to a range of 9.37% – 13.17%. Ejinkeonye et al (2018) reported an increase in the moisture content of water melon seeds from (12.04-34.25%) as fermentation period increased and Abiola and Ekunrin, (2016) also reported a significant increase in the moisture contents of fermented melon husk from 2.46% to 5.84% after 72hours**.** This general increase in moisture content is obviously due to boiling which is part of the fermentation process and has also been attributed to contribution by micro-organisms during the breakdown of the organic components of the food sample during fermentation. (Oladele and Oshodi, (2008); Oseni and Ekperigin (2007).

The ash content of the raw and fermented melon samples ranges from 0.90%-2.40% and 1.58%-2.93% respectively, with *C. Lanatus (Thunb)* having the highest ash content in both raw and fermented samples. An (8.13% - 24.99%) increase in ash content was observed after fermentation across the melon samples analysed. This agrees with the reports of Jokotagba et al (2015) who recorded a 23.74% increase in the ash content of *Phoenix dactylifera* L Flour after fermentation and also Oladele Ebun-Oluwa (2008) who reported a 14.90% and 19.14% increase in ash content of fermented Berlandier Nettle Spurge (*Jatropha cathartica*) and Physic Nut (Jatropha curcas) Seeds respectively after fermentation.

The crude protein content of the fermented samples were found to be significantly higher compared to the raw samples, ranging from 23.98% -27.95% and 28.81% – 29.10% respectively in unfermented and fermented samples. This increase in protein contents could be attributed to the ability of the micro organism to secrete some extra cellular enzymes (proteins) which degrade the materials during fermentation. There are lots of controversial results as per the effect of fermentation on proteins in food. In a study in 2013, David and colleagues reported an increase in protein content of *Citrullus lanatus* from (28.30±2.5)% - (31.50±0.1)% while they observed a decrease in *Cucumeropis manni* and *Colocynthis vulgaris* following fermentation.

Crude fibre was also observed to increase following fermentation but the increase was only significant in Citrullus lanatus (thunb).

The crude fat content (ether extract) of the raw samples were found to be lower (23.33 - 30.00)% than the fermented samples (24.17-31.71)%. The increase in crude fat could be as a result of extensive breakdown of large molecules of fat into simple fatty acids. On the contrary decrease in crude fat values were observed for the fermented products of jatropha seed and jatropha carthatica seeds and was attributed to the utilization of lipids by fermenting microbes for energy (Oladele and Oshodi 2008**).**

Carbohydrate content of the melon seeds examined generally decreased with fermentation as follows: *C.Lanatus* (Thumb) (23.60-16.00%), *C.Lanatus* (thumb) (21.00-11.40%), *L.Siceraria* (18.80-10.60%) and *C. Manni* (26.00-14.00%). Similar trend of result was obtained by; Ibukun and Anyasi, (2013) who reported a significant ($p<0.5$) reduction of carbohydrate in the fermented seeds of *S. indicum, C. melo* and *C. mannii* from 13.0% to 3.4%, 20.60% to 5.10% and 10.30% to 2.50% respectively, Oladele Ebun-Oluwa (2008) reported a decrease in carbohydrate content from 16.89 % to 15.71% and 6.45 to 3.81% for *Jatropha curcas* and *jatropha carthatica* seeds respectively and Jokotagba et al (2015) also reported a decrease from 64.50-76.23% in *Phoenix dactylifera L* Flour. This decrease may be as a result of possible utilization of carbohydrate as carbon source for microbial growth and production of secondary metabolites like ethanol from carbohydrate (Akindahunsi et al 1999). However, Some others reported an increase in carbohydrate content, among these are Wakshama et al (2010) who reported an increase from (2.3)-33.6)%) for C. pepo, while Ouoba 2005 reported increase in carbohydrate content of *Citrullus lanatus* (13.30-25.20%) and *Citrullus vulgaris* (7.60-23.60%) during fermentation, Asagbara (2012) also reported increased

carbohydrates content for *Citrullus lanatus* (16.31-23.43%).

ANTINUTRIENT ANALYSES

The results of the anti-nutrient contents of both raw and fermented melon samples are presented graphically below.

The phytate, tannin and oxalate contents of the melon samples analysed are represented graphically in figure 1 above.

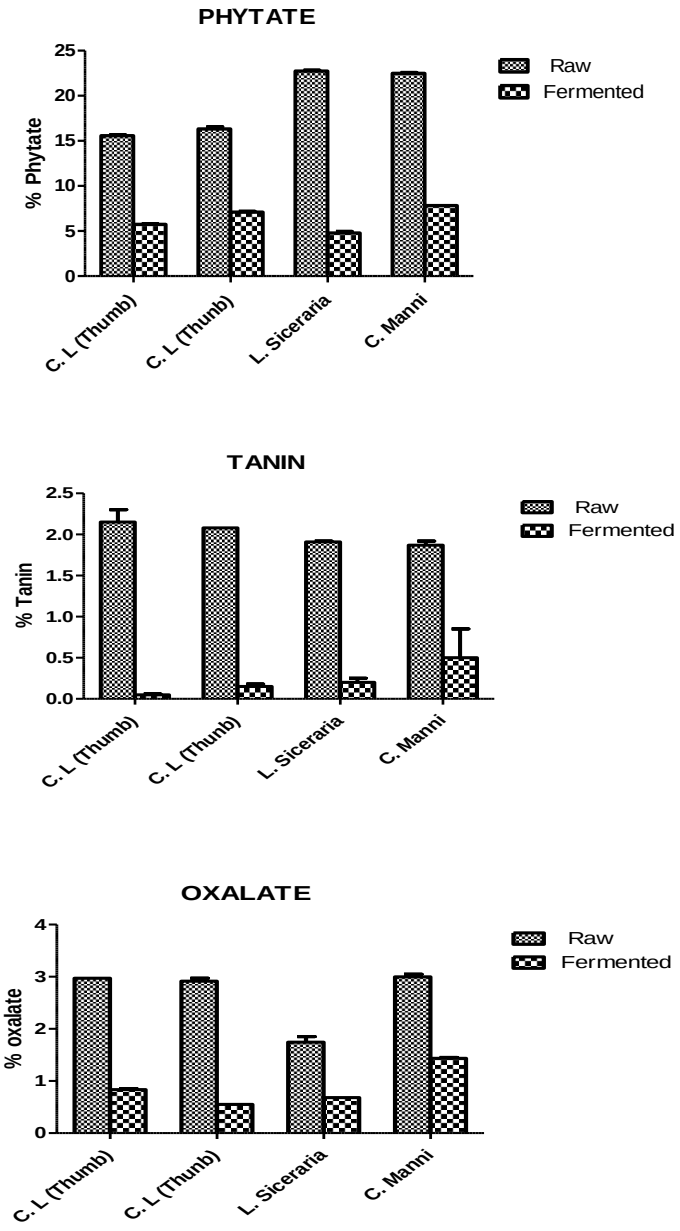

Figure 1: The anti-nutrient contents of raw and fermented Citrullus Lanatus (Thumb), Citrullus Lanatus (Thunb), Lagereria Siceraria and Citrullus Manni

***Mineral analyses*: Table 2 gives the mineral contents of both raw and fermented melon samples in mg/100g**

Sample	Zn		Fe		Ca		Mn	
	Raw	Fermented	Raw	Fermented	Raw	Fermented	Raw	Fermented
C. L (thumb)	0.30±0.01[a]	0.33±0.03[b]	0.18±0.03[a]	0.21±0.00[b]	0.38±0.01[a]	1.76±0.20[b]	0.79±0.03[a]	0.84±0.03[b]
C. L (thunb)	0.25±0.10[a]	0.43±0.06[b]	0.20±0.04[a]	0.22±0.02[a]	0.59±0.06[a]	2.24±0.00[b]	1.00±0.00[a]	1.23±0.02[b]
L. Siceraria	0.33±0.02[a]	0.47±0.02[b]	0.21±0.07[a]	0.25±0.15[b]	0.45±0.20[a]	1.98±0.10[b]	1.00±0.30[a]	1.21±0.02[b]
C. manni	0.25±0.00[a]	0.35±0.03[b]	0.15±0.02[a]	0.20±0.20[b]	0.46±0.04[a]	1.90±0.00[b]	0.05±0.10[a]	0.59±0.01[b]

Values are means ± standard deviations of triplicate determinations. Means on the same row with different superscripts are significantly different @ (P<0.05)

Phytate content was highest in the melon samples analysed with values ranging between (15.58-22.74)% in raw melon samples and (4.78 to 7.83)% in fermented melon samples while tannin content was the lowest across the four melon samples with values between (1.87-2.15)% in raw and (0.05-0.20)% in fermented samples. This correlates with the reports of Abiola and Ekunrin (2016) in which Tannin was the lowest anti-nutrient detected in the raw sample of Melon (*Cucumis melo* L.) Husk with a value of 0.05 mg, tannin content of S. indicum, C.melo and C. mannii were also considered very low as reported by Ibukun and Anyasi (2013).

However, there was a general significant decrease in the anti-nutrient contents after fermentation. This result is in agreement with several literatures: Ibukun and Anyasi (2013) observed a progressive reduction of anti-nutrient factors in S. indicum, C.melo and C. mannii seeds as fermentation progresses, Damaris et al (2015) reported a decrease in *Citrullus vulgaris, Citrullus lanatus, Colocynthis citrullus, Cucurbita pepo, Cucurmeropis edulis* melon seeds after secondary fermentation etc. Dietary phytates are considered undesirable because they form insoluble complexes with important cations, protein and starch therefore reduction in phytate in diets improves the availability and absorption of minerals such as calcium and phosphorus (Enujiugha and Agbede, 2000; Oatway et al (2001).

4. CONCLUSIONS

From the reports above, it is evident that fermentation of melon seeds of African origin results in improvement of essential nutrients, reduction in anti-nutrient and enhancement of the overall food quality. The changes in nutrient composition following fermentation of melon seed could be a result of the enzymatic activities of the fermenting organisms.

Thus fermented condiments like ogiri in addition to their flavoring properties are good sources of proteins and minerals and thus can be utilized to produce complementary food supplements. However, there is need for more optimization and microbiological studies of their production process with the aim of establishing standardized protocols for their production and utilization.

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