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Comparative Soluble Nutrient Value of Ogiri Obtained From Dehulled and Undehulled Boiled Melon Seeds(Cucumeropsis mannii)

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Abstract

The amount of residual soluble nutrient (sugars and amino acids) in ogiri obtained from dehulled (A) and undehulled (B) boiled melon seeds (*Cucumeropsis mannii*) fermented in the leaves of *Musa spp* were studied. Proximate compositions of the major components in ogiri obtained from dehulled and undehulled boiled melon seeds revealed a decrease in the values of fat (A 38.61%), (B 38.42%) and crude protein (A 18.64%), (B 18.36%) but an increase in carbohydrate values (A 25.83%), (B 25.26%) relative to the raw seeds. Significant daily higher soluble sugar levels were obtained in ogiri from undehulled seeds with fluctuations, coming to approximate starting values of (A 2.67 ± 0.02), (B 10.02 ± 0.03) at the end of fermentation. Steady increases without fluctuations were observed in levels of free amino acids in ogiri obtained from both seeds (A 42.83 ± 0.29), (B 60.96 ± 0.41), with significant higher retention from undehulled seeds.

Key words: dehulled, undehulled, fermentation, boiled melon seeds, ogiri, soluble nutrient, testa.

1. Introduction

Ogiri is a product of the fermentation of boiled melon seeds (*Cucumeropsis manii)*. It is a food flavouring condiment used in sauces and stews that serve as accompaniment to starchy root and vegetable diets. It is also added to other preparations as seasoning e.g. in boiled meat and staple foods such as ikokore – a Nigerian local pottage. It is consumed in the Eastern, South-Western and Middle belt regions of Nigeria. The melon seed contain a seed coat (testa/hull) which forms its outer covering and it is derived from the integument tissue, originally surrounding the ovule. The seed coat in the mature melon seed is a transparent and very thin layer. It is a multifunctional organ that plays an important role in embryo nutrition during seed development and in protection against detrimental agents from the environment afterwards (Mohamed-Yasseen et al., 1994; Weber et al., 1996).

The traditional preparation of ogiri from melon seeds is by the method of uncontrolled solid state fermentation, Achi, (2005) and it involves boiling the raw seeds after which they are dehulled, and then boiled again to soften seeds for fermentation. The softened seeds are wrapped in leaves, kept in sacks and incubated near the earthen pot for a period of three to five days or longer after which the mash is dried and milled to a smooth paste, the ogiri. The dehulling process is the separation of the seed coat of the melon seeds from the cotyledons, and it requires an abrasive action. This abrasive removal of the testa/hulls is carried out manually and because of its tedious nature when done with the hands, the locals have resorted to dehulling the boiled seeds with the aid of the bare feet as this is easier and faster; thus dehulling is always done manually and with the aid of the feet. However, this method of dehulling may introduce a myriad of organisms into the seeds prior to fermentation (some of which could be pathogenic and/or spoilage). This development coupled with an unhygienic fermentation and environment of preparation could result in the production of an ogiri with variable quality and unacceptable aroma, short shelf - life and one that can pose health hazards to the consumers.

Various studies have been documented on ogiri viz: Microbiology and amino acid composition of ogiri (Odunfa 1981(b), the biochemical changes taking place during the production of ogiri (Odunfa,1983), Microbiology of ogiri production (Barber and Achinewu 1992) and Soluble nutrient production during the fermentation of melon varieties into ogiri using different leaf types (Onawola *et al,* 2011) to mention a few; however, there has been no work documented on the soluble nutrient value of ogiri obtained from dehulled and undehulled boiled melon seeds and hence, the need for the dehulling process of boiled melon seeds during ogiri preparation, considering its unhygienic nature as carried out by the traditional producers/village entrepreneurs. This study therefore aims to investigate the need for a dehulling process with respect to the residual soluble

nutrients available in the ogiri.

2. Materials and Methods

2.1 Materials:

Melon seeds (*Cucumeropsis mannii*) were obtained from a local market in Lagos, Nigeria.

Leaves from the tree of *Musa spp* were obtained from the garden of the Federal Institute of Industrial Research Oshodi, Lagos, Nigeria.

Ogiri (A) obtained after fermentation of dehulled boiled melon seeds.

Ogiri (B) obtained after fermentation of undehulled boiled melon seeds.

2.2 Methods:

2.2.1. Preparation and fermentation of melon seeds: This was done on two portions of 400g melon seeds according to the method of Onawola *et al*, 2011 but with a slight modification. While one portion was dehulled during the boiling process, the other portion was left undehulled during the boiling process.

2.3 Analyses:

2.3.1. Proximate and mineral compositions: These were determined on raw melon seeds and on ogiri obtained from fermentation of dehulled and undehulled boiled melon seeds by the method of the Association of Official Analytical Chemists (AOAC, 1995)

2.3.2. Statistical Analyses: This was carried out using the student's T-test at 5% level of significance. T at 5% level of significance is $\pm$2.042 (two tails) and $\pm$1.697(one tail) respectively.

2.3.3. Extraction and Quantitation of Residual soluble sugar present

2.3.3.1. Extraction: To 1.0g ground samples of ogiri A, (each collected at different fermentation periods and dried at 60^{o}C to constant weight before grinding) was added 10mls of cold 50% methanol in a conical flask and shaken for about 10minutes to allow for extraction of total soluble / reducing sugars after which it was filtered. To the filtrate in a separating funnel was added 5ml of chloroform for the removal of organic matter and other alcohol soluble extracts such as proteins. This was shaken vigorously after which the layers were allowed to settle, the chloroform extract forming the lower layer was discarded. To the extract in the separating funnel was added 10ml petroleum ether and shaken together to remove lipids. The methanol lower layer was collected and stored as the carbohydrate extract for soluble / reducing sugar determination. The same procedure was adopted for ogiri B.

2.3.3.2. Quantitation*:* This was estimated as follows.

To 1ml each of ogiri A and glucose standard was added 1ml each of Dinitrosalicylic acid (DNS) solution after which they were heated in a boiling water bath for five minutes to develop colour. The O.D. was immediately measured at 540nm against a reference blank and the amount of soluble sugar present in mg glucose/g ogiri was calculated as follows.

The same procedure was adopted for the quantitation of sugar in ogiri B. Determinations were carried out in triplicates.

2.3.4. Extraction and Quantitation of Residual free amino acids present:

2.3.4.1. Extraction: To 2.0g of ogiri A and B were added 20ml of 80% ethanol in a conical flask and the resulting suspension shaken for maximum extraction. The suspension was allowed to clarify/settle under cooling after which it was centrifuged at 5000rpm for seven minutes. The supernatant was harvested and used for amino acid quantitation.

2.3.4.2. Quantitation: This was determined by the Ninhydrin reaction method of Rosen, (1957.) The total amino acid present as mg leucine/g ogiri was obtained from a standard curve based on known concentrations of leucine and calculated as follows in triplicate determinations:

2.3.5. pH: This was determined on a 10% suspension of fermenting mash at intervals of 24hours over a period of 120hours.

2.3.6. Microbial Count: This was determined according to the pour plate method of Harrigan and MacCance, (1976).

2.3.7. Moisture content: This was determined as follows on daily fermenting mash. About 5.0g portions of ogiri A and B were weighed into empty, dry and previously weighed petri dishes using an analytical balance. The ogiri

samples were dried at 105^{o}C until a constant weight was attained in each case. The loss in weight (moisture content) was calculated as a percentage of the wet mash as follows:

3. Results and Discussions

Table 1 shows the result of proximate composition of raw melon seeds (*Cucumeropsis mannii)* and ogiri obtained from dehulled and undehulled boiled seeds after fermentation.

A decrease in the fat and crude protein contents after fermentation into ogiri was observed in the dehulled and undehulled seeds; however, an increase was observed in the carbohydrate levels in both cases. These findings are in agreement with the findings of Omafuvbe *et al.,* (2004) in fermentation of melon seeds into condiment. The decrease in fat content was expected as some oil was observed to have leaked into the water during boiling of the seeds. This agrees with the observations of Odunfa, (1981) during preparation of melon seeds for fermentation into ogiri. In addition the decrease observed in fat content may also be attributed to the breakdown of fat into free fatty acids, some of which might have been used in flavor and aroma generation when in reaction with other components of the mash to form esters which produced the characteristic aroma of the food. Ouoba *et al.*, (2005) and Whitaker, (1978) reported on beneficial effects of lipase in the developments of characteristic flavours and aromas. Lipase activity was reportedly observed in the fermentation of boiled melon seeds by Onawola *et al.,* (2011) and in the fermentation of *Prosopis Africana* by Ogunshe *et al.,* (2007). The decrease observed in crude protein content has been attributed to a high proteolytic activity of the fermenting organisms, generating free amino acids (Onawola *et al.,* 2011), (Ogunshe *et al.,* 2007). However, some of the free amino acids might have been used up in cell generation and possibly for aroma, flavor and texture geneneration in a reaction with other components. Whitaker (1978) reported that in most fermented high - protein products, the extent of protein hydrolysis is one of the most important factors in the changes in texture and flavor. However, it was observed that fat, crude protein and carbohydrate retention was judged to be about the same in the ogiri obtained from the dehulled and undehulled boiled seeds, (this was not subjected to statistical analysis).

Table 2 shows the results of pH, moisture content and microbial count during fermentation of dehulled (A) and undehulled (B) boiled melon seeds into ogiri.

Steady pH increases were observed throughout the period of fermentation and this is in accordance with the observations of Ogunshe *et al.,* (2007) in *Aisa* production, Omafuvbe *et al.,* (2004) in *Iru* and *Ogiri* production, and Odunfa (1981) in ogiri production respectively. However, pH increases was more pronounced in dehulled seeds than in the undehulled. Sarkar *et al.,* (1997a), Barber and Achinewu (1992) reported that amino acids produced due to protein metabolism are responsible for gradual pH increase leveling off toward 7.5-8.0. pH increases may also be due to the formation of ammonia from amino acids. The microbial count was also observed to increase in both cases throughout the period of fermentation as was observed by Ogunshe *et al.,* (2007) and Odunfa (1981) respectively but with higher values in the dehulled seeds. However, a reversal of this trend was observed for moisture content, with a drastic reduction observed from day two unto the end of fermentation but yet with higher moisture retention in the dehulled seeds during fermentation.

The lower values of pH, moisture content and microbial count observed in the fermenting mash from undehulled boiled melon seeds could be as a result of the presence of the testa on the melon cotyledons. The presence of the testa might have resulted into a reduced multiplication of microorganisms and penetration of the cotyledons for nutrient use in the undehulled seeds. This is evidenced by the lower microbial count observed during fermentation and as microbial count increased from day to day, so did the pH, due to an increased release of amino acids and ammonia signifying the further breakdown and usage of nutrients encased within the testa. In contrast, the moisture content which was of high value at the start of fermentation dropped sharply at day two and further reduced toward the end of fermentation in both cases. Moisture retention in dehulled seeds was however higher than in the undehulled, right from day zero unto the end of fermentation, thus providing a more favourable environment for microbial growth and hence, digestion of nutrient. This is evidenced by a higher count as observed in the dehulled seeds. The sharp drop observed in moisture level with further reduction could be as a result of high activity of the organisms and possibly some evaporation at the temperature of incubation (37^{o}C).

Table 3 shows the result of residual soluble sugars and free amino acids in ogiri obtained from dehulled and undehulled boiled melon seeds after each day of fermentation.

It was observed that residual soluble sugar levels in both cases fluctuated from day to day during fermentation and this is in agreement with the findings of Onawola *et al.,* (2011), Ogunshe *et al.,* (2007), Omafuvbe *et al.,* (2004) and Odunfa, (1983) in melon seed and other leguminous seed fermentations respectively. However residual soluble sugar levels obtained from dehulled boiled seeds were much lower than those in undehulled boiled seeds. The fluctuations observed in residual soluble sugar levels may be attributed to high amylolytic activity coupled with high usage of sugar by fermenting organisms for metabolic activity. Onawola *et*

al., (2011) and Omafuvbe *et al.,* (2004) reported that the fluctuations in soluble sugar level with fermentation may be related to its utilization by fermenting organisms for their metabolic activities. The higher residual sugar level observed in ogiri from undehulled seeds may be attributed to a lower usage/consumption by fermenting organisms, possibly due to a lower microbial count and possibly also, an inherent property of the testa (perhaps, its permeability) on the melon cotyledons which may have lead to a higher retention of soluble sugars.

Residual free amino acids were found to increase from day zero of fermentation unto the last day without any fluctuations. This is in agreement with the observations of Omafuvbe *et al.,* (2004). Also similar increases in the levels of free amino acids with fermentation have been reported in other seeds by Omafuvbe *et al.,* (1999), (2000). The increase observed in residual free amino acids is due to the ability of the fermenting organisms to degrade protein, leading to increases in pH, free amino acids and increased microbial count (fermenting and unfermenting) as some of the free amino acids would have been used up in cell generation. A similar trend in free amino acid generation and usage has also been reported in fermentation of boiled melon seeds by Onawola *et al.,* (2011). A daily increase in proteinase activity during fermentation has been reported by Onawola *et al.,* (2011) and Omafuvbe *et al.,* (2004) and this is a reflection of rapid increases in free amino acids generated. Similar proteinase activity and hence, free amino acid increases have been reported in similar protein rich foods by Sarkar *et al.,* (1993) and Omafuvbe *et al.,* (2002). As it was with soluble sugar generation, lower values of residual free amino acids were observed in ogiri from dehulled seeds than from the undehulled. This may also be due to an inherent property of the melon seed coat leading to a higher retention of free amino acids in ogiri from undehulled boiled melon seeds.

Statistical analysis however reveal that the differences observed in the residual daily values of soluble nutrients (sugars and free amino acids) in ogiri obtained from dehulled and undehulled boiled melon seeds were significant and that the higher values observed in ogiri obtained from undehulled boiled seeds are actually higher than those obtained from dehulled seeds at the level of test $t_{\alpha = 0.05}$

4.Conclusion

The most significant aspect of melon seed fermentation is the increase in the soluble nutrients and of particular importance, the free amino acids liberated, thereby increasing the digestibility and utility of the melon seeds (Onawola *et al.,* 2011). However, it is apparent that during fermentation, the seed coat of the melon seed plays a great role in determining the digestibility of the food reserve, the amount of residual soluble nutrients available for human consumption, and regulating the amount of nutrient uptake by microorganisms for growth, metabolic processes and conversion to flavor and aroma components. Ogiri produced from undehulled boiled melon seeds has a higher retention of soluble nutrients, and therefore, the risk of pathogen ingestion from ogiri could be greatly reduced as the need for dehulling (with bare feet) may no longer be necessary. Also, the requirement for a mechanized dehulling for the purpose of easing the production process may no longer be necessary as this may increase the cost of production borne by the traditional entrepreneurs. It is therefore suggested that the dehulling stage of melon processing into ogiri (whether by manual or mechanical means) be possibly omitted as a more nutritious and health-safe ogiri can be made available from such process.

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Table 1. Proximate composition of raw melon seeds (*Cucumeropsis mannii)* and ogiri obtained from dehulled (A) and undehulled (B) boiled melon seeds.

Parameter (%)	**Raw melon seeds**	**Ogiri from A**	**Ogiri from B**
Fat	55.93	38.61	38.42
Crude protein	24.42	18.64	18.36
Carbohydrate	11.83	25.83	25.26
Moisture	6.69	7.25	7.15
Ash	3.13	3.67	3.95
Crude fibre	2.60	6.28	6.58
Phosphorous	0.04	0.0053	0.0045
Calcium	0.06	0.019	0.015
Magnessium	0.52	0.300	0.440

Table 2. pH, microbial counts and moisture content levels during the fermentation of dehulled (A) and undehulled (B) boiled melon seeds.

ND= Not Determined

Table 3. Residual soluble sugar and free amino acid levels in ogiri obtained from dehulled (A) and undehulled (B) boiled melon seeds.

Fermentation (days)	**Soluble sugar (mg glucose/g) A**	**Soluble sugar (mg glucose/g) B**	**Free amino acids (mg leucine/g) A**	**Free amino acids (mg leucine/g) B**
0	2.61 ± 0.04	11.21 ± 0.03	4.74 ± 0.21	7.78 ± 0.05
1	0.09 ± 0.02	6.97 ± 0.02	15.98 ± 0.72	23.35 ± 0.57
2	2.09 ± 0.03	21.73 ± 0.07	26.14 ± 0.39	50.40 ± 1.20
3	6.81 ± 0.03	13.35 ± 0.03	29.52 ± 0.53	50.21 ± 0.68
4	3.67 ± 0.01	9.78 ± 0.04	41.53 ± 0.45	60.68 ± 0.80
5	2.67 ± 0.02	10.02 ± 0.03	42.83 ± 0.29	60.96 ± 0.41