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Effect of Fermentation on Physicochemical Properties and *in vitro* Radical Scavenging Ability of *Citrullus vulgaris*

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AOI and TRO designed the study. Author AOI performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AOI and TRO managed the analyses of the study. Authors AOI and TRO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Ogiri, a fermented product of melon seed (*Citrullus vulgaris),* is a fermented condiment rich in vitamins and protein commonly consumed in South-East and Western Nigeria. It was produced by sorting the dehulled seed by hand to remove the spoilt seeds, washing, boiling for 4 h, aseptically pouring into the fermenting can and incubating at 35 °˚C for 120 h. The fermented and unfermented cotyledons were analyzed for physicochemical properties and vitamins. pH and total titrable acidity increased from 6.34 to 7.28 and 0.4 N to 0.23 N respectively. The moisture content increased from 7.78 % to 32.77 %. Both *Citrullus vulgaris* and *Ogiri* exhibited antioxidant efficacy. The vitamins and 2,2-diphenyl-1-picrylhydrazyl (DPPH)increased significantly which showed changes in the radical scavenging ability. *Ogiri* had improved nutritional composition, as a condiment, it is therefore recommended for consumption in preference of melon seed.

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1. INTRODUCTION

Fermentation started in Babylon 7,000 years ago and has been assumed to be the oldest means of food preservation [1]. Fermentation in food processing can be said to be the means of by which carbohydrates are being converted to alcohol and carbon dioxide or organic acids by making use of the microorganisms; yeasts, bacteria and molds [2]. The process of fermentation increases the nutritional value of food, as well as its shelf-life and flavor [3]. It also makes food to be easily digested in the body. *Citrullus vulgaris* (melon seed) is a plant which belongs to the family of *Cucurbitaceae*. Members of the family include Melon, Cucumber and Pumpkin among others. They are useful both medicinally and nutritionally. *Citrullus vulgaris* produces oil wused in the preparation of food for human consumption because of their special flavor. It is consumed in some African countries as well as in the Middle East [4].

Ogiri is an oily paste produced mainly from *Citrullus vulgaris* and consumed within the West African countries [5]. The production process is still a traditional family art and the fermentation is by chance inoculation and alkaline fermentation of the melon seeds [6]. *Ogiri* serves as a cheap soup condiment particularly among the poor rural dwellers. In the South-East Nigeria, *ogiri* can also be produced from castor oil seeds (*Ricinus cummunis)* [7] and fluted pumpkin (*Telfariaoccidentalis*) [8,9].

Free radicals are highly reactive substances containing unpaired electrons. They are taught to be responsible for producing irreversible damage to biomolecules and may cause agents of slower, ubiquitous ageing process. Intermediates produced during reduction of oxygen in reactive oxygen species (ROS) include superoxide anion radical (O_2.), hydroxyl radical (OH.) and hydrogen peroxide (H_2O_2) [10]. According to free radical theory of aging, cells continuously produce free radicals due to oxidative stress, and constant radical damage eventually kills the cell. When radicals kill or damage enough cells in an organism, the organism ages [11]. Antioxidants vitamins are free radical scavengers needed in most body metabolic functions. Antioxidants interact with and stabilize free radicals and may prevent some of the damages free radicals might otherwise cause. Free radical damage may lead to cancer. Antioxidant vitamins include vitamins A, C and E among others [12]. *Citrullus vulgaris* is a good source of vitamin [13].

Ogiri has been used as condiment in South East and West Nigeria because of its high protein contents, hence, this study embarked upon the determination of physicochemical properties and radical scavenging ability of fermented and unfermented *Citrullus vulgaris* juxtaposing *Ogiri* a better Scavenger to melon seed.

2. MATERIALS AND METHODS

2.1 Source of Seeds and Preparation of *Ogiri*

The shelled melon seeds of *Citrullus vulgaris* were purchased at Ado- Ekiti, Ekiti State, Nigeria. The equipment employed in the process of production and analysis of the product *ogiri* were obtained from the laboratory of the Department of Microbiology, Ekiti State University, Ado-Ekiti and College of Agriculture, Federal University of Technology, Akure, Ondo State, Nigeria. All the reagents used in this study were of analytical grade. The method of Omafuvbe *et al*. (2004) was adopted in the processing of the melon seeds to *ogiri*. Two hundred grams (200 g) of the shelled melon seeds were sorted in order to remove dirt, spoilt and decomposing seeds. It was then washed thoroughly with water and boiled for four hours (4 h) and 72 hundred grams (100 g) of the boiled melon seeds were wrap ped tightly and left to ferment in the fermenting cans for five days (120 h) at a temperature of 35°C.

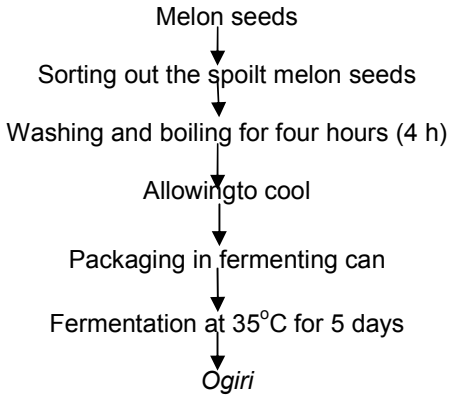

Fig. 1. Flowchart for the processing of *ogiri* from melon [14]

2.2 pH Determination

According to AOAC [15], twenty grams (20 g) of the sample was taken and mashed using a sterile mortar and pestle. Thereafter, 250 ml of distilled water was added to the sample in order to homogenize it and then the probe of the pH meter (which has been calibrated with buffer (7.0) was rinsed with distilled water and placed into the solution in order to determine the pH.

2.3 Total Titratable Acidity

According to AOAC [15], the slurry formed when the mashed sample was dissolved in Distilled water was filtered (using whatsman no.1 filter paper). Two drops of phenolphthalein was added to 10ml of the filtrate. The total titrable acidity was determined by titrating against 0.1M NaOH. The end point was the appearance of a pink colour. TTA is calculated as:

$$NaVa = N_bV_b \quad (1)$$

2.4 Moisture Content

This was determined according to AOAC [15] by weighing a clean and well labeled petridish that has been oven-dried (W1). Five grams (5 g) of the sample was added to the dish and this was reweighed (W2). The dish and its content was transferred to the thermo setting oven at about 105^{o} C for about 24 h. After which it was transferred to the desiccator and cooled for about one hour (1h) and weighed again. This was repeated severally to get a constant weight (W3).

$$\% \text{ Moisture} = [(W_2 - W_3)100]/(W_2 - W_1) \quad (2)$$

2.5 Determination of Vitamin A

According to Pearson [16], a weighed quantity (2 g) of the sample containing not more than 1 g of fat was mixed with 30 ml absolute alcohol and 3 ml of 5 % potassium hydroxide. This was boiled gently under reflux for 30 minutes in a stream of oxygen free nitrogen. It was cooled rapidly and 30 ml of water was added after which it was transferred to a separator. It was washed in 3 x 50 ml ether and the vitamin A was extracted by shaking for 1 minute. After complete separation, the lower layer was discarded and the extract was washed with 4 x 50 ml water, mixing it cautiously in order to avoid emulsion formation during the first two wash. The washed extract was evaporated down to about 5 ml and the remaining ether was removed in a stream of nitrogen at room temperature. After which the residue was dissolved in sufficient isopropyl alcohol to give a solution that contains 9-15 units per ml and the extinctions were measured at 300, 310, 325 and 334 nm with the maximum wavelength.

2.6 Determination of Vitamin B1 (Thiamin)

According to Okwu and Josiah [17], 5 g of the sample was homogenized with 50 ml ethanolic sodium hydroxide. It was filtered into a 100 ml conical flask and 10 ml of the filtrate was pipetted. The colour was developed by the addition of 10 ml of 1% potassium dichromate and the absorbance read at 360 nm. A blank solution was also prepared.

2.7 Determination of Vitamin C (Ascorbic Acid)

Ascorbic acid reduces the 2,6-dichlorophenol indophenol dye to a colourlessleuco-baseand it is oxidized to dehydroascorbic acid. The end point is the appearance of a pink colour because the dye is a pink coloured acid medium. According to Pearson [16], five milliliters (5 ml) of the working standard solution was pipetted into a 100 ml conical flask and ten milliliters (10 ml) of 4 % oxalic acid was added. This was titrated against the dye (V1 ml). The end point was the appearance of a pink colour which persisted for a few minutes. “The amount of the dye consumed is equivalent to the amount of ascorbic acid”. The sample was extracted in 4 % oxalic acid and made up to a known volume (100 ml) and it was centrifuged and five milliliters (5 ml) of the supernatant was pipetted, ten milliliters (10 ml) of 4% oxalic acid was added and titrated against the dye (V2 ml). It was calculated as follows:

$$AA\ (mg/100mL) = [(0.5mg \times V_2) / (V_1 \times 5mL \times W_{sample})] \times 100 \quad (3)$$

Where AA is ascorbic acid. V_1 is the volume consumed by the standard solution, and V_2 is the volume consumed by the sample solution, and W_{sample} is the weight of sample taken.

2.8 Determination of Vitamin D

According to Pearson [16], Vitamin D content was determined by mixing freshly prepared carr-price reagent (20 % m/v of antimony trichloride in chloroform with 40% pureacteyl chloride) that is free from alcohol. Nine millitres (9 ml) of the carr-price reagent was added to 1 ml of the sample extracted with chloroform and the extinction was measured at 500 nm against the blank reagent. The concentration was extrapolated from the standard curve using vitamin D standard.

2.9 Determination of Vitamin E

According to Pearson [16], 1.0 g of sample was placed in 100 ml flask fitted with a reflux condenser, then 10 ml of absolute alcohol and 20 ml of 1M alcoholic sulphuric acid was added. It was refluxed for 45 minutes and cooled. Fifty milliliters (50 ml) of water was added; it was transferred to a separating funnel of low actinic glass with the addition of a further 50 ml of water. The saponifiable matter with 5x30 ml diethyl ether was extracted, after which the combined ether extract free from acid was washed and dried over anhydrous sodium sulphate.

The extract was evaporated at low temperature of about 30° C. Protecting it from sunlight, the residue was dissolved in 10 ml absolute alcohol, both the standard and the sample were transferred to a 20 ml volumetric flask and 5 ml of absolute alcohol and 1ml concentrated nitric acid was added. The flask was placed on a water bath at 90°C for 3 minutes. It was cooled under running water and made up to the volume of 20 ml with absolute alcohol. The absorbance was measured at 470 nm against blank containing absolute alcohol.

$$C_{sample}\ (mg/mL) = [(A_{sample} \times C_{standard})/A_{standard}] \quad (4)$$

Where $C_{sample}$is the concentration of sample solution.$C_{standard}$ is the concentration of standard solution, $A_{sample}$is the absorbance of the sample solution, and $A_{standard}$ is the absorbance of the standard solution.

2.10 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The method described by Garcia *et al.*, [18] was modified, 500 microliters of prepared samples were respectively mixed with 500 microliters of methanolic solution containing 0.3mmol·L−1 DPPH radicals. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 516 nm against blank (methanol). The percentage scavenging effect was calculated according to the equation:

$$\%DPPH = [(A_{standard} - A_{sample})100]/A_{standard} \quad (5)$$

$A_{standard}$ = Absorbance of reference, A_{sample} = Absorbance of sample

3. RESULTS AND DISCUSSION

The results of the analysis show the physicochemical property, vitamins and DPPH content of both the fermented and unfermented samples.

Table 1 shows the result of the pH, total titrable acidity and moisture content of the melon seed and *Ogiri* in which there was an increase in pH from 6.34 to 7.28 after the melon seeds were fermented indicating that *Ogiri*is slightly alkaline. This trend in pH agrees favorably with the earlier studies carried out on *Ogiri* by Ogunsanwo *et al.* [19] and Njoku *et al.* [20]. The high pH value of the *Ogiri* as compared to melon seed might have been attributed to higher protein contents of these seeds and the ammonia released due to the proteolytic activity that occurred during fermentaion [21]. Wang, and Fang, [22] referred to such fermentation as alkaline fermentation and this aids in prolonging the shelf-life of melon seeds.

Table 1 further shows the total titrable acidity of the melon seed and *Ogiri.* The table showed that an inverse relationship is established between the pH and total titrable acidity. The total titratable acidity of the raw melon seeds decreased from 0.4 N to 0.23 Nat the end of the fermentation. Liberation of ammonia is a common phenomenon [6]. This might be due to hydrolysis of protein and abundant production of ammonia. The values of the total titrable acidity were appreciably small values and these low acidic level of the products aids the growth of fermenting organisms and inhibits the growth of other contaminants. Table 1 shows the moisture content of the melon seed and *Ogiri.* The increase in moisture content of *Ogiri*might be as result of the moist nature of the fermentation process [14]. It may also be possible that the hydrolytic composition of the fermenting melon seeds had contributed to the increased moisture content.

Table 1. Table of pH, total titrable acidity and moisture content of melon seed and *Ogiri*

Samples	pH	Total titratable acidity (N)	Moisture Content (%)
Melon seed	6.34 ± 0.02	0.4 ± 0.02	7.78± 0.93
Ogiri	7.28 ± 0.02	0.23 ± 0.01	32.77±0.96

Note: Data are expressed in Mean±SD from triplicate experiments (n=3) at p<0.05

Table 2. Table of vitamins

Samples	Vitamin A (mg/100 g)	Vitamin B_1 (mg/g)	Vitamin C (mg/100 g)	Vitamin D (mg/g)	Vitamin E (mg/g)	DPPH (%)
Melon seed	23.33± 0.01	0.376± 0.00	3.43± 0.01	1.660± 0.00	0.110± 0.00	68.02 ±0.09
Ogiri	24.23± 0.02	0.509± 0.00	4.12± 0.02	2.579± 0.02	0.555± 0.01	84.71±0.02

Note: data are expressed inMean±SD from triplicate experiments (n=3) at p<0.05

Table 2 above show that both melon seed and *Ogiri*exhibited antioxidant efficacy assessedby DPPH assay. The result obtained presents the changes in radical scavenging ability of these samples. DPPH content was found to be higher in *Ogiri*.It is shown that *Ogiri*consist of good antioxidant activity as compared to melon seed. Thus, indicating that fermentation process increases the DPPH free radical scavenging capacity. The vitamin content of the raw melon seeds increased after fermentation. Vitamin A increased from 23.33mg/100g to 24.23mg/100g, vitamin B increased from 0.376 mg/g to 0.509 mg/g, vitamin C increased from 3.43 mg/100 g to 4.12 mg/100 g, vitamin D increased from 1.660 mg/g to 2.579 mg/g while vitamin E increased from 0.110 mg/g to 0.555 mg/g. This is an indication that *Ogiri*is richer in vitamins, a better anti-oxidant nutrient and as such have a better radical scavenging potential compared to melon seed. Vitamin E is the most powerful natural antioxidant. It is involved in removal of free radicals and prevents their peroxidative effects on unsaturated lipids of membranes and thus helps maintain the integrity of cell membrane and reducing the risk of atherosclerosis [23,24]. Vitamin E also protects red blood cells from hemolysis by preventing peroxidation. Vitamin E (*α*-tocopherol) reacts with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction, acting as free radical trapping antioxidant [25]. The tocopheroxy-free radical product, formed in the process, is relatively unreactive and ultimately forms non-radical compounds.

Usually the tocopheroxyl radical is reduced back to *α*-tocopherol again by reaction with vitamin C from plasma or reduced glutathione (G-SH). The fact that vitamin C is very sensitive to reversible oxidation, Ascorbic acid to Dehydroascorbic acid, suggests that it may be involved in cellular oxidation- reduction reactions, perhaps serving as hydrogen transport agent. Ascorbic acid is required for functional activities of fibroblasts and osteoblasts [26].

4. CONCLUSION

This study shows that processing of *citrullus vulgaris* to *Ogiri* will produce a high nutritive food Condiment and as well serve as good nutraceutical against free radicals that could otherwise cause damage in human body. This implies that melon seed is more beneficially taken as *Ogiri* by humanfor its higher nutrient and pharmacological purpose. Therefore, the choice of quality raw material, hygienic fermentation conditions and the use of starter culture instead of natural fermentation may be encouraged for high quality product and to avoid contamination of such product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Battcock M, Azam-Ali S. Fermented fruits and vegetables: A global perspective. Food and Agriculture Organisation of the United Nations. F.A.O. Rome; 1998.
2. William CF, Dennis CW. Food microbiology. Fourth Edition, McGraw Hill, India. 2011;330.

3. Adeleke RO, Abiodun OA. Physicochemical properties of commercial local. Pakistan Journal of Nutrition. 2010;9(9):853-855.
4. Al-Khaliffa AS. Physicochemica characteristics, fatty acid composition and lipoxygenase activity of crude pumpkins and melon Seed oils. Journal of Agricultural 219 Food Chemistry. 1996;44:964-966.
5. Odunfa SA. Microbiology and amino acids composition of *Ogiri* a food condiment from fermented melon seeds. Die Nahrung. 1981;25:811-813.
6. Odunfa SA. Microbiological and toxicological aspect of fermentation of castor oil seeds for *Ogiri* production; 1985.
7. Enujiugha VN. Nutrient changes during the fermentation of African Oil Bean (*Pentaclethra macrophylla* Benth) Seeds. Pakistan Journal of Nutrition. 2003;2(5): 320-323.
8. Odibo FJC, Nwabunia F, Osuigwe DI. Biochemical changes during Fermentation of *Telfairia occidentalis* seeds for production. World Journal Microbial Biotechnology., 1990;6:425-427.
9. Omafuvbe BO, Oyedapo OO. Observed biochemical changes during Natural fermentation of African oil bean (*Pentaclethra macrophylla*) seeds. Nigerian Journal of Nutritional Science. 2000;21:19-23.
10. Mehlhorn RJ. In physiological basis of aging and geriatrics, edited by 238 Paola S. Timiras (CRS Press, Ann Arbor). 1994;61-72.
11. Takayuki Ozawa in understanding the process of Aging. Edited by Enrique Cadenas and Lester Packer (Marcel Dekker, New York). 1999;265-292.
12. Sies H. Oxidative Stress: Oxidants and Antioxidants. Exp. Physiol. 1997;82(2):291 -295.
13. Sodeke VA. Extraction of oil from water melon seeds and analysis. Quarterly Research Service. 2005;25-30.
14. Omafuvbe BO, Falade OS, Oshuntogun BA, Aduwusi SRA. Chemical and biochemical changes in African locust bean (*Parkia biglobosa*) and melon (*Colocynthis vulgaris*) during fermentation of condiments. Pakistan Journal of Nutrition. 2004;3(3):140-145.
15. AOAC. Official methods of Analysis. 14th 220 edition, Association of Analytical Chemists. Washington D.C.; World Journal Microbial Biotechnolog. 2005;367-384.
16. Pearson D. Chemical analysis of food, 7th edition. Churchill Ligstone, 269 London. 1975;419-620.
17. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006;5(4):357-351.
18. Garcia EJ, Oldoni TLC, Alencar SM, Reis A, Loguercio AD, Grande RHM. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian Dental Journal, 2012;23:22-27.

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19. Ogunsanwo BM, Faboya OO, Ikotun T, Idowu R. Fate of aflatoxins in soybeans during the preparation of Soyogi. Molecular Nutrition & Food Research. 1989;33:5485-487.
20. Njoku HO, Ogbulie JN, Nnubia C. African oil bean (*Pentaclethra macrophylla* Bentham) for 'ugba' production. Journal of Food Science. 1990;49:13-26.
21. Lopez-paredes O, Harry GI. Food biotechnology review: Traditional solidstate fermentation of plant raw materials application, nutritional significance and future prospects. CRC. Critical Review in Food Science and Nutrition. 1991;27:159-187.
22. Wang J, Fang DY. Critical review microbiology. 1996;22:101-139.
23. Arinathan V, Mohan VR, De-Britto AJ, Chelladurai V. Studies on food and medicinal plants of western ghats. Journal of Economic and Taxonomic Botany. 2003;27:750-753.
24. Arun AB, Sridhar KR, Raviraja NS, Schmidt E, Jung K. Nutritional and antintritional components of canavaliaspp. Seeds from the West Coast Sand Dunes of India. Plant Foods for Human Nutrition, 58, 1-13." "Takayuki Ozawa in understanding the process of Aging, edited by Enrique Cadenas and Lester Packer (Marcel Dekker, New York, 1999). 2003;265-292.
25. Aletor VA, Aladetimi OO. Compositional evaluation of sov me cowpea varieties and

some under-utilised edible legumes in Nigeria. Die Nahrung. 1989;33:999-1007. DOI:http://dx.doi.org/10.1002/food.19890331023

26. Oboh G, Akindahunsi AA. Change in the ascorbic acid, total phenol and antioxidant activity of some sun-dried green leafy vegetables in Nigeria. Nutrition and Health. 2004;18:29-36.
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