

Original Research Article

EFFECT OF FERMENTATION ON PHYSICOCHEMICAL PROPERTIES AND IN-VITRO RADICAL SCAVENGING ABILITY OF *Citrullus vulgaris*

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. Its production involve sorting the dehulled seed by hand to remove the spoilt seeds, washing, boiling for 4hrs, aseptically pouring into the fermenting can and incubating at 35⁰C for 120hrs. The fermented and unfermented cotyledon were analysed physicochemical properties and vitamins. pH and total titrable acidity equally increased from 6.34 to 7.28 and 0.4N to 2.38N respectively. The moisture content increased from 7.78% to 32.77%. Both *Citrullus vulgaris* and *Ogiri* exhibited antioxidant efficacy. The Vitamins 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.

Keywords: Fermentation, ‘ogiri’, melon, *Citrullus vulgaris*, vitamins.

INTRODUCTION

Fermentation started in Babylon 7,000 years ago and has been assumed to be the oldest means of food preservation (Battcock and Aza-Alli, 1998). The process of fermentation increases the nutritional value of food, as well as its shelf-life and flavor. It also makes food to be easily digested in the body. 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 (William and Dennis, 2002; Adeleke *et al.*, 2010). Compared to the size of those microorganisms, their effect is usually felt on the nature of

32 food especially the flavor and other organoleptic properties, which are usually very obvious
33 (Okafor, 2009).

34 *Citrullus vulgaris* (melon seed) is a plant which belongs to the family of *Cucurbitaceae*.
35 The family *Cucurbitaceae* can also be called the Cucurbit family. The gourd, Melon, Cucumber
36 or Pumpkin are commonly found in the same Cucurbit family. They are useful both medicinally
37 and nutritionally. *Citrullus vulgaris* produces oil which is used in the preparation of food for
38 human consumption because of their special flavor and it is consumed in Nigeria and other
39 African countries and in the Middle East (Al-Khaliff, 1996).

40 ‘Ogiri’ is an oily paste produced mainly from *Citrullus vulgaris* and consumed within the
41 West African countries (Odunfa, 1981a). The production process is still a traditional family art
42 and the fermentation is by chance inoculation and alkaline fermentation of the melon seeds
43 (Odunfa, 1985a). ‘Ogiri’ serves as a cheap soup condiment particularly among the poor rural
44 dwellers. In the South-East Nigeria, ‘ogiri’ can also be produced from castor oil seeds (*Ricinus*
45 *cummunis*) (Enujiugha, 2003) and fluted pumpkin (*Telfaria occidentalis*) (Odibo *et al.*, 1990;
46 Omafuvbe and Oyedapo, 2000).

47 Free radicals are highly reactive substances containing unpaired electrons. They are taught to be
48 responsible for producing irreversible damage to biomolecules and may cause agents of slower,
49 ubiquitous ageing process. Intermediates produced during reduction of oxygen in reactive
50 oxygen species (ROS) include super-oxide anion radical (O_2^-), hydroxyl radical (OH.) and
51 hydrogen peroxide (H_2O_2) (Mehlhorn, 1994). According to free radical theory of aging, cells
52 continuously produce free radicals, and constant radical damage eventually kills the cell. When
53 radicals kill or damage enough cells in an organism, the organism ages (Takayuki, 1999).
54 *Citrullus vulgaris* is a good source of vitamin (Sodeke, 2005). Antioxidants vitamins are free
55 radical scavengers needed in most body metabolic functions. Antioxidants interact with and
56 stabilize free radicals and may prevent some of the damage free radicals might otherwise cause.
57 Free radical damage may lead to cancer. Antioxidant vitamins include vitamins A, C and E
58 among others (Sies, 1997).

However, *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*.

Materials and methods

2.1 Source of seeds and preparation of 'ogiri'

The shelled melon seeds also known as *Citrullus vulgaris* was 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, and 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 (200g) of the shelled melon seeds were sorted in order to remove dirt, spoilt and decomposing seeds. It was then washed thoroughly and boiled for four hours (4h) and hundred grams (100g) of the boiled melon seeds were wrapped tightly and left to ferment in fermenting cans for five days (120h) at a temperature of 35°C.

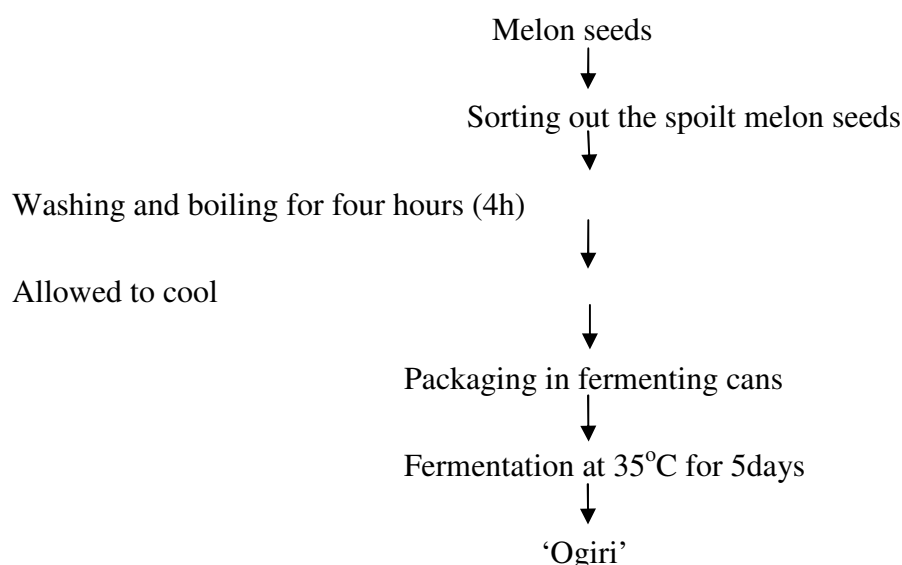


Fig. 1: Flowchart for the processing of 'ogiri' from melon

(Omafuvbe *et al.*, 2004)

83

84 **2.3 pH determination**

85 According to AOAC (2005), twenty grams (20g) of the sample was taken and mashed
86 using a sterile mortal and pestle, after which 250ml of distilled water was added to the sample in
87 order to homogenize it and then the probe of the pH meter (which has been calibrated with buffer
88 7.0) was rinsed with distilled water and placed into the solution in order to determine the pH.

89 **2.3 Total titratable acidity**

90 According to AOAC (2005), the slurry formed when the mashed sample was dissolved in
91 distilled water was filtered (using whatman no. 1 filter paper). Two drops of Phenolphthalein
92 was added to 10ml of the filtrate. The total titratable acidity was determined by titrating against
93 0.1M NaOH. The end point was the appearance of a pink colour.

94 **2.4 Moisture content**

95 This was determined according to AOAC (2005) by weighing a clean and well labelled
96 petri-dish that has been oven-dried (W_1). Five grams (5g) of the sample was added to the dish
97 and this was reweighed (W_2). The dish and its content was transferred to the thermo setting oven
98 at about 105°C for about 24h. After which it was transferred to the dessicator and cooled for
99 about one hour (1h) and weighed again. This was repeated severally to get a constant weight
100 (W_3).

101 **2.5 Determination of vitamin A**

102 According to Pearson (1975), a weighed quantity of the sample containing not more than
103 1g fat and atleast 240unit of vitamin A was mixed with 30ml absolute alcohol and 3ml of 5%
104 potassium hydroxide. This was boiled gently under reflux for 30 minutes in a stream of oxygen
105 free nitrogen. It was cooled rapidly and 30ml of water was added after which it was transferred
106 to a seperator. It was washed in 3X50ml ether and the vitamin A was extracted by shaking for
107 1minute. After complete seperation, the lower layer was discarded and the extract was washed
108 with 4X50ml water, mixing it cautiously in order to avoid emulsion formation during the first
109 two washes. The washed extract was evaporated down to about 5ml 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 was measured at 300, 310, 325 and 334nm with the maximum wavelenght.

2.6 Determination of vitamin B₁ (thiamin)

According to Okwu and Josiah (2006), Five grams (5g) of the sample was homogenized with 50ml ethanoic sodium hydroxide. It was filtered into a 100ml conical flask and 10ml of the filtrate was pipetted. The colour was developed by the addition of 10ml of 1% potassium dichromate and the absorbance was read at 360nm. 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 coulourless leuco-base and 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 (1975), Five millitres (5ml) of the working standard solution was pipetted into a 100ml conical flask and ten millilitres (10ml) of 4% oxalic acid was added. This was titrated against the dye(V₁ml). The end pont was the appearance of a pink colour which persists 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 (100ml) and it was centrifuged and Five millitres (5ml) of the supernatant was pipetted, ten millitres (10ml) of 4% oxalic acid was added and titrated against the dye (V₂ml). It was calculated as follows:

$$\text{Ascorbic acid mg/100ml} = \frac{0.5\text{mg}}{\overline{V_1\text{mL}}} \times \frac{V_2}{\overline{5\text{mL}}} \times \frac{100\text{mL}}{\text{Sample weight}} \times 100$$

2.8 Determination of vitamin D

According to Pearson (1975),Vitamin D content was determined by mixing freshly prepared carr-price reagent (20% m/v of antimony trichloride in chloroform with 40% pure acteylchloride) that is free from alcohol. Nine millitres (9ml) of the carr-price reagent was added to 1ml of the sample extracted with chloroform and the extinction was measured at 500nm

against the blank reagent. The concentration was extrapolated from the standard curve graph using vitamin D standard.

2.9 Determination of vitamin E

According to Pearson (1975), a suitable weight of sample 1.0g was placed in 100ml flask fitted with a reflux condenser, then 10ml of absolute alcohol and 20ml of 1M alcoholic sulphuric acid was added. It was refluxed for 45 minutes and cooled. Fifty millilitres (50ml) of water was added; it was transferred to a separating funnel of low aciditic glass with the addition of a further 50ml of water. The saponifiable matter with 5x30ml 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. Protecting it from sunlight, the residue was dissolved in 10ml absolute alcohol, both the standard and the sample was transferred to a 20ml volumetric flask and 5ml 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 20ml with absolute alcohol. The absorbance was measured at 470nm against blank containing absolute alcohol.

III. Results

The effect of fermentation on the nutritional quality of fermented and unfermented *Citrullus vulgaris* ('ogiri'). The results of the analysis shows the number of colonies isolated, cultural, morphological and biochemical characterization of the organisms isolated, as well as the physicochemical and vitamins 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 a slight increase in pH from 6.34 to 7.28 after the melon seeds were fermented indicating that *Ogiri* is slightly alkaline. These trend in pH agrees favourably to the earlier studies carried out on 'ogiri' by Ogunsanwo *et al.* (1989) and Njoku *et al.* (1990). 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 fermentation (Lopez-Paredes and Harry, 1991), Wang and Fang (1996)

referred to such fermentation as alkaline fermentation and this aids in prolonging the shelf-life of melon seeds.

Table 1: Table of pH, Total Titrable Acidity and Moisture Content of Melon seed and *Ogiri*

samples	pH	Total Titerable Acidity	Moisture Content
Melon seed	7.28	0.40	7.78%
<i>Ogiri</i>	7.28	2.38	32.77%

Table 1 further shows the total titrable acidity of the melon seed and *Ogiri*. The total titratable acidity of the raw melon seeds increased from 0.40 to 2.38 at the end of the fermentation due to the production of organic acids (Chelule *et al.*, 2010). This is in agreement with the research work carried out by David and Aderibigbe (2010). The values of the total titratable 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 also 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 (Omafuvbe *et al.*, 2004). It may also be possible that the hydrolytic composition of the fermenting melon seeds had contributed to the increased moisture content.

To assess the changes in the radical scavenging ability in this study **Table 2** showed that both melon seed and *Ogiri* exhibited antioxidant efficacy. 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 0.376mg/g to 0.509mg/g, vitamin C increased from 3.43mg/100g to 4.12mg/100g, vitamin D increased from 1.660mg/g to 2.579mg/g while vitamin E increased from 0.110mg/g to 0.555mg/g. These are an indication that *Ogiri* is richer in vitamins, a better anti-oxidant nutrient and as such having 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 (Arinathan *et al.*, 2003; Arun *et al.*, 2003). Vitamin E also protects red blood cells from hemolysis by preventing

peroxidation. Vit 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 (Aletor *et al.*, 1989). 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, and consequently for formation of MPS of connective tissues, osteoid tissues, dentine and intercellular cement substance of capillaries (Oboh and Akindahunsi, 2004).

Table 2 : Table of Vitamins

Samples	Vitamin A (mg/100g)	Vitamin B ₁ (mg/g)	Vitamin C (mg/100g)	Vitamin D (mg/g)	Vitamin E (mg/g)
Melon seed	23.33	0.376	3.43	1.660	0.110
<i>Ogiri</i>	24.23	0.509	4.12	2.579	0.555

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.

208 Therefore, the choice of quality raw material, hygienic fermentation conditions and the use of
209 starter culture instead of natural fermentation may be encouraged for high quality product and to
210 avoid contamination of such product.

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