Microbiology and Proximate Composition of 'Ogiri', A Pastry Produced From Different Melon Seeds

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Abstract: The total bacterial load in fermented *Cucumeropsis manii* (Naud); *Citrullus lanatus* (L) and *Colocynthis vulgaris* (Schrad) ranged from 2.12 x 10^8 to 2.15 x 10^8; 1.35 x 10^8 to 2.00 x 10^{10} and 2.05 x 10^8 to 2.10 x 10^{10} cfu/g respectively. Six bacterial species were isolated from the fermented products which were tentatively identified to belong to the genera: *Bacillus, Micrococcus, Leuconostoc, Streptococcus, Pediococcus* and *Lactobacillus.* The proximate composition of both fermented and unfermented samples of the three melon seeds were determined. Results showed that unfermented samples had higher amounts of dry matter (91.9 to 93.4g/mg) and crude fiber (2.61 to 3.85g/100g) than corresponding fermented products. The ash content decreased in the fermented samples, except in *Colocynthis vulgaris.* Fermented samples had higher amounts of moisture and carbohydrate; a higher pH and titratable acidity during fermentation. Potassium was the predominant mineral in the samples. It ranged between 1075.00 and 1834.42 mg/100g of dried fermented samples. The fermented products were challenged with four pathogenic organisms: *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Klebsiella* sp. The results indicated a prebiotic potential of freshly-fermented 'ogiri' against some of the pathogens. [New York Science Journal. 2010;3(4):18-27]. (ISSN: 1554-0200).

Key words: Proximate composition, pathogens, melon seeds, 'ogiri', fermentation, prebiotic

Introduction

'Ogiri' is an oily paste produced mainly from melon seeds and consumed within the West African countries (Odunfa, 1981a). The production process is still a traditional family art and the fermentation is by chance inoculation (Odunfa, 1985). '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* (Enujiugha, 2003) and fluted pumpkin (*Telfairia occidentalis)* (Odibo *et al.*, 1990; Omafuvbe and Oyedapo, 2000). Obizoba and Atti (1991) studied the chemical properties of fluted pumpkin, as the mostly used food condiment in some parts of Nigeria.

Apart from *Citrullus lanatus* which is the regular substrate used for the production of 'ogiri; there are other varieties of melon seeds which are readily available in South-West Nigeria. These other melon seeds which are underutilized by fermentation processes can serve as alternative substrates for the production of 'ogiri'. Contamination of foods by pathogenic organisms remains one of the major public health problems worldwide (Nester *et al.*, 1998). Food-borne diseases are endemic in many developing countries and constitute a major cause of mortality in these areas (Adam and Moss, 1999).

The objective of this study was to investigate the microbiology of two other types of melon seeds, *Cucumeropsis manii* (Naud), 'ito' and *Colocynthis vulgaris* (Shard) 'sewere' with the regular substrate, *Citrullus lanatus* (L.) 'bara' during production of 'ogiri'. The biochemical changes that occur during fermentation of the seeds and the probiotic potential of the three types of 'ogiri' products against some pathogenic bacteria were also monitored.

Materials and methods

Source of seeds and preparation of ogiri

The shelled melon seeds: *Citrullus lanatus* (L.) 'bara', *Cucumeropsis manii* (Naud), 'ito' and *Colocynthis vulgaris* (Shard) 'sewere' were bought at an open market in Ibadan, Nigeria (Bodija Market). The seeds were sorted to remove grit, dirt and decomposing ones washed and boiled for one hour in 10 times its volume of water. Then the water was drained and replaced with another after which the seeds were boiled for about six hours until the seeds were soft. The melon seeds were transferred into a clay pot and covered with *Thaumatococcus danielii* and wrapped with jute bag for five days. The fermented product was then mill to a pulp as described by Omafuvbe *et al.* (2004).

Microbiological Analyses

The total viable counts of the samples were analyzed daily by the method of Olutiola *et al.* (1991). Serial dilutions were done in sterile distilled water and platings were on plate count agar (PCA, Lab M). The mean of replicate platings were calculated and the total number expressed as cfu/g.

Pure cultures of isolates were stored on nutrient agar slants in a refrigerator (at 4^{o}C). The isolates were characterized by cultural, morphological and biochemical tests, which included Gram stain, motility, spore stain, catalase, coagulase and sugar fermentation tests as described by Olutiola *et al*, (1991).

Microbiological Challenge Test (MCT).

The four pathogenic organisms used for MCT were obtained from the stock culture of the Department of Microbiology, University of Ado-Ekiti, Nigeria. It was reported that they were the mostly encountered pathogens in many African fermented foods (Gadaga *et al.,* 2004). The pathogens are *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* sp. and *Staphylococcus aureus.* Eosine methylene Blue (EMB) Agar (Oxoid), Plate Count Agar (PCA Lab M), MacConkey Agar (Oxoid) and Mannitol Salt Agar (MSA Oxoid) were used respectively for the enumeration of the pathogens in both sterilized and freshly fermented 'ogiri' samples.

Sterilization of the 'ogiri' samples were done at 121^{o}C for 15 min. Ten grams (10g) of each sample in a screw-cap bottle was inoculated with 1 ml of the culture of the pathogenic organism, which had been standardized according to the method of Bauer *et al.*, (1966). This was mixed thoroughly with surface-sterilized spatula. Population of isolates were determined daily for seven days.

Proximate Analyses

The proximate composition of both fermented and unfermented melon seeds were analyzed by the method of Association of Official Analytical Chemists (1990). Samples were analyzed for fatty acids, crude fiber, soluble proteins, ash, titratable acidity and moisture contents. The pH of the sample was determined using a digital pH meter (ELE model, No

34). The energy values were calculated by adding up the values obtained for carbohydrates (x 17 kJ), crude protein (x17kJ) and crude fat (x37kJ) for each of the samples (Kilgour 1987). Calcium/phosphorus (Ca/P) and sodium/potassium (Na/K) ratios were calculated for all the samples as described by Nieman *et al*. (1992).The ash was digested with 3M HCl and mineral contents (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) were determined by atomic absorption spectrophotometry (Vogel, 1962) values recorded were the mean of triplicate determinations.

Statistical analyses

Data obtained were statistically analyzed using SPSS (version 11). Multiple comparisons of mean±SEM were carried out by correlation and two-way ANOVA. A probability level of less than 5% was considered significant.

Results

The microbial load increased progressively with days of fermentation in *C. manii,* except for the day 3, which had lower bacterial load when compared to day 2 (Figure 2). The microbial load in *C. lanatus* peaked on the 4th day of fermentation. During the fermentation of the melon seeds, six different types of bacteria were isolated. Characterization was based on their cultural, morphological and biochemical properties. Using the Bergey's Manual of Determinative Bacteriology (Bucchanan and Gibbons, 1974), the six isolates were tentatively identified as species belonging to the genera *Bacillus*, *Leuconostoc*, *Streptococcus*, *Pediococcus* and *Lactobacillus*. Figures 2, 3 and 4 show the population of the bacterial isolates during fermentation of *C. manni*, *C. lanatus* and *Co. vulgaris* respectively. *Bacillus* sp. had the highest population followed by *Micrococcus* sp., while *Lactobacillus* sp had the least count (Figure 2).

Table 1: Mineral contents of both fermented and unfermented melon and watermelon seeds (mg/100g)

	SAMPLES					
MINERAL	*Cucumeropsis manni*		*Citrullus lanatus*		*Colocynthis vulgaris*	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
Calcium	85.15± 0.10	78.36±0.31	52.71± 1.17	78.60± 0.38	77.16± 1.93	140.57± 2.90
Copper	6.54± 1.73	11.38± 2.55	13.67± 0.58	10.14± 2.12	16.28 ± 2.30	22.60 ± 1.53
Iron	24.60± 0.35	12.07 ± 0.36	24.44 ± 0.91	14.50 ± 0.83	18.08 ± 0.42	28.53 ± 0.72
Magnesium	80.21± 0.51	12.07 ± 0.36	114.39 ± 0.50	58.72 ± 0.46	85.19 ± 0.27	124.72 ± 0.77
Manganese	1.85± 0.05	7.56 ± 0.20	1.09 ± 0.26	1.15 ± 0.09	1.10 ± 0.09	1.60 ± 0.05
Phosphorus	179.02± 0.23	105.25 ± 0.18	169.31 ± 2.00	91.17 ± 0.23	130.23 ± 1.15	200.06 ± 4.61
Potassium	1671.34± 6.24	1691.34 ± 6.24	1741.37 ± 1.73	1075.00 ± 3.00	1623.43 ±1.73	1834.42 ± 4.58
Sodium	550.37±5.20	560.37 ± 5.29	631.82 ± 4.16	369.36 ± 1.73	497.67 ± 0.58	793.23 ±1.53
Zinc	1.91± 0.05	1.89 ± 0.05	1.19 ± 0.04	1.17 ± 0.02	1.85 ± 0.06	2.26 ± 0.04
Na/K	0.33	0.33	0.36	0.34	0.31	0.43
Ca/P	0.46	0.74	0.03	0.07	0.05	0.08

The values are the means ± SEM of triplicate determinations

Table 2: Proximate composition of both unfermented and fermented melon seeds samples (g/100g of dry matter).

	Cucumeropsis manni		*Citrullus lanatus*		*Colocynthis vulgaris*	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
Moisture content	8.1±1.5*	34.6±1.3	6.7±0.1	38.3±1.3	6.6±0.1	33.4±0.4
Dry matter	91.9±2.5	65.4±3.7	93.3±2.0	60.7±5.2	93.4±2.8	66.6±3.4
Ash	3.05±0.5	2.91±0.7	2.82±0.3	2.97±0.2	2.68±0.2	4.80±0.3
Soluble protein	36.20±0.8	32.00±1.5	28.30±2.5	31.50±0.1	32.30±0.2	24.60±0.3
Fatty acid	44.80±1.5	20.24±1.3	52.10±3.5	38.40±2.1	53.50±0.1	43.20±2.1
Crude fiber	3.30±0.1	1.99±0.1	3.43±0.2	1.98±0.1	3.85±0.2	3.75±0.2
Carbohydrate	12.20±0.3	24.50±1.3	13.30±0.2	25.20±1.3	07.60±0.2	23.60±0.5
pH	6.1±0.03	7.6±0.01	6.4±0.05	7.8±0.02	6.2±0.02	6.8±0.01
Titratable acidity	020±0.02	2.00±0.01	0.11±0.01	2.40±0.01	0.10±0.02	1.60±0.0
Metabolizable Energy (kJ/100g)	1865.0	1165.38	2153.8	1849.2	2108.7	1999.6

*The values are the means ± SEMs of triplicate determinations

Results of *C. lanatus* showed different trend than others. Population of bacterial isolates in *Co. vulgaris* and *C. manii* continued to increase during fermentation after 3-4 days. Similar results were obtained in the other two types of seeds (Figures 3 and 4)

Results of the MCT showed that the pathogens thrived or grow in the sterilized ‘ogiri’ samples (Figures 5, 6 and 7) but did not in the un-sterilized (freshly fermented) samples (Figures 8, 9, 10). The population of the pathogens decreased at different rates in the freshly fermented ‘ogiri’ samples; except for *Pseudomonas aeruginosa* whose population increased throughout the time of the study.

Moisture contents in the melon seeds were lower than their corresponding fermented products. The fatty acids contents were higher in the fermented melon samples (44.80 - 53.50g/100g) than in the fermented products (20.24 - 43.20g/100g). Similarly, the values of crude fibre; ash and soluble protein were higher in unfermented melon seeds than in the fermented products; except for ash content in *C. vulgaris.* In all the three samples the values of carbohydrate were higher in the fermented products than the substrates.

The pH of the unfermented substrates (ranged between 6.1 and 6.4) increased slightly during fermentation (pH 6.8 to 7.8). Titratable acidity of substrates also increased significantly during fermentation. The amount of metabolizable energy decreased after fermentation.

Table 1 shows the mineral contents of both unfermented and fermented melon seeds. The most abundant mineral was potassium, its value varied between 1075.00 and 1834.42 mg/100g dry matter in the fermented melon seeds substrates. The quantity of potassium increased in fermented *Cu. manni* and *Co. vulgaris* seeds after fermentation. Similarly the amount of iron, magnesium and phosphorus are higher in fermented *C. vulgaris* seed. Though calcium content was higher in unfermented *Cu. manii* than in the corresponding fermented product, the reverse was the case in *C. lanatus* and *Co. vulgaris.*

There was negative correlation between *Bacillus* sp and soluble protein (r=-0.84), and carbohydrate (r=-0.866). In addition, the relationship between total bacterial load and dry matter was also correlated with correlation coefficient of 0.972.

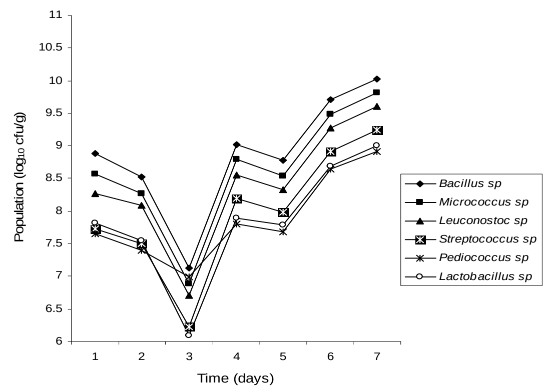

Fig 2: Population of bacterial isolates during fermentation of *Cu. manii* ($\log_{10}$ cfu/g)

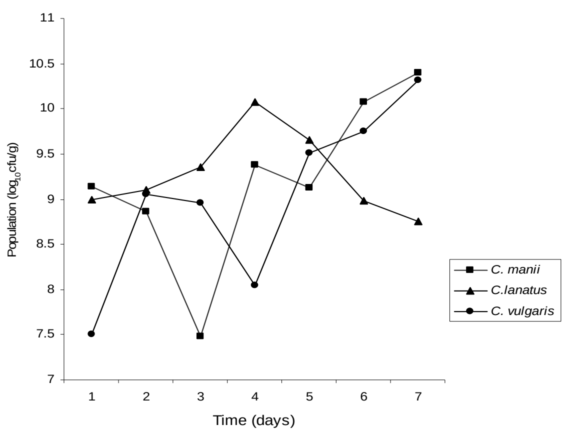

Fig 1: Total aerobic bacterial (plate) count during fermentation of melon and watermelon seeds ($\log_{10}$ cfu/g).

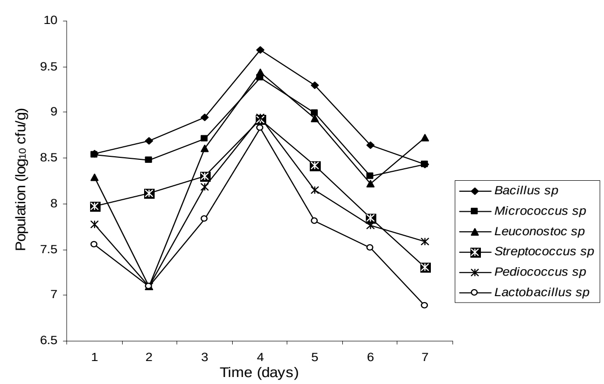

Fig 3: Population of bacterial isolates during fermentation
of *C. lanatus* ($\log_{10}$ cfu/g).

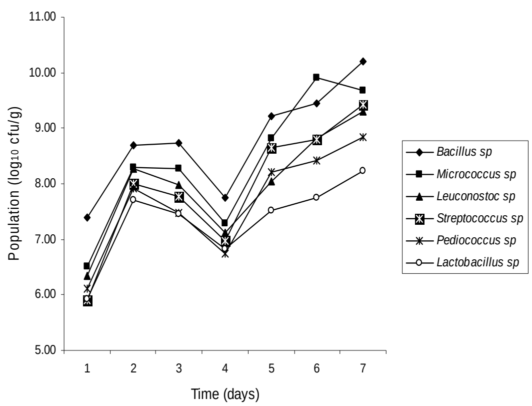

Fig 4: Population of bacterial isolates during fermentation of *Co. vulgaris* (log_{10} cfu/g).

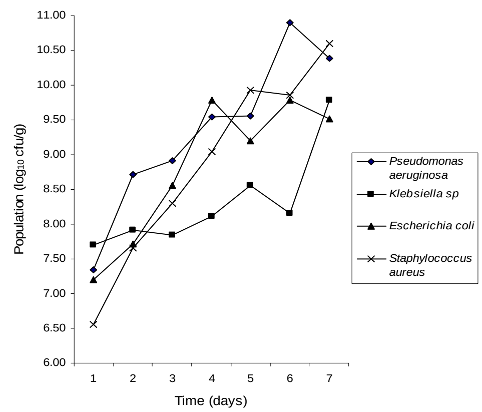

Fig 7: The population of pathogenic organisms in sterilized 'ogiri' produced from *Co. vulgaris* (log_{10} cfu/g).

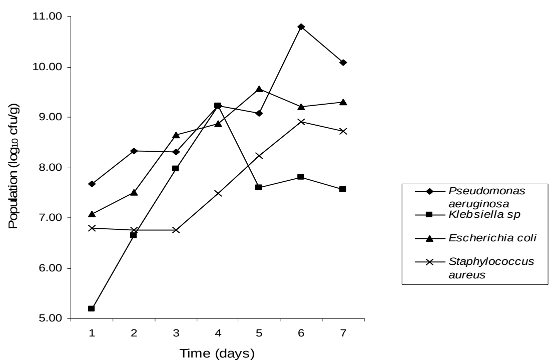

Fig 5: Population of pathogenic organisms in sterilized 'ogiri' produced from *Cu. manii* (log_{10} cfu/g).

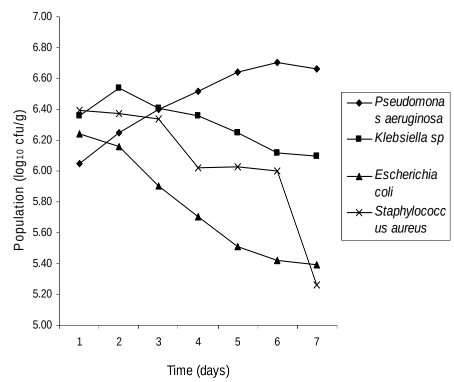

Fig 8: The population of pathogenic organisms in freshly fermented 'ogiri' produced from *Cu. manii* (log_{10} cfu/g).

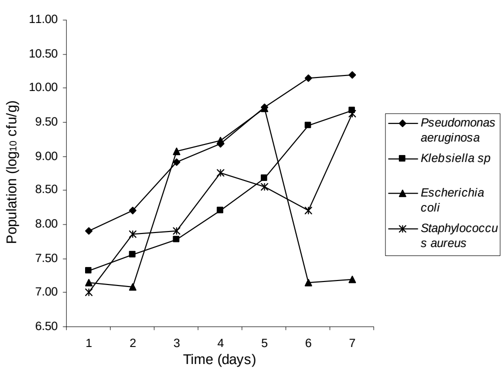

Fig 6: Population of pathogenic organism in sterilized 'ogiri' samples produced from *C. lanatus* (log_{10} cfu/g).

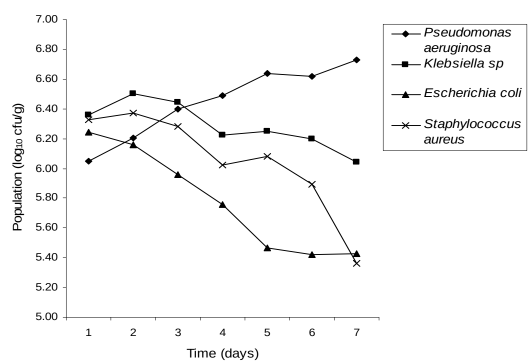

Fig 9: The population of pathogenic organisms in freshly fermented 'ogiri' produced from *C. lanatus* (log_{10} cfu/g).

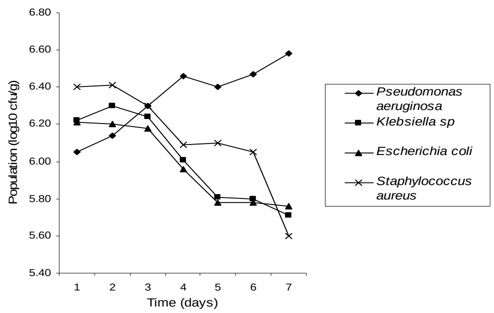

Fig 10: The population of pathogenic organisms in freshly fermented 'ogiri' produced from *Co. vulgaris* (log_{10} cfu/g).

Discussion and conclusion

The steady increase in the microbial load during fermentation of the melon seeds could have been influenced by the accumulation of toxic compounds such as organic acids and other metabolites. Yong and Wood (1976) also observed fluctuations in microbial load during the fermentation of soy sauce. Bacterial species belonging to the genera *Bacillus, Leuconostoc* and *Streptococcus* were found to be predominant during fermentation. This agrees with the earlier reports by Odunfa (1981 a, b). The increase in pH during fermentation of melon seeds could also have contributed to the poor growth of *Lactobacillus* sp, which had been reported to be aciduric (Aderiye and Ojo, 1987). Increase in pH during fermentation of protein-rich oil seeds has been reported (Onukwo, 1992; Aderibigbe and Adebayo, 2002). Population of bacterial isolates during fermentation continuously increased in *Co. vulgaris* and *Cu. manii* after 3-4 days but it decreased in *C. lanatus* this may be as a result of the intrinsic properties of the seed to support bacterial growth.

The increase in moisture contents of the fermented products agree closely with the report of Omafuvbe *et al.,* (2004).This may be as a result of the decomposition of the fermenting bacteria on the products. The large surface area of *Cu. manii* could have aided the loss of mineral contents during boiling as proffered by Omafuvbe and Oyedapo (2000) and Omafuvbe *et al.* (2000).The crude fiber values (1.98-3.75g/100g) obtained in this study were higher than the 0.2g/100g reported for soybean (Suarez *et al.*, 1999).

There was an increase in the soluble protein of *C. lanatus*, while a decrease was observed in that of *Cu. manii* and *Co. vulgaris.* The changes in nutrient composition during fermentation of melon seed could have been facilitated by the enzymatic activities of the fermenting organisms (Enujiugha, 2003; Odibo *et al.* 1990). The decrease in amount of soluble protein and carbohydrate with increase in population of *Bacillus* sp., suggests utilization by the organism. In this study, the most abundant mineral was potassium, which agrees with the report of Olaofe *et al.* (1993), and Olaofe and Sanni (1988). However the amounts of sodium in the samples differed from values reported by Olaofe *et al.*, (1994). The differences in sodium contents could be as a result of soil composition and the rates of uptake of the mineral by the plant.

In traditional fermented food preparations, microbes are used to prepare and preserve food products (Achi, 2005). Fermentation of food has many advantages such as improvement of nutritional value and ‘protection’ against bacterial pathogens (Gadaga *et al.*, 2004). The populations of the four pathogenic organisms inoculated into the sterile ‘ogiri’ samples increased during the seven-day incubation. The growth might have been supported by intrinsic factors of the ‘ogiri’ sample such as availability of nutrients, pH, water activity (a_w), lack of competing organisms and extrinsic factors which include the temperature of storage (Diet-Gonzalez *et al.*, 1988).

To avoid hypertension from food sources, the ratio of Na:K should be about 1:0.6 (Kilgour, 1987). This study has shown that fermentation has not significantly increased the Na:K ratio. Though the level was higher in fermented samples, the low levels of Ca:P (below 1:0.5) in both fermented and unfermented melon seeds might not allow strong bone development because absorption of calcium under this situation would be low (Nieman *et al.*, 1992).

Pseudomonas aeruginosa continued to increase on freshly fermented ogiri samples but other bacteria decreased. This may be as a result of its ability to metabolize varieties of substrates ranging from organic to inorganic. The relative reduction in the viable counts of the pathogenic organisms in the freshly fermented 'ogiri' during the storage at room temperature ($30 \pm 2^{o}C$) suggests a prebiotic potential of fresh 'ogiri' against the tested pathogens.

Based on microbial and biochemical changes observed in this study the two other types of melon seeds is safe and can also be used as substrates for commercial production of 'ogiri'.

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