Production and Proximate Composition of *Ogiri* Condiments from Different Substrates

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Abstract: Production and proximate analysis of *Ogiri* condiment from Melon, Castor oil, Pumpkin and Watermelon seeds were carried out usingSolid Sate Fermentation(SSF). Six (6)bacteria genera identified as *Staphylococcus* spp, *Klebsiella* spp, *Bacillus* spp, *Lactobacillus* spp, *Escherichia coli* and *Serretia* species and three fungi: *Aspergillus niger*, *Rhodotorula* spp and *Rhizopus stolonifer* were isolated from fermenting seeds. These isolates were recovered during the fermentation, but at the end of the fermentation, only *Bacillus* and *Lactobacillus* species were isolated from the *Ogiri* condiments. The pH of the Melon seed at the beginning of the fermentation was 5.5 while that of the *Ogiri* at the end of fermentation was 6.4.*Ogiri*producedfrom castor oil seed has the highest moisture content (10.84±0.05), Water melon had highest crude protein (38.32± 0.10), Melon has highest crude fat (44.86 ±0.50), water melon recorded the highest value in Ash content (6.36± 0.11), Castor oil seed has the highest crude fibre(10.17± 0.05) and highest carbohydrate value (45.98±0.04). The *Ogiri* condiments were found to be free from pathogens and spoilage organismsand also rich in nutrients such as protein and minerals. The *Ogiri* condiments could be good substitutes for animal proteins in rural areas where animal proteins are scarce.

Key words: Fermentation, microorganisms, *Ogiri*, proximate analyses, substrates

INTRODUCTION

A condiment is a substance applied to food in the form of a sauce, powder, spread or anything similar, to enhance or improve the flavour. In Nigeria and most African countries, condiments such as fermented locust bean (*Iru*), fermented melon seed (*Ogiri)*, fermented soybeans (*Dawadawa*), fermented cotton seed (*Ogiri*) and fermented pigeon pea are widely used to season food. The production of condiments is largely on a traditional small-scale, household basis under highly variable conditions (Odunfa, 1981).

Fermented food seasonings or local seasonings are those food seasonings which undergo traditional food processing method that involves biochemical changes brought about by microbes inherent in grain or derived from a starter culture and their enzymes (Smid and Hugenholtz, 2010).Fermented or local seasonings are good source of nutrients and could be used to produce complementary food supplements. Macro-nutrient in fermented legumes contributes to enhance food quality. Traditional fermented seasonings used in Nigeria includes African oil bean (*Pentaclethra macrophylla* Benth),*Ogiri* (fermented castor oil or melon paste), African locust bean (*Dawadawa*), *Okpeye* (*Prosopis Africana*), watermelon seed (*Citrillus lanatus*)and fluted pumpkin (*Telfairia occidentalis*).

These traditional fermented seasonings used in Nigeria are of great benefits, which include toxin removal, preservation, easing marketing and distribution tasks, and increasing food flavour. Local seasonings can also add extra nutrients such as vitamins B to traditional processes which it undergoes. In addition, it increases seasonal availability of many foods, enables transporting of delicate perishable foods across long distances and makes many kinds of food safe to eat by de-activating spoilage and pathogenic microorganisms.

Ogiri is a seasonings produced from Castor oil bean (*Ricinus communis*) by traditional fermentation processes (Barber and Achinewhu, 1992). *Ogiri* is use as a flavouring agent in soups and sauces. This research aimed at the production and proximate analyses of *Ogiri* condiments from different substrates.

MATERIALS AND METHODS

Collection of Samples

The Melon, Castor, Fluted Pumpkin and Watermelon seeds used in this research was purchase from New Umuahia Main market in Abia State, Nigeria.

Preparation of substrates for fermentation

Castor oil bean seeds

The traditional method of preparing *Ogiri* according to Odibo and Umeh (1989) was used in the spontaneous fermentation of castor oil bean into *Ogiri.*Three hundred grams (300g) of castor bean seed were boiled for two hours, dehulled, and drained and boiled again for about six hours. The seed were ground in a clean mortar into paste, wrapped with aluminum foil and allowed to ferment for four days at room temperature (Odibo and Umeh, 1989).

Melon seed

Three hundred grams (300g) of melon seed was washed and boiled for 12hour and left over night. They were ground into paste using a blender. The resulting paste was mixed into a big pasty mass with ash made from the tree of lined and covered with flamed banana leaves and left by the fire side for about 1- 2 days during which the melon oil drains into another container put underneath it. The big pasty mass was then cut into smaller sizes of flattened paste and wrapped with flamed banana leaves. The smaller wraps were cooked together and wrapped with a much bigger banana leaves and suspended over fire to maintain a temperature of 20 - 40^{o}C for four days after which the fermented products was ready for consumption (Odibo and Umeh, 1989).

Fluted Pumpkin seed

The cotyledons of pumpkin seed were removed manually after which 300 g of the sample was boiled for 2 hours. They were then washed, mashed in a mortar and wrapped in small balls with plantain leave and kept close to fire for fermentation to occur in for 3-4 days. After the fermentation, the fermented product was ready for consumption (Achi, 2005).

Fresh watermelon Seed

Watermelon Fruits (*Citrullus lanatus*) bought at Ubani Market, Umuahia were sliced open and the seeds were manually separated from the pulp. The seeds were washed with potable water to remove adhering pulp, dried at 45 ℃ in an oven for 1 hour and were manually shelled to obtain the dehulled seed Omafuvbe *et al.* (2004). Exact 300 g of the seeds was dehulled, washed and boiled for 1 hour in 10 times its volume of water. Then the water was drained and replaced with another after which the seeds were boiled for 4 hours until the seeds became soft. The boiled seeds were then washed, mashed in a mortar and wrapped in small balls with plantain leave and kept close to fire for fermentation to occur in 5 days (David and Aderibigbe, 2010).

Microbiological analysis of *Ogiri* Condiments

One gram of each of the fermenting seeds was diluted serially and 0.1ml aliquot of suitable dilution was inoculated onto Nutrient, De Man Rogosa and Sharpe (MRS) Agar for isolation of bacteria and Potato Dextrose Agars for isolation of fungi respectively in duplicates. The bacterial plates were incubated at 35^{o}C for 48 hrs while the fungal plates were incubated at 22^{o}C for 5 days. Various microbial counts were taken after. The isolates were sub-cultured and later characterized following Gram staining, biochemical and sugar fermentation tests (Cowan and Steele, 1965)

Proximate analysis of *Ogiri* Condiments

The samples were processed by milling into smooth powder and then analyzed for moisture, total ash, crude protein, fat, crude fibre (AOAC, 2005) and carbohydrate contents (James, 2005). The pH and temperature of the fermenting samples were also determined.

RESULTS

Table 1 shows the bacterial isolates recovered from the fermenting samples for *Ogiri* production. They include *Staphylococcus aureus, Klebsiella* spp,

Serratia spp*, Bacillus* spp, *Lactobacillus* spp and *Escherichia coli.*

The following Fungi: *Aspergillus niger*, *Rhodotorula* spp and *Rhizopus stolonifer* were isolated from the fermenting seed samples (Table 2).

From the viable cell count of isolates from fermenting seed samples, the total heterotrophic plate count was in the range:7.1 x 10^2 from melon after 48 hrs to 2.1 x 10^2CFU/g while the total coliform plate count was in the range: 6.1 x 10^2 from Melon seed to 1.5 x 10^2CFU/g. The Lactic acid bacteria plate count was in the range: 6.7 x 10^2 to 6.0 x 10^1CFU/g while the Fungal plate count was in the range: 6.2 x 10^2 to 1.2 x 10^2CFU/g. These are shown in Table 3.

Results of the pH and temperature values of fermenting seed samples are indicated in Table 4.The highest temperature recorded was 33^{o}C while the lowest was 28^{o}C. The highest and lowest pH values were 6.6 and 5.5 respectively.

The results of the microbial succession during fermentation of the seed samples are presented in Table 5. The following bacteria: *Streptococcus* spp*, Bacillus* sp, *Escherichia coli*, *Enterobacter*sp, *Proteus* sp, *Serratia marcesens* and *Lactobacillus* sp were isolated from the substrates within the first 23 hrs of the fermentation. On the other hand, these fungi: *Aspergillus flavus*, *Rhodotorula* sp and *Rhizopus stolonifer* were isolated within the same time. But at the end of the fermentation, only two bacteria: *Bacillus* sp, *Lactobacillus* sp were isolated while no fungus was isolated from the *Ogiri.*

The result of the Proximate Composition of *Ogiri*s amples is presented in Table 6. Castor has the highest moisture content (10.84) while pumpkin seed has the lowest value (5.63). For crude protein, watermelon has the highest value (38.32) while castor oil seed has the lowest value (26.39). Melon seed had the highest fat content (44.86) while Castor seed had the lowest (2.44). For ash content, Water melon seed had the highest value (6.36) while castor oil seed had the lowest. Crude fibre was found highest (10.17) in Castor oil seed while pumpkin seed had the lowest value (2.53). Castor oil seed had the highest vale (45.98) while the least was from watermelon.

Table 1: Morphological and Biochemical Characteristics of Bacteria isolates from *Ogiri*

Morphology	Microscopy		Catalase	Coagulase	Indole	Citrate	Oxidase	Motility	Glucose	Lactose	Sucrose	Manitose	Probable Organisms
Pink colony	-ve	Short rods	+	-	+	-	-	+	AG	AG	AG	A	*Escherichia coli*
Creamy mucoid colony	-ve	Single short rods	+	-	-	+	-	-	AG	AG	AG	AG	*Klebsiella* sp
Moistured milk colour	-ve	Straight short rods	+	-	-	-	-	+	NAG	NAG	AA		*Bacillus* sp
Red smooth colony	-ve	Short rods	+	-	-	-	-	+	A	A	AGAG		*Serretia* sp
Yellow to green pigment	-ve	Short thick rods	+	-	-	-	-	-	NAG	NAG	NAGNAG		*Lactobacillus* sp
Golden yellow, smooth colony	-ve	Cocci in cluster	+	-	-	-	-	+	A	A	AA		*Staph. aureus*

KEY: (+) present (-) absence; A: Acid production; AG: Acid and Gas production; NAG: No Acid or Gas production, - ve = Gram negative

Table 2: Fungal Isolates from Fermenting Substrates

Cultural characteristics	Microscopy	Isolates
Dark-brown Mycelium	Dark brown comidia and conidiosphere long	*Aspergillus niger*
Creamy colonies of profuse growth	Viable size and shaped cornidophore. Slender, simple, stunt etc. cornidia were seen as hyaline and slightly curved at both ends in a curved shaped form	*Rhodotorula* sp
Round and creamy colonies on SDA.	Single oval cells were seen, some in pairs and elongate, Also budding was pronounced, spores seen	*Rhizopus stolonifer*

Table 3: Viable Cell count of Isolate of Fermenting *Ogiri* (CFU/g)

Fermentation time (hr)	Samples	THPL	TCC	TLABC	TFPC
0	Melon seed	6.0×10^2	1.6×10^2	1.2×10^2	No growth
	Castor seed	2.1×10^2	2.9×10^2	1.9×10^2	No growth
	Pumpkin seed	4.6×10^2	3.6×10^2	6.0×10^1	No growth
	Water melon	2.9×10^2	No growth	2.2×10^2	No growth
24	Melon seed	4.2×10^2	1.8×10^2	2.1×10^2	No growth
	Castor seed	6.4×10^2	2.6×10^2	2.9×10^2	No growth
	Pumpkin seed	3.3×10^2	1.5×10^2	3.1×10^2	No growth
	Water melon	4.0×10^2	3.2×10^2	3.4×10^2	1.2×10^2
48	Melon seed	7.1×10^2	6.1×10^2	4.4×10^2	3.4×10^2
	Castor seed	5.4×10^2	3.2×10^2	5.8×10^2	4.1×10^2
	Pumpkin seed	5.9×10^2	4.6×10^2	3.9×10^2	2.6×10^2
	Water melon	6.2×10^2	3.9×10^2	4.3×10^2	3.0×10^2
72	Melon seed	4.9×10^2	4.1×10^2	5.6×10^2	4.4×10^2
	Castor seed	5.7×10^2	4.9×10^2	6.7×10^2	6.2×10^2
	Pumpkin seed	6.0×10^2	3.1×10^2	5.9×10^2	5.7×10^2
	Water melon	4.3×10^2	3.5×10^2	4.3×10^2	4.9×10^2

Key: THPL: Heterotrophic plate counts; TCC: Total coliform counts; TLABC: Total Lactic acid bacterial counts; TFPC: Total fungal plate counts

Table 4: Temperature and pH of Fermenting Seeds

	Temperature (^oC)				pH			
Fermentation period (h)	Melon seed	Castor seed	Pumpkin seed	Watermelon seed	Melon seed	Castor seed	Pumpkin seed	Watermelon seed
0	28	28	28	28	5.6	5.6	5.5	5.5
24	30	30	30	30	6.2	6.1	6.1	6.2
48	31	31	31	31	6.4	6.2	6.2	6.3
72	33	32	32	32	6.6	6.4	6.4	6.4

Table 5: Microbial Succession during Fermentation of Substrates

Fermentation period	Bacterial Succession	Fungal Succession
0	*Streptococcus* sp, *Bacillus* sp, *Escherichia coli*, *Enterobacter* sp, *Proetus* sp, *Serratia marcesens* and *Lactobacillus* sp	*Aspergillus flavus*, *Rhodotorula* sp and *Rhizopus stolonifer*
24	*Bacillus* sp, *Enterobacter* sp, *Proteus* sp, *Escherichia coli* and *Lactobacillus* sp	*Aspergillus flavus,* *Rhodotorula* sp and *Rhizopus stolonifer*
48	*Bacillus* sp, *Enterobacter* sp, *Lactobacillus* sp	*Rhodotorula* sp and *Rhizopus stolonifer*
72	*Bacillu s* sp, *Lactobacillus* sp	Not detected

Table 6: Proximate Composition of *Ogiri* from Various Substrates (g/100g)

Sample	Moisture	Crude Protein	Fat	Ash	Crude fibre	Carbohydrate
Melon seed	$6.00\pm^{c}$ 0. 28	34.50^{b} ±0.14	44.86^{a}±0.50	4.66^{c}±0.08	6.57^{b}±0.06	3.75^{d}±0.02
Castor seed	10.84^{a}±0.05	26.39^{d}±0.01	2.44^{d}±0.03	4.21^{d}±0.14	10.17^{a}±0.05	45.98^{a}±0.04
Pumpkin seed	5.63^{d}±0.03	33.00^{c}±0.19	36.96^{c}±0.36	5.33^{b}±0.01	2.53^{d}±0.05	16.54^{b}±0.45
Watermelon seed	8.60^{b}±0.12	38.32^{a}±0.10	41.50^{b}±1.14	6.36^{a}±0.11	5.26^{c}±0.04	6.77^{c}±0.06

Values with the same superscript down the column is not significantly different ($p \leq 0.05$)
Mean with± standard deviation N= 2

DISCUSSION

The following bacteria: *Staphylococcus aureus*, *Klebisella* spp, *Lactobacillus* spp, *Bacillus* spp, *Escherichia coli* and *Serratia* spp and the following fungi: *Aspergillus niger*, *Rhodotorula* spp and *Rhizopus stolonifer* were isolated from the fermenting seeds during the course of the fermentation. The plantain leaves used to wrap the fermenting seeds are presumably a major source of the isolates. Odibo and Umeh (1989) reported similar bacterial flora in fermentation of pumpkin seeds**.** Other authors have also reported similar isolates in the fermentation of the substrates used here for *Ogiri* production (David and Aderibigbe 2010).

The high bacteria load could be attributed to conditional factors of questionable personal and environmental hygiene during processing (Sebudde *et al.,* 2012).

It was observed that the possibility of several condiment contamination by microorganisms is during production process. Sources of microbial contamination of certain condiment from various substrates during production process includes the water used in washing of utensils and other materials as well as some airborne bacterial spores which eventually germinate to vegetative cells.

The trend in pH as observed in this study is in contrast with previous studies on *Ogiri* production from pumpkin and castor seeds (Odibo and Umeh, 1989) and from melon seeds (Odibo *et al.,* 1990). It is however in line with the trend in pH observed by Odunfa (1981) and David and Aderibigbe, (2010) for Ogiri produced from other substrates. Odunfa (1981) described the fermentation process as essentially putrefactive, noting that the increase in pH was probably due to the formation of ammonia by the deaminase enzyme of *Bacillus* and *Lactobacillus* species.

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).

The bacterial succession study shows that all the bacteria reported here were isolated from the first hour of the fermentation towards the end of the fermentation. However, only *Bacillus* and *Lactobacillus* species were isolated at the end of the fermentation. *Lactobacillus* spp are known to produce metabolites with antimicrobial properties such as organic acids, bacteriocins, peroxides, diacetyl and these help to eliminate pathogens and spoilage bacteria during the fermentation (Foegeding *et al.,* 1992). The production of bacteriocins by these lactic acid bacteria is thought to support domination of the producer in the microbial community (competitive exclusion)

The three fungi isolated during the earlier hours of the fermentation were not isolated at the end of the fermentation. These too must have been eliminated by the antagonistic metabolites produced by Lactic acid bacteria **(**Foegeding *et al.,* 1992**).**The elimination of pathogens namely: *E. coli, S. aureus, Klebsiella* spp and *A. niger* from the fermented product means that the product is safe for human consumption as fermentation has been found to confer safety on food products (Achi, 2005).

Lactic acid bacteria are generally fastidious on artificial media, but they grow readily in most food substrates and lower the pH rapidly to a point where competing organisms are no longer able to grow (Daeschel *et al*., 1987). The low growth rate of *Lactobacillus plantarum* in this study for both fermented condiments used signifies that the organism was not able to lower the pH of the condiments and therefore could not suppress the growth of *B. subtilis* which invariably affects its growth rate. Nevertheless, lactic acid fermentations have other distinct advantages in that the foods become resistant to microbial spoilage and toxin development.

The isolation of coagulase-positive *S. aureus* from the fermenting seeds is of public health concern as the organism is known to cause food poisoning (Frazer and Westhoff, 2000). Also, the presence of *Klebsiella,* a coliform could constitute a health risk since some species of this genus are associated with diseases of man (Collins et al., 2004). However, Odibo and Umeh (1989) and Odibo et al. (1992) expressed the expectation that the high heat treatment subjected to Ogiri and Ogiri-okpei, respectively during cooking will destroy these microorganisms and possibly any toxin elaborated in the condiment. Similarly, Odunfa (1981) noted that if Ogiri is well boiled in soup, the danger of microbial infection is eliminated.

Members of the genus *Bacillus* have consistently been reported to be responsible for the fermentation of some vegetable proteins in West Africa especially *iru* and

Ogiriegusi (Odunfa, 1981; Odunfa and Oyewole, 1986; Enujiugha, 2009; Osho *et al*., 2010). Bacilli have proteolytic ability and are able to degrade oil and the proteins are hydrolysed to peptides and amino acids. *Bacillus* spp were the most predominant in the fermentation process and this is in agreement with the observation of Beaumont that *Bacillus* spp constitute over 95 g/100g of the total microbial population density in *iru* and *Ogiri* fermentation because *Bacillus* cells exhibit very high protease activity compared with the other bacterial isolates.

Furthermore, species of *B. subtilis* group have been reported to be generally regarded as safe (GRAS) by the U.S. Food and Drug Administration and their role in the fermentation of locust bean has been thoroughly investigated (Prabir *et al*., 2014).

The disappearance of spoilage organisms namely *Serratia*sp, *Proteus* sp, *Rhodotorula* spp and *Rhizopus stolonifer* at the end of the fermentation was due to the production of antagonistic metabolites by Lactobacillus species. 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 disappearance of these organisms from the fermented product shows that the product is safe from food spoilage organisms and their metabolites. This will enable the *Ogiri* to last for some time without spoilage especially in the rural areas where storage by refrigeration is greatly lacking.

There was significant difference ($P \leq 0.05$) in the moisture content of the *Ogiri* samples. 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.

There was a significant difference ($P \leq 0.05$) in the ash content of the samples (4.21to 6.36g/100g). Ash content gives the reflection of the mineral composition of the samples and shows that the samples were rich in mineral. There was a significant difference ($P \leq 0.05$) also in crude protein content of the samples. Watermelon had 38.32 g/100gfollowed by melon 34.50 g/100g. This indicates that the *Ogiri*samples are rich in amino acids which are the building block of protein. This result is in line with the report of Odunfa (1981).

There was also a significant difference ($P \leq 0.05$) in the crude fibre content of the *Ogiri* condiments. Castor oil substrate gave *Ogiri* sample with the highest fibre content of 10.17 g/100g. The high fibre content will have for reaching effect on human nutrition such as increase in faecal bulk and lowering of gastric cholesterol (Agomuo *et al.,* 2011). It has been reported that diet low in fibre is understandable as it could cause constipation. There was also a significant difference ($P \leq 0.05$) in the crude fatty acid content of the samples. Melon had the highest fatty acid content of 44.86 g/100g while castor has the least among the samples. A significant difference ($P \leq 0.05$) in carbohydrate content of the samples was recorded in which castor oil seeds gave the highest carbohydrate content of 45.98 g/100g.This shows that the *Ogiri* from this substrate will be a good source of energy.

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 changes in amount of soluble nutrients during the fermentation suggests utilization by the organisms.

CONCLUSION

This work has shown that *Ogiri* condiment can be produced from the four substrates namely melon, castor, pumpkin and water melon seeds. The condiments produced were absent of pathogenic and spoilage microorganisms making them safe for human consumption. The condiments are also of good nutritional quality and will be good sources of protein to the people especially in the rural areas where animal protein is scarce.

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