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Microbiological Profile of 'Ogiri' Condiment Made from Seeds of Watermelon (*Citrullus lanatus*)

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Aim: This research work was conducted to evaluate the microbiological profile of 'ogiri' condiment made from the seeds of watermelon (*Citrullus lanatus*).

Study Design: This work was a laboratory experimental design study.

Place and Duration of Study: Dept. of Microbiology (Food and Industrial unit), Nasarawa State University, Keffi, between March and April, 2017.

Methodology: Traditional method of 'ogiri' production was adopted to prepare the sample in replicates to facilitate the 24-hourly microbiological evaluations. Microbial isolation and identification were done using standard microbiological techniques. Also, laboratory-controlled fermentation was carried out using the isolates obtained from traditional fermentation as starter-cultures.

Results: The result of the traditional fermentation of the watermelon seeds yielded an oily brownish paste that has a strong characteristic pungent aroma. The result of the microbial enumeration showed that bacteria were present throughout the period of fermentation in an increasing population that ranged from 32×10^1 cfu/g at the starting time (Day 0) to 288×10^6 cfu/g at the end of the fermentation period (Day 5). There was no fungal growth at the beginning of the fermentation, till on Day1 (8×10^3 cfu/g) to the Day 5 (6×10^6 cfu/g). The isolation of the coliform group of bacteria showed an unusual growth pattern: no coliform isolated from the freshly boiled seeds, coliform was present at Day 1 and 2, and no isolation of coliform bacteria from Day 3 to the

end of the fermentation period (Day 5). Over the 5-day period of fermentation, the organisms isolated and identified are *Bacillus subtilis, Corynebacterium xerosis, Lactobacillus fermenti, Staphylococcus saprophyticus, Staphylococcus aureus, Citrobacter freundii,* coliform bacteria, yeast and mould.

Conclusion: Hence, it was concluded that 'ogiri' condiment can be made from watermelon seeds, using *Lactobacillus fermenti*, *Corynebacterium xerosis* and/or *Bacillus subtilis* as starter cultures.

Keywords: Microbiological profile; condiment; 'ogiri'; watermelon seed; fermentation; starter culture isolates.

1. INTRODUCTION

Condiments are essential part of the diet of various cultures in different parts of the world. Common condiments of the world include ketchup, mayonnaise, mustard, salsa, chutney, and fermented soy sauce and bean paste. Fermented condiments used in soup are common in West Africa, and are usually made from protein-rich leguminous plants and oilseeds; while fermented condiment from fish (fish sauce) appear to be common in many parts of Asia Japan, Thailand, Vietnam, the including Philippines, Indonesia and Malaysia, and some parts of Northern Europe, including France [1]. Fermented soup condiments eaten in different parts of Nigeria include 'ogiri' from castor bean (Ricinus communis) or melon seed (Citrullus vulgaris), 'iru' or 'dawadawa' from African locust bean (Parkia bialobosa), 'okpei' from mesquite seed (Prosopis africana) and 'ugba' from African oil bean (Pentaclethra macrophylla).

'Ogiri' refers to a fermented oily paste that is used as soup condiments for its strong smell. It is a product prepared by traditional method of uncontrolled solid state fermentation of castor bean (Ricinus communis) and/or melon seeds (Citrullus vulgaris), involving the use of natural inoculation or chance fermentation. This fermentation process is known to enhance the palatability, increases protein value, vitamin content and mineral levels of such condiments. It increases variety in the diet, improves nutritional value, reduces anti-nutritional compounds and in some cases, it improves functional properties [2]. Members of the genus Bacillus and coagulasenegative Staphylococcus have been reported to be involved in the fermentation processes such as in the production of 'ogiri', 'ugba', 'ogiri-igbo', 'dawadawa' and 'iru' [3]. Also, various groups of bacteria comprising species of Bacillus, Micrococcus, Leuconostoc, Staphylococcus and Enterobacteriaceae were reported by [4] as contributing to the fermentation of three different species of melon seeds.

Although, fermented food condiments have constituted significant proportion of the diet of many people, Nigerians have exhibited great differences in preference of consumer tastes and preferences for such foods [4]. Also, the prevailing population pressure in Nigeria has resulted in an increasing demand for wild underexploited nutritious plant products with organoleptic appeal in the daily diet [5]. This led to the focus on uncommon seeds for possible development and use, in the search for novel sources to complement the traditional ones. One of such unexploited protein-rich seed with high potential for condiment production is the usually discarded seeds of watermelon fruit. Hence, this research work was conducted to evaluate the microbiological profile of 'ogiri' condiment made from watermelon seeds.

2. METHODOLOGY

2.1 Sample Preparation

The traditional method of 'ogiri' preparation was adopted. Sun-dried watermelon seeds (*Citrullus lanatus*) were shelled and washed. The seeds were boiled in excess volume of water for 8hrs in a pressure cooker, and then the excess water was allowed to evaporate to dryness, leaving moist boiled seeds that were aseptically mashed and wrapped in flamed warm banana leaves to ferment for 5 days at ambient room temperature $(28\pm2^{\circ}C)$ according to the documented traditional method of [4], which include de-hulling of the melon seeds, washing and boiling, mashing of the seeds, packaging in banana leaves, and fermentation at $28^{\circ}\pm2^{\circ}C$ to produce 'ogiri'.

The 'ogiri' condiment was made in seven replicates to facilitate the 24-hourly microbiological evaluations.

2.2 Determination of Microbiological Profile of the Samples

Microbial isolation and identification were done using standard microbiological techniques. One wrap/replicate of fermenting samples was taken aseptically at 24-hour interval over a period of 5 days for the microbiological analysis. The microbiological properties conducted were total bacterial counts, total coliform counts, total fungal counts, microscopic examination of bacterial cells and biochemical characterization of isolates.

The steps involved in microbiological enumeration of the samples, as described by [6] are as follows: One (1) gram of each sample was aseptically weighed into sterile bottle and nine (9) ml of sterile water was added. The mixture was shaken thoroughly to obtain the stock solution, which also serve as the first dilution (10^{-1}) . Subsequently, serial dilutions were made from this stock solution by adding 1ml of solution from preceding concentration to 9ml of the diluent (sterile water), using sterile syringe. Total viable count was made on Nutrient Agar (NA), coliform count was made on MacConkey agar (MA), while fungal count was made on Sabouraud Dextrose Agar (SDA), to which chloramphenicol antibiotic was added to prevent growth of bacteria.

The plates for total viable count and coliform count were labeled appropriately, inverted and incubated at $37^{\circ}\pm2^{\circ}$ C for 24 hrs in a Gallenkamp incubator [5]. The plates for fungal counts were incubated at $28^{\circ}\pm2^{\circ}$ C for 72 hrs. Colonies obtained after incubation were streaked on nutrient agar plates which were incubated for 24 hours at 37° C, to obtain pure cultures.

2.3 Characterization and Identification of Isolates

The cultural characteristics of isolates on the agar plates were observed. Gram staining and spore staining reactions and cell morphology from heat fixed smears were conducted. Pure cultures of the different organisms isolated were sub-cultured and preserved on agar slants at refrigeration temperature (4°C). Bacterial isolates were characterized based on cultural morphology, microscopic and biochemical properties as outlined in [7,8].

2.4 Laboratory Inoculation of Sterile Seeds with Identified Isolates

The fermentation was carried out as earlier explained above. The seeds of *C lanatus* were mechanically shelled and boiled in excess water for about 8 hours. The boiled seeds were packed in washed banana leaves, about 30g in each

wrap, replicated according to the different types of organisms involved. The wraps were packed inside a container and sterilized in the autoclave at 121°C for 15 minutes.

For the isolates inoculation, ten (10) ml each of Nutrient broth was poured into test tubes and sterilized at 121° C for 15 minutes. They were allowed to cool down to 45° C, a loopful of the organisms from the agar slants was aseptically transferred into the broth and incubated at 37° C for 24 hrs. The cell suspensions obtained were shaken together and 1ml was taken for inoculation of each of the sterilized samples, using a sterile pipette for each of the organisms obtained from the traditionally fermented seeds. Thereafter, the wraps were allowed to ferment for 3 days at 37° C in an incubator.

3. RESULTS AND DISCUSSION

3.1 The Produced 'Ogiri' Condiment

The traditional fermentation of the watermelon seeds yielded an oily brownish paste, which has a strong characteristic pungent aroma that is known to impacts the desired flavor in soups. The brownish color of the product deferred greatly from the original creamy color of the raw seeds used. Also the 'smell' of the condiment obtained was completely different from that of the starting seeds. Fermentation of the seeds completely transformed the raw material (seeds of *Citrullus lanatus*) into the finished product ('ogiri' condiment) in a favorable manner.

A similar fermented seed product 'ogiri-saro' is described as brown, soft and sticky condiment with an ammoniacal odor that is usually used as a flavoring ingredient in soups [9]; 'ogiri-okpei' is characteristically dark-brown in appearance and is said to play a major role as a nutritive protein substitute, as well as containing some phytochemicals that are the reason behind its health functions to humans [10].

3.2 Microbiological Profile of the Samples

Table (1) shows the enumeration of the microbial counts of the organisms that were isolated from the samples over a period of 5-day fermentation. It can be seen that bacteria were isolated throughout the period of fermentation in an increasing population that ranged from 32×10^{1} cfu/g at the starting time (Day 0) to 288×10^{6} cfu/g at the end of the fermentation period (Day 5). This is an expected outcome, as the seeds

provide the required conditions for rapid growth and multiplication of the bacteria population. This result is similar to the work of [11] which stated that only bacterial isolates were recovered from climbing fermenting seeds of melon (Cucumeropsis mannii Naud) 'ogiri' for production; and that the presence of bacteria in the fermenting seeds was attributed to the low oxygen tension within the packet of the fermenting seeds.

Table 1. Enumeration of microbial counts (cfu/g)

Days	Total bacterial count	Total fungi count	Total coliform count		
0	32x10 ¹	0	0		
1	316x10 ¹	8x10 ³	54x10 ³		
2	67x10 ⁶	1x10 ³	21x10 ⁶		
3	111x10 ⁶	1x10 ³	0		
4	248x10 ⁶	5x10 ⁶	0		
5	288x10 ⁶	6x10 ⁶	0		

There was no fungal growth at the beginning of the fermentation, till from Day 1 that ranges from 8×10^3 cfu/g to 6×10^6 cfu/g on Day 5. The isolated fungi could have been introduced from the leaves used in wrapping the samples during the traditional fermentation process. Conversely, the fungi may have developed from the micro-flora of the watermelon seeds, considering the initial decrease in their population from 8x10³ cfu/g at Day 1 to 1x10³ cfu/g at Day 2 and 3, and subsequent increase to 5×10^6 cfu/g and 6×10^6 cfu/g at Day 4 and 5, respectively. Specifically, mould growth was observed only from the Day 5 period of fermentation. The mould growth could most probably be a spoilage agent, in which case, it should be noted that after 3-4 days of fermentation, the aromatic product should be adequately preserved to inhibit the growth of mould and other spoilage agents. Meanwhile, the high presence of fungi obtained for the Day 1 (8x10³ cfu/g), which most probably was from external contamination by the unsterilized packaging material used got reduced by Day 1 and 2, as a result of unfavorable conditions within the fermenting mash. Such conditions nay include inhibitory substances that the fermentative organisms synthesized during their metabolic activities to suppress the growth of contaminants and other unwanted microorganisms. On the other hand. the subsequent increase in population of fungi by Day 4 and 5 may be accounted for by the development of a succession of fungi that are inherent in the sample, or by some spoilage fungi that later developed in the mash as fermentation process of the major organisms got completed and the accumulated metabolites in the batch fermentation system allows the proliferation of other microorganisms.

The isolation of the coliform group of bacteria showed an unusual growth pattern. There was no coliform isolated from the freshly boiled seeds that was sampled before wrapping in leaf for fermentation. However, there was isolation of the coliform bacteria at Day 1 and 2 (with a decreased population). Thereafter, there was no isolation of coliform bacteria from Day 3 to the end of the fermentation period (Day 5). It is indicated in the work of [12] that one common application of coliform bacteria as indicator organisms is in their association with hygienic conditions and overall guality of food processing steps. Therefore, their total absence in the freshly boiled sample at Day 0 confirms the good sanitary condition of the preparation process, while their isolation at Day 1 and 2 indicated post-processing contamination from the leaves used in packaging the seeds, as the leaves are not sterilized in the traditional process of 'ogiri' making.

The coliform contaminants later went into extinction, as the prevailing conditions within and around the substrate did not favor their growth.

Table (2) shows the cultural and microscopic characteristics of microbial colonies isolated from the samples. From the table, the probable genera of the suspected organisms are as indicated. Some of the suspected organisms are of multiple genera (for example, suspected organisms for colony isolate 3 are *Bacillus* spp., *Lactobacillus* spp. and *Corynebacterium* spp.), while others has only one suspected genus due to their distinct macroscopic and microscopic characteristics. Such example is colony isolate 14 (*Bacillus* spp.) that produced spore.

Table (3) shows the result of biochemical tests conducted to specifically identify the organisms. Over the 5-day period of fermentation, the organisms isolated are *Bacillus subtilis*, *Staphylococcus saprophyticus*, *Corynebacterium xerosis*, *Lactobacillus fermenti*, *Staphylococcus aureus*, *Citrobacter freundii*, coliform and fungi. Amongst these isolates, there are organisms that participated throughout the fermentation period (e.g *Bacillus subtilis*), while some appeared as

contaminants that were later phased out (e.g. coliform). The Staphylococcus spp. are similar in morphology, but in biochemical characteristics, S. aureus hydrolyses gelatin while S. saprophyticu is negative to the test. Also S. aureus hydrolyses starch while S. saprophyticus does not. On the other hand, S epidermidis does not ferment mannitol to produce acid and is novobiocin sensitive; whereas, S saprophyticus is resistant to novobiocin and can produce acid from mannitol and trehalose [7]. The mould growth that was observed at the fifth day of fermentation could be spoilage organisms, as it is generally peculiar to filamentous fungi to grow on prolong storage of damp carbohydrate and/or fatty foods such as the sample used. This implies that processing of the condiment could be stopped and the product could be properly preserved (e.g by drying) after 3-4 days, if the desired organoleptic attributes have been attained at such period.

The isolation of Bacillus subtilis, Staphylococcus spp., Corvnebacterium xerosis and Lactobacillus fermenti from the fermenting mash agreed with some other research work, as these bacteria are among common microbial genera that have been isolated from different fermented protein condiment by various researchers [13-16]. Amongst these, Bacillus spp. are said to be the major group of fermenting organisms as a result of their presence and population throughout the period of fermentation, and because Bacillus cells are known to exhibit very high protease activity compared with the other bacteria isolates [15]. Although, Staphylococcus spp. are usually contaminants, they have been regularly isolated from different fermenting mash by various researchers such as [11,17,18]. The presence of pathogenic contaminants like Staphylococcus aureus and Citrobacter freundii suggest potential health risk of the product.

Table (4) shows the daily distribution of the isolates throughout the period of fermentation. It can be seen that Bacillus subtilis was isolated throughout the fermentation period from the fermenting mash. This fact agrees with the work of several authors that indicated that there is a predominance of Bacillus species during the fermentation processes of various legumes. authors include Such [11,15,18]. The Staphylococcus saprophyticus that was isolated from the raw sample could have resulted from external contamination during the preparation of the melon seeds for fermentation. Isolation of same on the 2nd and 4th day of fermentation period may be part of the consortia of organisms responsible for the process, since Staphylococcus spp. have been regularly reported to be part of the fermentative organisms by different authors. Conversely, the pathogen S. aureus that was isolated on the 3rd day of fermentation period could be external contaminants during the microbial analyses. Similarly, isolation of the pathogen Citrobacter freundii from the 3rd to 5th days of fermentation period could be external contaminants during the microbial analyses, as this genus is not reported to be part of the fermentative organisms of condiments. However, a genus (Enterobacter spp.) that is in the same family with the Citrobacter has been reported in condiment fermentation [4].

As stated earlier, the appearance of the coliform bacteria in the fermenting mash on 1st and 2nd days of the process could have resulted from post-processing contamination by the leaves used for wrapping the mash. By the 3rd day of fermentation period, the disappearance of coliform may be attributed to the action of some anti-microbial agents like acid that might have been secreted by fermentative organisms such as the Lactobacillus fermenti that appeared in the fermenting mash by the 2nd dav. Corynebacterium xerosis also appeared as part of the fermentative organisms on the 2nd day of the process, as it was reported by some researchers such as [13] to be involved in fermentation of condiment.

Similar to the occurrence of *Bacillus subtilis*, fungi was isolate throughout the fermentation period. However, the ecology of the fungi in the fermenting mash differed towards the end of the process, as the cultural features of the growth on the SDA plates on the 5th day indicated growth of filamentous fungi, along with the fungi (yeast) that were isolated from day to 4.

3.3 Laboratory Inoculation of Sterile Seeds with Identified Isolates as Stater-Culture

An inspection of the six products obtained in laboratory-controlled fermentation shows slight differences in color and odor of the various products, with the exception of the *Citrobacter*fermented product that yielded a highly offensive smelling mash. According to some researchers, only the *Bacillus* spp. starter culture yielded 'ogiri' condiment of acceptable attributes. For example, [19] indicated that the use of pure isolates to

Colony isolates	Size (mm)	Shape	Elevation	Color	Margin	Surface appearance	Gram rxn	Cell shape	Association	Endospore	Probable organism
1	1.5	Round	Raised	Cream	Entire	Moist/shiny	+	Rods	Single		Bacillus spp., Lactobacillus spp.,
									-		Corynebacterium spp.
2	2.5	Round	Raised	Cream	Entire	Moist/shiny	+	Cocci	Cluster		Staphylococcus spp.
3	3.5	Round	Raised	Cream	Entire	Moist/shiny	+	Rods	Single/chain		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
4	1.0	Round	Raised	Cream	Entire	Moist/shiny	+	Rod	Single		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
5	4.5	Irregular	Raised	Cream	Serrated	Moist/shiny	-	Rod	Single		Enterobacter spp., Citrobacter spp.
6	2.5	Irregular	Raised	Cream	Serrated	Moist/shiny	+	Rod	Single		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
7	1.0	Round	Raised	Cream	Entire	Moist/shiny	+	Rod	Single		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
8	4.5	Round	Raised	Cream	Entire	Moist/shiny	+	Cocci	Cluster		Staphylococcus spp., Micrococcus spp.
9	2.3	Round	Raised	Cream	Entire	Moist/shiny	+	Cocci	Single/cluster		Staphylococcus spp.,
10	2.0	Round	Raised	Cream	Entire	Moist/shiny	+	Rod	Single/chains		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
11	3.8	Round	Raised	Cream	Entire	Moist/shiny	-	Rod	Single		Enterobacter spp., Citrobacter spp.
12	3.8	Round	Raised	Cream	Entire	Moist/shiny	+	Rod	Single		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
13	1.0	Round	Raised	Cream	Entire	Moist/shiny	+	Rod	Single		Bacillus spp., Lactobacillus spp.,
											Corynebacterium spp.
14	2.0	Round	Raised	Cream	Entire	Moist/shiny	+	Rod	Single	+	Bacillus spp.
15		Round	Raised	Cream	Entire	Moist/shiny	+	Ellipsoidal	Single/chain/cluster		Yeast
16	4.0	Round	Raised	Cream	Entire	Moist/shiny	+	Cocci	Single		Staphylococcus spp., Micrococcus spp.
17	0.5	Round	Raised	Cream	Entire	Moist/shiny	+	Cocci	Cluster/chain		Staphylococcus spp.,

Table 2. Cultural and microscopic characteristics of microbial colonies isolated from the samples

Isolates Catalase	Oxidase	6.5%NaCl	Starch hyd	MSA	Nvb	Triple sugar iron agar tests						Identified isolate	
				-			Glucose	Lactose	Sucrose	H₂S	Motility	Gas	_
	-	+	+	+			+	-	-	-	-	-	Bacillus subtilis
2	-	-			+/pig		+	-	-	-	-	-	Staphylococcus aureas
3	+	-	-	-			+	-	-	-	-	-	Corynebacterium xerosis
1	-	-	-	-			+	+	+	-	-	+	Lactobacillus fermenti
5	+	-					+	+	+	+	+	+	Citrobacter freundii
3	+	-	+	+			+	-	-	-	-	-	Bacillus subtilis
7	-	+			+		+	+	+	-	-	+	Lactobacillus fermenti
3	+	-			+	-	+	+	+	-	+	+	Staphy. saprophyticus
9	+	-			+	-	+	+	+	-	+	+	Staphy. saprophyticus
10	-	-	+	+			+	+	+	-	+	+	Bacillus subtilis
11	+	-					+	+	-	+	+	+	Citrobacter freundii
12	+	-	+	+			+	-	-	-	-	-	Bacillus subtilis
13	+	+	+	+			+	+	+	-	-	-	Bacillus subtilis
14	+	-			+	-	+	+	+	-	+	-	Staphy. saprophyticus
15	+	-			+	-	+	+	+	-	+	+	Staphy. saprophyticus

Table 3. Biochemical characterization and identification of isolates

Key: + positive , - negative, pig pigmentation, MSA mannitol salt agar

Table 4. Occurrence of the organisms during fermentation period

Isolates		Time	(days)							
	0	1	2	3	4	5				
1	S. saprophyticus	Bacillus subtilis	Bacillus subtilis	Citrobacter freundii	Staph. saprophyticus	Bacillus subtilis				
2		Fungi	Staph. saprophytic.	Bacillus subtilis	Bacillus subtilis	Citrobacter freundii				
3		Coliform	Lact. fermenti	Lact. fermenti	Fungi	Filamentous fungi				
4			Corynebact. xerosis	Staph. aureas	,	C C				
5			Fungi	fungi						
6			Coliform	-						

ferment the sterile seeds showed that only *Bacillus* sp. could ferment the seeds in pure cultures during the fermentation of castor seeds (*Ricinus communis*) by the traditional method. In a study on fermented products of locust bean seeds (*Parkia biglobosa*), castor bean seeds (*Ricinus communis*), African oil bean seeds (*Pentaclethra macrophylla*) and mesquite seeds (*Prosopis africana*) by [20], only *B. subtilis* was found to give the products with acceptable quality attributes.

The combined isolates-fermented product yielded the most ideal product. Its virtual inspection showed an identical product with the traditionally fermented (chance-inoculated) 'ogiri' product. This is an expected outcome since the desirable organoleptic attributes of condiment is often achieved by action of a consortia of microorganisms. More so, some researchers like [21,22,23] recommended mixed starter cultures that include *Bacillus* species in fermented condiment making.

4. CONCLUSION AND RECOMMENDA-TION

The main aim of this research work was to conduct the microbiological profile of fermented condiment made from the seeds of watermelon (*Citrullus lanatus*).

The results obtained from the study have shown the prevalence of bacteria throughout the period of fermentation in an increasing population. Fungi and coliform group of bacteria were not isolated at the beginning of the experiment till after 24 hour of commencing the fermentation process. Filamentous fungi (mould) growth was obtained only after the fifth day of fermentation, thereby suggesting it to be spoilage growth. Bacillus spp. was isolated throughout the fermentation period, thereby proving to be a major fermentative organisms. The result of the laboratory-controlled fermentation confirmed that 'ogiri' condiment could be obtained with Lactobacillus fermenti, Corynebacterium xerosis and/or Bacillus subtilis starter cultures.

However, it is recommended that the products of this study should be further assessed for any possible toxicology study before it can be wholly acceptable for human consumption.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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