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PRODUCTION OF OGIRI (A LOCAL FERMENTED FOOD CONDIMENT FROM CASTOR OIL SEEDS) BY THE USE OF BACTERIAL STARTER CULTURE

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Abstract
Fermented foods are products processed through the activities of microorganisms, where the weight of the microorganisms in the food is minimal. Ogiri is a paste derived from the local fermentation of castor oil seeds (Ricinus communis). It is a traditional food common among Easterners, serving as a condiment and flavoring material in various dishes. The substrate was obtained from vendors at New and Ogbete main markets located in Enugu North, Enugu State. It was promptly transferred to the Godfrey Okoye University Microbiological Laboratory, where it underwent anaerobic fermentation both in a laboratory setting and traditionally. The sample was diluted, pour-plated, sub-cultured, and colonies were counted. Bacillus sp yielded 150 viable colonies, while Lactobacillus and Micrococcus sp yielded 130 and 115 colonies, respectively. Pure cultures believed to be Bacillus sp, Lactobacillus sp, and Micrococcus sp, isolated from the traditional type using different media (Hi-crome bacillus agar, nutrient agar, and enrichment agar), were used to ferment fresh ogiri in the lab, and the results were evaluated. Bacillus sp produced the best results among the various strains.
Temperature and pH variations of different ogiri samples (traditionally fermented ogiri, ogiri fermented with isolates, unfermented or fresh ogiri, and commercial ogiri) were determined and represented graphically. The proximate composition of these various ogiri samples was determined. The results, along with sensory evaluations, were subjected to ANOVA, which showed the significance level to be 95%.

INTRODUCTION

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Ogiri is a paste made from fermented oil seeds, serving as a native seasoning in Nigeria, West Africa. It is a protein-based food that functions as a nutritive non-meat protein substitute. Additionally, Ogiri can serve as a condiment and flavoring material in various dishes and soups. It has an oily gray pasty consistency and a very strong pungent smell, which is significantly reduced when frozen. There are several types of ogiri, with the most common varieties being Ijebu, Igbo, and Nwan, named after their regions of origin (Odunfa, 2001).

Ogiri Igbo, a fermented castor oil seed, is of Igbo origin, belonging to the southeastern Nigeria tribe. Proximate analysis of Ogiri reveals its major constituents as protein, fats and oil, moisture, ash, fiber, and carbohydrates. This condiment is prepared using the traditional method of uncontrolled solid-state fermentation, resulting in extensive hydrolysis of protein and carbohydrate components (Achi, 2005). Microorganisms play a crucial role in modifying the substrate physically, nutritionally, and sensorially during ogiri fermentation (Njoku and Okemadu, 2001). The fermentation process is influenced by factors such as temperature, pH, type of microorganism, nature of the substrate used, and time (Achi, 2005).

Obtained from the castor oil plant, botanically known as Ricinus communis, castor oil is a species of flowering plant in the Spurge family, Euphorbiaceae. Castor oil contains 40% to 60% oil rich in triglycerides, primarily Ricinolein. The seeds also contain Ricin, a water-soluble toxin, albeit in lower concentrations. Originating in Africa, castor plants grow wild in East and North Africa, with relatively large, black, and glossy seeds. These seeds are poisonous if ingested raw and require a warm climate, being sensitive to frost. Castor oil is utilized in various industries, including machine manufacturing, high-grade lubricants, soap and ink production, textile dyeing, leather preservation, and paint production (Chelule et al., 2010; Nester et al., 2007).

The nutritional value of castor seeds increases during fermentation due to the breakdown of complex components, making digestion easier (Adeyemi, 2008). Ogiri, as a food condiment, can be preserved at a low pH of 4, inhibiting the growth of pathogenic organisms responsible for food spoilage. It is particularly used in the production of bitter leaf soup for its enhanced aroma and flavor.

Despite fermented food condiments like ogiri being a significant part of many diets, Nigerians have shown ambivalence in terms of consumer tastes, aroma, and preferences for such foods. The introduction of foreign high-tech products, especially processed ones due to globalization and economic liberalization, has transformed Nigerian food culture into a mix of both foreign and local dishes. Imported foods are often chemically treated or genetically modified, leading to increased cases of terminal illnesses such as cancer, kidney failure, diabetes, and viral infections, which were uncommon in the past.

These health challenges may be attributed to differences in genetic makeup between Africans and other populations. Traditional foods in Nigeria are nutrient-rich, but their poor keeping quality limits their consumption. To address this, efforts should focus on stimulating and standardizing processing operations to enhance the nutritional and keeping qualities of traditional foods. Studies have identified various bacteria, including Bacillus, Micrococcus, Lactobacillus, Streptococcus, Leuconostoc, Staphylococcus, and Enterobacteria, involved in the fermentation of these condiments through natural inoculation (Enujiugha and Badejo, 2007).

To obtain hygienic and safe products, there is a growing need to develop microbial starters for these local fermentations. The samples used in this study were collected from the New Market in Enugu North Local Government Area of Enugu State. Castor oil bean seeds were purchased from a seller at the specified site and immediately transported to the laboratory for the fermentation process.

MTERIALS AND METHODS

Preparation of Ogiri:

The castor oil bean seeds were sorted to remove unwanted ones, followed by de-hulling, washing, and boiling for 8 hours. After boiling, the products were sieved and fried for a few minutes. The seeds were then allowed to cool, wrapped in banana leaves, and packed into a basket. The mixture was left to ferment for 5 days at room temperature and under sunlight. Once fermentation was complete, the fermented seeds were mashed into a paste, resulting in the final product, ogiri. The ogiri was placed near a fireplace to develop its characteristic flavor.

Sterilization of Glassware:
Glassware used for the experiment was washed, thoroughly rinsed, and then subjected to the hot air oven at 160^{o}C for 1 hour.

Preparation of Media for Isolation:
Preparation involved creating 180ml each of Nutrient agar, Bacillus Hi-crome agar, and Mineral salt agar. The media were sterilized using an autoclave at 121^{o}C for 15 minutes. After sterilization, the media were poured into petri dishes to solidify, and they were used for isolating Bacillus and Lactobacillus species.

Serial Dilution:
Test tubes were sterilized in a hot air oven at 160^{o}C for 1 hour. Fifteen sterilized test tubes were filled with 9ml of distilled water each. One gram of the ogiri sample was added to the first test tube (10^{-1}) using a micropipette. From this solution, 1ml was transferred to a second test tube (10^{-2}) following the method described by Chessbrough (2005) and Ezigbo et al. (2014).
For the dilution of 10, 1ml was inoculated on the media by pour-plating in triplicate, adhering to aseptic conditions as per Harigan (1998). The plates were then incubated at 35^{o}C ± 2^{o}C for 24 hours.

Preparation of pure cultures.
After good growth of colonies, distinct colonies were purified on Nutrient agar, Hi-crome bacillus agar and mineral salt agar by streaking. The plates were incubated at 35^{o}C for 24hours.

Microscopic observation and storage of sample culture
The morphology of the colonies were observed to be Bacillus species and Lactobacillus species, by checking their colony shapes, pigment, elevation, edge and surface appearance. Gram staining of Nutrient agar and Mineral salt agar was done by use of. Air dry and heat fix, place the slides on a staining rack with a pan underneath. Flood each slide with crystal violet, leave for about 30-60seconds, and wash the slides with tap water by draining. Flood each smear again with iodine solution, leave for 1minute and wash again with tap water. Decolorize with acetone until the color from the slide appear colorless. Wash the slide briefly with tap water, drain off excess water, and counter stain the smear with safranin for 20-30seconds, wash briefly with tap water and blot dry. Observe slide under oil- immersion objective lens (Tasie and Okafor).
Spore staining of Hi-crome Bacillus agar, Nutrient agar and Mineral salt agar were done using prepared smear from the culture. A thin smear was made, allowed to dry and passed over the flame with small heat to fix the organism. Malachite green was added, boiled for 5-8minutes. The slide was left to cool for five minutes and was run off with water to get rid of excess stain. Methylene blue was added for 60seconds, washed off with water, hot dried and passed out flame for further drying. It was then viewed under the microscope. (Tasie and Okafor, 2005).
Spore staining of organisms on Hi-crome Bacillus agar, Nutrient agar and Mineral salt agar was done by used of prepare smear from the culture. Thin smear, allow to dry and Passover the flame with little heat to fix the organism. Add malachite green, boil for 5-8minutes. Leave slide to cool for 5minutes, and run out with water to get rid of excess stain. Apply methylene blue for 60seconds.Wash with water, blot dry and pass over flame for further drying. Observe under the microscope (Tasie and Okafor, 2005).
Each colony was observed to be form spores and gram positive bacteria with rods by examining it under a light microscope from high to low power objective after staining. Pure isolates were placed on Nutrient agar slants, Hi-crome bacillus agar slants and Mineral salt agar slants and stored at 4^{o}C until needed.

Endospore staining
After a week, endospore staining was done on Nutrient agar, Mineral salt agar and Hi-crome bacillus agar. A prepared smear organism was placed on separate microscopic slides. Leave to air dry and heat fix by passing over the Bunsen burner for about three times. Saturate the smear with malachite green stain. Place the slide over a beaker of boiling water and leave for about 5-8minutes. After boiling, leave the slide to cool for 5minutes and flood with water to get rid of excess stain. Add safranin leave for 30seconds.Wash under the tap and blot dry using a blotter. Examine with the oil immersion lens.(Tasie and Okafor).

Biochemical characteristics of isolates

Observing motility in bacteria, bijou bottles, Nutrient agar, Hi-crome Bacillus agar, Mineral salt agar, dissecting pin, autoclave, weighing balance and beaker. Through soft agar method, been prepared was poured into bijou bottles and allowed to cool. Stab inoculate a soft agar tube Incubate overnight (24hours) Occurrence of growth away from the line of the stab indicates presence of motility. But occurrence of growth along the stab indicate absence of motility (non-motile) (F.C. Ogbo, 2005).

Catalase analysis of the isolates

2mls of hydrogen peroxide solution was poured into a test tube. Using a glass rod, remove several colonies of the test organism and immerse in hydrogen peroxide solution. Look for immediate bubbling, active bubbling indicate positive test, No bubbles indicates negative test (Cheesbrough, 2005).

Citrate utilization test of the isolate

Prepare a slope of the media Using a wire loop, streak the slope with a saline suspension of the test organism and then stab. Incubate at 35^{o}C for 48hours. Look for a bright blue color in the media which indicate positive result (Chessbrough, 2005).

Indole test of the isolates

Inoculate the test organisms in a bijou bottle containing 3ml of sterile peptone water. Incubate at 35^{o}C-37^{o}C for up to 48hours. Test for indole by adding 0.5ml of Kovac reagent. Shake gently and examine for a red color in the surface layer within 10minutes. (Cheesbrough, 2005).

Starch hydrolysis of the test isolates

Prepare a media. Pour it in petri dishes and allow to cool Isolates were inoculated and allowed to grow at 30^{o}-35^{o}C within24hours. After 24hours, the plates were flooded with iodine.

A clear zone around the isolate indicates that the starch was utilized and that the organism contains amylase.

Sugar fermentation test of the isolates

Preparation of media, sterilization of the test tubes. After adding the sugars carefully, poured into test tubes, autoclaved at 121^{o}C for 15minutes. Allowed to cool at 45^{o}C before inoculating the organisms.The positive results were cloudy which do not have the same color with the control While the negative was clear.

Glucose fermentation of the isolates

Glucose anhydrous powder was weighed and sterilized for 15minutes at 121^{o}C; allowed to cool.

Small quantity of methyl orange was added into the solution and dissolved thoroughly. Sterilized wire loop was used to pick a colony of the organism and put into the solution. The Durham's tube was inserted upside down into the bottle, covered and incubated for 24hours. Blue litmus paper was also stripped into the solution containing those samples. Color change in the litmus paper indicated the presence of acids and the ring which formed inside the Durham's tube indicated the presence of gas.

pH Determination of the samples of ogiri

Turn on pH. Wait around 30minutes for the electronics to warm up. Take electrode out of storage solution. Rinse the electrode with distilled water. Do not wipe the electrode membrane, but instead dab the electrode with kinwipe. Submerse the electrode into the pH 7buffer. Note that buffer and samples should be read at room temperature. Press the calibrate button. Wait until the pH Icon stops flashing and press the calibrate button again. Rinse the electrode with distilled water and wipe with Kinwipe. Submerse the electrode into your samples. Note: The sample and buffer should be read at room temperature. Press the measure button; wait until the pH stops flashing and record the pH of your sample.

Proximate analysis

Proximate composition (moisture, ash, fat, protein, crude fiber and carbohydrate) of the r castor oil seeds (*Ricinuscommunis*) was determined using the method of Association of Official Analytical Chemists.

Moisture content determination

Two grams (2g) of the sample was weighed into a dried crucible of known weight, fed into the oven at 105^{o}C for 3hours, withdrawn into a desiccator to cool, weighed, then re-heated, cooled and reweighed, and re-heated. The process was repeated until relatively constant weight was realized. The difference in the weights before and after drying was recorded as moisture content.

Ash content determination

After moisture content determination, the dried samples were transferred into the muffle furnace, ignited heat at 55^{o}C until they were carbonized and calcinated until black particles were no more. The samples were withdrawn and cooled in a desiccator, then the weight of the residual incinerate was calculated as ash content.

Crude fat (ether extract) determination

Two grams (2g) of sample was wrapped in a filter paper and gradually lowered in the thimble which was fitted to a flask containing the solvent, n-hexane. The round-bottomed flask, in a soxhlet extraction unit was slowly heated with thermostically controlled mantle, during which the solvent evaporated and passed through the side's tube of the extract to the reflux condenser where it condensed and ran back into the samples dissolved in the n-hexane and the mixture of the hexane and fat. The filter paper with the spent (defatted sample) was removed from the extractor and the refluxed solvent distilled out and recovered. The flask containing the fact and residual solvent was dried at 85^{o}Cfor 3hours, cooled and weighed.

Crude protein determination

Two grams (2g) of samples, 0.10gCuSO4 and 2.50Na_2SO_4were put into a micro Kjeldahl flask, followed with addition of 20ml concentrated of H_2SO_4 solution together with anti- bumping clips. The flasks were heated using heating mantle in the unit until initial black color formed turned to light green. The flasks, covered (plugged) with cotton wool were cooled prior to distillation. After cooling, the digest was mounted on the distillation apparatus which the distillation arm was connected such that the condenser stipe was below the surface of a 20ml of 2% boric acid solution in conical flask. Using funnel, 35ml of 40%NaOHand 80ml of distilled water were added into the digest flask. The contents of the flask were distilled until 30ml of distillate were obtained after about 15minutes, to this was added 2 drops of methyl-red indicator and titrated with 0.1NHCLsolution to a pink end point. A blank test was carried out by repeating the processes of digestion, distillation and titration without the presence or involvement of the test raw and fermented samples in order to give a blank value for any trace of nitrogen that may be present in the reagent and handling material (analytical chemicals/media).

Crude fiber determination

Two grams (2g) of sample was weighed and placed in a hot200ml of 1.25%H_2SO_4 and boiled for 230mins. It was then filtered through a buckner funnel equipped with muslin cloth and held with elastic band. The funnel was made hot by pouring boiling water on to it. The residue was washed thrice with hot water, scooped into a conical flask, digested with 200ml of 1.25%NaOHsolution for30mins boiling. It was filtered and progressively washed with boiling water, 1%Hcl and boiling water to remove acid from it. The residue was washed twice with alcohol and three times with petroleum ether using small quantities. The residue was scooped into a clean, dry and weighed porcelain crucible, dried in the oven at 85^{o}C to a constant mass. It was then cooled and weighed. The crucible with its content was placed inside muffle furnace at 6000^{o}C for 2hours, withdrawn, cooled in desiccator and weighed.

Carbohydrate content determination

Carbohydrate content was calculated as Nitrogen Free Extract (NFE), determined by the difference obtained by subtracting the values of all the nutrients measured from100

Sensory evaluation of sample

Sensory evaluation was carried out using eleven panelists to assess the sensory attribute (color, aroma, texture and overall acceptability) of the produced food condiment. The fresh samples fermented with different isolates were presented to the panelist using commercial ogiri as a reference. The panelists were selected randomly cutting across students and workers of GoddfreyOkoye University community which include people who have knowledge of ogiri and those who do not. The samples were presented in coded identical plates. The panelists were instructed to rate the samples for the parameters based on a seven (7) point hedonic scale ranging from7-liked extremely to one (1) disliked extremely. The raw scores were assembled and statistically analyzed using the SPSS Version 16.

Data obtained from the study of the sensory evaluation and proximate analysis were subjected to analysis of variance (ANOVA) and the means were separated using fisher LSD and judged significantly different at 95% confidence level (i.e. $p<0.05$).

RESULTS

The samples collected from market (New market and Old Park) in Enugu were inoculated in culture plates containing three different media.

Inoculation identified

In total, there were many colonies, pure cultures of the isolates were prepared and their morphology was observed and identified. The statement of the result in tables showed that studies on the isolation and identification of the fermenting microorganisms of castor oil bean seeds identified the following groups of microorganisms as being present during the fermentation of castor oil bean seeds into OGIRI; *Bacillus species, Micrococcus species and Lactobacillus species.* It was designed to identify the organisms responsible for the fermentation of castor oil bean seeds into ogiri, and ultimately select starter culture for its production. Bacillus is a gram positive long rods with central spores in which the morphology showed that the organism is greenish/ pinkish transparent colonies in Bacillus agar and cream, circular, opaque, flat, and rough colonies on mineral salt enrichment agar (Agar Agar supplement media). The micrococcus species were mixed culture of gram positive cocci that appeared single; morphology showed that the organism was milkish-white colonies on nutrient agar. The Lactobacillus species were mixed culture of gram positive bacillus and cocci which appeared milkish-white colonies.

Table 3. Microbial mean count of the isolates

Isolates	No of isolates positive	Colony forming unit (cfu)
Bacillus sp.	131	1.3×10^5
Micrococcus sp.	95	9.5×10^4
Lactobacillus sp.	55	5.5×10^4

Table no 3 showed the microbial mean count of the isolate in the counted colonies by calculation of colony forming unit per milliliters. It was only three plates that showed positive of the colony counted.

Table 4. Results for the colony characteristics of the isolates

Sample and isolate	Color	Texture	Shape	Elevation	Characterization	Spore stain	Suspected organism
Sample 1							
NA1	Cream	Rough	Circular	Flat	Gram positive long rods in clusters.	Cocci (+)	*Lactobacillus sp.*
NA3	Cream	Rough	Circular	Flat	Gram positive long rods in clusters.	Clusters (+)	*Lactobacillus sp.*
NA5	Yellow	rough	Circular	Raised	Gram positive long rods.	Central spore (+)	*Bacillus sp.*
Sample 2							
AA1	Yellow	rough	Lobate	Flat	Gram positive rods.	Central spore	*Bacillus sp*
AA3	Cream	rough	Lobate	Flat	Gram positive rods.	Cocci/clusters	*Micrococcus sp.*
AA5	Yellow	rough	Circula	Raised	Gram positive	Central spore	*Bacillus sp.*

			r		rods.		
Sample 3							
BA1	pinkish/greenish	rough	Lobate	Raised	Gram positive long rods.	Central spore	*Bacillus sp*
BA3	Pinkish/greenish	Smooth	Lobate	Flat	Gram positive long rods.	Elongated spore	*Bacillus sp*
BA5	Pinkish/greenish	Smooth	Lobate	Flat	Gram positive long rods.	Elongated spore	*Bacillus sp*

Table no 4 showed the result of the colony morphology of the isolates, its color characterization, texture, shape and probable organisms in all the samples was determine after subculturing and streaking method to achieved a pure culture.

Table 5: Biochemical properties of isolates

Isolate	**Catalase**	**Oxidase test**	**Motility**	**Coagulase**	**fructose**	**Glucose**	**Maltose**	**Sucrose**	**manitol**	**lactose**	**Galactose**	**xylase**	**Glucose anhydrase**	**Starch hydrolysis**	**Citrate utilization**
NA1	+	+	+	–	A	A	AG	AG	A	A	AG	A	AG	+	+
NA3	+	–	+	–	A	A	A	A	-	-	-	A	AG	+	+
NA5	–	–	–	–	A	A	A	A	A	A	A	A	AG	+	–
BA1	+	+	+	–	A	A	AG	AG	A	A	AG	A	AG	+	+
BA3	–	+	+	–	A	A	A	A	A	A	A	A	AG	+	+
BA5	+	+	+	–	A	A	AG	A	A	A	AG	A	AG	–	+
AA1	–	–	+	–	A	A	A	A	A	A	A	A	AG	+	–
AA3	+	-	+	-	A	A	AG	A	A	A	A	A	AG	+	+
AA5	+	-	+	-	A	A	A	A	-	-	-	A	AG	+	+

Table no 5 showed the biochemical activities of the isolate to determine the intracellur and extracellular enzyme of the cell isolates.

Keys

A= acid, AG= acid and gas, +=positive, -=negative

Table 6: Sensory evaluation of various ogiri samples expressed as mean ± standard error

Isolates	**Texture**	**Aroma**	**Colour**	**P- values**
Bacillus sp	8.8± 0.207	8.84± 0.207	8.82± 0.122	0.284
Lactobacillus sp	6.18± 0.325	7.00 ± 0.191^{a}	6.18 ± 0.296^{b}	0.015
Micrococcus sp	3.09± 0.392	2.00 ± 0.270^{a}	1.82 ± 0.263^{a}	0.70

Legend: [a] implies significant difference with *Bacillus sp;* [b] implies significant difference with *Lactobacillus sp*and [c] implies significant difference with *Micrococcus sp*.

Table 7: Proximate analysis of various ogiri samples expressed as mean ± standard error

Analysis	Ogiri commercial	Ogiri with isolates	Fresh ogiri	Traditional fermented ogiri	P- values
Moisture%	46.39± 0.03	45.15± 0.90	40.32±0.31[ab]	42.68±0.52[bc]	0.005
Fats%	19.53± 0.90	9.6315±0.38[a]	3.2375±0.13[a]	10.3945±0.42[ac]	0.001
Protein%	14.28± 0.09	12.9060±0.04[ac]	4.1000±0.003[bc]	16.0730±0.14[abc]	0.000
Ash%	3.52± 0.09	3.0380±0.07	3.2720±0.16[ab]	3.5375±0.21[ab]	0.179
Fibre%	2.43± 0.01	2.4015±0.01	2.3840±0.04	2.2735± 0.057[a]	0.119
Carbohydrates%	13.87±0.69[a]	26.4175±0.95[a]	26.6905±0.64[a]	25.0385±0.02[a]	0.000

Legend:[a] implies significant difference with ogiri commercial; [b] implies significant difference with ogiri isolates; [c] implies significantly difference with fresh ogiri and [d] implies significantly difference with traditionally fermented ogiri.

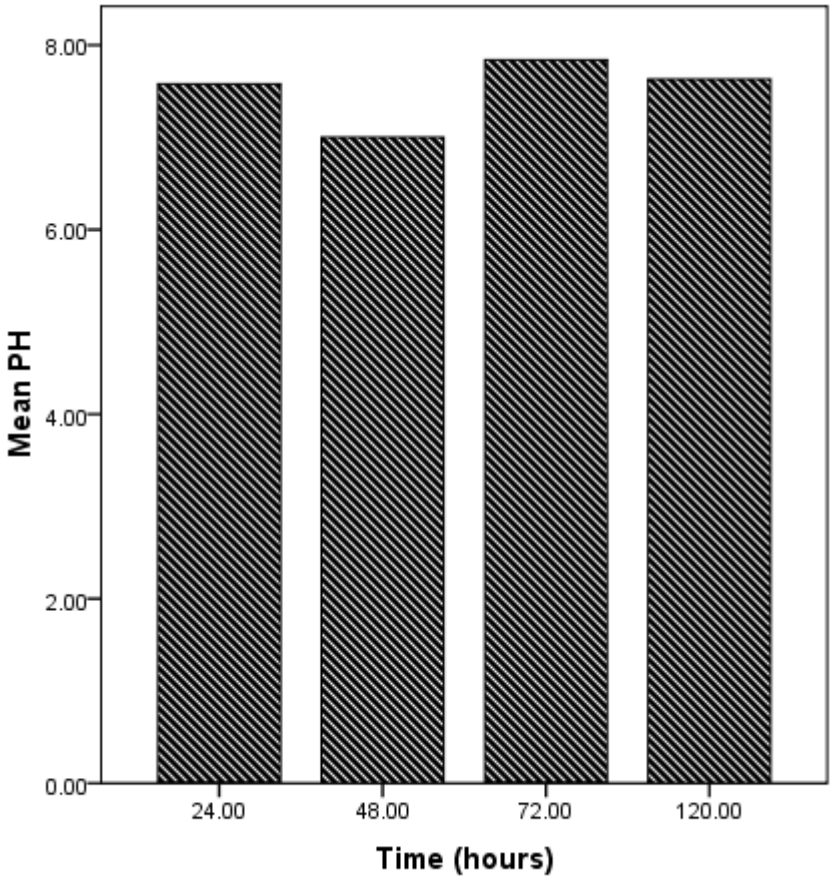

Figure 1: pH variation of various ogiri samples with time.

Figure no 1 showed the fermentation pH of the ogiri for four days which determine the high increase of the fermented ogiri to range from 7.0 to 7.84.

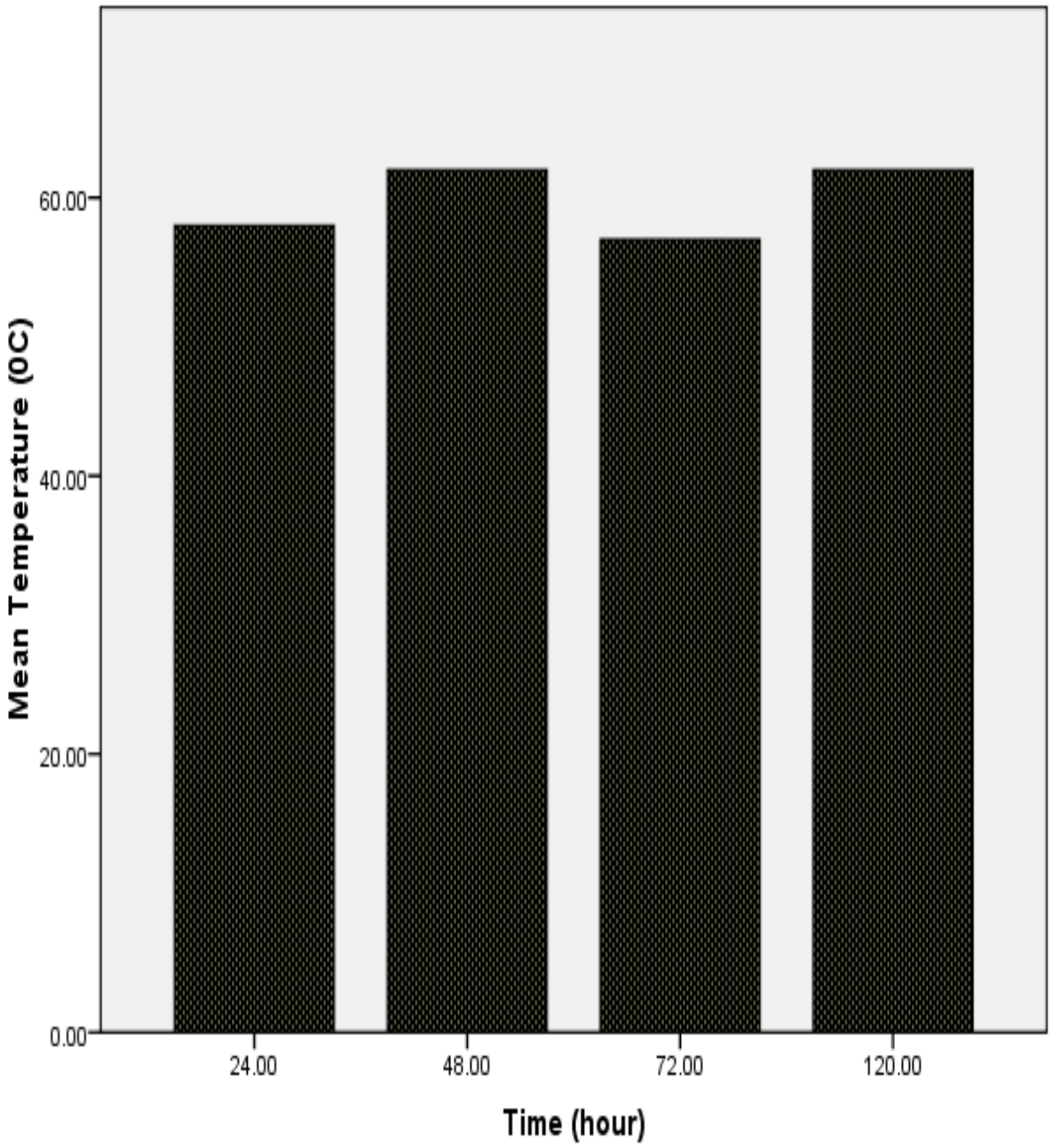

Figure 2: Temperature variation of various ogiri samples with time. This showed the temperature of the various fermentation of the ogiri. The temperature variation rnaged from 57ºc to 62ºc. Traditional fermented ogiri and market ogiri has the same temperature 62ºc while ogiri isolate and ogiri fresh has slight differences 57ºc and 58ºc

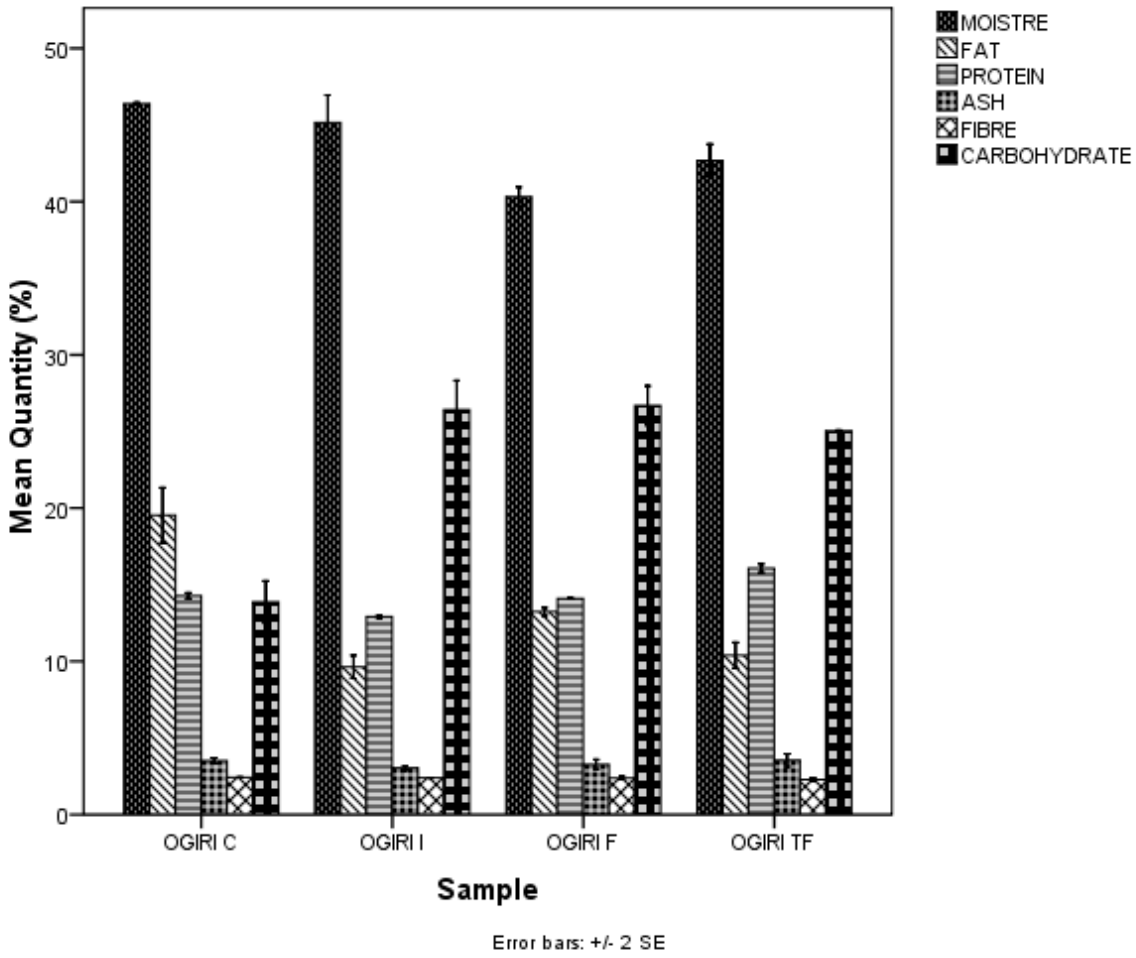

Figure 3: Proximate analysis of various ogiri samples expressed as mean ± standard error of the fat, protein, ash, fibre, carbohydrate and moisture content of the isolate.

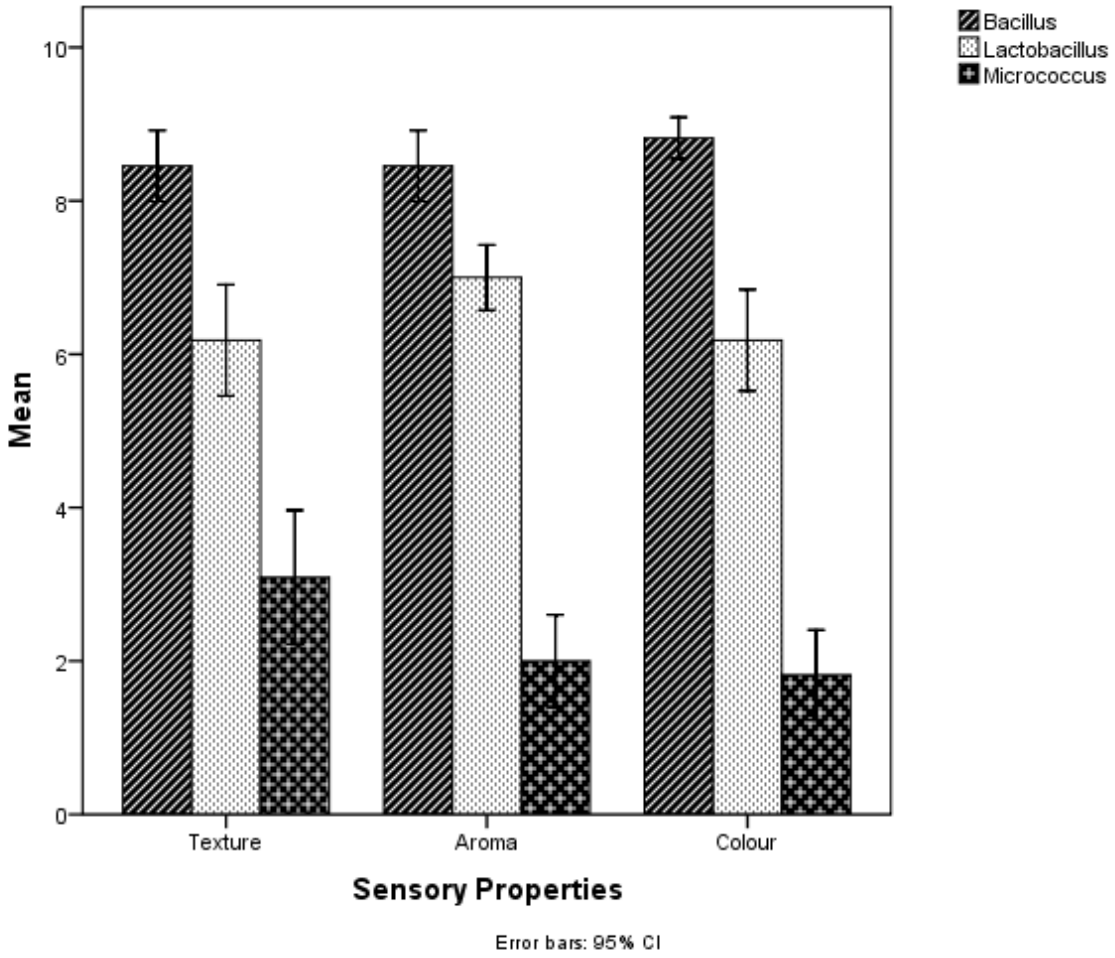

Figure 4: Sensory properties of various ogiri samples there was a significant difference (P ≤0.05) in the texture, aroma, and color.

DISCUSSIONS

Ogiri is a paste obtained from castor oil seeds (*Ricinus communis*) gotten through local fermentation processes. It is indigenous to eastern Nigerians. It serves as a nutritive non-meat protein substitute and as a flavouring material in food. Fermentation of castor oil seeds into ogiri is possible as a result of activities of some microorganisms. Hence, this study evaluated some microorganisms associated with the fermentation of castor oil seeds for the production of ogiri and assessed the biochemical properties, proximate composition and sensory parameters of ogiri.

Various bacterial species that were isolated from fermented ogiri include Bacillus species, Micrococcus species and Lactobacillus species as shown in table 3.These organisms has been reported on the previous works done by early researchers like (Enujiugha and Badejo, 2002) Bacillus species has also been reported to be responsible for the fermentation of African locust bean to produce dawadawa (Aderibigbe and Adebayo 2002), some Asian fermented foods (Sarkar*et al.*, 1997) and other oil seeds (Achi 2005).. The presence of *Bacillus* in fermentation may be due to their ability to initiate fermentation of both nitrogen and carbohydrate products (Omafuvbe, 1994). Their metabolic activities can contribute to flavours and aroma generating reactions and degradation of amino acids (Ouoba*et al.*, 2003). The number of microbes increased tremendously with increase in fermentation period (day1 to 5thday) as shown in figure 3. Since the major constituents of castor oil seeds are proteins, fats and carbohydrates, the microorganisms responsible for ogiri fermentation must be capable of utilizing these food sources (Pierson, et al., 1996). The predominant organism was *Bacillus* species and was present throughout the 72hours of fermentation. *Lactobacillus* was low during the first 48hours but generally increased at the end of fermentation period.

Since protein hydrolysis is the major biochemical change in ogiri fermentation, it can be suggested that *Bacillus* species are the major fermenting organism for the production of ogiri as previous work has shown bacillus to be responsible for protein hydrolysis (Achi, 2005). The dominance of *Bacillu*s species in fermenting samples during the first 24hours was typical of the micro flora in African fermenting bean seed (Njoku and Okemmadu, 2010). The co-dominance of *Bacillus* and *Lactobacillus* species was observed towards the end of fermentation period could be attributed to the production of bacitracin- an antibiotic which may have inhibited the growth of some pathogenic bacteria and also organic acids especially bacteriocins which acts as a preservative to preserve ogiri. The bacterial species responsible in the local fermentation of ogiri were probably introduced by contamination.

During fermentation, some biochemical reaction change occurred producing acids as shown in table 5. pH of various ogiri samples (ogiri market, ogiri isolate, fresh ogiri and traditional fermented ogiri) were determined. The sample pH value ranged from 7.00 to 7.84. When the sample were compared, it was observed that ogiri isolate after 72hours of fermentation, had the highest pH value, followed by fresh ogiri which lasted 24hours for its fermentation and traditional fermented 120hours. Market ogiri yielded the least value after 48hours of fermentation as shown in fig 1. This result is in accordance with early researchers who recorded the fermented food condiment from vegetable proteins to be alkaline in nature (Aeribigbe and Adebayo 2002). The increase in pH could be attributed to the production of ammonia and amine during fermentation (Achi, 2005) which inhibits contamination (Steinkraus, 2002; and Sarkar 1997). Increase in pH could also have contributed to the poor growth of *Lactobacillus sp.* Which had been reported to be aciduric (Aderiye et al., 2007).

Variations in temperature during the fermentation periods of ogiri were also noted. The temperature variation ranged from 57^{o}C to 62^{o}C. Traditional fermented ogiri and market ogiri has the same temperature (62^{o}C) while ogiri isolate and ogiri fresh has slight differences (57^{o}C and 58^{o}C) respectively as shown in figure 2. The rise in temperature is attributed to heat or liberation of Carbondioxide by the fermenting organisms. This ight be as a result of exerted pressure on the substrate (Ojimelukwe et al., 2011). Temperature increase has been reported by (Odunfa 1981). Similar trends have been observed in other plant materials (Achi, 2005; Ogueke and Nwagwu, 2007). The rise in temperature indicates that fermentation reaction is exothermic which changes being due to metabolic activities of microorganisms (Achi, 2005).

The proximate composition of fermenting castor oil bean seeds was evaluated as shown in 6.

The nutritional values (moisture, fats, proteins, ash, fibre and carbohydrates) were determined in this research work. The moisture content of ogiri sample, ranged from 40.32±0.31 to 46.39±0.03%. Ogiri commercial had the highest value, followed by ogiri isolates and traditional fermented ogiri. The result showed that fresh ogiri had the least moisture content. Increment in moisture content can be attributed to boiling of the seeds in water as reported by (Omafuvbe *et al*., 2004).Also, there was a significant difference in the sample with P value= 0.005.

The fat content of ogiri sample ranged from 3.2375±0.13 to 19.53±0.90% and was significantly difference among the samples. Ogiri commercial had the highest , followed by traditional fermented type and ogiri fermented with isolates. Fresh ogiri had the least fat content. This shows that microorganisms utilize fat contents more in fermented ogiri than in fresh ogiri .

Protein content which is one of the major constituents of the seed was also analyzed between the samples. After the fermentation, it was observed that the protein increased from 4.1000±003 to 16.0730±0.14. Traditional fermented ogiri had the highest value, followed by ogiri commercial and ogiri isolate. Ogiri fresh had the least value. This result shows that the nutritional values of food can be increased by fermentation. Protein content of the product is appreciable and can add to the protein of food preparation, hence a good protein supplement for both adult and children (Achi, 2005).Also, the nutritional qualities of condiments fermented using starters are higher than the nutritional values of condiments fermented without starter. Significant difference was also observed.

The ash content of the samples were given as follows (Traditionally fermented ogiri = 3.5375±0.21, ogiri commercial =3.52±0.09, ogiri fresh = 3.2720±0.16 and ogiri isolate =3.0380±0.01). Traditional fermented ogiri had the highest value while ogiri fermented with isolate had the least value. Traditional fermented ogiri having the highest value indicates that it contains more mineral products than ogiri isolate. Decrease in mineral of ogiri

isolate can be attributed to loss of mineral during processes of fermentation or it may mean that the mineral components actually resides in the hull of the seed and are thus removed during processing (Ogueke and Nwagwu, 2007).There was no significant difference in the sample.

The fibre content of the compared samples is reduced and there was no significant difference, showing that it does not contribute much in the nutritional value of ogiri.

Carbohydrate content of various ogiri samples was compared and the values ranged from 26.6905±0.64% to 13.87±0.69%. Fresh ogiri (unfermented) had the highest value as 26.6905%, followed by ogiri fermented with starter (isolate) and traditional fermented ogiri (10.0385±0.02%). Commercial ogiri had the least value. This showed that carbohydrate content decrease with increase in fermentation period (Parkoudaet et al., 2008; Ibrahim and Antai, 1986). This could be as a result of metabolization of carbohydrate by microorganisms of responsible for fermentation.(Ogbonnya et al., 2010) also reported a reduction in total carbohydrate in fermented locust bean.

Sensory evaluation of various ogiri samples fermented with *Bacillus, Lactobacillus* and *Micrococcus species* respectively, were compared in terms of texture, aroma and colour by eleven panelists. There was no significant difference ($P \leq 0.05$) in the texture, aroma and color of ogiri fermented with *Bacillus species*. There was a significant difference in ogiri fermented with *lactobacillus* and *Micrococcus species* respectively in terms of aroma and colour. Ogiri fermented with *Bacillus species* was mostly preferred by the panelists followed by the ogiri fermented with *Lactobacillus species*. *Micrococcus* was least accepted. Ogiri fermented with *Bacillus species* being the most preferred, can be attributed to the action of the organism on the protein contents of the seeds producing the best aroma and flavor (Enujiugha, 2003: Odibo et al., 1990). *Micrococcus species* being the least accepted showed that it did not contribute much to the fermentation.

CONCLUSION

Present findings indicated that a number of bacterial species are involved in the fermentation of the castor oil seed to obtain ogiri. The most predominant microbial species in the fermenting castor oil seed include *Bacillus and Lactobacillus* species. The results of this study have shown that fermentation of castor oil seed to produce ogiri respectively increases the nutritional content of the product. The liberal use of this condiment is expected to increase the intake of the essential dietary components appreciable. This work has also indicated the possibility of upgrading ogiri production to a college industry.

Pure culture of *Bacillus subtilis* is encouraged to be produced in the industry and used as starter culture for any local condiment, just as yeast (*Sacharomycessp)* are used in yoghurt production and alcoholic fermentation, since the organism is the chief fermenter of castor oil seeds into ogiri and is non-pathogenic. This will help to reduce the rate of food borne illness especially when compared with the condiments produced commercially and also improve the shelf life of the products.

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