

# Comparative Study on Microbial Loads of Fermented Castor Oil Seeds (*Ricinus communis*) and Melon Seeds (*Citrullus vulgaris*)

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**Abstract:** Various types of microorganisms were isolated from fermented products of castor oil seeds and egusi. The fermented products are mainly used as condiment soup among populations in Nigeria. The total bacteria load in fermented castor oil seed and egusi was in average of  $195.0 \pm 9.6 \times 10^7$  cfu/g and  $145.7 \pm 12.5 \times 10^7$  cfu/g respectively, while the total viable count of fungi cells show that castor oil seed ogiri contained  $3.3 \pm 1.5 \times 10^2$  cfu/g and  $2.7 \pm 0.6 \times 10^2$  cfu/g for egusi seed ogiri. The bacterial and fungi loads of ogiri made from castor oil seeds are more than ogiri from egusi. The bacteria flora species obtained include *Lactobacillus*, *Bacillus*, *Enterobacter*, *Streptococcus*, *Pseudomonas* and *Escherichia*. Fungi flora include *Rhizopus*, *Penicillin*, *Aspergillus* and *Yeast species*. They were characterized based on microscopy, colony features, biochemical reactions and sugar utilization. The result from this study confirmed that different microorganisms are associated in fermentation of castor oil seeds and egusi in the production of ogiri.

**Keywords:** Ogiri, Castor Oil, *Pseudomonas*, *Escherichia*, Fermentation

## 1. Introduction

Fermentation is the change of substrate to a final product by means of chemical reaction involving microorganisms (Achi, 2005). It is one of the oldest ways of food processing. It is an operation carried out by using microorganisms and their enzymes to achieve desirable quality characteristics of food and food products (Ogundana, 2002).

During the process, microorganisms utilize biochemical constituents of the food changing them from one form to another with the aid of microbial enzymes (Odunfa, 1995). This process enhances the palatability, increases protein value, vitamin content and mineral levels of such foods.

It also improves food preservation, food safety, enhances flavor and acceptability, it increases variety in the diet, improves nutritional value, reduces anti-nutritional compounds and in some cases, improves functional properties (Muller and Tobin, 1980).

Ogiri, a dark brown food condiment popular among people of West Africa is prepared by the fermentation of boiled leguminous oil seeds such as melon (*Citrullus vulgaris*) and castor oil seeds (*Ricinus communis*). It is a traditional food generally prepared in homes, used as flavoring agent in food (Reddy, 1986). The fermenting seeds may be occasionally exposed to sun to accelerate this process. The fermented seeds are ground into a paste, molded into balls and sun-dried before it is consumed or sold. This food is consumed throughout Western and Central Africa, but predominantly by the Ibos, a smaller ethnic group of Eastern Nigeria where it becomes a popular important food delicacy serving as sauce.

Ogiri is among the numerous legume-based food product that owe their production and characteristics to the activities of microorganisms. All fermented foods have aroma and flavor characteristics directly or indirectly from the fermenting organisms. Many of food fermentation processes are natural or mixed culture fermentation consisting of different species and genera of yeast, fungi or bacteria.

According to Singleton (1997), Bacteria are minute organisms which occur everywhere. Most bacteria do little or no harm and, many are positively useful to man. Perhaps, bacteria are usually employed in the production and processing of food for instance, the fermentation of ogiri with castor oil seeds or melon. It is necessary to ascertain the nutritive value of the product and species of bacteria and fungi involved in ogiri production, since almost every class of people like this food product.

The present study is aimed at comparing the microbial loads of fermented foods by isolating and identifying lactic acid bacteria in ogiri that can be used as starter cultures. The benefit of fermentation may include improvement in palatability and acceptability by developing improved flavor and texture, preservation through formation of acidulates, alcohol and antibacterial compounds. It also improves enrichment of nutritive content by microbial synthesis of essential nutrients and improving digestibility of protein and carbohydrates, removal of anti-nutrients, natural toxicants and mycotoxins and decreased cooking time (Steinkraus, 1995; Knout, 1994).

Adieus, (2004) has shown ogiri to be a protein rich condiment available to those in developing countries, Nigeria in particular. But short shelf life, stickiness,

characteristics putrid odour, objectionable packaging materials, lack of proper hygienic standards in processing of this product has made it difficult to handle effectively.

As a consequence of this, this study attempts to develop a convenient ogiri condiment from the seeds of the castor oil plant and melon that are of acceptable quality. To assess the nutritional quality of the condiment produced with different microorganisms.

To assess its acceptability by comparing the microbial loads of ogiri condiment produced with castor oil seeds and melon. Microorganisms need a source of nitrogen for normal growth which is met by proteins in the food. Thus, microbes attack proteins in food through proteolysis which breaks down proteinous materials to release foul smelling odour by putrefaction and smaller molecular compounds like amino acids (Oyewole, 1990). Also, according to the same author it has reported by some workers that microbial growth encourages the synthesis several vitamins and amino acids in order that fermented products should have an increased nutritive value than the parent substrate.

Naturally occurring fermentation operation is plagued with many problems, which include non-reproducible quality of products, lack of uniformity in taste, flavor and short shelf life. This is because, fermentation generally depends on inoculation from the environment and starter cultures are not used, thus, encouraging spoilage organisms, contamination and unhygienic products.

In Nigeria, as with most African countries, the problem of food security is not just that of inadequacy of food but also a problem of loss of food due to spoilage (Knout, 1994). Lack of inadequacy food preservation methods is a major problem contributing to food security in Africa. The high cost and infrastructural requirement of many advanced food preservation methods such as refrigeration, freezing, canning and irradiation have greatly reduced their application in the developing world (Cooke, 1987). This implies that promoting fermentation and fermentation technology in Africa, is helping to promote food security in Africa.

## 2. Literature Review

Plant or animal tissue subjected to the action of microorganisms or enzymes to give desirable biochemical changes and significant modification of food quality are required to as fermented food (Campbell, Platt, 1994). Fermented foods are of great significance because, they provide and preserve vast quantities of nutritious foods in a wide diversity of flavor, aroma and texture which enrich the human diet (Steinkraus, 1997). Fermented foods are also essential components of the diet in many countries and are consumed either as main dishes or as condiments (Steinkraus, 1996).

Indigenous fermented foods were developed through traditional technologies which were prepared over the years in order to maintain their uniqueness and identity (Valyasevi and Rolle, 2002). They are prepared from both plant and animal materials, using processes in which

microorganisms play active roles in the physical, nutritional and organoleptic modification of the starting materials (Aidoo, 1994). Nigeria is endowed with wide range of fermentable indigenous staple foods that serve as raw materials for agro-allied cottage industries.

These industries utilize small-scale equipment while adding value to such local produce (Latunde Dada, 2000).

The fermented food in Nigeria can be classified into groups according to the substrates or raw materials employed (Odunfa, 1985). These include products from tubers (Lafun and Fufu), all products of cassava, cereals (Ogi, Pito and burukutu), all products of maize, sorghum or millet, legumes (iru and dawadawa), products of locust beans and soya bean respectively, fruits (ogiri), a product of castor oil seeds or melon, beverages (palm wine) and milk (warakishi)

According to Steinkraus (1995), fermented foods have a very food safety record even in the developing world where foods are manufactured by people without training in microbiology or chemistry.

Ogiri is a fermented soup condiment of flavoring agent whose character and organoleptic properties depend on microbial activity (Ihekoronye Ngoddy, 1985). This condiment is used in relish dishes and may be prepared from pumpkin seeds (Achinewhu, 1983; Achinewhu, 1986), castor oil seeds and melon. The odour of this flavouring agent meets with mixed reception among people of different background, which has discouraged wide use by elite Nigerians despite its highly acceptable taste. There is therefore, a need to identify the microorganisms responsible for the undesirable odour production and to improve processing methods to enhance acceptability and prolong the shelf life of this condiment.

Ogiri is a fermented product of melon seeds (*Citrullus vulgaris*) or castor oil seeds (*Ricinus communis*). The fermented products are used as condiment in soup, sauces and porridges among consuming population in Nigeria. The fermentation process involves varying groups of microorganisms (mainly bacteria). Fungi were also reported by (Ogundana, 1980), but was considered questionable (Odunfa, 1985). Bacteria involved include *Lactobacillus spp*, *Streptococcus spp*, *Eschenchia coli*, *Bacillus spp*, *Pediococcus spp* (Odunfa, 1981), *Proteus spp*, *Micrococcus spp* and *Klebsiella spp* were isolated from the fermented melon seeds.

*Proteus spp* has been implicated as a spoilage organism of protein containing foods (Frazier and Hoggman, 1979; Jay, 1996). Microbial spoilage of ogiri is a major problem. It was however, observed that the organism responsible for the fermentation of a given product also accounted for the spoilage, for example, the spoilage of ogiri has been attributed to saccharolytic, proteolytic and lipolytic organisms (Odunfa, 1981).

The common edible portion of most under-utilized plants are the seeds, which in some cases are cooked or roasted and eaten directly as snack foods example, conophor nut

and bambara groundnut while some are fermented and used as food in some rural parts of Nigerian, among which are ogiri from castor oil seeds (*Ricinus communis*), melon (*Citrullus vulgaris*) and ugba from Africa oil bean (*Pentaclethra macrophylla*).

Diverse group of bacteria comprising species of *Bacillus*, *Micrococcus*, *Leuconostoc*, *Escherichia coli*, *salmonella enteritidis*, *shigella* etc. have been reported by various authors (Enujiugha, 2002) as contributing to the individual fermentation. However, the indigenous fermentation is mostly achieved via natural inoculations.

Important enzymatic qualities with nutritional affects had been reported in many lactic acid bacteria and yeast in indigenous formation. These enzymes allow the breakdown of many complex substances such as starch, oligosaccharide, protein and phylic acid complexes thus, increasing the quantities and qualities of easily digestible nutrient in foods (Gobbetti, 1994; Olasupo, 1996). Lactic acid bacteria particularly *lactobacillus spp* are involved in the fermentation of many African food.

Melon seeds belong to the family cucurbitacea and are mostly cultivated in the Southern part of Nigeria and are usually interplant with yam and cassava where they serve as cover crops. The seeds may be the only source of protein for some groups where they are used to substitute meat or fish (Aidoo, 1986).

Melon seeds have been reported to contain 3.3% moisture, 15.5% and 3.6% ash (Akonbundu, 1982; Omafuvble, 2004). Various workers have identified different microorganisms in fermented melon seeds. These include *Bacillus spp*, *Proteus spp*, *Pediococcus spp* and *Alcaligonus spp* (Sannie, 2002).

However, *Bacillus subtilis* and *Bacillus licheniformis* have been identified as the main bacteria present (Barimalla, 1989).

Castor tree is a large leguminous woody plant which belongs to the family leguminosae. It is a perennial tropical tree crop found mostly in the Southern and middle belt regions of Nigeria and in the other coastal parts of West and Central Africa where it is believed to have originated from. The tree is recognized by pleasant farmers in these parts of the country for its soil improvement properties and as a component of an forest system (Fernandez, 1987).

Okafor, (1982) recognized castor oil seed (*Ricinus communis*) as a food tree specie for outlying farms in the forest zone. Presently, they grow either wild or semi-wild with no organized cultivation in plantation or chards on Nigeria.

The plant is relatively fast growing to about 40-60 feet height. The main flowering season is between the months of March and April with a smaller new fresh growth in June and July. The flowers are green in colour, with offensive smelling. The fruit is a round green pod which darkens at maturity.

Fruits are available at most period of the year because, the round woody pods bearing the seeds are always available (Hess, 1983). The number of seeds in pod depends on the size of the pods. The seeds explode when mature and dried, dispersing the seeds. The mature dispersed seeds are harvested by gathering them manually from around the tree. These seeds are the source of ogiri used in food condiments.

### Fermentation of Ogiri

Fermentation, which is changing of substrate to a final product by means of chemical reaction involving micro-organisms, is one of the oldest and economical food processing methods that could be used in home to improve the nutritive values of plant foods (Heseltine, 1980).

It is probably the oldest method of processing legumes (Siegel and Fawcett, 1976). Legume-based fermented food originated centuries ago.

The various types of fermentation have been used by nearly every civilization since historical times (Reddy, 1980), and this includes solid state fermentation used for preparation of ogiri. Foods are fermented for so many reasons including enhancement of nutritive value, keeping quality of products and texture, characteristics (flavor, aroma, appearance and consistency), improvement in digestibility, improved safety (absence of toxins and partial or complete elimination of anti-nutritional factors) and reduced cooking time. The increased nutritive value of fermented food is due to the breakdown of complex compounds such as carbohydrates, protein and lipids, to easily digest sugar, free fatty acids, amino acids as well as synthesis certain vitamins (Reddy, 1986).

The fermentation process reduces the toxicity of some food (gari), while others may become extremely toxic during fermentation (Bongkrek Coconut Press cake, product of central Indonesia).

The fermented process of castor oil seeds or melon removes bitterness, remarkably softens the cotyledons and improves digestibility and nutrient availability (Enujiugha and Akunbi, 2002).

Fermentation of castor oil seeds or melon is usually by chance inoculation with desirable fermentation agents example, bacteria which are ubiquitous.

This product may also be contaminated during fermentation process and the sources of contamination are natural contaminants from air, the leaves used for wrapping, from handling, from flies and from the utensils used in cooking the cotyledon (Reddy, 1986).

### Lactic acid bacteria and Lactic acid fermentation

Lactic acid bacteria are a group of gram positive bacteria, non-respiring, non-spore forming cocci or rods that are associated by their common metabolic and physiological characteristics. The bacteria, usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. This

trait has throughout history linked lactic acid bacteria with food fermentation, as acidification inhibits the growth of spoilage agents.

Lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item. The industrial importance of the lactic acid bacteria is further evidence by their generally recognized as safe status, due to their ubiquitous appearance in food and their contribution to the healthy micro flora of human mucosal surfaces.

Historically, bacteria from the genera, *Lactobacillus*, *Streptococcus* and *Pediococcus* are the main species involved. Several more have been identified but, play a minor role in lactic acid fermentation. Lactic acid bacteria were recently reviewed by Axels (1992). Species of the genera *Streptococcus* and *Leuconostoc* produce the least acid.

Next are the heterofermentative species of *Lactobacillus* which produce intermediate amounts of acid followed by the *Pediococcus* and lastly, the homofermenters of the *Lactobacillus* species which produce the most acid (Axels, 1998).

Lactic acid fermentation is a biological process by which sugar as glucose and sucrose are converted into cellular energy and the metabolite lactate. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells such as muscle cells in the absence of oxygen. If oxygen is present in the cell, many organisms will bypass fermentation and undergo cellular respiration. However, facultative anaerobic organisms will both ferment and undergo respiration in the presence of oxygen.

### 3. Materials and Method

#### Source of materials

The castor oil and melon seeds used in this project work were purchased at Nkwueke village market in Onitsha. Laboratory and other facilities were obtained from the Central laboratory services unit of National Root Crops Research Institute, Umudike, Abia State.

#### Preparation of sample

Prior to use in the production of ogiri, the seeds were first prepared. The castor oil seeds were dehaulled manually by breaking the pod to extract the cotyledons. In the process, diseased and bad ones were sorted out and removed such that healthy seeds were used in the process. This method is applicable in the preparation of egusi (Ogundana, 1980).

The media used in the enumeration of microbes in the ogiri sample were Nutrient Agar (for bacteria culture) and Potato Dextrose Agar (for fungi culture). These were prepared in accordance with the manufacturer's instructions. 28g of the Nutrient Agar powder and 39g of the Potato Dextrose Agar were weighed out separately and dispersed in 800mls of distilled water in separate heat stable flasks. The suspensions were heated in a water bath until the Agar melted to form a homogenous clear solution. They were made up to 1 liter each and their respective PH levels

measured with a PH ratio to ensure compliance with the standard  $7.2 \pm 0.2$  for Nutrient Agar and  $5.4 \pm 0.2$  for Potato Dextrose Agar. The media were transferred to a separate and labeled Duram bottle and sterilized by autoclaving at  $121^\circ\text{C}$  for 15 minutes. The sterile media were aseptically dispensed in approximately 15ml portion into sterile petri dishes inside the inoculation chamber and allowed to cool and solidify for use in microbial culture (Bucchanan and Gibbons, 1974).

#### Production of Ogiri

The Ogiri samples both castor oil and egusi seeds were produced in accordance with the traditional method of Abatete people of Anambra State.

After preparing the seeds, they were put in separate pots and covered completely with water and were strongly boiled. The castor seeds were boiled for six (6) hours to soften the seed coat and eliminate anti nutrients after which it was drained of the boiled water. On the other hand, the egusi seeds were boiled for three (3) hours and drained of the water as well. The boiled and drained seeds were wrapped with plantain leaves for 48hrs at ambient temperature. Then the softened seeds were separately ground into a smooth pulp and 10g portion was later wrapped in flamed large plantain leaves. They were allowed to ferment for another 48hrs also at ambient temperature in the wrapped form.

This final product was ready for use as food condiment and it was used for analysis. The procedure was extracted from Bergey's manual according to Bucchanan and Gibbons, 1974.

#### Determination of microbial load

The plate count technique described by the International Commission on Microbiological Specification of Foods ICMSF (1998) was used. The microbial load was determined as the total viable count (TVC) and expressed as the number of colony forming unit of microorganisms per gramme of the sample (cfu/g).

Aseptic principals were used throughout the work to avoid possible introduction of contaminating microbes from outside the samples. The Ogiri sample was pounded in a sterilized porcelain laboratory mortar to form a uniform pulp out of which 1g sample was collected and mixed with 9mls of sterile distilled water in a test tube. From this mixture, 1ml portion was aseptically transferred to another test tube containing 9mls of distilled water and was thoroughly mixed to form a second 10-fold dilution. In this sequence, the sample mixtures were diluted serially in 10 folds down to the sixth diluents ( $10^{-6}$ ).

All through the process of dilution, fresh sterile pipettes were used at each turn of dilution. The serially diluted sample mixtures (suspension) were used for microbial culture and subsequent enumeration is described below.

The actual culture and count of the microbes (bacteria and fungi) was done using the spread plate technique (Fawoce and Oso, 1998; Cheesbrough 2000). An Inoculum of 0.1ml was carefully and aseptically collected from the sixth

diluents ( $10^{-6}$ ) and deposited on the surface of the sterile Nutrient Agar plate. With the aid of a flamed glass hockey (spreader), the inoculum was spread evenly all over the surface of the medium in plate. The inoculated plates were labeled appropriately, sealed at the edges and incubated in the incubator at  $37^{\circ}\text{C}$  for 24-48 hours. They were observed daily for growth. In a similar way, a 0.1ml inoculum was collected from the first diluent ( $10^{-6}$ ) and cultured on Potato Dextrose Agar using the same method for bacteria. However, the inoculated plates for fungi culture were incubated at room temperature ( $28-32^{\circ}\text{C}$ ) and for 2-5 days. They were accordingly observed daily for growth.

On establishment of growth, the number of microbial colonies in each plate was counted with the aid of the colony counter. A mean count was taken from the three (3) plates cultured for each sample.

The formula below was used to calculate the total viable count of bacteria and fungi in the test samples.

$$\text{TVC (cfu/g)} = 1 - v \times N \times D$$

V = Volume of inoculums

N = Number of colonies counted

D = Dilution factor

#### Isolation and characterization of microbes

Following the establishment of growth in the initial culture, the resulting culture was examined closely for distinct colonies. From such distinct colonies, inocula were aseptically collected and transferred to the surface of sterile Agar media (Nutrient Agar and Potato Dextrose Agar). The sub-cultured plates were incubated accordingly. When growth was established in the sub-cultured plates, each was examined for uniformity as a form of purity. The resulting pre-cultures were used for characterization and subsequent identification.

Characterization of bacteria and fungi isolates was based on established colony, other structure which were matched against existing taxa in standard identification manuals. Bacteria isolated from the test samples were subjected to a four (4) step tests to determine their various characteristics. The steps include the following:

#### Colony features

Colonies in each pure culture isolates was observed very closely and their features such as extent of growth, colony elevation, consistency, pigmentation, nature and edge of colonies observed features were recorded accordingly.

#### Microscopy

This step involved conducting test to determine the reaction of each isolate to general dye (Gram stain) as well as specific dyes which showed the presence or absence of features such as flagella, spores, capsule etc. in addition, the shape and arrangement of the bacteria cell as well as their respective motility status were observed and recorded.

#### Biochemical Reactions

This step included tests which showed the ability of the bacteria isolated to produce enzymes such as catalase, oxidase, coagulase, urease etc. methyl red and voges proskauer tests were done which showed the ability to

reduce nitrate, produce indole, utilize citrate etc. records of findings were kept.

#### Sugar Utilization Test

This step included those which should whether the different bacteria isolates are able to utilize different sugars in broth cultures with the introduction of acid or gas or both. The test sugars included glucose, maltose, lactose, mannitol, etc.

## 4. Result

Results of laboratory analysis of the ogiri samples are shown in Tables 1-3. Table 1 show the microbial load (bacteria and fungi) of the samples while Table 2 and 3 show the bacteria and fungi occurrence in the samples.

#### Microbial Loads of Bacteria and Fungi

Table 1 above shows the microbial load of the two ogiri samples. The result shows that the bacteria load was an average of  $145.7 \pm 12.5$  colony forming unit per gramme (cfu/g) of the egusi seed ogiri and  $195 \pm 9.6$  (cfu/g) of the castor oil seed ogiri. The total viable count of fungi cells show that castor oil seed ogiri contained  $3.3 \pm 1.5$  cfu/g of the sample.

**Table 1: Microbial Load**

Bacteria load					
Samples	A	B	C	Mean load $\times 10^7$	Remarks
Castor Oil Seeds	191	206	188	195	1.0g sample was used
Egusi Seeds	146	133	158	145.7	

#### Fungi load

Samples	A	B	C	mean load $\times 10^2$	Remarks
Castor Oil Seeds	3	2	5	3.3	1.0g sample was used
Egusi Seeds	2	3	3	2.7	

#### The Occurrences of microbial isolates from the two sources (Castor Oil seed and Egusi)

The bacteria isolates obtained from the two samples and their occurrences show that six (6) bacteria species were isolated and identified as belong to species of *Bacillus*, *Streptococcus*, *Pseudomonas*, *Escherichia*, *Enterobacter* and *Lactobacillus*. All the isolates were found in the castor seed ogiri except *Proteus* species while all but *Enterobacter* species were found in the egusi seed ogiri.

Four (4) fungi species were isolated from the ogiri samples including *Rhizopus*, *Penicillin*, *Aspergillus* and yeasts. All the isolates were found present in the egusi seed ogiri.

**Table 2: Occurrence of bacteria Isolates**

Castor oil seeds	Egusi seeds	Isolates
+ve	+ve	<i>Bacillus spp</i>
+ve	+ve	<i>Pseudomonas spp</i>
-ve	+ve	<i>Proteus spp</i>
+ve	+ve	<i>Escherichia spp</i>
+ve	-ve	<i>Enterobacter</i>
+ve	+ve	<i>Lactobacillus</i>

+ve = Present (Isolated), -ve = Absent (Not Isolated)

**Table 3:** Occurrence of fungi isolates

Castor seed ogiri	Egusi seed ogiri	Isolates
+ve	+ve	<i>Rhizopus</i>
+ve	+ve	<i>Aspergillus</i>
+ve	+ve	<i>Penicillin</i>
+ve	+ve	Yeasts

+ve =Present (Isolated), -ve = Absent (Not Isolated)

**Table 4:** Characterization of Bacterial Isolates

Colony Features	Creamy elevated colonies on Nutrient Agar	Creamy w slimy elevation translucent with visible green pigments on Nutrient Agar	Large irregular colonies creamy on Nutrient Agar	Dull white colonies on Nutrient Agar	Large swarmy creamy moist flat on Nutrient Agar	Small and whitish colonies
<b>Microscopy</b>						
Gram stain	-ve	-ve	+ve	-ve	-ve	-ve
Spore	-ve	-ve	+ve	-ve	-ve	+ve
Flagella	+ve	+ve	+ve	-ve	+ve	+ve
Motility	+ve	+ve	+ve	-ve	+ve	-ve
<b>Biochemical reaction</b>						
Catalase	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	+ve	-ve	-ve	-ve	-ve
Coagulase	-ve	-ve	+ve	-ve	-ve	+ve
<b>Sugar Utilization Test</b>						
Glucose	AG	AG	+ve	+ve	+ve	+ve
Sucrose	-ve	+ve	+ve	+ve	+ve	+ve
Maltose	+ve	AG	AG	+ve	+ve	+ve
Mannitol	+ve	+ve	-ve	+ve	-ve	-ve
	<i>Escherichia coli</i>	<i>Pseudomonas areginosa</i>	<i>Bacillus Spp</i>	<i>Enterobacter</i>	<i>Proteus</i>	<i>Lactobacillus</i>

Keys +ve = Positive -ve = Negative AG = Acid and Gas

## 5. Discussion

The result shows a high bacteria load of  $195.0 \pm 9.6 \times 10^7$  and  $145.7 \pm 9.64 \times 10^7$  colony forming unit per gramme (cfu/g) of the castor oil seeds and egusi seed ogiri respectively. Both seeds are known to be rich in protein and carbohydrate which are essential supportive growth factors for most bacteria and this corroborates with the findings of Odunfa, 1985.

Most bacteria are reported to possess proteolytic and lipolytic ability and as such thrive well in oil seed of high protein content (Forarty, 1994). On comparative grounds however, the bacteria load of the castor oil seed ogiri was found to be higher than that of the egusi seed ogiri. The lower bacteria load of the egusi seed ogiri may not be unconnected with the presence of anti nutrients in the seed, some of which may be toxic nutrients in the organisms thus, retarding growth and multiplication. The fungi load show an average of  $3.3 \times 10^2$  and  $2.7 \times 10^2$  colony growing units of fungi per gramme of the samples. Their figures are samples, intra and inter species antagonism exist in which some microbes produce exudates that antagonize the other types (Olasupo, 1997). The high bacteria load has contributed to suppression of fungi proliferation in the ogiri samples. The bacteria flora include the species of lactobacillus, bacillus etc.

The bacteria flora of the ogiri was found to agree with results of various research works. Odunfa (1985) reported that there are varieties of lactic acid bacteria in fermenting ogiri while Jay (1996) implicated *Proteus* species as spoilage organism in protein rich foods.

The fungi flora centered *Rhizopus*, *Aspergillus*, *Penicillium* and Yeast species. Ogundana (1980) had earlier implicated several fungi species in fermentation process. However, *Rhizopus* is known to be saprophytes which are generally found in fermenting or decaying environment. *Aspergillus* and *Penicillium* as well as wild yeasts are more or less ubiquitous as they are found everywhere in our environmental. In spite of fungi presence, it is supportive that the fermentation process involved mainly the bacteria isolates while most of the fungi may be transient.

## 6. Conclusion

A wide variety of microorganisms are involved in the fermentation of ogiri irrespective of the seed used. Also, outside the lactic acid bacteria, other species of bacteria found their way by chance inoculation and contribute in fermentation of ogiri. The wide range of species of bacteria involved in the ogiri fermentation was considered to be due to the rich protein and oil content of castor oil seeds and egusi seeds.

Fermentation of food has many advantages such as improvement of nutritional value and protection against bacterial pathogens (Gadage, 2004). Meanwhile, it is very necessary to maintain good sanitary conditions during the processing of ogiri production since there is a possibility of getting food borne diseases from it.

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