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(RESEARCH ARTICLE)

Isolation and identification of bacteria associated with fermentation of melon seed for Ogiri Sold in Owerri, Nigeria

Joy Nkeiruka Dike-Ndudim, Chizaram Winners Ndubueze *, Juliet Chidinma Ezihe and Emeka Okechukwu

Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria.

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Abstract

In order to ascertain the public health implication of the consumption of the fermented melon (Cucumismelo), known as Ogiri, consumed in all South Eastern Nigeria and beyond as a food condiment, thirty samples of locally fermented melon seeds, Ogiri, were randomly purchased from three markets in Owerri metropolis in Imo State. These samples were microbiologically analysed using pour plate technique on nutrient agar, MacConkey and Salmonella Shigella agar at 370C for 24 hours. The viable and mean counts were determined and the data obtained were statistically analyzed. There was no significant difference between the contamination of the samples ($p>0.05$). These organisms, following the order of predominance were isolated; Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus. The presence of these microorganisms of public health importance in food condiments pose a risk of enteritis and other food borne diseases in some individuals after its intake. Thus, the health organizations should embark on public and personal enlightenment programs targeted at both the producer and the consumer.

Keywords: Ferment; Melon Seed; Ogiri; Food; Contamination; Bacteria

1. Introduction

Ogiri is a food condiment produced from the fermentation of melon seed and is adjudged to be an indigenous fermented soup condiment which is used as flavoring agent whose character and organoleptic properties depend on microbial activities [1]. It has gray colour with porous structure and sharp smell when in a raw state, but the dried form has less pungent smell. The production of 'Ogiri' has been limited to household level and mostly women are involved in its production.

This condiment is consumed among the 'Yorubas' and 'Igbos' who are largely found in South Western and Eastern part of Nigeria. The production of 'Ogiri' involves solid fermentation of melon seeds. The step by step production involves boiling of raw melon seeds and the water is drained. The seeds are peeled and allowed to ferment naturally for three to five days in clay pots. The fermented seeds are then mashed into pastes, wrapped in leaves and kept over a fire place to dry. Because of the natural fermentation, the products vary in pungency depending on fermentation time and melon seeds [2]. Apart from regular melon seed (*Citrulus lanatus*) used for 'Ogiri' preparation, it can also be produced from castor oil seeds (*Ricinuscummunis)* as reported by Enujiugha [3] and fluted pumpkin (*Telfairia occidentalis)* by Omafuvbe and Oyedapo [4]. These other melon seeds which are underutilized can serve as alternative substrates for the production of 'Ogiri' thereby increasing their utilisation.

Fermentation processes play an important role in the production of Ogiri. In traditional fermentation process, natural microorganisms are used in the fermentation of different types of food. Fermented foods have great potential as key protein, carbohydrate and fatty acids [1].

* Corresponding author: Chizaram Winners Ndubueze
Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria.

Fermented foods in general, enhance flavouring, aid digestibility, edibility and are basic ingredients for food supplements and other socio-economic importance in Nigeria. The fermentation techniques are characterized by the use of simple non-sterile equipment, natural inoculums, unregulated conditions, sensory fluctuation, unclean and unattractive packages. The food flavoring condiments are prepared by traditional method of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate component [3]. The fermented foods ofNigeria are classified into staple food and condiments like Ogiri [5].

Microorganisms however are involved in the fermentation process of these fermented foods. The microorganisms are also involved in the spoilage of these foods and also cause food poisoning. Therefore the need to discover the various micro-organisms associated with this process becomes sacrosanct and forms the basis for this study.

2. Materials

2.1. Samples used

The samples used were obtained from three major markets in Owerri, Imo State, Nigeria. The three markets are Eke Ukwu Owerri market, Relief market and Nkwo-Orji market.

2.2. Sample Collection

A total number of 30 wrapped Ogiri samples were randomly purchased from the three markets earlier mentioned; 10 from each market. The samples were transported in sterile polythene bag to the Medical Microbiology Laboratory, department of Medical Laboratory Science, Imo State University.

2.3. Laboratory processing and analysis

All the materials used were sterilized using the autoclave at 121^{o}C (15 psi) for 15 minutes. Each of the samples was opened and emptied into a sterile beaker and carefully mixed with a sterile glass rod. 1 gram of each sample was weighed out and added in respective test tubes and serial dilution (10 fold) done in each of the samples.

2.4. Cultivation of Samples

All the materials used were sterilized and the work area disinfected to avoid undue contamination of samples to be cultured which will yield a false positive result. 1ml of the diluted samples from each of the test tubes were inoculated onto Nutrient agar, MacConkey and Salmonella Shigella agar (SSA) by pour plate method and mixed gently for uniformity. The plates were incubated for 24hours at room temperature under aerobic condition. Duplicate plates were incubated for 24 hours at room temperature under an anaerobic condition in an air tight container.

2.5. Isolation and Identification

After an overnight incubation of the media, pure isolates of the discrete colonies were identified using their morphological and microscopic features. Gram staining was done on each of the isolates. The identity of the organisms was confirmed by employing some biochemical tests which includes catalase test, motility test, oxidase test, citrate, urease test and indole test.

2.6. Statistical Analysis

Data generated from practicals and results of the laboratory analysis were entered into Microsoft Excel and analyzed using SPSS software (version 20; IBM Corporation, Armonk, NY, USA). Results obtained were reduced to tables.

3. Results

The following results gotten from the isolates obtained from the sixty samples of ogiri obtained randomly from the three markets in Owerri local government area and the results were recorded in tables as shown below.

Table 1 shows the colonial morphologies and microscopic evaluation of the bacterial isolates. From the table, four groups of isolates with distinct morphological appearance were observed. Isolate 1 to 3 were rod shaped and Gram negative. Isolate 4 on the other hand was Gram positive and cocci shaped. Motility was observed in isolate 1 and 2, whereas isolate 3 and 4 were non-motile.

Table 1 Colonial morphologies and microscopic evaluation of the bacterial isolates

	Gram reaction/shape	**Colour**	**Motility**
Isolate 1	- / bacilli	Large, circular, low convex, colourless colonies on NA	Motile
Isolate 2	- / bacilli	Large, smooth, translucent colonies with characteristic fruity odour on NA	Motile
Isolate 3	- / bacilli	Large circular, dome-shaped, greyish white, mucoid colonies	Non-motile
Isolate 4	+ / cocci	Small, round, smooth, glistening yellow colonies	Non-motile

Table 2 shows the biochemical reactions of the various bacterial isolates obtained after 24 hours culture. After relevant biochemical investigations were performed on the isolates, it was confirmed that isolate 1 was *Escherichia coli,* isolate 2 was confirmed *Pseudomonas aeruginosa,* isolate 3, *Klesiella pneumonia* and Isolate 4, *Staph. aureus*.

Table 2 Biochemical Reactions of Test Organisms

Organism	**Indole**	**Methyl red**	**Vogues Proskauer**	**Citrate utilization**	**Urease**	**Nitrate**	**Catalase**	**Oxidase**	**Coagulase**	**Name of Isolates**
Isolate 1	+	+	-	-	-	+	+	-	-	*Escherichia coli*
Isolate 2	-	-	-	+	-	+	+	+	-	*Pseudomonas aeruginosa*
Isolate 3	-	-	+	+	+	+	+	-	-	*Klebsiella pneumonia*
Isolate 4	-	+	+	+	+	+	+	-	+	*Staphylococcus aureus*

Table 3 shows the minimum and maximum log $_{10}$ CFU of microorganisms per gram of Ogiri obtained from different markets in Owerri metropolis. *E. coli* from Relief market showed the lowest count at 0.38 $\log_{10}$CFU whereas *Pseudmonas* per gram of Ogiri from Nkwo orgi showed the highest count at 3.46 log $_{10}$ CFU.

Table 3 Minimum and maximum values of count of microorganisms isolated from different markets in Owerri metropolis (expressed as log CFU/g of Ogiri)

MARKET	***E. coli*** **($\log_{10}$CFU/g)**	***P. aeruginosa*** **($\log_{10}$CFU/g)**	***K. pneumoniae*** **($\log_{10}$CFU/g)**	***S. aureus*** **($\log_{10}$CFU/g)**
Eke Ukwu	1.97-7.69	3.01-6.83	1.27-6.83	2.23-5.31
Relief	0.38-6.95	0.79-8.74	1.42-8.70	2.77-5.78
Nkwo-orji	1.69-8.63	3.46-8.18	2.84-6.67	0.84-6.33

Table 4 shows the Mean ± SD bacterial counts (CFU/g) of Ogiri from different markets in Owerri metropolis. *Pseudomomas* count per gram of Ogiri from Eke Ukwu market had the highest mean value of 6.39±2.36 with the lowest count of 3.51±1.38 expressed in *Klebsiella* from Eke Ukwu.

Table 4 Mean ± SD bacterial counts (CFU/g) of Ogiri from different markets in Owerri metropolis

MARKET	*E. coli* (log_{10}CFU/g)	*P. aeruginosa* (log_{10}CFU/g)	*K. pneumonia* (log_{10}CFU/g)	*S. aureus* (log_{10}CFU/g)
Eke Ukwu	4.57±2.18	6.39±2.36	3.51±1.38	3.67±1.09
Relief	4.19±2.44	4.92±2.53	4.53±2.26	3.82±1.32
Nkwo Orji	4.74±2.07	5.65±1.99	4.05±1.25	3.62±1.94

Table 5 shows a comparison of the mean of the log 10 CFU/g of Ogiri from the different markets. From the table, it was shown that there was statistically no significant difference (p=0.858, 0.374, 0.415and 0.950 respectively) in the means.

Table 5 Comparison of mean ± SD bacterial counts of Ogiri from different markets in Owerri, Imo State

	Eke ukwu	Relief	Nkwo Orji	F	P
E.Coli (log_{10}CFU/g)	4.57±2.18	4.19±2.44	4.74±2.07	0.154	0.858
P.aeruginosa (log_{10}CFU/g)	6.39±2.36	4.92±2.53	5.65±1.99	1.020	0.374
K. pneumonia (log_{10}CFU/g)	3.51±1.38	4.53±2.26	4.05±1.25	0.909	0.415
S.aureus (log_{10}CFU/g)	3.67±1.09	3.82±1.32	3.62±1.94	0.051	0.950

4. Discussion

Food borne diseases are endemic in many developing countries and constitute the cause of mortality in these areas [3]. The fact that Ogiri is produced locally through uncontrolled fermentation by people with little or no knowledge and application of healthy practices pose a limitation on the use of the product and risk when consumed. The contamination of food by human pathogens during fermentation process remains a major public health problem worldwide [7]. Ogiri can be contaminated in a number of ways including use of unsterilized native leaves for wrapping, increased number of *Musca domestica* (housefly), in environment where the product is been produced increases the chances of the mechanical transfer of human pathogens into the product by this vectors, unclean utensils (e.g muter) used in the process when pounding it into pulps unhygienic practices such as talking and sneezing into the products. Improper ways of storage of the products at room temperature can also be a factor.

From the study, *Escherichia coli*, *Pseudomonas aeruginosa*, *K. pneumoniae* and *Staphylococcus aureus*were identified as the major micro-organisms associated with the fermentation of Ogiri. This agrees with the report of Gadaga *et al.,* [8] in his study on the major bacterial contaminants involved in fermentation of food products.

Though there was no report by the above named researchers on why the isolated micro-organisms were predominant but this could be as a result of the conditions in which the Ogiri was prepared, the environmental conditions, vectors predominant in the area, storage conditions and other factors.

Achi *et al.*, [9] reported thatthe presence of these microorganisms in fermented foods may be as a result of contamination from food handlers and unhygienic materials used for the packaging as *Staphylococcus*spp. Were normal flora from the food handlers in his research?

The current study showed counts as high as 3.46 log $_{10}$ CFU in *Pseudomonas* from Nkwo Orji. With the lowest count of at 0.38 log $_{10}$ CFU/g of Ogiri from *E. coli* from Relief Market. *Pseudomomas* count per gram of Ogiri from Eke Ukwu market had the highest man at 6.39±2.36 with the lowest count of 3.51±1.38 expressed in *Klebsiellaspp.* From Eke Ukwu.

Comparison of the mean of the log $_{10}$ CFU/g of Ogiri from the different markets showed no significant difference (p=0.858, 0.374, 0.415and 0.950 respectively) in the means.

The reason for no significant observable difference in the mean resides in the fact that all sites were equally exposed to such health hazards as being close to gutters and use of unsterilized laves as a conventional procedure.

5. Conclusion

Fermented foods such as Ogiri form the staple food of developing countries such as Nigeria. Microorganisms are involved in the fermentation process of these fermented foods. Given the fact that microorganisms are involved in the spoilage of these foods and also act as food poison, emphasis should be placed on hygiene and closely controlled practices. The presence of these microorganisms in fermented foods may be as a result of contamination from food handlers and unhygienic materials used for the packaging.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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