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Aspect of Microbiology and Amino Acid Composition of "ogiri" Produced from Seeds of *Telfairia Occidentalis*.

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ABSTRACT

Aspect of microbiology and amino acid composition of "ogiri" produced from seeds of *Telfairia occidentalis* was studied. The samples were bought from open market in Anambra State. Local/traditional producers of "ogiri" were engaged in the production and the resultant paste was used in the analysis. The ogiri samples were analyzed microbiologically using standard microbiological methods which included pour plate, Gram staining, biochemical tests and gene sequencing. Amino acid profile was carried out using standard method for determination of amino acids. The total viable bacterial counts ranged from 2.5±0.1 x 10^6 cfu/g to 4.1±0.1 x 10^6 cfu/g while the total fungi counts ranged from 1.5± 0.01 x 10^6 cfu/g to 3.2±0.001 x 10^6 cfu/g. Among the bacteria isolated are *Pseudomonas plecoglossicida, E. coli, Staphilococcus* spp, *Lactobacillus* spp, *Streptococcus* spp, *Shigella* spp, *Bacillus fusiformis* and *Enterobacter cloacae*, while fungi isolated include *Penicillium* spp, *Muco*r spp, *Aspergillus* spp and *Trichoderma reesei*. The amino acid composition ranged from 1.23±0.01g/100g protein to 13.11±0.01g/100g protein with methionine recording the least concentration and Glutamic acid recording the highest concentration. Personal hygiene can help to address food safety issues through reduction of microbial load.

Key words: Ogiri, *Telfairia* seed, Microbiology, amino acids.

INTRODUCTION

Ogiri is an oily paste produced mainly from oil seeds such as melon and castor oil. Apart from melon seeds (*Citrulus vulgaris*) and castor oil seeds (*Ricinus communis*) which are common substrates for the production of "ogiri" climbing melon seeds (*Cucumeropsis manni*) and fluted pumpkin seeds (*Telfairia occidentalis*) are also used as alternative substrates for the production of "ogiri" [1] [2].

Ogiri constitute major soup condiment in Anambra, Ebonyi, Enugu, Abia and Imo States of Nigeria. The traditional production of "ogiri" from *Telfairia occidentalis* is by the method of uncontrolled fermentation. [3] dehulling of the raw seeds after which they are wrapped in plantain leaves and boiled to soften the seeds for fermentation. Traditional fermentation of food serves several functions which include enhancement of diet through development of flavour, aroma and texture in food substances, preservation and shelf life extension through lactic acid, alcohol acetic acid and alkaline fermentation, enhancement of food quality with protein, amino acids, essential fatty acids and vitamins [4]. The fermentation of the seeds is by species of microorganisms which may be indigenous to the seeds or occur in their production environment, [5].

Unhygienic fermentation and operational environment could result in the production of "ogiri" with variable quality that may be unacceptable and unhealthy to the consumers. Various bacteria and fungi genera have been isolated from "ogiri", [6] and these include *Bacillus* spp, *Seratia* spp, *Pseudomonas* spp, *Klebsiella* spp, *Staphylococcus* spp, *Pediococcus* spp and *Leuconostic* spp.

[7] also isolated *Streptococcus* spp, *Bacillus* spp, *Pediococcus* spp, *Micrococcus*

spp and *Lactobacillus* spp from fermenting *Citrullus vulgaris* seeds for "ogiri" production.

Microorganism in "ogiri" are not artificially included but found their way into "ogiri" through a variety of sources such as air, water used in mixing, leaves used in wrapping, the handlers and equipment and utensils, [8].

Amino acids the building block of protein are very important in the body because of the numerous functions they perform. They are needed in the body for vital processes such as building of proteins and synthesis of hormones and neurotransmitters. [9] emphasized on the consumption of local and home based complementary foods rich in protein to ensure adequate nutrition and energy intake to prevent protein-energy malnutrition.

The aim of this study is to isolate and characterize microorganisms from "ogiri" produced from *Telfairia occidentalis* seeds and also to determine the amino acid contents.

MATERIALS AND METHODS

Seeds of *Telfairia occidentalis* were brought from open markets in Anambra State of Nigeria. Ten different producers were engaged in the production of "ogiri" from seeds of *Telfairia occidentalis*.

The traditional method of processing "ogiri" as described by Nzelu 2010 was adopted as shown in Figure 1. Wrap of each sample was boiled for four hours.

The boiled seeds were drained and left to ferment at room temperature. One wrap of each sample wash mashed in a mortar and the resultant paste, "ogiri" was finally wrapped in blanched plantain leaves (*Musa sapientum*)

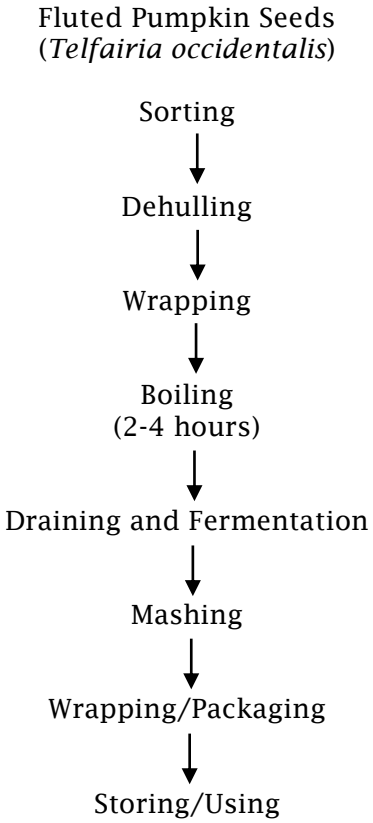

Fig. 1: Process Flow Chart for Traditional Production of "ogiri"

Microbial Analysis of Ogiri Samples

This was carried out by pour plate method as described by [9]. One gram of each sample was weighed using electronic weighing balance (0106-1) and dissolved in 9ml of peptone water and diluted using a ten-fold serial dilution. Zero point one millilitre of each sample suspension was inoculated on nutrient agar. MacConkey agar, Salmonella shigella agar and sabouraud dextrose agar and incubated at room temperature (Maximum of 35°C) for 24-72 hours. The mean of the replicate plating was calculated and the total viable count (TV) obtained using the formula TV = N/V x D where N = mean colony, V = volume plated, D = Dilution. The result was expressed in colony forming unit per gram (cfu/g).

The pure bacteria cultures were characterized by their cultural, morphological and biochemical tests which included Gram staining, motility test, catalase test, Coagulase test, citrate test, oxidase test, indole test, methyl-red test, Voges-Proskauer test, Nitrogen reduction test, and sugar fermentation test as described by [10]. Fungi isolates were also identified by slide culture method. The isolates were also identified molecularly using gene sequencing by Centre for Agriculture and Bioscience International (CABI).

Determination of Amino Profile

This was done by the method of Benitez (1989). Each sample was dried to a constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequencing Multisystem Amino Acid Analyzer (TSM).

Defatting of samples was done using the method described by (AOAC, 2006).

RESULTS AND DISCUSSION

The high microbial count may be attributable to poor hygienic practices and poor sanitary quality of processing utensils, water used in mixing and packaging materials. A similar observation was made by [11] [12]. Bacteria identified include *Pseudomonas, Staphylococcus, Lactobacillus, Streptococcus, Proteus, Escherichia, Klebsiella, Salmonella, Shigella, Bacillus*, *Eneterobacter*, *Klebsiella Pneumoniae, Leclerecia adecaboxylata, Flavobacterium, Micrococcus* and *Virbio* while the fingi genera isolated include *Penicillium*, *Mucor* and *Aspergillus*.

The population of pathogenic organisms may be as a result of intrinsic and extrinsic factors of "ogiri" samples such as availability of nutrient, pH, water activity (aw) and temperature. This observation agrees with that of [13]. The isolation of coagulase positive *Staphylococcus* aureus from "ogiri" samples is of a public health concern as the organisms is known to cause food poison [7].

The presence of *E. coli* an indicator organism reveals faecal contamination.

Glutamic acid and aspartic acid are the most concentrated amino acids (13.11±0.10 and 7.92±0.01 g/100g protein and this observation is in agreement with the earlier report trend of glutamic and aspartic acid being the most concentrated amino acids [3]. Methionnie was the least concentrated in all the sample (1.23±0.01 g/100g protein) and the low concentration of methionnie is in consonance with the earlier report of [6]

Table 1: Total Viable Count

	Total Viable Count (cfu/g)	
Sample	***Bacteria (x10^6)***	***Fungi (x10^6)***
A	3.7±0.2	2.2±0.001
B	2.8±0.2	3.2±0.001
C	4.0±0.1	1.5±0.01
D	3.5±0.1	2.0±0.1
E	3.4±0.2	2.5±0.01
F	2.9±0.2	2.3±0.01
G	2.5±0.1	2.1±0.001
H	3.2±0.1	2.7±0.001
I	2.7±0.2	2.9±0.01
J	4.1±0.1	3.0±0.1

Table 2: Characteristics of Bacterial Isolates

Isolates	Cultural Morphology	Microscopic Morphology	Gram Reaction	Catalase	Citrate	Coagulase	Oxidase	Methyl Red	Nitrate	Indole	Voges Proskauer	Urease	H_2S	Motilitty	Lactose	Maltose	Glucose	Sucrose	Xylose	Manitol	Sorbitol	Probable organism
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1	On nutrient and MacConkey agar, colonies are large, low convex, rough and oval in shape. Some are irregularly round about 2-3mm in diameter and emit fruity odour and also pigmented (green-yellow, blue-green	Straight and slightly curved rods	-	+	-	-	+	-	+	-	-	+	+	+	-	-	A	-	-	-	-	*Pseudomonas Plecoglossicida*
2	Colonies are yellowish, moist and have smooth glistering surface on nutrient agar, appears pinkish on MacConkey agar and about 1-2mm in size.	Cocci in grape-like cluster with some singe and paired	+	+	-	+	-	+	+	-	+	+	-	-	A	+	A	A	+ A	A	A	*Staphylococcus* spp
3	Colonies are round, entire, low convex, smooth, transluscent, colourless and about 2-3mm in diameter on MacConkey agar	Slender irregular rods	+	-	-	-	-	-	-	-	-	-	-	-	+ A	+ A	+ A	-	A	A	A	*Lactobacillus* spp
4	Low convex discrete colonies about 0.5-	Spherical cocci in short																				

	1.0mm in diameter	chains	+	-	-	-	+	-	-	-	-	+	-	-	+	+ A	+ A	+ A	A	-	-	*Streptococcus* spp
5	Very large swarming (spreading) growth (surface), emit putrefactive fishy odour and creamy in colour about 3-5mm in diameter	Coccobacilli in short chain and some are in pairs	-	+	-	-	-	+	+	+	-	+	+	+	-	-	A	A	A	-	-	*Proteus* spp
6	Colourless to greyish smooth colonies on nutrient agar, rose pink, large colonies of MacConkey agar about 2-3mm in diameter	Rod shaped	-	+	-	-	-	+	+	+	-	-	+	+	+ A	A	+ A	+ -	A	+ A	-	*Escherichia coli*
7	On MacConkey agar, colonies appear large, mucoid and red, colourless to grey on nutrient agar.	Rod shaped	-	+	+	-	-	-	+	-	+	+	-	-	+ A	A	+ A	+ A	A	+ A	A	*Klebsiella Pneumoniae*
8	Colonies are greyish to white circular, moist, convex and transluscent in nutrient agar Pale yellow on MacConkey agar, colourless with black centre on SSA, about 2-3mm in diameter	Rod shaped	-	+	+	-	-	+	+	-	-	-	+	+	+ A	+ A	+ A	A	+ A	+ A	A	*Salmonella* spp
9	Smooth greyish colour, transluscent colonies on nutrient agar, colourles on MacConkey agar, colourless without	Short rods in pairs	-	+	-	-	-	+	+	-	-	-	-	-	+ A	+ A	+ A	+ A	+ A	+ A	+ A	*Shigella* spp

	blackening on SSA																					
10	Large, greenish, raised, wide-spreading with irregular fingerlike edges and opaque about 2-3mm in diameter	Long straight rods in single, some in pairs	+	+	+	-	+	-	+ -	-	+	-	-	+	-	+ A	+ A	+ A	A	A	A	*Bacilus Fusiforms*
11	Small, smooth, yellow and transluscent colonies about 1-2mm in diameter	Short slender rods	-	+	+	-	+	-	-	-	-	-	-	-	+ A	+ A	+ A	+ A	+ A	+ A	+ A	*Flavobacterium* spp
12	Smooth yellow colonies, shinny, raised and some with elevated centres, about 1-2mm diameter	Small irregular rods in pairs and tetrads	+	+	-	-	-	-	-	-	+	+	-	+	-	A	-	+ -	A	-	-	*Micrococcus* spp
13	White to yellow colonies that are star-shaped with irregular edges	Fetted mass of branding filament which are interlaced.	+	-	-	-	+	+	+	-	-	+ -	-	A	A	A	A	A	A	A	A	*Actinomyces* spp
14	Round , greenish to bluish colonies about 2-3mm in diameter	Curved and straight	-	+	+	-	+	+	+	+	+	+	-	+	-	A	A	-	A	A	A	*Vibrio*
15	Very small flat colonies, white to colourless colonies on nutrient agar, about 1mm in diameter.	Cocci in tetrad or in short chain	+	-	-	-	-	+	+	-	-	-	+	-	A	+ A	A	A	A	A	A	*Pediococcus* spp
16	Greyish smooth colonies	Gram negative Rods	-	+	-	-	-	+	+	+	-	+	+	+	-	-	A G	A G	-	-	A G	*Leclercia Adecarboxylata*
17	Large mucoid	Rod shaped	-	+	+	-	-	-	+	+	-	+	+	-	-	+	A	A	A	A	A	*Enterobacter*

colonies on red Mackonkey agar	G	G	G	G	*Cloacae*

Table 3: Characteristics of Fungi Isolates

Isolate	Colony Morphology	Microscopic Morphology	Probable Identity
1	Greenish white, Flat, irregular shaped, dry and dull	Septate, hyphae, conidia arranged like mob-heead	*Penicillium* spp
2	White filamentous colonies	Non-septate hyphae, spores enclosed in a sporangium	*Mucor* spp
3	Granular to wooly colonies that have some shade of yellow or yellow-brown	Long conidiophores	*Aspergillus* spp
4	1-2 concentric rings with green conidal production which is denser in the centre with irregular yellow zones	Conidia shape glubose to subglobose	*Trichoderma reesei*

CONCLUSION AND RECOMMENDATION

Microorganisms associated with fermentation of "ogiri" from the seeds of *Telfairia occicentalis* has been established. The amino acid profile of fermented "ogiri' from the seeds of *Telfairia occidentalis* has also been elucidated from the study. It is therefore recommended that the local producers of "ogiri" should be enlightened on the most hygienic way of production of "ogiri" so that the product will be safe to the consumers.

Table 4: Amino Acid Profile of "Ogiri" produced from *Telfairia* seeds

Amino Acid	*Concentration of Amino Acid (g/100g) Protein*
Lysine	3.10±0.01
Histidine	2.20±0.01
Arginnie	7.92±0.01
Aspartic acid	6.85±0.01
Threonine	2.30±0.02
Serine	3.16±0.01
Glutamic acid	13.11±0.10
Proline	2.25±0.02
Glycine	2.30±0.10
Alanine	4.02±0.01
Cystine	1.34±0.02
Valine	4.06±0.01
Methronine	1.23±0.01
Isoleucine	3.01±0.01
Leucine	5.48±0.01
Tyrosine	2.55±0.01
Phenylalanine	3.78±0.01
Tryptophan	6.50±0.01

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