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HAZARD ANALYSIS CRITICAL CONTROL POINTS ON "OGIRI" PRODUCED FROM DIFFERENT SUBSTRATES

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ABSTRACT

The study was on the identification of hazards in the production of "ogiri" from creeping melon (*Citrullus vulgaris*), climbing melon (*Cucumeropsis manii*), castor oil (*Ricinus communis*) and fluted pumpkin (Telfairia occidentalis) seeds and establishment of Critical Control Points in the course of production. The processed and unprocessed samples as well as utensils, packaging materials and water used in the production of "ogiri" were analyzed using standard methods. Hazard analysis was carried out at each stage of production. Samples were collected from different States in Nigeria. Microbial isolates were obtained from different sources (raw seeds, processed "ogiri", handlers, utensils, packaging materials as well as each stage of production. The bacterial and fungal isolates were identified by DNA sequencing 165rDNA and ITSrDNA. The Critical Control Points were determined using decision tree and the results analyzed statistically using Analysis of Variance (ANOVA). The mean viable microbial counts of raw and processed samples significantly ($P<0.05$) increased from 8.3×10^4 to 3.7×10^8 cfu/g, 9.5×10^4 to 5.2×10^8 cfu/g, 9.8×10^4 to 4.7×10^8 cfu/g and 7.6×10^4 to 4.5×10^8 cfu/g in climbing melon, castor oil, creeping melon and fluted pumpkin respectively. The coliform counts from different sources were higher than the acceptable limits 10^4 cfu/g and 10^2 cfu/g for heterotrophic bacteria and coliform respectively. The heavy metal contents were higher in the raw than in the processed samples. The hazard analysis revealed sorting, washing,

fermentation, mixing, boiling and packaging as Critical Control Points. Awareness campaign on production and personal hygiene can help to address food safety problems. Boiling of "ogiri" before use in preparing cold ready-to-eat foods such as "abacha" is recommended.

Keywords: Ogiri, creeping melon seeds, climbing melon seeds, castor oil seeds, fluted pumpkin seeds, hazards, heavy metals, heterotrophic bacteria, coliforms, critical control points

INTRODUCTION

Ogiri is a paste-like substrate produced mainly from oil seeds. The alarming increase in the demand and prices of various substrates for "ogiri" resulted in the use of *Cucumeropsis*, an uncommon substrate which is relatively cheaper for "ogiri" production **[1, 2]**. Apart from melon seeds (*Citrullus vulgaris*) which is a regular substrate for "ogiri" production, castor oil seeds (*Ricinus communis*), fluted pumpkin (*Telfairia occidentalis*) seeds are also used as alternative substrates for "ogiri" production **[3]**.

"Ogiri" constitute a major soup condiment in Anambra, Enugu, Abia and Imo States of Nigeria. The production is based on uncontrolled fermentation which involves dehulling of the raw seeds, boiling to soften the seeds and fermentation for three to four days prior to drying and mashing to a smooth paste "ogiri" **[3, 4]**.

Fermentation of condiments play a vital role in the diet of many Africans. Fermentation of food condiments serves several functions which include enhancement of diet through development of flavor, aroma and flavor in food substrates, preservation and shelf life extension, enhancement of food quality with protein, amino acids and vitamins, improvement of digestibility and vitality and detoxification of anti-nutrients **[4]**.

The traditional methods of preparing food condiments are generally very labourious, time and energy consuming and are usually carried out with rudimentary utensils **[5]**. The use of leaves to wrap "ogiri" predisposes the product to cross-contamination by undesirable microorganisms and results in short shelf life **[6]**. Fermentation remains of interest since they do not require refrigeration during distribution and storage **[5]**. Dehulling of seeds is usually done manually by local producers and this introduces a myriad of organisms into the seed prior to fermentation some of which may be pathogenic and this coupled with unhygienic fermentation and operation environment could result in the production of "ogiri" with variable quality and unacceptable aroma, short shelf life and the one that pose health hazard to the consumers **[4]**. Various bacteria and fungi genera which include *Aspergillus, Muccor, Bacillus, Staphylococcus, Pseudomonas,*

Streptococcus, Klebsiella, Escheuchia and *Pediococcus* have been isolated from "ogiri" **[6]**. These organisms are not artificially inoculated but found their way into "ogiri" through variety of sources which may include air, water used in mixing, leaves used in wrapping, the handlers as well as utensils and equipment used in the processing **[4]**.

Hazard Analysis Critical Control Point (HACCP) is a systematic approach to ensure food safety by U.S. National Advisory Committee on Microbiological Criteria for Foods **[7]**. It is a worldwide recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention rather than through end products inspection and testing **[8]**. Critical Control Points (CCPs) are located at any step of production process where these hazards can either be prevented, eliminated or reduced to acceptable levels.

Public Health and Food Authorities worldwide have promoted the concept of HACCP for providing safe and healthy foods **[9]**.

Considering the production of "ogiri" and its unhygienic nature as carried out by the traditional producers or village entrepreneurs, there is every need to employ a management system in which food is addressed through the analysis and control of biological, chemical and physical hazards right from the raw materials production, procurement and handling to manufacturing/production process, distribution and consumption of the finished product. The aim of the study is to establish the hazards and critical control points of "ogiri" produced from castor oil seeds (*Ricinus communis*), melon seed (*Citrullus vulgaris*), fluted pumpkin seed (*Telfairia occidentalis*) and climbing melon (*Cucumeropsis*).

MATERIALS AND METHODS

The samples, climbing melon (*Cucumeropsis*), creeping melon (*Citrullus vulgaris*), castor oil seeds (*Ricinus communis*) and fluted pumpkin seeds (*Telfairia occidentalis*) were bought from open markets from different states in Nigeria.

Plate 1: Various substrates for "ogiri" production
Key: 1 = Climbing melon seeds
2 & 3 = Creeping melon seeds
4 & 5 = Castor oil seeds
6 = Fluted pumpkin fruits
7 = Fluted pumpkin seeds

Production of "Ogiri"

The traditional method of "Ogiri" production as described by **[10, 11]** was modified to become appropriate in this study as shown in **Figure 1**.

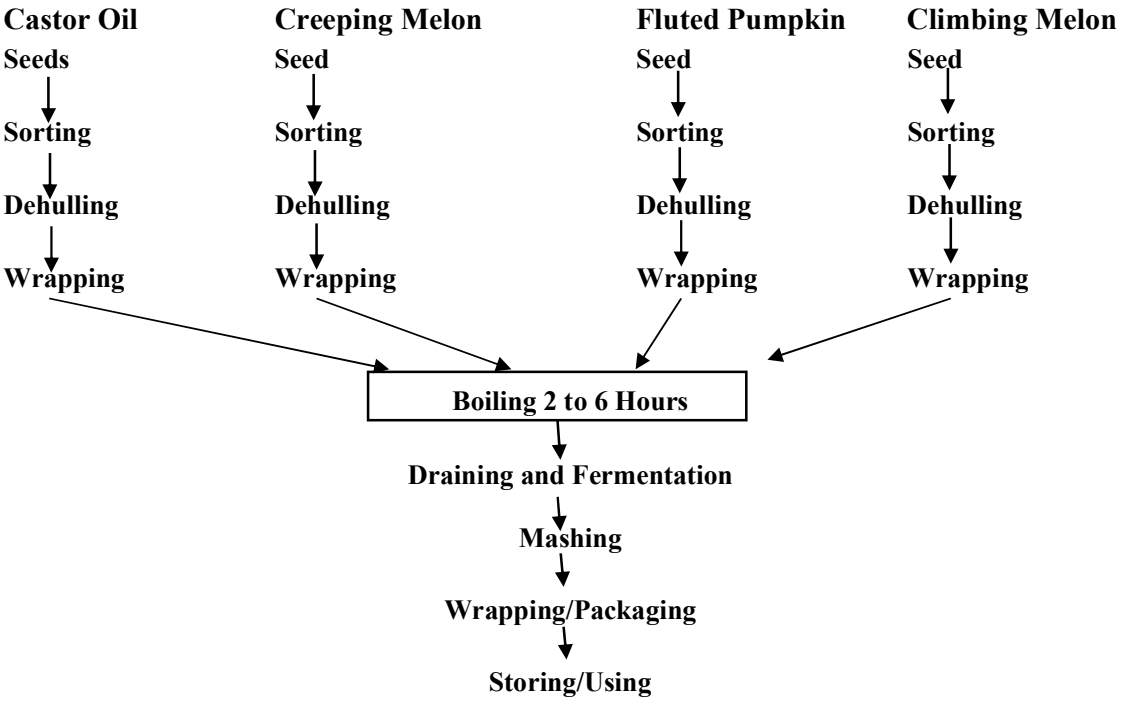

Figure 1: Process Flow Chart for the Laboratory Production of "Ogiri"

Total Viable Count

This was carried out using pour plate method as described by **[3]**.

One gram (1g) of each sample was weighed out 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 taken from dilutions 10^{-4}, 10^{-5} and 10^{-6} and incubated on different media (Nutrient Agar, Sabauraud Dextrose Agar, MacConkey Agar, Salmonella Shigella Agar and Potato Dextose Agar) and incubated at 35ºC for 24 to 72 hours. The mean of the replicate plating was calculated using the formula: TVC = N/V x D where TVC = Total Variable Count, N = Mean Colony, V = Volume plated and D = Dilution and expressed in colony forming unit per gram (cfu/g). The isolations were made at raw material collection, before sorting during fermentation, after mashing and storing/using. The organisms were sub-cultured and characterized by the methods of Association of Official Analytical Chemists (AOAC), 2006 using colony morphology, microscopic morphology, biochemical tests – catalase, motility, Hydrogen Sulphate test, coagulase, idole, methyl red, citrate utilization, Vogas Proskauer, Urease, Oxidase nitrate

reduction, and sugar fermentation tests. Fungi isolates were characterized by bactophenol cotton blue, wet mount and slide culture. All the isolates were further purified prior to molecular identification.

The molecular identification of the isolates was carried out by the method of Center for Agricultural and Bio Science International (CABI). All procedures were validated and processing undertaken in accordance with CABI's in-house methods as documented in TPs 61-68 and TP 70 for bacteria and TPs 72-80 for filamentous fungi.

Determination of Heavy Metals

This was determined using smart spectrophotometer (Lamotte) and different kits for heavy metals. The samples were extracted using soxhlet apparatus (GT 301). The samples were scanned by selecting the menu, scan sample and the results recorded in part per million (Ppm)

Determination of Critical Control Points

This was done using the method described by **[10]**.

Does the step involve a sufficient harzard likelihood of occurrence and severity to warrant its control?

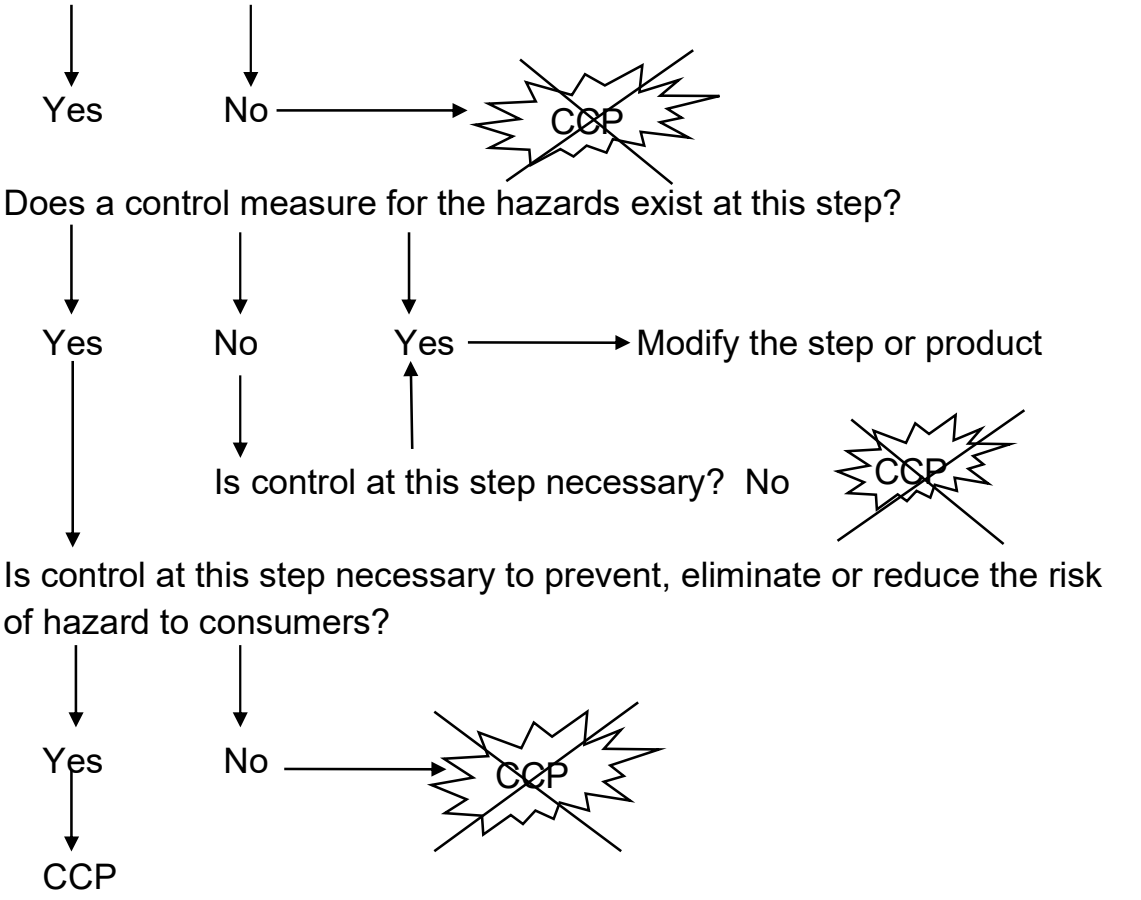

Figure 2: Critical Control Point Decision Tree
Source: **(Rabi *et al.*, 2013)**

Statistical Analysis

The obtained was subjected to Analysis of Variance (ANOVA) using computer, SPSS (2015) version 20. Differences in means were evaluated at 5% (0.05) level of significance and direction of interaction between treatment using Turkey HSD POST HOC Test.

RESULTS

Table 1: Mean Viable Counts of the Utensils, Packaging Materials, Water and Handlers involved in "ogiri" Production

Sample	Mean Viable Count (cfu/ml)			
	NA	MA	SDA	SSA
Leaves for wrapping	2.5×10^6	2.5×10^6	2.0×10^4	4.4×10^5
Water for mixing	1.2×10^6	2.1×10^6	1.0×10^4	2.0×10^4
Mortar and Pestle	4.5×10^6	2.4×10^6	2.0×10^4	6.5×10^6
Handlers (Nasal Swab)	5.0×10^3	2.8×10^3	4.0×10^2	NG
Handlers (Skin Swab)	3.4×10^3	3.3×10^3	2.0×10^2	Ng
String	3.8×10^3	1.2×10^4	2.0×10^2	1.0×10^4

Key: SSA = *Salmonella-Shigella* Agar; NG = No Growth; NA = Nutrient Agar; MA = MacConkey Agar; SDA = Sabouraud Dextrose Agar

Table 2: The mean viable counts of processed and unprocessed samples

Samples	Mean Heterotrophic counts (cfu/g)	
	Unprocessed (Seeds)	Processed (Ogiri)
Creeping melon	8.3×10^4	3.7×10^4
Castor oil	9.5×10^4	5.2×10^4
Fluted pumpkin	9.8×10^4	4.7×10^4
Climbing melon	7.6×10^4	4.5×10^4

Table 3: Bacterial and Fungi counts (cfu/g/ml) of samples from the production stages and sources

Production Stage	Heterotrophic Bacterial Count ($x10^6$)	Coliform count ($x10^6$)	*Salmonella-Shigella* Count ($x10^6$)	Fungal Counts ($x10^4$)
Raw seed	5.0	4.2	3.5	4.2
Boiled seed	4.8	3.4	3.0	3.7
Fermented seed	4.9	4.3	3.4	4.3
Nasal swab	5.5	3.4	NG	5.2
Skin swab	3.5	3.2	NG	3.0
Mortar	4.5	2.4	6.5	1.4
Pestle	4.4	2.2	5.8	1.5
Water	1.2	2.3	2.1	2.0
Leaves	2.5	2.5	4.4	1.2
Strings	3.8	1.2	1.0	2.0

FAO (1979) Maximum acceptable limit is 1.00×10^5 cfu/g/ml

Table 4: Bacterial and Fungi Isolates at various stages

Production Stage	Bacteria Isolated	Fungi Isolated
Raw seeds	*E. coli, Staph. aureus*, *Bacillus fusiformis*, *Pseudomonas* spp and *Proteus* spp	*Aspergillus fumigatus*, *Penicillium* spp, *Mucor* spp, *Trichoderma reesei*, and *Aspergillus terreus*
Boiled seeds	*Staphylococcus aureus* and *Streptococcus* spp	*Mucor* spp
Fermented seeds	*E. coli, Staph. aureus*, *Flavobacterium* spp, *Micrococcus* spp, *Bacillusfusiformis, Shigella* spp, and *Lactobacillus*	*Mucor* spp
Nasal swab	*Staph. aureus, Streptococcus* spp, *Bacillus* spp and *Pseudomonas* spp	*Penicillium* spp, *Aspergillus terreus*
Skin swab	*Staph. aureus* and *E. coli*	*Mucor* spp
Mortar	*Salmonella* spp, *Staphaureus*,	*Mucor* spp, *Penicillium* spp, *Aspergillus fumigatus*
Pestle	*Salmonella* spp, *Shigella* spp, *Staph. Aureus*	*Mucor* spp, *Penicillium* spp
Water	*Pseudomonas, E. coli*, *Proteus spp*, *Enterobactercloacae, Leclercia adecarboxylata, Vibrio*	*Aspergillus* spp, *Penicillium* spp
Leaves	*Actinomyces* spp, *Klebsiella pneumoniae, E. coli*	*Trichoderma ressei*, *Mucor* spp, *Aspergillus fumigatus*

Table 5: Mean Heavy Metal Contents (ppm) of the samples at different stages of "Ogiri" Production

Production Stage	*Copper*	*Lead*	*Cadmium*
Raw material	0.48	0.82	0.53
Sorted seeds	0.40	0.75	0.45
Boiled seeds	0.33	0.35	0.30
Fermented seeds	0.33	0.35	0.30

NAFDAC acceptable limits for heavy metals in food contaminations: Copper = 5.0ppm, Lead = 0.2ppm, Cadmium = 0.5ppm

Table 6: Mean Heterotrophic Bacterial Count of "Ogiri" Produced in the Laboratory and that Produced by Local Producers

Samples	Mean Heterotrophic Counts (cfu/g)	
	Locally Processed ($x10^8$)	Laboratory Processed ($x10^6$)
Creeping melon	4.8±0.1	1.1±0.2
Castor oil	5.6±0.3	0.5±0.2
Fluted pumpkin	3.7±0.2	1.8±0.1
Climbing melon	2.7±0.7	1.0±0.3

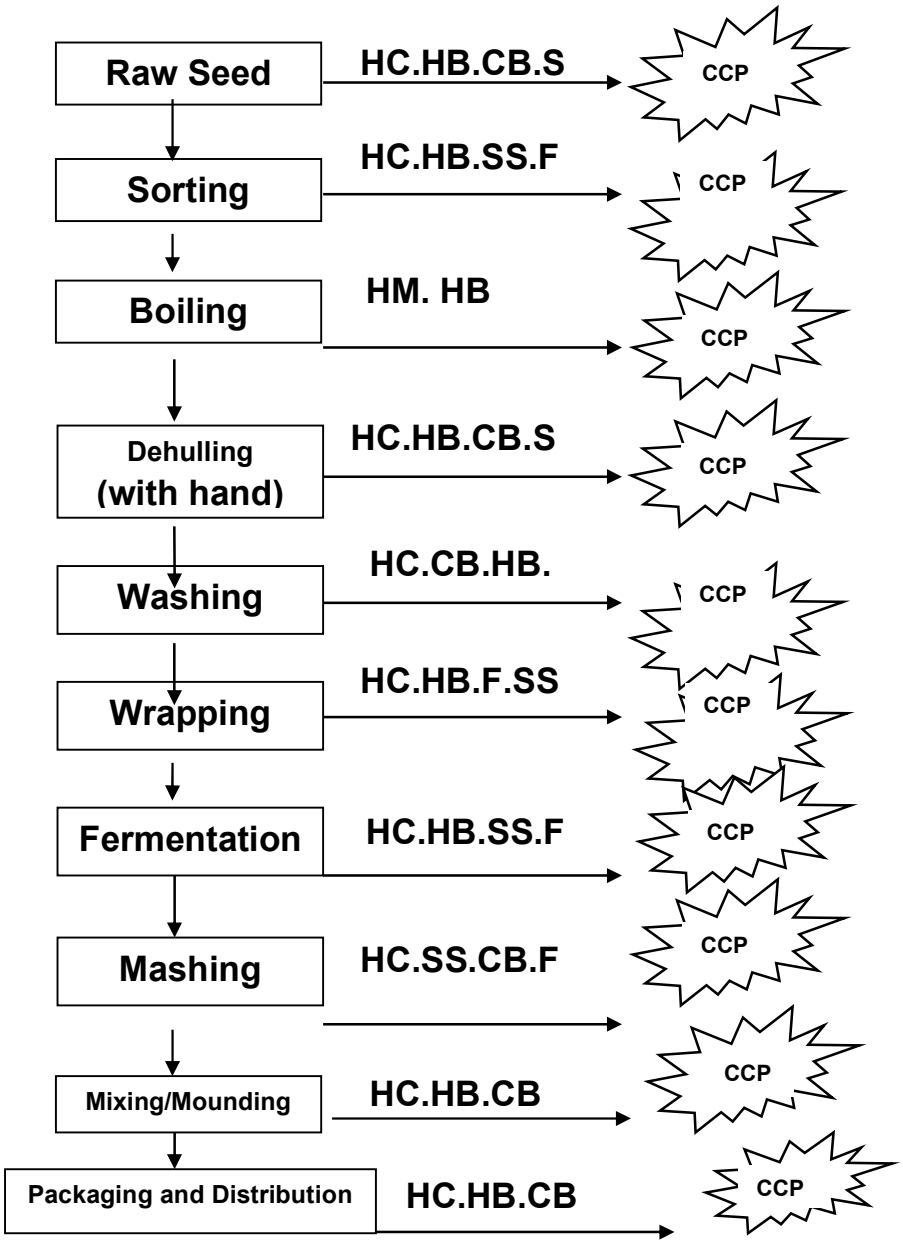

Figure 2: Critical Control Points from the Production of "Ogiri" from creeping melon, fluted pumpkin, castor oil and climbing melon seeds

Key: **CCP = Critical Control Point, HC = High Count, HB = Heterotrophic Bacteria, CB = Coliform bacteria, SS = Salmonella Shigella, F = Fungi, HM = Heavy Metal**

DISCUSSION, CONCLUSION AND RECOMMENDATION

The higher bacterial count in the "ogiri" obtained from the local producers is higher than that obtained in the laboratory produced "ogiri". The high bacteria count in the locally produced "ogiri" may be attributable to poor hygienic practices and poor sanitary quality of processing utensils, water and packaging materials. A similar observation was made by **[3, 4]**.

A great number of bacteria and fungi genera isolated were identified as *Pseudomonas plecoglossida*, *Bacillus fusiformis*, *Klebsilla pneumonia*, *Enterobacter cloacae, Lactobacillus* spp*, Lecleracia adecarboxylata*, *Vibrio, Flavobacterium, Escheuchia coli, Micrococcus* spp, *Actinomycess* spp, *Aspergillus fumigatus*, *Trichoderma reesei*, *Aspergillus terreus*, *Penicillin* spp and *Mucor* spp.

The leaves used in wrapping, the mortar and pestle used in mashing the seeds constitute the major source of bacteria particularly *Actinomyces* spp, and *Salmonella* spp.

The population of pathogenic organisms may be attributable to intrinsic factors of "ogiri" such as availability of nutrients, pH water activity (aw), lack of competing organisms and extrinsic factors such as storage temperature. This observation agrees with the findings of **[5]**.

The isolation of coagulase positive *Staphylococcus aureus* from the fermenting seeds is of public concern as the organism is known to cause food poisoning **[12]**. The presence of *Klebsiella, Coliform* could constitute a health risk since some species of this genius are associated with the diseases of man. This observation was also made by **[4]** who also expressed the expectation that heat treatment subjected to "ogiri" and "ogiri okpei" respectively during cooking will destroy these organisms and possibly any toxin in the condiment. Therefore there is the risk of using "ogiri" in the preparation of cold ready-to-eat foods such as "abacha".

The presence of *Escherichia coli*, an indicator organism was observed to come from the water used in the reconstitution of "ogiri".

Actinomycess, a filamentous anaerobe to micro aerophilic, Gram positive, non-spore forming rods us typically in the soil, decaying organic matter and this was observed to come from leaves used in wrapping "ogiri" and these leaves are usually collected from the bush and dirty environment.

Salmonella spp isolated were typically from the mortar and pestle used in mashing and it was also observed that the

local producers of "ogiri" used these utensils several times without washing and the cracks in these utensils serve as hiding place for these organisms and probably other contaminants.

The high level of lead may be attributable to the source of raw materials because most of the substrates used in producing "ogiri" are produced in the affected areas and are distributed all over the country. Lead and copper are used for making pesticides used in crop preservation and pest control. These elements penetrate into the food stuff which when consumed can cause cancer.

The presence of Lead and Cadmium poses a public health hazard to the consumers. Cadmium exposure has been reported to cause kidney stone and kidney damage. The possible source could be from soil contaminated with these elements, poor mining and hygienic practices **[5]**. The concentrations of Lead in unprocessed samples are higher than that in the processed samples with that of *Cucumeropsis* (2.80ppm) above the NAFDAC Limit (0.2ppm).

Heat has little or no effect on mineral content of food hence, they are said to be heat stable. The slight decrease in the mineral content may be as a result of leaching out of these minerals during cooking of food in boiling water. Boiling has been reported to decrease iron and copper contents **[12, 13]**. The significant decrease in the copper content from unprocessed (<0.05) samples is in line with the finding of **[14, 15, 16]**.

The hazard analysis revealed sorting, washing, fermentation, mixing, boiling, packaging and raw materials as Critical Control Points. Apart from these controls specified as Critical Control Points for "ogiri", the quality of the water used in reconstitution, the cleanliness and sterilization procedures as well as personal traffic hygiene were also analyzed within the HACCP system to improve the effectiveness of system.

Since women who are largely involved in the production of "ogiri" are ignorant of good house-keeping, good hygienic practices and good manufacturing practices, which are prerequisite operations needed for a successful implementation of HACCP, it becomes a challenge. It is therefore recommended that the regulatory agencies relating to food safety such as NAFDAC, SON, NCC among others should join hands to enlighten the local producers and ensure compliance.

Finally, people should boil "ogiri" before using it to prepare ready-to-eat foods such as "abacha".

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