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Microbiological and Sensory Quality of Saponified Dishes (*Otong*) from Ash Solutions of Oil Palm (*E. guineensis*) Bunches and Unripe Plantain (*M. paradisica*) Peels

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Abstract: This study investigated the microbiological quality and sensory properties of saponified dishes (*otong*) prepared with unripe plantain peel and empty palm bunch ash solutions. Dried unripe plantain peels and palm bunches were incinerated using open pan (charring) and muffle furnace combustion methods. Commercially acquired potash was used as a control. The usage of these peels and palm bunches, as an alternative to limestone was to reduce the heavy dependence on this synthetic bicarbonate and limestone. Instant emulsion (*otong*) was prepared from the resulting alkaline solutions from the waste resources with palm oil. Commercial alkaline solution (potash) served as control. The formulated instant emulsions (*otong*) were analyzed for microbiological and sensory properties. The microbial populations of all the samples ranged from 1.0×10^3-3.0×10^5cfu/mg for bacteria and 1.3×10^3-8.6×10^5cfu/mg for fungi. Total of fourteen microorganisms comprising of four bacterial and eight fungal species were isolated from the raw oil and the formulated emulsions. Semi-trained panelists made up of 20 persons scored the emulsions (*otong*) when used for the preparation of goat head delicacy (*Isiewu*) on a nine-point hedonic scale for appearance, taste, flavor, smoothness and general acceptability. Results indicated significant acceptance ($P<0.05$) for *otong* prepared from the laboratory ash extracts while the one made with limestone extract (potash) was scored least accepted by the panelists ($P<0.05$).

Keywords: Saponification, Formulation, Potash, Palm Bunches, Plantain Peel, Emulsion

1. Introduction

Saponified dish otherwise known as *'Otong'* is a local delicacy in Akwa Ibom State prepared locally by mixing the filtrate of ash and water mixture (potash) with palm oil. It is characterized by a brown coloration and forms emulsion when palm oil is added [1]. The slippery nature of the "otong" give an alkali impression pointing to the saponification of the fatty acids in the palm oil [1, 2] stated that as a result of its alkaline nature arising from the high level of potash (KOH), the ashes from both palm bunch and plantain peel when leached with water, the filtrate reacts with oil to produce a yellow alkaline mixture known in *Efik* local language as '*otong*'.

'*Otong*' is a special type of soup among the many delicacies found in Southern Nigeria. In Akwa Ibom State, "*Otong*" has been locally commercialized and is widely consumed by the Annang and Ibibio tribe. The key to making a good "*otong*" is to ensure that all the ingredients such as onions, nutmeg, salt, pepper, maggi, 'utazi' leave (*Gongronema latifolium*), 'oziza' leave (*Piper guineense*) etc are well incorporated. The ingredient added is dependent on one's choice, purchasing power and availability. The dish when prepared can be used as sauce for goat meat head (*Ise-ewu), Nkwobi, Kpomo* (Cow skin), *Ekporoko* (Stock fish), Dried bonga fish, *Ncha-Abacha* (shredded cassava), Tapioca and *Ugba* (*Pentaclethra macrophylla*) etc.

In the Eastern parts of Nigeria, instant emulsion called "*Ncha*" is basically used as an African Salad (*Abacha*) dressing water-in-oil emulsion [3]. The authors further

pointed out that, this emulsion can also be used in the preparation and consumption of bitter yam and processed oil bean seed (*Ugba*), which is served in most traditional ceremonies. The making of "*otong*" from ash-derived alkali has been an age-long practice in Akwa Ibom State, but it lacks scientific information on the production, sensory properties and microbiological safety.

Microorganisms are known to cause chemical characteristics that lead to deterioration in quality of vegetable oils derived from the seeds or fruits pulps of plants. The keeping quality of the oils is basically dependent on their chemical compositions, for instance, the percentages of the degree of unsaturation [4]. Some slight deterioration at least is expected in any commercial oil-bearing material and is, in fact, inherent in the process by which fat is formed. In the living plants and animals, fats, carbohydrates and proteins are synthesized in a complicated series of steps with the aid of certain enzymes. These enzymes are capable of assisting the reverse as well as the forward reactions and hence under proper conditions may promote the degradation of the very substances that, they have previously been instrumental in synthesizing [5]. Oils in general are known to be susceptible to microbial attack. The composition of the various oils determines the extent and type of organisms likely to thrive in them [6]. Palm oil is known to support the growth of fungi and bacteria especially when it contains moisture. Therefore, knowledge of the microbiological quality of these instant saponified dishes is an important factor in appreciating the safety problems related to palm oil and its products.

Previous research efforts had indicated the viability of vegetable matter especially the agricultural biogenic waste for alkali production [7-9]. Presently the Federal Government policy on sourcing for local raw materials which are non-toxic and potentially suitable for alkali generation has given rise to an increased interest in research effort geared towards exploiting locally available vegetable materials [10]. The first time "*otong*" was mentioned in scientific literature was in [1, 11]. There is paucity of information on the production, sensory properties, and microbiological safety of *otong* delicacy in scientific literatures.

Thus, this study is aimed at assessing the microbiological and sensory properties of the different instant saponified dishes (*otong*) prepared from the ash solutions of biogenic wastes (plantain peel and palm bunches) along side with limestone (sodium sesquicarbonate). It is hoped that the results of this findings will cause a distinct shift from the heavy dependence on synthetic and unhealthy chemicals (biocarbonates) towards the use of agricultural biogenic wastes for obvious advantages of good potash yield and quality.

2. Materials and Methods

2.1. Collection of Raw Materials

Unripe plantain peels were collected from Food Affairs Limited, Uyo and Oil palm fruit bunches from oil palm mill at *Ikot Ayan* Itam, Itu Local Government Area. Crude palm oil was obtained from Agro-Ideas, *Ibesikpo* Local Government Area and Limestone (Sodium sesquicarbornate) was purchased at *Etaha Itam* Market, Itu Local Government Area all in Akwa Ibom State and transported to the Department of Food and Science and Technology Processing Laboratory for onward processing and analysis.

2.2. Extraction of Ash from Empty Oil Palm Fruit Bunch

The method of [9] was used for the extraction process. Two thousand grams (2000g) of the collected empty oil palm fruit bunch wastes were sun-dried and later oven dried at 105°C for two days to ensure adequate removal of moisture from the sample. The drying continued until it became "bone-dried". One thousand grams (1000g) of the bone dried bunches were charred for 1 hour to ensure uniform combustion which yielded about 100g of charred ash.

Another, 1000g of the dried palm bunch was ground with mortar and pestle to increase its surface area, and was burnt in a temperature controlled furnace (Model 5XL) set at temperature of 550°C for about 6 hours for proper ashing yielding approximately 70g of ash. The ashed samples were homogenized by crushing between fingers and then sieved with analytical sieve of mesh size of 0.105mm (US sieve No. 140 Tyler equivalent 150 Mesh) to obtain particle of uniform size.

2.3. Extraction of Ash from Unripe Plantain Peel

Two thousand (2000) gram of the plantain peels waste was washed with distilled water and sundried for three days to constant weight. The peels were sun-dried and later oven dried at 105°C for two days to ensure adequate removal of moisture from the sample. The drying continued until it became "bone-dried". One thousand grams (1000g) of the bone-dried peels were charred for 1 hour to ensure uniform combustion, which yielded about 100g of charred ash [12]. The ash samples were powdery but it was sieved with 0.105 mm sieve (US Sieve No. 140 Tyler equivalent 150 Mesh) to remove large particles.

Another, 1000g of the dried peels was ground with mortar and pestle. The grounded waste peels were burnt in a temperature-controlled furnace (Model 5XL) set at temperature of 550°C for about 6 hours for proper ashing yielding approximately 70g of ash. This was cooled in desiccators before being sieved with a mesh size of 0.105 mm (US sieve No. 140 Tyler equivalent 150 Mesh).

2.4. Preparation of the Aqueous Crude Extract from Limestone (Akang)

Limestone (*akang*) was ground into fine powder using laboratory mortar and pestle, after which 2.5 grams of the powder was dissolved in 100ml with distilled water to make a 2.5% solution. The flask was covered with aluminum foil paper and left for 3 weeks (21 days) for digestion. After 21

day, the mixture was mixed vigorously using an electric mechanical shaker for 4 hours and allowed to settle for 48 hours before being filtered using poplin cloth and re-filtered with whatman No. 1 filter paper (125cm) to obtain a clearer extract.

2.5. Preparation of the Instant Emulsion "otong"

One hundred milliliters (100ml) of Red Palm Oil was carefully dispensed into a 500ml capacity beaker. The beaker was then placed in a water bath (60°C) for 5 minutes to warm. Then, known quantity of the "*otong*" solution was added with intermittent stirring until it saponified becoming yellowish in colour. The amount of the solution, which saponified the oil, was measured.

2.6. Microbiological Analysis

2.6.1. Enumeration of Microbial Loads

Precisely 1ml of the desired diluents (10-3 ml) was taken and plated out on a standard microbiological analytical media (Nutrient Agar and Sabourand Dextrose Agar) in triplicates using the pour plate technique [13]. The bacterial population was determined using Nutrient Agar (Lab tech, India) while fungal population was determined using Sabouraud Dextrose Agar (Lab Tech, India). The bacteria culture plates were incubated at 37°C for 48 hours, the fungal plates was incubated at room temperature (28 ± 2°C) for 3-5 days. The emerging colonies were enumerated using the Quebec colony counter and recorded as colony forming unit per milliliter of *otong* (CFU/ml).

2.6.2. Maintenance of Pure Microbial Isolates

Discrete colonies were purified using by repeated sub-culturing on freshly prepared Nutrient agar and Sabouraud Dextrose agar for bacteria and fungi respectively. The pure isolates were maintained on agar slants bottle and stored at 4°C for further use.

2.6.3. Characterization of Bacterial Isolates

The bacterial isolates were characterized based on their cultural and morphological characteristics as well as their responses to standard biochemical test as described by [14]. Twenty four (24) hours old cultures of bacteria obtained were subjected to Gram's staining and several biochemical test such as Catalase test, Citrate Utilization test, Oxidase test, Motility test, Urease test, Starch hydrolysis, Methyl red and Vogues Proskauer test, Indole test, as well as sugar fermentation test as described in Bergey's manual of determinative bacteriology [15] for identification.

2.6.4. Characterization and Identification of Fungal Isolates

Yeast isolates were characterized on the basis of their morphological and biochemical characteristics as presented by [15, 16], while mold isolates were characterized on the basis of their cultural attributes, and identified by consulting various taxonomic books and monographs available on various groups of fungi [17].

2.7. Sensory Evaluation

Sensory evaluation was carried out on the freshly prepared emulsion "*otong*" samples using a nine (9) point hedonic Scale for scoring; Where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely [18]. The samples were presented to a twenty (20) semi-trained panelists of the Department of Food Science and Technology who were requested to assess the samples for Colour, Flavor, Taste, Smoothness and General acceptability. All the samples were presented at the same time and the identities of the samples were not revealed to the panelists. Each panelist was provided with sufficient privacy to ensure that his/her result would be arrived at independently and without being influenced by other panelists. The panelists were asked to rinse their mouths with water after assessing the first sample.

2.8. Statistical Analysis

Statistical Package for Social Science (SPSS, version 20) was used for the statistical analysis. The differences between samples in each parameter tested was done using One Way Analysis of Variance (ANOVA) and New Duncan's Multiple Range Test as a post-hoc test when the analysis of variance indicates significant difference in their means. A significant level of $P<0.05$ was used throughout the study.

3. Results and Discussion

3.1. Microbial Analysis

The result of analysis (Table 1) revealed that the heterotrophic bacterial load of the instant emulsion samples prepared with different ash solutions ranged between 1.0×10^3 to 1.7×10^3 cfu/ml, with emulsion (*otong*) made from furnace plantain peel ash solution having the highest bacterial load (1.7×10^3cfu/ml). The microbial density was significantly ($P<0.05$) reduced among the delicacies prepared from the different alkaline solutions. Fungal load of the emulsion (*otong*) ranged between 1.3×10^3 to 3.7×10^3 cfu/ml with emulsion prepared with furnace empty palm bunch ash solution having the highest fungal load. The low microbial loads obtained from the freshly prepared emulsions (*otong*) may be attributed its alkaline properties, which has been reported to have inhibitory and surface-active cleansing (surfactant) potentials [3]. The flash pasteurization treatment of the raw oil before formulation might have also contributed to the reduced level of microbial load. National Agency for Food Drug and Control (NAFDAC) stipulate that microbial load in food should be in the order of 10^{-4} as maximum allowable limit. The results of this study are in consonance with the minimum acceptable limit of microbial for food sample especially palm oil [19]. The results of microbial load of the samples were within the acceptable limit by NAFDAC.

The present study has shown that freshly prepared emulsion (*otong*) contained several microorganisms including pathogens. Table 2 shows the bacterial species

associated with the saponified dishes (*otong*). *Bacillus subtilis* is a heterotrophic bacterial that is not harmful but is responsible for food spoilage [19]. Further reported that some *Bacillus sp.* are pathogenic and can cause food poisoning, bacteremia and endocarditis, Their presence in some palm oil may be due to the fact that most palm oil are sold in the open markets where they may be exposed to the spores of the organisms from the atmosphere. Spores of this organism are highly resistant to the lethal effects of heat drying with radiation. By extension, the microorganisms in the raw oil contaminate the instant emulsion prepared with the alkaline solution also known as potash. *Proteus sp* has been implicated in urinary tract infection. *Micrococcus species* are heterotrophic bacteria some of which may be responsible for food spoilage. [20] Reported that some *Micrococcus* spices are saprotrophic pathogens and spoilage organisms. *Pseudomonas aeruginosa* are the commonest microorganism that causes infection and diseases. It is an opportunistic pathogen [19]. The isolation of these bacteria from some samples of the *otong* delicacies may be an indication of unhygienic handling of the palm oil by the sellers [21] used in the preparation of the delicacies. The fungal species associated with the freshly prepared saponified dishes (*otong*) include *Microsporium gypsium, Aspergillus fumigatus, Penicillium notatum, Botrytis cineria, Microsporium americana, Aspergillus glacus, Penicillium expansum, Aspergillus parasiticus, Candida albicans and Candida pseudotropicalis,* which are similar to those obtained in previous works on raw palm oil [19, 22]. There are indicators that these fungi are able to survive the anaerobic nature of the palm oil through lipase production and spore formulation [23]. These organisms are believed to aid fast deterioration of palm oil as well as toxin production (aflatoxin), which causes health challenge when consumed. *Penicillium* however are known to produce antibiotics therefore, *Penicillium notatum* and *Penicillium expansium* obtained in this study may have the ability of producing beneficial antibiotic effects in the food. *Aspergillus species,* the most prevalent microbial species are largely responsible for increased incidence of invasive aspergillosis in immune compromised plants. *Candida species* causes candidiasis and oval thrust.

Table 1. *Total microbial counts of the samples.*

Samples	THBC (CFU/mg)	TFC (CFU/mg)
SAPD1	$1.7^{a}+2.00\times10^{3}$	$3.0^{b}+1.00\times10^{3}$
SAPD2	$1.1^{b}+1.00\times10^{3}$	$1.3^{f}+0.00\times10^{3}$
SAPD3	$1.3^{b}+3.00\times10^{3}$	$3.7^{a}+2.00\times10^{3}$
SAPD4	$1.0^{b}+1.00\times10^{3}$	$2.0^{d}+2.00\times10^{3}$
SAPD5	$1.2^{b}+1.00\times10^{3}$	$1.7^{e}+1.00\times10^{3}$
SAPD6	$1.1^{b}+2.00\times10^{3}$	$2.9^{c}+1.00\times10^{3}$

Values are mean+Standard deviation of triplicate determinations, means with the same superscript on the same column are significant (P<0.05) different.
THBC = Total Heterotrophic Bacterial Counts.
TFC = Total Fungal Counts.
SAPD1 -Saponified dish prepared from aqueous extract of furnace ashed plantain peel.
SAPD2 - Saponified dish prepared from aqueous extract of of charred plantain peel.
SAPD3 - Saponified dish prepared from aqueous extract of furnace ashed palm bunch.
SAPD4 - Saponified dish prepared from aqueous extract of charred palm bunch.
SAPD5 - Saponified dish prepared from aqueous extract of sodium sesquicarbonate.
SAPD6 - Saponified dish prepared from Commercial potash extract bought from the market.

Table 2. *Frequency occurrence for bacterial isolates.*

Oraganisms	SAPD1	SAPD2	SAPD3	SAPD4	SAPD5	SAPD6
Micrococcus sp	+	-	-	+	-	+
Bacillus subtilis	-	+	+	+	+	+
Proteus sp	+	+	-	+	-	-
Pseudomonas aeruginosa	+	-	+	-	+	+

Key+= Present, - = Absent.

Table 3. *Frequency occurrence for fungal isolates.*

Organisms	SAPD1	SAPD2	SAPD3	SAPD4	SAPD5	SAPD6
Microsporium gypsium	-	-	+	-	-	+
Aspergilus fumigates	-	+	-	-	+	-
Penicillium notatum	+	+	-	-	-	-
Botrytis cineria	-	-	-	+	+	-
Microsporium americana	+	-	-	+	-	-
Aspergillus glaucus	+	-	+	+	+	-
Penicillium expansum	-	+	-	+	-	+
Aspergillus parasiticus	+	-	+	-	-	+

Organisms	SAPD1	SAPD2	SAPD3	SAPD4	SAPD5	SAPD6
Candida albicans	-	+	-	-	-	+
Candida pseudotropicalis	+	-	+	+	-	-

Key.
+= Present.
- = Absent.
SAPD1 -Saponified dish prepared from aqueous extract of furnace ashed plantain peel.
SAPD2 - Saponified dish prepared from aqueous extract of of charred plantain peel.
SAPD3 - Saponified dish prepared from aqueous extract of furnace ashed palm bunch.
SAPD4 - Saponified dish prepared from aqueous extract of charred palm bunch.
SAPD5 - Saponified dish prepared from aqueous extract of sodium sesquicarbonate.
SAPD6 - Saponified dish prepared from Commercial potash extract bought from the market.

Table 4. *Percentage occurrence frequency of bacteria.*

Organisms	Occurrence frequency	% Occurrence frequency
Micrococcus sp	4	20
Bacillus substilis	6	33.3
Proteus sp	4	20
Pseudomonas aerogenosa	5	26.7

Table 5. *Percentage occurrence frequency of fungal species.*

Organisms	Occurrence Frequency	% Occurrence Frequency
Microsoprium gypsium	2	8
Aspergillus fumigatus	2	8
Penicillium notatum	2	8
Botrytis cineria	2	8
Microsporium americana	2	8
Aspergillus glaucus	4	16
Penicillium expansum	3	12
Aspergillus parasiticus	3	12
Candida albicans	2	8
Candida pseudotropicalis	3	12

3.2. Sensory Evaluation of the Otong Delicacy

The results of the sensory evaluation of the *otong* delicacies prepared with the alkaline solution including commercial potash from the open market are presented in Table 6. Results obtained revealed that, there was no significant (P<0.05) difference amongst samples SAPD1, SAPD2, SAPD3 and SAPD4 but there was significant difference among samples SAPD5 and SAPD6 in terms of appearance. This may be attributed to the fact that sample SAPD5 was prepared using limestone extract and sample SAPD6 was prepared with commercially purchased potash from an open market. Similar trend was observed throughout the parameters measured. There were no significant (P<0.05) differences among samples SAPD1, SAPD2, SAPD3 and SAPD4 but there was significant difference among samples SAPD5 and SAPD6 in terms of taste, flavor, smoothness and general acceptability, but there was significant difference among samples SAPD5 and SAPD6 in all the parameters tested. The reason for such a similar observations amongst the laboratory treated samples could be attributed to the carefully selection of the method of processing and also sanitary condition of the processing equipment and environment compared with the commercial potash and limestone extract that was heavily contaminated with impurities. In terms of appearance, taste and flavor and smoothness, sample SADP rated highest while sample SADP5 rated lowest followed by SADP6. Similar trend was observed in overall acceptability as sample SADP1 was highly rated by the panelist and sample SADP5 was poorly rated. Also overall acceptability indicated that instant emulsion (*otong)* delicacies prepared from furnace plantain peel was highly acceptable together with furnace/charred palm bunch. *Otong* prepared from limestone crude extract was rated the lowest followed by *otong* from commercial potash extract.

Table 6. *Sensory evaluation of the saponified dishes 'otong'.*

Samples	Appearance	Taste	Flavour	Smoothness	General Acceptability
SAPD1	7.70^{a}+0.65	7.55^{a}+0.60	7.50^{a}+0.51	7.40^{a}+0.59	7.80^{a}+0.41
SAPD2	7.40^{ab}+0.59	7.10^{b}+0.44	7.20^{a}+0.52	7.00^{bc+}0.32	7.15^{b}+0.36
SAPD3	7.65^{a}+0.58	7.50^{a}+0.60	7.45^{a+}0.60	7.30^{ab}+0.57	7.50^{a}+0.51
SAPD4	7.15^{b}+0.58	7.05^{b}+0.39	7.20^{a}+0.52	6.75^{c}+0.44	7.10^{b}+0.30
SAPD5	4.40^{d+}0.50	4.65^{c}+0.48	4.65^{c}+0.81	4.80^{d}+0.52	4.65^{d}+0.69
SAPD6	6.70c+0.80	6.85^{b}+0.67	6.65^{b}+0.58	6.70^{c}+0.47	6.60^{c+}0.75

Values are mean+Standard deviation of triplicate determinations, means with the same superscript on the same column are significant (P<0.05) different.
SAPD1 -Saponified dish prepared from aqueous extract of furnace ashed plantain peel.
SAPD2 - Saponified dish prepared from aqueous extract of of charred plantain peel.
SAPD3 - Saponified dish prepared from aqueous extract of furnace ashed palm bunch.
SAPD4 - Saponified dish prepared from aqueous extract of charred palm bunch.
SAPD5 - Saponified dish prepared from aqueous extract of sodium sesquicarbonate.
SAPD6 - Saponified dish prepared from Commercial potash extract bought from the market.

4. Conclusion

This study has shown that agricultural waste such as plantain peel and empty palm bunch could conveniently be used as an alternative to limestone, which had been reported to have adverse effect in humans and animals. This will make our environment free of those agricultural wastes that often render them untidy and unhealthy. It will also save the environment from the potential harmful effects of pollutions that are commonly associated with these wastes. In addition, the heavy dependence on synthetic and unhealthy chemicals (biocarbonates) for the local delicacy "*otong*" and other dishes that requires saponification or emulsification would drastically reduce.

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