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Full Length Research Paper

# Effects of glucose and clove treatment on the microbial load of *Bunyi Youri* during fermentation and solar tent drying of the product

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"Bunyi youri" is a Nigerian fermented sun-dried fish product used as condiment in soups. This study was carried out to determine the effects of glucose and clove treatment on the microbial quality of the fermented and solar tent-dried product. A total of 24 isolates were obtained from the "Bunyi youri" samples analyzed, *Micrococcus* sp. was the predominant constituting 45.83% of the isolates, while *Bacillus* sp., *Staphylococcus* sp., *Leuconostoc* sp. and *Streptococcus* sp. constituted 29.17, 12.5, 8.33 and 4.17% of the isolates, respectively. Treatment of the samples with glucose resulted in an increase of the total aerobic plate count from an initial  $2.11 \times 10^3$  cfu/g in unfermented raw fish samples to an average of  $2.05 \times 10^5$  cfu/g during a 24 hour fermentation; the mean total plate count decreased to  $9.73 \times 10^2$  cfu/g after the solar tent drying of the product. An improvement in the fermentation was observed as a result of the incorporation of glucose which increased the growth rate of the lactic acid bacteria (LAB) and which, in combination with clove resulted in a reduction of the spoilage bacteria in the final processed products.

Key words: "Bunyi youri", fermentation, microbial, glucose, clove.

# INTRODUCTION

Fish is one of the most important animal protein foods available in the tropics (Eyo, 2001). The less developed countries (LDCs) capture about 50% of the world's harvest and a large proportion of that catch are consumed locally (FAO, 1985). In many Asian countries, over 50% of the animal protein intake comes from fish while in Africa the proportion is only 17.5% (William et al., 1998). Fish and other seafood constitute an important and popular part of the diet of many Nigerians which is made up of 60% of animal protein intake, particularly in regions where livestock is relatively scarce (Adeniyi 1987; Igwegbe, 2015). This means that shortfall in fish availability will affect the animal protein intake of people in Nigeria and other tropical countries. In addition, the

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Author(s) agree that this article remains permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> harvesting, handling, processing and distribution of fish provide livelihood for millions of people across the globe (Eyo, 2001; Aljufaili and Opara, 2006). Furthermore, well processed fish from the tropics have a ready market in developed countries and are thus good foreign exchange earners (Akande and Odogbo, 2005).

The domestic fish production in Nigeria in the year 2007 was 615,000 metric tonnes (FAOSTAT, 2009). Nigeria was reported to spend over ₩100 billion on the importation of frozen fish in 2010. Estimated annual demand was about 2.66 million metric tonnes as against the annual domestic production of about 0.70 million metric tonnes giving a demand-supply gap of about 1.8 million metric tonnes (Tiamiyu, 2012). Despite the fact that Nigeria has a compact landmass of 923,762 km<sup>2</sup>; 860 km of coastline on a major gulf of the South Atlantic, abundant water resources with major rivers of the Niger and the Benue traversing its territory in addition to numerous smaller rivers and streams crisscrossing its vast terrains (Olaosebikan and Raji, 1998; Igwegbe et al., 2014), the country imports over 780,000 metric tonnes of frozen fish annually from Europe, Latin America and eastern countries. The shortfall in fish supply in the country had led to a low annual per capita fish consumption rate of only 7.5 kg as against 15 kg per annum recommended by the FAO. There are many health benefits that can be derived from fish consumption. One of such merits is that fish is less tough and more digestible compared to beef, mutton, chicken and bush meat. This is possible because of the greater ratio of muscle protein to connective tissue protein in fish as compared with other animals (Alais and Linden, 1999) thus making fish acceptable to infants and adult alike (Eyo, 2001). Because of its greater digestibility, fish is usually recommended to patients with digestive disorders such as ulcers (Eyo, 2001); cardiovascular benefits which reduce the risk of heart attack, and anti-inflammatory properties of fish oil due to the presence of omega-3 fatty acid (Igwegbe, 2015). Apart from this, fish is abundant and to some extent occurs "free" in nature. This may account for its relatively low cost compared with other animal products. In addition, there is hardly any religious taboo affecting the consumption of fish, unlike pork and beef. Immediately the fish dies, a number of physiological and microbial deterioration set in and thereby degrade the fish. Most of the fish harvested in the tropics are either used in direct human consumption or processed into fish meal for use in compounding feeds. A significant quantity is also lost through the absence of adequate technology to prevent post-harvest losses common in most tropical countries. An estimated 20 to 50% of the fish produced in the remote coastal centers and hinterlands of many tropical countries deteriorate before they get to the consumers due to poor handling, preservation and processing practices adopted by the artisanal fishermen, fish farmers and fisheries entrepreneurs in those areas (Negbenebor, 1990; Eyo, 2001;

Igwegbe, 2015). The gap between fish demand and supply is unfortunately widening due to increasing population, drop in meat and fish supply, thus prompting the search for methods of improving fish quantity and quality. Thus, fish spoilage could be reduced if fish are traditionally processed to products that will have extended shelf life such as "Bunyi youri".

"Bunyi youri" is a putrefied sun dried fish product prepared form Nile perch (Lates niloticus) or Clarias species and is used as condiment in the flavouring of soups and is very popular among the Kanuri Tribe of North Eastern Nigeria (Negbenebor et al., 1995). These authors reported the proximate composition of this traditional product as 32.63±1.05% protein, 40.59±0.12% fat, 24.80±2.55% moisture and 1.88±0.32 ash. It is therefore evident that "Bunyi youri" is a highly nutritious fish product that could be processed very easily using the abundant sun light energy in the tropics including Nigeria. The processing and distribution of "Bunvi vouri" can also serve as a source of income as well as offer employment opportunities to many Nigerians that may be engaged in the business. The traditional "Bunyi youri" processing involves the removal of the fish scale, gutting, washing and fermentation of the fish for 6 to 8 days at ambient temperatures of between 31 and 49°C.

Since partial putrefaction of the fish is involved during the traditional processing of "Bunyi youri", uncontrolled chemical and microbial changes in the product can be expected. Also, the production of very strong odour which can attract flies and even rodents, during the processing, could lead to a serious contamination by these agents. Therefore, the control of both chemical and microbial activities during the processing is paramount to obtaining a shelf-stable product. Idakwo et al. (2016) reported the proximate composition and acceptability of "Bunyi youri" treated with glucose and clove stored for 24 weeks in which the product was acceptable within these weeks. The aim of the present study is therefore to determine the possible effects of combination of glucose and clove particularly on microbial activities during fermentation and subsequent solar tent-drying of "Bunyi youri". This is targeted at improving the shelf-stability of the product as influenced by the microbial load. Although glucose and clove have been used either separately or in combination with other spices to enhance the quality of meat and fish products, there is paucity of information on the combined effects of the two ingredients on enhancing the quality of fermented fish and this is thus the major reason for the present study.

# MATERIALS AND METHODS

# Acquisition of the raw materials

Fresh fish samples of Nile perch (*Lates niloticus*) species were obtained directly from the River Benue fish landing sites in Yola, Adamawa State Nigeria. Samples were collected using a clean



Plate 1. The fresh fish samples.



Plate 2. The prepared samples.

plastic container ( $80 \times 20 \times 45$  cm) with tight lid, and packed with crushed ice to minimize deterioration of the fish during transportation to the laboratory in Maiduguri. For the purpose of comparison, samples were equally divided and a portion of it was taken to a local processor in Gamboru ward, for "Bunyi your" to be produced using the normal traditional method, this product served as the commercial control sample. Cloves (*Eugenia carryophyllata*) were obtained from Maiduguri Monday Market while glucose of analytical grade (BDH Chemicals Ltd, U.K.) was obtained from chemicals supplier in Maiduguri.

The materials for the construction of the solar tent drier were

obtained from Maiduguri Timber and Monday markets. Finally, plastic buckets of five litre capacity were purchased from the Gamboru plastic container depot in Maiduguri and used for the fermentation. The constructed solar tent-drier used in this study is presented in Plate 3.

# Preparation of fish samples

The fresh fish samples (Plate 1) were gutted and thoroughly washed with potable water. Five treatments were used in each experimental processing. For each of the five treatments, three replicates were made. The prepared fish (Plate 2) was divided into five different groups and four groups were treated separately with concentrations 2.0% glucose and 0.1, 0.3, 0.5 and 0.6% of clove, dipping the fish samples into the glucose-clove solution for 20 min (Adams et al., 1987); while the fifth group, which served as the control, was treated by dipping it only in distilled water. The prepared samples were allowed to ferment for 24 h at mean ambient temperature and relative humidity (RH) of 38.5±1.7 and 25.9 ± 2.1% respectively (Adams et al., 1987). The fermentation was carried out in covered plastic buckets of 5 L capacity in the laboratory. The fermented fish were dried in the solar tent-drier (Plate 4) at the mean temperature and RH of 65.0  $\pm$  5.2 and 21.9  $\pm$ 0.35%, respectively, for two to three days until the samples were crisp dried (Plate 4). The humidity was measured using an electronic temperature/humidity meter (ELE, EA 506-124). The dried samples were stored in plastic containers until analyzed.

### Sequence of operation in "Bunyi youri" processing

The traditional method of "Bunyi youri" processing was utilized with modifications by including the dipping of the fish samples in mixtures of glucose and clove for 20 minutes prior to fermentation for 12 to 24 h (Figure 1), and drying in a solar tent drier instead of the usual traditional practice of spreading on mats and direct exposure to the sun. The drying was continued for two to five days until the "Bunyi youri" was crispy-dried. The clove samples were ground using a hammer mill, wrapped in aluminum foil and then autoclaved at a temperature of 121°C and pressure of 15 psi for 15 min, to ensure its freedom from mould and bacterial spores before its application to the fish samples.

### Construction of solar tent drier

The solar tent-drier was constructed as described by Clucas and Ward (1996) but with some modifications to include raising the drier on a platform table to prevent dust contamination and attacks by domestic animals like cats and dogs. The dimensions of the drier were as described by FAO (1983) and this is shown in Plate 3 as improved solar tent-drier. This consisted of a rectangular table measuring 210 × 240 cm as the base and a tent roof with a height of 40 cm. The top of the table was covered with a black polythene sheet for absorption of heat while the roof was vented to allow the exit of saturated air from the tent. These vents were covered with wire net in order to prevent flies and insect infestation of the drying products. The table was supported on 100 cm woods at the four angles. A window was also fitted on one side of the tent-drier to facilitate loading and offloading of the samples (Plate 3).

### Microbiological media

The bacteriological and mycological media which included de-man Rogosa Sharpe agar (Oxoid Ltd, Basingstoke, UK), Plate count agar (Oxoid) nutrient agar (Biotec Laboratories, Suffolk, UK), Brain-



Plate 3. The solar tent-drier during drying.

heart infusion agar (Oxoid) Sabourand agar (Topley House, England), Lauryl sulphate tryptose broth, Brilliant green lactose broth and Eosin methylene blue agar (Biotec) were obtained and used for the isolation, enumeration and identification of the microorganisms associated with "Bunyi youri" processing. All glass wares including petri-dishes, test tubes, pipettes, flasks and bottles, were sterilized in a hot air oven at  $170 \pm 5^{\circ}$ C for at least two hours, while the media and distilled water were sterilized by autoclaving at 121°C for 15 min and at 15 psi (Marshall, 1992; Quinn et al., 2002), and each medium was prepared and used according to the manufacturer's instructions. The media were allowed to cool to about 40°C before pouring or plating.

### **Microbiological analysis**

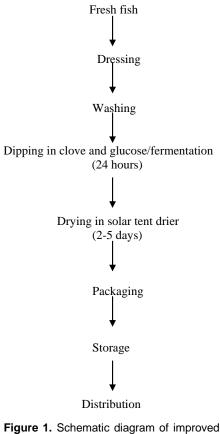
"Bunyi youri" sample (25 g) was aseptically transferred to a sterile plastic bag and pummeled for 1min in a stomacher (IUL Instrument, Spain), with 225 ml of 0.1% sterile peptone water. Appropriate decimal dilutions of the sample were prepared using the same diluents and 0.1 ml of each dilution was plated in triplicate on

different growth media.

Lactic acid bacteria (LAB) were enumerated on de-man Rogosa Sharpe agar (Oxoid) using the pour plate technique (Harrigan and McCance, 1976), catalase negative, gram positive, opaque, white colonies were counted as lactic acid bacteria (Adams et al., 1987). Spoilage organisms were enumerated on plate count agar (Oxoid) using the pour plate technique as described by Harrigan and McCance (1976). Catalase positive, large and medium sized colonies were counted as spoilage organisms (Adams et al., 1987), on the same plate count agar, lactic acid bacteria were enumerated as very translucent or white, catalase negative colonies. These small colonies were enumerated so as to determine the proportion of lactic acid bacteria involved in the fermentation process. All the plates were incubated at 37°C for 24 h. Staphylococcus aureus were enumerated as described by FAO (2013) utilizing 0.25 ml of the fish homogenate and the subsequent decimal dilutions on the surface of Baird Parker agar. A confirmatory test or coagulase test was used to establish the identity of Staphylococcus aureus. Also, the enumeration of coliforms was as described in FAO (2013) utilising the Lauryl sulphate tryptose broth, brilliant green lactose broth and Eosin methylene blue agar (EMBA) which consisted of



Plate 4. Solar tent-dried samples.



**Figure 1.** Schematic diagram of improved "bunyi youri" processing (improved from traditional method).

presumptive, confirmatory and completed tests. Sabouroud agar was incubated at 25°C for 3 to 4 days for fungal isolation

The other inoculated plates were inverted and then incubated at 37°C for 24 h, after which the cultural and morphological characteristics of colonies were observed. Colonies were selected at random and sub-cultured to obtain pure isolates on fresh plates

containing nutrient agar and then incubated again at 37°C for 24 h. The stock cultures were obtained, labeled carefully and were used for conventional identification of the organisms using Gram's staining, motility, indole production, urease, carbohydrate utilization, catalase, citrate utilization, oxidase, coagulase and methyl red tests.

### **Biochemical identification of Isolates**

The colonies of bacteria were isolated, purified and subjected to different biochemical tests using methods described by Harrigan and McCance (1976). However, mould colonies appearing on the plates were sub-cultured to give pure colonies employing the method of Onions et al. (1980). The moulds were identified following the guidelines prescribed by Gupta *et al.*, (2013) and Onions et al. (1980).

### Statistical analysis of the data

The statistical package for Social Science (SPSS version 10) was used. Data analysis involved one-way analysis of variance (ANOVA). The mean differences were determined using Duncan's multiple range test. A significant difference was established.

## **RESULTS AND DISCUSSION**

### Microbial analysis

The microbial contents described as the total aerobic plate count (TAPC), lactic acid bacteria (LAB) and spoilage bacteria (SB) counts in Bunyi youri are presented in Table 1. The mean total bacterial counts recorded in the control samples ranged between 1.76 ×  $10^3$  and  $1.803 \times 10^3$  cfu/g whereas that of the treated samples ranged between  $1.79 \times 10^5$  and  $2.08 \times 10^5$  cfu/g. Increase in the viable count of micro flora from 10<sup>6</sup> to 10<sup>9</sup> during the 72 h of fermentation using starter culture was reported in African fermented fish (Aseidu and Sanni, 2002). Suchitra and Sarojinalini (2012) also reported a viable count of up to 10<sup>6</sup> cfu/g in samples of "Ngari" Aryanta et al. (1991) also observed a population of  $10^{\prime}$  to 10<sup>8</sup> cfu/ g after 48 h in fermented fish sausage using Pediococcus acidilactici as starter culture. ICMSF (1986) mentioned the limit for standard plate count (SPC) for microorganisms per gram weight of different fish samples to be  $10^5$  cfu/g while Cho et al. (1988) reported a range of viable bacterial count between  $10^3$  and  $10^7$  cfu/g in dried fish. In this study, the significant increase in total bacterial counts recorded is an indication of the bacterial role in fermentation, which ultimately results in the production of flavours.

Generally, there was an increase in the microbial population in all the treated samples because the microorganisms responsible for fermentation were multiplying as a result of abundant substrate particularly glucose and suitable conditions of low pH for their growth and this observation is in agreement with that made by Pelczar et al. (1986). Thapa et al. (2004) also fermented

Treatment <sup>2</sup>	Microbial Group (cfu/g) / Percentage <sup>1</sup>					
	TAPC	LAB	LAB %	SB	SB%	
Control 1	1.76×10 <sup>3</sup> ±4.9×10 <sup>2b</sup>	$4.43 \times 10^{2} \pm 6.8 \times 10^{1b}$	25.71±2.9 <sup>c</sup>	1.32 x 10 <sup>3</sup> ±4.2×10 <sup>b</sup>	74.29±2.9 <sup>a</sup>	
Control 2	1.80×10 <sup>3</sup> ±2.9×10 <sup>2b</sup>	5.09×10 <sup>2</sup> ±1.8×10 <sup>1b</sup>	28.72±4.6 <sup>c</sup>	1.29×10 <sup>3</sup> ±2.8×10 <sup>2b</sup>	71.29± 4.6 <sup>a</sup>	
А	1.79×10 <sup>5</sup> ±4.0×10 <sup>4a</sup>	1.22×10 <sup>5</sup> ±1.5×10 <sup>4a</sup>	68.16±15.8 <sup>a</sup>	$5.70 \times 10^4 \pm 3.5 \times 10^{4a}$	31.84±15.8 <sup>b</sup>	
В	2.05×10 <sup>5</sup> ±4.0×10 <sup>4a</sup>	1.55×10 <sup>5</sup> ±4.6×10 <sup>4a</sup>	74.39±8.2 <sup>ª</sup>	$5.03 \times 10^4 \pm 6.1 \times 10^{3a}$	25.54±8.1 <sup>b</sup>	
С	1.92×10 <sup>5</sup> ±7.5×10 <sup>4a</sup>	1.69×10 <sup>5</sup> ±9.0×10 <sup>4a</sup>	74.80±10.0 <sup>a</sup>	$5.30 \times 10^{4} \pm 3.7 \times 10^{4a}$	24.53± 9.7 <sup>b</sup>	
D	2.08×10 <sup>5</sup> ±5.0×10 <sup>4a</sup>	$1.48 \times 10^{5} \pm 4.0 \times 10^{4a}$	71.27±11.1 <sup>a</sup>	$5.97 \times 10^{4} \pm 2.9 \times 10^{4a}$	28.73±11.2 <sup>b</sup>	

**Table 1.** Microbiological counts (cfu/g) of "Bunyi youri" after fermentation.

<sup>1</sup>In any column, means bearing similar alphabetical superscripts are not significantly different ( $P \ge 0.05$ ); TAPC = Total aerobic plate count; LAB = lactic acid bacteria; SB = spoilage bacteria. <sup>2</sup>Control 1 = commercial "Bounyi youri"; Control 2 = laboratory prepared "Bounyi youri"; A = 2% glucose + 0.1% clove; B = 2% glucose + 0.3% clove; C = 2% glucose + 0.5% clove; D = 2% glucose + 0.6% clove.

fish into 'Ngari', 'Hentak' and 'Tungtap', which are traditional fermented fish products, and recorded increased microbial load in the fish samples. The predominant microorganisms were in the order of *Micrococci* followed by *Bacilli* spp. Anihouvi et al. (2006) similarly reported high microbial population during the fermentation of "Lanhoun" a traditionally processed fermented fish product popularly consumed in Benin Republic. The presence of this similar micro flora 'Momone' has also been reported by many researchers including Yankah (1988), and Abbey et al. (1994) and in other fermented fish products (Essuman, 1992).

Furthermore, no significant difference ( $P \ge 0.05$ ) was observed in the total aerobic plate count, Lactic acid bacteria and spoilage bacteria counts of the treated samples. Although a similar trend was observed for the control samples, there was however, a significant difference ( $P \le 0.05$ ) in the TAPC, LAB and SB counts of the controls when compared with the treated samples. This could be due to the favourable environment created for the proliferation of Lactic acid bacteria in the treated samples, which was reflected in the increase in the load of LAB in the treated samples and a reduction in the spoilage bacteria as well. There was no significant difference ( $P \ge 0.05$ ) in the level of spoilage bacteria among the treated samples, however, the two control samples (1 and 2) recorded higher loads of spoilage bacteria, 74.29 and 71.29%, respectively (Table 1), when compared with the samples treated with various levels of glucose and clove. This finding is similar to that recorded on 'Sharrmud' which is a fermented meat product common in Nigeria, which usually has a pH level of 6.4 (Idakwo, 2004).

The pH of the treated samples may have played a significant role in controlling the type of bacteria found in the treated and untreated control samples. Visessanguan et al. (2006) also reported that as source of lipolytic and proteolytic enzymes, these organisms may contribute to flavour formation of 'Nham' a Thai fermented pork sausage. Similarly Beddows et al. (1980) reported that

the complex interaction of enzymatic activity and oxidation during the fermentation along with bacterial production of volatile fatty acids may be responsible for the characteristic flavour and aroma of fermented fish products.

As regards to the microbial characteristics of the raw fish materials, the total aerobic plate count recorded was 2.11  $\times$  10<sup>3</sup> cfu/g in the fresh fish samples, which decreased during the fermentation and drying of the products to a range between  $1.02 \times 10^3$  to  $1.24 \times 10^3$ cfu/g, and 0.87 to 0.92  $\times$  10<sup>3</sup> cfu/g in the control and all the treated samples, respectively (Table 2). This observation is within the microbiological limit of 10<sup>2</sup> to 10<sup>4</sup> cfu/g stipulated by FAO (1979. Also, the Staphylococcus count ranged between 3.98  $\times$  10<sup>2</sup> and 4.10  $\times$  10<sup>2</sup> cfu/g for the controls and  $0.45 \times 10^2$  and  $0.54 \times 10^2$  cfu/g for the treated samples (Table 2). High count of Staphylococci  $(10^5 - 10^7)$  was recorded in fermented fish sausage by Arvanta et al. (1991). Staphylococcal count exceeding 10<sup>6</sup> cfu/g is considered to be hazardous and a count of 100 million  $(10^8)$  cfu/g in any food item is considered unfit for human consumption (Almas, 1981), Iver (1998), however, noted that the occurrence of a smaller number of Staphylococcus in the fishery products is not a serious problem but that food poisoning may occur if the products are handled carelessly during processing. On the other hand, the low counts of coliforms and faecal coliform in all the treated samples showed that "Bunyi youri" was prepared in hygienic environment. The presence of coliforms and Staphylococci, even though in few numbers in some of the samples is still significant and shows that there is need for improved handling and processing procedures in the preparation of "Bunyi youri". Although the absence of Salmonella sp. is good sign of the safety the products for consumers, yet the public health importance of the above findings cannot be ignored. It is therefore important to stress here the need to strictly adopt the good manufacturing practices right from the preparation of raw materials, use of utensils, handling practices and processing of "Bunyi youri" to the storage

	Type of microorganism <sup>1</sup>					
Treatment <sup>2</sup>	TAPC (cfu/g×10 <sup>3</sup> )	Staphylococcus (cfu/g×10 <sup>3</sup> )	Total coliforms (mpn/g)	Fecal coliform (mpn/g)	Mould count (cfu/g×10 <sup>2</sup> )	
Fresh Fish	2.110	4.60	23	11	ND	
Control 1	1.020	4.10	15	11	1.25	
Control 2	1.240	3.98	11	10	1.18	
А	0.920	0.54	< 3	< 3	0.39	
В	0.884	0.48	< 3	< 3	0.40	
С	0.872	0.45	< 3	<3	0.30	
D	0.900	0.52	< 3	< 3	0.45	

**Table 2.** The Microbial Characteristics of the Fresh Fish and "Bunyi youri" prepared with different Levels of Glucose and Clove.

<sup>1</sup>TAPC = Total aerobic plate count; ND = not detected. <sup>2</sup>Control 1= commercial "Bounyi youri;" Control 2 = laboratory prepared "Bounyi youri;" A = 2% glucose + 0.1% clove; B = 2% glucose + 0.3% clove; C = 2% glucose + 0.5% clove; D = 2% glucose + 0.6% clove.

Type of organism <sup>1</sup>	Frequency of occurrence (n) <sup>2</sup>	Number of positive isolates (%)
Micrococcus	24	11 (45.83)
Bacillus	24	7 (29.17)
Staphylococcus	24	3 (12.50)
Leuconostoc	24	2 (8.33)
Streptococcus	24	1 (4.17)

Table 3. Types and frequency of occurrence of microbial isolates from "Bunyi youri".

<sup>1</sup>Total number of Isolates from nutrient agar = 120; <sup>2</sup>n = pooled number of isolates from six sampling.

of the product in order to safeguard the health of the consumers. The use of glucose and clove in this study had greatly reduced the proportion of fecal coliform, thus this practice should be encouraged to ensure the safety of the consumers.

# The microbial isolates from "Bunyi youri"

The microorganisms isolated from "Bunyi youri" during fermentation and after solar tent-drying are presented in Tables 3 and 4. Among the bacteria identified were Gram positive rod, namely *Bacillus* and Gram positive cocci, namely *Micrococcus* which were the predominant bacterial flora. Sarojnalini and Suchitra (2009) also isolated similar microbial flora from 'Ngari' of Manipur. The presence of *Bacillus* suggested that spore forming bacilli might play an active role during the fermentation of "Bunyi youri". The occurrence of *Micrococcus* species also indicates the possible involvement of non-spore forming microorganisms (Rose, 1982). These bacteria may contribute to the development of flavour and odour in fermented fish products due to their proteolytic

activities and they may also assist in the breakdown of the fish tissue and the development of flavour and aroma (Beaumont, 2002) which are essential for the desirable quality of the final product.

A few numbers of Staphylococci were also identified and further subjected to coagulase test which confirmed all the isolates to be coagulase negative, thus indicating that they were Staphylococcus epidermidis. Micrococcus and Staphylococcus are catalase positive and Gottschalk (2012) noted that catalase production inhibits peroxide formation and, in a similar development, Liepe (1983) showed this reaction to also inhibit rancidity. Similarly Steinkraus (1997)reported that Staphylococcus epidermidis as well as Micrococcus play some roles in meat fermentation by inhibiting rancidity. Thus Micrococcus and Staphylococcus might actually improve the quality of the final products as a result of the production of flavour compounds (Visessanguan et al., 2006). Among the fungi identified were Aspergillus, Penicillium and Cladosporum species. All the fish samples, irrespective of treatment, contained Aspergillus and *Penicillium* spp. In addition *Clasdosporum* spp was recorded in the two control samples (1 and 2) but not in

Treatment <sup>2</sup>	Type of organism <sup>1</sup>				
Treatment	Aspergillus spp.	Penicillium spp.	Cladosporum spp.		
Control 1	+	+	+		
Control 2	+	+	+		
А	+	+	ND		
В	+	+	ND		
С	+	+	ND		
D	+	+	ND		

**Table 4.** Effect of glucose and clove treatments on the mycological flora of "Bunyi youri".

<sup>1</sup> += positive; ND = Not detected; <sup>2</sup> Control 1= commercial "Bounyi youri"; Control 2 = laboratory prepared "Bounyi youri"; A = 2% glucose + 0.1% clove; B = 2% glucose + 0.3% clove; C = 2% glucose + 0.5% clove; D = 2% glucose + 0.6% clove.

# any of the treated samples (Table 4).

Aspergillus and Penicillium spp. constituted the dominant genera as shown in Table 4 and these two genera have been shown to be very common in various fermented fish products including fish sauces and fish pastes (Suchitra and Sarojnalini, 2012). The treatment appeared to have inhibited *Clasdosporum* growth and activity in the processed samples. Thus, the use of a combination of glucose and clove has helped in the improvement of the microbial quality of the final product.

# Conclusion

In conclusion, this study has shown that a shelf-stable and acceptable "Bunyi youri" could be produced from Nile perch treated with a combination of different levels of glucose and clove one hand and fermentation and drying under strict hygienic conditions on the other hand. The use of glucose in the processing of "Bunyi youri" reduced the fermentation time, and the use of solar tent drier operated at a higher temperature, when compared to the ambient temperature, resulted to an accelerated process as well as a safer product with regards to the microbial load. Such accelerated processing will lead to an increase in the turnover of the product and more financial benefits to the local fish processors. It will also help in increasing the protein supply which hitherto remains a serious problem in most developing countries, including Nigeria.

# **Conflict of Interests**

The authors have not declared any conflict of interests.

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### REFERENCES

- Abbey LD, Hodari-Okae M, Osei-yaw A (1994).Studies on traditional processing and quality of fermented fish 'momone' Ghana/Netherlands Artisanal fish Processing and Applied Res. Project Report. Food Research Institute, Accra, Ghana. P 48.
- Adams MR, Cooke RD, Twiddy DR (1987). Fermentation parameters involved in the production of lactic acid preserved fish-glucose substrates. Int. J. Food Sci. & Tech. 22:105-114.
- Adeniyi JP (1987). Fish consumption in Nigeria: Implications for fishery development policies. J. West Afr. Fish. 3(2):151-161
- Akande GR, Odogbo D (2005). Field testing technologies for evaluating post-harvest losses in the artisanal fisheries of Dorobaga fishing community and Baga fishmarket in Maiduguri. Report and Papers presented at the Seventh FAO Expert Consultation on Fish Technology. http://www.fao.org/docrep/008/y9155b/y9155b/oc.htm.
- Alais C, Linden G (1999). Food Biochemistry, English Edition Aspen Publishers Inc. Gaitherburg
- Al-jufaili MS, Opara IU (2006). Status of Fisheries Postharvest in the Sultanate of Oman: Part 1:handling and Marketing System of fresh fish. J. Fish. Int. 1(2-4):144-149.
- Almas AK (1981). Chemistry and Morphology of fish and fish processing. Department of Biochemistry Norwegian Institute of Technology, University of Trondhain, Norway.
- Anihouvi VB, Ayernor GS, Hounhouigan JD, Sakyi-Dawson E (2006). Quality characteristics of Lanhoun.- A traditionally processed fermented fish product in the Republic of Benin. AJFAND 6 (1):1-15
- Aryanta RW, Fleet GH, Buckle KA(1991). The occurrence and growth of micro organisms during the fermentation of fish sausage. Intl.J.Food. Microbiol.13:143-155.

Aseidu M, Sanni AI (2002). Chemical composition and microbiological changes during spontaneous and starter culture fermentation of 'enamne-setraky' a West African fermented fish-carbohydrate product. Eur. Food Res. Technol. 215:8-12.

- Beaumont M (2002). Flavouring composition prepared by fermentation with *Bacillus* spp. Int. J. Food Microbiol. 75:189-196.
- Beddows CG, Ardeshir AG, David WJ. (1980). Development and origin of volatile fatty acids in 'Budu', J. Sci. Food Agric. 31:86-92.
- Cho HO, Byun MW, Kwon JK (1992). Preservation of dried fish powders and mixed condiments by gamma irradiation. Asian Regional Cooperative Project on Food Irradiation: Technology Transfer, 51.

- Clucas IJ, Ward AR (1996). Post-harvest fisheries development: Processing and quality. NRI, ODA Kent, UK
- Essuman KM (1992). Fermented fish in Africa. A study on processing, marketing and consumption. FAO Fisheries Technical Paper No. 329, 80p Food and Agriculture Organization, Rome, Italy.
- Eyo AA (2001). Fish processing in the tropics. Published by National Institute for fresh Water Fisheries Research, NIFFR, New Bussa, Nigeria, University of Ilorin Press 403 p.
- FAO (1983). The production and storage of dried fish. Proceedings of the workshop on the production and storage of dried fish. University Pertanian Malaysia Sedrdang (Malaysia) November. FAO Fisheries report No. 279 (Supplement) Rome: Food and Agriculture Organization of the United Nations, Rome, Italy.
- FAO (1985). World Catch and Trade of Fishery Products in 1984. Info fish Marketing Digest No.25, Food and Agriculture Organization of the United Nations, Rome, Italy.
- FAO (2013). Quality assurance for microbiology in feed analysis laboratories, by R.A. Cowie. Edited by Harinder P.S. Makkar. FAO Animal Production and Health Manual No. 16. Rome
- FAOSTAT (2009). World production of Fish, Crustaceans and Molluscs by major fishing areas. Fisheries Information Data and Statistics Unit (FIDI), Fisheries Department, FAO, Rome
- Gottschalk G (2012). Bacterial Metabolism (Spriger Science and Business Media. Science 281 p.
- Gupta VK, Tuohy MG, Ayyachay M, Turner KM, Anthonia O (2013). Laboratory Protocols in Fungal Biology (Electronic resource) Current Methods in Fungal Biology, Springer, New York, NY Harrigan WF, McCance ME (1976). Laboratory Methods in Food and
- Harrigan WF, McCance ME (1976). Laboratory Methods in Food and Dairy Microbiology. Academic Press, London. pp. 58-100
- ICMSF (1986). Microorganisms in Foods vol. 2. Sampling for Microbiological Analysis: Principles and Specific Applications. International Commission on Microbiological Specifications for Foods. University of Toronto Press, Toronto, Canada 213 p.
- Idakwo PY (2004). Effects of sugar types and concentrations on the quality of solar tent dried "Sharrmud" (A fermented sun dried Nigerian Meat Product). An M.Sc. Dissertation of Department of Food Science and Technology, University of Maiduguri, Nigeria.
- Idakwo PY, Badau MH, Igwegbe AO, Negbenebor CA (2016). Proximate composition and acceptability of Bunyi youri: A traditional Nigerian fermented solar tent dried fish condiment. Int. J. Appl. Microbiol. Biol. Res. 4:15-20.
- Igwegbe AO, Negbenebor CA, Chibuzo EC, Badau MH (2014). Effects of season and location on heavy metal contents of fish species and corresponding water samples from Borno State of Nigeria. Glo. Adv. Res. J. Med. Med.Sci. 3(3):64-74.
- Igwegbe AO (2015). Effects of Location and Processing on Heavy Metal Contents in Fish. Scholar Press Publishers, OmniScriptum GmbH & Co. KG, Germany, 153 p.
- Iyer TSG (1998). Pathogens and faecal organisms in fish and fishery products. In: Quality control in fish processing. Central Institute of Fish Technology; Cochin (ICAR), pp. 45-59.
- Liepe HU (1983). Starter culture in meat production In: Biotechnology, Vol. 5 ed. G.Reed, Verlagchemie Weinheim, pp. 400-424.
- Marshall RT (1992). Standard Methods for the Examination of Dairy and Vegetable Products, 16th Ed., American Public Health Association (APHA), Washington DC.
- Negbenebor CA (1990). Raw material supply for fishery and animal products processing industry. In: Strategies for agro-based industry, raw material supply, processing and sanitation. Eds. I. Nkama and P.A. Sopade. In: Proceedings of a two-day workshop by North-Eastern Chapter NIFST, University of Maiduguri, Maiduguri.
- Negbenebor CA, Kadaura SG, Igene JO (1995). Preliminary studies in the production of "Bunyi youri" (A putrified sun-dried fish production) Agrosearch 1:25-33
- Olaosebikan BD, Raji A (1998). Field Guide to Nigerian Freshwater Fishes. Federal College of Freshwater Fisheries Technology, New Bussa, Nigeria.

- Onions AHS, Allsopp D, Eggins HOW (1980). Smiths Introduction to Industrial Mycology. 7<sup>th</sup> Edition, John Wiley, Sons NY, pp. 50-57
- Pelczar MJ Jr, Chan EC, Noel RK (1986). Microbiology 5<sup>th</sup> Edition McGraw-Hill, New York..
- Quinn PJ, Carter ME, Markey B, Carter GR (2002). Clinical Veterinary Microbiology, Mosby International Ltd., London pp. 42-49.
- Rose AH (1982). Fermented Foods. In: Rose AH, Marmon J (Eds) Economic Microbiology\_Academic press London. 17:1-13
- Sarojnalini CH, Suchitra T (2009). Microbial profile of starter culture fermented fish product 'Ngari' of Manipur. Indian J. Fish. 56(2):123-127.
- Steinkraus KH (2002). Comprehensive Reviews in Food Science and Food Safety, Inst. of Food Technologist 1:23-32
- Suchitra T, Sarojinalini C (2012). Effect of temperature on biochemical and microbiological qualities of "Ngari" Nature Science 10 (2):32-40
- Tiamiyu N (2012).Providing fish needs of the Nigerian market. Business day Monday 28<sup>th</sup> May.
- Thapa N, Joydeb P, Iyoti PT (2004).Microbial diversity in 'Ngari', 'Hentak' and 'Tungtap', Fermented fish products of Northeast India. World J. Microbiol. Biotech. 20(6):599 -607.
- Visessanguan W, Benjakul S, Riebroy S, Yarchai M, Tapingkae W (2006). Changes in lipid composition and fatty acid profile of 'Nham', a Thai fermented pork sausage, during fermentation. Food Chem. 94:580-588.
- Williams R, Halwart M, Barg U (1998). Integrating fisheries and Agriculture to enhance fish production and food security. FAO Aquaculture Newsletter No. 20, December pp. 3-8.
- Yankah VV (1988). Studies on 'momone' A Ghanaian fermented fish product. B.Sc. Thesis. Department of Nutrition and Food Sciences, University of Ghana-Legon.