

Physicochemical and Microbiological Qualities of African Catfish (*Clarias gariepinus*) Sauce Produced by Salt Fermentation

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Abstract

Fish sauce was made from African catfish, *Clarias gariepinus* with the objectives of determining its suitability for sauce production and standard operating procedure which can support good manufacturing practice (GMP). Whole African catfish were washed, minced, mixed with 20% (w/w) salt and incubated in air-tight sterilized plastic containers at room temperature for six months. Thereafter, the mixture was manually drained using muslin cloth pore size 2mm to separate the supernatant liquor, the fish sauce. The sauce had moisture range of 57.49 -68.25%, protein 11.35%- 24.29%, lipid 2.53% - 6.87%, ash content 2.87% - 7.42%, crude fibre 1.53-9.36%. There were significant differences ($p<0.05$) between the weekly values of the proximate composition. The pH values ranged between 5.6 and 8.4. Four strains of gram-positive bacteria namely *Bacillus spp*, *Micrococcus luteus*, *Clostridium perfringens* and *Staphylococcus epidermidis* and a fungus *Candida albicans* were isolated from the sauce. The study showed that the African catfish is a safe and nutritious potential material for utilization in sauce production and that the procedure used complied with GMP.

Keywords: Proximate, characteristics, bacteria, *Candida albicans*.

Introduction

Fish and fish products play a significant role in the diets of the populations of West African countries. It is estimated that between 15 and 20 percent of all animal proteins come from aquatic sources (FAO, 2018). However, post-harvest losses of fresh fish are estimated to be about 20% in West African countries (Horemans, 1998). In developed countries the practice of cold storage limits the problem posed by the extreme perishability of fish while in tropical regions, particularly in West

Africa, traditional processes such as drying, salting, smoking, fermentation and combinations of these treatments are used for fresh fish preservation (FAO, 1981).

Fish sauce, is a clear brown liquid, produced by fermenting fish or shrimp or by-products from aquatic products with salt (Sanceda *et al.*, 2003a; 2003b). Fermentation results from the action of endogenous enzyme or additional microbial enzymes (Fukami *et al.*, 2004a; 2004b; Siringan *et al.*, 2006; Dissaraphong *et al.*, 2006). The regions of fish sauce production and consumption are

mainly in the southeast of Asia, east coast of China, Japan, and the north of Philippines. It is called by different names and is prepared differently, with the method of processing depending upon various factors and availability of raw materials, consumers' preference and the climatic conditions of the region (Al-Jedah *et al.*, 2000). Fish sauce contains a mixture of amino acids and other protein degradable products (Ijong and Ohta, 1995; Gildberg, 2001; Sanceda *et al.*, 2001; Aquerreta *et al.*, 2001; Fukami *et al.*, 2002; Ichimura *et al.*, 2003).

The importance of the nutritive value of fermented fish products in the daily diet of the Southeast Asian population has been discussed to a large extent by various investigators (Amano, 1962; van Veen, 1965; Areekul *et al.*, 1974; Garby and Areekul, 1974; Suwanik, 1979). However, literature on fermented fish in Nigeria is scarce because little or no fish sauce is produced in West Africa. Currently, more attention is being given to improving traditional fermented fish products in order to increase the availability of low-cost protein food for local consumption. Based on this, fish sauce production is being introduced to Africa countries, especially Nigeria.

The African catfish, *Clarias gariepinus*, a freshwater species is of great economic interest. It is generally considered to be one of the most important tropical catfish species for aquaculture (FAO 2010). In Nigeria, culture of African catfish by private and public sectors is beginning to gain ground as a result of large-scale fisheries development projects, mechanization, and improved technology to increase production capacities in fisheries to support food security and availability of protein for the increasing human population. With increasing production, comes the problem of marketing. To prevent gluts as well as economic and postharvest losses, there is the need to for innovative ways of value addition and fish utilization.

Therefore, this work sought to investigate the potentials of utilizing catfish for sauce production by determining its characteristics, evaluating the nutritional value and suitability for human consumption.

Materials and Methods

Collection and preparation of fish sauce

Fresh catfish, *Clarias gariepinus* samples were obtained from the Aquaculture unit, Department of Marine Sciences, University of Lagos. Fish samples of average weight of 480 ± 0.99 g were used. Whole sample of each *Clarias gariepinus* was cleaned, minced, homogenized and thoroughly mixed by gloved hands with 20% finely refined sodium chloride of the weight of the fish. They were packed in sterile plastic containers with screw caps and were tightly sealed for fermentation process. All containers were incubated at room temperature (27°C) for 180 days. After which the mixture was manually drained using muslin cloth of pore size 2mm to separate supernatant fish sauce liquor sample which was stored in air-tight plastic containers and used for the analysis.

Analytical Methods of *Clarias gariepinus* sauce Proximate analysis

Moisture and protein contents analysis were determined using the procedure described in the Association of official Analytical Chemists (AOAC) (2016). Moisture content was determined by drying known weighed sauce in cleaned porcelain dish to constant weight in an oven at 110°C for 12 hours. Nitrogen was assayed using Kjeldahl method and the nitrogen content converted to protein by a multiplication factor of 6.25. The ash content was determined using the procedure described by Pearson (1976). The Soxhlet extraction method was used for the lipid content determination. The crude fibre content was determined using the procedure described by Kirk and Sawyer (1991). Carbohydrate content was determined using the method described by Pearson, (1976). Energy value of the sample was estimated (Kcal; 100g) using modified method of Vadivel and Janardhanan, (2000). Proximate analyses were carried out in triplicate and results expressed as percentage of the sauce analysed.

pH and temperature determination

The pH and temperature of the fish sauce samples were measured fortnightly in the laboratory with a pH-meter (Hanna Instrument HI 9318) and a Mercury-in-glass thermometer respectively.

Determination of minerals in ash

Standard solutions of sodium, potassium, calcium, magnesium, iron and zinc were made from the chloride salts of each of the mineral into a stock solution of 1000 ppm of the cation. A standard curve for each mineral was plotted from dilutions of 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 20.0, 40.0 ppm made from the stock solutions; each dilution of the respective mineral was aspirated into an Atomic Absorption Spectrophotometer (AAS). The corresponding readout signal was noted and used to plot the standard curves. The ash obtained from the proximate analysis was dissolved in distilled water and aspirated into the AAS and the amount of minerals in the samples corresponding to the integrator readout were determined from the respective standard curve. The determination of each mineral in the solution was done in triplicate (AOAC 2016).

Microbiological analysis

About 10 ml of sauce was transferred aseptically to 90 ml of sterile 0.1% (w/v) peptone water. Serial decimal dilutions in 0.1% peptone water were prepared and triplicate 1ml of appropriate dilutions was poured on selective agar plates. Plate count agar (PCA, Oxoid) for total bacterial count (mesophilic bacteria), and Potato-Dextrose Agar (PDA, Oxoid) for yeasts and moulds count were incubated at 37°C for 48h (FAO, 1979).

Microbiological results were converted into log₁₀ CFU/ml. Morphological characteristics of the various bacterial isolates in the agar plates were noted after microscopy. After staining reactions and several biochemical tests, individual microbial species were identified. Representative isolates were re-plated on various selective media to observe their specific colonial attributes so as to further separate the aerobic bacterial species (Okoro *et al.* 2010).

Statistical Analysis

The results of chemical composition, biochemical and microbial aspects were expressed as means ± SE. The values of the means were statistically compared using Duncan Multiple Range Test and values were considered statistically significant at P<0.05.

Results

The mean weekly moisture, protein, lipids, ash, crude fibre, carbohydrate and energy contents of the sauce are shown in Table 1. The mean moisture and crude fibre contents significantly increased (p<0.05) with time while the protein, lipid, ash and energy contents significantly decreased with time. It was only the carbohydrate content that shows no significance difference (p>0.05) in the values obtained with time.

Table 1: Proximate composition (%) of *Clarias gariepinus* sauce after fermentation at 20% salt (w/w)

		Moisture	Protein	Lipid	Ash	C/Fibre	CHO	Energy (Kcal; 100g)
Week 1	Range	57.49-63.31	20.65-24.29	3.67-6.85	3.78-7.11	0.61-3.27	2.80-6.29	146.61-171.90
	Mean ± SE	^a 60.63±0.61	^d 22.07±0.40	^c 5.49±0.32	^b 5.30±0.39	^a 1.53±0.26	^a 4.97±0.36	^c 157.55±3.35
Week 2	Range	56.91-65.46	17.93-21.67	2.53-5.46	3.51-7.42	3.09-6.29	3.44-6.47	83.44-150.85
	Mean ± SE	^a 61.12±0.84	^c 19.35±0.38	^{a,b} 4.20±0.29	^{sb} 5.31±0.46	^b 4.59±0.32	^a 5.09±0.34	^b 131.99±6.65
Week 3	Range	60.56-65.82	17.52-20.66	3.34-6.05	3.41-7.36	2.49-4.71	3.08-5.75	128.28-149.89
	Mean ± SE	^b 63.15±0.58	^c 18.68±0.34	^{a,b,c} 4.74±0.28	^b 5.29±0.43	^b 3.71±0.28	^a 4.74±0.32	^b 135.93±2.68
Week 4	Range	60.36-66.20	14.26-16.60	3.26-6.62	3.77-6.58	4.49-7.46	2.74-6.12	112.14-140.69
	Mean ± SE	^{b,c} 63.91±0.62	^b 15.11±0.28	^{b,c} 5.13±0.35	^b 5.19±0.35	^c 5.76±0.34	^a 4.82±0.38	^b 125.64±3.51
Week 5	Range	61.72-68.25	11.50-13.70	2.68-5.50	2.87-5.90	6.23-10.62	2.76-5.83	97.63-119.51
	Mean ± SE	^c 65.41±0.67	^a 12.53±0.24	^{a,b} 4.41±0.29	^{a,b} 4.33±0.34	^d 8.53±0.44	^a 4.61±0.32	^a 108.12±2.78
Week 6	Range	62.36-68.25	11.35-12.83	2.44-5.47	2.87-5.17	6.23-12.17	2.76-7.05	94.89-117.42
	Mean ± SE	^c 65.77±0.61	^a 12.01±0.19	^a 3.88±0.31	^a 3.88±0.29	^d 9.36±0.63	^a 5.14±0.45	^a 103.26±2.56

Values are mean of triplicate analyses. Mean with same superscript letter are not significantly different (P>0)

The pH and temperature values of the *Clarias gariepinus* sauce samples range were presented in Table 2. The mean pH remained virtually

Table 2: pH values and Temperature profile of *Clarias gariepinus* sauce at 20% salt (w/w)

Week		pH	Temp(C)
1	Range	6.4-8.4	22.9-23.9
	Mean	7.3	23.58
	SD	0.62	0.36
3	Range	5.6-8.4	22.9-24.0
	Mean	7.24	23.58
	SD	0.93	0.31
5	Range	6.4-7.8	23.7-24.8
	Mean	7.31	24.2
	SD	0.49	0.34
7	Range	6.0-7.0	23.6-24.2
	Mean	6.64	24.09
	SD	0.42	0.48

neutral, consequently there was no significant difference ($p > 0.05$) in the values during the storage period.

There was variation in the mineral composition of the sauce during the storage period. The mineral values of the fish sauce shown in Table 3 indicated significant difference ($p < 0.05$) within the weekly zinc values while no significant difference ($p > 0.05$) was observed in the calcium, iron, magnesium, potassium and sodium values.

The number of colonies of bacteria and fungi on each fish sauce sample is shown in Table 4. The total bacteria count of samples varied from 25.8-40.0 cfu/ml and fungi from 38-112 cfu/ml. All the fungi colonies were identified as *Candida albicans*, making it the dominant fungi in the sauce. The descriptive chart identifications and various bacteria isolates were shown in Table 5. The bacteria identified were *Micrococcus luteus*, *Bacillus species*, *Clostridium perfringens* and *Staphylococcus epidermidis*. The morphological and biochemical characteristics of the bacteria isolates were shown in Table 6. *Bacillus species* was the highest isolates in the fish sauce samples.

Table 3: Mineral ($\mu\text{g g}^{-1}$) values of *Clarias gariepinus* sauce after fermentation at 20% salt (w/w)

Week		Ca	Fe	Mg	Zn	K	Na
1	Range	0.89-2.97	14.37-74.12	2.79-4.99	0.06-0.10	0.12-0.36	0.02-0.42
	Mean \pm SE	^a 1.89 \pm 0.29	^a 39.53 \pm 8.28	^a 3.69 \pm 0.28	^c 0.08 \pm 0.01	^a 0.22 \pm 0.02	^a 0.08 \pm 0.04
3	Range	0.91-3.02	14.01-74.02	2.84-4.97	0.06-0.09	0.11-0.36	0.03-0.31
	Mean \pm SE	^a 2.03 \pm 0.32	^a 39.14 \pm 8.26	^a 3.56 \pm 0.24	^b 0.07 \pm 0.00	^a 0.21 \pm 0.02	^a 0.08 \pm 0.03
5	Range	1.01-3.00	13.99-72.57	2.00-4.67	0.04-0.07	0.02-0.41	0.02-0.11
	Mean \pm SE	^a 1.89 \pm 0.26	^a 36.29 \pm 8.18	^a 3.38 \pm 0.30	^a 0.06 \pm 0.00	^a 0.18 \pm 0.04	^a 0.06 \pm 0.01
7	Range	1.12-3.01	12.50-71.00	1.58-3.99	0.02-0.07	0.07-0.44	0.02-0.11
	Mean \pm SE	^a 1.96 \pm 0.26	^a 34.81 \pm 8.10	^a 3.03 \pm 0.26	^a 0.05 \pm 0.00	^a 0.20 \pm 0.04	^a 0.07 \pm 0.01

Values are mean of triplicate analyses. Mean with same superscript letter are not significantly different ($P > 0.05$)

Table 4: Mean count of bacteria and fungi isolated in *Clarias gariepinus* sauce samples

Samples	Mean number of colony (log ₁₀ CFU/ml)	
	Bacteria	Fungi
1	40.0±28.55	96
2	33.8±27.36	85
3	26.8±20.58	112
4	27.4±23.82	72
5	34.8±25.85	38
6	31.4±20.96	66
7	33.6±29.52	84
8	33.4±28.99	70
9	25.8±18.99	101

Table 5: Descriptive chart for bacteria identification

Macroscopic	Microscopic	Isolates
Yellowish colour	Gram+ve, catalase-ve, cocci in clusters.	<i>Micrococcus luteus</i>
Cream white	Gram+ve, catalase+ve, long and short rods.	<i>Bacillus species</i>
White in colour	Gram+ve, catalase –ve, short rods.	<i>Clostridium perfringens</i>
Milkfish white round edges	Gram+ve, catalase+ve, cocci in clusters.	<i>Staphylococcus epididemis</i>

Table 6: Morphological and biochemical characteristics of the bacteria isolates from *C.gariepinus* sauce

Samples	Gram's reaction	Catalase	Oxidase	Coagulase	Maltose	Mannitol	Sucrose	Glucose	Lactose	Xylose	Suspected organisms
Sample 1											
Isolate 1	+	-	+	-	+	-	-	-	-	-	<i>Micrococcus luteus</i>
Isolate 2	+	+	-	-	+	+	-	+	+	-	<i>Bacillus species</i>
Sample 2											
Isolate 1	+	+	-	-	+	+	-	+	+	-	<i>Bacillus species</i>
Isolate 2	+	-	-	-	+	-	+	+	-	-	<i>Clostridium perfringens</i>
Sample 3	+	-	-	-	+	-	+	+	-	-	<i>Clostridium perfringens</i>
Sample 4	+	+	-	-	+	-	-	+	+	-	<i>Bacillus species</i>
Sample 5											
Isolate 1	+	+	-	-	+	-	-	+	+	-	<i>Bacillus species</i>
Isolate 2	+	-	-	-	+	-	+	+	-	-	<i>Clostridium perfringens</i>
Isolate 3	+	-	+	-	+	-	-	-	-	-	<i>Micrococcus luteus</i>
Sample 6											
Isolate 1	+	+	-	-	+	+	+	+	+	+	<i>Staphylococcus epididemis</i>
Isolate 2	+	+	-	-	+	-	-	+	+	-	<i>Bacillus species</i>
Sample 7	+	+	-	-	+	-	-	+	+	-	<i>Bacillus species</i>
Sample 8	+	+	-	-	+	-	-	+	+	-	<i>Bacillus species</i>
Sample 9	+	+	-	-	+	-	-	+	+	-	<i>Bacillus species</i>

Discussion

The results of the proximate composition of the fish sauce agreed with those reported for moisture and protein by Ibrahim (2010) and Park *et al.* (2001) who reported that the range of proximate composition of fish sauce was 61.40-79.20% for moisture; 0.9- 13.70% for crude protein, however the values (18.20-25.80%) of ash contents were higher than the values (3.88-5.30%) reported in this study. The differences in the values of ash may be due to the species of fish used for the sauce. However, according to Ünlüsayın *et al.*, (2007) the chemical composition of traditional pates made with sea bass, sea bream and rainbow trout ranged from 56 to 59% for moisture, 15 to 18% for lipid, 13-14% for protein and 7-9 % for carbohydrate, all these were within the values obtained in this study. The difference in the moisture contents reported could be as a result of the type of salt used in fermentation, because different salt has different ability to draw out moisture from samples. The increase in the moisture content of the sauce could be as a result of the salt absorbing moisture from the surrounding and also drawing out moisture from the fish samples. The decrease in the values of the protein, lipid and ash may be as a result of the fermentation process, which involved the breaking down of the complex substances to other simpler substances.

The mean temperature of the sauce remained virtually constant being at room temperature. The almost constant neutral pH throughout the experiment indicates that the fermentation was homogenous throughout the storage period. The pH value of 6.08 reported by Ibrahim (2010) was within the values (5.6-8.4) reported in this study, but higher than the values (4.66- 5.91) reported by Cho *et al.*, (2000) and Aquerreta *et al.*, (2001). It is however in agreement with the values

reported by Ijong and Ohta (1996), Cho *et al.*, (1999) and Park *et al.*, (2001). Aquerreta *et al.*, (2001) reported that the pH values of a sauce may reflect bacterial activity during fermentation and probably a consequence of the accumulation of some basic compounds from the fermentation of nitrogen-based components. The almost constant temperature during the storage period is consistent with the room temperature and the uniform hydrolysis of the fish sample. The room temperature used in this study may aid fermentation.

The number and quantity of mineral elements in the sauce is an indication that the *Clarias gariepinus* can be used to make sauce that is fit for human consumption. The fact that there were no significant differences in the concentrations of the mineral elements with time except zinc indicated that the sauce had completely fermented.

The total microbial counts obtained from this study (Table 4) were found to be lower than the specified standard limits (1×10^5 cfu/g) for bacteria and fungi and 1×10^2 cfu/g for coliforms) by the International Commission on Microbiological Specification for foods (ICMSF) (2011) and the United States Food and Drug Administration (USFDA) (1991). Some of the species of bacteria found in these samples were also reported in the work of Sangjindavong *et al.*, (2009) on fish sauce from surimi waste using pineapple. The micro-organisms might have contributed to the hydrolysis of proteins as well as possible flavour development of the sauce. The organism with the highest count isolated in this study was *Bacillus cereus* (Tables 5 and 6). This is a common air and dust borne contaminant that multiplies readily in foods. The spores survive short periods of cooking and reheating, when the food is stored at room temperature, the spores germinate and release enterotoxins, but care can be taken to prevent contamination by sterilizing all materials used for the sauce.

The variation in the bacterial count in this study (Tables 5 and 6) could be as a result of the quantity of salt, temperature, age of the fish, environment from where the fish was obtained and the period of fermentation. The study also revealed the presence of *Candida albicans*, a ubiquitous fungus, which may have been introduced during preparation of the fish sauce, the absence of other species may be due to unfavourable environmental condition for their development.

Available literature on fish sauce is scarce, this does not allow for adequate comparison of the results obtained in this investigation with other works. However, it was observed that the African catfish sauce contains important protein and lipid contents; it is also rich in minerals and contained moderately halophilic bacteria that help in the breakdown of the chemical constituents of the fish. It is also safe for human consumption, because of the non-pathogenic microorganisms present in the sauce, therefore the sauce can contribute to protein availability for the populace.

Conclusion

In Nigeria, culturing of African catfish is gaining ground to support food security and availability of protein for increasing human population. Parameters generated from this study can be utilized to support fish sauce production, so that consumers can use them in their diets.

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