African Journal of Food Science Vol. 6(14), pp. 381-385, 29 July, 2012
Available online http://www.academicjournals.org/AJFS
DOI: 10.5897/AJFS12.039
ISSN 1996-0794 ©2012 Academic Journals

Full Length Research Paper

The influence of fermentation and salting on the bacterial, chemical and sensory characteristics of catfish *(Clarias buthupogon)* based marinate in Nigeria

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Accepted 13 June, 2012

Catfish *(Clarias buthupogon)* was treated with 10% w/w NaCl and 15% w/w NaCl with or without the addition of spices (1% w/w garlic powder and 1% w/w red pepper) and allowed to ferment naturally and at room temperature (28 ± 2℃) for two weeks. Bacte rial, chemical and sensory analyses were carried out on the unfermented non-salted and fermented salted samples of the fish species. Results obtained revealed that fermentation and salting caused a remarkable reduction in the total bacterial count from 2.29×10^6 to 3.80×10^5 cfu/g. There was also reduction in pH from 7.29 to 5.26. The predominant bacteria isolated from the samples were *Lactobacillus* sp*, Staphylococcus* spp for the unfermented non salted and fermented salted samples respectively while total titratable acidity increased from 0.63 to 1.44%. Chemical analysis result showed that fat and ash contents increased with fermentation and salting while moisture, protein and Nitrogen free Extracts (NFE) contents decreased with fermentation and salting of *C. buthupogon*. Free fatty acid, peroxide value and total volatile base (TVB) also showed increase with fermentation. Peroxide value ranged from 5.22 mEq/kg for unfermented non-salted to 8.43 mEq/kg for fermented 15%w/w salted products while total volatile base increased from 5.63 mgN/100 g unfermented non-salted to 16.85 mgN/100 g for fermented 10%w/w salted products. Sensory analysis indicates that stew containing fermented samples treated with 10%w/w NaCl and spices were more preferred than others.

Key words: Catfish, microbial, chemical, sensory, marinate, fermentation.

INTRODUCTION

Fermented fish have for many years been considered as a value added product. However, since the raw fish are sometimes from poor quality or underutilized species which are very cheap, product price are affordable to many low income consumers. Some examples of fermented fish products include Shushi of Japan, Patis of Philippines and Budu of Malaysia (Klinic, 2003).

It is obvious that some fish species in Nigeria especially in the coastal areas are of smaller size and are also underutilized. These ranges from by-products in sea fishing to the stunted Tilapia which are often discarded in fish farms because of their numerous bones and small sizes which render them of little acceptance for human consumption, thus posing the problem of post-harvest losses (Eyo, 2001). Furthermore, nutritional losses also occur due to spoilage or exposure of fish to high temperature during smoking especially that of traditional hot smoking and fish burning.

Considering the fact that these underutilized fish species, including Tilapia (*Chromidotilapia guentheri*) and Catfish (*Clarias buthupogon*) are readily available and abundant in the Eastern part of Nigeria, they could be transformed into fermentable products which are far more stable, safer, enjoyable and with improved shelf life than the raw material.

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Though fish fermentation is not common and really appreciated in Nigeria, the benefit inherent in the consumption of this product will be enormous, more especially in salvaging the fish farmer and marketers from post-harvest losses. The objective of this work is to improve the utilization of unconventional fish species through fermentation for human consumption and to determine the bacterial, proximate and chemical properties of the product. The sensory properties of stew prepared with the products were also assessed.

MATERIALS AND METHODS

Collection of samples

The fish species *C. buthupogon* used in this study were bought fresh from Imo River Market, Owerrinta, Abia State, stored in plastic coolers containing ice cubes and transported to the laboratory. The size ranged between 7.6 to 17.3 cm.

Preparation of samples

The fishes were beheaded, gutted, scaled and thoroughly washed in running water and divided into five triplicate portions of 100 g each. One portion was unfermented non salted fresh fish (control). To the second portion, 10% w/w of sodium chloride was added while the third had 15% w/w of NaCl added to it. The fourth portion was treated with 10% w/w NaCl and spices (1% w/w red pepper and 1% w/w garlic powder).

The fifth portion was treated with 15%w/w NaCl and spices (1%w/w red pepper and 1% garlic powder) .The spices were ground and added to the fish portions respectively. The treated samples apart from the fresh fish (unfermented and untreated) were placed in plastic containers, well covered and allowed to ferment at room temperature (28± 2℃) for two weeks. The pH, total titratable acidity and total viable counts were determined on the raw (fresh) samples. The pH, titratable acidity and other microbiological analysis were carried out weekly on the fermented samples. After two weeks, the fermented samples were dried in an oven at 65℃ for 24 h, milled and stored in plastic containers. Portions of the ground samples were used for further microbiological, chemical and sensory analyses.

Microbiological analysis

Isolation and enumeration of heterotrophic bacteria was carried out using the pour plate method as described by Ezeama (2007). Using the taxonomic schemes as described by Cowan (1985), the isolates were characterized and identified. Gram staining, spore staining and motility tests were carried out for the microscopic characterization of the isolates while the following biochemical tests: coagulase, catalase, citrate utilization, oxidase, methyl red, Voges Proskauer and carbohydrate fermentation tests were carried out according to methods described by Cruickshank et al. (1975).

Chemical analysis

Free fatty acid content, peroxide value and total titratable acidity of the unfermented and fermented fish were determined using AOAC (2000) method while total volatile base content was determined by volumetric method for the determination of volatile bases in fish as described by Pearson (1976).

Proximate composition

Moisture content, crude protein determination using Kjeldahl method as described by AOAC (2000) was used. The ash and lipid contents were determined by AOAC (2000) while crude fibre was estimated using AOAC (2000) method as modified by Okon (2005). The “Difference” method was used to estimate the percentage Nitrogen free extract of each of the fish samples.

Sensory evaluation

The products incorporated into blank stew (without salt and spices) where subjected to sensory parameters of odour, saltiness, colour, taste and overall acceptability by 10 semi-trained panelists using a 5-point hedonic scale were 5= excellent, 4= very good, 3= good, 2= fair and 1= poor as described by Achinewhu and Oboh (2002).

Statistical analysis

Analysis of variance (ANOVA) in completely randomized design was performed on the data obtained using SPSS (2006). Significant means were compared at 5% probability level using Duncan’s Multiple Range Test (DMRT) as provided in the same SPSS (2006).

RESULTS AND DISCUSSION

Table 1 shows the total bacterial count, pH and total titratable acidity (TTA) of unfermented non-salted and fermented salted samples of *Clarias buthupogon*. The total bacterial count for unfermented non-salted *C. buthupogon* was 2.29×10^6 cfu/g. The bacterial load reduced to 3.80×10^5 cfu/g at the end of fermentation. This was attributed to the reduction in pH during fermentation. The alteration in pH affected enzyme function and created an acidic environment destructive to some bacteria. Erichen (1983) had stated that the two main factors controlling food poisoning bacteria in fermented fish product were high salt concentration and low pH values. It was also observed that the fermented samples with 15% w/w NaCl and spice (red pepper and garlic powder) had relatively low bacterial count. There was remarkable reduction in bacterial count at the end of fermentation. A reduction in pH from 7.29 to 5.26 was observed in the unfermented non salted and fermented salted samples of the fish product. Consequently, there was corresponding increase in total titratable acidity from 0.63 to 1.44 for unfermented non salted and fermented salted samples respectively. These could be attributed to the utilization of carbohydrate in the medium by *Lactobacillus* sp thus producing lactic acid strong enough to reduce the pH and increase the TTA. The bacterial count, pH and total titratable acidity showed significant difference ($P<0.05$) in the unfermented non salted and fermented salted products respectively. Table 2 shows the spatial occurrence of bacterial isolates in the fermented catfish (*C. buthupogon*). Results revealed that though *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus* sp, *Lactobacillus* spp and

Table 1. Total bacterial count, ph and total titratable acidity of fermented catfish (*C. buthupogon*).

Sample	Total bacterial count (CFU/g)		pH		TTA (%) lactic acid	
	Fermentation period (weeks)					
	Week1	Week 2	Week 1	Week 2	Week 1	Week 2
Unfermented non-salted fish	$2.29\pm10^{a}\times10^{6}$	–	7.29 ± 0.06^{a}	–	0.63 ± 0.11	–
A	$1.85\pm0.0.5^{b}\times10^{6}$	$1.38\pm0.06^{b}\times10^{6}$	5.62 ± 0.08^{b}	5.47 ± 0.05^{b}	1.04 ± 0.05^{a}	1.44 ± 0.04^{a}
B	$6.6\pm0.14^{c}\times10^{6}$	$4.9\pm0.11^{c}\times10^{6}$	5.23 ± 0.05^{c}	5.29 ± 0.05^{c}	1.06 ± 0.09^{a}	1.37 ± 0.05^{a}
C	$1.66\pm0.14^{b}\times10^{6}$	$1.30\pm0.00^{b}\times10^{6}$	5.46 ± 0.09^{bc}	5.32 ± 0.00^{bc}	1.06 ± 0.19^{a}	1.33 ± 0.07^{a}
D `	$6.3\pm0.06^{c}\times10^{5}$	$3.80\pm0.08^{c}\times10^{5}$	5.21 ± 0.09^{c}	5.26 ± 0.03^{c}	1.05 ± 0.16^{a}	1.31 ± 0.09^{a}

Mean on the same row with different superscripts are significantly different ($p<0.05$). Values are means of two independent determinations, sample means ± SEM, A=Fermented fish + 10% NaCl, B=Fermented fish + 10% NaCl + 1% garlic powder + 1% red pepper, C=Fermented fish + 15% NaCl, D=Fermented fish + 15% NaCl + 1% garlic powder + 1% red pepper, TTA= Total titratable acidity.

Table 2. Spatial occurrence of bacterial isolates in fermented catfish (*C. buthupogon*).

Isolated organisms	Week 1 fermented sample				Week 2 fermented sample				
	UNS	A	B	C	D	A	B	C	D
S. aureus	+	+	-	+	+	+	-	+	+
S. epidermidis	+	+	+	+	+	+	+	+	-
Bacillus sp	+	+	+	-	-	-	-	-	-
Lactobacillus spp	+	+	+	+	+	+	+	+	+
Proteus sp	+	-	-	-	-	-	-	-	-

A= Fermented fish 10% NaCl, B= Fermented fish 10% NaCl + 1% garlic powder + 1% red pepper, C= Fermented fish + 15% NaCl, D= Fermented fish + 15% NaCl + 1% garlic powder + 1% red pepper, UNS =Unfermented and non-salted.

Table 3. Proximate composition (%) of fermented catfish (*C. buthupogon*).

Nutrients (%)	Fish samples				
	A	B	C	D	E
Moisture	77.57 ± 0.04^{a}	74.52 ± 1.13^{b}	74.46 ± 1.23^{b}	73.25 ± 1.57^{b}	73.30 ± 1.45^{b}
Protein	69.56 ± 0.21^{a}	67.83 ± 0.12^{b}	67.25 ± 0.62^{b}	65.60 ± 0.32^{c}	64.60 ± 0.26^{c}
Fat	12.30 ± 0.44^{a}	14.85 ± 0.11^{a}	14.20 ± 0.47^{a}	14.38 ± 0.50^{a}	14.40 ± 0.22^{a}
Ash	11.85 ± 0.13^{a}	11.55 ± 0.32^{b}	12.05 ± 0.50^{b}	13.72 ± 0.13^{a}	13.74 ± 0.24^{a}
NFE	8.39 ± 0.38^{a}	5.85 ± 0.49^{b}	6.51 ± 0.50^{a}	6.30 ± 0.55^{a}	7.26 ± 0.39^{a}

Mean on the same row with different superscripts are significantly different ($p<0.05$). Values are means of two independent determinations, Sample means ± SEM, A=Non-salted unfermented, B=Fermented fish + 10% NaCl, C=Fermented fish + 10% NaCl + 1% garlic powder + 1% red pepper, D=Fermented fish + 15% NaCl, E=Fermented fish + 15% NaCl + 1% garlic powder + 1% red pepper, NFE= Nitrogen free extract.

Proteus sp were isolated from the unfermented non salted samples of the product, the growth of *Proteus* was not observed during fermentation probably due to fermentation stress and environment. *Bacillus* sp growth was sparingly observed during the fermentation. *Lactobacillus* spp*, S. aureus* and *S. epidermidis* were the predominant bacterial isolate during the first week of fermentation. During the second week of the fermentation, the growth of *Bacillus* sp was no longer observed on the fermenting samples of catfish *(C. buthupogon)* probably due to the changing brine environment. However, *Lactobacillus* spp, *S. epidermidis* and *S. aureus* were consistently observed in the fermenting samples. *S. aureus* according to Oetterer *et al*. (2003) is quite resistant to high salinity (salt tolerant), surviving in media with up to 20% of NaCl.

Table 3 shows the proximate composition of fermented catfish (*C. buthupogon*). The moisture content ranged between 73.30% and 77.57% for the fermented 15%w/w salted and unfermented non salted samples of the product receptively, showing a decrease with fermentation and salting. This decrease may be attributed to the

Table 4. Free acid peroxide value and total volatile base of fermented catfish (*C. buthupogon*).

Parameter	Fish samples				
	A	B	C	D	E
Free fatty acid (%)	9.76±0.15^{c}	11.29±0.45^{a}	10.88 ± 0.60^{b}	10.68 ± 0.14^{b}	9.45 ± 0.14^{d}
Peroxide value m/Eq/kg	5.22±0.10^{e}	8.02±0.11^{b}	6.83±0.09^{d}	8.43±0.10^{a}	7.65±0.05^{c}
Total volatile base (MgN/100 g)	5.63±0.10^{e}	16.85±0.05^{a}	16.28±0.06^{b}	12.63±0.11^{c}	11.23±0.11^{d}

Mean on the same row with different superscripts are significantly different ($p<0.05$). Values are means of two independent determinations, Sample means ± SEM, A=Non-salted unfermented, B=Fermented fish + 10% NaCl, C=Fermented fish + 10% NaCl + 1% garlic powder + 1% red pepper, D=Fermented fish + 15% NaCl, E=Fermented fish + 15% NaCl + 1% garlic powder + 1% red.

action of salt which reduced the water activity of the fish tissues through osmosis, as the salt extracted water from the cells the osmotic affinity of the dissolved chloride, minerals, sugars, amino acids and other solutes in the cell increased resulting in a two way transport. Since the salt sets more osmotic pressure than the cell liquor, there was loss in weight of the salted fish due to the uptake of moisture (Eyo, 2001). These findings agreed with the work of Achinewhu and Oboh (2002) who showed that fermented fish product from *Sardinella* sp in Nigeria had slightly lower moisture content that the unfermented due to salt driving out water. There was significant difference ($p>0.05$) in the moisture content between the unfermented non-salted and fermented salted samples of *C. buthupogon.*

There was significant reduction in crude protein from 69.56% for the unfermented to 64.60% for the fermented 15%w/w salted samples. Fermentation and salting may have been responsible for the decrease. This finding disagreed with the work of Achinewhu and Oboh (2002) who showed a slight increase in crude protein from 16 to 18% during fermentation of *Sardinella* sp. Thus, the significant crude protein decrease could also be attributed to the activities of proteolytic enzymes and differences in species of fish used. Visessanguan *et al*. (2004) had reported that the protein and oil contents of fish species vary widely from species and with other factors. Eyo (1991) also in his work showed a decrease in crude protein of fermented fish due to the action of proteolytic enzymes. There was significant difference ($p<0.05$) in crude protein content of fermented salted and unfermented non-salted samples of the product. The products also showed significant increase in lipid from 12.30 for the unfermented non salted to 14.85% for the fermented salted samples respectively. This increase could be attributed to dehydration as reported by Oetterer *et al*. (2003) in monitoring the sardine *(Sardinella brasiliensis)* fermentation to obtain anchovies. This findings however disagreed with the work of Achinewhu and Oboh (2002) who showed no difference between the lipid content of unfermented and fermented fish product from *Sardinella* sp. It also disagreed with the work of Eyo (2001) who showed a considerable reduction in lipids by fermentation of fish product. This therefore implies that the lipid content of fermented fish product varies widely with species of fish. Increase in ash content (11.85 to 13.74%) was observed with the sample treated with 15% w/w NaCl and even without spices. This could be attributed to salt uptake during fermentation as reported by FAO (1992). This finding agreed with the work of Oetterer *et al*. (2003) who showed that increase in ash content was directly related to the presence of salt in sardine muscle.

Table 4 shows the result of the free fatty acid, peroxide value and total volatile base (TVB) content of catfish *(C. buthupogon).* The peroxide value increased with fermentation with value ranging from 5.22 to 8.43 mEq/kg for the unfermented non-salted and fermented salted samples respectively. Since peroxide value is used to estimate the degree of rancidity, the product is within acceptable limit as values corresponding to incipient spoilage are usually in the order of 20-40 mEq/kg of sample (Eyo, 2001). Furthermore, the high salt concentration may have decreased the solubility of oxygen thereby retarding fat oxidation. The significant increase from 9.76 to 11.29% for the unfermented non-salted to fermented 10%w/w salted samples of the product observed in free fatty acid content may be attributed to the effect of salt and the curing mixture (garlic and pepper) that were used as spices. This agrees with the work of Achinewhu and Oboh (2002). However, salting up to 15%w/w had significant decreas on free fatty acid of the product. There also existed significant difference ($p<0.05$) in TVB between the unfermented non-salted (5.63 mgN/100 g) and fermented salted samples (16.85 mgN/100 g) of the product. The values were in agreement with the work of Klinic (2003). The results obtained from the fresh samples were good indices of the freshness of the raw materials.

Table 5 shows the sensory evaluation of unfermented and fermented catfish *(C. buthupogon)* incorporated into blank stew. The result revealed that stew containing fermented samples treated with 10% NaCl and spices (1% w/w garlic powder and 1% w/w red pepper) as condiment were preferred. These samples had significantly higher scores in all parameters tested irrespective of the NaCl content. This was attributed to the use of garlic and red pepper (spice) during the fermentation. Achinewhu

Table 5. Mean sensory score of stewed catfish (*C. buthupogon*).

Sensory attributed	Fish samples				
	A	B	C	D	E
Colour	3.45±0.40^{c}	3.38±0.10^{c}	3.86 ± 0.09^{b}	3.82 ± 0.16^{b}	4.37 ± 0.85^{a}
Odour	2.36±0.10^{c}	3.67±0.12^{ab}	3.14±0.25^{b}	3.69±0.11^{ba}	4.15±0.26^{a}
Saltiness	3.39±0.07^{b}	3.77±0.05^{a}	2.62±0.00^{c}	3.75±0.10^{a}	2.07±0.15^{d}
Taste	3.09±0.20^{a}	3.49±0.09^{a}	2.51±0.19^{b}	3.54±0.00^{a}	2.21±0.01^{b}
Overall acceptability	3.08±0.14^{b}	4.03±0.19^{a}	3.06±0.21^{b}	3.83±0.11^{a}	1.44±0.11^{c}

Mean on the same row with different superscripts are significantly different ($p<0.05$). Values are means of two independent determinations, Sample means ± SEM, A=Stew with fermented fish + 10% NaCl, B=Stew with fermented fish + 10% NaCl + 1% garlic powder + 1% red pepper, C=Stew with fermented fish + 15% NaCl, D=Stew with fermented fish + 15% NaCl + 1% garlic powder + 1% red pepper,E=Stew with unfermented non-salted fish.

and Oboh (2002) had earlier stated that the use of locally available spices in fermentation of fish will no doubt enhance food flavour as well as provide some antibacterial effect on the fermented product.

Conclusion

This research work reveals that fermentation combined with salting, spicing and or drying as methods of production of fermented fish product would help to salvage the fish farmers and marketers from post harvest losses since small sized, underutilized, by catches, sales left over could easily be converted to fermented products under controlled and hygienic environment. It has proved that salting, spicing and drying could be used as processing and preservation methods to extend the shelf-life of fermented fish product. Since microorganisms are the major cause of fish spoilage, the salt used inhibited the growth of the non-salt tolerant, putrefying bacteria while allowing the growth of the halophilic ones. Spicing also enhanced the flavour of the fermented fish product thus solving the problem of taste complained by many consumers. However, depending on the degree of fermentation, fermented fish products are not eaten as such but are used either as condiment to enhance food flavour or as source of protein.

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