



## Influence of Brine Salting on the Quality Attributes of Smoke-dried Catfish (*Clarias anguillaris*) Stored at Ambient Temperature

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**ABSTRACT:** Salting is a method of fish preservation in many countries of the world. It can be used in combination with drying or smoking. Salting of fish removes water and lowers the water activity, thereby reducing the water available to support microbial growth which causes spoilage. The objective of this study is to investigate the influence of brine salting on the quality attributes of smoke-dried catfish (*Clarias anguillaris*) stored at ambient temperature. The quality attributes of the smoke-dried fish stored for 0-6 weeks at ambient temperature ( $28 \pm 2^\circ\text{C}$ ) were evaluated. Smoking was done using a traditional kiln, with charcoal as the source of energy. The proximate composition, total volatile base nitrogen (TVB-N), peroxide value (PV), microbiological and sensory characteristics of smoked catfish were assessed using standard methods. The smoking process reduced the moisture content of catfish from 73.6 to 8.65%, and increased the protein, fat, and ash contents significantly ( $P \leq 0.05$ ). The TVB-N of unsalted catfish increased from 4.30 to 36.4mg N/100g within 6 weeks of storage while the PV increased from 1.61 to 24.0 meq g/kg. The TVB-N and PER values for unsalted smoked fish were within the acceptable limit at 4 weeks of storage, but exceeded the limit at 5 weeks. Brine salting reduced the total viable count (TVC) and yeast and mould count (YMC) of the smoked product; the extent of reduction depended on the concentration of brine. A concentration of 6% brine was found to be a suitable level for catfish. The bacteria isolated from the smoked fish samples belonged to the genera *Bacillus*, *Klebsiella*, *Lactobacillus*, and *Staphylococcus* while the fungi belonged to *Aspergillus*, *Fusarium* and *Penicillium*. Sensory scores showed that unsalted smoked catfish stored from 0 to 4 weeks, and the brine salted samples stored from 0 to 6 weeks, had good quality.

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*Clarias anguillaris* is a species of catfish that is widely consumed in Nigeria. It is one of the two clariid catfishes preferred in the country because of their large sizes; the other species being *Clarias gariepinus* (Offem *et al.*, 2010). Fish is a good source of high-quality protein, vitamins, minerals and fat. However, fish deteriorates readily after capture due to microbial and enzymatic activity. Post-harvest losses have been estimated at 20 – 50% of domestic fish catch in Nigeria (Eyo, 2001). Therefore, the use of proper handling practices, prompt application of preservation techniques and suitable storage conditions are required

to reduce losses. Several methods are available for fish preservation, including smoking, drying, freezing and canning. Fish smoking is an age long practice that is being used globally. The fish may be smoked with or without salting. The temperatures used during smoking are sufficiently high to obtain smoke-dried fish that is cooked and has a desirable flavor. The preservative effect of smoking is due to a combination of dehydration, and antimicrobial and antioxidant activities of compounds present in smoke, such as formaldehyde, phenol and carboxylic acids (Doe, 1998). Smoking is a commonly used method in

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Southern Nigeria. The process involves the use of traditional, and improved smoking kilns and the fish is mostly smoked without salting. George *et al.*, (2014) reported that only 29.9% of smoked fish assessed in Lagos State was salted while 69.1% was smoked without salting. Fish can be salted with dry salt or a salt solution (brine). The addition of salt (sodium chloride) to fish enhances flavour and also has a preservative role. Although smoking preserves fish, the quality of smoked fish decreases during storage, due to the decomposition of components by microorganisms, enzymes and fat oxidation (Brainerd and Junianto, 2021). Smoked fish is often stored and marketed under conditions that favour absorption of moisture from the atmosphere. The product is not packaged and is kept at ambient temperature. A moisture content exceeding 12% in fish products allows moulds to grow within a few days (FAO/PHCA, 1989). Information on the quality of salted smoke-dried fish is lacking in the literature. Hence, the objective of this study is to investigate the influence of brine-salting on the quality attributes of *Clarias anguillaris* stored at ambient temperature.

## MATERIALS AND METHODS

**Sample Collection:** Catfish (*Clarias anguillaris*) of  $400 \pm 30$ g weight was purchased fresh from Agbura market in Agbura, Bayelsa State. The fish was collected in sterile polythene bags and transported on ice to the laboratory.

**Sample Preparation:** The fish was gutted, washed and divided into eight portions. Fish in one portion was not smoked, and fish in a second portion was smoked without salting. The other six portions were immersed separately in sodium chloride solution of varying concentrations (2 – 15%) for 30 min at  $28 \pm 2^\circ\text{C}$ , drained and smoked. Smoking was done in a traditional steel kiln (drum) for 6 hours, with charcoal as the source of energy. The fish was turned at intervals during smoking to obtain a uniformity smoked product. The catfish samples were stored at ambient temperature ( $28 \pm 2^\circ\text{C}$ ) for 0-6 weeks.

**Microbiological Analysis:** Ten grammes of fish muscle was homogenized with 90ml sterile distilled water for 3 min using a Kenwood blender. Tenfold serial dilutions were prepared and pour-plated in nutrient agar (NA) and potato dextrose agar (PDA) for bacterial and fungal growth, respectively. NA plates were incubated at  $37^\circ\text{C}$  for 24 hr and PDA plates at ambient temperature ( $28 \pm 2^\circ\text{C}$ ) for 3 – 5 days, and the colonies were counted. Bacterial and fungal isolates were subcultured and preserved in NA and PDA, respectively. Bacteria were identified based on morphology and biochemical tests (Holt *et al.*, 1994).

Yeasts and moulds were identified based on cultural and microscopic characteristics (Barnet and Barry, 1972).

**Chemical Analysis:** Proximate composition of the fish samples was determined by standard AOAC methods (1990). Total volatile base nitrogen (TVB-N) was determined using the magnesium oxide method (AOAC, 1990).

Peroxide value (PV) was determined volumetrically, as described by Pearson (1981).

**Sensory Evaluation:** The colour, aroma, texture and general appearance of the smoked fish samples were evaluated by 20 panelists who were familiar with the product. A modification of the scoring system of Achinewhu and Oboh (2002) was used, where 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = unacceptable.

**Statistical Analysis:** The data obtained was analyzed with SPSS statistical software (Version 19) using analysis of variance (ANOVA), means were analyzed using paired samples t-test.

## RESULTS AND DISCUSSION

**Biochemical Attributes:** The proximate composition of fresh, and unsalted smoked catfish (*Clarias anguillaris*) is presented in Table 1. The protein, fat and ash contents of the smoked fish were significantly higher ( $P \leq 0.05$ ) than the values for fresh fish. The moisture content of the fish reduced from 73.6% to 8.65% due to the smoking process. These changes in proximate composition were expected since the loss of moisture during smoking caused an increase in dry matter.

**Table 1:** Proximate composition (w/w%) of fresh and unsalted smoked catfish (*Clarias anguillaris*)

Component	Fresh catfish	Freshly smoked catfish (unsalted)
Moisture (%)	73.6 <sup>a</sup>	8.65 <sup>b</sup>
Protein (%)	18.4 <sup>b</sup>	52.5 <sup>a</sup>
Fat (%)	4.60 <sup>b</sup>	25.7 <sup>a</sup>
Ash (%)	2.80 <sup>b</sup>	9.60 <sup>a</sup>

Means with the same superscript within a row are not significantly different ( $P \leq 0.05$ ).

The moisture content of unsalted smoked fish increased during storage and reached 15.2% at 3 weeks (Table 2). This level of moisture exceeds 12%, which allows the growth of moulds (FAO/PHCA, 1989). The increase in moisture content was more pronounced during the first 3 weeks of storage, and slowed down from the 4<sup>th</sup> week. The total volatile base nitrogen (TVB-N) level in the unsalted freshly smoked fish was 4.30 mgN/100g. During storage at ambient

temperature the TVB-N increased to 27.3 mgN/100g at 4 weeks, and exceeded the acceptable limit of 30 mgN/100g EC, 2008) by the 5<sup>th</sup> week. The increase in TVB-N was more rapid in the 1<sup>st</sup> week of storage. It has been reported that the smoking process increased TVB-N levels in fish (Nahid *et al.*, 2013, 2016). These volatile compounds are produced by hydrolysis of proteins into simpler compounds such as ammonia, trimethylamine and free amino acids (Viji *et al.*, 2014). Volatile compounds such as ammonia and trimethylamine can have adverse effects on the sensory properties of the product. Apart from the smoking process, TVB-N is affected by the activity of bacteria. Therefore, this parameter is a useful fish spoilage indicator that is related to bacterial spoilage (Connell, 1995). The peroxide value (PV) of the smoked fish samples also increased significantly ( $P \leq 0.05$ ) during storage. PV is a useful indicator of fat oxidation (rancidity) in food. The acceptability limit for PV of fish oil is 20 meq O<sub>2</sub>/kg (Connell, 1995). The PV of smoked catfish exceeded this limit slightly by the 5<sup>th</sup> week of storage. Based on the TVB-N and PV values, unsalted smoked catfish was acceptable up to the 4<sup>th</sup> week of storage.

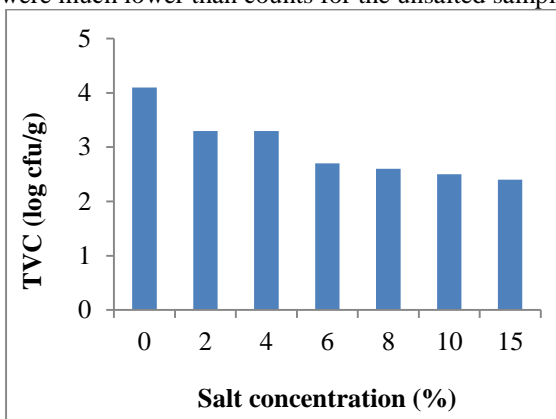
**Table 2:** Changes in the biochemical properties of unsalted smoked catfish (*Clarias anguillaris*) during storage at ambient temperature (28 ± 2°C).

Storage time (weeks)	Moisture content (%)	Total volatile base-nitrogen	
		(mg N/100g)	(meq g/kg)
0	8.65 <sup>e</sup>	4.30 <sup>g</sup>	1.61 <sup>h</sup>
1	10.1 <sup>d</sup>	13.1 <sup>f</sup>	3.80 <sup>f</sup>
2	12.0 <sup>c</sup>	18.1 <sup>e</sup>	6.61 <sup>e</sup>
3	15.2 <sup>b</sup>	22.0 <sup>d</sup>	10.0 <sup>d</sup>
4	16.5 <sup>a</sup>	27.3 <sup>c</sup>	13.2 <sup>c</sup>
5	16.7 <sup>a</sup>	34.2 <sup>b</sup>	21.3 <sup>b</sup>
6	17.0 <sup>a</sup>	36.4 <sup>a</sup>	24.0 <sup>a</sup>

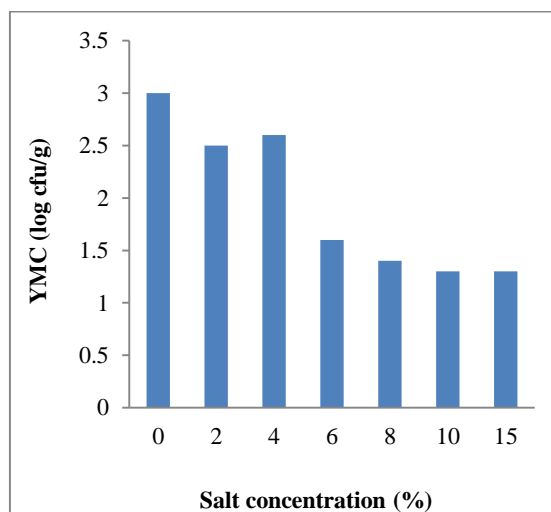
Means with the same superscript within a column are not significantly different ( $P \leq 0.05$ ).

**Microbiological Attributes:** The effect of brine-salting on the microbial load of smoked catfish is shown in Figs 1 and 2. Salting reduced the total viable count (TVC), and the yeast and mould count (YMC) of smoked catfish. Generally, the higher the salt concentration, the lower the microbial counts. The TVC for brine salted smoked fish reduced from 4.08 to 2.41 logcfu/g and YMC from 3.00 to 1.32 logcfu/g as the salt concentration increased from 2 to 15%. The reductions in TVC and YMC exceeded 1 log unit at 6% brine salting, after which the reduction was minimal. A brine concentration of 6% was therefore considered suitable for application to fish prior to smoking. The changes in TVC of unsalted and 6% brine-salted catfish during storage are shown in Fig 3. The counts increased steadily till the 6<sup>th</sup> week of

storage but the counts for the 6% brine-salted catfish were much lower than counts for the unsalted sample



**Fig. 1:** Effect of salt concentration on the total viable count (TVC) of smoked (*Clarias anguillaris*) stored at ambient temperature (28±2°C) for one week.



**Fig. 2:** Effect of salt concentration on the yeast and mould count (YMC) of smoked (*Clarias anguillaris*) stored at ambient temperature (28±2°C) for one week.

While the unsalted sample had a TVC of 5.51 logcfu/g at 4 weeks of storage, the brine-salted sample had a lower TVC (3.82 logcfu/g) at 6 weeks. These counts are below the maximum recommended bacterial count (5.7 logcfu/g) for good quality food products (ICMSF, 1989). The microorganisms isolated from fresh and stored smoked catfish samples are shown in Table 3. The predominant bacteria and fungi isolated from fresh, and unsalted smoked fish belonged to 3 genera each. The 6% brine-salted fish had bacteria and fungi belonging to 2 genera each. *Bacillus* and *Staphylococcus* species were present in the fresh and unsalted smoked fish samples. *Bacillus* species was also isolated from the 6% brine-salted fish but *Staphylococcus* species was not. Bacteria in these two genera were reported to be the most predominant

bacteria in smoke-dried seafood marketed in Port Harcourt, Rivers State (Aminigo and Okoro, 2002).

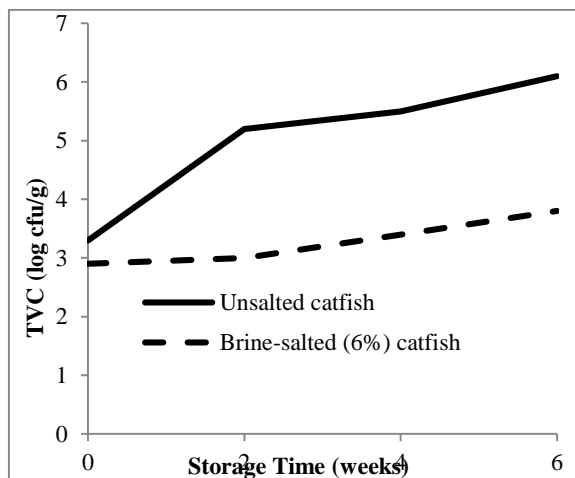


Fig. 3: Total viable count (TVC) of smoked *Clarias anguillaris* stored at ambient temperature (28±2°C).

*Pseudomonas* species were also isolated from fresh catfish. Bacterial species in this genus have been reported to be frequently associated with fish (Cahill, 1990). Other bacteria isolated from smoke-dried catfish were *Klebsiella* and *Lactobacillus* species. The moulds, *Aspergillus* species and *Fusarium moniliformis* were isolated from the smoked fish samples stored for 6 weeks. *Penicillium* species were isolated from the fresh and unsalted smoked fish. Some species of fungi belonging to these three genera (i.e. *Aspergillus*, *Fusarium* and *Penicillium*) have been reported in smoked fish sold in Southern Nigeria (Aminigo and Okoro, 2002; Adelaja *et al.*, 2013). A yeast belonging to the genus *Candida* was found in fresh fish but not in the smoked samples. Certain species of bacteria and fungi that belong to the genera identified in this study are pathogenic to humans. It is therefore important that fresh catfish intended for smoking should be of good microbial quality, and the smoked products should be stored properly to minimize microbial contamination.

Table 3: Predominant bacteria and fungi isolated from smoked *Clarias anguillaris* stored at ambient temperature (28±2°C) for 6 weeks

Sample	Storage Time (weeks)	Bacteria	Fungi
Fresh catfish	0	<i>Bacillus</i> spp <i>Staphylococcus</i> spp <i>Pseudomonas</i> sp	<i>Aspergillus</i> spp <i>Penicillium</i> sp <i>Candida</i> sp
Unsalted smoked fish	6	<i>Bacillus</i> spp <i>Lactobacillus</i> sp <i>Staphylococcus</i> spp	<i>Aspergillus</i> spp <i>Fusarium moniliformis</i> <i>Penicillium italicum</i>
Brine salted (6%) smoked catfish	6	<i>Bacillus</i> sp <i>Klebsiella</i> sp	<i>Aspergillus</i> spp <i>Fusarium moniliformis</i>

**Sensory Attributes:** The highest scores for colour, Odour, texture and general appearance were generally obtained for freshly smoked catfish, and the scores reduced gradually during storage (Table 4). The scores for unsalted catfish at 4 weeks of storage were generally above 3.0 (fair), and generally below 3.0 at 6 weeks of storage. This is in agreement with the findings of Akise *et al.* (2013) that smoked fish stored

at ambient temperature was marginally acceptable by the 6<sup>th</sup> week. The sensory scores for 6% brine-salted smoked fish were similar for the 4<sup>th</sup> and 6<sup>th</sup> weeks of storage; 3.2-3.7 and 3.2-3.5, respectively. This indicates that unsalted smoked catfish was of good quality up to 4 weeks of storage, and 6 weeks for the 6% brine salted sample.

Table 4: Sensory attributes of smoked catfish (*Clarias anguillaris*) stored at ambient temperature (28±2°C).

Sample	Storage time (weeks)	Colour	Odour	Texture	General Appearance
Unsalted smoked catfish	0	3.8 ± 0.2	3.6 ± 0.4	3.5 ± 0.2	3.2 ± 0.3
	1	3.5 ± 0.5	3.2 ± 0.4	3.5 ± 0.5	3.0 ± 0.7
	2	3.6 ± 0.3	3.6 ± 0.5	3.6 ± 0.4	3.2 ± 0.4
	4	3.3 ± 0.4	3.2 ± 0.7	3.2 ± 0.6	2.9 ± 0.5
	6	2.9 ± 0.6	2.8 ± 0.5	3.0 ± 0.7	2.6 ± 0.6
Brine salted (6%) smoked catfish	0	3.6 ± 0.2	3.6 ± 0.7	4.0 ± 0.8	3.5 ± 0.5
	1	3.5 ± 0.2	3.4 ± 0.6	3.8 ± 0.6	3.2 ± 0.7
	2	3.6 ± 0.2	3.4 ± 0.6	3.8 ± 0.4	3.2 ± 0.5
	4	3.4 ± 0.2	3.4 ± 0.4	3.7 ± 0.6	3.2 ± 0.4
	6	3.2 ± 0.2	3.2 ± 0.5	3.5 ± 0.5	3.2 ± 0.6

**Conclusion:** The result of this study indicated that 6% salt (sodium chloride) concentration was suitable for application to catfish intended for smoke-drying. During storage at ambient temperature ( $28 \pm 2^\circ\text{C}$ ) for 6 weeks, unsalted hot smoked catfish had good quality from 0-4 weeks while it was 0-6 weeks for 6% brine salted smoked catfish.

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