EFFECT OF STORAGE METHODS ON THE FATTY ACID PROFILE OF AFRICAN CATFISH (*Clarias gariepinus*)

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Abstract: This study was carried out to determine the changes in fatty acid profile of African Catfish (*Clarias gariepinus*) using different storage methods (frozen and heated environment) for 6 weeks. Fresh (live) fish samples were purchased from Oba's market Akure. The fish samples were divided into three equal parts. One part was stored fresh in a freezer at -6^{o}C the other two parts were smoked at 70^{o}C until the equilibrium moisture content was attained, and was further divided into two parts. One part of the smoked fish was stored in continuous heated environment at temperature of 40 ± 4^{o}C while the second part was packaged in polyethylene and stored in freezer also at -6^{o}C. Each of the samples was analyzed on weekly basis for quality assessment over a period of six (6) weeks. The result indicated that Palmitic acid (C16:0), Oleic acid (C18:1) and Docosahexanoic Acid (DHA) (C22:6) were the most dominant saturated, monounsaturated and polyunsaturated fatty acids. Smoked fish stored in freezer showed the least reduction in omega-3 fatty acids *n*3/omega-6 fatty acids *n*6 ratio compared with fresh frozen and smoked heated environment during the six weeks storage period. It is recommended that smoked fish in freezer over a period of time, is better in terms of fatty acids.

Keywords: *Clarias gariepinus*, freezing, smoking, storage method

Introduction

Fish is an important source of protein to millions of people worldwide. It is a less expensive animal protein that provides vital nutrients required in human diets (Sadiku and Oladimeji, 1991). Fish oil offers outstanding health benefits to consumers by lowering the danger of cardiovascular disease due to the presence of omega-3 polyunsaturated **fatty acid**s (PUFA), Eicosapentaenoic acid (EPA C-20:5) and Docosahexaenoic acid (DHA C-22:6) (Neil, 1997; Minis *et al*., 2006; Leaf and Kang, 1996). A daily intake of 500 mg EPA plus DHA per day is recommended for the primary prevention of coronary heart disease (ISSFAL, 2004). However, quality of harvest is markedly affected by the ease of deterioration and spoilage in fresh fish. Therefore, processing and preservative measures are important immediately after harvest to extend shelf life and prevent economic losses (Okonta and Ekelemu, 2006). This can be attained by diverse preservation methods; i.e., refrigeration, freezing, salting, brining (wet salting), icing, smoking, glazing, drying, frying etc.

Smoking is a long standing and universal method of preserving fish in many developing countries (Kumolu-Johnson *et al*., 2010). More than 95% of the artisanal landings are preserved by smoke drying. The primary aim of smoking is to preserve, add flavour and colour to the product. In addition, refrigeration and freezing can help in preserving fish by reducing temperature. During which a variety of changes, such as denaturation and aggregation of the myofibrillar proteins in fish muscle occur. Deterioration in the quality of fish and fish products during frozen storage is caused by lipid oxidation (Mackie, 1993; Verma *et al*., 1995). Oxidative deterioration can directly affect quality characteristics such as color, flavor, texture, nutritive value and safety (Wang, 2009). During frozen storage, the shelf life of marine products are influenced by enzymatic and non-enzymatic rancidity as a result of the unsaturated lipid composition and the presence of pro-oxidant molecules in their muscles (Aubourg *et al*., 2004). Thus, the objective of this study is to determine the changes in the fatty acid profiles of *Clarias gariepinus* under different storage conditions.

Materials and Methods

Collection of materials

Twenty four pieces of catfish (*Clarias gariepinus*), each weighing 450 ± 5 g were purchased from Johnny-Beth Fish Farm. Turkey Cold room at Oba's market Akure was used for freezing, while smoking kiln was obtained from the Food Science and Technology Laboratory of the Federal University of Technology, Akure.

Sample preparations

Fish samples were killed immediately after capture and carefully degutted. The blood and slime were removed by washing with clean water according to the method of Ogbonnaya and Ibrahim (2009). The samples were then divided in two equal parts. The first part was stored fresh in freezer while the second part was smoked at 65 ± 3^{o}C for 24 h and further divided into two. One portion of the smoked fish was stored in continuous heated environment at temperature of 40 ± 4^{o}C while the other portions was packaged in polyethylene and stored in freezer. Storage was carried out for six weeks. Samples were being taken for analyses on weekly basis.

Fatty acid analyses

The extracted fat (500 mg) from samples was saponified at 95^{o}C using 3.4 ml of the 0.5 M KOH in dry methanol at for 5 minutes to form a mixture. This was neutralized with 0.7M HCL. 3 ml of the 14% boron triflouride was added to methanol and heated for 5 min at the temperature of 90^{o}C (AOAC, 1990). The fatty acid methyl esters (FAMEs) were extracted with redistilled n-hexane from the mixture. This was then concentrated to 1 ml for gas chromatography analysis and 1 µl was injected into the port of HP 6890 gas chromatography with HP Chemstation Rev. A09.01 [1206] software attached to a flame ionization detector (FID). Separation of esters was done using an HP INNOWax capillary column (30 m x 0.25 µm x 0.20 µm film thickness), using split mode (split ratio, 20:1). The inlet temperature was 250^{o}C. Column

oven temperature was programmed at 60°C with first ramping at 12°C min^{-1} for 20 min and second ramping at 15°C min^{-1} for 3 min, respectively. The hydrogen pressure was at 22 psi and compressed air was at 35 psi. Nitrogen was used as a carrier gas. All experiments were carried out in triplicates.

Statistical analyses

Statistical analysis was performed on the replicate data by one-way analysis of variance (ANOVA) laid in completely randomised design using SPSS 17.0. Mean were separated using Duncan New Multiple Range Test (DNMRT) at ($p \leq 0.05$) level of significance.

Results and Discussion

The fatty acid compositions of catfish under different storage methods for six weeks are summarized in Table 1. Atotal of 14 fatty acids were present in the fish oil. The result indicated that Palmitic acid (C16:0), Oleic acid (C18:1) and Docosahexanoic Acid (DHA) (C22:6) were the most dominant saturated, monounsaturated and polyunsaturated fatty acids. The total saturated fatty acid were 44.55% and 45.05% for fresh and smoked fish in week 0.This increased significantly in fresh fish frozen (FFF) 45.82% and smoked frozen (SFF) 45.22% but decreased in smoked fish stored in heated environment (SFH) 44.90% in week 6. The total monosaturated fatty acids (MUFA) were 40.22% and 38.91% for fresh and smoked fish in week 0, this significantly ($p \leq 0.05$) decreased in FFF but increased in SFH and SFF during storage. The decrease in total saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) of smoked fish suggests that smoking was responsible for their decrease in *Clarias gariepinus*. However, there was increase in saturated fatty acid (SFA) fresh frozen and smoked stored in freezer during storage. The increase may have been as a result of unsaturated fatty acids in fish being prone to oxidation and enzymatic hydrolysis.

Palmitic acid was found to be highest level among all saturated fatty acids with Oleic acid and Palmitoleic acid showing the high levels among monounsaturated fatty acid (MUFA) respectively. However, Erucic acid was not found in smoked fish samples. The high levels of Palmitic (C16:0), Oleic (C18:1) and Palmitoleic acid (C16:1) in the samples are similar to reports by Passi *et al*. (2002) and Osman *et al*. (2001), that 16 and 18 carbon atoms are the main saturated and monounsaturated fatty acids found in most marine lipids. Availability of high levels of palmitic acid has been described as characteristics of fresh water fish (Osibona *et al*., 2009). Also these results (high proportion of palmitic acid) are in concurrence with the reports by Aggelousis and Lazos (1991); Okonji and Daniel (2013) on the fatty acid composition of both tilapia and catfish fillets. Oleic acid (C18:1) and Palmeiteolic acid (C16:1) were the main fatty acids among the MUFAs recorded. This is in accordance with the findings of Osibona *et al*. (2009). Their presence has been suggested to be from an exogenous source and an indication of the diet of that particular fish (Ackman, 1980).

Table 1: Fatty acid profile of *Clarias gariepinus* (%)

Fatty Acid	Fresh fish		Smoked Fish		
	Week 0	Week 6	Week 0	WEEK 6	
				SFH	SFF
Caprylic (C8:0)	ND	ND	ND	ND	ND
Capric (C10:0)	ND	ND	ND	ND	ND
Lauric (C12:0)	ND	ND	ND	ND	ND
Myristic (14:0)	4.68^{b}±0.02	4.69^{b}±0.02	4.59^{c}±0.05	4.31^{d}±0.02	4.76±0.01^{a}
Palmitic(C16:0)	34.22^{a}±0.02	34.19^{a}±0.06	34.06^{b}±0.05	33.73^{c}±0.03	32.36±0.04^{d}
Margaric (C17:0)	0.70^{b}±0.02	0.65^{bc}±0.03	0.63^{c}±0.04	0.66^{bc}±0.04	0.95±0.05^{a}
Stearic (C18:0)	4.19^{d}±0.03	4.37^{c}±0.04	4.23^{d}±0.03	4.47^{b}±0.02	4.75±0.05^{a}
Arachidic (C20:0)	0.36^{c}±0.04	0.49^{b}±0.03	0.35^{c}±0.05	0.37^{c}±0.05	0.56±0.02^{a}
Behenic (C22:0)	0.78^{b}±0.02	0.79^{b}±0.04	0.77^{b}±0.05	0.79^{b}±0.05	0.91±0.04^{a}
Lignoceric (C24:0)	0.62^{bc}±0.04	0.64^{b}±0.05	0.42^{d}±0.03	0.57^{c}±0.03	0.73^{a}±0.04
ΣSFA	45.55^{e}	45.82^{a}	45.05^{c}	44.90^{d}	45.22^{b}
Palmitoleic (C16:1)	10.60^{a}±0.05	10.57^{a}±0.02	10.42^{b}±0.02	10.26^{c}±0.07	10.28±0.06^{c}
Oleic (C18:1)	29.30^{c}±0.02	29.24^{c}±0.01	28.49^{d}±0.01	30.21^{a}±0.06	29.62±0.04^{b}
Erucic (C22:1)	0.32^{a}±0.02	0.15^{b}±0.01	ND	ND	ND
ΣMUFA	40.22^{b}	39.96^{c}	38.91^{d}	40.47^{a}	39.90^{c}
Linoleic (C18:2*n-6*)	1.97^{b}±0.03	1.94^{b}±0.04	1.89^{b}±0.03	2.39^{a}±0.03	2.00±0.40^{b}
Linolenic (18:3*n*-3)	0.68^{ab}±0.04	0.65^{b}±0.03	0.38^{c}±0.01	0.72^{a}±0.04	0.42±0.05^{c}
Arachidonic (C20:4*n*-6)	0.62^{b}±0.04	0.60^{b}±0.04	0.58^{b}±0.07	0.99^{a}±0.02	0.62±0.04^{b}
Docosahexanoic (22:6*n*-3)	11.20^{c}±0.2	11.03^{c}±0.25	13.18^{a}±0.02	11.59^{b}±0.05	10.68^{d}±0.07
ΣPUFA	14.47^{c}	14.22^{d}	16.03^{a}	15.69^{b}	13.72^{e}
Σ*n*3	11.88	11.68	13.56	12.31	11.10
Σ*n*6	2.59	2.54	2.47	3.38	2.62
*n*3/*n*6	4.58	4.59	5.48	3.64	4.23
PUFA/SFA	0.32	0.31	0.36	0.35	0.30

Values are represented as means ± SD; Values with the same superscripts in the row are not significantly different ($p \leq 0.05$); ND= Not detected, FF0: fresh fish week 0, FS0: smoked fish week 0, FFF: fresh fish frozen at week six, SFH: Smoked fish stored in heated environment (week six), SFF: Smoked fish stored in freezer (week six)

The Polyunsaturated fatty acid (PUFA) recorded were Linoleic (C18:2*n-6*), Arachidonic (C20:4*n*-6), Linolenic (18:3*n*-3) and Docosahexanoic (22:6*n*-3). DHA had the highest presence in fresh and smoked fish during storage. DHA is needed in fish nutrition and health for maintaining the structure and functioning of fish cells. Significant difference ($p<0.05$) in total polyunsaturated fatty acids (PUFA) was recorded between 14.47% fresh and 16.03% smoked fish in week 0. This decreased to 14.22% in fresh fish stored freezer, and 15.69% and 13.72% in smoked fish stored in heated environment and in freezer in week 6. The decrease in total PUFA in smoked fish suggests that smoking must have been responsible for the reduction of PUFAs. According to Yi-Chen *et al*. (2008), their

reduction in all fish samples during storage could be due to oxidative and hydrolytic reactions as a result of the presence of long chain hydrocarbon and high level of unsaturation.

In this study, fish samples were higher in *n*3 than *n*6 PUFAs. The *n*3/*n*6 ratio is a useful key for evaluating relative nutritional value of fish oils (Pigott and Tucker, 1990). According to reports by the HMSO, 1994, a ratio 4.0 will promote healthy human diet. In this study, the *n*3/*n*6 ratio was 4.58% for fresh frozen and 5.48% for smoked fish in week 0, which increased to 5.59% in fresh frozen fish and decreased to 3.64% and 4.23% in smoked fish stored in heated environment and freezer for 6 weeks respectively. In this study, ratios of n3/n6 in fresh and smoked *Clarias gariepinus* were slightly above the recommended value. However, the higher reduction in smoked stored in heated environment was as a result of the presence of higher value of *n-6* PUFA (Linoleic and Arachidonic) in the sixth week of storage.

Conclusion

This research work has shown that with any of the storage conditions- heated environment and frozen storage, there were significant reductions in fatty acids of smoked fish. However, smoked fish in freezer showed the least reduction in *n*3/*n*6 ratio compared with fresh frozen and smoked heated environment *Clarias gariepinus* observed during the six weeks storage period. It is recommended based on the results that smoked fish in freezer over a period of time, is better in terms of fatty acids.

References

Ackman RG 1980. Fish Lipids. Part 1. In: JJ. Connell. Ed. *Advances in Fish Sciences and Technology*. Fishing News Books Ltd. Farnham, Surrey, 86 -103.

Aggelousis G & Lazos SE 1991. Fatty acid composition of the lipids from eight freshwater fish species from Greece. *J. Food Compos. Anal.*, 4: 68-76.

AOAC 1990. *Official Methods of Analysis*. 15th ed. Association of Analytical Chemists. Washington, DC.

Aubourg SP, Pineiro C & Gonzalez MJ 2004. Quality loss related to rancidity development during frozen storage of horse mackerel (*Trachurus trachurus*). *J. Amer. Oil Chem. Soc.*, 81: 671–678.

Chávez-Mendoza C, García-Macías JA, Alarcón-Rojo AD, Ortega-Gutiérrez JA, Holguín-Licón C & Corral-Flores G 2014. Comparison of fatty acid content of fresh and frozen fillets of rainbow trout (*Oncorhynchusmykiss*) Walbaum. *Brazilian Archives of Bio. Techn.*, 57(1): 103-109.

HMSO 1994. Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subject No. 46. London, England. International society for the study of fatty acid and lipids 2004. Recommendations for intake of polyunsaturated fatty acids in healthy adults, UK (www.issfal.org.uk/), Retrieved 04/06/2010.

Kumolu-Johnson CA, Aladetohun, NF & Ndimele, PE 2010. The effects of smoking on the nutritional qualities and shelf-life of *Clarias gariepinus* (BURCHELL 1822). *Afri. J. Biotech.*, 9(1): 73-76.

Leaf A & Kang JX 1996. Prevention of cardiac sudden death by n-3 fatty acids: A review of the evidence. *J. Int. Mod.*, 240: 5-12.

Mackie IM 1993. The effects of freezing on flesh proteins. *Food Reviews International*, 9(4): 575- 610.

Minis R, Haq I, Jackson PR, Yeo W & Ramsay L 2006. Oily fish and fish oil supplements in the prevention of coronary heart disease. *J. Hum. Nutr. Dietetics*, 5: 449-459.

Neil JS. 1997. Fish consumption, fish oil, lipids and coronary heart disease. *Am. J. Clin. Nut*, 65: 1083-1086.

Nielsen J & Jessen F 2007. Quality of frozen fish. In: Handbook of meat, poultry and seafood quality. Nollet, L. M. L. (Ed.) Blackwell Publishing, Iowa, pp. 577-586.

Ogbonnaya C & Ibrahim MS 2009. Effectof drying methods on proximate composition of Catfish (*Clarias gariepinus*). *World J. Agric. Sci.*, 5(1): 114-116.

Okonji VA & Daniel E. 2013. Fatty acids variation in *Oreochromis niloticus* and *Clarias gariepinus* from semi-intensive fish tank. *World J. Fish & Marine Sci.*, 5(2): 169-175.

Okonta AA & Ekelemu JK 2006. A preliminary study of micro-organisms associated withfish spoilage in Asaba, Southern Nigeria. *Proc. of the 20th Annual Conference of Fisheries Society of Nigeria. (FISON), Port Harcourt*, 14th-18th November,pp. 557 - 560.

Osibona AO, Kusemiju K & Akande GR 2009. Fatty acid composition and amino acidprofile of two freshwater species, african catfish (*Clariasgariepinus)* and tilapia (*Tilapia zilli). Afri. J. Agric. Nutr. Devt.* 9. 609-621.

Osman H, Suriah AR & Law EC 2001. Fatty acid composition and cholesterol contents of selected marine fish in Malaysian waters. *Food Chem*, 73: 55-60.

Passi S, Cataudella S, Marco PD, De Simone F & Rastrelli L 2002. Fatty acid composition and antioxidant level in muscle tissue of different Mediterranean marine species of fish and shellfish. *J. Agric. Food Chem.*, 50: 7314-7322.

Pigott GM & Tucker BW 1990. Seafood effects of technology on nutrition. Marcel Dekker, Inc. New York.

Sadiku SOE & Oladimeji AA 1991. Relationship of proximate composition of *Latesniloticus* and *Synodontisschall* and *Sarotherodongalilaeus* (Trewavas) from Zaria Dam, Nigeria. *Biosci. Res. Comm.*, 3(1): 29-40.

Verma JK, Srikar LN, Sudhakara NS & Sarma J 1995. Effects of frozen storage in lipid freshness parameters and some functional properties of oil sardine (*Sardinellalongiceps*) mince. *Food Res. Int.*, 28: 87–90.

Wang T 2009. Oxidative deterioration of lipids can directly affect quality characteristics such as colour, flavour, texture, nutritive value and safety. Ph.D Thesis, Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Island, pp. 5-10.

Yi-Cheng CH, Nguyen J, Semmens K, Beamer S & Jaczyanski J 2008. Chemical changes in omega-3 enhanced farmed rainbow trout (*Oncorhynchusmyskiss*) fillets during abusive-temperature storage. *Food Control*, 19: 599 - 608.