PROXIMATE AND LIPID PROFILE ANALYSIS OF CULTURED AND WILD AFRICAN CATFISH, *Clarias gariepinus* [BURCHELL]

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ABSTRACT

As part of ongoing studies on the lipid profile of farmed fishes in our laboratory, this research work is aimed at investigating the proximate chemical composition and lipid profile of the muscles of the African catfish found in Nigerian waters. Crude protein, ash, moisture, and lipid content of the muscles of wild and cultured African catfish were determined using standard methods. Caloric value was also calculated from the lipid and protein content. Lipids in the muscles and livers of the cultured and wild catfish were extracted using the Bligh & Dyer method of lipid extraction. Extracted lipids were then analyzed by gas chromatography to determine the composition and relative abundance of the fatty acids present. The wild catfish contained 77.83 ± 0.88% moisture, 1.20 ± 0.02% ash and 18.76 ± 0.45% protein while the cultured catfish had 75.58 ± 0.88% moisture, 1.20 ± 0.03% ash and 19.33 ± 0.25% protein content, with no significant difference between the wild and cultured catfish. Both wild and cultured African catfish were found to be lean fish (fat content below 5%). Average caloric value was found to be 1204.4 cal/g and 1200.8 cal/g in the wild and cultured catfish respectively. The percentage of total saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) was higher in the muscles of the cultured catfish (38.86% and 42.83% respectively) than in the muscles of the cultured catfish (36.96% and 41.57% respectively). The cultured African catfish also had more omega-6 polyunsaturated fatty acids (PUFA) than the wild ones, whereas its total omega-3 PUFA was lower. Omega-3 to omega-6 ratios in the wild and cultured catfish were 0.81 and 0.52 respectively. Conclusively, the proximate composition, percentage lipid content and indices of fatty acid content of the wild and the cultured African catfish indicated it to be of both nutritional and physiological benefits to humans.

Key Words: *Clarias gariepinus*, Fish oil, Omega 3, Omega 6, African catfish.

INTRODUCTION

The nutritional benefits attributed to fish are particularly obtained from its exceptionally advantageous fatty acid profile. In recent years, increasing attention has been focused on the significance of polyunsaturated fatty acids in human nutrition, particularly eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) that are practically only found in fish. This increased attention is as a result of their positive effect in ameliorating some disease conditions, cardiovascular diseases high up on that list (Kris-Etherton *et al.,* 2002).

The -3 polyunsaturated fatty acids (PUFAs) also play a vital role in the development and function of the nervous system (brain) and the reproductive system (SanGiovanni and Chew, 2005; Bourre, 2007). Eicosapentaenoic acid and docosahexaenoicacid, along with other monounsaturated and polyunsaturated fatty acids have demonstrated benefits in the prevention and treatment of cardiovascular diseases (CVDs) (Kris-Etherton *et al.,* 2002; Mattson and Grundy, 1985), stroke, lupus nephropathy, hypertension, rheumatoid arthritis, breast cancer, colon cancer, prostate cancer (Levine and Barbara, 1997; Woods, 2008), autoimmune diseases, preventing weight loss in cancer patients (Uauy-Dagach and Valenzuela, 2000), eye sight and the improvement of learning ability (Nielsen *et al.,* 2005).EPA and DHA are particularly proven as the precursors of composite hormones known as eicosanoids, which are involved in several metabolic processes of the human body (Harris, 2004; Inhamuns and Franco, 2008).

Generally, when lipids are stored mostly in the liver, with little or nothing being stored in the muscles of the fish, they are termed as lean fish. If the excess stored in the muscles are much, they are termed as fatty fish (Sutharshiny and Sivashanthini, 2011). Based on fat content of the muscles, fish could be either lean fish (<5% fat by weight), medium-fat (5-10% fat by weight) and high-fat fish (>10% fat by weight) (Gurr, 1992).

Fish farming, or aquaculture, has been the fastest growing sector of animal food production in the world since 1970. Due to a decline in wild fish populations and a growing human population, aquaculture is expected to fill the gap in supplies of fish as food for humans, as demand continues to increase (FAO, 2008).

The fatty acid profile of fish vary with species and could be affected by many factors such as temperature, salinity, season, size, age, species habitat, life stage, and the type and abundance of food, especially whether a species is carnivorous, herbivorous or omnivorous (Ackman, 1989; Saito *et al.*, 1999; Sargent et al., 1999). As the world's fish stocks are getting limited, cultured fish is now being proposed as an alternative for consumption.

Clarias gariepinus, or the African sharp-tooth catfish is a species of the family Clariidae, the air-breathing catfishes. The African catfish is cultured mainly in Africa and Europe (Hecht *et al.*, 1985) although it is now also receiving attention in India, China and some East European countries (Huisman and Ritcher, 1987). It however has grown in importance in Nigeria over the years (Idodo-Umeh, 2003). The African catfish is considered to be one of the most important tropical catfish species for aquaculture because of a number of characteristics/factors, among which are its good meat quality, ability to tolerate adverse environmental conditions far better than other fishes do and its high growth rate even at high stocking densities. These factors contribute to its geometric rise in preference for commercial aquaculture (Eding and Kamstra, 2001; Pruszyński, 2003).

In Nigeria, the present knowledge of the chemical proximate composition of fish species from Nigerian waters is very limited. Most of the work done so far has focused primarily on proximate analysis. While this is also an important factor of nutrition, it does not directly address the health value of the fish, with respect to its fatty acid profile. In this study, wild and cultured African catfish were analyzed to determine the composition and relative abundance of fatty acids in their muscles.

MATERIALS AND METHODS

Sampling

Seven (7) cultured catfish were obtained from Ajanla Farms, Ibadan and seven (7) wild catfish were caught from the Opa River, Ile-Ife.

Fish fillets of required weights for analysis were cut from the fish muscles. The liver was also removed and weighed. Muscle samples were cut in triplicates from each fish shortlyafter the fish was immobilized by cervical dislocation. All samples were stored in the freezer until required for analysis.

Proximate Analysis

Proximate analysis of the muscles was carried out according to standard methods of the Association of Official Analytical Chemists (AOAC, 1994)

Moisture determination

Moisture was determined gravimetrically by desiccation at 105°C and 77 mmHg for 5 h.

$$\% \text{ Moisture} = \frac{\text{initial weight (g) - final weight (g)}}{\text{initial weight (g)}} \times 100\,\%$$

Ash content

Ash content of the sample was determined by incinerating in a muffle furnace at 600°C for 3 h.

$$\% \text{ Ash} = \frac{\text{weight of ash after heating}}{\text{weight of fresh sample}} \times 100\,\%$$

Protein content

The total nitrogen was determined by the Kjedahl method (Vlieg, 1984). Total protein content was obtained by multiplying protein content by 6.25.

Crude fibre content

Crude fibre was determined as loss in weight on ashing after acid and base digestion of the sample.

Lipid Extraction

Lipid was extracted from the muscles of the fish, in triplicates, according to methods as described by Bligh and Dyer (1959) with slight modifications, as described by Widjaja *et al.* (2009). Five grams (5 g) of the muscle sample (1 g of liver) was homogenized with 80 ml methanol, 40 ml chloroform and 28 ml of distilled water for 2 minutes. Chloroform (40 ml) and distilled water

(40 ml) was added and homogenization continued for about 2 minutes. After homogenization, it was filtered in a glass funnel, using a Whatmann No. 1 filter paper. The residue was put back in a fresh beaker and was re-homogenized with 40 ml chloroform:methanol (1:1 v/v) for about 30 seconds, then filtered. Filtrates were then combined and transferred to a separating funnel to allow for phase separation. The bottom chloroform layer was then collected after being passed through a 2.5 cm thick layer of anhydrous sodium sulphate (Na_2SO_4). The remaining aqueous layer was washed with 20 ml chloroform. The collected organic chloroform layer, containing the extracted lipids was then evaporated under vacuum at 40°C to remove the solvent and the obtained lipid kept in the refrigerator. The weight of the extracted lipid was recorded.

Lipid Content (%)

$$\text{Lipid content (\%)} = \frac{\text{amount of lipid extracted (g)}}{\text{weight of original sample (g)}} \times 100\,\%$$

Caloric Value

The total protein and lipid content (in grammes) was multiplied with the corresponding energy coefficient (Usydus *et al.,* 2011).

1 g of protein	= 4.1 kilocalories	=	17 KJ
1g of lipid	= 9.3 kilocalories	=	37 KJ

Fatty Acid Methyl Ester (Fame) Derivatization For Gas Chromatography Analysis

Methyl esters were prepared by transmethylation. Fifty milligrams (50 mg) of the extracted fat content of the sample was esterified for 5 mins at 95°C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralized by using 0.7 M HCl and then 3 ml of 14% boron trifluoride in methanol was added. The mixture was heated for 5 minutes at 90°C to achieve complete methylation. The fatty acid methyl esters were extracted thrice from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for gas chromatography analysis and 1 µl was injected into the injection port of the gas chromatography machine(Hewlett-Packard HP 6890).

Gas Chromatography Analysis

FAMEs were analyzed by capillary gas chromatography (column: 30m × 0.25m × 0.25µm HP-Innowax; flame ionization detect temperature at 320°C; carrier gas N_2 at 1 ml/min; injector temperature 250°C; oven temperature programmed from 60°C to 345°C) using a Hewlett-Packard HP 6890 powered with a HP ChemStation integrator.

The identification of fatty acid methyl esters was performed by external standards submitted to the same process of manipulation as the biological samples.

Statistical Analysis

Statistical analyses were conducted with the GraphPad statistical software package (GraphPad Prism®, Version 5.01).

Moisture content, ash content, crude protein content, malondialdehyde level and lipid content of the muscle of the cultured and wild catfish were analysed by t-test with Welch's correction. Statistical difference at $p < 0.05$ was considered significant.

RESULTS

Proximate Analysis

Proximate composition of the muscles of the wild and cultured African catfish is shown in Table 1.

The muscles of the wild catfish have 77.83 ± 0.49% moisture, 1.20 ± 0.02% ash and 18.76 ± 0.45% crude protein content; while the muscles of the cultured catfish have 75.58 ± 0.88% moisture, 1.20 ± 0.03% ash and 19.33 ± 0.25% crude protein content. The wild catfish showed higher moisture content and lower protein content than the cultured catfish. Crude fibre was negligible in both fish.

However, the differences were not significant, with $p > 0.05$.

Table 1: Proximate composition (%) of muscles of the cultured and wild African catfish, *Clariasgariepinus.*

Fish	% Moisture		% Ash		% Crude Fibre		% Crude Protein	
Sample description	*Cultured*	*Wild*	*Cultured*	*Wild*	*Cultured*	*Wild*	*Cultured*	*Wild*
n1	76.00	77.83	1.16	1.26	nil	nil	19.49	19.37
n2	75.57	80.34	1.22	1.23	nil	nil	19.23	16.19
n3	74.63	78.63	1.22	1.13	nil	nil	19.69	18.59
n4	72.15	76.35	1.33	1.17	nil	nil	20.56	19.67
n5	76.56	77.04	1.22	1.21	nil	nil	18.68	19.50
n6	79.74	77.35	1.17	1.22	nil	nil	18.99	18.79
n7	74.38	77.29	1.09	1.16	nil	nil	18.70	19.23
Mean ± S.E.M	**75.58 ± 0.88^{a}**	**77.83 ± 0.49^{a}**	**1.20 ± 0.03^{b}**	**1.20 ± 0.02^{b}**	**-**	**-**	**19.33 ± 0.25^{c}**	**18.76 ± 0.45^{c}**

n=7; Values with same superscript are not significantly different (p>0.05)

Lipid Content

Percentage lipid content of the muscles of the wild and cultured African catfish on extraction with the Bligh & Dyer method is shown in Table 2.

Table 2: Percentage (%) Lipid Content of Cultured and Wild *Clariasgariepinus*

Fish	% Lipid Content			
Sample description	Muscle		Liver	
	Cultured	*Wild*	*Cultured*	*Wild*
n1	4.28 ± 0.55	3.89 ± 0.35	8.74	7.96
n2	3.67 ± 0.81	4.13 ± 0.37	5.30	6.47
n3	4.85 ± 1.40	5.65 ± 1.28	8.15	8.15
n4	5.53 ± 0.33	5.53 ± 0.33	10.66	6.16
n5	3.53 ± 0.21	4.02 ± 1.16	5.42	7.64
n6	2.02 ± 0.37	6.43 ± 1.24	4.45	5.60
n7	6.87 ± 2.12	3.16 ± 0.29	8.76	13.00
Mean ± S.E.M	**4.39 ± 0.59^{a}**	**4.68 ± 0.45^{a}**	**7.35 ± 0.87^{b}**	**7.85 ± 0.93^{b,}**

n=7; Values with same superscript are not significantly different (p>0.05)

Lipid content in the muscles of the wild catfish (4.68 ± 0.45%) was found to be higher than that in the muscle of the cultured catfish (4.39 ± 0.59%). The difference in mean values was not significant ($p > 0.05$). Liver lipid content was 7.85 ± 0.93% and 7.35 ± 0.87% in the wild and cultured catfish respectively. The difference was also not significant at $p > 0.05$.

Caloric Value

The caloric value, a factor of the lipid (9,300 cal/g or 37,000 J/g) and protein (4,100 cal/g or 17,000 J/g) content, of the muscles of the cultured and wild variety of the *Clarias gariepinus* is 4910.4 J/g and 4920.8 J/g respectively. This is shown in Table 3.

Table 3: Caloric Value of the Muscles of the Cultured and Wild African Catfish

Fish	Cultured		Wild	
Sample description	Caloric value (J/g)	Caloric value (cal/g)	Caloric value (J/g)	Caloric value (cal/g)
n1	4896.9	1197.1	4737.2	1156.0
n2	4627.0	1129.7	4208.4	1047.9
n3	5141.8	1258.4	5250.8	1287.7
n4	5541.3	1357.3	5390.0	1320.8
n5	4481.7	1094.2	4802.4	1173.4
n6	3972.3	965.7	5573.4	1368.4
n7	5720.9	1405.6	4438.3	1082.3
Mean	**4910.4**	**1200.8**	**4920.8**	**1204.4**

Gas Chromatography Analysis (Fatty Acid Composition)

The fatty acid profile of the cultured and wild African catfish (shown in Table 4) which was revealed on gas chromatography analysis, was grouped as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The percentage of total SFA was higher in the muscles of the cultured catfish (38.86%) than in the muscles of the wild catfish (36.96%). Total MUFA was found to be 42.83% and 41.57% in the muscles of the cultured and wild catfish respectively. The major SFA and MUFA identified in the samples were C16:0 (palmitic acid) and C18:1 (oleic acid). Higher percentage share of PUFA was noted in the lipids of the muscles of the wild African catfish.

Table 4: Fatty Acid Composition (%) of the Lipids Extracted from the Cultured and Wild Variety of the African Catfish, *Clarias gariepinus*.

Fatty acid	Cultured	Wild
C12:0	1.06	1.52
C14:0	3.69	4.30
C16:0	24.06	23.37
C18:0	10.00	7.73
C20:0	0.03	0.02
C22:0	0.02	0.02
∑SFA	**38.86**	**36.96**
C14:1	0.11	0.09
C16:1	4.84	5.12
C18:1	32.72	31.17
C20:1	4.01	3.94
C22:1	1.15	1.25
∑MUFA	**42.83**	**41.57**
C18:2n6	7.16	6.72
C18:3n6	1.01	0.99
C18:3n3	0.13	0.10
C20:3n6	0.32	0.33
C20:3n3	0.02	0.01
C20:4n6	3.18	3.70
C20:5n3	0.73	0.53
C22:2n6	0.34	0.11
C22:6n3	5.42	8.98
PUFA	**18.31**	**21.47**
∑n6 PUFA	12.01	11.85
∑n3 PUFA	6.30	9.62
EPA	0.73	0.53
DHA	5.42	8.98
EPA + DHA	6.15	9.51
n3:n6	0.52	0.81
DHA:EPA	7.42	16.94

Of the PUFAs in the cultured fish (18.31%), 12.01% was n-3 and 6.30% was n-6, while in the wild catfish, 11.85% was n-3 and 9.62% was n-6 PUGAs, making a total of 21.47% PUFA content (in the wild catfish).

DISCUSSION

Fish farming has been the fastest growing sector of animal food production in the world, Nigeria not left out. Due to stagnating wild fisheries and a growing human population, aquaculture is

expected to fill the gap in supplies of fish as food for humans, as demand continues to increase (Naylor *et al.*, 2000; FAO, 2008). The nutritional properties of fish render them valuable foodstuffs that are beneficial for human health (Usydus *et al.,* 2011)

Proximate analysis results showed that the wild and cultured African catfish (Table 1) have an average moisture content of 77.83% and 75.58% respectively while protein contents were 18.76% and 19.33% respectively. This agrees with data showing the average moisture content of marine animals (Gallagher *et al.*, 1991). Ash content was found to be 1.20% in both wild and cultured species. This tallied with data from other researchers of the African catfish (Marcu *et al.*, 2010, Ayeloja *et al.*, 2011), the tilapia (Zelibe, 1989) and other fish species (Hendersen and Tocher, 1987; Andrade *et al.,* 1995). The observed moisture content compares well with other fish species reported as having moisture content of around 70% (Exler, 1987; Gallagher *et al.,* 1991; Eun *et al.*, 1994). High ash content in a fish suggests the fish is a good source of minerals (Osibona *et al.,* 2006). No significant differences were observed between the proximate composition of the cultured African catfish and that of the wild African catfish.

The percentage lipid content obtained by the Bligh and Dyer method of extraction in the wild and cultured African catfish range from 3.16% to 6.43% and 2.02% to 6.87% respectively, with a mean content of 4.68% and 4.39% respectively. This indicates that the African catfish is a lean fish, with less than 5% of its muscle content being fat. The higher levels of lipids in the livers than in the muscles of the fish is indicative of the notion that lean fish stores 50-80% of its fat in the form of triacylglycerols in the liver (Jacquot, 1961). The lipid storage site in fish is, primarily, the liver and muscle tissues (Arrington *et al.,* 2006). In a fish, once the lipid content exceeds the needed quantity of metabolizable energy, the excess will be deposited in muscle tissues.

The fish specie examined, the African catfish, *Clarias gariepinus*, belong to a high-protein (15-20%) low oil (<5%) category. The muscle tissues of the African catfish have a high protein content that is above 98% digestible (negligible crude fibre content). The protein and lipid contents are linked to the caloric value of the muscle tissues of the fish, and in the wild and cultured African catfish, the average caloric values are 4920.8 J/g (1204.4 cal/g) and 4910.4 J/g (1200.8 cal/g) respectively (Table 3). Given these values, the catfish could be incorporated into diet, with 500g portions (per week) sufficient to meet Food and Drugs Administration (FDA) recommendations (Recommended Daily Intake, RDI, values).

The fatty acid profile of fish vary between and within species, even in dark and white muscle and are affected by many factors such as temperature, salinity, size, age, habitat and diet (Saito *et al.*, 1999). Numerous clinical and epidemiological studies have correlated the long term consumption of PUFAs of the n-3 series with a reduced incidence of wide range of common degenerative diseases in humans, as well as the alleviation of some symptoms (Harris, 2004). The percentage of total SFA was higher in the muscles of the cultured catfish than in the muscles of the wild fish. Total MUFA was also higher in the muscles of the cultured fish. The major SFA and MUFA identified in the samples, both wild and cultured fish muscles, were C16:0 (palmitic acid) and C18:1 (oleic acid). This follows observations made by other researchers on the gilthead sea bream (Mnari *et al.*, 2007), the tilapia and sutchi catfish (Usydus *et al.,* 2011) among other fish.

Typically, the higher percentage share of PUFA was noted in the lipids of the muscles of the wild African catfish (21.47%). The results also revealed that the cultured African catfish contained more n6 PUFAs than the wild one, whereas its total n3 PUFA was lower. This difference could be due to diet that each fish is exposed to. The wild specie gets to feed on various plant materials, phytoplankton, zooplankton and other smaller fishes that have high n3 PUFA content, whereas the diet of the cultured specie is more controlled and could contain varying levels of n3 and n6 PUFAs. In the two varieties, the content of PUFAs is observed to be lower than that of the SFAs and MUFAs. This can be attributed to the fact that freshwater fishes feed largely on vegetation and plant materials and less on zooplanktons that are rich in PUFAs (Osman *et al.*, 2001).

Observed levels of eicosapentaenoic acid (EPA) are found to be lower than levels of docosahexaenoic acid (DHA) in both the wild and cultured African catfish. DHA is the main component of phosphoglycerols in fish membranes and is critical for normal development of the brain and retina. EPA is more likely to be catabolized for energy and less likely to be retained in membranes than DHA (Subhadra *et al.*, 2006). This could be the reason for the lower levels of EPA than DHA in the muscles of the fishes. The percentage share of DHA in the lipid exceeded that of EPA in both the wild and cultured variety, with the ratio of DHA to EPA being higher in the wild (16.94) than in the cultured variety (7.42). This difference could be as a result of diet. A high ratio of DHA/EPA is advantageous for consumer health and DHA is more efficient than EPA in reducing the risk of coronary heart diseases (Hossain, 2011). The biological significance of dietary DHA/EPA can be viewed in terms of competitive interactions between fatty acids for incorporation into phospholipids, especially for the esterifying enzymes (Sargent *et al.*, 1999).

According to Sargent (1997), the optimum ratio of n3:n6 should be 1:5 (0.2). the higher the n3:n6 ratios, the more able the body is to use the n3 fats. A lower ratio means the enzymes that convert these fatty acids into the forms in which they are active in the body are more likely to be used up by the n6 PUFAs. Both the wild and cultured African catfish showed high ratios of n3:n6 PUFAS, however, the ratio was higher in the wild variety than the cultured variety (0.81 and 0.52 respectively). As mentioned earlier, diet is the main factor affecting the PUFA content in fish. Location, season and environmental conditions may also play a role (Saito *et al.,* 1999). Generally, fish lipids have much higher n3:n6 ratio than recommended (1:5) and from a physiological stand point, this is highly beneficial and desirable for the daily human diet.

In conclusion, both the cultured and wild African catfish are of nutritional and physiological benefits to humans on consumption. Further work is ongoing in our laboratories to investigate the effect of various factors – such as diet, temperature, season, stage of maturity etc. – on the nutritional quality of the African catfish.

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