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Fatty Acids, Phospholipids and Sterols Levels of the Skin and Muscle of Tongue Sole Fish

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Abstract. The levels of fatty acids, phospholipids and sterols were determined in the skin and muscle of Tongue sole fish on dry weight basis. Results showed crude fat varied from 0.027-0.360 g/100 g; SFA varied from 35.0-36.9% of total fatty acids, total unsaturated fatty acids varied from 42.6-47.3%, PUFA ranged from 0.068-0.149. In the phospholipids, phosphatidylcholine was highest in both skin and muscle, with respective values of 12.7 and 16.1 mg/100 g. The sterols level in the skin varied between 6.86-6.94 (6.90±0.04) mg/100 g and muscle was n.d.-0.961 mg/100 g. Samples had low levels of n^{-6} fatty acids [4.20% (skin) and 0.140% (muscle)] and n^{-3} fatty acids [1.20% (skin) and 2.36% (muscle)].

Keywords: lipid profiles, skin, muscle, tongue sole fish

Introduction

Fish and meat from wild animals are the chief source of animal protein in the diets of the rural communities, especially in the southern states of Nigeria (Petrides, 1962). The FAO calculation for apparent annual per capita consumption of fish and shellfish for human food, by region and country (2001-2008) put the expected estimate for 2008 as 26.6 kg or 58.8 pounds in Nigeria (Adeyeye, 2009). Hence work on the determination of the chemical composition of fish should be an important part of aquaculture research.

Fish are widely recognized as a nutrition source, due to their high content of proteins, phospholipids and polyunsaturated fatty acids, as well as essential minerals (Simopoulos, 2002). In particular, fish are an important source of essential polyunsaturated fatty acids, which contribute to the reduction of cardiovascular disease (Kris- Etherton *et al*., 2003), inflammatory diseases (Tapiero *et al*., 2002), colon cancer (Roynettle *et al*., 2004), and disorders of the immune system (Belluzi, 2001).

Sole is the common name for various species of flatfish. Generally speaking, they are the members of the family Soleidae, but outside Europe, the name 'sole' is also applied to various other similar flatfish. The main aim of this paper was to investigate the lipid composition (fatty acids, phospholipids and sterols) of Tongue sole fish (Cynoglossidae), commonly found in the fish markets of Nigeria. These fish are sold after drying and with their skin peeled off for storage, hence the skin and the muscle of the fish in this study were separately evaluated, to determine the potential loss of nutrition from the consumption of fish without their skin.

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Materials and Methods

Sample collection and treatment. Five Tongue sole fish were purchased from the local fish market and brought to the laboratory; all bones and viscera were carefully removed and oven-dried at 55 °C for 5 h. The cooled dried samples were further separated into the skin and muscle, ground using mortar and pestle into a fine powder. The ground portions were kept in plastic rubbers in the freezer (-4 °C) pending analysis.

Determination of ether extract. An aliquot (0.25 g) of each part was weighed in an extraction thimble and 200 mL of petroleum ether (40-60 °C boiling range) was added. The covered porous thimble containing the sample was extracted for 5 h using a Soxhlet extractor. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether, oven dried at 105 °C for 1 h, cooled in a desiccator and the weight of dried oil was determined.

Preparation of fatty acid methyl esters and analysis. A 50 mg aliquot of the dried oil was saponified for 5 min at 95 °C with 3.4 mL of 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl and 3 mL of 14% boron triflouride in methanol was

added. This mixture was heated for 5 min at 90 °C to achieve complete methylation. The fatty acid methyl esters were thrice extracted from the mixture with redistilled *n*-hexane and concentrated to 1 mL for analysis. The fatty acid methyl esters were analysed using an HP 5890 gas chromatograph (GMI, Inc., Minnesota, USA) fitted with a flame ionization detector and using ChemStation software. Nitrogen was used as the carrier gas with a flow rate of 20-60 mL/min. The oven program was: initial temperature at 60 °C, ramping at 10 °C/min for 20 min, held for 4 min, with a second ramping at 15 °C/min for 4 min and held for 10 min. The injection temperature was 250 °C and the detector temperature was 320 °C. A polar (HP INNOWAX) capillary column (30 m × 0.25 mm × 0.25 µm) was used to separate the esters. A split injection was used with a split ratio of 20:1. The peaks were identified by their relative retention time compared with known standards.

Sterols analysis. Aliquots of the dried oil were added to screw-capped test tubes. The sample was saponified at 95 °C for 30 min, using 3 mL of 10% KOH in ethanol, to which 0.20 mL of benzene was added to ensure miscibility. Deionised water (3 mL) was added and 2 mL of hexane was used in extracting the non-saponifiable materials. Three extractions, each with 2 mL of hexane, were carried out for 1 h, 30 min and 30 min respectively to achieve complete extraction of the sterols. Hexane was concentrated to 1 mL for gas chromatographic analysis.

Phospholipids analysis. Using a modified method of Raheja *et al*. (1973), 0.01 g of the dried oil was added to test tubes. Any remaining solvent was removed by passing a stream of nitrogen gas over the oil. Then 0.40 mL of chloroform was added, followed by addition of 0.10 mL of the chromogenic solution. The tube was heated to 100 °C in a water bath for 1 min 20 sec., cooled to room temperature, 5 mL of hexane was added and the tube was shaken gently several times. After separation of the solvent and aqueous layers, the hexane layer was recovered and concentrated to 1.0 mL for analysis. Analysis was performed using the gas chromatograph with a polar (HP5) capillary column (30 m × 0.25 mm × 0.25 µm). The oven programme was: initially at 50 °C, ramping at 10 °C/min for 20 min, held for 4 min, a second ramping at 15 °C/min for 4 min and held for 5 min. The injection temperature was 250 °C, and the detector temperature was 320 °C. As previously described, a split injection type was used having a split ratio of 20:1. Peaks were identified by comparison with the known standards.

Quality assurance. Standard chromatograms were prepared for cholesterol, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient was determined for each fatty acid (28), sterol (4) and phospholipid (5). Correlation coefficient > 0.95 was considered acceptable.

Fatty acid values were also subjected to the calculation of uncertainty interval percentage. Some CRMs values were available for sterols and phospholipids but none in food samples relevant to this study. The CRMs used here were from Wolf (1993).

Calculation of fatty acid per 100 g in samples. Crude fat level was multiplied by a conversion factor of 0.70 to convert it to total fatty acids (Paul and Southgate, 1978). For fatty acids expressed in g per 100 g total fatty acids, precision is best limited to the 0.1 g/100 g level, with trace being set at < 0.06 g/100 g to fatty acids (Greenfield and Southgate, 2003).

Statistical analysis. Statistical analysis (Oloyo, 2001) was carried out to determine the mean, standard deviation, coefficient of variation in percent. Also calculated were linear correlation coefficient (r_{xy}), coefficient of determination (r_{xy}^2), linear regression coefficient (R_{xy}) and coefficient of alienation (C_A) in percent and index of forecasting efficiency (IFE) in percent. The r_{xy} was subjected to the table (critical) value at r=0.05 to see if significant differences existed in the values of fatty acids, sterol and phospholipids between the skin and muscle of Tongue sole.

Results and Discussion

Fatty acids. Table 1 depicts the crude fat and the calculated total acid levels of the fish parts on dry weight basis. The values between the skin and muscle were wide spread with the coefficient of variation of 122 and a ratio of skin: muscle as 13.3:1 (crude fat) and 13.3:1 (total fatty acids), showing that virtually all the fat was concentrated in the skin of the Tongue sole. The crude fat in the skin was lower than the values reported for three different types of land snails found in Nigeria with values of 1.12-1.42 g/100 g (wet weight basis) (Adeyeye, 1996) and also lower than all parts of male and female common West African fresh water crab *Sudananautes africanus africanus* with values of

Table 1. Crude fat and total fatty acid levels of skin and muscle (g/100 g) of Tongue sole fish

Parameter	Skin	Muscle	Mean	SD	CV %
Crude fat	0.360	0.027	0.194	0.235	122
Total fatty acid*	0.252	0.019	0.136	0.165	122

*Crude fat × 0.70; SD = standard deviation; CV % = coefficient of variation.

1.69-8.88 g/100 g (dry weight basis) (Adeyeye, 2002), lower than in insects: 52.7 g/100 g (dry weight) in winged termites (Adeyeye, 2005) and 13.3 g/100 g (dry weight) in grasshopper (Olaofe *et al*., 1998). The concentration of fat in the skin was similar to the observation in the exoskeleton of *Penaeus notabilis* where the value was greater than in the muscle (54.0-40.4 g/100 g dry weight) (Adeyeye and Adubiaro, 2004); the epicarp of *Chrysophyllum albidum* than its mesocarp (15.6-0.7 g/100 g wet weight) (Adeyeye and Agesin, 1999) and in the hull of two varieties of African yam bean than their cotyledons (17.0-8.15 and 16.5-10.8 g/100 g dry weight respectively) (Adeyeye and Agesin, 2007). The energy density from the skin (due to fat) was 14.0 kJ/100 g whilst it was 1.05 kJ/100 g from the muscle.

Table 2 shows the saturated fats (SFA) and the monounsaturated fats (MUFA) of the samples. The following members were found in traces: C22:0, C24:0, C14:1 n^{-5}, *cis*, C20:1 n^{-9}, *cis*, C22:1 n^{-9}, *cis*, C24:1 n^{-9}, *cis* and C18:1 n^{-11}, *trans*. Both SFA from skin and muscle was with coefficient of variation (CV %) of 3.74. C16:0 was the most concentrated fatty acid in the two samples; whilst C18:0 level was the second most concentrated in both samples. SFA with C12:0, C14:0 and C16:0 are the primary contributors to elevated blood cholesterol, and so contribute to cardiovascular diseases; C14:0 is the main culprit. SFA with 12, 14, or 16 carbons generally constitute about 25% - 50% of the total fat in animal foods. C18:0 is also thought to increase the risk of cardiovascular disease.

Like in SFA, C16:1n^{-7}, *cis* was the most concentrated fatty acid in the group of monounsaturated fatty acid (MUFA) in both skin and muscle. It was followed by C18:1 n^{-9}, *cis* in both samples with a value of CV % of 13.8. In the *trans* MUFA group, C18:1 n^{-9}, *trans* was the most concentrated in both samples; all *trans* MUFA value was 10.8% (skin) and 11.4% (muscle) but the total [MUFA (*cis*) + MUFA (*trans*)] was 42.1% (skin) and 45.2% (muscle) and CV% of 3.44 which are very close in the present results. The natural trans fatty acids in butter are said not to be harmful and may even have health-promoting properties, such as preventing certain forms of cancer (Wardlaw, 2003).

Table 2. Saturated and monounsaturated fatty acid composition of the skin and muscle of Tongue sole fish (% total fatty acid)

Fatty acid	Skin	Muscle	Mean	SD	CV %
C12:0	1.12	1.05	1.09	0.049	4.56
C14:0	4.60	5.45	5.03	0.601	12.0
C16:0	20.3	21.2	20.8	0.64	3.07
C18:0	8.96	9.17	9.07	0.148	1.64
C20:0	Tr	Tr	-	-	-
C24:0	-	-	-	-	-
SFA	35.0	36.9	36.0	1.34	3.74
C14:1 n^{-5}, *cis*	Tr	Tr	-	-	-
C16:1 n^{-7}, *cis*	21.7	19.1	20.4	1.84	9.01
C18:1 n^{-6}, *cis*	3.17	1.81	2.49	0.96	38.6
C18:1 n^{-9}, *cis*	6.44	7.83	7.14	0.98	13.8
C20:1 n^{-9}, *cis*	Tr	Tr	-	-	-
C22:1 n^{-9}, *cis*	-	Tr	-	-	-
C24:1 n^{-9}, *cis*	Tr	-	-	-	-
MUFA (*cis*)	31.3	28.7	30.0	1.84	6.13
C18:1 n^{-6} *trans*	1.19	1.80	1.50	0.43	28.8
C18:1 n^{-9}, *trans*	9.59	9.57	9.58	0.014	0.15
C18:1 n^{-11}, *trans*	Tr	Tr	-	-	-
MUFA (*trans*)	10.8	11.4	11.1	0.424	3.82
MUFA (totals)	42.1	40.1	41.1	1.41	3.44

Tr = trace; - = not detected; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

Table 3 contains the polyunsaturated fatty acids (PUFA) composition of n^{-6} and n^{-3} of the samples. Among the n^{-6} family, C20:2 n^{-6}, *cis* was the most concentrated with a value of 3.86% of the total fatty acids in the skin but not detected in the muscle. Whilst total PUFA n^{-6}, *cis* was 4.20% in the skin, it was 0.140% in the muscle. The C18:2 n^{-6}, *trans* was in traces. C18:2 n^{-6}, *trans* is known as conjugated linoleic acid (CLA) which occurs naturally. The bacteria that live in the rumens of some animals, for example produce trans fatty acids that eventually appear in foods such as beef, milk and butter (Wardlaw and Smith, 2009), this may have happened in case of Tongue sole. The n^{-3} that was observed in the sample was only C22:6 n^{-3} in both samples: 1.20% (skin) and 2.36% (muscle); this brought the total PUFA (*cis* + *trans*) in skin as 17.8% and 12.1% in the muscle. These results showed that the eicocanoids in the samples were only in traces (less than 0.06% each). The relative values of PUFA in both the skin and muscle made the two parts important in the fish flesh. However, C20:2

n^{-6}, *cis* constituted the highest levels of PUFA in both the samples. The eicosanoids help regulate blood clot formulation, blood pressure, blood lipid (including cholesterol) concentrations, the immune response, the inflammation response to injury and infection and many other body functions (Whitney *et al*., 1994). A deficiency of n^{-6} fatty acids in the diet leads to skin lesions. A deficiency of n^{-3} fatty acids leads to subtle neurological and visual problems. Deficiencies in PUFA produce growth retardation, reproductive failure, skin abnormalities and kidney and liver disorders. However, people are rarely deficient in these fatty acids (Tapiero *et al*., 2002). Both the skin and muscle of the fish were good sources of the PUFA (in combination).

Total unsaturated fatty acids in the skin were 47.3% and 42.6% in the muscle; these were made up by MUFA and PUFA. The essential fatty acids (EFA) are not unique in their ability to supply energy. The ß-oxidation of fatty acids in fish is basically the same as in mammals. The EFA, SFA and monoenoic fatty acids are all equally utilized for energy production. The relative amounts of PUFA and SFA in oils is important in nutrition and health. The ratio of PUFA/SFA (P/S ratio) is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by saturated fats and polyunsaturated fats (Honatra, 1974; Keys, 1972). The present PUFA/SFA in skin was 0.149 and 0.068 in the muscle, the value of P/S in the skin was poor to discourage atherosclerotic tendency whilst the muscle would support the skin in this action. N^{-3} fatty acids were all in traces in the samples. The n^{-6} and n^{-3} fatty acids have critical roles in the membrane structure (Kinsella, 1990; Lynch and Thompson, 1984) and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the n^{-6} and the n^{-3} fatty acids in the diet can be of considerable importance (WHO/FAO, 1994). The ratio of n^{-6} to n^{-3} in the diet should be between 5:1 and 10:1 (WHO/FAO, 1994) or 4-10 g of n^{-6} fatty acids to 1.0 g of n^{-3} fatty acids (Canadian Government Publishing Centre, 1990; Nestel, 1987). However, strictly speaking the C18 polyunsaturated fatty acids, linoleic or *cis* -9, *cis*-12-octadecadienoic acid [18:2(n^{-6})] and α-linolenic or *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid [18:3(n^{-3})], are the main essential fatty acids in that they cannot be synthesized in animal tissues. On the other hand, as linoleic is almost always present in foods, it tends to be relatively abundant in animal tissues. This is supported in the present report as follows: C18:2 (n^{-6}) in skin 0.06% and in muscle it was 0.14% whereas C18:3 (n^{-3}) in skin and muscle was in traces (Tr). In turn, these fatty acids are the biosynthetic precursors in animal systems of C20 and C22 polyunsaturated fatty acids, with three to six double bonds, *via* sequential desaturation and chain -elongation steps (desaturases in animal tissues can only insert a double bond on the carboxyl side of an existing double bond) (Steyer *et al*., 2007). Whilst it would be easy for the body to synthesize arachidonic acid [20:4 (n^{-6})] from [18:2 (n^{-6})], it would be a bit difficult to synthesize the n^{-3} PUFA series: especially eicosapentaenoic acid [20:5 (n^{-3}) or EPA] because of the low level of C18:3 (n^{-3}) and so the diet must be enhanced in this PUFA if this fish is to serve as the only dietary oil source. However, docosahexaenoic acid [22:6 (n^{-3}) or DHA], was present in both the samples.

Table 3. PUFA n^{-6} and n^{-3} fatty acid composition of the skin and muscle of Tongue sole fish (% total fatty acids)

Fatty acid	Skin	Muscle	Mean	SD	CV %
C18:2 n^{-6}, *cis*	0.06	0.14	0.10	0.06	56.6
C18:3 n^{-6}, *cis*	Tr	Tr	-	-	-
C20:2 n^{-6}, *cis*	Tr	Tr	-	-	-
C20:3 n-6, *cis*	-	Tr	-	-	-
C20:4 n^{-6}, *cis*	0.283	Tr	-	-	-
C22:2 n^{-6}, *cis*	3.86	-	-	-	-
n^{-6} PUFA (*cis*)	4.20	0.140	2.17	2.87	132
C18:2 n^{-6}, *trans*	Tr	Tr	-	-	-
n^{-6} PUFA (totals)	4.20	0.14	2.17	2.87	132
C18:3 n^{-3}	Tr	Tr	-	-	-
C18:5 n^{-3}	Tr	-	-	-	-
C22:6 n^{-3}	1.20	2.36	1.78	0.82	46.1
n^{-6} + n^{-3} (PUFA)	5.20	2.50	3.85	1.91	49.6
Totals (SFA+MUFA + PUFA	82.3	79.5	80.9	1.98	2.45
Totals (MUFA+PUFA)	47.3	42.6	45.0	3.32	7.39
PUFA/SFA	0.149	0.068	0.109	0.06	52.8
2 n^{-6}/3 n^{-3}	-	-	-	-	-
Ratio	1.03:1		-	-	-

PUFA = unsaturated fatty acid (essential fatty acid).

The results in Tables 2 and 3 were further subjected to statistical analysis (Table 4). Results showed highly positive and significant linear correlation coefficient (r_{xy}) at r=0.05 and n^{-2} degrees of freedom. The coefficient

Table 4. Statistical analysis of the results from Table 2 and 3

Parameter	Skin(X)	Muscle(Y)	r_{xy}	r_{xy}^2	C_A	R_{xy}	IFE	Remark
SFA	35.0	36.9	-	-	-	-	-	-
MUFA (totals)	42.1	40.1	-	-	-	-	-	-
$n^{-6} + n^{-3}$PUFA	5.20	2.51	-	-	-	-	-	-
Totals	82.3	79.5	0.9973	0.99	0.05	-0.81	0.95	*
MUFA+PUFA	47.3	42.6	-	-	-	-	-	-
PUFA/SFA	0.15	0.07	-	-	-	-	-	-

r_{xy} = correlation coefficient; r_{xy2} = coefficient of determination; C_A = coefficient of alienation; R_{xy} = regression coefficient; IFE = index of forecasting efficiency; * = result significantly different at n^{-2} and r = 0.05.

of determination (r_{xy}^2) was also high showing that 99.0% of variance in the muscle (Y) was associated with the variance in the skin (X). The linear regression coefficient (R_{xy}) showed that for every unit increase in the skin fatty acid, there was a corresponding decrease of 0.81 in the fatty acid of the muscle. The coefficient of alienation (C_A) was low at 5.0% with a corresponding high value of index of forecasting efficiency (IFE) with a value of 95.0%. The IFE is actually a value of reduction in the error of prediction of relationship between the skin and muscle fatty acids; this meant that the error in the prediction of relationship was just 5.0%. The implication of this was that the skin fatty acids could carry out adequately the functions of the muscle fatty acids of Tongue sole.

Table 5 shows the values of fatty acids per 100 g of skin and muscle distribution in Tongue sole as food. The values in the skin were consistently higher than the corresponding values for the muscle; this was mainly due to the total fatty acids (calculated) which were more in the skin than in the muscle. This calculation accounted for 0.209 g/100 g or 79.5% in skin and 0.015 g/100 g or 75.5% in muscle, the balance being due to trace levels of other fatty acids.

Phospholipids. Table 6 shows the levels of various phospholipids in the samples. Phospholipids are not essential nutrients; they are just another lipid and, as such, contribute 9 kcalories per gram of energy. Cephalin (phosphatidylethanolamine, PE) was the second largest concentrated entity in muscle and in skin. PE is found

Table 5. Fatty acids level in the Tongue sole fish per 100 g skin and muscle samples as food

Fatty acid	Skin	Muscle	Mean	SD	CV %
C12:0	0.003	0.0002	0.0016	0.002	124
C14:0	0.012	0.001	0.0065	0.008	120
C16:0	0.051	0.004	0.028	0.033	119
C18:0	0.023	0.002	0.013	0.015	114
C16:1n^{-7}, *cis*	0.055	0.004	0.03	0.036	122
C18:1n^{-6}, *cis*	0.008	0.0003	0.004	0.005	131
C18:1n^{-9}, *cis*	0.016	0.001	0.009	0.011	125
C22:1n^{-9}, *cis*	-	-	-	-	-
C18:1n^{-6}, *trans*	0.003	0.0003	0.002	0.002	116
C18:1n^{-9}, *trans*	0.024	0.002	0.013	0.016	120
C18:2n^{-6}, *cis*	0.0002	0.00003	0.0001	0.0001	105
C18:2n^{-6}, *trans*	-	-	-	-	-
C18:3n^{-6}, *cis*	-	-	-	-	-
C20:2n^{-6}, *cis*	-	-	-	-	-
C20:4n^{-6}, *cis*	0.001	-	-	-	-
C22: 2n^{-6}, *cis*	0.10	-	-	-	-
C20:5n^{-3}, *cis*	-	-	-	-	-
C22:6n^{-3}, *cis*	0.003	0.0004	0.002	0.002	108
Totals	0.209	0.015	0.112	0.137	122
Difference	0.043(20.5%)	0.004(24.3%)	0.023	0.03	117

in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neural tissue and in spinal cord (Adeyeye, 2011).

Phosphatidylserine (Ptd-L-Ser or PS) is a phospholipid usually kept on the inner-leaflet, the cytosolic side, of cell membranes by an enzyme called flippase. When a cell undergoes apoptotic cell death, PS is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell. PS has been demonstrated to speed up recovery, prevent muscle soreness, improve well-being, and might possess ergogenic properties in athletes involved in cycling, weight training and endurance running. PS supplementation promotes a desirable hormonal balance for athletes and might attenuate the physiological deterioration that accompanies overtraining and/or overstretching (Starks *et al*., 2008). In recent studies, PS has been shown to enhance mood in a cohort of young people during mental stress and to improve accuracy during tee-off by increasing the stress resistance of golfers (Alter, 2006). The US Food and Drug Administration (USFDA) had stated that consumption of PS may reduce the risk of dementia and cognitive dysfunction in elder perons (Adeyeye, 2011). PS can be found in meat, but most abundant in the brain and innards such as liver and kidney. The present results recorded 6.33 mg/100 g in the skin, and 3.49 mg/100 g in the muscle which were lower then the value in beef (69) and pork (57); but both were also lower than the value in European pilchard (sardine) of 16.0 mg/100 g (Alter, 2006). Phosphatidylcholine (lecithin) is usually the most abundant phospholipid in animal and plants, often amounting to almost 50% of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin value in the muscle (16.1 mg/100 g or 66.8%), and in the skin (12.7 mg/100 g or 46.7%). Phosphoinositides (P1, P2, P3) play important role in lipid signaling, cell signaling and membrane trafficking (Adeyeye, 2011). PI was of minor concentration in both samples. Partial hydrolysis of lecithin with removal of only one fatty acid yields a lysophosphatidylcholine (White *et al*., 1973). An example of alterations in enzymic activity related to association of a membrane -bound protein with lipid is that of phenylalanine hydroxylase, which catalyzes the conversion of phenylalanine to tyrosine. The activity of this enzyme, which is attached to the endoplasmic reticulum, is enhanced fifty fold in the presence of lysophosphstidylcholine, with which it is probably complexed in the hepatic cell (White *et al*., 1973). Lysophosphatidylcholine was of low level in both samples. Table 7 depicts the statistical analysis of the results from Table 6. Both r_{xy}, r_{xy}^2 and IFE were low. The R_{xy} was high and negative. The r_{xy} was significant at r = 0.05 and n^{-2} degrees of freedom.

Table 6. Phospholipids level (mg/100 g) of skin and muscle of Tongue sole fish

Phospholipids	Skin	Muscle	Mean	SD	CV %
Cephalin (PE)	8.23 (30.3)	4.48 (18.6)	6.36	2.65	41.7
Lecithin	12.7 (46.7)	16.1 (66.8)	14.4	2.40	16.7
Ptd-L-Ser (PS)	6.33 (23.3)	3.49 (14.5)	4.91	2.01	40.9
Lysophosphatidylcholine	0.06 (0.221)	0.007 (0.029)	0.034	0.04	112
PtdIns (PI)	0.002 (0.007)	0.011 (0.046)	0.007	0.006	97.9
Totals	27.2	24.1	25.7	2.19	8.55
Ratio	1.13:1				

PE = phosphatidylethanolamine; Lecithin = phosphatidylcholine; PS = phosphatidylserine; PI = phosphatidylinosotol; Values in parentheses are in percentages.

Table 7. Statistical analysis of the results from Table 6

Phospholipids	Skin(X)	Muscle(Y)	r_{xy}	r_{xy}^2	C_A	R_{xy}	IFE	Remark
PE	8.23	4.48	-	-	-	-	-	-
Lecithin	12.7	16.1	-	-	-	-	-	-
PS	6.33	3.49	0.9092	0.83	0.42	-1.19	0.58	*
Lysophosphatidylcholine	0.06	0.007	-	-	-	-	-	-
PI	0.002	0.011	-	-	-	-	-	-

Sterols. The sterol levels are shown in Table 8. The values in the cholesterol, cholestanol, stig-masterol and sitosterol range were close in both samples as: 6.86-6.94 mg/100 g (6.90 ± 0.04 mg/100 g) in skin. Cholestanol was not detected in the muscle. The skin predominantly had higher levels of all the sterols detected than in the muscle. On the whole the total sterol ratio in the skin to the muscle was 14.1:1 or 7.60 mg/100 g to 1.96 mg/100 g. This showed that the skin could be discarded to have lower sterol levels; however this might not be necessary since both samples contained high PUFA levels. The total dietary fats and oils range from 0.01-2% (Itoh *et al.*, 1973); the present levels were 0.360% in the skin and 0.027% in the muscle which were within the literature values. Stig-masterol shared first position in the two samples with respective values of 6.93 mg/100 g (skin) and 0.961 mg/100 g in the muscle. Stigmasterol is used as a precursor in the manufacture of synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast and colon cancers. Studies have also indicated that a diet high in phytosterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30% respectively over a 6 week period (Adeyeye, 2011). Stigmasterol is also known as Wulzen antistiffness factor. The levels of cholestanol in the skin and muscle could have come from cholesterol breakdown or to both cholesterol breakdown and liver transformation of cholestenone. Both cholestanol and sitosterol shared close positions in the skin, cholestanol was not detected in the muscle but sitosterol occupied the second position in the muscle. Results from Table 8 were analyzed statistically and shown in Table 9. The r_{xy}, r_{xy}^2 and IFE were high. The r_{xy}^2 showed that 75.0% variance in the muscle was related to the variance in the skin. R_{xy} was high and negative, C_A was high and the r_{xy} was lower than the critical value (table value) at $r_{=0.05}$ and n^{-2}, showing no significant difference existed in the samples.

Quality assurance. Table 10 shows the uncertainty interval percent (UIP) for the fatty acids. Most of the literature Table UIP levels were correspondingly higher than the present results in both skin and muscle. Also the correlation determined for all the standards: fatty acids, phospholipids and sterols, all had values ranging as follows: 0.99833-0.99997 (fatty acids), 0.99909-0.99999 (phospholipids) and 0.99920-0.99994 (sterols); all the correlation values were greater than 0.95 which is the critical correlation for acceptance of these types of analytical results. Both the correlation values and the UIP values attested to the quality assurance of the determinations.

Table 8. Sterols level (mg/100 g) of skin and muscle of Tongue sole fish

Sterols	Skin	Muscle	Mean	SD	CV %
Cholesterol	6.86 (24.9)	0.303 (15.5)	3.58	4.64	129
Cholestanol	6.87 (24.9)	-	-	-	-
Stigmasterol	6.93 (25.1)	0.961 (49.0)	3.95	4.22	107
Sitosterol	6.94 (25.1)	0.698 (35.6)	3.82	4.41	116
Totals	27.6	1.96	14.8	18.1	123
Ratio	14.1:1				

Table 9. Statistical analysis of the results from Table 8

Sterols	Skin (X)	Muscle (Y)	r_{xy}	r_{xy}^2	R_{xy}	CA	IFE	Remark
Cholesterol	6.86	0.303	-	-	-	-	-	-
Cholestanol	6.87	-	-	-	-	-	-	-
Stig-masterol	6.93	0.961	0.8662	0.75	-44.8	0.50	0.50	NS
Sitosterol	6.94	0.698	-	-	-	-	-	-

NS = results not significantly different at n^{-2} and r = 0.05.

Table 10. Uncertainty intervals as percent of analytical results

Fatty acid	UIP (table)	UIP (skin)	UIP (muscle)
C12:0	3.0	0.585	0.624
C14:0	2.8	6.87	5.80
C16:0	3.3	6.01	5.75
C18:0	4.2	2.94	2.87
C20:0	12	-	-
C16:1	3.2	3.76	4.27
C18:1	3.0	-	-
C18:1n^{-6}			
-cis	-	-	-
-trans	-	-	-
C18:1n^{-9}	10.2	-	-
-cis	-	0.152	0.125
- trans	-	0.102	0.102
C18:1n^{-11}	1.3	-	-
C20:1n^{-9}	-	-	-
C22:1	22.6	-	-
C22:1n^{-9}	-	-	-
C24:1	-	-	-
C18:2	6.6	-	-
-cis	-	-	-
-trans	-	-	-
C18:3	11.3	-	-
C18:3n^{-6}	-	6.44	4.97
C18:3n^{-3}	-	-	-
C20:4	9	-	-
C20:4n^{-6}	-	-	-
C22:2	3	-	-
C22:2n^{-6}	-	23.8	-
C22:6n^{-3}	-	-	-

UIP = uncertainty interval in percent.

Conclusion

The findings of this study showed that the samples contained unequal distribution of all the parameters determined. Both samples were high in n^{-6} fatty acids but low in n^{-3} fatty acids. Both samples had unsaturated acids as the predominant fatty acids. Significant difference occurred in the fatty acid levels. Both samples could serve as average source of lecithin but are much lower in sterols particularly in the muscle. Quality assurances of the determinations were highly satisfactory.

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