BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **46(4)**, 523-532, 2011

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

Lipid Profile of the Skin and Muscle of Freshwater Sardine (*Pellenula afzeliusi*): Nutritional /Dietary Implications

E. I. Adeyeye[a] and M. A. Oyarekua[b]

[a]Department of Chemistry, University of Ado Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria and
[b]Department of Chemistry, University of Education, Ikere-Ekiti, Nigeria

Abstract

The levels of fatty acids, phospholipids and sterols were determined in the skin and muscle of *Pellenula afzeliusi* using gas chromatograph. Results showed crude fat varied from 0.697-7.476 g/100 g; SFA varied from 30.3-34.8 % of total fatty acids, total unsaturated fatty acids varied from 57.3-61.2 %, PUFA range was 12.1-17.8 % and PUFA/SFA ranged from 0.348-0.587. Both skin and muscle had high levels of n-6 fatty acids but low in n-3 fatty acids. The correlation coefficient was significantly and positively high at $r_{=0.05}$ in the fatty acids. In the phospholipids, cephalin was highest in skin but lecithin was highest in the muscle with respective values of 965 and 432 (mg/100 g). The sterol values in the skin varied between 111-112 (111.5±0.58) mg/100 g and muscle was 19.5-19.6 (19.6±0.058) mg/100 g. Whilst 100 g skin would provide 5.233 g fatty acids, 100 g muscle would provide 0.488 g fatty acids.

Key words: Lipid profile, Skin and muscle, *Pellenula afzeliusi,* Gas chromatograph, Fatty acid, Phospholipid, Sterol

Introduction

Animal protein intake by Nigerians has been very low due to a decrease in animal production per capita (Olayide *et al.,* 1972) and the rising growth in the human population (Oyenuga, 1968). The price of fish has stabilized in recent times, thus fish have become the major source of animal protein (Adeyeye, 2009).

Petrides (1972) had indicated that fish and meat from wild animals are the chief sources of animal protein in the diets of the rural communities especially in the Southern States of Nigeria. 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 fishes should be an important part of aquaculture research.

The freshwater fish species of Nigeria is the richest in West Africa, with more than 268 known presently. They comprise Nilo-Sudanian, Guinean and Zairean fishes (Olaosebikan and Raji, 1998).

Fish is known for its high nutrition due to its high protein content, phospholipids and polyunsaturated fatty acids as well as the covering percentage of the essential minerals RDA/RDI (recommended daily allowance/ intake) (Simopoulos, 2002). Polyunsaturated, especially n-3 and n-6, fatty acids are particularly important in fish, since their consumption contributes to the reduction of appearance of cardiovascular disease (Kris-Etherton *et al.,* 2003). Also, n-3 PUFA have been shown to be very beneficial in the prevention of inflammatory diseases (Tapiero *et al.,* 2002), colon cancer (Roynette *et al*., 2004) and disorders of the immune system. Phospholipids are the main constituents of biological membranes and play an essential role in the regulation of biophysical properties, protein sorting and cell signalling pathways. They are essential components of the human diet and their absence can lead to a number of serious diseases (Turkmen *et al.,* 2005).

Cholesterol is the principal sterol of animal products. It is required to build and maintain cell membranes; it regulates membrane fluidity over the range of physiological temperatures. In this structural role, cholesterol reduces the permeability of the plasma membrane to protons and sodium ions (Haines, 2001). Within the cell membrane, cholesterol also functions in intercellular transport, cell signalling and nerve conduction. In many neurons as myelin sheath, rich in cholesterol provides insulation for more efficient conduction of impulse (Pawlina and Ross, 2006).

*Corresponding author. E-mail: eiadeyeye@yahoo.com

The freshwater sardine fish belongs to the family of Clupeidae and genus *Pellonula*: premaxilla and dentary lacking canniform teeth. Two species in Nigeria. *Pellonula leonensis*. Synonym: *Pellonula afzeliusi.* Local name: Taga rana, Banabanagi, Isoun. Maximum size: 86 mm. Description: No pelvic scutes in front of the origin of the first pectoral fin ray. Premaxillary teeth small and inwardly pointed. Habitat and fisheries of Kanji, Jebba Lake. Introduced into Tiga Lake (Olaosebikan and Raji, 1998). Studies have been carried out on the determination of the mineral components important for health in *P. afzeliusi* parts (flesh, bone and head) (Adeyeye and Faleye, 2004).

The main aim of this paper was to investigate the lipid composition (fatty acids, phospholipids and sterols) of *P. afzeliusi*. Most skin of fish in Nigeria were peeled off after drying and storage, hence the skin and the muscle of the fish under study were separately evaluated for their lipid composition to find out if any food value was lost when the skin was discarded.

Materials and Methods

Sample collection and treatment

Dried samples of *P. afzeliusi* were purchased at the market in Ado Ekiti, Nigeria. The samples were brought into the laboratory, all bones and viscera carefully removed and further oven-dried at 55 ^{o}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. Five fish samples were purchased for this experiment and the ground portions were kept in plastic bags in the freezer (-4 ^{o}C) pending analysis.

Extraction of lipid

0.25 g of each sample part was weighed into the extraction thimble. 200 ml of petroleum ether (40-60 ^{o}C boiling range) was measured and then added to the dried 250 ml capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled (AOAC, 2005). The sample was extracted for 5 h. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with the oil was oven dried at 105 ^{o}C for the period of 1 h. The flask containing the dried oil was cooled in the desicator and the weight of the cooled flask with the dried oil was measured.

Preparation of methyl esters and analysis

50 mg of the extracted fat was saponified for 5 min at 95 ^{o}C with 3.4 ml of the 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl. 3 ml of 14 % boron triflouride in methanol was added (AOAC,2005). The mixture was heated for 5 min at 90 ^{o}C to achieve complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for analysis and 1 µl was injected into the injection port of the GC. The fatty acid methyl esters were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. AO9.01 (1206) software (GMI, Inc, Minnesota, USA)) fitted with a flame ionization detector. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven program was: initial temperature at 60 ^{o}C, first ramping at 10 ^{o}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{o}C/min for 4 min and maintained for 10 min. The injection temperature was 250 ^{o}C whilst the detector temperature was 320 ^{o}C. A polar (HP INNOWAX) capillary column (30 m x 0.25 mm x 0.25 µm) was used to separate the esters. Split injection type was used having a split ratio of 20:1. The peaks were identified by comparison with standard fatty acid methyl esters.

Sterol analysis

The sterol analysis was as described by AOAC (2005). The aliquots of the extracted fat were added to the screw-capped test tubes. The sample was saponified at 95 ^{o}C for 30 min, using 3 ml of 10 % KOH in ethanol, to which 0.20 ml of benzene had been 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. The hexane was concentrated to 1 ml in the vial for gas chromatographic analysis and 1 µl was injected into the injection port of GC. The peaks were identified by comparison with standard sterols. The sterols were analysed using similar conditions as for fatty acid methyl ester analyses.

Phospholipids analysis

Modified method of Raheja *et al.* (1973) was employed in the analysis of phospholipids. 0.01 g of the extracted fat was added to the test tubes. To ensure complete dryness of the oil for phospholipids analysis, the solvent was completely

removed by passing stream of nitrogen gas on the oil. 0.40 ml of chloroform was added to tube followed by the addition of 0.10 ml of the chromogenic solution.The tube was heated at 100 ^{o}C in water bath for about 1 min 20 sec. The content was allowed to cool to the laboratory temperature and 5 ml of hexane was added and the tube shaken gently several times. The solvent and the aqueous layers were allowed to be separated. The hexane layer was recovered and concentrated to 1.0 ml for analysis. The phospholipids were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. AO9.01 (1206) software (GMI, Inc, Minnesota, USA)) fitted with a pulse flame photometric detector. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven program was: initial temperature at 50 ^{o}C, first ramping at 10 ^{o}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{o}C/min for 4 min and maintained for 5 min. The injection temperature was 250 ^{o}C whilst the detector temperature was 320^{o}C. A polar (HP 5) capillary column (30 m x 0.25 mm x 0.25 µm) was used to separate the esters. Split injection type was used having a split ratio of 20:1.

The peaks were identified by comparison with standard phospholipids.

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 parameter, same for sterol and phospholipids. Correlation coefficient should be > 0.95 for the result to be acceptable. It was performed with the Hewlett. Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Bunker Lake Blvd Ramsey, Minnesota, 55303 USA).

Further on quality assurance, the fatty acid values were subjected to the calculation of uncertainty interval percentage. Certified reference materials (CRMs) play a critical role in validating the accuracy of nutrient data. A range of food CRMs with assigned values and uncertainty intervals (UIs) for many nutrients are currently supplied by several organizations (Phillips *et al.,* 2007). The fatty acids evaluated in certified reference materials (CRMs) were: C12:0, C14:0, C16:0, C18:0, C16:1, C18:1, C18:2, C18:3, C20:0, C20:5, C22:6, C22:0, C22:5 and C24:0 (AOAC, 2005; Phillips *et al.,* 2007). Some CRMs values were available for sterol and phospholipids but none in food samples relevant to this study. The CRMs used here from Wolf (1993).

At the data source and reference database levels, values for individual fatty acids are usually expressed as percentages of total fatty acids. At the user database levels, values per 100 g of food are required. A conversion factor derived from the proportion of the total lipid present as fatty acids is required (Paul and Southgate, 1978) for converting percentages of total fatty acids to fatty acids per 100 g of food. Total lipid 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). Hence all values below 0.06 g/100 g in the samples were reported as trace and not used in any calculation.

Statistical analysis

Statistical analysis (Oloyo, 2001) was carried out to determine mean, standard deviation, coefficient of variation in percent. Also calculated were linear correlation coefficient (CC), coefficient of determination (CD), linear regression coefficient (RC) and coefficient of alienation (C_A) in percent and index of forecasting efficiency (IFE) in percent. The CC was subjected to the table (critical) value at $r_{=0.05}$ to see if significant differences existed in the values of fatty acids, cholesterol and phospholipids between the skin and muscle of *Pellenula afzeliusi.*

Results and Discussion

Fatty acids

Table II depicts the total lipid and the calculated total fatty acid levels of the fish parts on dry weight basis. The values between the skin and muscle were very wide with the coefficient of variation of 117 and a ratio of skin: muscle as 10.7:1, showing that virtually all the fat was concentrated in the skin of the *P. afzeliusi*. The total lipid found in the skin was higher 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) but closer to most parts of male and female common West African fresh water crab *Sudananautes africanus africanus* with values of 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 grasshop-

per (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 energy density in the skin (due to fat) was 277 kJ/100 g whilst it was 25.8 kJ/100 g from the muscle.

Table I shows the saturated fats (SFA) and the monounsaturated fats (MUFA) of the samples. The following members were found in traces: C12:0, C22:0, C24:0, C14:1 n-5, cis, C20:1 n-9, cis and C18:1 n-11, trans. Both SFA from skin and muscle was with coefficient of variation (CV %) of 9.81. C16:0 was the most concentrated fatty acid in both skin and muscle: whilst C18:0 level was the second most concentrated in the skin (5.126 %), C14:0 was the second most concentrated in the muscle (6.756 %). SFA with C12:0, C14:0 and C16:0 are the primary contributors to elevated blood cholesterol, and so contribute to cardiovascular disease; 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. The negative effect on the heart is probably due in part to an increase in blood clotting (Wardlaw, 2003). However, C18:0 may not be as hypercholesterolemic as the other SFA (apparently because it is converted to oleic acid) (Bonanome and Grundy, 1988). This is done by the desaturation of stearic acid by stearoyl-CoA desaturase-1 which produces oleic acid. The pathways of fatty acid metabolism have been reviewed by Mead and Kayama (1967). Fish are able to synthesize, *de novo* from acetate, the even-chain SFA. Radio tracer studies have shown that fish can convert C16:0 to monoene (Mead and Kayama, 1967).

Unlike in SFA, C18:1n-9, cis was the most concentrated fatty acid in the group of monounsaturated fatty acid (MUFA) for both skin and muscle (12.067 % (skin), 9.966 % (muscle)). It was followed by C18:1n-6, cis in both samples with CV % of 9.75. In the trans MUFA group, C18:1n-9, trans was the most concentrated in both samples; all trans MUFA value was 18.8 % in skin and 15.3 in muscle but the total MUFA (cis + trans) was 43.4 % in skin and 45.2 % in muscle with CV % of 2.78 showing the very closeness of the results. Literature shows that flaky pastry contained 31.08 µg/ml of 18:1n-9, trans whilst egg contained 22.98 µg/ml of the same fatty acid (LECO Corporation, 2008). 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). Most results on the fatty acid composition are favourably comparable to the results obtained by Yusuf Harun *et al.* (1993) who worked on fatty acid composition of the body oils of 12 marine fish species of the Bay of Bengal and two other freshwater fishes for comparison.

Table I also shows the polyunsaturated fatty acids (PUFA) composition of n-6 and n-3 in skin and muscle. Among the n-6 family, C20:2n-6, cis was the most concentrated with a value of 13.9 % of the total fatty acids in the skin but 7.51 % in the muscle. While total PUFA n-6, cis was 15.3 % in the skin, it was 10.3 % in the muscle. The only C18:2n-6, trans had a value of 2.33 % in skin and 1.77 % in the muscle. C18:2n-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 could also have happened in *P. afzeliusi.* The n-3 that was observed in the sample was only C18:3n-3 in both samples: 0.138 % (skin) and 0.058 % (muscle); this brought the total PUFA (cis + trans) in skin as 17.8 % and 12.1 % in the muscle. These results showed that the eicosanoids in the samples were only in traces (less than 0.06 % each). The relative values of PUFA in both the skin and muscle make the two parts important in the fish flesh. However, C20:2n-6, cis constituted the highest levels of PUFA in both samples. The eicosanoids help to regulate blood clot formation, 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). Both the skin and muscle of the fish are good sources of the PUFA (in combination).

Total unsaturated fatty acids in the skin were 61.2 % and 57.3 % in the muscle. 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 (Adeyeye *et al.,* 1999; Honatra, 1974). The present PUFA/SFA in skin was 0.587 and 0.348 in the muscle, the value of P/S in the skin was good enough to discourage atherosclerotic tendency while the muscle would

Table I: Fatty acid composition of the skin and muscle of *P. afzeliusi* (% total fatty acid).

Fatty acid	Skin	Muscle	CV %	CC	CD	RC	C_A	IFE	Remark
C12:0	Tr	Tr	-						
C14:0	4.643	6.756	26.2						
C16:0	16.924	18.237	5.28						
C18:0	5.126	6.018	11.3						
C20:0	3.609	3.808	3.79						
C22:0	Tr	Tr	-						
C24:0	Tr	-	-						
SFA	30.302	34.819	9.81						
C14:1n-5, cis	Tr	Tr	-						
C16:1n-7, cis	4.293	5.317	15.1						
C18:1n-6, cis	8.303	9.537	9.75						
C18:1n-9, cis	12.067	9.966	13.5						
C20:1n-9, cis	Tr	Tr	-						
C22:1n-9, cis	-	5.048	-						
C24:1n-9, cis	-	-	-						
MUFA (cis)	24.663	29.868	13.5						
C18:1n-6, trans	6.845	5.878	10.7						
C18:1n-9, trans	9.595	7.662	15.8						
C18:1n-11, trans	Tr	Tr	-						
MUFA (trans)	18.77	15.31	14.4						
MUFA (totals)	43.433	45.178	2.78						
C18:2n-6, cis	0.622	1.495	61.7						
C18:3n-6, cis	0.847	1.276	28.6						
C20:2n-6, cis	13.861	7.514	42.0						
C20:3n-6, cis	Tr	-	-						
C20:4n-6, cis	Tr	-	-						
C22:2n-6, cis	-	-	-						
n-6 PUFA (cis)	15.33	10.285	27.9						
C18:2n-6, trans	2.33	1.77	19.3						
n-6 PUFA (totals)	17.66	12.055	26.7						
C18:3n-3	0.138	0.058	57.7						
C20:5n-3	-	-	-						
C22:6n-3	Tr	Tr	-						
n-6+n-3 (PUFA)	17.798	12.113	26.9						
Totals (SFA+ MUFA+PUFA)	91.533	92.109	0.44						
Totals (MUFA + PUFA)	61.231	57.29	4.70						
PUFA/SFA	0.587	0.348	36.1						
2n-6/3n-3	17.07	8.083	50.5						
Ratio(skin:muscle)		0.99:1.00							
SFA	30.302	34.819							
MUFA (totals)	43.433	45.178							
n-6+n-3PUFA	17.798	12.113	0.9935	0.9871	-0.86	0.11	0.89	*	
Totals	91.533	92.109							
MUFA+PUFA	61.23	57.290							
PUFA/SFA	0.587	0.348							

Tr = trace(less than 0.06 %); - = not determined; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid (essential fatty acid); CC = correlation coefficient; CD = coefficient of determination RC = regression coefficient; C_A = coefficient of alienation; IFE = index of forecasting efficiency; * = result significantly different at n-2 and $r_{=0.05}$; CV % = coefficient of variation. Determinations were in duplicate.

support the skin in this action. The ratio of 2n-6/3n-3 value in the skin was 17.1 and it was 8.08 in the muscle. The n-6 and n-3 fatty acids have critical roles in the membrane structure (Lynch and Thompson, 1984; Kinsella, 1990) 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 acid (18:2(n-6)) and a-linolenic 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 (Nestel, 1987). This is supported in the present report as follows: C18:2 (n-6) in skin 0.622 % and in muscle it was 1.495 % whereas C18:3 (n-3) in skin was 0.138 % and 0.058 % in muscle. 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) (Stryer *et al.*, 2007). In the present study C18:2 (n-6) (0.622 in skin and 1.495 in muscle) and (18:3 (n-3)) (0.138 in skin and 0.058 in muscle) ratios would then be: 4.51:1 in skin and 25.8:1 in muscle; this meant that the skin value was within range whilst the muscle value was outside the range. While it would be easy for the body to synthesise arachidonic acid (20:4 (n-6)) from (18:2 (n-6)), it would be a bit difficult to synthesise the n-3 PUFA series: especially eicosapentaenoic acid (20:5 (n-3) or EPA) and docosahexaenoic acid (22:6 (n-3) or DHA) because of the low level of C18:3 (n-3) and so the diet must be enhanced in this PUFA if this fish serves as the only dietary oil source.

The fatty acids were further subjected to statistical analysis (Table I). Result showed a highly positive and significant linear correlation coefficient (CC) at $r_{=0.05}$ and n-2 degrees of freedom. The coefficient of determination (CD) was also high showing that 98.71 % of variance in the muscle (Y) was associated with the variance in the skin (X). The linear regression coefficient (RC) showed that for every unit increase in the skin fatty acid, there was a corresponding decrease of 0.86 in the fatty acid of the muscle. The coefficient of alienation (C_A) was low at 11.0 % with a corresponding high value of index of forecasting efficiency (IFE) with a value of 89.0 %. 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 11.0 %. The implication of this was that the skin fatty acids could carry out adequately the functions of the muscle fatty acids of *P.afzeliusi.*

Table II shows the fatty acids distribution per 100 g of skin and muscle in *P. afzeliusi* as food. The values in skin were consistently higher than in the corresponding values in the muscle; this was due mainly to the total fatty acids (calculated) which were more in the skin than in the muscle. This calculation accounted for 4.62 g/100 g or 86.8 % in skin and 0.44 g/100 g or 89.8 % in muscle, the balance being due to trace levels of other fatty acids.

Table II: Fatty acid per 100 g skin and muscle as food in *P. afzeliusi*

Fatty acid	Skin	Muscle	CV %
C14:0	0.24	0.033	107
C16:0	0.89	0.089	116
C18:0	0.27	0.029	114
C20:0	0.19	0.019	115
C16:1n-7, cis	0.22	0.026	112
C18:1n-6, cis	0.43	0.047	133
C18:1n-9, cis	0.63	0.049	121
C22:1n-9, cis	-	0.025	-
C18:1n-6, trans	0.36	0.029	120
C18:1n-9, trans	0.50	0.037	122
C18:2n-6, cis	0.03	0.007	85.6
C18:2n-6, trans	0.12	0.009	121
C18:3n-6, cis	0.01	0.0003	137
C20:2n-6, cis	0.73	0.037	128
C22:2n-6, cis	-	-	-
C22:5n-3, cis	-	-	-
Totals	4.62	0.44	117
Difference	0.61(13.2%)	0.05(10.2 %)	120
Crude fat	7.476	0.697	117
Total fatty acid*	5.233	0.488	117

*Crude fat x 0.70.

Phospholipids

Table III shows the levels of the various phospholipids in skin and muscle of *P. afzeliusi.* Phospholipids are not essential nutrients; they are just another lipid and, as such, contribute 9 kcalories per gram (Kilgour, 1987). Among the phospholipids cephalin (PE) was the second largest concentrated entity in muscle but largest in skin. PE is found in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neutral tissue and in spinal cord (http:// en. wikipedia. org/ wiki/phosphatidy lethanolamine). Phosphatidy lserine (Ptd-L-Ser or PS) supplementation pro-

Table III: Phospholipid levels (mg/100 g) of skin and muscle of *P. afzeliusi*

Phospholipid	Skin	Muscle	CV %	CC	CD	RC	C_A	IFE	Remark
Cephalin (PE)	965 (51.0)	133 (20.3)	107						
Lecithin (PC)	769 (40.7)	432 (66.0)	39.6						
(PS)	150 (7.92)	78.6 (12.0)	44.3						
(LPC)	7.62 (0.402)	5.79 (0.884)	19.3						
(PI)	1.36 (0.072)	5.64 (0.862)	86.5						
Total	1893	654							
Ratio (skin: muscle)		2.89:1							
PE	965	133							
Lecithin	769	432							
PS	150	78.6	0.6980	0.4873	28.5	0.72	0.28	NS	
(LPC)	7.62	5.79							
PI	1.36	5.64							

PE = phosphatidylethanolamine/cephalin; Lecithin = phosphatidylcholine/lecithin;
PS = phosphatidylserine; PI = phosphatidylinositol; LPC = Lysophosphatidylcholine
Values in parentheses are in percentages; NS = results not significantly different at n-2 and $r_{=0.05.}$

motes 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 in the elderly and may also reduce the risk of cognitive dysfunction in the elderly (http://en. wikipedia.org/wiki/phosphatidy lethanolamine). The present results recorded 150 mg/100 g in the skin, and 78.6 mg/100 g in the muscle which is close to the value in beef (69 mg/100 g) and pork (57 mg/100 g) (Alter, 2006); but both were much better than the value in European pilchard (sardine) of 16.0 mg/100 g (Alter, 2006). Phosphatidylcholine (lecithin) is usually the most abundant phospholipids in animal and plants, often amounting to almost 50 % of the total, and as such it is the key buildingblock of membrane bilayers. While this observation is true for lecithin value in the muscle (432 mg/100 g or 66.0 %), it is in the second position in the skin (769 mg/100 g or 40.7 %). Lecithin is also the principal phospholipids circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL. Large doses of lecithin may cause gastrointestinal upsets, sweating, salivation and loss of appetite (Whitney *et al.,* 1994). Phosphatidylinositol (PtdIns, PI) is a negatively charged phospholipid and a minor component in the cytosolic side of eukaryotic cell membranes. The inositol can be phosphorylated to form phosphatidylinositol phosphate (PIP), phosphatidylinositol bisphosphate (PIP_2) and phosphstidylinositol trisphosphate (PIP_3). PIP, PIP_2, and PIP_3 are collectively called phosphoinositides. Phosphoinositides play important roles in lipid signalling, cell signaling and membrane trafficking (http://en. wikipedia.org/wiki/phosphatidylethanolamine). PI was of minor concentration in both skin and muscle. 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 these enzymes, 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 skin and muscle. Both CC, CD and IFE were low. The RC was high and positive. The CC was not significant at $r_{=0.05}$ and n-2 degrees of freedom.

Sterols

The sterol levels are shown in Table IV. The values in the cholesterol, cholestanol, stig-masterol and sitosterol range was close in both samples as: 111-112 mg/100 g (112±0.58 mg/100 g) in skin and 19.5-19.6 mg/100 g (19.6±0.058 mg/100 g) in muscle. 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 7.58:1. This showed that the skin may be discarded to have lower sterol levels; however this might not be necessary since both skin and muscle contain high PUFA levels. However, the good aspects of cholesterol included being present in mammalian cell membranes where it is required to establish proper membrane permeability and fluidity, a precursor molecule for the biosynthesis of bile acids, steroid hormones and several fat soluble vitamins. Cholesterol does exert one negative influence in the body, however. On its way into cells from the blood stream, some cholesterol forms deposits in the artery walls. These deposits lead to atherosclerosis, a disease that causes heart attacks and strokes. Complex lipids are bonded to other types of molecules. Because lipids are mostly insoluble in water, the movement of lipids from organ to organ through the bloodstream is facilitated by plasma lipoproteins. Dietary patterns can also affect the metabolism of cholesterol. However, diet low in saturated fat, trans fat and cholesterol encourage the uptake of LDL by the liver, thereby removing LDL from the blood stream and decreasing the ability of scavenger cells to form atherosclerotic plagues in the blood vessels. Likewise, diets high in saturated fat, trans fat and cholesterol reduce the uptake of LDL by the liver, increasing cholesterol in the blood and the risk for cardiovascular disease (Whitney *et al.,* 1994). The total sterol content of dietary fats and oils ranges from 0.01-2 % (Itoh *et al.,* 1973); the present levels were 0.445 % in the skin and 0.059 % in the muscle which were within the literature values. Cholesterol and stig-masterol shared first positions in the two samples with respective values of 112 mg/100 g (skin) and 19.6 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 (http:// en.wikipedia.org/ wiki/stigmasterol). Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23 % and 30 % respectively over a 6 week period (http://en.wikipedia.org/ wiki/stigmasterol). Stigmasterol is also known as Wulzen antistiffness factor.

Table IV: Sterol levels (mg/100 g) of skin and muscle of *P. afzeliusi*

Sterol	Skin	Muscle	CV % CC CD RC C_A IFE Remark
Cholesterol	112(25.1 %)	19.6(33.4 %)	99.3
Choslestanol	111(25.0 %)	-	-
Stig-masterol	112(25.1 %)	19.6(33.4 %)	99.3
Sitosterol	111(25.0 %)	19.5(33.3 %)	99.1
Totals	445	58.7	108
Ratio (skin: muscle)		7.58:1	
Cholesterol	112	19.6	
Cholestanol	111	-	0.577 0.3333 -1100 0.82 0.18 NS
Stig-masterol	112	19.6	
Sitosterol	111	19.5	

* Values in parentheses are in percentages.

Cholesterol enters the intestinal tract by excretion across the intestinal mucosa as well as via the bile. In the lumen of the gut a portion is reduced microbially to coprostanol and cholestanol and thereby is excluded from reabsorption. These two stanols, together with cholesterols, constitute the bulk of the fecal sterols. Certain of these transformations, e.g., from cholestenone to cholestanol, also occur in the liver (White *et al.,* 1973). 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 the second position in the skin, cholestanol was not detected in the muscle but sitosterol occupied the second position in the muscle. The CC, CD and IFE were low. The CD showed that only 33.3 % variance in the muscle was related to the variance in the skin. RC was high and negative, CA was high and the rxy 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 V shows the uncertainty interval percent (UIP) for the fatty acids. All the 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 V: Uncertainty intervals as percent of analytical results

Fatty acid	UIP (table)	UIP (skin)	UIP (muscle)
C14:0	2.8	0.194	0.133
C16:0	3.3	0.236	0.219
C18:0	4.2	0.215	0.183
C20:0	12	0.033	0.032
C16:1	3.2	0.606	0.489
C18:1n-6	3.0	-	-
-cis	-	0.964	0.839
-trans	-	1.169	1.361
C18:1n-9	10.2	-	-
-cis	-	0.008	0.010
-trans	-	0.010	0.013
C18:1n-11 (trans)	1.3	-	-
C20:1n-9	-	-	-
C22:1n-9	-	-	-
C18:2	6.6	-	-
-cis	-	3.70	1.54
-trans	-	0.987	1.30
C18:3	11.3	2.95	1.96
C18:3n-3	-	-	-
C20:2	4.3	0.031	0.06

UIP = uncertainty interval in percent from Wolf (1993).

Conclusion

The findings of this study showed that both skin and muscle of *P. afzeliusi* show unequal distribution of all the parameters (fatty acids, phospholipids and sterols) determined. Both skin and muscle were high in n-6 fatty acids but low in n-3 fatty acids. Both skin and muscle had unsaturated acids as the predominant fatty acids. Significant difference occurred in the fatty acid levels. Both samples would serve as good source of lecithin but much lower in sterols particularly the muscle.

References

Adeyeye E. I. (1996). Waste yield, proximate and mineral composition of three different types of land snails found in Nigeria. *Int J Food Sci Nutr* **47:**111-116.

Adeyeye E. I. (2002). Determination of the chemical composition of the nutritionally valuable parts of male and female West African fresh water crab *Sudananautes africanus africanus. Int J Food Sci Nutr* **53:** 189-196.

Adeyeye E. I. (2005). The composition of the winged termites, Macrotermes bellicosus. *J Chem Soc Nigeria* **30** (2): 145-149.

Adeyeye E. I. (2009). Amino acid composition of three species of fish: Clarias anguillaris, Oreochromis niloticus and Cynoglossus senegalensis. *Food Chemistry* **13:** 43-46.

Adeyeye E. I. and Adubiaro H. O. (2004). Chemical composition of shell and flesh of three prawn samples from Lagos lagoon. *J Sci Food Agric* **84**: 411-414.

Adeyeye E. I. and Faleye F. J. (2004). Mineral components important for health from animal sources. *Pak J Sci Ind Res* **47**(6): 471-477.

Adeyeye E. I., Oshodi A. A. and Ipinmoroti K. O. (1999). Fatty acid composition of six varieties of dehulled African yam bean (*Sphenostylis stenocarpa*) flour. *Int J Food Sci Nutr* **50:** 357-365.

Altar T. (2006). More than you wanted to know about fat and oil. Sundance Natural Food Online retrieved 2006-08-31.

AOAC (2005). Official Methods of Analysis, 18th ed. Ass Off Anal Chem., Washington DC, 920.39 (A); 969.33 and 996.06; 970.51.969.33 and Belluzi A. (2001) N-3 and n-6 fatty acids for the treatment of autoimmune diseases. *Eur J Lipid Sci Technol* **103**: 399-407.

Bonanome A. and Grundy S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England J Med* **318:** 1244-1248.

Canadian Government Publishing Center (1990). Scientific Review Committee, Nutrition Recommendations: The Report of the Scientific Review Committee. Canadian Government Publishing Center, Ottawa. p.45.

Greenfield H. and Southgate D. A. T. (2003). Food composition data, production, management and use. FAO, Rome, pp.165-170.

Haines T. H. (2001). Do sterols reduce proton and sodium leaks through lipid bilayers? *Prog Lipid Res* **40** (4): 299-324.

Honatra G. (1974). Dietary fats and arterial thrombosis. *Heamostasis* **2:** 21-52.

Itoh T., Tamura T. and Matsumoto T. (1973). Sterol composition of 19 vegetable oils. *J Am Oil Chemists' Soc* **50:** 122-125.

Keys A. (1972). Diet as a risk factor in cardiovascular disease. Paper presented at the 148th Scientific Meeting of the Nutrition Society, 6-7 July 1972, University of Surrey, Guilford.

Kinsella J. E. (1990). Possible mechanisms underlying the effects of n-3 polyunsaturated fatty acids. *Omega-3 News* **V**: 1-5.

Kris-Etherton P., Harris W. S., and Appel L. J. (2003). Fish consumption, fish oils, omega-3 fatty acids and cardiovascular disease. Arteriosclerosis, *Thrombosis and Vascular Biology* **23:** 20-31.

LECO Corporation (2008). Determination of fatty acid methyl esters by GC x GC-TOFMS. LECO Corporation, Michigan, pp. 1-4.

Lynch D. V. and Thompson G. A. (1984). Pie-tailored lipid molecular species: a tactical mechanism for modulating membrane properties, *Trends Biochem Sci* **9:** 442-445.

Mead J. F. and Kayama M. (1967). Lipid metabolism in fish. In: Stansby, M.E. (Ed.), Fish oils. Avi Publ Co, Westport, Conn, pp. 289-299.

Nestel P. J. (1987). Polyunsaturated fatty acids (n-3, n-6). *American J Clin Nutr* **45:** 1161-1167.

Olaofe O., Arogundade L. A., Adeyeye E. I. and Falusi O. M. (1998). Composition and food properties of the variegated grasshopper, *Zonocerus variegatus. Trop Sci* **38:** 33-237.

Olaosebikan B. D. and Raji A. (1998). Field guide to Nigerian freshwater fishes. Federal College of Freshwater Fisheries Technology, New Bussa, Nigeria, pp.88-89.

Olayide S. O., Olatunbosun D., Idusogie E. O. and Abiagom J. D. (1972). A quantitative analysis of food requirements, supplies and demands in Nigeria, 1968-1985. The Federal Department of Agriculture, Lagos, Nigeria, pp. 9-14.

Oloyo R. A. (2001). Fundamentals of research methodology for social and applied sciences. ROA Educational Press, Ilaro, Nigeria, pp.53-200.

Oyenuga A. (1968). Nigeria's foods and feeding-stuffs. Ibadan University Press, Ibadan, Nigeria, pp.6-93.

Paul A. A. and Southgate D. A. T. (1978). McCance and Widdowson's The Composition of foods (4th edition). HMSO, London. In: Greenfied H., and Southgate D.A.T. (2003) Food composition data, production, management and use. FAO, Rome, p.77 pp.169-170.

Pawlina W. and Ross M. W. (2006). Histology: a text and atlas: with correlated cell and molecular biology. Lippincott Williams and Wilkins, Philadelphia, pp.1-230

Petrides G. A. (1972). Advisory report on wild life and national parks in Nigeria. In: Olayide, S.O., Olatunbosun, D., Idusoye, E.O., Abiagom, J.D. (Eds.), A quantitative analysis of demands in Nigeria, 1968-1985. The Federal Department of Agriculture, Lagos, Nigeria, p. 2.

Phillips K. M., Wolf W. R., Patterson K. Y., Sharpless K. E., Amanna K.R. and Holden J.M. (2007). Summary of reference materials for the determination of the nutrient, composition of foods. *Accred Qual Assur* **12:**126-133.

Raheja R. K., Kaur C., Singh A. and Bhatia I. S. (1973). New colorimetric method for the composition of foods. *Accred Qual Assur* 12: 126-133. quantitative estimation of phospholipids without acid digestion. *Journal of Lipid Research* **14:** 695-697.

Roynette C. E., Calder P. C. and Dupertuis Y. M. (2004). N-3 polyunsaturated fatty acids and colon cancer prevention. *Clin Nutr* **23:** 139-151.

Simopoulos P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* **56:** 365-379.

Starks M. A., Starks S. L. and Kingsley M. (2008). The effects of phosphatidylserine on endocrine response to moderate intensity exercise. *J Int Soc Sports Nutr* **5:**11

Stryer L., Berg J. M. and Tymoczko J. L. (2007). Biochemistry (6th edition). W.H., Freeman, San Francisco., pp.974-976.

Tapiero H., Nguyen Ba G., Couvreur P. and Tew K. D. (2002) Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomed Pharmacother* **56:** 215-222.

Turkmen A., Turkmen M., Tepe Y. and Akyrt I. (2005). Heavy metals in three commercially valuable fish species from Iskenderum bay, Northern East Mediterranean Sea, *Turkey. Food Chem* **91:** 167-172.

Wardlaw G. M. (2003). Contemporary nutrition: issues and insights (5th edition). McGraw-Hill, Boston., pp.178-186.

Wardlaw G. M. and Smith A. M. (2009). Contemporary nutrition (7th edition). McGraw-Hill Higher Education, New York., pp.160-199.

White A., Handler P. and Smith E. L. (1973). Principles of Biochemistry (5th edition) McGraw-Hill Kogakusha Ltd, Tokyo, pp.966-970.

Whitney E. N., Cataldo C. B. and Rolfes S. R. (1994). Understanding normal and clinical nutrition. West Publishing Company, New York, pp.132-168.

Wolf W. R. (1993). In: Sullivan D.M., and Caroenter D.E. (eds) Methods of analysis for nutrition labeling. AOAC International, Arlington, Virginia, pp 111-122.

WHO/FAO (1994). Fats and oil in human nutrition. (Report of a joint expert consultation.) *FAO Food and Nutrition Paper* 57: WHO/FAO, Rome, pp.3-107.

Yusuf Harun K.M., Rumi A. S., Quazi R. R. and Anwar S. H. (1993). *J Food Comp Anal* **6** (4): 346-353.

Received : February 24, 2010;
Accepted : February 17, 2011