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Alestes macrolepidotus: nutritional implications of the lipid profile of its skin and muscle

Emmanuel Ilesanmi Adeyeye

Department of Chemistry, University of Ado Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria.

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ABSTRACT

The levels of fatty acids, phospholipids and sterols were determined in the skin and muscle of *Alestes macrolepidotus*. Results showed crude fat varied from 0.488-5.46 g/100 g; SFA from 16.0-29.5 % of total fatty acids, total unsaturated fatty acids varied from 61.6-80.9 %, PUFA range was 33.1-37.4 % and PUFA/SFA ranged from 1.27-2.07.Skin and muscle had low levels of n-6 fatty acids but high in n-3 fatty acids. Correlation coefficient was significantly and positively high at $r_{=0.05}$ in the fatty acids. In the phospholipids, lecithin was highest both inskin and muscle with respective values of 734 and 313 (mg/100 g). The sterol values in the skin varied from81.6-81.7 mg/100 g and muscle was not detected (–) to11.4 (9.89±2.62) mg/100 g. Whilst 100 g skin would provide 3.82 g fatty acids, 100 g muscle would provide 0.342 g fatty acids.

Introduction

Animal protein intake by Nigerians has been very low due to a decrease in animal production per capita and the rising growth in the human population. The price of fish has stabilised in recent times, thus fish have become the major source of animal protein[1].

Petrides [2] 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[1]. 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[3].

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)[4]. 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[5]. Also, n-3 PUFA have been shown to be very beneficial in the prevention of inflammatory diseases[6], colon cancer[7] 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[8].

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[9]. Within the cell membrane, cholesterol also functions in intercellular transport, cell signaling and nerve conduction. In many neurons as myelin sheath, cholesterol provides insulation for more efficient conduction of impulse[10].

The silversides belong to the order Characiformes (Characins) with these characters: 3-5 branchiostegals; teeth usually well developed; adipose fin present. Silversides belong to the family Characidae with characters: adipose fin higher than long; large scales; strong multi-cuspid teeth; no spines in dorsal fin. Seven genera and 20 species are found in Nigeria. A specie of the silversides (a freshwater fish) is *Brycinus macrolepidotus* (Valenciennes, 1849); synonym: *Alestes macrolepidotus* (Bilhaz, 1852) with Nigeria local names: *Kawara* (Hausa), *Egbagi* (Nupe), *Elei* (Ijaw), *Kaya* (Kanuri); it has maximum size of 120 mm. The fish has this description: body depth more than three times in the standard length for adults; snout long, less than three times in the length of head, dorsal fin plainly behind pelvic fins; head large; no sexual dimorphism of the anal fin and it is widely distributed[3].

The main aim of this research was to investigate the lipid composition (fatty acids, phospholipids and sterols) of *A. macrolepidotus*. Most skin of fish in Nigeria mostly peeled off after drying and storage thereby making the skin unavailable for consumption; 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 unavailable for consumption.

Materials and methods

Sample collection

Dried samples of *A. macrolepidotus* were purchased at the market in Ado Ekiti, Nigeria.

Sample treatment

The samples were brought into the laboratory, all bones and viscera carefully removed and further oven-dried at 55 ^{0}C for 5 h. The cooled dried samples were further separated into the skin and muscle, ground using mortar and pestle into fine powder.

Tele:
E-mail addresses: eiadeyeye@yahoo.com

Ten fish samples were purchased for this experiment and the ground portions were kept in plastic bags in the freezer (4 ^{0}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 ^{0}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[11]. The lipid 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 ^{0}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 ^{0}C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralised by 0.7 M HCl. 3 ml of 14 % boron triflouride in methanol was added[11]. The mixture was heated for 5 min at 90 ^{0}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 programme was: initial temperature at 60 ^{0}C, first ramping at 10 ^{0}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{0}C/min for 4 min and maintained for 10 min. The injection temperature was 250 ^{0}C whilst the detector temperature was 320 ^{0}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[11]. The aliquots of the extracted fat were added to the screw-capped test tubes. The sample was saponified at 95 ^{0}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*.[12] was employed in the analysis of phospholipids. 0.01 g of the extracted fat was added to each test tube. 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 the tube followed by the addition of 0.10 ml of chromogenic solution. The tube was heated at 100 ^{0}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 programme was: initial temperature at 50 ^{0}C, first ramping at 10 ^{0}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{0}C/min for 4 min and maintained for 5 min. The injection temperature was 250 ^{0}C whilst the detector temperature was 320 ^{0}C. A polar (HP 5) capillary column (30 m x 0.25 mm x 0.25 µm) was used to separate the phospholipids. 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 sterols, 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 sterols 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 BlvdRamsey, 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[13]. The fatty acids evaluated in certified reference materials (CRMs) were: C14:0, C16:0, C18:0, C16:1, C18:1, C18:2, C18:3, C20:2, C20:4, C22:6 and C22:1[11,13]. 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[14].

Calculation of fatty acid per 100 g in samples

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 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. For fatty acids expressed in g per 100 g total fatty acids, precision is best limited to 0.1 g/100 g level, with trace being set at < 0.06 g/100 g of fatty acids[15]. 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[16] 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), 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, sterols and phospholipids between the skin and muscle of *Alestes macrolepidotus*.

Results and discussion

Total lipid

Table I depicts the total lipid and the calculated total fatty acid levels as food of the fish parts on dry weight basis. The values of the total lipid between the skin and muscle are very different with the coefficient of variation percent of 118 and a ratio of skin: muscle as 11.2:1, showing that virtually all the fat was concentrated in the skin of the *A. macrolepidotus*. The total lipid found in the skin is close 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)[17]. The concentration of fat in the skin is 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)[18]. The energy density in the skin (due to fat) was 202 kJ/100 g whilst it was 18.1 kJ/100 g from the muscle. The only n-3 fatty acid in the samples is docosahexaenoic acid (DHA) with a value of 1.06 g /100 g (skin) and 0.067 g/100 g (muscle) as food. The 1990 Canadian RNI (Recommended Nutrient Intakes) included specific amounts for 3n-3 fatty acids and 2n-6 fatty acids. For n-3 fatty acids, the RNI is 0.5 % of total energy or 0.55 g/1000 kcalories; for n-6 fatty acids, the RNI is 3 % of total energy or 3.3 g/1000 kcalories[19]. From the present results, the DHA would contribute much higher than 0.5 % of total energy being produced by the samples.

Fatty acids as food

Table I shows the fatty acids distribution per 100 g of skin and muscle in *A. macrolepidotus* as food. The values in skin were consistently higher than in the corresponding values in the muscle except in C22:1n-13, cis, C20: 2n-6, cis, and C22:6n-3, cis; this was due mainly to the total fatty acids (calculated) which were more in the skin than in the muscle. This calculation accounted for 3.33 g/100 g or 87.2 % in skin and 0.321 g/100 g or 93.9 % in muscle, the balance being due to trace levels of other fatty acids.

Table II shows the saturated fats (SFA) and the monounsaturated fats (MUFA) of the samples. The following members were found in traces: C12:0;C20:0;C22:0; C24:0; C14:1n-9, cis; C20:1n-11, cis;C24:1n-15, cisand C18:1n-11, trans. Both SFA from skin and muscle were with coefficient of variation (CV %) of 42.0. 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 and in the muscle. 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 major 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 that might be caused by the SFA[20]. However, C18:0 may not be as hypercholesterolemic as the other SFA (apparently because it is converted to oleic acid)[21]. This is done by the desaturation of stearic acid by stearoyl-CoA desaturase-1 which produces oleic acid. Fish are able to synthesise, *de novo* from acetate, the even-chain SFA. Radio tracer studies have shown that fish can convert C16:0 to monoene.

Unlike in SFA, C18:1n-9, cis was the most concentrated fatty acid in the group of monounsaturated fatty acid (MUFA) for the skin (6.76 %) andC16:1n-9, cis for the muscle (3.90 %). It was followed by C16:1n-9, cis in skin and C18:1n-9, cis in muscle. In the trans MUFA group, C18:1n-9, trans was the most concentrated in both samples; all trans MUFA value was 8.72 % in skin and 6.25 % in muscle but the total MUFA (cis + trans) was 43.4 % in skin and 47.8 % in muscle with CV % of 46.4 showing the unequal distribution of the fatty acids. 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[20]. Most results on the fatty acid composition are favourably comparable to the results obtained by Yusuf *et al*.[22] 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 II 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 9.00 % of the total fatty acids in the muscle but not detected in the skin. Whilst total PUFA n-6, cis was 5.75 % in the skin, it was 10.3 % in the muscle. C18:2n-6, trans had a value of 3.90 % in skin and 3.32 % 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[23]. This could also have happened in *A. macrolepidotus*. The only n-3 fatty acid that was observed was C22:6n-3 in both samples: 27.7 % (skin) and 19.5 % (muscle); this brought the total PUFA (cis + trans) in skin as 37.4 % and 33.1 % in the muscle. These results showed that the eicosanoids in the samples were the major fatty acids. The relative values of PUFA in both the skin and muscle made the two parts important in the fish flesh. However, C22:6n-3, 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[19]. Both the skin and muscle of the fish would be good sources of the PUFA (in combination).

Total unsaturated fatty acids in the skin were 61.6 % and 80.9 % 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 utilised 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[24]. The present PUFA/SFA in skin is 1.27 and 2.07 in the muscle, the values of P/S in the skin and muscle are good enough to ameliorate atherosclerotic tendency. The ratio of n-6/n-3 value in the skin was 0.35 and it was 0.70 in the muscle. The n-6 and n-3 fatty acids have critical roles in the membrane structure[25] 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[26]. The ratio of n-6 to n-3 in the diet should be between 5:1 and 10:1[26] or 4-10 g of n-6 fatty acids to 1.0 g of n-3 fatty acids. However, strictly speaking the C18 polyunsaturated fatty acids, linoleic acid [18:2(n-6)] and α-linolenic acid [18:3(n-3)], are the main essential fatty acids in that they cannot be synthesised in animal tissues. On the other

hand, as linoleic acid 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 was 1.35 % and in muscle it was 0.75 % whereas C18:3 (n-3) in skin was< 0.06 % and < 0.06 % 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) [26]. Whilst it would be easy for the body to synthesise arachidonic acid [20:4 (n-6)] from [18:2 (n-6)], it would be impossible 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. Fortunately, the samples produced high levels of DHA. Many body tissues contain this important omega – 3 fatty acid; DHA is especially active in the retina of the eye and the cerebral cortex of the brain. About half of the DHA accumulates in the brain before birth, and half after birth, an indication of the importance of lipids during pregnancy and lactation. Based on the high levels of DHA in the samples more C18:2 (n-6) must be consumed with this fish to maintain the normal ratio of n-6 to n-3 for normal physiological functions of the fatty acids.

The fatty acids were further subjected to statistical analysis (Table II). 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 85.9 % 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 increase of 1.68 in the fatty acid of the muscle. The coefficient of alienation (C_A) was low at 37.0 % with a corresponding high value of index of forecasting efficiency (IFE) with a value of 63.0 %. 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 37.0 %. The implication of this is that the skin fatty acids could carry out adequately the functions of the muscle fatty acids of *A. macrolepidotus*.

Phospholipids

Table III shows the levels of the various phospholipids in skin and muscle of *A. macrolepidotus*. Among the phospholipids, cephalin (PE) was the second largest concentrated entity in muscle and 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, neural tissue and in spinal cord [28]. Phosphatidylserine (Ptd-L-Ser or PS) supplementation promotes a desirable hormonal balance for athletes and might attenuate the physiological deterioration that accompanies overtraining and/or overstretching [29]. 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. 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 [28]. The present results recorded 123 mg/100 g in the skin, and 18.5mg/100 g in the muscle which is close to the value in beef (69 mg/100 g) and pork (57 mg/100 g) particularly in the skin; but both are much better than the value in European pilchard (sardine) of 16.0 mg/100 g. 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 values in the muscle (313 mg/100 g or 74.3 %), it is also true in the skin (734 mg/100 g or 55.1 %). Lecithin is also the principal phospholipid 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 [19]. 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 phosphatidylinositol trisphosphate (PIP_3). PIP, PIP_2, and PIP_3 are collectively called phosphoinositides. Phosphoinositides play important roles in lipid signalling, cell signalling and membrane trafficking [28]. PI was of minor concentration in both skin and muscle. Partial hydrolysis of lecithin with removal of only one fatty acid yields a lysophosphatidylcholine. An example of alterations in enzymic activity related to association of a membrane –bound protein with lipid is that of phenylalanine hydroxylase, which catalyses 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 lysophosphatidylcholine, with which it probably complexes in the hepatic cell. Lysophosphatidylcholine was of low level in both skin and muscle. CC, CD and IFE were low. The RC was high but negative. The CC was 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: 81.6-81.7 mg/100 g (81.7±0.05 mg/100 g) in skin and not detected(–) to 11.4 mg/100 g (9.89±2.62 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 11.0:1. 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.

Table I. Fatty acid (g/100 g) skin and muscle as food in *A. macrolepidotus*

Fatty acid	Skin	Muscle	CV %
C14:0	0.040	0.002	128
C16:0	0.806	0.036	129
C18:0	0.279	0.017	125
C16:1n-9, cis	0.211	0.013	125
C18:1n-6, cis	0.123	0.003	135
C18:1n-9, cis	0.258	0.010	131
C22:1n-13, cis	-	0.116	-
C18:1n-6, trans	0.123	0.006	128
C18:1n-9, trans	0.210	0.015	123
C18:2n-6, cis	0.052	0.003	126
C18:3n-6, cis	0.005	0.001	94.3
C20:2n-6, cis	-	0.031	-
C20:4n-6,cis	0.003	0.001	70.7
C22:2n-6, cis	0.160	-	-
C22:6n-3, cis	1.06	0.067	125
Total	3.33	0.321	117
Difference	0.49(12.8%)	0.021(6.14 %)	130
Crude fat	5.46	0.488	118
[a]Total fatty acid	3.82	0.342	118

[a]Crude fat x 0.70.

Table II. Fatty acid composition of the skin and muscle of A. macrolepidotus (% total fatty acid)

Fatty acid	Skin	Muscle	CV %	CC[c]	CD[d]	RC[e]	C.[b]	IFE	Remark
C12:0	T	T	-						
C14:0	1.06	0.49	52.0						
C16:0	21.1	10.5	47.4						
C18:0	7.30	5.05	25.8						
C20:0	T	T	-						
C22:0	T	T	-						
C24:0	T	-	-						
[b]SFA	29.5	16.0	42.0						
C14:1n-9,cis	T	T	-						
C16:1n-9,cis	5.53	3.90	24.4						
C18:1n-6,cis	3.21	0.85	82.2						
C18:1n-9,cis	6.76	2.83	58.0						
C20:1n-11,cis	T	-	-						
C22:1n-13,cis	-	33.9	-						
C24:1n-15,cis	-	T	-						
[c]MUFA (cis)	15.5	41.5	64.5						
C18:1n-6,trans	3.22	1.77	41.1						
C18:1n-9,trans	5.50	4.48	14.5						
C18:1n-11,trans	T	T	-						
MUFA (trans)	8.72	6.25	23.3						
MUFA (total)	24.2	47.8	46.4						
C18:2n-6,cis	1.35	0.75	40.4						
C18:3n-6,cis	0.13	0.21	33.3						
C20:2n-6,cis	-	9.00	-						
C20:3n-6,cis	T	T	-						

Table II continuation

C20:4n-6, cis	0.070.30	87.9							
C22:2n-6, cis	4.20-	-							
n-6 PUFA (cis)	5.75	10.3	40.1						
C18:2n-6(trans)	3.903.32	11.4							
n-6 PUFA(total)	9.65	13.6	24.0						
C18:3n-3	T	T	-						
C20:5n-3	-	-	-						
C22:6n-3	27.719.5	24.6							
n-6+n-3 (PUFA)	37.4 33.1	8.63							
Total (SFA+ MUFA+PUFA)	91.196.9	4.36							
Total (MUFA + PUFA)	61.6	80.9	19.2						
PUFA/SFA	1.27	2.07	56.6						
n-6/n-3	0.35	0.70	47.1						
Ratio(skin:muscle)	0.94	1.00							
SFA	29.5	16.0							
MUFA (totals)	24.2	47.8							
n-6+n-3PUFA	37.4	33.1		0.9270	0.8592	1.68	0.37	0.63	*
Totals	91.1	96.9							
MUFA+PUFA	61.6	89.9							
PUFA/SFA	1.27	2.07							

[a]T = trace(less than 0.06 %); - = not determined; [b]SFA = saturated fatty acid; [c]MUFA = monounsaturated fatty acid; [d]PUFA = polyunsaturated fatty acid (essential fatty acid); [e]CC= correlation coefficient; [f]CD = coefficient of determination;[g]RC = regression coefficient; [h]C_A = coefficient of alienation; [i]IFE = index of forecasting efficiency; * = results significantly different at n-2 and $r_{=0.05}$; [j]CV % = coefficient of variation. Determinations were in duplicate.

Table III. Phospholipid levels (mg/100 g) of skin and muscle of *A. macrolepidotus*

Phospholipid	Skin	Muscle	CV %	CC	CD	RC	C_A	IFE	Remark
Cephalin (PE)[a]	388	89.6	88.4						
	(29.2)	(21.3)							
Lecithin (PC)[b]	734	313	55.0						
	(55.1)	(74.3)							
(PS)[c]	123	18.5	104						
	(9.24)	(4.39)							
(LPC)[d]	76.1	0.11	141						
	(5.72)	(0.026)							
(PI)[e]	10.4	0.061	140						
	(0.78)	(0.014)							
Total	1331	421	73.5						
Ratio (skin:muscle)		3.2:1.0							
PE	388	89.6							
Lecithin	734	313							
PS	123	18.5		0.9739	0.9484	31.5	0.23	0.77	*
LPC	76.1	0.11							
PI	10.4	0.061							

[a]PE = phosphatidylethanolamine/cephalin; [b]PC = phosphatidylcholine/lecithin; [c]PS = phosphatidylserine; [d]LPC = lysophosphatidylcholine; [e]PI = phosphatidylinositol; [f]Values in parentheses are in percentages; * = results significantly different at n-2 and $r_{=0.05}$.

TableIV. Sterol levels (mg/100 g) of skin and muscle of *A. macrolepidotus*

Sterol	Skin	Muscle	CV %
Cholesterol	81.7(25.0)[a]	11.4(38.5)	107
Choslestanol	81.7(25.0)	-	-
Stig-masterol	81.7(25.0)	6.87(23.2)	119
Sitostero!	81.6(25.0)	11.4(38.5)	107
Total	327	29.6	118
Ratio (skin: muscle)		11.0:1.00	

[a]Values in parentheses are in percentages.

Table V. Uncertainty intervals as percentage of analytical results

Fatty acid	[a]UIP (table)	UIP (skin)	UIP (muscle)
C14:0	2.8	0.849	1.84
C16:0	3.3	0.190	0.381
C18:0	4.2	0.151	0.218
C16:1	3.0	-	-
: ln-9, cis	-	0.470	0.667
C18:1	3.0	-	-
:ln-6, cis	-	2.49	9.41
:ln-6, trans	-	2.48	4.52
C18:ln-9	10.2	-	-
-cis	-	0.015	0.035
-trans	-	0.018	0.022
C22:1	22.6	-	-
:ln-13, cis	-	-	0.018
C18:2	6.6	-	-
:2n-6, cis	-	1.70	3.07
:2n-6, trans	-	0.590	0.693
C18:3	11.3	-	-
:3n-6, cis	-	19.2	11.9
C20:2	4.3	-	-
:2n-6, cis	-	-	0.048
C20:4	9.0	-	-
:4n-6, cis	-	27.1	6.33
C22:6	10.9	-	-
:6n-3, cis	-	0.361	0.513

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

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 [19]. The total sterol content of dietary fats and oils ranges from 0.01-2 % [30], the present levels were 0.327 % in the skin and 0.0296 % in the muscle which are within the literature values. Cholesterol, cholestanol and stig-masterol shared first positions in the skin with respective values of 81.7 mg/100 g but cholesterol and sitosterol in the muscle with respective values of 11.4 mg/100 g. 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 [28]. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23 % and 30 % respectively over a 6 week period [28]. Stigmasterol is also known as Wulzen antistiffness factor. 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. The level of cholestanol in the skin could have come from cholesterol breakdown or to both cholesterol breakdown and liver transformation of cholestenone. Cholestanol and sitosterol shared the first and second positions in the skin respectively, cholestanol was not detected in the muscle but sitosterol occupied the first position in the muscle.

Quality assurance of the determinations

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 except C18:1n-6, cis (muscle), C18:3n-6, cis (skin and muscle) and C20:4n-6,cis. 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.

Conclusion

In conclusion, the findings of this study showed that both skin and muscle of *A. macrolepidotus*showed unequal distribution of all the parameters (fatty acids, phospholipids and sterols) determined. Both skin and muscle were low in n-6 fatty acids but high in n-3 fatty acids. Both skin and muscle had unsaturated acids as the predominant fatty acids. Significant differences existed in the fatty acid levels on one hand and the phospholipid levels on the other hand. Both samples would serve as good sources of lecithin but much lower in sterols particularly the muscle.

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