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Full Length Research Paper

Total lipid, phospholipid and cholesterol contents of six commercially important fishes of Tuticorin, south east coast of India

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Total lipid, phospholipid and cholesterol content of six commercially important fishes commonly consumed by the local population were assessed. Total lipids in the flesh of fishes were extracted and classes of lipid such as phospholipids, cholesterol contents were estimated. Values obtained for total lipid, phospholipids and cholesterol content indicates good nutritional values of essential lipid on these fishes. Highest and lowest total lipid content and phospholipids were observed in *Scombermorus commerson* and *Sardinella albella* where cholesterol content was high in *S. commerson* and low in *Atule mate* respectively. Analysis of variance (ANOVA) for total lipid, phospholipids and cholesterol content of six different fishes showed a significant difference ($p < 0.05$) between the fishes.

Key words: Fresh fishes, lipid classes, total lipids, phospholipid, cholesterol, nutritional values.

INTRODUCTION

Fish is a major source of human nutrition providing important amount of dietary protein and lipid in many countries (Bouriga et al., 2010). Compared with red meat, fish flesh is easily digestible (Pirestani et al., 2010). It is well known for its nutritional value of protein with essential amino acids and its consumption is ever increasing but no knowledge about lipid classes. Lipids are the predominant source of energy for fish. The mechanisms by which fish allocate energy from lipids for metabolism, development, growth and reproduction are critical for understanding key life history strategies and transitions (Leaver et al., 2008). Lipids are the major source of nutrition in marine fishes (Sargent, 1976; Sargent et al., 1989) and they are considered as an efficient of stored energy in large amounts at a small space. The cells of white fat tissue called adipocytes are responsible for lipid synthesis, release and storage in the organism (Szkudelski et al., 2009). The major storage sites of fish are mesenteric fat, muscle and Liver (Sheridan, 1988). These marine natural products especially fish lipids are known to be beneficial in preventing attack from coronary heart diseases (CHD), cancer, diabetes, high blood pressure, gout and other diseases that are associated with consumption of excess foods containing high levels of cholesterol (Jitender Kumar Jakhar et al., 2012). The lipids in fish muscle have received much attention as a source of Omega-3 fatty acids in human diets (Ackman and Takeuchi, 1986). All lipids are hydrophobic in nature (Shirai et al., 2001) and fish lipids constitute a wide range of important constituents and may also be classified into lipid fraction such as; wax esters (WE), Cholesterol (CHL), steryl esters (STE), triacylglycerides (TAG), diacylglycerides (DAG), diacylglyceryl esters (DAGE), monoacylglycerides, phospholipids (PL), and free fatty acids (FAA) (Kinsella et al., 1978). The quantity of the total lipid may differ between various tissues and species (Ackman, 1990).

The fish lipids may be divided into neutral (saponifiables) and polar lipids (non saponifiables). The variation in the amount of polar lipids is small and somewhat constant. Changes in the amount of neutral lipid content of fish flesh vary depending on the amount of triglycerides and energy status. (Hanukoglu, 1992).

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Neutral lipids consisting mainly of saturated and monounsaturated fatty acids (MUFA) (Oxley et al., 2005), where as phospholipid consisting mainly of poly unsaturated fatty acids (PUFA) (Moriya et al., 2007) Exler and Weihrauch (1976) reported that phospholipids contain high level of polyunsaturated fatty acids that are useful to the human nutrition. Cholesterol is a waxy, fat like substance that is present in all animals but not in plants. Cholesterol alone is not very soluble in blood, so it is carried in the bloodstream by molecules called lipoproteins (Olson, 1998). Lipoproteins are named based on their density. We know from the careful study of cholesterol metabolism that high density lipoprotein (HDL) is "good" - it transports cholesterol to the liver. Low density lipoprotein (LDL) is "bad" - oxidized LDL gets deposited in the walls of arteries, contributing to arteriosclerosis (Hickley et al., 2003). High density lipoprotein is present in higher amount in fish lipid, where as low density lipoproteins were dominant in crude plant oil (Lecerf and De lorgeril, 2011). Every cell in our body contains cholesterol; we cannot live without it (Ohvo-Rekila et al., 2002). According to the Weston Price Foundation, fish lipid cholesterol is a potent antioxidant that protects against free radical damage to the cell membrane (Smith, 1991) and promotes myelin formation and neuronal plasticity in brain (Yeagle, 1991).

India is the third largest producer of fish in the world, and second in inland fish production (Anon, 2008). Marine water fish species viz., *Scomberomorus commerson* (Cheela), *Sphyraena jello* (Uuli), *Lethrinus nebulosus* (velameen), *Atule mate* (Manjaparai), *Stolephorus indicus* (nethili), *Sardinella albella* (Chooda) are the major fish species in Tuticorin next to crustaceans. All these fish species gave good economic supports to the local fishery department. These fishes formed the major species of the total fish production (953106 tonnes) in India in 2008 (Anon, 2010). No study on lipid class analysis of fish has been done so far so in the present study investigation was carried out to understand the lipid class such as total lipid, phospholipid and cholesterol content of six commercially important fishes of Tuticorin and commonly consumed by the local population.

MATERIALS AND METHODS

Sampling of fishes

The commercially important fishes such as *S. commerson, S. jello, L. nebulosus, A. mate, S. indicus, S. albella* were selected for the present study and collected from Tuticorin fish landing center during April 2013 and brought to laboratory in an ice box. The species identification was confirmed using the FAO species identification guide (De Bruin et al., 1994). The samples were dissected and the muscle tissue was cut from the fresh fish, rinsed with distilled water and dried to constant weight in a drying oven at 60°C for 24 h and the dried samples were used for the total lipid class analysis and the results were given on dry weight basis.

Total lipid analysis

Total lipid was extracted from each sample by the method of Folch et al. (1957). 1 g of the sample was homogenized with chloroform/methanol (2:1) to a final volume of 20 ml. After dispersion, the whole mixture was agitated for 20 min in a shaker at room temperature. The homogenate was then centrifuged at 2800 rpm to recover the liquid phase. The solvent was washed with 4 ml of 0.9% Nacl solution. After vortexing for few seconds, the mixture was centrifuged at low speed (2000 rpm) to separate the two phases. After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids was evaporated under vacuum in a rotary evaporator. Total lipid content was estimated by difference between pre and post weighed beaker.

Cholesterol analysis

The non-saponifiable lipids such as cholesterol were analyzed spectrometrically according to the method of Zlatkis et al. (1953). 3 ml of total lipid was extracted from each sample and it was saponified by adding 10 ml of 12% potassium hydroxide solution in ethanol and then kept in water bath at 80°C with agitation for 15 min and then cooled by the addition of 5 ml of distilled water and double extracted using 10 ml of hexane. 4 ml of the hexane extract was taken and evaporated using desiccators. With the evaporated sample colour developing reagents of 6 ml of a saturated solution of ferrous sulphate in 1% glacial acetic acid and 0.1 N of 2 ml of concentrated sulfuric acid (H_2SO4) were added. The calibration curve was constructed based on 50, 100, 150, and 200 mg of standard cholesterol solutions and subjected to the saponified and colour development stages.

Phospholipid analysis

The total phospholipid in the tissue was estimated as described by Wagner et al. (1968). The stock solution of colour reagent was prepared by dissolving 27 g of ferric chloride ($FeCl_3$ $6H_2O$) and 30 g of ammonium thiocyanate (NH_4SCN) in 1 l water and this solution is stable for months at room temperature. For the standard preparation, standard solution of phosphatidylcholine in chloroform (1 mg/ml). Extracted lipid residues dissolved in 2 ml chloroform and 1 ml of thiocyanate reagent. Vortex 1 min and centrifuge at low speed (2000 rpm) and

Table 1. Composition of lipid classes in the fishes (% of dry weight basis).

Scientific names of the fishes	Local names of the fishes	Mean total lipid (%)	Mean Phospholipid (%)	Mean cholesterol (mg/100g)
Scombermorus commerson	Cheela	9.11±0.49[a]	27.0±1.06[g]	107±2.64[m]
Sphyraena jello	Uuli	8.58±0.61[b]	26.1±0.96[h]	60±26.45[n]
Lethrinus nebulosus	Velameen	6.70±0.30[c]	17.0±1.40[i]	49±6.70[o]
Atule mate	Manjapaari	4.0±0.30[d]	9.0±0.94[j]	38±2.49[p]
Stolephorus indicus	Nethili	4.2±0.73[e]	9.2±0.95[k]	45±5[q]
Sardinells albella	Chooda	3.54±0.43[f]	6.50±1.50[l]	47±2.78[r]

Values are mean of triplicate analyses ± SD.
Value in the same column with different subscript letters (a – f) (g – j) (m - r) with in a same nutritional component are significant different ($p < 0.05$).

removed the red lower layer (chloroform) with a pipette. The absorbance of this solution was read at 488 nm and compare with known amounts of a standard phospholipid solution (range: 10-100 µg per tube). The total phospholipid concentration was then determined from a standard curve.

Statistical analysis

The total lipid, phospholipid and cholesterol content obtained for the different fishes were analyzed by two way analysis of variance (ANOVA) and the level of statistical significance was set as $p < 0.05$.

RESULTS

Total lipid, phospholipid and cholesterol content of six selected fishes are presented in Table 1.

Total lipid, phospholipid and cholesterol content of eighteen fish samples of the six selected species [*S. commerson* (Cheela), *S. jello* (Uuli), L. *nebulosus* (Velameen), *A. mate* (Manjaparai), *S. indicus* (Nethili), *S. albella* (Chooda)] were subjected in the computation of mean value based on the dry weight are presented. Mean total lipid content ranged from 3.54 ± 0.43% (*S. albella) to* 9.11 ± 0.49% (*S. commerson)* and mean phospholipid ranged from 6.5 ± 1.5% (*S. albella) to* 27.0 ± 1.06% *(S. commerson)* where as mean cholesterol content ranged from 38 ± 2.49 mg/100 g (*A. mate) to* 107 ± 2.64 mg/100 g *(S. commerson).* Values obtained for total lipid, phospholipid, cholesterol content of the different fishes was found to be good indication of essential lipids in nutritional values of commercial fishes. Highest total lipid content was found in muscle tissue of *S. commerson* and lowest was found in *S. albella.* However, highest and lowest phospholipids and cholesterol contents were recorded in *S. commerson* and *S. albella*, *A. mate* respectively.

Analysis of variance (ANOVA) for total lipid, phospholipid and cholesterol content of the six different fishes showed that there is significant difference ($p < 0.05$) between fishes. Analysis of variance (ANOVA) reveals non significance ($p > 0.05$) between lipid class of each fish but the lipid classes between fishes are significantly different ($p < 0.05$).

Discussion

The principle constituents of fish are water (66 – 84 %), protein (15 – 24 %), lipids (0.1 – 22 %), minerals (0.8 – 2 %) and sugar in very minute quantity (0.3%) (Bouriga et al., 2010). In this study selected fishes showed the lipid value ranges of 3.54 - 9.11%. Normally fishes were classified according to their lipid content (Ackman, 1990): Lean fish (< 2% fat), Low fat fish (2 - 4 % fat), Medium fat fish (4 - 8 % fat) and High fat fish (> 8% fat). Based on the above classification the selected experimental fishes were classified according to the lipid content, *S. albella* comes under low fat fish. *S. indicus, A. mate, L. nebulosus* are medium fat fish and *S. commerson* and *S. jello* were high fat fish. The lipid extracted by solvents is also called “crude fat.” or “extractable fat”. Crude fat is heterogeneous material, consisting of a mixture of triacylglycerols, phospholipids, fatty acids, cholesterol, waxes and pigments. The gravimetrically determined content of crude lipids is usually referred to as “total lipid”. Total lipid, as an estimate for energy content and nutritional values in biological material has been criticized because of the content of non-fat and non-digestible substances (Johnson and Barnett, 2003). Fish flesh is an important part of the fish, which people eat and is considered only as the important source of protein. Lipids are generally regarded as the most important constituent in fish muscle used to evaluate the quality of fish meat (Keriko et al., 2010). According to Zhou and Ackman (1995), lipids are primarily located in the subcutaneous tissue, belly flap, muscle tissue, liver, mesenteric tissue and in the head. The amount of lipid dispersed

throughout the body and it is high in muscle tissue decreases in the head and the tail. So in this study lipid is extracted from the muscle tissue for the analysis of lipid composition (Caulton and Burselle, 1977; Love et al., 1984). In the present study lipid were extracted from the muscle tissue of the commercially important fishes by the standard method of Folch et al. (1957), (Livia Facuri Araujo Macedo et al. (2012).

The estimated total lipid content in *S. commerson* muscle was 7.46% (Al-Jedah et al., 1999) which is lower than the value estimated for *S. commerson* (9.11%) in this study. Jitender Kumar Jakhar et al. (2012) reported *Magur* fresh water fish lipid content is 7.90% followed by *Pangas* 4.98%, *Rohu* 2.9% and *Catla* 1.2%. They also reported fresh water fish having low lipid in their muscle compared to marine water fish and it was agreed with our results. Dikel (1999) showed higher lipid content in fish grown in salt water (1.45%) than in freshwater (0.96%). Lipid contents in the muscle of freshwater and marine water fishes from Bangladesh ranged between 3.45 and 7.90% respectively (Kamal et al., 2007). Tapas mukhopadhyay and Ghosh 2003 reported total lipid content of commom carp (*Cyprinus carpio*) was 9.8% and also major portion of the lipid is composed of mainly phospholipid, about 64.3%, triglycerides about 30.4% and cholesterol about 5.2%. Besides these components trace amount of fatty acids, monoglycerides and diglycerides are observed in chromatographic plate. Watts (1957) reported total lipid content of west African shad (*Ethmalosa dorsalis*) shows a range in lipid content of 2 - 7%. Corvina (*Micropogon furnieri*) and pescada-foguete (*Marodon ancylodon*) have a lipid content range of 0.2 - 8.7% and 0.1- 5.4% respectively (Ito and Watanabe, 1968). Our results of total lipid content of economically important selected fish muscles fall within these ranges. Mangold and Mukherjee (1975) reported that total lipid varied as, 2.5, 8.9 and 4.9% in catfish, dogfish and Basking shark muscle respectively. Mangold and Mukherjee (1975) found that the black shark contained 4.6 ± 0.4% of total lipids in the liver tissues. The levels of the muscle lipids of Alaska Pollack (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) were between 0.4 - 0.7 and 0.5 - 4.8% respectively (Ackman, 1989b). Our results indicated that all the commercially important fishes are having >3% lipid. This fish lipid has a special importance to the animal body, which are primarily a source of energy in the diet (Gehring et al., 2009). Total lipids offer higher calories also vary between body organs and place. They offer higher calories percentage energy than that offer by protein and carbohydrate. Moreover, fats give the diet its particular flavors (Phleger, 1998). Intensive feeding in *Ambassis commersoni* coincides with the occurrence of high fat content in the muscle of fish gave good flavor compared to same fish with low lipid value (Bumb, 1992).

Principal total lipids constitute classes such as glycerides, phospholipid and cholesterol. Among those glycerides is a product of enzymatic or chemical hydrolysis of phospholipid. Cholesterol is a relatively stable lipid (Skipski, 1968). De Koning 2005 has estimated that one fifth of the total cholesterol of fish passes into oil and four fifth remains in the muscle. The ratio may vary with raw material but is convenient natural index for recoveries of the non polar or neutral lipid class. While total lipid content is often used as an indicator of biochemical condition. Shuter and Post, 1990 reported the ability to endure energy deficits may be dependent on the magnitude of endogenous stores of specific lipid classes (Norton et al., 2001). Among these classes, phospholipid and cholesterol are principal energy reserves for most fin fishes beyond the yolk-sac stage and have been used as a principal indicator of biochemical condition (Doucett et al 1999), than other neutral lipids, such as mono- and di-acylglycerols, free fatty acids (FFA).

Phospholipids are a class of lipids that are a major component of all cell membranes as they can form lipid bilayers. Mukhopadhyay et al. (2004) reported phospholipids are the major lipid fraction in the body tissue of fish. Keriko et al. (2010) reported lipid content of different parts of *Cyprinus carpio*, *Cyprinus specularis*, *Micropterus salmoides* and *Oreochromis leucosticus* fishes and the total lipid content was highest in the muscle tissue of the fish and phospholipids were the dominant in the total lipid classes in tissues. In our present study also phospholipid was the most dominant lipid class. Fraser et al. (1988) showed that phospholipid was a source of metabolic energy and a source of essential n-3 fatty acids. So our results indicated all the experimental fishes had good source of essential fatty acids. Buckley et al. (1989) studied the lipid class analysis of Heterotis *niloticus*, *Brycenus nurse*, *Gnathonemus cyprinoids* and *Sarotherodon galilaeus* and reported phospholipids as the most predominant lipid class as evidence of good PUFA contents. Talwar and Jhingran (1991) reported fish is composed with essential nutrient, good fish having the characteristics of high phospholipid with essential fatty acid. The presence of higher percentage of phospholipid fraction was reported in fresh water and marine fish species namely *O. pabda* and *W. attu* (Mukhopadhyay and Ghosh, 2007), *Cyprinus carpio* (Tapas Mukhopadhyay and Ghosh, 2003), *Notopterus pallas* (Mukhopadhyay et al., 2004) and *Theragra chalcogramma* (Bechtel et al., 2007). However, the phospholipids content was almost identical in both catla (27.6%) and mrigal (27.4%) roe lipids (Prabhakara Rao et al., 2013). They reported because the high content of phospholipid in muscle is responsible for muscle with good palatability. Our study results coincided with the above statement. Vila nova et al. (2005) reported phospholipids from 17.1 to 31.0% in tilapia fishes *(Oreochromis niloticus)* with low percentage of cholesterol. In our study 17.0% phospholipid was observed for *Lethrinus nebulosus.* El-Sayed et al. (1984)

reported that marine fish contained higher muscular lipids than the freshwater fish. In our study all the fishes from marine environment coincide with the above statement. The dehydrated fish egg powders of rohu (*Labeo rohita*) and murrel (*Channa striatus*) studied for lipid classes and fatty acid composition (Prabhakara Rao et al., 2010) showed 43.8, 72.9% neutral lipids; 12.7, 9.4% glycolipids and 43.5, 17.7% phospholipids. Kinsella et al. (1978) reported phospholipids usually contain significantly higher levels of unsaturated fatty acids. Mukhopadhyay and Ghosh (2007) reported phospholipid present in eggs of *W. attu* 28.6%. Johannes (1985) reported phospholipid content of Capelin fish was 24%. Based on the above reports indicates phospholipid was present in considerable amount indicates all the fishes good amount of essential polyunsaturated fatty acids responsible for good palatability of the experimental selected fishes. Cholesterol is by far the most common member of a group of sterols in animal tissues and it has an essential role in maintaining membrane fluidity. Cholesteryl esters important forms of long-term energy stores (Lochmann et al., 1995). Within cells, cholesterol is the precursor molecule in several biochemical pathways. In the liver, cholesterol is converted to bile, which is then stored in the gall bladder. Bile contains bile salts, which solubilize fats in the digestive tract and aid in the intestinal absorption of fat molecules as well as the fat-soluble vitamins, A, D, E and K. Cholesterol is an important precursor molecule for the synthesis of vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone, as well as the sex hormones progesterone, estrogens and testosterone and their derivatives. Some research indicates cholesterol may act as an antioxidant (Smith, 1991; Hanukoglu, 1992). Tapas Mukhopadhyay and Santinath Ghosh (2003) reported Fatty fish (salmon, sardines, herring, mackerel, whitefish, and bluefin tuna) raise good cholesterol (HDL) and lower triglycerides and fish oil capsules are having the same. In this present study, also all the commercially important fishes showed good cholesterol value. Mendez et al. (1992) reported the lipid content of 6.6% was reported in southwest Atlantic Hake (*Merluccius hubbsi*), of which 27.6% were waxes, 42% triacylglycerols, 14% phospholipids and 5.7% cholesterol. In our study also cholesterol content was lower than compared to phospholipid. Borgstrom and George (1961) reported cholesterol content of *Callinectes sapidus*, crab, *Melanogrammus aeglefinus* (haddock) , lobsters (*Panulirus spp*) , *Scomber scomberus* (maceral) , oyster (*Ostreidae spp*) , *Crassostrea virginica*, *Pecten irradians* and shrimp was 84 mg/100g, 57.5 mg/100 g, 66.3mg/100g, 260 mg/100g, 47.5 mg/100g, 105.5 mg/100g, 159.5 mg/100g respectively. In our study, cholesterol content of *S. commerson*, *S. jello*, *L. nebulosus*, *A. mate*, *S. indicus*, *S. albella* was 107 ± 2.64, 60 ± 26.45, 49 ± 6.70, 38 ± 2.49, 45 ± 5, 47 ± 2.78 mg/100 respectively. Mukhopadhyay and Ghosh (2007) reported cholesterol content of *O. pabda* and *W. attu* is 9.6 and 4.1% respectively. Ejike and Emmanuel (2009) studied distribution of cholesterol in the bovine meat parts studied, the cholesterol was found to be highest in the liver (6.5 ± 0.15 mg/g) followed by the kidney (5.6 ± 0.25 mg/g) and colon (5.1 ± 0.10 mg/g), and lowest in the intestines (small intestine, 1.3 ± 0.05 mg/g, and large intestine, 1.0 ± 0.01 mg/g). In our study only muscle tissue was tested for cholesterol content, highest was noted on the muscle tissue of *S. commerson* (107 ± 2.64mg/100g). In general fish lipid contains cholesterol level least down to a level of 140 mg/100g (Javitt, 1994). Our results coincide with the above statement. The lipid class of cholesterol in the six commercially important fishes indicated that all the fishes having high density lipoprotein is very good for human health and also help to synthesizing of essential hormone for maintain membrane fluidity and act as natural antioxidant in human body.

Conclusion

The fishes selected in the present study are high consumer demand food fishes having good taste. Among the selected fishes *S. indicus*, *S. albella* are relatively cheaper than the other fishes and therefore people living in coastal regions frequently consume these fishes even though they did not have any idea about the nutritional composition of these fishes. Now the results show that all the selected commercially important fishes are the good source of total lipid and very rich in phospholipids and good cholesterol. Present study will provide a detailed understanding the necessary of the healthy life for the fish consuming people. This information also may be helpful to evaluate the nutritional significance of the fishes. We concluded that fish lipid should not be avoided in human nutrition; its consumption should be minimized by individuals and populations at risk of cardiovascular diseases and its associated sequelae.

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