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# Seasonal variation in the proximate composition of sardine (*Sardinella gibbosa*) from Thoothukudi coast

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Seasonal variation in the proximate composition (moisture, protein, fat, carbohydrate and ash) of sardine (*Sardinella gibbosa*) was analysed for one year (June 2013 to May 2014). In addition, the fatty acid profile and protein fractions (sarcoplasmic, myofibrillar and stoma proteins) of the sample collected in the month of May 2014 were also analysed. Protein, fat, carbohydrate and ash content were varied significantly ($P<0.05$) from 15.43 to 22.76%, 1.25 to 6.77%, 0.47 to 0.89% and 1.78 to 3.21% respectively. Lipid content of sardine was high during November 2013 to January 2014 (6.21 – 6.77%) and least in the month of May 2014 (1.25%). Sardine sample collected in May 2014 was rich in PUFA especially EPA (C20:5) and DHA (C22:6). Analysis of protein fraction in sardine collected in the month of May 2014 showed that the sardine muscle contains 23.81% sarcoplasmic, 71.97% myofibrillar and 3.20% stroma proteins. The results showed no marked the variation in the protein, carbohydrate and ash contents, whereas, greater seasonal variation in the lipid content of the sample. Lipid content increased from the month of June 2013, reaching a maximum of 6.77% in November 2013, was almost stable in December 2013 (6.33%) and January 2014 (6.21%), after where it decreased as low as 1.25% in May 2014.

**[Keywords:** Sardine, proximate composition, seasonal variation]

## Introduction

Fish is a rich source of omega 3 polyunsaturated fatty acid (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[1]. They provide a range of health a benefit that are not present in any other food, including prevention of coronary heart disease, improvement of retina and brain development; reduction of incidence of breast cancer, rheumatoid arthritis, multiple scelerosis, psoriasis and inflammation[2-4]. The omega 3 fatty acids are associated with the synthesis of eicosanoids, thromboxanes and leucotrienes[5]. Moreover, these omega 3 fatty acids cannot be synthesised in the body and have to be obtained from the diet[6]. Therefore, the importance of consuming fish cannot be neglected and the promotion of fish as health food / brain food has become the global concern. The general recommendation for daily intake of DHA/EPA is 0.5 g for infants and 1 g for adults[7]. Recently the intake of n-6 fatty acids from vegetable oil and animal fats has been increasing and the WHO/FAO recommends n-6/n-3 ratio in the food products to be 5:1[8].

Principal composition of fish includes protein (16-21%), fat (0.2-25%), minerals (1.2-1.5%), carbohydrate (0-0.5%) and water (66-81%)[9]. The composition, however, varies greatly from species to species and also from individual to individual depending on age, sex, environment and season[10]. Furthermore, the variations in proximate composition of fish are closely related to the feed intake and the variations in the lipid content are much wider than that in protein. Lipids are the most important constituent of fish muscle providing energy reserve and components of cell biomembranes. Fish with fat content as low as 0.5% and as high as 16-18% are common. In many species, there is a build up of lipids during the feeding season and decrease during spawning. The lipid content in such fish show wide variations with season and sexual maturity. In fatty fish like sardine, mackerel, herring etc. the main site of storage of lipids is the muscle. Total lipid content, fatty acid proportions and

trace mineral compositions of fish depend greatly upon the diet consumed[6]. Throughout the year, fish are subjected to considerable environmental changes and fluctuations in availability and compositions of feed that will affect their proximate muscle composition[10]. Many other exogenous factors (temperature and salinity) may also affect the proximate and fatty acid compositions[11,12]. Furthermore, proteins and lipids are mobilised from muscle and transferred to the gonads in the reproductive period[13], in turn there is a change on the proximate composition.

Sardines are fatty fish with significant nutritional characteristics because of their high levels of omega 3 fatty acids. Sardine lipid varies widely with season and other factors such as temperature, feed intake, age, sex, size, etc affect the lipid composition[14]. The lipid content of the muscle of oil sardine (*Sardinella longiceps*) is about 3-4% in June-July, which increases to about 18% by November-December and in *S. pilchardus* the variation in lipid from 1.2 to 18.4%[11]. In *S. melanostidus* the lipid content was very low in February (1.8%) and high in July, September (7.2%).

No information is available till date on the seasonal variation in proximate composition of sardine (*S. gibbosa*) from Thoothukudi coast. Since the fishes are promoted as a means of improving health, the information on seasonal variation in the proximate composition of sardine would be of great use. Sardine (*S. gibbosa*) is a commercially important and highly consumed in Thoothukudi district of Tamil Nadu, India and is landed throughout the year in the landing centres of Thoothukudi. Therefore, considering the nutritional and health point of view, the variation in the proximate composition of sardine (*S. gibbosa*) from Thoothukudi coast was studied from June 2013 to May 2014.

## Materials and Methods

Sardine (*S. gibbosa*) was procured from the landing centre of Thoothukudi on the second week of every month for one year from June 2013 to May 2014. The fishes were placed in sterile polythene bags, kept in ice and brought to the laboratory within 30 mins. In the laboratory, fishes were washed and length (cm) and weight (g) were taken for each fish. Then fishes were dissected to remove skin, gills, fins and viscera and the muscle portion was collected for analysis. Moisture content was determined by the hot air oven method[15]. Nitrogen content was determined by the Kjeldhal method[15] using KEL PLUS – Elite ExVA – digestion and distillation apparatus and the protein content was calculated by multiplying the nitrogen content with a factor of 6.25. The crude fat was determined by Soxhlet method[15] using petroleum ether (60- 80° C) as solvent in a SOCS PLUS-SCS 08R system. Ash content was determined in a Muffle furnace at 500-550° C for 16 h[15]. Carbohydrate was estimated using anthrone reagent[16]. Sample collected in the month of May 2014 was also analysed for the fatty acid composition by Gas chromatography and for the protein fractions (sarcoplasmic, myfibrillar and stroma proteins)[17].

For the estimation of protein fractions, one gram of the sample was homogenised with 20 ml of 50 mM phosphate buffer (pH 7.0) and centrifuged at 5000 rpm for 5 min in a refrigerated centrifuge (Eppendorf, 5804 R, Germany). Supernatant was collected and the protein was estimated and reported as sarcoplasmic protein. To the pellet 20 ml of 50 mM phosphate buffer (pH 7.0) containing 0.5 M potassium chloride was added and kept overnight at 4$^{o}$C and centrifuged at 5000 rpm for 5 min in a refrigerated centrifuge. Supernatant was collected and the salt soluble protein was estimated. To the precipitate 10 ml of 0.1 M NaOH was added and kept overnight at 4$^{o}$C and centrifuged at 5000 rpm for 5 min. Supernatant was collected and the alkali soluble protein was estimated. 10 ml of acetic acid was added to the precipitate and kept at room temperature for 2 days, centrifuged and the supernatant constitutes the acid soluble protein fraction.

For the determination of fatty acid composition by Gas chromatography, total lipid was extracted from fish using chloroform and methanol[18], methylation of fatty acids by BF3-methanol and subsequent analysis of fatty acids methyl esters by capillary gas chromatography[19]. Fatty acid profile was analysed using Gas chromatography (Clarus 580, Perkin Elmer, USA) with a flame ionization detector and capillary column. Oven temperature was 50° C, held 3 min, raised to 150° C held 5 min and raised to 250° C. The injector and the detector temperature were set at 220° C and 280° C respectively. Sample size

was 1 µl and the carrier gas was controlled at 16 psi. Split was 1:10. Fatty acid was identified by comparing the retention times of FAME with a standard component FAME mixture (Supelco, USA). Two replicates GC analysis was performed and the results were expressed in GC area % as a mean value ± standard deviation.

Data collected in this study was analysed using SPSS (1999)[20] (Scientific package of social science, version 10). One way ANOVA test was used to find difference in the means of moisture, fat, protein, carbohydrate content of sardine (*S. gibbosa*) among the sampling months using Turkey HSD test.

**Results and Discussion**

The proximate composition of fish varies greatly due to physiological reasons and changes in environmental conditions[21]. Moisture content of the fish under study varied from 70.79% to 78.16% ($P<0.05$) (Table 1). Protein forms the largest quantity of dry matter in fish[19]. Protein content of sardine varied from 15.43% to 22.76%. Stransby (1976)[22] classified protein content as high when it is greater than 15 %. According to that, sardine cloud be classified as a high protein fish since, the protein content was greater than 15% throughout the year. The carbohydrate content in sardine was very low ranging from 0.47 to 0.89%. The carbohydrate content in fishes is generally low. Low carbohydrate content in fish suggest that glycogen in marine animals do not contribute significantly to the total reserves in the body[23]. Ash content in the fish is an indicator of mineral content of fish. Ash content varied from 1.78 % to 3.21 %. The sample collected in the month of May 2014 recorded the highest ash content.

Sardine (*S. gibbosa*) showed variation in fat content from 1.25% to 6.77%. Highest fat content was recorded in the months of November 2014 (6.77%), December 2013 (6.33%) and January 2014 (6.21%). A similar observation with high variation in the fat content (from 1.9% to 8.4%) of sardine (*S. gibbosa*) from Karachi coast, Pakistan[24] and with high concentrations of fat in sardine (*S. longiceps* and *S. fimbriata*) from Cochin waters[25] has been reported. The higher concentration of lipid content in the months of November, December 2013 and January 2014 observed in the present study mainly coincide with their spawning period. Moreover, during these three months period the sea water temperature falls to 26-27° C and fishes like sardine increase their lipid content to survive the low temperature. Similar observation has been observed in earlier studies[11,25-27]. Lipid content of sardine studied showed an increasing trend with decreasing moisture content in fish. The lipid content of fish varies with species, diet, geographic origin, season, reproduction[8]. Studies showed that plankton also influence the fat and fatty acid composition of sardine[25,27,28]. Krzynowek (1985)[29] reported about 10% variation in the content of fish according to season of capture. Fishes also tend to reduce their feed intake during sexual maturation as essential fatty acid and other nutrients needed for ovarian growth are taken from the reserves in their bodies. The study showed that the seasonal variation in the proximate composition of sardine is influenced by many factors including the nutrition of the organism.

The analysis of protein fraction in sardine collected in the month of May 2014 showed 23.81% sarcoplasmic, 71.97% myofibrillar and 3.20% stroma proteins. Sarcoplasmic protein content of fish constitutes 25-30 % of the protein, myofibrillar protein (actin, myosin, tropormyosin and actomyosin) 70-80% of the total protein content and connective tissue proteins (collagen) 3%[30].

Studies on the fatty acid profile of sardine (from C14:0 to C22:6N3) for the month of May 2014 (Table 2) revealed that the saturated fatty acid of 35.55%, dominated by palmitic acid contributing to approximately 20.03% (56.34% of the total SFA content) followed by Stearic acid (7.03%). Palmitic acid is the major saturated fatty acid in the lipids of many marine fish species (perch, sardine, anchovy etc.) constituting about 70% of the total SFA[31,32]. Predominance of palmitic acid (C16:0) in sardine (*S. gibbosa*) from Karachi coast of Pakistan has been documented[24]. Further C16:0 is a key metabolite of fish and did not seem to be influenced by diet. In the present study the major monounsaturated fatty acid was 8.02% of Elaidic acid (C18:1N9T) followed by 3.01% of oleic acid (C18:1N9C). However, earlier studies have shown oleic acid to be the predominant MUFA in the lipids of many species of marine fish accounting for 60-75% of the MUFA[6,33,34]. High level of C18:1N9T in the present studied

**Table 1. Seasonal variation in the proximate composition of *S. gibbosa***

| Proximate composition | June' 13 | July | August | September | October | November | December | January' 14 | February | March | April | May |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Length** | 10.10±0.02$^{b}$ | 9.20±0.05$^{a}$ | 12.2±0.11$^{c}$ | 13.8±0.11$^{e}$ | 13.2±0.05$^{de}$ | 9.8±0.11$^{ab}$ | 10.1±0.11$^{b}$ | 10.3±0.11$^{b}$ | 10.1±0.11$^{b}$ | 12.3±0.11$^{c}$ | 12.5±0.28$^{c}$ | 12.83±0.08$^{cd}$ |
| **Weight** | 23.00±0.57$^{de}$ | 24.00±1.15$^{e}$ | 24.00±1.73$^{e}$ | 30.00±0.57$^{f}$ | 26.00±0.57$^{ef}$ | 19.00±0.57$^{cd}$ | 14.00±1.15$^{ab}$ | 14.00±1.52$^{ab}$ | 13.00±0.57$^{a}$ | 17.00±1.54$^{abc}$ | 18.00±0.57$^{bc}$ | 18.00±0.28$^{bc}$ |
| **Moisture** | 74.41±0.10$^{bc}$ | 74.04±0.02$^{b}$ | 76.5±0.28$^{f}$ | 70.79±0.14$^{a}$ | 74.2±0.05$^{b}$ | 74.40±0.11$^{bc}$ | 75.16±0.10$^{cd}$ | 74.10±0.05$^{b}$ | 76.1±0.05$^{ef}$ | 76.47±0.28$^{f}$ | 75.4±0.23d$^{e}$ | 78.16±0.17$^{f}$ |
| **Protein** | 19.32±0.15$^{f}$ | 18.3±0.20$^{de}$ | 15.5±0.20$^{a}$ | 20.15±0.20$^{g}$ | 16.49±0.24$^{b}$ | 15.44±0.16$^{a}$ | 15.43±0.23$^{a}$ | 15.44±0.19$^{a}$ | 17.29±0.12$^{bc}$ | 17.5±0.14$^{cd}$ | 17.24±0.14$^{bc}$ | 19.14±0.09$^{ef}$ |
| **Fat** | 2.45±0.02$^{b}$ | 3.71±0.05$^{c}$ | 3.89±0.01$^{cd}$ | 3.90±0.01$^{cd}$ | 4.13±0.08$^{d}$ | 6.77±0.10$^{f}$ | 6.33±0.03$^{e}$ | 6.21±0.06$^{e}$ | 2.32±0.06$^{b}$ | 2.34±0.02$^{b}$ | 2.43±0.02$^{b}$ | 1.25±0.07$^{a}$ |
| **Carbohydrate** | 0.47±0.01$^{a}$ | 0.63±0.02$^{cde}$ | 0.52±0.01$^{ab}$ | 0.61±0.008$^{bcde}$ | 0.77±0.02$^{g}$ | 0.65±0.02$^{def}$ | 0.65±0.02$^{ef}$ | 0.81±0.005$^{gh}$ | 0.89±0.005$^{h}$ | 0.53±0.02$^{abc}$ | 0.75±0.02$^{fg}$ | 0.54±0.02$^{abcd}$ |
| **Ash** | 2.34±0.03$^{fg}$ | 2.36±0.02$^{f}$ | 2.11±0.04$^{cd}$ | 2.27±0.02$^{defg}$ | 2.15±0.02$^{cde}$ | 2.08±0.01$^{bc}$ | 2.19±0.005$^{cdef}$ | 2.08±0.008$^{c}$ | 2.3±0.05$^{efg}$ | 1.92±0.01$^{ab}$ | 1.78±0.01$^{a}$ | 3.21±0.05$^{h}$ |

Results are mean ± standard error, values within a row with different superscript letters are significantly different ($p<0.05$) in Tukey HSD test..

fish was in accordance with the finding of Ackman, 1982[14] who reported that MUFA in marine lipid usually contained 18 carbon atoms.

The percentage of n-3 PUFA in sardines in the present study was 28.86%, this was higher than the n-6 PUFA (5.125 %) as referred in the earlier research reports[34]. Among PUFA, the EPA and DHA have an important role in nutrition for human health and both are the dominant PUFA in sardine analysed, accounting for 11.5% and 17.9% respectively. Sardine (*S. gibbosa*) had low levels of Arachidonic acid (C20:4n-6) (3.27%) which may be advantageous to consumers with cardiovascular disease due to the antagonistic effects to the health benefits of n-3 fatty acids[4]. EPA is the most essential fatty acid of the n3 series in the human diet because it is the precursor to the 3-series eicosanoids[35]. A decrease in number of deaths due to coronary heart disease in people who consumed fish containing significant amount of n-3 fatty acids has been recorded[36]. EPA and DHA are found only in seafood's and possess several beneficial properties for the prevention of human coronary Artery diseases and therefore, fish diet is suggested as a key component for human health[12]. British Nutrition Foundation (1992)[36] has also recommended that people who have balanced and healthy diet consume 0.2 g EPA + DHA daily on a weekly basis.

**Table 2. Fatty acid profile of *S. gibbosa***

| Fatty acid | % | µg/g |
|---|---|---|
| **C14:0** | 3.11 | 539 |
| **C15:0** | 0.1 | 80 |
| **C16:0** | 20.03 | 3981 |
| **C17:0** | 3.02 | 500 |
| **C18:0** | 7.03 | 1407 |
| **C20:0** | 0.08 | 61 |
| **C21:0** | 0.1 | 83 |
| **C22:0** | 1.05 | 284 |
| **C24:0** | 1.03 | 216 |
| **TOTAL SFA** | 35.55 | 7151 |
| **C14:1** | 4.03 | 893 |
| **C15:1** | 1.01 | 104 |
| **C18:1N9T** | 8.02 | 1547 |
| **C18:1N9C** | 3.01 | 547 |
| **C20:1** | 0.01 | 85 |
| **TOTAL MUFA** | 16.08 | 3176 |
| **C18:2N6T** | 7.03 | 1350 |
| **C18:2N6C** | 2.5 | 481 |
| **C18:3N3** | 0.1 | 83 |
| **C20:3N6** | 2.01 | 451 |
| **C20:3N3** | 4.13 | 879 |
| **C20:4N6** | 3.2 | 637 |
| **C20:5N3** | 11.5 | 2107 |
| **C22:6N3** | 17.9 | 3472 |
| **Total PUFA** | 48.37 | 9459 |

## Conclusion

Present study inferred that Sardine, *S. gibbosa* showed variation in protein, fat, carbohydrate and ash content. Lipid content was high during November 2013 to January 2014 (6.21 – 6.77%) and least in the month of May 2014 (1.25%). The study has provided a base line data on the proximate composition of Sardine, *S. gibbosa* for one year suggesting greater variation in the lipid content compared to protein, carbohydrate and ash content. However, further studies on the fatty acid profile of Sardine, *S. gibbosa*, for one year is required to provide detailed information on the seasonal variation on the fatty acid profile of Sardine, *S. gibbosa*.

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