

Nutritional attributes in the fillet of skipjack tuna (*Katsuwonus pelamis*) from the Arabian Sea near the south-west coast of India

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*Inter-annual and seasonal variability in the nutritional parameters of the edible portion of skipjack tuna (*Katsuwonus pelamis*) collected from the Arabian Sea were determined for a period of 4 years. Greater levels of long chain n-3 fatty acids (35% during pre-monsoon), critical in the human diet for their anti-inflammatory properties with greater n-3:n-6 fatty acid ratio (8:12) demonstrated that this species may serve as an alternative to balance the greater amount of n-6 fatty acids. The present study demonstrated skipjack tuna as a significant source of protein, amino acids, minerals and vitamins. A balanced essential to non-essential amino acid ratio (1.2:1.4) in the fillets indicated that this species could provide well-balanced protein depositions. Vitamins A and K₁ demonstrated post-monsoon maxima, whilst vitamins D₃ and E showed pre-monsoon maxima. Greater calcium (172 mg 100 g⁻¹) and phosphorus contents (923 mg 100 g⁻¹) were recorded in the fillets of skipjack tuna during the pre-monsoon season. The chlorophyll-a concentration and sea surface temperature of its habitat were considered to understand their effect on the nutritional composition of skipjack tuna all through the study period. Significant correlation between long chain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid ($r^2 \sim 0.99$) of skipjack tuna alongside chlorophyll-a concentration was observed, particularly during the monsoon. The lesser atherogenic/thrombogenicity indices (<1), greater hypocholesterolaemic/hypercholesterolaemic ratio (>1.0), and lesser cholesterol contents (<50 mg 100 g⁻¹) of the fillets in skipjack tuna contributed towards its parameters to be qualified as a high value, balanced nutritional source.*

Abbreviations: AI, Atherogenicity index; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; FAMES, Fatty acid methyl esters; HH, Hypocholesterolaemic:hypercholesterolaemic ratio; HPLC, High performance liquid chromatography; PUFA, Polyunsaturated fatty acid; SFA, Saturated fatty acid; TAA, Total amino acids; TArAA, Total aromatic amino acid; TEAA, Total essential amino acid; TFA, Total fatty acids; TI, Thrombogenicity index; TNEAA, Total non-essential amino acid; TSAA, Total sulphur containing amino acid.

Keywords: polyunsaturated fatty acids, amino acids, vitamins, cholesterol, minerals, chlorophyll-a, inter-annual, seasonal

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INTRODUCTION

Marine fishes are rich in polyunsaturated fatty acids (PUFAs) containing n-3 fatty acids, for example, eicosatrienoic (ETE, 20:3n-3), eicosapentaenoic (EPA, 20:5n-3), and docosahexaenoic (DHA, 22:6n-3) acids. These fatty acids are thought to provide protection against rheumatoid arthritis, psoriasis, asthma and inflammatory bowel diseases, and have a role in improving visual function and brain development, and protecting against cardiovascular ailments (Inhamuns & Franco, 2008; Bulut *et al.*, 2012). Fish protein is easily digestible and favourably complements the dietary protein provided by beef, pork, chicken, cereals and legumes that are ordinarily consumed in many developing countries. Fish proteins have noteworthy nutritional qualities, and the amino acid composition is one of the most essential dietary characteristics of

protein (FAO & WHO, 1990). The dietary essential amino acid content of fish protein is about 30% greater than that of plant source, and compares favourably with egg, milk and meat proteins (Jan *et al.*, 2012). The sulphur-containing amino acids (methionine and cysteine) and lysine have been found to be deficient in cereal-based food items, whilst these constitute an overwhelming share of total amino acids in the fish fillets from marine origin (Iwasaki & Harada, 1985). Marine fishes are additionally known to be rich in minerals and vitamins (Erkan & Ozden, 2007; Özyurt *et al.*, 2009). When diets are dominated by the staple foods, as they are for people on low incomes, there is less uptake of mineral content. Fortunately, adding even a small amount of marine fish to a plant-based diet can greatly increase mineral intake. Other vital micronutrients supplied by marine fish include iron, calcium, phosphorus, selenium and vitamins, for example, A, D, E, K and C (Erkan & Ozden, 2007).

Tuna is considered as an epipelagic-to-midwater fish, occupying the upper and middle layers of ocean waters, to a depth of 1600 feet or more (500 m), contingent upon their size and species (Wild & Hampton, 1994). They are found in all seas,

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including the tropical seas and oceans, apart from in polar oceans. Maritime and coastal species of tuna, for example, albacore, big-eye, bluefin, skipjack and yellowfin tuna are commercially valuable, and are considered to be the principal market tuna species. Between 1980 and 1991, the yearly catch of tuna and tuna-like species in the Pacific expanded by 68% to about 3 million metric tonnes. The tuna species in the Indian Ocean and Arabian Sea are critical segments of food security, and also sustain noteworthy business exercises. Skipjack tuna (*Katsuwonus pelamis* Kishinouye, 1923) is the smallest and most plentiful of the major commercial tuna species, and is a significant group of finfish widely distributed in Indo-Pacific waters. They are found in the tropical regions of the Atlantic, Indian and Pacific waters, with the greatest abundance seen close to the equator (Matsumoto *et al.*, 1984). Fishery by islanders at the south-west shore of India is presently constrained to territorial waters and adjacent seas, and the catch is largely constituted of skipjack tuna (greater than 90% of the aggregate tuna landings). Proximate composition was determined in various tuna fishes (Bykov, 1983), which demonstrated the importance of tuna in the diet.

The seasonal variations of various biological factors, such as sexual development and environmental conditions, for example nutritional availability, temperature, salinity, etc. may alter the biochemical and nutritional compositions of fish (Dal Bosco *et al.*, 2012). The significance of marine fish as a potential health food having been acknowledged (Chakraborty *et al.*, 2014; Chakraborty & Joseph, 2015), studies on its biochemical composition have started to receive considerable attention. The Arabian Sea is the most productive marine fishery zone in India, representing around 70% of the aggregate tuna landings. While a few studies have been directed to the biological description and distribution of this species (Matsumoto *et al.*, 1984; Doulman & Kearney, 1987; Bayliff, 1988), no published studies are available on its nutritional composition, and their inter-annual and seasonal variations. Hence the present study aimed at the seasonal (pre-monsoon, monsoon and post-monsoon) and inter-annual (2008–2011) variations among the lipids, fatty acids, total cholesterol, protein, amino acids, minerals and vitamins in fillets of skipjack tuna collected from the Arabian Sea bordering the south-west coast of India. The relative abundance of chlorophyll-a concentration and sea surface temperature were considered in order to understand their impact on the nutritional composition in the fillet of this species all through the study period.

MATERIALS AND METHODS

Samples and study area

Fresh samples of skipjack tuna (total 5 kg varying from 0.5 to 2 kg) were harvested from the fishing harbours of Mangalore, Calicut and Cochin (south-west coast of India, SW) situated throughout the coastline of the Arabian Sea over a continuous period of 3 years (2008–2011). The samples were collected on the 15th day of each month. In order to acquire information on seasonal variations, the monthly data were grouped as pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January). Two pools of fish

per collection site, each one made out of 20 specimens of practically identical body size were collected within each sample, before being transported to the laboratory in an ice box (-20°C) for analysis. The results of the three centres were pooled, and the mean values were used in the present study. The whole fish after removal of skin were gutted, and the edible portion (fillet) was minced for analysis. Despite the fact that age and sex differences in nutritional composition are possible, we have viewed the fish as a whole food source, which is illustrative of the market, without any age or sex differences.

Lipid extraction and fatty acid analysis

Total lipids were extracted from the homogenized edible portion utilizing chloroform:methanol (2:1, v/v) (Folch *et al.*, 1957), determined gravimetrically in triplicate, and expressed as % w/w of the edible portion. The aliquots of the lipids extracted were utilized to prepare the fatty acid methyl esters (FAMES) and analysed using gas liquid chromatography (GLC) according to the procedure of Metcalf *et al.* (1966). GLC data were recorded on a Perkin-Elmer AutoSystem XL gas chromatograph (HP 5890 Series II, Perkin Elmer, Bridgeport Ave, Shelton, CT) connected with a SP 2560 (crossbond 5% diphenyl – 95% dimethyl polysiloxane) capillary column (100 m \times 0.25 mm i.d., 0.50 μm film thickness, Supelco, Bellfonte, PA) utilizing a flame ionization detector (FID) equipped with a split/splitless injector, which was used in the split (1:15) mode. The GLC analyses were accomplished using an oven temperature ramp program: 140°C for 1 min, rising at $30^{\circ}\text{C min}^{-1}$ to 250°C , where it was held for 1.0 min, followed by an increment of $25^{\circ}\text{C min}^{-1}$ to 285°C , where it was held for 2.0 min, until all peaks had showed up. The injector and detector were held at 285 and 290°C , respectively. Nitrogen (ultra high purity $>99.99\%$) was utilized as the carrier gas with a pressure of $5.6 \times 10^3 \text{ kg m}^{-2}$ and flow rate of 25 ml min^{-1} . The flow rate of hydrogen (45 ml min^{-1}) and air (450 ml min^{-1}) were kept at a pressure of $3.5 \times 10^4 \text{ kg m}^{-2}$. The injection volume was 0.2 μl . FAMES were recognized by comparison of retention times with known standards (SupelcoTM 37 Component FAME Mix, Catalogue no. 47885-U), and expressed as per cent of total fatty acids (% TFA).

Total cholesterol content

The total cholesterol content in the fillets of skipjack tuna was resolved spectrophotometrically (Varian Cary 50, Palo Alto, CA) as described elsewhere (Wanasundara & Shahidi, 1999) with suitable alteration utilizing *o*-phthalaldehyde (50 mg dl^{-1} in glacial acetic acid). The aggregate cholesterol content was ascertained from the standard curve of cholesterol, and expressed as mg 100 g^{-1} fillet.

Total protein and amino acids

The protein content of the fillets of skipjack tuna was assessed by the established method (Lowry *et al.*, 1951). The protein content of the sample was ascertained from the standard curve of bovine serum albumin, and expressed as g 100 g^{-1} fillet. Amino acid content was measured using the Pico-Tag method as described earlier (Heinrikson & Meredith, 1984) with suitable modifications. The samples (0.1 g) were hydrolysed with HCl (6N, 10 ml) at 110°C in sealed glass tubes

for 24 h on a multi-place heating mantle. The aliquot containing hydrolysed amino acids was treated with redrying solution (methanol 95%: water: triethylamine, 2.2:1 v/v/v), and thereafter pre-column derivatization of hydrolysable amino acids was performed with phenyl isothiocyanate (PITC, or Edman's reagent) to form phenylthiocarbamyl (PTC) amino acids. The reagent was freshly prepared, and the composition of derivatizing reagent comprised methanol 95%: triethylamine: phenylisothiocyanate (20 μ l, 7:1:1 v/v/v, 70 μ l methanol + 10 μ l distilled water + 10 μ l triethylamine + 10 μ l phenyl isothiocyanate). The derivatized sample (PTC derivative, 20 μ l) was diluted with sample diluent (20 μ l, 5 mM sodium phosphate NaHPO₄ buffer, pH 7.4: acetonitrile 95:5 v/v) before being injected into reverse-phase binary gradient HPLC (Waters Corporation, Milford, MA) fitted with a column maintained at 38 \pm 1°C in a column oven to be detected by its UV absorbance (λ_{max} 254 nm) with 2487 dual λ absorbance detector. A reverse phase C₁₈ column (dimethylcatadecylsilyl-bonded amorphous silica; Nova-Pak, 3.9 \times 150 mm) was used to separate the amino acids. The mobile phase comprised (A) sodium acetate trihydrate (0.14 M, 940 ml, pH 6.4) containing triethylamine (0.05%), mixed with acetonitrile (60 ml), and (B) acetonitrile: water (3:2, v/v). A gradient elution program, with increasing eluent B was employed for this reason. The quantification of amino acids was carried out by comparing the peak area of the sample with the standard (PIERS amino acid standard H; Thermo Scientific), and the amino acid content was expressed as g 100 g⁻¹ protein.

Nutritional health indices

A number of fatty acid- and amino acid-based health indices were calculated. The different ratios of fatty acid, indicating nutritional values of the fillets of skipjack tuna, for example, $\sum n-6$: $\sum n-3$ PUFAs, DHA/EPA, \sum PUFA: \sum SFA and LA/ALA were calculated in order to allow comparisons with the UK Department of Health recommendations (HMSO, 2001). The indices of atherogenicity (AI) and thrombogenicity (TI) (Ulbricht & Southgate, 1991; Barrento *et al.*, 2010) have been calculated as:

$$AI = \frac{4 \times C_{14} : o + C_{18} : o + C_{16} : o}{\sum MUFA + \sum n-3PUFA + \sum n-6PUFA}$$

$$TI = \frac{C_{14} : o + C_{18} : o + C_{16} : o}{(0.5 \times \sum MUFA) + (0.5 \times \sum n-6PUFA) + (3 \times \sum n-3PUFA) + (\sum n-3PUFA / \sum n-6PUFA)}$$

where \sum MUFA means the total monounsaturated fatty acids.

The hypocholesterolaemic:hypercholesterolaemic (HH) ratio were determined as:

$$HH = \frac{C_{18} : 1n - 9 + C_{18} : 2n - 6 + C_{20} : 4n - 6 + C_{18} : 3n - 3 + C_{20} : 5n - 3 + C_{22} : 5n - 3 + C_{22} : 6n - 3}{C_{14} : o + C_{16} : o}$$

(Santos-Silva *et al.*, 2002).

The amino acid score (AS) for the essential amino acids was calculated using the FAO/WHO formula: \sum amount of amino acid per sample protein (mg g⁻¹) / \sum amount of amino acid per protein in reference protein (mg g⁻¹) with respect to reference amino acid requirements for adults (FAO, WHO and UNU, 2007).

Fat-soluble (A, D₃, E, K₁) and water-soluble (C) vitamins

Estimation of the fat-soluble vitamins (A, D₃, E and K₁) was carried out by a modified method of Salo-Vaananen *et al.* (2000). The stock solutions (1, 10, 25, 50 and 100 ppm) of vitamin standards (Sigma-Aldrich Chemical Co. Inc, St. Louis, MO) were stored at -20°C except vitamin D₃, where the stock solutions were stored at 4°C. The lipids (0.1 g) were extracted utilizing the established method (Chakraborty *et al.*, 2016), before being hydrolysed (KOH/MeOH 0.5 N, 2 ml). The hydrolysed mixture (2 ml) was extracted with petroleum ether (fraction of 40–60°C, 15 ml) and washed with deionized water (2 \times 10 ml) to make it alkali-free. The non-saponifiable portion was concentrated under vacuum using a rotary evaporator (Heidolph Instruments GmbH & Co., Schwabach, Germany) at 50°C before being reconstituted in MeOH. The latter was filtered through a syringe filter (0.2 mm) before being injected (20 ml) in the HPLC (Shimadzu LC 20AD, Shimadzu Corporation, Nakagyo-ku, Japan). The HPLC system was equipped with a reverse phase column (Phenomenex, C₁₈ 250 mm length, 4.6 mm i.d., 5 mm) that was housed in a column oven (32°C) and connected to a photodiode array detector. The gradient program was as follows: 20% MeOH (HPLC grade) up to 3 min, which was increased to 100% in the next 5 min and held for 37 min with a complete run time of 45 min. The flow rate was 1 ml min⁻¹. The vitamin C was determined based upon the quantitative discolouration of 2, 6-dichlorophenol indophenol titrimetric method (AOAC International, 1995). In brief, ascorbic acid was extracted from the fish fillet (M, 15–20 g) using an acetic acid and metaphosphoric acid solution (HPO₃-CH₃COOH, 10 ml \times 2). The extracts were transferred with distilled water into a known volume (B, ml) and filtered rapidly. The known volume (C, ml) of the above solution was pipetted out and titrated with the redox dye, 2, 6 dichlorophenol indophenol solution until the faint pink colour persisted for 15 s.

$$\text{Ascorbic acid was calculated as: } \frac{A - A_0 \times D \times B \times 10}{M \times C},$$

where A = average volume for test solution titration (ml), A₀ = average volume for test blank titration (ml) and D = mg ascorbic acid equivalent to one ml indophenol standard solution. The vitamins A, D₃, E, K and C were expressed as mg 100 g⁻¹ fish fillet.

Mineral composition

Estimation of minerals was carried out by atomic absorption spectrophotometer (Chemito AA 203) following the di-acid (HNO₃/HClO₄) digestion method with suitable modifications (Chakraborty & Joseph, 2015). In brief, the samples (2 g) were

placed in digestion tubes, to which concentrated HNO_3 (7 ml) was added, and the content was kept for overnight digestion in a fume hood until no brown fumes appeared. The analyses of Ca, Na, K, Mn, Fe, and Zn were performed by flame atomic absorption spectrophotometry equipped with a hollow cathode lamp containing D_2 lamp background correction system. For Se, a continuous flow hydride generator coupled with an atomic absorption spectrometer was used. Phosphorus content was analysed by an alkalimetric ammonium molybdophosphate method as described in AOAC official method 995.11 (AOAC International, 2002).

Chlorophyll-a concentration and sea surface temperature

Chlorophyll-a concentration and sea surface temperature (SST) data were derived from the global 9 km monthly mean SeaWiFS (Sea-viewing Wide Field-of-view Sensor) and MODIS (Moderate Resolution Imaging Spectroradiometer) –AQUA, respectively for the period from January 2008 to December 2011 as described earlier (Chakraborty *et al.*, 2014). These were taken into account to indicate the distribution of the photosynthetic pigment chlorophyll-a, and to study the effect of temperature on nutritional composition.

Statistical analyses

Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, ver. 13.0). The descriptive statistics were calculated for all the studied traits. Analyses were carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance (ANOVA). Pearson correlation coefficient between biochemical compositions of samples collected was analysed. The level of significance for all analyses was $P < 0.05$.

RESULTS AND DISCUSSION

Inter-annual and seasonal variability of chlorophyll-a and sea surface temperature

There is a general assumption that phytoplankton are the major source of essential fatty acids in the marine environment and the ratio of typical fatty acids can be utilized as biomarkers for diverse classes of phytoplankton. Satellite colour images of the ocean, which were required to give information about the relationship between the chlorophyll-a concentration and plankton abundance, were statistically studied with respect to the different nutritional parameters. The nutritional qualities with respect to various biochemical indicators were assessed seasonally, and their signatures have been correlated with the chlorophyll-a concentration to understand their impact on the nutritional signatures of *Katsuwonus pelamis* all through the study period as described earlier (Chakraborty *et al.*, 2014). In the SW coast of India, chlorophyll-a showed relatively lesser values during the pre-monsoon season (4 years' mean of 0.3 mg m^{-3}), reached monsoon maxima (4 years' mean of 1.2 mg m^{-3}), and subsequently decreased throughout post-monsoon season (4 years mean' of 0.5 mg m^{-3}) (Chakraborty *et al.*, 2014). The sea surface temperature (SST) data derived from MODIS-AQUA

showed that high SST were seen during pre-monsoon all through the study period ($>30^\circ\text{C}$) along the SW coast of India, which decreased in monsoon (29.5°C), and showed a gradual decrease during the post-monsoon season ($<29^\circ\text{C}$) (Chakraborty *et al.*, 2014).

Inter-annual and seasonal variability in lipid content and fatty acid composition of skipjack tuna

No significant inter-annual variations of lipid were observed over the contemplated years ($P > 0.05$) (Table 1). However, significantly greater lipid content was observed during the monsoon season (4 years' mean of 1.6%) as contrasted with those acquired during the pre-monsoon and post-monsoon seasons (4 years' mean of 0.27 and 0.57%, respectively). The greater lipid content observed during the monsoon as compared with other seasons correlated well with the greater chlorophyll-a concentration (4 years' mean chlorophyll-a 1.2 mg m^{-3}) in the same season ($r^2 = 0.722$; Figure 1A). It is generally recognized that water temperature and differences in salinities are the foremost marine ecological components affecting development and gonadal change in marine fishes (Pazos *et al.*, 1996), which determine the lipid content. In a warm climate, as in the pre-monsoon season (4 years' mean SST of 30.6°C), water is poor in nutritional components (Njinkoue *et al.*, 2002), and the fish may utilize their energy depots in the form of lipids, thereby realizing a reduction of lipid content during the pre-monsoon as compared with the monsoon season. The greater ocean surface temperature during the pre-monsoon than in monsoon seemed to contribute towards greater lipid content during the monsoon season. Twenty-nine fatty acids, including saturated fatty acids (SFAs), MUFAs and PUFAs, were determined in the fillets of skipjack tuna. SFAs with their greater caloric content are essentially utilized as a storage form of energy. SFAs contributed a major proportion (35–53%) of the total fatty acid composition, which were significantly correlated with chlorophyll-a concentration during the monsoon ($r^2 = 0.935$; Figure 1B) and post-monsoon seasons ($r^2 = 0.882$; Figure 1C). Generally, insignificant seasonal and inter-annual disparities were apparent in the SFA composition of skipjack tuna ($P > 0.05$). The C_{16} palmitic acid, which is a source of potential metabolic energy in marine fish, was seen to be the most common SFA with monsoon and post-monsoon maxima (4 years' mean of greater than 25%). This result was similar to the prior study on the Mediterranean fishes (Zlatanov & Laskaridis, 2007) and other marine pelagic species (20–25%) (Njinkoue *et al.*, 2002). The MUFA content in the fillets of skipjack tuna ranged from 15–28% with a monsoon minimum (4 years' average of $\sim 19\%$). The predominant MUFA was found to be 18:1n-9, which is in concurrence with prior findings that the marine lipids of the pelagic fish species typically contain C_{18} fatty acids (Zlatanov & Laskaridis, 2007).

PUFAs are considered to be a critical dietary indicator directing the quality of marine fishes. The total PUFA content in the fillets of skipjack tuna showed significantly greater values during the pre-monsoon and monsoon seasons (4 years' mean of $\geq 35\%$) as contrasted with the post-monsoon season (4 years' mean of $\sim 28\%$) ($P < 0.05$). This seasonal change observed in the PUFA content was due to

Table 1. Lipid per cent (% w/w) and fatty acid distribution (% TFA) in the fillets of *K. pelamis* collected from the south-west coast of India during 2008–2011 in three different seasons (pre-monsoon, monsoon and post-monsoon).

	2008	2009	2010	2011	Mean	2008	2009	2010	2011	Mean	2008	2009	2010	2011	Mean
	Pre-monsoon					Monsoon					Post-monsoon				
Lipid	0.5 ± 0.02 ^a	0.2 ± 0.02 ^a	0.3 ± 0.02 ^a	0.1 ± 0.02 ^a	0.27	1.6 ± 0.01 ^a	1.8 ± 0.03 ^{ad}	1.2 ± 0.02 ^a	1.8 ± 0.02 ^{ad}	1.60	0.5 ± 0.02 ^a	0.3 ± 0.02 ^a	0.7 ± 0.02 ^a	0.8 ± 0.02 ^a	0.57
Saturated fatty acids															
12:0	0.12 ± 0.02 ^a	0.15 ± 0.02 ^a	0.13 ± 0.02 ^a	0.14 ± 0.02 ^a	0.13	0.76 ± 0.01 ^a	0.05 ± 0.03 ^{ad}	0.20 ± 0.02 ^a	0.14 ± 0.02 ^{ad}	0.29	0.15 ± 0.02 ^a	0.01 ± 0.0 ^a	0.14 ± 0.02 ^a	0.13 ± 0.02 ^a	0.11
14:0	2.04 ± 0.32 ^{ad}	2.88 ± 0.35 ^{acd}	2.26 ± 0.29 ^{ad}	2.46 ± 0.41 ^{ad}	2.39	3.61 ± 0.12 ^{ad}	0.81 ± 0.08 ^{ab}	4.00 ± 0.07 ^{ad}	2.60 ± 0.11 ^d	2.76	0.75 ± 0.07 ^{ab}	4.65 ± 0.33 ^{ad}	4.68 ± 0.02 ^a	2.34 ± 0.02 ^a	3.11
15:0	1.17 ± 0.15 ^{ac}	0.69 ± 0.13 ^a	1.06 ± 0.17 ^{ac}	0.94 ± 0.10 ^a	0.97	0.38 ± 0.03 ^b	0.18 ± 0.22 ^c	1.50 ± 0.09 ^{ab}	0.62 ± 0.05 ^{ab}	0.67	0.33 ± 0.14 ^a	0.95 ± 0.08 ^{ab}	0.95 ± 0.02 ^{ad}	0.56 ± 0.02 ^{ad}	0.70
16:0	20.8 ± 1.10 ^a	23.6 ± 1.22 ^a	21.6 ± 2.98 ^a	22.5 ± 0.46 ^{ab}	22.0	35.0 ± 0.10 ^a	21.6 ± 0.18 ^a	22.0 ± 0.11 ^{ab}	21.7 ± 0.81 ^a	25.1	26.6 ± 0.97 ^a	27.5 ± 0.80 ^{ab}	27.7 ± 0.15 ^{ac}	19.6 ± 0.15 ^{ac}	25.4
17:0	1.23 ± 0.17 ^a	1.14 ± 0.17 ^a	1.21 ± 0.08 ^a	1.18 ± 0.06 ^{ad}	1.19	ND	1.25 ± 0.26 ^{ac}	1.70 ± 0.15 ^a	1.03 ± 0.01 ^b	1.30	0.08 ± 0.21 ^a	0.95 ± 0.13 ^{ad}	1.46 ± 0.10 ^a	0.93 ± 0.03 ^a	0.86
18:0	11.7 ± 1.70 ^a	12.0 ± 1.71 ^a	11.8 ± 1.68 ^a	11.9 ± 1.75 ^a	11.83	12.4 ± 1.48 ^a	10.3 ± 1.39 ^a	9.70 ± 1.58 ^a	11.0 ± 1.92 ^a	10.85	13.4 ± 1.69 ^a	11.7 ± 1.42 ^a	11.7 ± 0.17 ^a	9.91 ± 0.17 ^a	11.68
20:0	0.47 ± 0.06 ^a	0.26 ± 0.05 ^a	0.42 ± 0.07 ^{ab}	0.37 ± 0.04 ^{ac}	0.38	ND	ND	0.60 ± 0.03 ^{ac}	0.23 ± 0.06 ^a	0.42	0.41 ± 0.10 ^b	0.70 ± 0.03 ^{ac}	0.71 ± 0.07 ^a	0.21 ± 0.07 ^a	0.51
22:0	0.32 ± 0.04 ^a	0.22 ± 0.04 ^a	0.30 ± 0.05 ^a	0.27 ± 0.03 ^{ab}	0.28	0.06 ± 0.01 ^b	0.09 ± 0.08 ^{ac}	0.50 ± 0.03 ^{ab}	0.19 ± 0.02 ^a	0.21	ND	0.23 ± 0.03 ^{ab}	0.23 ± 0.06 ^a	0.18 ± 0.06 ^a	0.32
24:0	0.06 ± 0.01 ^a	0.04 ± 0.01 ^a	0.06 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05	1.06 ± 0.06 ^d	0.41 ± 0.01 ^a	ND	0.04 ± 0.20 ^b	0.38	1.39 ± 0.05 ^{ad}	0.35 ± 0.00 ^a	0.35 ± 0.04 ^a	0.03 ± 0.04 ^a	0.53
∑SFA ^a	37.9 ± 2.58 ^a	40.9 ± 2.71 ^a	38.8 ± 2.44 ^a	39.8 ± 1.98 ^a	39.2	53.3 ± 0.96 ^a	34.7 ± 0.83 ^a	40.2 ± 0.38 ^a	37.6 ± 0.18 ^c	41.4	43.1 ± 6.88 ^a	47.0 ± 4.84 ^a	47.9 ± 0.01 ^a	33.9 ± 0.01 ^a	43.0
Mono-unsaturated fatty acids															
14:1n-7	0.13 ± 0.02 ^a	0.04 ± 0.01 ^{ab}	0.11 ± 0.02 ^a	0.09 ± 0.01 ^{ab}	0.09	ND	ND	0.20 ± 0.01 ^b	0.04 ± 0.01 ^b	0.12	ND	0.29 ± 0.00 ^b	0.12 ± 0.02 ^a	0.03 ± 0.02 ^a	0.14
15:1n-7	0.06 ± 0.01 ^a	0.13 ± 0.01 ^a	0.08 ± 0.01 ^a	0.10 ± 0.02 ^{ac}	0.09	ND	ND	ND	0.12 ± 0.01 ^b	0.12	ND	0.16 ± 0.02 ^a	0.05 ± 0.02 ^a	0.11 ± 0.02 ^a	0.10
16:1n-7	2.66 ± 0.40 ^a	3.30 ± 0.43 ^a	2.82 ± 0.38 ^a	2.98 ± 0.47 ^{ab}	2.93	2.55 ± 0.42 ^a	2.97 ± 0.60 ^{ab}	4.10 ± 0.42 ^a	2.97 ± 0.36 ^a	3.15	2.49 ± 0.73 ^b	5.07 ± 0.38 ^a	5.11 ± 0.01 ^a	2.67 ± 0.01 ^a	3.84
18:1n-7	0.20 ± 0.02 ^a	0.06 ± 0.02 ^a	0.16 ± 0.05 ^a	0.13 ± 0.0 ^b	0.14	ND	ND	0.10 ± 0.01 ^b	0.06 ± 0.01 ^a	0.08	ND	0.32 ± 0.01 ^b	0.32 ± 0.40 ^a	0.05 ± 0.00 ^a	0.17
18:1n-9	16.7 ± 0.15 ^a	9.77 ± 0.90 ^a	15.0 ± 0.40 ^{ac}	13.2 ± 0.40 ^a	13.82	14.8 ± 2.18 ^a	15.2 ± 1.65 ^a	11.0 ± 1.26 ^{ad}	8.80 ± 1.71 ^a	12.45	11.9 ± 2.36 ^{acd}	16.3 ± 1.13 ^{ab}	16.4 ± 0.02 ^a	7.92 ± 0.02 ^a	13.13
20:1n-9	0.10 ± 0.04 ^a	0.84 ± 0.07 ^a	0.29 ± 0.01 ^a	0.47 ± 0.02 ^b	0.41	0.18 ± 0.09 ^b	1.32 ± 0.11 ^b	0.70 ± 0.11 ^b	0.76 ± 0.16 ^b	0.74	1.14 ± 0.06 ^a	0.10 ± 0.01 ^a	0.39 ± 0.05 ^a	0.68 ± 0.01 ^a	0.58
22:1n-9	3.53 ± 0.51 ^a	3.69 ± 0.52 ^a	3.57 ± 0.51 ^a	3.62 ± 0.53 ^a	3.60	ND	ND	3.60 ± 0.47 ^a	3.32 ± 0.02 ^a	3.46	ND	3.18 ± 0.43 ^a	3.20 ± 0.04 ^a	2.99 ± 0.04 ^a	3.12
24:1 n-9	0.08 ± 0.02 ^a	0.18 ± 0.02 ^a	0.11 ± 0.01 ^a	0.13 ± 0.03 ^{ac}	0.12	0.59 ± 0.05 ^c	0.36 ± 0.01 ^a	0.10 ± 0.02 ^a	0.16 ± 0.11 ^b	0.30	0.75 ± 0.05 ^c	0.11 ± 0.02 ^a	0.35 ± 0.05 ^a	0.14 ± 0.01 ^a	0.34
∑MUFA ^b	23.5 ± 0.17 ^a	18.0 ± 0.97 ^a	22.1 ± 0.37 ^a	20.7 ± 0.57 ^a	21.2	18.1 ± 2.89 ^a	19.9 ± 2.95 ^a	19.8 ± 2.32 ^a	16.2 ± 2.37 ^a	18.5	16.3 ± 3.73 ^a	25.5 ± 2.09 ^a	25.9 ± 0.02 ^a	14.6 ± 0.02 ^a	20.6
Polyunsaturated fatty acids															
16:2n-4	0.41 ± 0.05 ^a	0.20 ± 0.04 ^{ac}	0.36 ± 0.06 ^a	0.31 ± 0.03 ^c	0.32	ND	ND	0.40 ± 0.03 ^c	0.18 ± 0.01 ^a	0.29	ND	ND	0.47 ± 0.07 ^a	0.16 ± 0.07 ^a	0.32
16:3n-4	0.20 ± 0.02 ^a	0.02 ± 0.00 ^a	0.15 ± 0.03 ^{ac}	0.11 ± 0.00 ^b	0.12	ND	ND	0.20 ± 0.00 ^b	0.02 ± 0.02 ^a	0.11	ND	ND	0.14 ± 0.05 ^a	0.02 ± 0.05 ^a	0.08
18:2n-6 LA	1.29 ± 0.18 ^a	1.23 ± 0.18 ^a	1.28 ± 0.18 ^a	1.26 ± 0.18 ^a	1.27	1.19 ± 0.22 ^a	1.51 ± 0.24 ^a	1.60 ± 0.16 ^a	1.10 ± 0.20 ^a	1.35	1.39 ± 0.19 ^a	1.31 ± 0.14 ^a	1.32 ± 0.02 ^a	0.99 ± 0.02 ^a	1.25
18:3n-6	0.97 ± 0.11 ^a	0.15 ± 0.08 ^a	0.77 ± 0.14 ^{ac}	0.57 ± 0.02 ^b	0.63	0.66 ± 0.09 ^{ac}	0.64 ± 0.08 ^a	0.50 ± 0.02 ^a	0.14 ± 0.07 ^{ab}	0.49	0.47 ± 0.12 ^{ac}	0.83 ± 0.02 ^b	0.83 ± 0.18 ^a	0.13 ± 0.18 ^a	0.57
18:3n-3 ALA	0.28 ± 0.03 ^a	0.21 ± 0.03 ^a	0.23 ± 0.04 ^a	0.18 ± 0.01 ^a	0.24	1.04 ± 0.29 ^c	2.02 ± 0.03 ^a	0.20 ± 0.01 ^a	0.22 ± 0.05 ^b	0.87	1.04 ± 0.11 ^b	0.78 ± 0.01 ^b	0.78 ± 0.11 ^a	0.22 ± 0.11 ^a	0.71
18:4n-3	ND	ND	ND	ND	ND	0.15 ± 0.13 ^b	0.92 ± 0.01 ^a	ND	ND	0.53	0.21 ± 0.02 ^a	0.10 ± 0.01 ^a	ND	ND	0.15
20:2n-6	0.60 ± 0.09 ^a	0.65 ± 0.09 ^a	0.62 ± 0.09 ^a	0.63 ± 0.09 ^a	0.62	0.06 ± 0.01 ^b	0.05 ± 0.15 ^c	1.00 ± 0.08 ^a	0.58 ± 0.01 ^b	0.42	ND	0.50 ± 0.08 ^a	0.50 ± 0.01 ^a	0.53 ± 0.02 ^a	0.51
20:3n-6	0.24 ± 0.03 ^a	0.13 ± 0.03 ^a	0.22 ± 0.03 ^a	0.19 ± 0.02 ^a	0.20	0.39 ± 0.05 ^{ab}	0.33 ± 0.02 ^a	0.10 ± 0.02 ^{ac}	0.12 ± 0.04 ^b	0.24	0.29 ± 0.04 ^{ab}	0.29 ± 0.02 ^{ac}	0.29 ± 0.09 ^a	0.11 ± 0.09 ^a	0.25
20:4n-6	0.43 ± 0.06 ^a	0.26 ± 0.05 ^a	0.39 ± 0.06 ^a	0.35 ± 0.04 ^{ac}	0.36	2.78 ± 0.57 ^c	4.02 ± 0.06 ^a	0.30 ± 0.03 ^a	0.23 ± 0.09 ^c	1.83	4.12 ± 0.04 ^a	0.25 ± 0.03 ^a	0.26 ± 0.03 ^a	0.21 ± 0.03 ^a	1.21
20:5n-3 EPA	3.69 ± 0.06 ^a	7.67 ± 0.81 ^{ab}	4.69 ± 0.53 ^{ac}	5.69 ± 1.10 ^a	5.35	3.84 ± 1.24 ^b	8.67 ± 1.02 ^b	7.10 ± 0.99 ^{ab}	6.91 ± 0.51 ^{ac}	6.63	3.54 ± 0.06 ^a	3.87 ± 0.09 ^{abc}	3.90 ± 0.06 ^a	6.22 ± 0.06 ^a	4.38
22:5n-3	3.25 ± 0.44 ^a	2.56 ± 0.42 ^a	3.09 ± 0.47 ^{ac}	2.91 ± 0.37 ^b	2.97	1.85 ± 0.33 ^{ab}	2.34 ± 0.28 ^{abc}	1.90 ± 0.33 ^{abc}	2.31 ± 0.32 ^{abc}	2.10	2.25 ± 0.18 ^b	1.22 ± 0.30 ^{abc}	1.23 ± 0.67 ^a	2.08 ± 0.67 ^a	1.70
22:6n-3 DHA	25.6 ± 0.74 ^a	27.9 ± 0.83 ^a	26.1 ± 0.66 ^a	26.7 ± 0.99 ^{ac}	26.53	15.6 ± 3.10 ^{ab}	21.6 ± 0.97 ^{ab}	20.0 ± 1.59 ^a	25.1 ± 0.88 ^{ab}	20.58	20.1 ± 1.74 ^b	12.1 ± 3.23 ^{ab}	12.2 ± 0.44 ^a	22.6 ± 0.44 ^a	16.75
∑PUFA ^c	37.0 ± 1.43 ^a	41.0 ± 0.56 ^a	37.9 ± 1.29 ^a	38.9 ± 1.84 ^a	38.6	27.6 ± 1.03 ^a	42.1 ± 1.95 ^a	33.3 ± 1.26 ^a	36.9 ± 1.78 ^a	35.44	33.4 ± 3.14 ^a	21.3 ± 4.73 ^a	21.9 ± 3.74 ^a	33.3 ± 3.74 ^a	27.5
EPA + DHA	29.3 ± 1.03 ^a	35.6 ± 0.43 ^a	30.8 ± 0.43 ^a	32.4 ± 2.01 ^a	31.9	19.4 ± 1.43 ^a	30.3 ± 1.43 ^a	27.1 ± 1.43 ^a	32.0 ± 1.43 ^a	27.2	23.6 ± 0.07 ^b	16.0 ± 0.07 ^b	16.1 ± 0.43 ^a	28.8 ± 0.23 ^a	21.1
∑n-3	32.8 ± 4.89 ^a	38.3 ± 5.08 ^a	34.1 ± 0.69 ^a	35.5 ± 0.47 ^{ac}	35.1	22.5 ± .09 ^a	35.6 ± 0.30 ^a	29.2 ± 1.22 ^a	34.5 ± 3.88 ^a	30.5	27.1 ± 2.59 ^a	18.1 ± 0.43 ^a	18.1 ± 0.09 ^a	31.1 ± 0.09 ^a	23.6
∑n-6	3.50 ± 0.47 ^a	2.40 ± 0.43 ^a	3.30 ± 0.50 ^a	3.00 ± 0.35 ^{ac}	3.07	5.10 ± 0.94 ^b	6.60 ± 0.55 ^a	3.50 ± 0.31 ^{ac}	2.20 ± 0.90 ^b	4.35	6.30 ± 0.46 ^a	3.20 ± 0.28 ^{ac}	3.20 ± 0.03 ^a	2.00 ± 0.03 ^a	3.68
∑n-3/∑n-6	9.3 ± 1.54 ^a	15.8 ± 1.75 ^a	10.4 ± 0.37 ^{ab}	11.8 ± 0.33 ^{ac}	11.83	4.4 ± 0.80 ^a	5.4 ± 1.15 ^{ab}	8.3 ± 2.09 ^{ac}	15.9 ± 0.64 ^b	8.50	4.3 ± 0.83 ^{ab}	5.7 ± 0.88 ^{ac}	5.7 ± 0.06 ^a	15.8 ± 0.06 ^a	7.88
∑n-6/∑n-3	0.11 ± 0 ^a	0.06 ± 0.01 ^a	0.10 ± 0.03 ^a	0.08 ± 0 ^a	0.09	0.23 ± 0.02 ^a	0.19 ± 0.01 ^a	0.12 ± 0.00 ^a	0.06 ± 0.00 ^a	0.15	0.23 ± 0.07 ^b	0.18 ± 0.07 ^b	0.18 ± 0.06 ^a	0.06 ± 0.00 ^a	0.16
∑PUFA/∑SFA	0.97 ± 0.14 ^a	1.0 ± 0.14 ^a	0.98 ± 0.14 ^a	0.98 ± 0.14 ^{ac}	0.98	0.52 ± 0.18 ^{ac}	1.21 ± 0.13 ^{abc}	0.83 ± 0.10 ^{abc}	0.98 ± 0.11 ^{ab}	0.89	0.77 ± 0.07 ^b	0.45 ± 0.12 ^{abc}	0.46 ± 0.04 ^a	0.98 ± 0.04 ^a	0.67
DHA/EPA	6.9 ± 0.82 ^{ab}	3.6 ± 0.69 ^{abc}	5.6 ± 0.02 ^b	4.7 ± 0.54 ^a	5.20	4.1 ± 0.33 ^{ac}	2.5 ± 0.43 ^{ac}	2.8 ± 0.08 ^a	3.6 ± 0.04 ^{ab}	3.25	5.7 ± 0.06 ^{ac}	3.1 ± 0.03 ^{ac}	3.1 ± 0.25 ^a	3.6 ± 0.74 ^a	3.88
LA/ALA	4.6 ± 0														

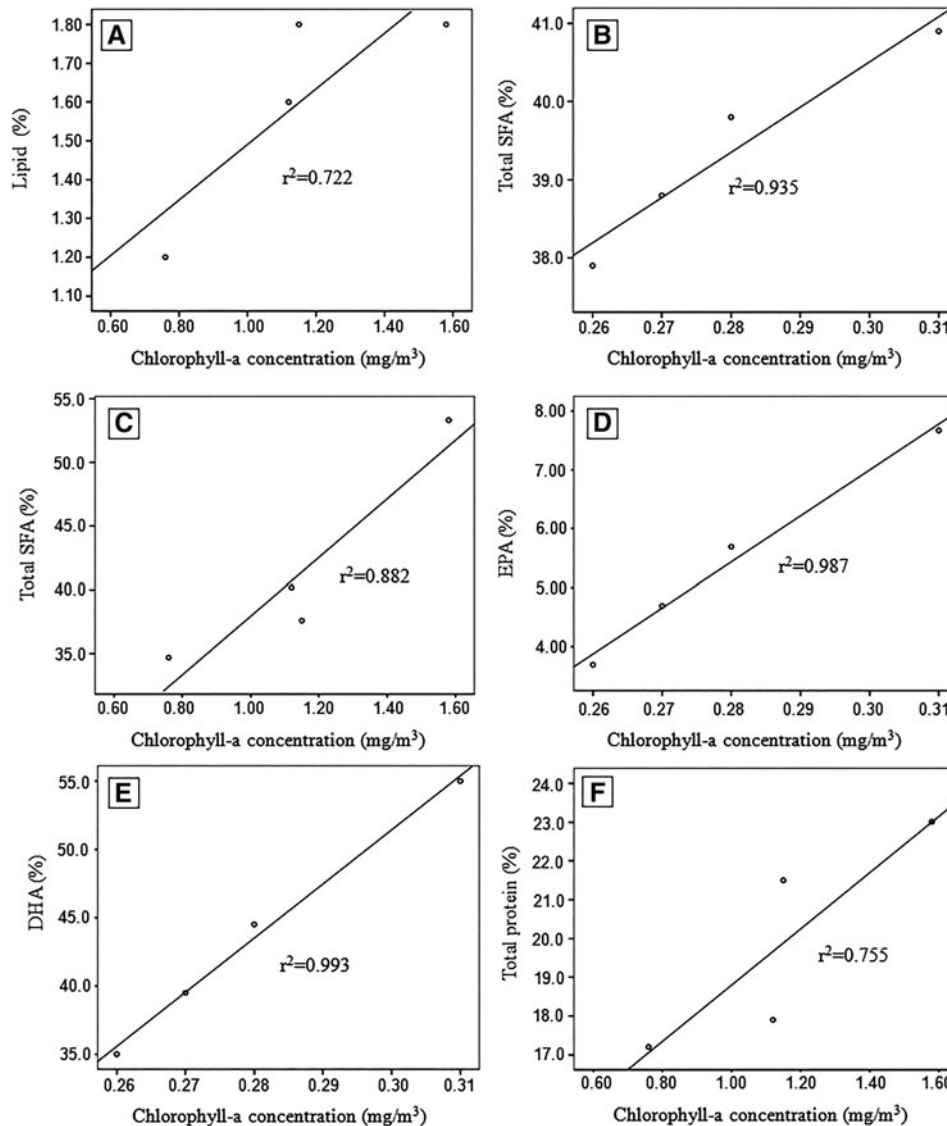


Fig. 1. The correlation plot between chlorophyll-a (mg m^{-3}) with (A) lipid (%) during monsoon, (B) \sum SFA (%) during monsoon, (C) \sum SFA (%) during post-monsoon, (D) EPA during monsoon, (E) DHA during monsoon and (F) total protein content during monsoon.

the substantial variation in DHA content of the fillets of skipjack tuna ($>20\%$) harvested during these seasons. The PUFAs in the fillets of skipjack tuna are mainly composed of $n-3$ fatty acids rather than $n-6$ fatty acids. An eating regimen containing high levels of $n-3$ PUFAs is beneficial for the treatment of obesity and diabetes as it lowers the plasma concentrations of triglycerides and insulin (Carrillo *et al.*, 2012). The total $n-3$ PUFAs in the edible portion of skipjack tuna showed seasonal variations, and was found to be significantly greater during the pre-monsoon (4 years' mean of 35%) followed by monsoon ($\sim 31\%$) and post-monsoon seasons ($\sim 24\%$) ($P < 0.05$). The PUFA composition of skipjack tuna was portrayed by the predominance of $n-3$ PUFAs especially EPA and DHA, which contributed major shares (<4 and $>16\%$, respectively) to the total $n-3$ PUFAs. A reasonable connection was seen between these long chain fatty acids and chlorophyll-a concentration during monsoon ($r^2 = 0.987$ and 0.993 , respectively; Figure 1D and E). The significantly greater concentrations of long chain $n-3$ fatty acids during monsoon were ascribed to the increased EPA and DHA in the oceanic phytoplankton,

which correspond with greater chlorophyll-a concentration. These results were similar to the prior studies demonstrating that PUFA content in marine fish changes with water temperature (Shirai *et al.*, 2002; Mateos *et al.*, 2010). The increasing level of unsaturation of fish lipids with lower temperatures is a known method utilized by the oceanic organisms to adjust the fluidity and permeability of their cell membranes to the temperature variations of the water (Caramujo *et al.*, 2008). Then again, when the chlorophyll-a concentration was lower during the pre-monsoon season, a decline in PUFA content in the fillet of skipjack tuna was noted. A relatively greater content of DHA and DHA:EPA ratio characterized this species, perhaps because of a greater affinity to retain DHA. A prior investigation of Intarasirisawat *et al.* (2011) had comparable findings in which skipjack tuna roe had a more prominent content of DHA (23%) than EPA (4%). In any case, the DHA content of skipjack tuna collected during the pre-monsoon season was greater than those observed for bluefin tuna (23%), bigeye tuna (20%) and yellowfin tuna (17%) (Peng *et al.*, 2013). The fatty acid DHA, which has the

longest side-chain and most elevated level of unsaturation among the PUFAs, is a unique structural and practical component in phospholipids, especially those in the retina and the neuronal neurotransmitters in the cerebrum. More prominent EPA and DHA contents during the pre-monsoon season (4 years' mean of 32%) are vital due to their role in the therapy and prevention of cardiovascular diseases (Dal Bosco *et al.*, 2012). The *n*-6 PUFAs in the fillet of skipjack tuna recorded monsoon maxima (4 years' mean of 4.4%). Limiting content of arachidonic acid (20:4*n*-6) in this fish species could be related to the lower content of 18:2*n*-6 in the fillets. It is of note that this *n*-6 fatty acid biotransformed to the inflammatory prostaglandins (PGG₂ and PGH₂) leading to oxidative stress-induced inflammatory diseases. Interestingly, anti-inflammatory resolvins biosynthesized from EPA (18R-HEPE series, E-series of resolvins, RvE₁) and DHA (17R-HDHA series, D-series of resolvins RvD₁) lead to potent inhibitors of inflammatory mediator recruitment *in vitro* and *in vivo* (Gilroy *et al.*, 2004). The *n*-3:*n*-6 proportion is an indicator of the biomedical significance of marine fish. The filets of skipjack tuna collected during different seasons indicated a more noteworthy *n*-3:*n*-6 ratio (8:12) than suggested (1:5), which highlighted its good quality. The fatty acid ratio was considerably greater than the health foods available in the market, and in this way this marine fish species may serve as a good option to adjust the greater amount of *n*-6 unsaturated fatty acid in the daily diet. Fitting parity of dietary *n*-3:*n*-6 unsaturated fatty acid ratio is fundamental to prevent chronic inflammation and cardiovascular disorders by decreasing the plasma lipids (Calder, 2004). A prior study directed in this line reasoned that a 20% decrease in general mortality and a 45% lessening in sudden death were accounted for in subjects with previous cardiovascular ailments when given 850 mg *n*-3 fatty acids (Cordain *et al.*, 2005). It seems that greater dietary amounts of skipjack tuna might forestall inflammatory diseases (Cleland *et al.*, 2006). The *n*-3:*n*-6 ratio noted pre-monsoon maxima (4 years' mean of ~12) may be because of the extensive variability of the lipid content of the fish, which depends upon the species, season, reproduction period and the fatty acid composition of the eating regimen (Dal Bosco *et al.*, 2012). Despite the fact that there are broadened metabolic capacities of *n*-3 and *n*-6 PUFAs, the right balance between them is recommended. Prior reports claimed that large amounts of *n*-6 PUFA in the human diet bring about numerous health disorders, while *n*-3 PUFAs appear to alter the adverse effects of *n*-6 PUFAs (Cleland *et al.*, 2006). Of note is that the *n*-6:*n*-3 fatty acid ratio within 0.2:1.5 would go towards a healthy human diet as stipulated by the UK Department of Health (HMSO, 2001), and the fillets of skipjack tuna collected during the study period met the suggested ratio. The prescribed minimum of PUFA:SFA proportion for a healthy eating regimen is 0.45 (HMSO, 2001). The skipjack tuna collected during different seasons had the capacity to meet the minimum threshold PUFA:SFA ratio (0.7:1.0) throughout the studied periods. Essentially, the DHA:EPA proportion recorded pre-monsoon maxima (4 years' mean of 5.2) followed by those in the post-monsoon and monsoon seasons (4 years' mean of 3.3 and 3.9, respectively) ($P < 0.05$). Atherogenicity and thrombogenicity indices of the fillets of skipjack tuna during the study period were considered to examine its efficacy to protect against atherosclerosis and platelet aggregation due to the anti-atherogenic and anti-thrombogenic action of the *n*-3

PUFAs. The lower values of the AI and TI indices are desirable from a dietary perspective (Ulbricht & Southgate, 1991). The AI and TI values were recorded from 0.5–1.1 and 0.3–0.6, respectively, and post-monsoon maxima in the fillets of skipjack tuna were observed (Figure 2A). The more noteworthy *n*-6:*n*-3 fatty acid proportion in the fillets of this species apparently contributed to lesser AI and TI's, particularly during the pre-monsoon season. These results furnished us with the important data in regards to skipjack tuna to be qualified as an ideal health food. Due to their anti-atherogenic and anti-thrombogenic properties, the *n*-3 PUFAs assume a major role to protect individuals from atherosclerosis and platelet aggregation (Barrento *et al.*, 2010). The HH ratio is specifically identified with cholesterol metabolism, and a greater value is desirable from a health perspective (Santos-Silva *et al.*, 2002). In the present investigation, the fillets obtained from skipjack tuna demonstrated a significantly greater HH ratio during the pre-monsoon season (4 years' mean of 2.1) (Figure 2A).

Total cholesterol content

Total cholesterol forms the building blocks of several compounds (e.g. bile, sex hormones, adrenal hormones and vitamin D₃) with critical physiological functions and major structural component of the cell membranes. It is one of the constituents of the lipid fraction and can be expected to vary with season. The aggregate cholesterol content in the fillets of skipjack tuna ranged between 17–30 mg 100 g⁻¹ fillet, which demonstrated lower values compared with a prior report by Stephen *et al.* (2010) who observed that tuna contained 82 mg cholesterol 100 g⁻¹ sample. In the present study, skipjack tuna showed maximum total cholesterol content during the monsoon season (4 years' mean of 26 mg 100 g⁻¹) (Figure 2B). Interestingly, the fillets of this marine pelagic fish species recorded significantly lower aggregate cholesterol content (≤ 30 mg%) than eggs (424 mg%), cheese (105 mg%), chicken (85 mg%) and beef (84 mg%) ($P < 0.05$) (Bowman *et al.*, 1998). These results show that this species is a low cholesterol food item for human consumption.

Total protein content and amino acid composition

Proteins are a fundamental nutritional component of skipjack tuna, and are essential for development and survival. The present study indicated significant seasonal variation in the protein content with a monsoon maximum (20 g 100 g⁻¹) and pre-monsoon minimum (~8 g 100 g⁻¹). A prior study reported that the protein content of different species of oceanic tuna ranged between 15–30 g 100 g⁻¹ (Bykov, 1983). These discrepancies in the total protein content could be attributed to food availability and climatic changes, which impact the general biochemical composition of the fish (Chakraborty *et al.*, 2014). A reasonable correlation was noted between protein and chlorophyll-a content off the south-west coast of India during the monsoon season ($r^2 = 0.76$) (Figure 2F).

The amino acid profile of skipjack tuna from the south-west shoreline of India bordering the Arabian Sea demonstrated that the key amino acids had altogether greater concentrations, when compared with the reference pattern (Kim & Lall, 2000), which suggested that the proteins

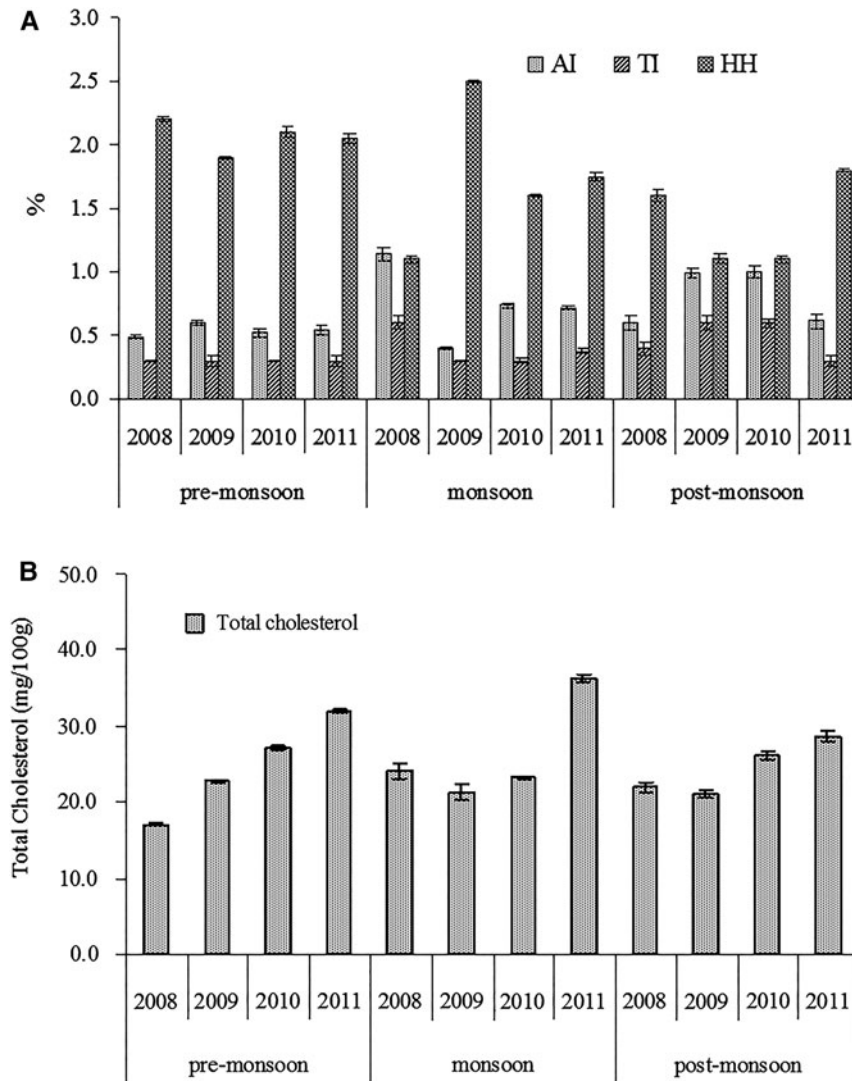


Fig. 2. Seasonal variations in (A) atherogenicity index (AI); thrombogenicity index (TI); HH ratio and (B) total cholesterol content ($\text{mg } 100 \text{ g}^{-1}$) of the fillets in *K. pelamis*.

present had a high biological value (Table 2). No significant variations in the amino acid composition between the fillets of skipjack tuna were seen during the study period (2008–2011) ($P > 0.05$). The EAA controlled the protein content in this species during diverse seasons, and demonstrated a monsoon maximum (4 years' mean of $\sim 12 \text{ g } 100 \text{ g}^{-1}$ fillet), primarily because of the noteworthy increment of leucine, lysine and arginine contents ($P < 0.05$).

The predominant EAA found during pre-monsoon was lysine and during post-monsoon was valine. This species can serve as a viable alternative to the plant proteins, which are deficient in lysine content. The most common EAAs in skipjack tuna were lysine ($0.5\text{--}2.0 \text{ g } 100 \text{ g}^{-1}$), arginine ($0.5\text{--}2.2 \text{ g } 100 \text{ g}^{-1}$) and leucine ($0.50\text{--}1.80 \text{ g } 100 \text{ g}^{-1}$) independent of the seasons and years. These branched chain EAAs (BCAA), which are critical to human life, and are particularly involved in stress, energy and muscle metabolism, comprised a critical share of the fillets of skipjack tuna. The lysine contents noted in this species was comparable with the reference egg protein ($0.63 \text{ mg } 100 \text{ g}^{-1}$). A decreased supply of lysine in the diet may lead to mental and physical handicaps as it is a

vital precursor for the *de novo* synthesis of glutamate, the major neurotransmitter in the mammalian central nervous system (Usyduš *et al.*, 2009). Arginine is included in numerous metabolic actions and important in the treatment of heart diseases and hypertension (Furuya *et al.*, 2012). It is, therefore, anticipated that the fillets of skipjack tuna are a reasonable source to supplement the inadequate amino acids in cereals (Iwasaki & Harada, 1985). The total non-essential amino acid (TNEAA) level was found to be at its maximum during monsoon (4 years' mean of $8.2 \text{ g } 100 \text{ g}^{-1}$) with a greater content of glutamic acid, which was the prevalent NEAA during each of the three seasons ($0.90\text{--}3.20 \text{ g } 100 \text{ g}^{-1}$). Peng *et al.* (2013) additionally demonstrated greater content of glutamic acid in *Thunnus albacares* (yellowfin tuna) and *T. obesus* (bigeye tuna) from the Pacific Ocean. The amino acid-based nutritional indices of skipjack tuna with respect to the different amino acids is indicated in Table 2. The protein content of this species included a wide assortment of amino acids and their isomers with an especially high proportion of EAA, which was greater than 56% of TAA, aside from the samples collected during the post-monsoon

Table 2. Protein (g 100 g⁻¹ fillet), amino acid composition (g 100 g⁻¹ fillet) and essential amino acid scores (%) of *K. pelamis* collected from the south-west coast of India during 2008–2011 in three different seasons (pre-monsoon, monsoon and post-monsoon).

	2008				2009				2010				2011				Mean
	Pre-monsoon				Monsoon				Post-monsoon								
Protein	7.2 ± 0.02 ^a	6.9 ± 0.02 ^a	7.8 ± 0.02 ^a	8.1 ± 0.02 ^a	7.5	23.01 ± 0.1 ^a	17.2 ± 0.03 ^{ad}	17.9 ± 0.02 ^a	21.5 ± 0.02 ^{ad}	19.9	9.02 ± 0.02 ^a	7.3 ± 0.02 ^a	8.80 ± 0.02 ^a	10.02 ± 0.02 ^a	8.8		
Histidine ^x (1.9 mg 100 g ⁻¹)	0.24 ± 0.02 ^a	0.22 ± 0.02 ^a	0.25 ± 0.02 ^a	0.17 ± 0.02 ^a	0.2	1.28 ± 0.01 ^a	1.26 ± 0.03 ^{ad}	0.25 ± 0.02 ^a	0.93 ± 0.02 ^{ad}	0.9	0.82 ± 0.02 ^a	0.12 ± 0.02 ^a	0.44 ± 0.02 ^a	0.46 ± 0.02 ^a	0.5		
Arginine ^x	0.70 ± 0.02 ^a	0.60 ± 0.05 ^a	0.81 ± 0.29 ^a	0.39 ± 0.41 ^a	0.6	2.18 ± 0.02 ^b	2.22 ± 0.58 ^b	2.78 ± 0.37 ^b	1.59 ± 0.11 ^b	2.2	0.11 ± 0.01 ^a	0.15 ± 0.01 ^a	0.92 ± 0.02 ^a	0.75 ± 0.02 ^a	0.5		
Threonine ^x (3.4 mg 100 g ⁻¹)	0.32 ± 0.05 ^{ac}	0.28 ± 0.13 ^a	0.36 ± 0.07 ^{ac}	0.20 ± 0.01 ^a	0.3	1.38 ± 0.03 ^b	1.56 ± 0.22	1.76 ± 0.09 ^b	1.00 ± 0.05 ^b	1.4	0.25 ± 0.14 ^a	0.12 ± 0.08 ^a	0.41 ± 0.02 ^a	0.53 ± 0.03 ^a	0.3		
Valine ^x (3.5 mg 100 g ⁻¹)	0.40 ± 0.01 ^a	0.36 ± 0.22 ^a	0.44 ± 0.08 ^a	0.27 ± 0.06 ^{ab}	0.4	1.09 ± 0.1 ^a	1.02 ± 0.08 ^a	1.40 ± 0.11 ^{ab}	0.79 ± 0.01 ^a	1.1	0.91 ± 0.07 ^a	0.90 ± 0.08 ^{ab}	0.53 ± 0.05 ^{ac}	0.49 ± 0.05 ^{ac}	0.7		
Methionine ^x	0.21 ± 0.07 ^a	0.18 ± 0.07 ^a	0.24 ± 0.08 ^a	0.11 ± 0.06 ^{ad}	0.2	0.70 ± 0.08 ^a	0.14 ± 0.06 ^{ac}	0.88 ± 0.15 ^a	0.52 ± 0.01 ^b	0.6	0.68 ± 0.21 ^a	0.20 ± 0.03 ^{ad}	0.27 ± 0.01 ^a	0.38 ± 0.01 ^a	0.4		
Isoleucine ^x (2.8 mg 100 g ⁻¹)	0.36 ± 0.05 ^a	0.33 ± 0.05 ^a	0.38 ± 0.04 ^a	0.27 ± 0.07 ^a	0.3	0.98 ± 0.01 ^a	0.14 ± 0.02 ^a	0.85 ± 0.03 ^a	0.70 ± 0.01 ^a	0.7	0.79 ± 0.06 ^a	0.74 ± 0.02 ^a	0.48 ± 0.17 ^a	0.43 ± 0.17 ^a	0.6		
Leucine ^x (6.6 mg 100 g ⁻¹)	0.59 ± 0.06 ^a	0.52 ± 0.05 ^a	0.65 ± 0.07 ^{ab}	0.37 ± 0.04 ^{ac}	0.5	1.77 ± 0.01 ^{ab}	1.84 ± 0.09 ^{ab}	2.20 ± 0.03 ^{ac}	1.35 ± 0.06 ^a	1.8	0.15 ± 0.01 ^b	0.13 ± 0.03 ^{ac}	0.71 ± 0.01 ^a	0.84 ± 0.07 ^a	0.5		
Phenylalanine ^x	0.32 ± 0.04 ^a	0.28 ± 0.04 ^a	0.36 ± 0.05 ^a	0.20 ± 0.03 ^{ab}	0.3	0.83 ± 0.01 ^b	0.87 ± 0.08 ^{ac}	1.04 ± 0.03 ^{ab}	0.62 ± 0.01 ^{ab}	0.8	0.84 ± 0.03 ^a	0.29 ± 0.03 ^{ab}	0.40 ± 0.06 ^a	0.51 ± 0.06 ^a	0.5		
Lysine ^x (5.8 mg 100 g ⁻¹)	0.75 ± 0.01 ^a	0.67 ± 0.01 ^a	0.84 ± 0.01 ^a	0.49 ± 0.01 ^a	0.7	2.39 ± 0.06 ^d	2.20 ± 0.01 ^a	1.26 ± 0.01 ^a	2.03 ± 0.20 ^b	2.0	0.08 ± 0.00 ^{ad}	0.08 ± 0 ^a	0.95 ± 0.04 ^a	0.94 ± 0.04 ^a	0.5		
Alanine ^y	0.52 ± 0.08 ^a	0.45 ± 0.01 ^a	0.58 ± 0.04 ^a	0.31 ± 0.08 ^a	0.5	1.05 ± 0.08 ^a	0.36 ± 0.01 ^a	0.36 ± 0.02 ^a	0.82 ± 0.18 ^c	0.6	0.09 ± 0.0 ^a	0.55 ± 0.04 ^a	0.64 ± 0.01 ^a	0.59 ± 0.01 ^a	0.5		
Cysteine ^y	0.04 ± 0.02 ^a	0.03 ± 0.00 ^a	0.04 ± 0.01 ^a	0.01 ± 0.0 ^a	0.0	2.14 ± 0 ^b	2.23 ± 0.03 ^b	2.86 ± 0.01 ^b	1.43 ± 0 ^b	2.2	0.24 ± 0.02 ^a	0.21 ± 0 ^b	0.04 ± 0.0 ^a	0.11 ± 0.0 ^a	0.2		
Glutamic acid ^y	1.28 ± 0.01 ^a	1.10 ± 0.01 ^a	1.46 ± 0.01 ^a	0.76 ± 0.02 ^{ac}	1.2	3.25 ± 0.02 ^b	3.21 ± 0.01 ^b	3.02 ± 0.02 ^c	3.41 ± 0.02 ^b	3.2	0.36 ± 0.01 ^{ab}	0.32 ± 0.02 ^a	1.42 ± 0.02 ^a	1.56 ± 0.02 ^a	0.9		
Glycine ^y	0.36 ± 0.04 ^a	0.30 ± 0.03 ^a	0.42 ± 0.08 ^a	0.18 ± 0.07 ^{ab}	0.3	0.64 ± 0.02 ^a	0.14 ± 0.06 ^{ab}	0.81 ± 0.02 ^a	0.47 ± 0.06 ^a	0.5	0.76 ± 0.03 ^b	0.78 ± 0.08 ^a	0.44 ± 0.01 ^a	0.43 ± 0.01 ^a	0.6		
Proline ^y	0.33 ± 0.02 ^a	0.27 ± 0.02 ^a	0.40 ± 0.03 ^a	0.15 ± 0.01 ^b	0.3	1.11 ± 0 ^b	1.25 ± 0.02 ^a	1.44 ± 0.01 ^b	0.78 ± 0.01 ^b	1.1	0.57 ± 0.05 ^c	0.44 ± 0.01 ^b	0.33 ± 0.04 ^a	0.36 ± 0.04 ^a	0.4		
Serine ^y	0.29 ± 0.05 ^a	0.26 ± 0.09 ^a	0.32 ± 0.04 ^a	0.20 ± 0.04 ^a	0.3	0.49 ± 0.08 ^a	0.27 ± 0.06 ^a	0.57 ± 0.26 ^a	0.42 ± 0.01 ^a	0.4	0.92 ± 0.06 ^a	0.25 ± 0.10 ^a	0.44 ± 0.02 ^a	0.54 ± 0.02 ^a	0.5		
Tyrosine ^y	0.15 ± 0.04 ^a	0.13 ± 0.07 ^a	0.18 ± 0.01 ^a	0.09 ± 0.02 ^b	0.1	0.28 ± 0.19 ^b	0.14 ± 0.01 ^b	0.32 ± 0.11 ^b	0.24 ± 0.06 ^b	0.2	0.60 ± 0.06 ^a	1.19 ± 0.10 ^a	0.18 ± 0.05 ^a	0.66 ± 0.05 ^a	0.7		
TEAA	3.89 ± 0.51 ^a	3.44 ± 0.52 ^a	4.33 ± 0.51 ^a	2.47 ± 0.53 ^a	3.5	12.6 ± 0.02 ^b	11.25 ± 0.02 ^a	12.42 ± 0.07 ^a	9.53 ± 0.02 ^b	11.5	4.63 ± 0.06 ^a	2.73 ± 0.03 ^a	5.11 ± 0.04 ^a	5.33 ± 0.04 ^a	4.5		
TNEAA	2.97 ± 0.02 ^a	2.54 ± 0.02 ^a	3.40 ± 0.01 ^a	1.70 ± 0.03 ^{ac}	2.7	8.96 ± 0.05 ^c	7.60 ± 0.01 ^a	9.38 ± 0.02 ^a	7.57 ± 0.11 ^b	8.4	3.54 ± 0.05 ^c	3.74 ± 0.02 ^a	3.49 ± 0.51 ^a	4.25 ± 0.51 ^a	3.8		
TAA	6.86 ± 0.17 ^a	5.98 ± 0.97 ^a	7.73 ± 0.07 ^a	4.17 ± 0.07 ^a	6.2	21.56 ± 0.09 ^a	18.85 ± 0.95 ^a	21.8 ± 0.02 ^a	17.1 ± 2.37 ^a	19.8	8.17 ± 0.03 ^a	6.47 ± 0.09 ^a	8.60 ± 0.02 ^a	9.58 ± 0.02 ^a	8.2		
TEAA/TAA	0.57 ± 0.05 ^a	0.58 ± 0.04 ^{ac}	0.56 ± 0.06 ^a	0.59 ± 0.03 ^c	0.6	0.58 ± 0.02 ^a	0.59 ± 0.07 ^a	0.57 ± 0.03 ^c	0.56 ± 0.02 ^b	0.6	0.57 ± 0.07 ^a	0.42 ± 0.02 ^{bc}	0.59 ± 0.17 ^a	0.56 ± 0.17 ^a	0.5		
TNEAA/TAA	0.43 ± 0.02 ^a	0.42 ± 0.02 ^a	0.44 ± 0.03 ^{ac}	0.41 ± 0 ^b	0.4	0.41 ± 0.02 ^a	0.40 ± 0.04 ^c	0.43 ± 0.02 ^b	0.46 ± 0.01 ^c	0.4	0.43 ± 0.02 ^a	0.58 ± 0 ^b	0.40 ± 0.05 ^a	0.44 ± 0.05 ^a	0.5		
TEAA/TNEAA	1.31 ± 0.18 ^a	1.35 ± 0.18 ^a	1.27 ± 0.18 ^a	1.45 ± 0.18 ^a	1.3	1.41 ± 0.22 ^a	1.48 ± 0.24 ^a	1.32 ± 0.16 ^a	1.26 ± 0.2 ^a	1.4	1.31 ± 0.19 ^a	0.73 ± 0.14 ^a	1.46 ± 0.02 ^a	1.25 ± 0.02 ^a	1.2		
TArAA (Ph + His + Tyr)	0.71 ± 0.11 ^a	0.63 ± 0.08 ^a	0.79 ± 0.14 ^{ac}	0.46 ± 0.02 ^b	0.6	2.39 ± 0.09 ^b	2.27 ± 0.08 ^b	1.61 ± 0.02 ^b	1.79 ± 0.07 ^b	2.0	2.26 ± 0.12 ^b	1.6 ± 0.02 ^b	1.02 ± 0.18 ^b	1.63 ± 0.18 ^b	1.6		
TSAA	0.25 ± 0.03 ^a	0.21 ± 0.03 ^a	0.28 ± 0.04 ^a	0.12 ± 0.01 ^a	0.2	2.84 ± 0.29 ^c	2.37 ± 0.03 ^a	3.74 ± 0.01 ^a	1.95 ± 0.05 ^b	2.7	0.92 ± 0.11 ^b	0.41 ± 0.01 ^a	0.31 ± 0.01 ^a	0.49 ± 0.01 ^a	0.5		
Arg:Lys	0.93 ± 0 ^a	0.89 ± 0 ^a	0.96 ± 0 ^a	0.79 ± 0 ^a	0.9	0.91 ± 0.03 ^a	1.01 ± 0 ^a	2.21 ± 0.01 ^a	0.78 ± 0.03 ^c	1.2	1.37 ± 0 ^a	1.85 ± 0 ^a	0.97 ± 0.03 ^a	0.79 ± 0.03 ^a	1.3		
Amino acid Score																	
His	175	168	169	110	156	293	386	74	228	245	478	87	263	242	267		
Thr	131	119	136	73	115	176	267	289	137	217	82	48	137	156	106		
Val	159	149	161	95	141	135	169	223	105	158	288	352	172	140	238		
Met + Cys	139	122	144	59	116	494	551	639	363	512	408	225	141	196	242		
Ile	179	171	174	119	161	152	29	170	116	117	313	362	195	153	256		
Leu	124	114	126	69	108	117	162	186	95	140	25	27	122	127	75		
Phe + Tyr	104	94	110	57	91	77	93	121	63	88	253	322	105	185	216		
Lys	180	167	186	104	159	179	221	121	163	171	15	19	186	162	96		

^xEssential amino acids; ^ynon-essential amino acids; TEAA- total amino acids; TNEAA - total non-essential amino acids; TAA - total amino acids; TArAA - total aromatic amino acids (histidine + phenyl alanine + tyrosine); TSAA - total sulphur containing amino acids (methionine + cysteine); data are expressed as mean ± standard deviation (N = 3); different superscripts (a-c) within a row denote significant differences (*P* < 0.05). FAO/WHO reference pattern (1990) for evaluating proteins (mg 100 g⁻¹) are indicated in parentheses (FAO & WHO, 1990). Tryptophan was not determined.

season of 2009 (42%). The TEAA:TAA ratio in skipjack tuna was greater than the adequate limits of 11% for ideal protein sustenance for adults, 26% for children and 39% for infants (Oluwaniyi *et al.*, 2010). The TEAA:TNEAA ratio (1.2:1.4), which indicated greater value than that recommended (1.0) (Iwasaki & Harada, 1985) in the samples harvested during the three seasons, showed that skipjack tuna can be an alternative source of amino acids with greater nutritional value. The average TEAA:TNEAA ratio of numerous fish species, as clarified by Iwasaki & Harada (1985), was considerably lower than the present study. Apparently, the total aromatic amino acids (TArAA), which are the antecedents of adrenaline and thyroxin hormones, indicated monsoon maxima (4 years' mean of $2.0 \text{ g } 100 \text{ g}^{-1}$). The sulphur-containing amino acid, methionine cannot be synthesized *de novo* in humans. Moreover, cysteine can be produced using homocysteine, but cannot be synthesized on its own. Accordingly the TSAA (methionine + cysteine, $2.7 \text{ g } 100 \text{ g}^{-1}$) and Cys:TSAA proportion (4 years' mean of 0.6) showed monsoon peaks, which were typical of the balance of amino acid composition of the fish fillet (Iwasaki & Harada, 1985). The leucine:isoleucine ratio demonstrated greater value during the monsoon season (4 years' average of ~ 5), and were typical of the ideal ratio suggested by the FAO, WHO and UNU (FAO and WHO, 1990; FAO, WHO and UNU, 2007). The amino acid scores were greater during post-monsoon with respect to histidine, valine, isoleucine and phenylalanine (Table 2). However, amino acid scores with respect to threonine, TSAA, leucine and cysteine observed monsoon maxima. It is of note that the amino acid score is demonstrative of the greater content of protein, which may be retained for development (García & Valverde, 2006).

Vitamin composition

The inter-annual and seasonal variations in the fat-soluble vitamins A, D₃, E, K₁ and the water-soluble vitamin C in the fillets of skipjack tuna are presented in Table 3. The lipid of this marine pelagic fish species is a rich source of fat-soluble vitamins, which may be taken on a regular basis because of their key roles in human wellbeing and metabolism. Skipjack tuna collected during the post-monsoon season demonstrated significantly greater vitamin A content (4 years' mean of $7.6 \mu\text{g } 100 \text{ g}^{-1}$, $P < 0.05$). Vitamin A assumes a crucial role in the process of photoreception, and regulates gene expression, cell division, reproduction, etc. Vitamins D₃ and E showed pre-monsoon maxima (4 years' mean of 494 and $1.4 \mu\text{g } 100 \text{ g}^{-1}$, respectively). Vitamin D₃ fortifies the bones, and is crucial for the upkeep of typical blood levels of calcium and phosphate (Trivedi *et al.*, 2003). The antioxidant vitamin (vitamin E) helps to shield the cells by protecting cell membranes from peroxidative damage by free-radical-intervened pathology (Packer, 1991). Vitamin K₁ was found to be considerably greater during the post-monsoon (4 years' mean of $2.2 \mu\text{g } 100 \text{ g}^{-1}$). Vitamin K₁ assumes an essential part in blood coagulation and bone metabolism relating to the prevention of osteoporosis and carotid artery elasticity. Vitamin C was found to be greater during monsoon (4 years' mean of $23 \mu\text{g } 100 \text{ g}^{-1}$). This water-soluble vitamin is a vital antioxidative supplement for humans, but an additional external dietary source is required because it is not synthesized by human metabolism (Jeevitha *et al.*, 2013). It additionally helps the body to absorb iron

and calcium, supports in wound healing and contributes to brain function (Iqbal *et al.*, 2004). The occasional dissimilarity observed in these vitamin levels could be the result of the season, life stage, age or availability of nutrition in the ocean.

Mineral composition

Minerals are nutritional supplements that are conserved by the body and assume a critical part in metabolism in the human body. The macro minerals (Na, K, Ca and P) and micro minerals (Zn, Fe, Mn and Se) in the fillets of skipjack tuna collected from the south-west coast of India are indicated in Table 3. The potassium content was significantly greater during monsoon (4 years' mean of $1821 \text{ mg } 100 \text{ g}^{-1}$, $P < 0.05$). Potassium (K), together with its close relative sodium (Na), is vital in keeping up normal osmotic pressure inside cells (Ensminger *et al.*, 1995). It is important to study the Na content, since excessive sodium consumption has been connected with hypertensive disorder. Erkan & Ozden (2007) likewise reported significantly greater values of K content compared with the current findings ($158\text{--}310 \text{ mg } 100 \text{ g}^{-1}$), at the mean normal of about $460 \text{ mg } 100 \text{ g}^{-1}$ in sea bass (*Dicentrarchus labrax*) and $394 \text{ mg } 100 \text{ g}^{-1}$ in sea bream (*Sparus aurata*). Both Na and K are necessary to maintain osmotic balance and the pH of body fluids, thus directly affect muscle and nerve functionalities, control glucose assimilation and increase normal retention of protein during the processes of growth and development. The greater K:Na ratio in the samples during different seasons gave off an impression of being imperative on the grounds that physiological and epidemiological information recommend that a greater K:Na ratio can be connected with a diminished danger of hypertensive and cardiovascular diseases (Ensminger *et al.*, 1995). Skipjack tuna collected during the post-monsoon showed greater calcium content ($172 \text{ mg } 100 \text{ g}^{-1}$). The abundance in phosphorus level ($923 \text{ mg } 100 \text{ g}^{-1}$ during pre-monsoon) in the fillets of this species ascribed to the certainty that phosphorus is a segment of protein. Phosphorus has been for the most part connected with the phospholipid content, and the vicinity of phosphoprotein (Mahmoud *et al.*, 2008). The variations in the concentration of the mineral elements in the fish fillet could have been an after-effect of the rate at which these are accessible in the water body, and the capacity of the fish to absorb and convert them from the diet or the water bodies where they live. Zinc was found to be the most abundant micro element followed by iron in the edible fillets of skipjack tuna. The Zn content in the present study varied from $2\text{--}4 \text{ mg } 100 \text{ g}^{-1}$, and showed pre-monsoon maximum (4 years' mean of $\sim 4 \text{ mg } 100 \text{ g}^{-1}$). The Zn level in skipjack tuna was found to be acceptable ($15 \text{ mg } 100 \text{ g}^{-1}$) according to the FAO and WHO (FAO & WHO, 1984). This microelement is known to be included in most metabolic pathways in humans, and its role in the pathophysiology of various ailments has been demonstrated (Coudray *et al.*, 2006). Saadettin *et al.* (1999) reported that the most abundant microelements in fish were Zn and Fe followed by Cu with the remaining elements present in amounts below toxic levels. The Fe content showed pre-monsoon maximum (4 years' mean of $\sim 4 \text{ mg } 100 \text{ g}^{-1}$), whilst only traces of Mn were observed in skipjack tuna. The Fe content in the present study was found to be more prominent in contrast with some chosen marine fish inspected by Nurnadia *et al.* (2013). The Mn content in the edible fillets was found to be lower than the permissible

Table 3. Vitamin ($\mu\text{g } 100 \text{ g}^{-1}$), macro, and micro mineral compositions in the fillets of *K. pelamis* collected from south-west coast of India during 2008–2011 in three different seasons (pre-monsoon, monsoon and post-monsoon).

	2008	2009	2010	2011												
	Pre-monsoon				Mean	Monsoon			Mean	Post-monsoon			Mean			
Vitamins																
Vit A	3.16 ± 0.45 ^a	3.21 ± 0.46 ^a	3.11 ± 0.40 ^a	3.31 ± 0.47 ^a	3.20	2.97 ± 0.42 ^a	2.27 ± 0.32 ^a	3.19 ± 0.46 ^a	2.73 ± 0.39 ^a	2.80	9.23 ± 1.32 ^b	9.37 ± 1.34 ^b	9.48 ± 1.36 ^b	2.46 ± 0.35 ^a	7.60	
Vit D ₃	452.4 ± 64.7 ^a	508.4 ± 72.7 ^a	396.5 ± 56.7 ^a	620.2 ± 88.7 ^{ac}	494.4	422.3 ± 60.4 ^a	422.3 ± 60.4 ^a	422.3 ± 60.4 ^a	422.3 ± 60.4 ^a	422.3	334.7 ± 47.8 ^{ad}	218.3 ± 31.2 ^{ab}	641.6 ± 91.77 ^{abc}	380 ± 54.36 ^a	393.7	
Vit E	1.41 ± 0.20 ^a	1.46 ± 0.21 ^a	1.36 ± 0.19 ^a	1.56 ± 0.22 ^a	1.40	0.16 ± 0.02 ^b	0.16 ± 0.02 ^b	0.16 ± 0.02 ^b	0.16 ± 0.02 ^b	0.16	0.13 ± 0.02 ^b	0.13 ± 0.02 ^b	0.26 ± 0.04 ^b	0.15 ± 0.02 ^b	0.17	
Vit K ₁	0.07 ± 0.001 ^a	0.07 ± 0.01 ^a	0.07 ± 0.01 ^a	0.07 ± 0.01 ^a	0.10	0.35 ± 0.05 ^a	0.26 ± 0.04 ^a	0.37 ± 0.05 ^a	0.32 ± 0.05 ^a	0.30	3.10 ± 0.44 ^b	2.63 ± 0.38 ^b	2.64 ± 0.38 ^b	0.29 ± 0.04 ^a	2.20	
Vit C	19.5 ± 2.79 ^a	19.5 ± 0.25 ^a	19.5 ± 0.09 ^a	19.5 ± 2.79 ^a	19.5	23 ± 3.29 ^a	23 ± 0.19 ^a	23 ± 0.09 ^a	23 ± 0.19 ^a	23.0	19.8 ± 0.84 ^a	20.6 ± 0.45 ^a	20.1 ± 0.88 ^a	20.7 ± 0.76 ^a	20.3	
Macro minerals																
Na	40.3 ± 5.77 ^a	52.6 ± 7.53 ^a	28 ± 4.01 ^a	77.2 ± 11.04 ^a	49.5	1745 ± 249.5 ^b	1705 ± 243.9 ^b	1758 ± 2.6 ^b	1731 ± 24.7 ^b	1734.8	291 ± 41.71 ^a	399 ± 57.16 ^a	167 ± 9.67 ^a	155 ± 222.93 ^b	253.0	
K	156.2 ± 22.35 ^a	193.2 ± 27.6 ^a	119.2 ± 17.05 ^a	267.3 ± 38.23 ^a	184.0	1809 ± 258.7 ^b	1856 ± 265.5 ^b	1793 ± 2.4 ^b	1825 ± 261.04 ^b	1820.8	534.3 ± 76.4 ^a	298.4 ± 42.6 ^a	230.7 ± 18.7 ^a	264.6 ± 34.93 ^b	332.0	
Ca	144 ± 20.6 ^{ac}	181 ± 5.90 ^a	106 ± 15.21 ^{ac}	257 ± 36.77 ^a	172.0	221 ± 31.75 ^a	227 ± 32.5 ^a	220 ± 31.5 ^a	223 ± 31.9 ^a	222.8	486 ± 23.7 ^c	728 ± 104.15 ^b	292 ± 41.86 ^a	201.0 ± 28.79 ^a	426.8	
P	923.4 ± 13.1 ^a	923.4 ± 13.06 ^a	923.4 ± 13.2 ^a	923.4 ± 13.6 ^a	923.4	606.4 ± 86.4 ^a	532.1 ± 76.1 ^a	631.2 ± 90.2 ^a	581.6 ± 83.19 ^a	587.8	618.9 ± 45.9 ^b	650.6 ± 64.4 ^a	735.9 ± 105.2 ^a	523.5 ± 74.87 ^a	632.2	
Na/K	0.26 ± 0.04	0.27 ± 0.04	0.23 ± 0.03	0.269 ± 0.04	0.30	0.96 ± 0.14	0.92 ± 0.14	0.98 ± 0.14	0.95 ± 0.14	0.95	0.54 ± 0.08	1.34 ± 0.2	0.72 ± 0.08	0.58 ± 0.14	0.80	
Ca + P	1067 ± 157.2	1104.4 ± 16.7	1029.4 ± 11.69	1180.4 ± 13.9	1095.4	827.4 ± 12.0	759.1 ± 11.8	851.2 ± 15.44	804.6 ± 11.6	810.6	1104.95 ± 57.0	1378.6 ± 13.6	1027.9 ± 15.5	724.5 ± 12.78	1059.0	
Ca/P	0.16	0.2	0.115	0.278	0.20	0.364	0.427	0.349	0.383	0.38	0.785	1.119	0.397	0.384	0.70	
Micro minerals																
Fe	2.26 ± 0.32 ^a	2.29 ± 0.33 ^a	2.21 ± 0.32 ^a	2.35 ± 0.34 ^a	2.30	1.88 ± 0.27 ^a	1.88 ± 0.27 ^a	1.88 ± 0.27 ^a	1.88 ± 0.27 ^a	1.88	5.86 ± 0.84 ^b	5.65 ± 0.81 ^b	3.11 ± 0.44 ^a	1.7 ± 0.24 ^a	4.10	
Mn	0.14 ± 0.02 ^a	0.1 ± 0.00 ^{ad}	0.18 ± 0.03 ^a	0.02 ± 0 ^{ab}	0.10	ND	ND	ND	ND	ND	0.21 ± 0.03 ^c	0.24 ± 0.03 ^{ac}	0.08 ± 0.01 ^a	ND	0.53	
Zn	2.53 ± 0.36 ^a	4.14 ± 0.59 ^c	0.93 ± 0.13 ^a	7.34 ± 1.05 ^b	3.70	2.11 ± 0.3 ^a	2.18 ± 0.31 ^a	2.08 ± 0.3 ^a	2.13 ± 0.3 ^a	2.10	0.74 ± 0.11 ^a	2.98 ± 0.43 ^{ac}	3.47 ± 0.5 ^{ac}	1.92 ± 0.17 ^a	2.30	
Se	0.08 ± 0.01 ^a	0.06 ± 0.01 ^{ab}	0.10 ± 0.01 ^{ab}	0.02 ± 0 ^{ab}	0.10	0.07 ± 0.01 ^{ab}	0.07 ± 0.01 ^{ab}	0.07 ± 0.01 ^{ab}	0.07 ± 0.01 ^{ab}	0.07	0.39 ± 0.05 ^d	ND	0.11 ± 0.02 ^{ab}	0.06 ± 0.01 ^{ab}	0.19	

Vitamin A, D₃, E, K₁ and C are represented in $\mu\text{g } 100 \text{ g}^{-1}$. Data are expressed as mean ± standard deviation (N = 3); Different superscripts (a–c) within a column denote significant differences ($P < 0.05$). The minerals are expressed as mg 100 g^{-1} wet fillet sample.

limit set by the FAO and WHO (FAO & WHO, 1984) as 5.4 ppm (or 540 µg 100 g⁻¹ food). These discrepancies might be explained in part by seasonal changes in metal concentrations, or the different stages of maturity and differences in the annual reproductive cycle of the specimens (Chafik *et al.*, 2001). In addition, differences in the mineral elements of the seawater could impact the mineral levels in the fish fillets. Increased dietary intake of selenium has been linked to protection against various cancers. The selenium content in skipjack tuna were demonstrated to be in the range of 0.02–0.1 µg 100 g⁻¹, and was significantly greater than in cereals, fruits and vegetables (Levander & Burk, 1994). Selenium functions essentially as selenoproteins, which maintain the oxidative balance in the physiological and metabolic framework in the body. Some of the selenoproteins have enzyme activities and selenocysteine is a key segment of the catalytic cycle (Liu *et al.*, 2012). The abundance of micro minerals among the skipjack tuna was likely to be due to high bioavailability of these elements arising in the fishes by a high mineral absorption from the food chain as a consequence of feeding activities.

CONCLUSION

The long-term study of nutritional parameters established skipjack tuna, an important marine pelagic finfish species of commercial importance, as a desirable food item from the consumer health perspective. This study shows how the change in the chlorophyll-a concentration and sea surface temperature impact the lipids and different nutritional parameters of the fish. The significant correlation between the *n*-3 polyunsaturated fatty acids of the edible fillets of skipjack tuna alongside chlorophyll-a concentration revealed that it is conceivable to correlate the important nutritional characteristics of this marine species with chlorophyll-a concentration. These results demonstrate that skipjack tuna is a promising source of essential fatty acids, particularly long chain *n*-3 fatty acids, for example, docosahexaenoic acid, essential amino acids, vitamins, minerals, and fatty acid and amino acid-based health markers. This commercially important fish species is available throughout the year, and is the most abundant species of tuna on the Arabian coast of the Indian subcontinent. Skipjack tuna can be considered as an important dietetic parameter, and can conceivably be utilized for the preparation of food ingredients and nutraceuticals for nutritional and pharmaceutical industries.

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REFERENCES

- AOAC International** (1995) Vitamin C (ascorbic acid) in vitamin preparations and juices. 2, 6-Dichloroindophenol titrimetric method procedure No. 967.21. In *Official methods of analysis of AOAC International*, 15th edition. Arlington, VA: Association of Analytical Communities, pp. 1058–1059.
- AOAC International** (2002) Official method 995.11. Phosphorus (total) in foods. In *Official methods of analysis of AOAC International*, 17th edition. Gaithersburg, MD: Association of Analytical Communities, p. 2.
- Barrento S., Marques A., Teixeira B., Mendes R., Bandarra N., Vaz-Pires P. and Nunes M.L.** (2010) Chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab *Cancer pagurus*. Ecological and human health implications. *Journal of Food Composition and Analysis* 23, 716–725.
- Bayliff W.H.** (1988) Growth of skipjack, *Katsuwonus pelamis*, and yellowfin, *Thunnus albacares*, tunas in the eastern Pacific Ocean, as estimated from tagging data. *Bulletin Inter American Tropical Tuna Commission* 19, 311–385.
- Bowman S.A., Lino M., Gerrior S.A. and Basiotis P.P.** (1998) *The healthy eating index: 1994–96*. Washington, DC: U.S. Department of Agriculture, Center for Nutrition Policy and Promotion, CNPP-5.
- Bulut S., Uysal K., Cemek M., Gok V., Kuş S.F. and Karaçali M.** (2012) Nutritional evaluation of seasonal changes in muscle fatty acid composition of common carp (*Cyprinus carpio*) in Karamik Lake, Turkey. *International Journal of Food Properties* 15, 717–724.
- Bykov V.P.** (ed.) (1983) Chemical composition and processing properties. In *Marine fishes*. New Delhi: Amerind Publishing Co. Pvt. Ltd, pp. 75–86.
- Calder P.C.** (2004) Long-chain fatty acids and cardiovascular disease: further evidence and insights. *Nutrition Research* 24, 761–772.
- Caramujo M., Boschker H.T.S. and Admiraal W.** (2008) Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. *Freshwater Biology* 53, 77–90.
- Carrillo S., Rios V.H., Calvo C., Carranco M.E., Casas M. and Perez-Gil F.** (2012) *N*-3 fatty acid content in eggs laid by hens fed with marine algae and sardine oil and stored at different times and temperatures. *Journal of Applied Phycology* 24, 593–599.
- Chafik A., Cheggour M., Cossa D. and Sifeddine S.B.M.** (2001) Quality of Moroccan Atlantic coastal waters: water monitoring and mussel watching. *Aquatic Living Resources* 14, 239–249.
- Chakraborty K., Joseph D. and Chakkalal S.J.** (2014) Seasonal and inter-annual lipid dynamics of spiny cheek grouper (*Epinephelus diacanthus*) in the southern coast of India. *Journal of the Marine Biological Association of the United Kingdom* 94, 1677–1686.
- Chakraborty K. and Joseph D.** (2015) Inter-annual and seasonal dynamics of amino acid, mineral and vitamin composition of silver belly *Leiognathus splendens*. *Journal of the Marine Biological Association of the United Kingdom* 95, 817–828.
- Chakraborty K., Joseph D. and Chakkalal S.J.** (2016) Inter annual and seasonal dynamics in lipidic signatures of *Sardinella longiceps*. *Journal of Aquatic Food Product Technology*. doi: 10.1080/10498850.2014.895918.

- Cleland J., Bernstein S., Ezeh A., Faundes A., Glasier A. and Innis J. (2006) Family planning: the unfinished agenda. *Lancet* 18, 1810–1827.
- Cordain L., Eaton S.B., Sebastian A., Mann N., Lindeberg S., Watkins B.A., Okeefe J.H. and Brand-Miller J. (2005) Origins and evolution of the Western diet: health implications for the 21st century. *American Journal of Clinical Nutrition* 81, 341–354.
- Coudray C., Feillet-Coudra C., Rambeau M., Tressol J.C., Gueux E., Mazur A. and Rayssiguier Y. (2006) The effect of aging on intestinal absorption and status of calcium, magnesium, zinc, and copper in rats: a stable isotope study. *Journal of Trace Elements in Medicine and Biology* 20, 73–81.
- Dal Bosco A., Mugnai C., Mourvaki E. and Castellini C. (2012) Seasonal changes in the fillet fatty acid profile and nutritional characteristics of wild Trasimeno Lake goldfish (*Carassius auratus* L.). *Food Chemistry* 132, 830–834.
- Doulman D.J. and Kearney R.E. (1987) Domestic tuna fisheries. In Doulman D.J. (ed.) *The development of the tuna industry in the Pacific islands region: an analysis of options*. Honolulu, Hawaii: East-West Center Press, pp. 3–32.
- Ensminger A.H., Ensminger M.E., Konlande J.E. and Robson J.R.K. (1995) Potassium. In *The concise encyclopedia of foods and nutrition*. London: CRC Press, pp. 865–866.
- Erkan N. and Ozden O. (2007) Proximate composition and mineral contents in aquacultured sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) analyzed by ICP-MS. *Food Chemistry* 102, 721–725.
- FAO and WHO (1984) *List of maximum levels recommended for contaminants by the Joint FAO/WHO Codex Alimentarius Commission, Second Series*, Volume 3. Rome: CAC/FAL, pp. 1–8.
- FAO and WHO (1990) *Report of the joint FAO/WHO expert consultation on protein quality evaluation*. Bethesda, MD: FAO/WHO.
- FAO, WHO and UNU (2007) *Protein and amino acid requirements in human nutrition*. Report of a Joint WHO/FAO/UNU Expert Consultation, WHO Technical Report Series 935. Geneva: WHO.
- Folch J., Lees M. and Stanley G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226, 497–509.
- Furuya W.M., Graciano T.S., Vida L.V.O., Xavier T.O., Gongora L.D., Righetti J.S. and Furuya V.R.B. (2012) Digestible lysine requirement of Nile tilapia fingerlings fed arginine-tolysine- balanced diets. *Revista Brasileira de Zootecnia – Brazilian Journal of Animal Science* 41, 485–585.
- García G.B. and Valverde C.J. (2006) Optimal proportions of crabs and fish in diet for common octopus (*Octopus vulgaris*) on-growing. *Aquaculture* 253, 502–511.
- Gilroy D.W., Lawrence T., Perretti M. and Rossi A.G. (2004) Inflammatory resolution: new opportunities for drug discovery. *Nature Reviews Drug Discovery* 3, 401–416.
- Heinrikson L. and Meredith S.C. (1984) Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Analytical Biochemistry* 136, 65–74.
- HMSO (2001) *Nutritional aspects of cardiovascular disease: report on health and social subjects*. London: Department of Health, pp. 37–46.
- Inhamuns A.J. and Franco M.R.B. (2008) EPA and DHA quantification in two species of freshwater fish from Central Amazonia. *Food Chemistry* 107, 587–591.
- Intarasirisawat R., Benjakul S. and Visessanguan W. (2011) Chemical compositions of the roes from skipjack, tongol and bonito. *Food Chemistry* 124, 1328–1334.
- Iqbal K., Khan A. and Khattak M.M.A.K. (2004) Biological significance of ascorbic acid (vitamin C) in human health – a review. *Pakistan Journal of Nutrition* 3, 5–13.
- Iwasaki M. and Harada R. (1985) Proximate and amino acid composition of the roe and muscle of selected marine species. *Journal of Food Science* 50, 1585–1587.
- Jan U., Shah M., Manzoor T. and Ganie S.A. (2012) Variations of protein content in the muscle of fish *Schizothorax niger*. *American Eurasian Journal of Scientific Research* 7, 1–4.
- Jeevitha M., Athiperumalsami T. and Kumar V. (2013) Dietary fibre, mineral, vitamin, amino acid and fatty acid content of seagrasses from Tuticorin Bay, Southeast coast of India. *Phytochemistry* 90, 135–146.
- Kim J. D. and Lall S. P. (2000) Amino acid composition of wholebody tissue of Atlantic halibut (*Hippoglossus hippoglossus*), yellowtail flounder (*Pleuronectes ferruginea*) and Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 187, 367–373.
- Levander O.A. and Burk R.F. (1994) Selenium. In Ziegler E.E. and Filer J.J. (eds) *Present knowledge in nutrition*, 7th edition. Washington, DC: ILSI Press, pp. 320–328.
- Liu H., Yin L., Board P.G. and Han X. (2012) Expression of selenocysteine-containing glutathione S-transferase in eukaryote. *Protein Expression and Purification* 84, 59–63.
- Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193, 265–275.
- Mahmoud K.A., Linder M., Fanni J. and Parmentier M. (2008) Characterization of the lipid fractions obtained by proteolytic and chemical extractions from rainbow trout (*Oncorhynchus mykiss*) roe. *Process Biochemistry* 43, 376–383.
- Mateos H.T., Lewandowski P.A. and Su X.Q. (2010) Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed Jade Tiger hybrid abalone in Australia. *Food Chemistry* 123, 436–441.
- Matsumoto W.M., Skillman R.A. and Dizon A.E. (1984) Synopsis of biological data on skipjack tuna, *Katsuwonus pelamis*. *National Oceanic and Atmospheric Administration Technical Report National Marine Fisheries Service Special Scientific Report Fisheries* 451, 92.
- Metcalf L.D., Schmitz A.A. and Pleka J.R. (1966) Rapid preparation of fatty acid esters from lipids for gas chromatographic analyses. *Analytical Chemistry* 38, 514–515.
- Njinkoue J. M., Barnathan G., Miralles J., Gaydoud E. M. and Sambe A. (2002) Lipids and fatty acids in muscle, liver and skin of three edible fish from the Senegalese coast: *Sardinella maderensis*, *Sardinella aurita* and *Cephalopholis taeniops*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 131, 395–402.
- Nurnadia A.A., Azrina A., Amin I., Mohd Yunus A.S. and Mohd Izuan Effendi H. (2013) Mineral contents of selected marine fish and shellfish from the west coast of Peninsular Malaysia. *International Food Research Journal* 20, 431–437.
- Oluwaniyi O.O., Dosumu O.O. and Awolola G.V. (2010) Effect of local processing methods (boiling, frying and roasting) on the amino acid composition of four marine fishes commonly consumed in Nigeria. *Food Chemistry* 123, 1000–1006.
- Özyurt G., Polat A. and Loker G.B. (2009) Vitamin and mineral content of pike perch (*Sander lucioperca*), common carp (*Cyprinus carpio*), and European catfish (*Silurus glanis*). *Turkish Journal of Veterinary and Animal Sciences* 33, 351–356.
- Packer L. (1991) Protective role of vitamin E in biological systems. *American Journal of Clinical Nutrition* 53, 1050S–1055S.

- Pazos A.J., Ruiz C., Garcia-Martin O., Abad M. and Sanchez J.L.** (1996) Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, N. W. Spain. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 114, 171–179.
- Peng S., Chen C., Shi Z. and Wang L.** (2013) Amino acid and fatty acid composition of the muscle tissue of yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*). *Journal of Food and Nutrition Research* 1, 42–45.
- Saadettin G., Barbaros D., Nigar A., Ahmet C. and Mehmet T.** (1999) Proximate composition and selected mineral content of commercial fish species from the Black Sea. *Journal of the Science of Food and Agriculture* 55, 110–116.
- Salo-Vaananen P., Mattila P., Lehikoinen K., Salmela-Molsa E. and Piironen V.** (2000) Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. *Journal of Agricultural and Food Chemistry* 71, 535–543.
- Santos-Silva J., Bessa R.J.B. and Santos-Silva F.** (2002) Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Science* 77, 187–194.
- Shirai N., Terayama M. and Takeda H.** (2002) Effect of season on the fatty acid composition and free amino acid content of the sardine *Sardinops melanostictus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 131, 387–397.
- Stephen N.M., Shakila R.J., Jeyasekaran G. and Sukumar D.** (2010) Effect of different types of heat processing on chemical changes in tuna. *Journal of Food Science and Technology* 2, 174–181.
- Trivedi D.P., Doll R. and Khaw K.T.** (2003) Effect of four monthly oral vitamin D₃ (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomized double blind controlled trial. *British Medical Journal* 326, 469–475.
- Ulbricht T.L.V. and Southgate D.A.T.** (1991) Coronary heart disease: seven dietary factors. *Lancet* 338, 985–992.
- Usyduz Z., Szlinder-Richert J. and Adamczyk M.** (2009) Protein quality and amino acid profiles of fish products available in Poland. *Food Chemistry* 112, 139–145.
- Wanasundara U.N. and Shahidi F.** (1999) Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. *Food Chemistry* 65, 41–49.
- Wild A. and Hampton J.** (1994) A review of the biology and fisheries for skipjack tuna, *Katsuwonus pelamis*, in the Pacific Ocean. In Shomura R.S. and Majkowski J. (eds) *Proceedings of the first FAO expert consultation on interactions of Pacific tuna fisheries*. Rome: Food and Agriculture Organization of the United Nations, 336/2, pp. 1–51.
- and
- Zlatanov S. and Laskaridis K.** (2007) Seasonal variation in the fatty acid composition of three Mediterranean fish, sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and picarel (*Spicara smaris*). *Food Chemistry* 103, 725–728.

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