

Seasonal variation in chemical composition of the Indian mackerel (*Rastrelliger kanagurta*) from Karachi Coast

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Abstract

The chemical composition variations of the Indian mackerel (*Rastrelliger kanagurta*) from Karachi coast were investigated seasonally over the period of one year (2004-2005). Moisture, crude protein, fat and ash contents varied from 70.11-74.41%, 16.02- 20.09%, 3.0-12%, and 0.89-1.35% respectively. The seasonal data indicated that the main fatty acids of the total lipid were C16:0, C16:1, C18:0, C18:1, C20:5 and C22:6. Total contents of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) percentages varied from 31.6-46.85%, 20.5-27.9%, and 26.8-40.85% respectively. Eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexaenoic acid (DHA, C22: 6n-3) of omega 3 series which have specific importance in nutritional values concept, were the major fatty acids. The lowest EPA and DHA were found to be in July and highest in November to March.

Keywords: Chemical composition, Seasonal variation, Fatty acid, *Rastrelliger kanagurta*

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Introduction

Marine food especially fish are an important part of the human diet across the world. Nutritional importance of fish is well documented and consumption is associated with not only protein and minerals but also largely with *omega*-3-polyunsaturated fatty acid (PUFA) content (Ackman, 1989). Owing to its numerous beneficial effects of *omega*-3-PUFA on human health, the nutritional content of fish has extensively been studied in the last decades. Seafood lipids are the best natural source of highly unsaturated fatty acid and are the major contributor of *omega*-3-PUFA, particularly eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexaenoic acid (DHA, C22: 6n-3). They provide a broad range of health benefits which are not found in any other fat (Ackman et al., 1974; Dyerberg and Bang, 1979; Kromann and Green, 1980; Hooper et al., 2006; Harris, 2007). It has been found that omega 3 PUFAs has a great contribution against heart and tissue related diseases. They have beneficial effects on hypertension, diabetes, macular degeneration etc. and that its deficiency results in disorders such as skin diseases anemia and defect in eyesight (Mehmet, 2008). Therefore the fat content and fatty acid composition is important when utilization of fish species is considered.

It is generally known that marine fish have seasonal variations in chemical composition especially in fatty acids. These variations are correlated generally with the environmental factors like temperature salinity, composition of their food as well as activities (reproduction and migration) including age, sex and size of fish (Standby 1981, 1986; Ackman, 1982). Data on proximate composition and fatty acid profile in fish and shellfish from

Karachi coast has been expressed in previous studies (Nisa et al., 1996; Nisa et al., 2001; Nisa and Asadullah, 2006) and recently seasonal changes in lipid composition in sardines from the same region have been studied (Nisa and Asadullah, 2008). However, no information is available on seasonal variation in fatty acids of this species of mackerel. Mackerel are commercially important fish of the coastal area of Pakistan and the total catch during 2003 was 31126 metric ton (Hand Book of Fisheries statistics of Pakistan). The Indian mackerel (*Restrelliger kanagurta*) is commonly harvested throughout shallow waters of Pakistani coastal area (Moazzum et al., 2005).

The aim of the present study was to determine the best time of year in which fish have eminent nutritional components especially protein and omega 3 PUFA particularly EPA and DHA.

Materials and methods

Mackerel samples were obtained from Karachi fish harbor, during the period May 2004 to April 2005, packed in ice for transport to the laboratory. The fish were washed with deionized water and stored at -40 °C until analyzed. Prior to analysis, fish were thawed at room temperature and were filleted. In order to determine the total lipid composite, 100 g sample weighed was homogenized for 2 minutes with a mixture of 100 ml chloroform and 200 ml methanol. 100 ml chloroform was added to the mixture and after homogenizing for 30 seconds, 100ml distilled water was added and homogenized for further 30 seconds. The homogenate was filtered and transferred to the separating funnel for complete separation of two layers. The

chloroform layer was removed and evaporated to dry in a rotary evaporator (Bligh and Dyer, 1959). In order to prevent oxidation, lipid was stored under nitrogen (N_2) in a screw capped test tube for further analysis at freezing temperature. The moisture, ash and protein content of fish were determined as described by AOAC (1984). The protein content was calculated by converting the nitrogen content determined by Kjeldahl method ($6.25 \times N$). All three composite samples were used in this study.

The preparation of the fatty acid methyl esters (FAME) from the total lipid was carried out by saponification and methyl esterification by Christie (1989). The fatty acids compositions were then determined by using chrompack CP 9001 gas chromatography, equipped with a polar 50 m capillary column, BP x 70 (SGE Australia, 50 m x 0.32 ID, 0.25 μ m layer thickness) with hydrogen FID and using helium as carrier gas. The oven was programmed to rise from the initial temperature of 85 °C to 150 °C at a rate of 30 °C /min, from 150 °C to 152 °C at 0.1% /min, from 152 °C to 172 °C at 0.65 °C /min, from 172 °C to 187 °C at 25% /min and stayed at 187 °C for 7 min. The injector was heated from 85 °C to 190 °C at 5 °C / sec and stayed at 190 °C for 30 min. The internal standard used was C20: 2(n-6) and individual FAME was identified as a relative percentage of comparison with authentic standard reference mixture (Nu-chek-prep.Inc; USA).

Results

The chemical composition of mackerel was determined over the period of one year and the results obtained are present in table 1. Fat content varied from 3.0% to 12.0% ($p <$

0.05), highest in December and lowest in January and June. Crude protein varied from 16.65% to 20.09%, highest in June and lowest in December. Moisture content varied from 70.11% to 74.41%, highest in May and lowest in December. The ash content varied from 0.89% to 1.35%, highest in March and lowest in December. Table 2 presents seasonal variations in fatty acid composition.

The total saturated fatty acid (SFA) varied from 31.6% to 46.85% ($p < 0.05$), highest in July and lowest in February. Among the SFA, Palmitic acid (C16: 0) varied from 23.0% to 32.0% ($p < 0.05$), highest in August and lowest in March. Amongst the unsaturated fatty acids, total MUFA showed a slight decrease in the month of May, June and July and an increase in November and December. The total MUFA content varied from 20.5 % to 27.9% ($p < 0.05$). The prominent MUFA was C18:1 which varied from 11.2% to 13.0 % and was lowest in February and reached the maximum content in December. C16: 1 was the next prominent MUFA, varying from 3.5% to 6.2%, lowest in September and highest in December. The seasonal changes of total PUFA were quite distinct being the major group attaining the lowest value in July, 23.97% and highest in February, 40.85%. The *omega*-3 fatty acid series, EPA and DHA which were dominant PUFA , showed some variation during the study year, EPA varied from 7.5% to 13.0% ($p < 0.05$), lowest in August and started to increase from September and reached its maximum in January and February. Whereas the DHA ranges were from 10.0% to 16.8% ($p < 0.05$), minimum in July and maximum in November, December, January and February.

Table-1: Seasonal variation in proximate composition of the Indian mackerel

	Jan	Feb	March	April	May	June	July	August	Sept	Oct	Nov	Dec
Moisture	73.31±2.4	73.10±3.05	73.56±2.88	74.22±2.29	74.41±2.17	74.36±3.01	74.12±2.16	73.01±2.55	73.07±2.36	73.20±2.84	72.32±2.28	70.11±3.05
Crude Protein	17.55±0.33	18.02±0.35	18.19±0.28	19.70±0.40	20.0±0.85	20.09±0.93	19.81±0.34	19.49±0.38	19.84±0.29	18.11±0.36	18.09±0.41	16.65±0.91
Lipid	3.00±0.05 ^a	6.80±0.11 ^a	5.40±0.51 ^a	3.50±0.32 ^a	3.00±0.09 ^a	3.00±0.11 ^a	3.50±0.08 ^a	5.00±0.30 ^a	4.50±0.27 ^a	6.50±0.40 ^a	7.50±0.48 ^a	12.00±0.96 ^a
Ash	1.09±0.02	1.12±0.02	1.35±0.04	1.33±0.03	1.28±0.04	1.24±0.01	1.22±0.02	1.30±0.02	1.34±0.01	1.15±0.03	1.04±0.04	0.89±0.01

^aData represent mean and standard deviation of 3 samples. Values in the same rows with different superscripts (abc) are significantly different (p < 0.05).

Table-2: Seasonal variation of fatty acid in Indian mackerel

Fatty acid	Jan	Feb	March	April	May	June	July	August	Sept	Oct	Nov	Dec
14:0	1.4±0.20	1.5±0.11	2.0±0.08	3.2±0.05	2.5±0.04	4.5±0.11	6.0±0.09	5.0±0.05	3.0±0.03	2.5±0.04	1.9±0.04	2.0±0.04
15:0	0.5±0.04	0.5±0.01	0.5±0.00	0.6±0.01	0.5±0.01	0.6±0.02	0.5±0.01	0.5±0.00	0.4±0.01	0.6±0.03	0.5±0.02	0.5±0.01
16:0	23.5±1.48 ^a	23.5±1.54 ^a	23.0±1.29 ^a	25.5±1.18 ^{ab}	29.0±1.21 ^{bc}	30.5±1.8 ^c	31.1±2.04 ^c	32.0±1.88 ^c	30.10±1.82 ^c	27.5±1.08 ^{bc}	26.0±0.79 ^a	23.8±0.68 ^a
17:0	1.0±0.07	1.2±0.05	1.4±0.04	1.5±0.04	1.5±0.03	2.1±0.02	1.8±0.03	1.9±0.03	1.9±0.02	1.7±0.02	1.8±0.01	1.2±0.01
18:0	5.7±0.62	4.5±0.47	5.5±0.38	6.0±0.51	5.5±0.49	6.0±0.44	6.8±0.5	5.5±0.36	5.8±0.37	5.5±0.33	5.7±0.23	4.5±0.15
20:0	0.3±0.01	0.4±0.01	0.3±0.00	0.3±0.00	0.5±0.01	0.3±0.00	0.6±0.02	0.5±0.01	0.6±0.01	0.4±0.00	-	0.3±0.00
Total SFA	32.4±0.40 ^a	31.6±0.37 ^a	32.7±0.30 ^a	37.1±0.30 ^{ab}	39.5±0.36 ^{bc}	44.0±0.40 ^{cd}	46.85±0.45 ^c	45.4±0.39 ^c	40.85±0.38 ^{bc}	38.2±0.25 ^{ab}	35.9±0.18 ^{ab}	32.3±0.15 ^a
14:1	3.4±0.2	3.5±0.18	2.5±0.13	2.3±0.09	2.1±0.08	1.9±0.04	2.5±0.05	2.2±0.02	3.0±0.02	3.5±0.01	5.5±0.04	5.1±0.05
15:1	2.0±0.04	1.9±0.02	1.5±0.01	1.5±0.05	1.5±0.05	1.5±0.03	1.2±0.01	0.9±0.01	2.5±0.09	1.9±0.01	1.5±0.00	1.6±0.01
16:1	5.2±0.50	5.4±0.47	4.5±0.36	4.7±0.19	4.5±0.17	4.6±0.18	4.0±0.20	4.5±0.31	3.5±0.40	4.5±0.22	6.0±0.21	6.2±0.30
17:1	1.0±0.01	0.9±0.01	1.0±0.00	0.9±0.01	1.0±0.01	0.8±0.00	0.75±0.01	0.8±0.02	0.9±0.01	1.0±0.02	1.0±0.01	1.0±0.00
18:1	12.3±1.01	11.2±0.99	12.5±1.1	12.5±1.08	12.0±1.10	12.0±0.97	11.8±0.8	11.5±0.76	11.6±0.55	12.9±1.01	12.5±1.11	13.0±1.08
19:1	0.4±0.00	0.5±0.00	0.4±0.00	-	0.1±0.00	0.2±0.00	0.19±0.01	0.02±0.00	0.5±0.00	0.5±0.00	0.19±0.01	0.5±0.00
20:1	0.7±0.01	0.7±0.02	0.5±0.01	0.6±0.01	0.85±0.04	0.55±0.01	0.56±0.01	0.6±0.00	0.5±0.02	0.4±0.01	0.5±0.00	0.5±0.01
Total MUFA	25.0±0.23 ^{bc}	24.1±0.24 ^{bc}	22.9±0.23 ^{ab}	22.5±0.20 ^{ab}	22.05±0.2 ^a	21.55±0.18 ^a	21.0±0.16 ^a	20.5±0.16 ^a	22.5±0.16 ^{ab}	24.7±0.18 ^b	27.19±0.20 ^c	27.9±0.21 ^c
18:2	1.5±0.01	1.5±0.01	2.0±0.04	1.4±0.05	1.5±0.05	1.6±0.05	1.1±0.08	1.2±0.08	1.0±0.02	1.2±0.00	1.2±0.07	1.32±0.09
18:3	0.7±0.01	1.0±0.04	1.2±0.03	0.9±0.03	0.9±0.01	0.8±0.01	0.6±0.00	0.5±0.01	0.35±0.00	0.33±0.0	0.7±0.01	0.85±0.04
18:4	4.0±0.16	2.5±0.13	4.0±0.15	0.45±0.02	0.3±0.05	0.4±0.00	0.39±0.01	0.3±0.00	0.3±0.01	0.4±0.01	3.4±0.04	3.5±0.03
20:3	0.5±0.00	0.35±0.01	0.4±0.01	0.34±0.00	0.35±0.01	0.2±0.00	0.31±0.01	0.35±0.01	0.3±0.00	0.35±0.01	0.4±0.00	0.32±0.01
20:4	3.5±0.05	4.0±0.07	3.0±0.06	3.0±0.05	2.0±0.05	2.2±0.05	2.1±0.03	1.2±0.00	2.0±0.03	2.1±0.01	2.9±0.02	3.5±0.04
20:5	12±1.02 ^{ab}	13.0±1.05 ^c	11.0±0.99 ^{bc}	8.5±0.71 ^{ab}	8.0±0.49 ^a	7.9±0.58 ^a	7.7±0.40 ^a	7.5±0.41 ^a	7.7±0.23 ^a	7.8±0.39 ^a	8.5±0.34 ^{ab}	9.6±0.41 ^{ab}
22:4	0.7±0.03	0.5±0.00	0.4±0.01	0.5±0.00	0.5±0.00	0.4±0.02	0.37±0.01	0.29±0.01	0.3±0.00	0.3±0.00	0.4±0.00	0.5±0.01
22:5	1.9±0.04	1.5±0.01	1.0±0.03	2.0±0.01	1.2±0.01	1.5±0.01	1.4±0.00	1.2±0.01	1.3±0.02	1.0±0.01	0.9±0.04	1.1±0.02
22:6	16.0±1.15 ^c	16.5±1.09 ^c	16.3±1.00 ^c	13.7±1.02 ^{bc}	13.0±1.00 ^b	11.8±1.01 ^{ab}	10.0±1.22 ^a	11.5±1.16 ^{ab}	13.4±1.90 ^{bc}	14.5±1.89 ^{bc}	16.0±1.83 ^c	16.8±1.78 ^c
Total PUFA	40.8±0.31 ^c	40.85±0.27 ^c	39.3±0.26 ^c	30.79±0.21 ^{ab}	27.75±0.19 ^{ab}	26.8±0.19 ^{ab}	23.97±0.19 ^a	24.04±0.19 ^a	26.65±0.24 ^a	27.98±0.26 ^{ab}	34.4±0.26 ^{bc}	37.49±0.27 ^{bc}
Others	1.80±0.9	3.45±0.94	5.10±1.05	9.61±2.04	10.70±1.97	7.65±2.11	8.18±2.57	10.06±1.41	10.00±1.27	9.22±1.66	2.51±0.11	2.31±0.44

^aData represent mean and standard deviation of 3 samples. Values in the same rows with different superscripts (abc) are significantly different (p < 0.05).

^aPresenting weight percent of total fatty acid methyl esters

Discussion

Seasonal data showed that even though fat content is somewhat high in the present study but the variation trend was to some extent in agreement with the values of Bandara et al. (2001) for the Indian horse mackerel. According to Borges and Gordo (1991) the spawning season of this species takes place during the first semester of the year, which would be the probable reason of low fat content in this time of year.

Protein percent reached its maximum in June and minimum in December. Low protein in this month may suggest that protein may utilize for metabolic energy. The recorded values for crude protein were not only in agreement with the values of Bandara et al. (2001) but also to some extent with findings of Mehmet (2008) for mackerel species.

The recorded results for moisture were somehow in agreement with the findings of Soyer and Sahin (1999) for chud mackerel. However Aubourg et al. (2002), Aubourg and Uglianol (2002) and Losada et al. (2005) identified moisture contents in horse mackerel as 75% to 79%, 78.2% and 77-81% respectively. The ash content varied from 0.89% to 1.35%, highest in March and lowest in December. These findings were in agreement with Mehmet (2008), as ash content of the under studied species was high in March (spring). The seasonal data on sardines indicated that the main fatty acids C16: 0, C18: 0, C16: 1, C18: 1, C20: 4, C20: 5, C22: 6 were common in all the seasons but their compositional percentage varied widely through out the year. These major fatty acids were not only same as the previous study on sardines from the same region (Nisa and Asadullah, 2008) but also the

same results were showed in other studies carried out by Bandarrra et al. (2001, 1997), Kostas (2007) and Spiros and Mehmet (2008). The saturated fatty acid (SFA) varied from 31.6% to 46.85% ($p < 0.05$), highest in July and lowest in February. The amount of SFA was not only in agreement with the findings of Spiros and Kostas (2007) and Mehmet (2008) but also with the previous study on sardine (Nisa and Asadullah, 2008). Amid the SFA, Palmitic acid (C16: 0) was the major fatty acid which showed seasonal variation like the one reported by Ackman (1964), Ackman and Eaton (1966) and Bandarrra et al. (2001) and reported similar findings and concluded that C16: 0 was a key metabolite in fish and did not seem to be influenced by diet.

MUFA content in the present study was comparatively high, however the seasonal variation trend (Table 2) was not only somehow in agreement with the findings of the previous study (Nisa and Asadullah, 2008) but also with the findings of Bandarrra et al. (2001). Amongst the MUFA, high level of C18:1 was in accordance with findings by Ackman (1982) who pointed out that the main MUFA in marine lipid usually contained 18 carbon atoms.

The seasonal changes of total PUFA had a considerable effect on the variation of unsaturated fatty acid content, from season to season which may suggest that PUFA may be responsible for the seasonal variation of total unsaturated fatty acids. The dominant PUFA are those of the *omega*-3-series, found chiefly were EPA and DHA of therapeutic importance (Sanders et al., 1997; Montori et al, 2002),

EPA showed some variation in percentage during the year. The DHA also showed seasonal prominent variations during the studied time. Both EPA and DHA have the same trend in seasonal variation which the content percentages were high in cold and low in hot seasons. These kinds of results have also been derived by Mehmet (2008). The seasonal variation in the lipid composition of marine organisms is generally influenced by many factors that may differ from year to year or season to season and may also depend on the feeding or preying of the organisms (Patton, 1975; Osako et al, 2003).

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References

- Ackman, R. G., 1982.** Fatty acid composition of fish oil. In: Nutritional evaluation of long chain fatty acids in fish oils. S. M Barlow and M. E. Standby, New York, Academic Press, p-25.
- Akman, R. G. and Eaton, C. A., 1966.** Some commercial Atlantic herring oils; fatty acid composition. 23,991.
- Akman, R. G., Epstein, S. and Kelleher, M. A., 1974.** composition of lipid and fatty acids of the ocean Quahuag, Arctica Islandica, from Nova Scotia and New Brunswick.. *Journal of the Fish Research Board of Canada*, 31, 1803-1811.
- Akman, R. G., 1964.** Structural homogeneity in unsaturated fatty acid of marine lipid (A review). *Journal of the Fisheries Research of Canada*, 21, 247.
- Akman, R. G., 1989.** Nutritional composition of fats in sea foods. *Progress in Food and Nutrition Science*, 13, 161-241.
- Association of Official Analytical Chemists, 1984.** Official Methods of analysis, 14th edn. Method, 24.003, 32.027 and 47.021, Washington DC: *Association of Official Analytical Methods*, 431, 610, 988.
- Association of Official Analytical Chemists, 1984.** Official Methods of analysis, 14th ed. Methods, Washington DC: *Association of Official Analytical Methods*, p-610.
- Association of Official Analytical Chemists, 1984.** Official Methods of analysis, 14th edn. Methods, Washington DC: *Association of Official Analytical Methods*, p-988.
- Aubourg, S. and Ugliano, M., 2002.** Effect of brine pre- treatment on lipid stability of frozen horse mackerel (*Trachurus trachurus*). *European Food Research and Technology*, 215, 91-95.
- Aubourg, S., Lehmann, I. and Gallardo, J., 2002.** Effect of previous child storage on rancidity development in frozen horse mackerel (*Trachurus trachurus*). *Journal of the Science of Food and Agriculture*, 82, 1764-1771.
- Bandarra, N. M., Batista, I., Nunes, M. L. and Empis, J. M. and Christie, W. W., 1997.** Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). *Journal of Food Science*, 62, 40-43.

- Bandarra, N. M., Batista, I., Nunes, M. L. and Empis, J. M., 2001.** Seasonal variation in the chemical composition of horse mackerel (*Trachurus trachurus*). *European Food research Technology*, 212, 535-539.
- Bligh, E. G. and Dyer, W. T., 1959.** A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
- Borge, F. and Gordo, L. S., 1991.** Spatial distribution by season and some biological parameters of horse mackerel (*Trachurus trachurus* L) in the Portuguese continental water (Division IXa). ICES. CM. 1991/H, 54, p-16.
- Christie, W. W., 1989.** Gas Chromatography and Lipids. A practical guide, the oil press. Ayr. Scotland. 64-84.
- Dyerberg, J. and Bang, H. O., 1979.** "Heamostaic function and platelet polyunsaturated fatty acid in Eskimos. *Lancet*, 2, 433-435.
- Hand Book of Fisheries Staistics of Pakistan, 2006.** Marine Fisheris Department Karachi Pakistan.19.
- Harris, W. S., 2007.** Omega 3 fatty acid and cardiovascular diseases: A case for omega 3 index as new risk factor. *Pharmaceutical Research*, 55(3), 217-223.
- Hooper, L., Rachel, L. T., Roger, A. H., Carolyn, D. S., Andy, R. N., Helen, J. M., Helen, V. W. et al., 2006.** Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *British Medical Journal*, 332, 752-760.
- Kroman, N and Green. A., 1980.** Epidemiological studies in the Upernavik District, Green Land. *Acta Scundinavica*, 208, 401-406.
- Losada, V., Pineuro, C., Barros-velazuez, J., and Aubourg, S. P., 2005.** Inhibition of chemical changes related to freshness loss during storage of horse makerel (*Trachurus trachurus*) in slurry ice. *Food Chemistry*, 93, 619-625.
- Mehmet, C., 2008.** Seasonal variation in the proximate chemical composition and fatty acid of chud mackerel (*Scomber japonicus*) and horse mackerel (*Trachurus trachurus*) from the north eastern Mediterranean Sea. *International Journal of Food Science and Technology*, 43, 933-938.
- Moazzum, M., Baber, H. O. and Zohra, K., 2005.** Indian mackerel (*Rastrelliger kanagurta*) from Pakistan-I. Some aspect of biology and fisheries. *Records Zoological Survey of Pakistan*, 16, 58-75.
- Montori, V. M., Farmer, A., Wollan, P. C. and Dinneen, S. F., 2002.** Fish oil supplementation in type 2 diabetes: a quantitative systematic review. *Diabetic Care*, 23(9), 1407-1415.
- Nisa, K. and Asadullah., 2006.** Lipid classes and fatty acid content in muscles of two shrimp species *F. penicillatus* and *F. merguensis* from Karachi coast. *Journal of the Chemical Society of Pakakistan*, 28, 600-605.
- Nisa, K. and Asadullah, 2008.** Seasonal Variation in Lipid Composition of Sardine (*Sardinella gibbosa*) from Karachi Coast. *Journal of the Chemical Society of Pakakistan*, 30(3), 436-441.
- Nisa, K., Qadri, R. B., Touheed, A. and Viqarudine, A., 1996.** Fatty acid profile of four marine species from Karachi coast. *Journal of the Chemical Society of Pakistan*, 18(1), 44-47.
- Nisa, K., Qadri, R. B., Touheed, A and Viqarudine, A., 2001.** Lipid and fatty

- acid profile of Lady fish, dhother, sua, sole, aal and khagga from Karachi coast. *Journal of the Chemical Society of Pakistan*, 23(3), 177-180.
- Osako, K., Yamaguch, A., Kurokawa, T., Kuwahara Saito, H. and Nozaki, Y., 2003.** Seasonal variation in docosahexaenoic acid content in horse mackerel caught in the East China Sea. 69, 589-596.
- Patton, J. S., 1975.** The effect of pressure and temperature on phospholipid and triglyceride. *Comparative Biochemical and Physiology*, 52B, 105-110
- Soyer, A. and Sahin, M. E., 1999.** Effect of glazing and storage time on lipid oxidation of frozen chud mackerel (*Scomber Japonicus*). *Turkish Journal of Veterinary and Animal Science*, 23, 575-584.
- Spiros, Z. and Kostas, L., 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(3), 725-728.
- Standby, M. E., 1981.** *Journal of the American Oil Chemists Society*, 58, 13.
- Standby, M. E., 1986.** Health effects of polyunsaturated fatty acid in seafood. Academic Press, Landon, p-389.
- Sanders, T. A., Oakley, F. R., Miller, G. J., Mitropoulos, K. A., Crook, D. and Oliver. M. F., 1997.** Influence of *n*-6 versus *n*-3 Polyunsaturated Fatty Acids in Diets Low in Saturated Fatty Acids on Plasma Lipoproteins and Hemostatic Factors. *Arteriosclerosis Thrombosis and Vascular. Biology*, 17, 3449-3460.