

Seasonal Variations in Cholesterol Content and Proximate Composition of Indian Mackerel (Rastrelliger kanagurta)

Hafsa Maqbool*, S. B. Patange, Mudassir Azhar, J. M. Koli and M. T. Sharangdhar College of Fisheries, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri - 415 629, India

Abstract

Dietary cholesterol levels are directly linked with risks of coronary heart disease (CHD). Food labeling is an important parameter for displaying the regulatory and safety level of cholesterol. Quadruplicate samples of Indian mackerel of different size and weight were collected on monthly basis for a period of one year and were analysed for cholesterol content, proximate composition and nonsaponifiable matter (NSM). Cholesterol content was not steady throughout the year and varied in relation to size and season; was found to be higher at 80.3 mg% and lower at 7.76 mg% during the month of October and December respectively. Protein content also showed wide variations throughout the period and the values ranged between 11.2 to 28.1%. The moisture content showed consistently inverse trend with fat content during the period of August to October. NSM content was negligible and undetectable. No correlation was observed between the content of cholesterol and NSM in fish samples for different months. The results of two way ANOVA showed that there was a significant difference in cholesterol content with in the months (p<0.05).

Keywords: Cholesterol, seasonal variations, mackerel, proximate composition

Introduction

Cholesterol has assumed great medical importance in recent years due to its role in human coronary heart diseases (CHDs) (Laker, 2003). CHD is the leading cause of death in most industrialized countries and its importance as a major public health problem is increasing in developing countries

Received 24 December 2016; Revised 20 July 2017; Accepted 26 August 2017

*E-mail: hafsamaqbool99@gmail.com

(Murray & Lopez, 1996). It is well known that increased cholesterol content in the blood can lead to cardiovascular diseases and the dietary cholesterol can affect the serum cholesterol levels (Sanchez-Muniz et al., 1991; Ammu et al., 1996). Indian Mackerel (*Rastrelliger kanagurta*) is a delicacy among the Indian consumers and it is landed in large quantities along the Indian coast mainly by purseseine and gillnet operations; therefore consumption of this fish has assumed importance in dietary cholesterol levels of Indian consumers.

Nutrition labeling-regulations which were issued since January 6, 1993, by the Food and Drug Administration (FDA) and the U.S. Department of Agriculture's Food Safety and Inspection Services (FSIS) have made food labeling. During the recent years nutrition labeling has become mandatory because of increasing consumer interest in food and nutrition and their relationship to health. Consumer awareness regarding the nutritional quality of fish has increased with reference to cholesterol content, their health issues and other hazardous compounds (Sehat & Niedwetizki, 1998). As the dietary cholesterol is known to effect serum cholesterol, the information regarding the daily dietary intake of cholesterol can be quite important, especially to those with cardiovascular problems (Ammu et al., 1996; Nair et al., 1985; Nair & Gopakumar, 1981; Sanchez et al., 1991). Disclosure of cholesterol content of food items including Packaged Seafoods along with related information on nutritional facts have made mandatory. Presence of meagre literature regarding the seasonal variations in cholesterol content of seafoods paved the way for this study.

Material and Methods

Fresh Indian mackerel (*Rastrelliger kanagurta*) were obtained from Ratnagiri fishing harbour as soon as they were landed by the fishing vessels. The fish samples were collected on monthly basis for a period of one year from March, 2015 to February,

2016. The fish samples were brought to the laboratory in iced conditions in insulated boxes. Analyses of cholesterol and proximate composition were carried out immediately. The total length (cm) and weight (g) of fish were recorded and analyzed for cholesterol content, proximate composition and nonsaponifiable matter (NSM). The analyses were carried out in quadruplicate each month to ensure variability in the size/length of samples. Fish mince from mackerel was prepared by using a homogenous mixture of edible portion of the muscle and an aliquot of the same was used for the further analysis. Cholesterol estimation was carried out as described by Zlatkis et al. (1953). The estimation of total lipids was carried out as per Folch method (Folch et al., 1957). The NSM, crude protein, moisture and ash content were determined as per the standard methods (AOAC, 2005). Cholesterol standard (Spinreact, Spain) was obtained from Siddhivinayak Diagnostic Laboratory, Ratnagiri. All the chemicals and reagents used were of analytical grade and were purchased from Hi Media, Mumbai.

Results and Discussion

The data on cholesterol content of fish samples with respect to month/season is detailed in Table 1. The highest level in cholesterol content was noticed in the month of October and the lowest was seen in the month of December (Fig. 1). The highest value of cholesterol was recorded at 80.3 mg% in a sample having total length (TL) 20 cm and weight 150 g during the month of October. The minimum cholesterol value of 7.76 mg% was recorded in a sample with TL 17.9 cm and weight 64 g during the month of December. Fish samples could not be procured in the month of June and July owing to closure of fishing activities. The results of two way ANOVA showed that there was a significant difference in cholesterol content between the months (p<0.05). Ackman (1994) reported the values of cholesterol in mackerel (Scomber scombrus) in the month of June, August and September as 61.9, 36.5 and 44.8 mg% respectively. Mathew et al. (1999) reported nearly similar values of cholesterol content in Indian mackerel as 69.7, 51.4, 55.6 and 65.4 mg% during the months of March, May, June and October with the respective fish size of 21 cm, 12 cm, 23 cm and 21 cm respectively. The significant variations in cholesterol content may be attributed to the food and feeding habits of fish. According to Bal & Rao (1984) mackerel feeds on phytoplankton and zooplankton. The phytoplanktonic elements comprising

the food are the diatoms represented chiefly by Coscinodiscus, Pleurosigma, Cheateoceros, Fragillaria, Nitzchia and Skeletonema. Among zooplanktonic items of food other than crustaceans, there are tintinids, polycheate larvae, molluscan larvae, fish eggs and fish larvae. According to Mathew et al., (1999) the cholesterol content of marine prawns ranged from 118 to 163 mg%, while in crabs, it was from 51.5 to 66.8 mg%; however extremely higher levels of cholesterol was observed in mackerel eggs (462 mg%). So, this might be the reason that the higher values of cholesterol found in mackerel as their food contained zooplankton and possibly due to pre-spawning status of the fish. However, it did not have any correlation with maturation and spawning activities of fish.

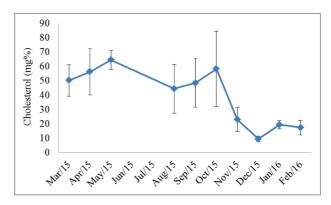


Fig. 1. Seasonal variations in cholesterol content of Indian mackerel

According to Yohannan & Sivadas (2003) the intensity of the spawning of mackerel starts by April/May and continues till July. By around December they start maturing and start spawning by February, reaching a peak by May, the age at first spawning being around one year. They grow to a size of around 230 mm by the end of the first year. Idler et al. (1964) reported a variation, during different seasons, in the cholesterol content of the scallop muscle, suggesting a relationship between the metabolism and biosynthesis of these sterols. Of the total sterols estimated as NSM (0.05 g%), the value of cholesterol (51 mg%) equalled the total sterols estimated in Indian mackerel (Mathew et al., 1999).

The data on cholesterol content if as in fish sample with respect to size/ weight of fish is organized in ascending order as detailed in Table 2. The statistical analysis showed that there was linear correlation between the cholesterol content of samples with

respect to the total length in all the months. The correlation coefficient values ranged between 0.85 and 0.67. As the size of fish increased, the cholesterol content also increased. In a study carried out to investigate seasonal variations in cholesterol content in different species of prawns, Mathew et al. (2005) noted negative correlation of cholesterol content between size and months of sampling from March to June.

The analysis of proximate composition of Indian mackerel showed seasonal variations with regard to the monthly values of moisture content. The moisture content of individual fish samples ranged from 69.00 to 76.19%. Moisture content was low during the month of September, November, December and January respectively. Highest moisture content was 76.19% from the sample with a total length of 22 cm and weight 149.5 g observed during the month of May (Table 1). The highest variation in moisture content was recorded in the month of May and the lowest variation was seen in the month of April as shown in the (Fig. 2). Results of two way ANOVA showed that there was significant difference in moisture content between the months (p<0.05). The inverse relationship between the degree of hydratation and the lipid content in the mackerel had been shown by Jacquot (1961) wherein the moisture content was 78.6, 62.7%; the respective value of fat contet was 2.2 and 16.4%. Jacquot (1961) reported 68.6% moisture content in fatty fishes. Gopakumar (1997) reported 71.19% moisture content in Indian mackerel. However, Nisa & Asadullah (2011) observed similar values for the Indian mackerel that varied from 70.11-74.41%, the highest being in May and lowest in December. Tzikas et al. (2007) reported similar seasonal variation in moisture content of the Mediterranean horse mackerel as significant decrease (p<0.05) in March (75.9%), April (75.3%) and May (75.4%) and a significant increase (p<0.05) in September and October.

The protein content of mackerel ranged from 11.2 to 28.1% (Table 1) with respect to total length. The protein content was observed to be minimum at 11.2% in mackerel fish sample with a total length of 16 cm and weight 45 g during the month of September. It was highest at 28.1% in a fish sample with a total length of 23.3 cm and weight of 128 g during the month of December. The highest seasonal variation in protein content was recorded in the month of December and the lowest level in the month of Febuary (Fig. 2). The results of ANOVA

showed that there was a significant difference in protein content between the months (p<0.05). The value of protein content of Indian mackerel agrees with observations of Gopakumar (1997) who reported nearly the similar value of 21.21%. According to Tzikas et al. (2007) protein content of the Mediterranean horse mackerel ranged from 19.7% (March) to 21.0% (January). Bhaware & Mane (2012) reported that the protein content of Indian mackerel increased in winter (22.24 ± 0.40) and decreased in summer (21.55 ± 1.23). Nisa & Asadullah (2011) reported the seasonal variations in protein content of the Indian mackerel which varied from 16.02-20.09%, highest in June and lowest in December. In the present study, the seasonal variations in protein content may be attributed to the fact that the fish protein comprises abundant of non-protein nitrogen (NPN) than nitrogen part of the protein; about 9-18% of the total nitrogen in teleosts, 20-25% in molluscs and crustaceans and 26% in case of cartilaginous fishes which includes the components such as free amino acids, TMAO (trimethylamineoxide), volatile nitrogen bases particularly ammonia, urea etc. Occurrence and distribution of NPN compounds vary widely between different fishes and also within same species depending on season, size, age, the stage of spawning (Balachandran, 2001).

The fat content of mackerel ranged from 1.1 to 15%. The minimum fat content was recorded at 1.1% in a sample with a total length of 22 cm and weight 149.5 g during the month of May. Highest value of 15% was obtained in a sample with a total length of 25 cm and weight 180 g during the month of April (Table 1). The highest variation in fat content was recorded in the month of April and the lowest variation was recorded in the month of May (Fig. 2).

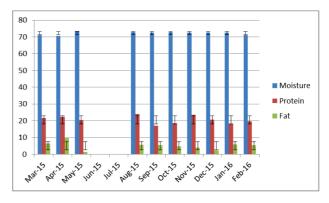


Fig. 2. Seasonal variations in moisture, protein & fat content of Indian mackerel

Table 1. Seasonal variations in cholesterol content and proximate composition of Indian mackerel

Sr. No.	Month & year	Fish sample		Cholesterol	Moisture	Protein	Fat	Ash
		Total Length (cm)	Weight (g)	(mg%)	(g%)	(g%)	(g%)	(g%)
	March,	24.0	160	55.5	73.11	18.5	8.60	2.0
	2015	22.0	140	62.6	72.18	20.0	7.20	2.0
	2010	15.0	58.0	45.3	70.27	22.5	5.01	2.0
		15.5	60.0	37.9	71.54	25.0	5.12	2.0
	Mean±	19.13	104.5	50.33	71.78	21.50	6.48	2.0
	SD	17.10	101.0	±10.91	±1.19	±2.86	±1.73	±0
		25.0	4.00					
	April	25.0	180	60.5	70.12	18.5	15.0	1.84
		23.0	140	74.5	71.19	21.0	10.0	2.19
		19.0	94.0	54.36	70.72	22.7	10.0	2.45
	M	19.0	94.0	35.72	70.72	26.7	5.00	2.42
	Mean± SD	21.50	127	56.27 ±16.08	70.69 ±0.44	22.23 ±3.45	10.0 ±4.08	2.23 ±0.28
	May	22.0	149.5	70.7	76.19	22.12	1.10	1.59
	,	21.5	140.9	69.5	76.12	22.37	1.20	1.31
		18.5	80.33	58.3	71.19	18.79	1.30	2.00
		18.0	77.40	59.7	70.72	17.95	1.30	1.40
	Mean±	20.0	112.03	64.55	73.56	20.31	1.23	1.58
	SD			±6.45	±3.01	±2.27	±0.10	±0.31
	June	NΑ	NΑ	N A	NΑ	NΑ	NΑ	NΑ
	July	NΑ	NΑ	N A	NΑ	NΑ	NΑ	NΑ
	August	21.0	119	69.1	70.11	23.0	10.0	1.60
		20.0	95.0	33.0	71.25	24.9	7.50	1.30
		18.0	68.0	43.1	74.43	20.5	2.50	2.45
	3.6	19.0	86.0	33.0	74.92	28.0	2.50	2.54
	Mean±	19.5	92.0	44.5	72.68	24.1	5.63	1.97
	SD			±17.05	±2.36	±3.16	±3.75	±0.62
	September	21.0	113	70.2	71.75	20.2	5.0	2.95
		20.0	117	53.5	69.0	20.9	2.5	1.7
		17.0	58.0	32.3	74.93	15.7	7.5	1.87
		16.0	45.0	38.2	74.8	11.2	7.2	2.85
	Mean±	18.5	83.25	48.55	72.62	17.0	5.55	2.34
	SD			±16.97	±2.83	± 4.50	±2.32	±0.65
	October	21.0	140	80.0	71.19	21.4	4.0	2.41
		20.0	150	80.3	71.57	21.1	2.5	2.80
		16.0	60.0	28.3	76.10	17.5	6.4	1.22
		17.0	70.0	44.6	71.8	13.5	6.0	3.00
	Mean±	18.5	105	58.31	72.67	18.3	4.73	2.36
	SD			±26.08	±2.30	±3.70	±1.82	±0.80
	November	21.0	130	34.56	71.75	25.3	6.84	1.38
		21.0	130	19.0	69.0	23.6	5.25	2.16
		18.0	88.0	23.68	74.93	24.9	2.08	1.19
		17.0	89.0	14.72	74.8	20.5	1.32	1.38
	Mean± SD	19.25	109.25	22.99 ±8.54	72.62 ±2.83	23.58 ±2.17	3.87 ±2.61	1.53 ±0.43
)	December	23.3	128.0	12.0	±2.65 71.75	±2.17 28.1	2.45	2.52
,	December							
		23.0 17.9	145.0 64.0	10.09 7.76	69.0 74.93	21.5 15.6	1.94 3.05	1.56 2.41
		16.0	51.0	8.54	74.93 74.8	17.0	1.50	3.12
	Mean±	20.0	97.0	9.60	74.6 72.62	20.55	2.24	2.40
	SD	20.0	97.0	±1.87	±2.83	±5.63	±0.67	±0.64
	January,	21.0	120	23.28	71.75	17.21	10	1.24
	2016	20.0	110	19.0	69.0	15.50	5.1	1.40
		17.0	60.0	16.29	74.93	18.04	7.5	1.47
		16.0	50.0	19.41	74.8	21.19	1.23	2.45
	Mean±	18.5	85.0	19.50	72.62	18.0	6.0	1.64
	SD			±2.88	±2.83	±2.38	±3.73	±0.55
12	February	21.0	100	12.8	70.30	18.26	6.84	1.36
		20.0	104	16.6	69.49	17.5	7.56	1.98
		17.5	54.0	15.5	73.00	21.0	2.08	1.42
		17.0	52.0	24.8	71.91	21.7	5.09	1.24
	Mean±	19.0	77.5	17.43	71.18	19.62	5.39	1.50
	SD			±5.17	±1.58	±2.05	± 2.44	±0.33

N A (not analysed)

Table 2. Variations in cholesterol content of Indian mackerel with respect to size

Sl.No.	Total length (cm)	Cholesterol (mg%)	Month & year of sampling
1	15.0	45.30	March 2015
2	15.5	37.90	March 2015
3	16.0	38.20	September 2015
4	16.0	28.34	October 2015
5	16.0	19.41	January 2016
6	16.0	8.54	December 2015
7	17.0	14.72	November 2015
3	17.0	16.29	January 2016
)	17.0	24.80	February 2016
10	17.0	32.30	September 2015
11	17.0	44.60	October 2015
12	17.5	15.50	February 2016
13	17.9	7.76	December 2015
14	18.0	23.68	November 2015
15	18.0	43.10	August 2015
16	18.0	59.70	May 2015
17	18.5	58.30	May 2015
.8	19.0	33.0	August 2015
19	19.0	35.72	April 2015
20	19.0	54.36	April 2015
21	20.0	16.60	February 2016
22	20.0	19.00	January 2016
23	20.0	33.00	August 2015
24	20.0	53.50	September 2015
25	20.0	80.30	October 2015
26	21.0	12.80	February 2016
27	21.0	19.00	November 2015
28	21.0	23.28	January 2016
<u>2</u> 9	21.0	34.56	November 2015
30	21.0	69.10	August 2015
31	21.0	70.2	September 2015
32	21.0	80.0	October 2015
33	21.5	69.5	May 2015
34	22.0	62.6	March 2015
35	23.0	71.0	May 2015
36	23.0	10.09	December 2015
37	23.0	74.5	April 2015
38	23.3	12.0	December 2015
39	24.0	55.5	March 2015
40	25.0	60.5	April 2015

The result of two way ANOVA shows that there was a significant difference in fat content between the months (p<0.05). (Jacquot, 1961) showed the values of the lipid content in fatty fishes at 10%. Gopakumar (1965) found that lipid content of oil sardine fluctuate from 2 g 100 g⁻¹ flesh weight (April, May) to 12-16 g 100 g⁻¹ (October to January). There is an inverse relation between muscle fat (depot fat) and moisture content. Increasing body fat is followed by lowering of moisture value. He also observed the increase in lipid content is contributed by triglycerides and phospholipid. Gopakumar (1997) reported nearly similar value of fat content of Indian mackerel as 7.51% which agreed with the findings of Mathew et al. (1999) who reported the values for fat content of Indian mackerel as 6.84%. Osman et al. (2001) reported the same values for the fat content of Spanish mackerel that is 1.46-5.77%. Tzikas et al. (2007) reported the increased values in lipid content of Mediterranean horse mackerel during the months of March, April and May. While as the decreased values were recorded in the month of September to October (0.4 and 0.6%) respectively. Nisa & Asadullah (2011) reported the seasonal variations in fat content of the Indian mackerel that varied from 3.0 to 12.0%, lowest in January & June and highest in December. Wan et al. (2012) reported the fat content of Indian mackerel less than 4% that is 3.2%.

The ash content of the individual fish samples varied from 1.19 to 3%. The minimum values of ash content was recorded at 1.19% in sample with a total length of 18 cm and weight 88 g recorded during the month of November. The highest variation in ash content was in the month of October and the lowest was in March. The result of two way ANOVA shows that there was a significant difference in ash content between the months (p<0.05). Jacquot (1961) has showed the same values of the ash content in fatty fishes at 1.4%. Gopakumar (1997) reported the ash content of Indian mackerel as 1.33%. Tzikas et al. (2007) reported the similar values for seasonal variation in ash content of Trachurus mediterraneus (Horse mackerel) as $(1.5 \pm 0.08\%)$ which was almost constant during the year. Nisa & Asadullah (2011) has also obtained nearly the similar value of 1.35% in Indian mackerel.

The present study on the variations in cholesterol content of Indian mackerel based on the data compiled can be concluded that the cholesterol content was found to be 7.76 mg% which is low in mackerel with respective to the season and size. The

monthly proximate composition of the seafood varied with respect to the total length and size; it is concluded that the moisture and fat showed the inverse relation. As the proteins in seafoods and the marine PUFA are known to be hypocholesterolemic, the fat content of Mackerel have cholesterol lowering effect on human blood serum.

Acknowledgement

Authors wish to thank the Associate Dean, College of Fisheries, Ratnagiri (Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli) for providing necessary facilities, and their kind encouragement and guidance during the course of the investigation.

References

- Ammu, K., Sankar, T. V. and Devadasan, K. (1996) Influence of vitamin E supplementation of dietary fish oils in lipid profile and blood glucose. J. Food. Sci. Technol. 33(2): 128-132
- AOAC (2005) Official Methods of Analysis 18th edn., Association of Official Analytical, Washington, DC, USA
- Ackman, R.G. (1992) Bioavailability of omega 3PUFA. In: Advances in Seafood Biochemistry Composition and Quality (Flick, G. J. and Martin, R. E., Eds) Lancaster and Basel; Technomic Publication, pp 269-289
- Bal, D.V. and Rao, V. K. (1984) Marine Fisheries. Tata Megraw Hill, New Delhi, 470 p
- Balachandran, K. K. (2001) Post-harvest Technology of Fish and Fish Product. Daya Publishing House, New Delhi, 440p
- Bhaware, G. B. and Mane, H. U. (2012) Seasonal variation in the protein composition of *Rastrelliger kanagurta* (Cuvier) from within PFZ and outside PFZ at Sakhri-Natye landing centre on the coast of Ratnagiri district at Maharashtra state. Recent. Res. Sci. Technol. 4(10): 01-04
- Folch, J. M., Lees, M. P. and Stanley, G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226: 497-509
- Gopakumar, K. (1965) Seasonal variations of lipid composition of oil sardine. Indian J. Fish XII (1).1
- Gopakumar, K. (1997) Biochemical Composition of Indian Food Fish. pp 01-04. Central Institute of Fisheries Technology, Cochin
- Idler, D. R., Tanura, J. and Wainai, J. (1964) Seasonal variations in the sterol fat and unsaponifiable components of scallop muscle. J. Fish. Res. Board Can. 21(1): 1035-1042

- Jacquot, R. (1961) Organic constituents of fish and other aquatic animal foods. In: Fish as Food (Borgstrom, G., Ed) pp 146-148. Academic Press, New York
- Laker, M. (2003) Understanding cholesterol. Family doctor publications, Dorset, United Kingdom, 116p
- Mathew, S., Ammu, K., Nair, P.G. V. and Devadasan, K. (1999) Cholesterol content of Indian fish and shellfish. J. Food Chem. 66: 455-461
- Murray, C. J. L. and Lopez, A.D. (1996) Evidence-based health policy, lessons from the global burden of disease study. Science. 274: 740-743
- Nair, P. G. V. and Nair, M. R. (1985) Fatty acid composition of muscle and skin lipids of oil sardine.
 In: Harvest and Post-Harvest Technology of Fish (Ravindran, K., Unnikrishnan, N., Perigreen, P.A., Madhavan, P., Gopalakrishna, Pillai. A.G., Panicker, P. A. and Thomas, M., Eds). pp 441-444. Society of Fishery Technologists. Cochin, India
- Nair, P. G. V. and Gopakumar, K. (1981) Effect of longterm feeding of a coconut oil-based and a combined coconut oil fish diet on serum cholesterol and fattyacid composition of different organs of albino rats. J. Food Sci. Technol. 18: 187-191
- Nisa, K. and Asadullah, K. (2011)Seasonal variation in chemical composition of the Indian mackerel (*Rastrelliger kanagurta*) from Karachi coast. Iranian J. Fish. Sci. 10(1): 67-74
- Osman, H., Suriah, A. R. and Law, E. C. (2001) Fatty acid composition and cholesterol content of selected marine fishes in Malaysian waters. Food Chem. 73: 55-60

- Sanchez-Muniz, F. J., Higon, E., Cava, F. and Viejo, T. M. (1991) Acceptability of diets containing olive oil fried sardine (*Sardina pilchardus*) in the prevention of dietary hypocholesterolemia. J. Sci. Food Agril. 56: 155-165
- Sehat, N. and Niedwetizki, G. (1998) Food labelling in Germany including European Union aspects. Food Technol. 52: 58-61
- Tzikas, Z., Amvroiadis. I., Soultos, N. and Georgakis, Sp. (2007) Seasonal variation in the chemical composition and microbilogical condition of Mediterranean horse mackerel (*Trachurus mediterraneus*) muscle from the North Aegean Sea. Food Control. 18: 251-257
- Wan, R. W. I., Rohana, A. J., Gan, S.H., Noor, F.H., Rosliza, H., Helmy, H., Mohd, N.S., Mohd, I. I., Shaiful, B. I., Wan, M. W.B. and Kamarul, I. M. (2012) Fat content and EPA and DHA levels of selected marine, freshwater fish and shellfish species from the east coast of Peninsular Malaysia. Int. Food. Res. J. 19(3): 815-821
- Yohannan, M.T. and Sivadas, M. (2003) Indian mackerel. In: Status of Exploited Marine Fishery Resources of India (Joseph, M.M. and Jayaprakash, A.A., Eds) pp 60-64. Central Marine Fisheries Research Institute, Kochi, India
- Zlatkis, A., Zak, B. and Boyle, A. J. (1953) A new method for determination of serum cholesterol by ferric chloride method. J. Lab. Clin. Med. 41: 486-488