

Vitamin A Content, Nutritional Value and Seasonal Variation of Proximate Composition of Indian Major Carps

¹Susmita Dey, ²Umesh C. Goswami

^{1,2} Department of Zoology, Fish Biology and Fishery Science, Centre for Aquaculture and aquatic Resources Monitoring Centre, Biodiversity and Bioinformatics Centre., DST-FIST (Govt. of India) & UGC-SAP Sponsored Department Gauhati University, Guwahati-781014, Assam, India.

Abstract: Three species of Indian major carp (*Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*) were collected from local fish market and fishing sites of river Brahmaputra for determining the nutritional value, seasonal variation of proximate composition and vitamin A content of these fishes. Results obtained show that there is no much significant seasonal variations in body composition of Indian major carp though exceptions are always there. The ratio of retinol: dehydroretinol is of not much difference. The lipid content is comparatively low with lowest in *Labeo rohita* but lipid subclasses vary in different parts of the body of same fish species. Protein content is seen maximum in *Cirrhinus mrigala* in comparison to the other two species.

Index Terms: Retinol, dehydroretinol, fatty acids, biochemical composition.

I. Introduction

The nutritional quality of food is very important for the human consumer. Consumption of fish provides important nutrients to a large number of people worldwide and makes a very significant contribution to nutrition. Infact fish is one of the main sources of protein and fat and has become a healthier alternative to meat for the last fifty years.

The biochemical composition of the whole body indicates the fish quality and so proximate biochemical composition of a species helps to assess its nutritional and edible value.

Consumption of fish provides important nutrients to a large number of people worldwide and makes a very significant contribution to nutrition.

Global fresh water fish production is mainly dependent on carps. In India, freshwater fishery is one of the major contributors to the animal protein for the population. Indian major carps (*Catla*, *Rohu*, *Mrigal*) are the major freshwater food fishes of India.

Fish stores the lipids in various organs particularly in muscle and liver. The lipids in fish muscles have received much attention as source of EPA and DHA fatty acid in human diets. Lipids and fatty acid composition of many marine fish and shell fish as well as the effect of different diets and factors on lipids composition of various species have been investigated. AcKman and Takeuchi (1986), Vishwanathan and Gopakumar (1984), Halver (1980), Suzuki *et al.*, (1986), Viola *et al.*, (1988), Bieniarz *et al.*, (2000) and observed some of the factors causing changes in the composition of fatty acids in various species.

The lipid in fish muscle can influence product quality through interaction with other components. Buckley *et al.*, (1989) also studied the lipid composition of certain fish and reported the common neutral fats as cholesterol, free fatty acids and cholesterol esters and also diphosphatidyl glycerol, phosphatidyl ethanolamine as the most predominant phospholipid.

An increasing amount of evidences suggest that due to its high content of poly unsaturated fatty acid fish flesh and fish oil are beneficial in reducing the serum cholesterol Stansby, (1985). In addition to that, the special type of fatty acid, omega-3 polyunsaturated fatty acid, recognized as an important drug to prevent a number of coronary heart diseases Edirisinghe *et al.*, (1998). It is recommended by cardiologists to use generous quantities of fish in food to obtain adequate protein without taking in excessive fatty acids and lipids. Dyerberg (1998), Kinsella (1977), AcKman (1989), Nair and Gopakumar, (1978).

Fish protein is regarded as quality protein being rich in essential amino acids with a high digestibility value Lavniegos and Lopez, (1997). Fish protein provides between 30% to 80% of the total animal protein intake of human and is rich in amino acid composition very well-suited to human dietary requirement.

This protein is relatively of high digestibility compared to other protein sources. It comprises of all the ten essential amino acids in desirable quantity for human consumption. High consumption of fish meat has a beneficial role on human health as it minimizes the appearance of cardio vascular diseases by decreasing the cholesterol and triglyceride level, moderates the inflammatory response as well as improves the carbohydrate metabolism.

The freshwater fishes provide a great amount of nutrient food source for human. Therefore, information about the chemical composition of various species and their nutritional properties, biochemical

structure and habitat condition is greatly needed. Fish flesh contains for basic ingredient and varying proportion of water (72%), protein (19%), fat (8%) and vitamin A,D,B and C (0.1%) etc. Guha, (1962). These values can also give a good estimate of seasonal nutritional variations, which can help consumer's choices and preferences for fish consumption.

The existence of commonly consumed fish belonging to the categories of very high vitamin A content containing preformed vitamin A as retinol and especially 3, 4 dehydroretinol offers a great unexploited potential for food based strategies to improve the vitamin A intake by promoting the production and consumption of these species. The preponderance of dehydroretinol over retinol in freshwater fishes remains obscure, with particular regard to the origin or biosynthesis of both retinol and dehydroretinol. Ever since the work of Moore (1930), several workers have shown the mechanism of conversion of β -carotene into vitamin A and it has been conclusively established that the carotenoid molecule cleaved either in central or in the terminal position of the molecule. Goswami and Bhattacharjee (1982) have showed that carotenoids like lutein, cryptoxanthin may also be cleaved in a similar fashion in the formation of different molecules of vitamin A.

The frequency of changes in the composition of biochemical constituents of any organism varies with the variation of the environmental changes

The objective of the present study was to screen commonly consumed Indian major carps for vitamin A content to evaluate the potential of fish as a vitamin A source in food, emphasis has been laid on the occurrence of diverse molecules of retinoids i.e. vitamin A in these Indian major carp, the seasonal variation of their proximate composition, total protein content, lipid content and free fatty acid composition.

II. Materials And Methods

Collection of fish:

The fishes were collected in Guwahati (Assam) from the local fishing sites of river Brahmaputra and were anaesthetized by applying diethyl-ether and then dissected to collect the muscle and liver tissue. The tissues were dried over a filter paper and immediately weighed and recorded.

The length and body weight of fish ranges from 15cm-60cm and 200 gm-1500 gm.

Proximate Composition Analysis:

i) Moisture : Estimation of the moisture content was carried out by drying the pre-weighed wet samples by hot air oven of Fischer (1971). Samples were dried at $105 \pm 1^\circ\text{C}$ to a constant weight. The difference in weight was calculated and expressed as percentage moisture content of the sample. Percentage was calculated by the following formula.

$$\text{Moisture\%} = \frac{\text{Wet Weight of tissue} - \text{Dry weight of tissue}}{\text{Wet weight of tissue}} \times 100$$

ii) Ash: Total ash content of the samples were determined by igniting moisture free samples at 550°C for 2-3 hours as described by I.S.I (1982). The ash content was expressed by percentage of the weight sample.

$$\text{Ash\%} = \frac{\text{Weight of dry sample}}{\text{Original weight of sample}} \times 100$$

iii) Dry matter: Dry matter recover was subtracted from original weight to estimate the weight of water evaporated during heating process. The dried samples were finely powdered using mortar and pestle and stored in desiccators for further analysis.

iv) Protein: Protein was estimated following the method of Lowry et al., (1951). To a 10 mg of sample 1 ml of 1N NaOH was added for protein extraction in water bath for 30 minutes. Thereafter, it was cool at room temperature at neutralization with 1ml of 1N HCL. The extracted samples was centrifuged at 2000 rpm for 10 minutes, and aliquote of the sample (1ml) was further diluted distilled water (1/9 v/v). From the diluted samples, 1 ml was taken and treated with 2.5 ml of mixed reagent (carbonate – tartrate – copper) and 0.5 ml of 1N followings regions. After 30 minutes sample absorbency was read at 550nm using spectrophotometer. The results were expressed as percentage.

$$\text{Protein \%} = \frac{\text{Weight of protein (mg)}}{\text{Weight of sample (mg)}} \times 100$$

v) Carbohydrate: Total Carbohydrate was estimated by the phenol sulphuric acid method Dubio set al., (1956). Samples of dried tissue (10mg) was treated with 2 ml of 80% sulphuric acid and was allowed to digest for about 20-21 hours at room temperature. Two ml of 5% phenol reagent followed by 5ml of concentrated sulphuric acid

Absorbency was measured at 490 nm. The concentrations were expressed as percentage. All the values of biochemical components were expressed as percentage dry weight basis.

Weight of Carbohydrate (mg)

Weight of Carbohydrate (mg)

$$\text{Carbohydrate \%} = \frac{\text{Weight of Carbohydrate (mg)}}{\text{Weight of sample (mg)}} \times 100$$

vi) Lipid: Lipid was estimated by the method of Folch [20]. Ten mg dried sample was homogenized in 10 ml of chloroform-methanol mixture (2/1 v/v). The homogenized was centrifuged at 2000 rpm. The supernatant then wash weight 0.9% saline solution (KCl) to remove the non-lipid contaminants and allowed to separate. The upper phase was discarded by siphoning. The lower phase was allowed to dry in an oven and the weight was taken. The muscle lipids were extracted by cold extraction using 2:1 mixture of chloroform and methanol. The homogenate was centrifuged at 2000 rpm. The supernatant then washed with 0.9% saline solution (KCL) to remove the non-lipid contaminants & allowed to separate. The upper phase was allowed to be dried and then the amount of lipid content is calculated. The lipid content was expressed as percentage by the following formula

$$\text{Lipid \%} = \frac{\text{Weight of lipid (mg)}}{\text{Weight of sample (mg)}} \times 100$$

vii) Free Fatty Acid or Acid Value: It was determined following the method of Morris(1959). Oil was extracted with ethyl ether and dissolved in 95% neutral alcohol followed by titration with 0.1N NaOH. The value was expressed in percentage as Oleic acid.

Viii) Isolation of lipid classes and Sub classes:

Lipid classes were separated by silica gel (1:30 w/w of lipid) open column chromatography by successive elution with chloroform (1:100 of w/v lipid), acetone-methanol (9:1 w/v: 1:150 w/v of lipid) and methanol (1:100 of w/v lipid) to get neutral lipid, glycolipid and phospholipids respectively

ix) Preparation of FAME (Fatty Acid Methyl Esters)

Boron trifluoride method

200mg of sample is taken in a flask and then added 4 ml of methanolic NaOH (0.5 M) and boiling chip. Condenser is attached and refluxed until fat globules disappear (10 min). 5.0 ml of BF₃ solution from auto pipette is added through condenser and boiled for 2 minutes. 2ml heptane is added through condenser and boiled for 1 minute. Heat is removed and 15ml saturated NaCl solution is added. Flask is stoppered and shooked vigorously for 15 seconds. Additional saturated NaCl solution is added to float heptanes solution into neck of flask. Upper heptane solution is transferred into glass stoppered test tube and small portion of Na₂SO₄ is added to remove H₂O. The solution is diluted to concentration of 10% for GC determination.

Chromatography

Relative retention times (vs FAME of triglyceride internal strength solution) and response factors of individual FAMES by GC analysis of individual FAME standard solutions and mixed FAME standard solutions is obtained. 1µl each of individual FAME standard solutions and 1µl each of mixed FAMES standard solution is injected. Mixed FAME standard solutions is used to optimize chromatographic response before injecting the test tube. 1µl of test solution is injected into GC column.

Calculation

Saturated fat % = $(\sum \text{saturated } W_i/W) \times 100$

Unsaturated fat % = $(\sum \text{unsaturated } W_i/W) \times 100$

W_i = weight of individual FAME in mixed FAMES standard solution.

W = weight of internal standard in mixed FAMES standard solution.

Preparation of lipid Extract

Head, meat & viscera portions of fish were separately analysed for the lipid content, lipid, classes and sub classes. Lipid extraction was carried out according to the method of Bligh and Dyer (1959). Different portions of fish were separately minced and homogenized using a homogenizer (Polytron PT 3100, Kinematica AG, Switzerland) in a solvent mixture of chloroform : methanol 2:1, kept over night and filtered. The lipid extract was dried over and hydro sodium sulphate to remove traces of moisture to get total lipids extract and evaporated to dryness using rotary flash evaporator (superfit Bangalore India).

Vitamin A content in terms of retinol,dehydroretinol:

UV -Visible Spectroscopy

Prior to the HPLC analysis uv-visible absorption maxima were taken and the $E^{1\%}_{1\text{cm}}$ values with the particular solvent system were considered in estimating the amount of retinol, dehydroretinol and other carotenoids (Goswami 1984; Plantaet al., 1962).

Ultraviolet and visible spectra were recorded in a ChemitoSpectran UV 2600 PC Double Beam UV-vis Spectrophotometer. The lipid extracts from each samples were first subjected to UV visible spectroscopy at 325nm $E^{1\%}_{1\text{cm}}$ value = 1832 (for retinol), 350nm $E^{1\%}_{1\text{cm}}$ value= 1430(for dehydroretinol) and 415-480nm $E^{1\%}_{1\text{cm}}$ value =2500 (for different carotenoids) and their spectra were recorded

HPLC Analysis

The metabolites of lutein and β -carotene with reference to the biogenesis of vitamin A in a number of fishes, were analysed through in vitro reaction in the Analytical Nutrition and chemistry Division of National Institute of Nutrition (ICMAR) Hyderabad .The extracts of different in vitro reaction were sealed under nitrogen and HPLC analysis was made. The HPLC system included a liquid chromatograph (various model 5000) and integration (No. 4270) an inject (Rheodyne model 725) with a 20 μ l loop and a various wave length detector. The coloumn was 300x3.9 mm Nova Pack C_{18} (4mm) with a guard packprecolumn module (water 5). All standard carotenoids and retinoids were procured from F. Hoffman La-Roche Switzerland.

Carotenoids and other retinoids (5.0mg) were dissolved in 100 ml of toluene / methanol mixture (1:1) containing 500 mg of BHT(butylatedhydroxytoluene)/litre to produce 50 μ g ml⁻¹ standard. The stock solution is stable and kept preserved for 4 months at -20⁰C. These were further diluted with the mobile phase to give working standards. HPLC grade solvents were degassed by vacuum filtration prior to use and water double distilled. Both retinoids and carotenoids were separated using acetonitrile/dichloromethane/ methanol/ water/ propionic acid (71:22:4:2:1; all are v/v) as mobile phase and the flow rate was maintained at 1.0 ml per minute run,detection of carotenoid pigments was performed at 450nm for dehydroretinol and retinol in352 and 326nm. 3-hydroxyretinol is isolated and purified from the liver oil of Wallagoattuas refered by Goswami,1984 and used as standard.

III. Discussion

The significance of the seasonal variation in biochemical constituents is complex and it is almost impossible to distinguish between the effects of many factors,which influence the biochemical composition of the fish.The biochemical constituents are also influenced by metabolism,mobility and geographical area. Non-consumption of fish during summer months is usually practiced by most consumers in the country.This behavior is attributed to the nutritional changes in fish due to onset of its breeding season.Studies were therefore,planned to assess the nutritional variations that might occur with the change of season and stages of fish.It has been seen that there is not much difference in nutritional values of major carps in seen in different seasons of the year inspite of seasonal variations.

The protein content of Catlacatla is found to be the highest value showing 18-19 % in the monsoon season and moisture content showed 79-80%.

C.mrigalashowed 17-18% protein where the monsoon season depicted about 18.5% which is higher than that of the other seasons.

L.rohita showed 17-18% protein and moisture content 80-81%. However,the ash content in all the three fish remain unchanged in all the seasons. From the total lipid content in different organ of the Indian Major Carp (Table-2), Catlacatla showed the maximum lipid content while Labeorohita showed the minimum lipid content.

There is no significant biochemical changes in other seasons except during the monsoon,where all the species showed good quality with reference to % of moisture,protein,lipids and ash content. Moreover, it can be stated that neither of these three species can be categorized under high fat content fish.

The biogenesis of dehydroretinol in these fishes having rich in dehydroretinol is not clearly known.It may be due to the fact that the carotenoids present in the food content play an important role,while there are some other factors which control the enzymatic conversion of retinol into dehydroretinol or from the carotenoids present in the foods converted into dehydroretinol.Therefore their ratio of retinol: dehydroretinol is of not much difference. Moisture content is maximum in Labeo rohita whereas minimum in Catlacatla (Table 1) is observed. Total protein content is maximum in Cirrhinus mrigal and lowest in Catlacatla and moderate in Labeorohita(Table 2). Total lipid content in Catla catla and Cirrhinus mrigal is comparatively higher than Labeorohita.(Table 2).The present study also depicts that the variations of the levels of lipids both in the liver and muscle tissues may be the result of seasonal variation which affect the fish diet.It has been found that lipid percentage is high in muscle than liver. Carbohydrate content of fish is affected by some environmental and seasons and feed intake.The carbohydrate percentage is low in pre-monsoon and slightly increases in monsoon and winter. Regarding the seasonal variation of free fatty acid in % Oleic acid there is not much fluctuation observed in different season of the year (Table 4). Lipid sub classes vary in different parts of the body of the

same fish species (Table 5). Previous and present studies further verify that changes in moisture, protein are a function of body weight, while lipid content appears to be a function of body weight and may be simultaneously affected by lipid contents of diet Javed et al., (1992).

Thus it can be concluded that there is no much significant seasonal variations in body composition of Indian major carp though exceptions are always there. Protein contents decrease and fat content increase with lowering of temperature. This appears that there is no hard and fast rule applicable universally to all the major carp fish. Body composition varies from species to species and changes with its own pre-determined set of principles.

The present work with seasonal variation may be useful in developing nutrient balanced, cost-effective production of studied fish by culture practices. PM-Premonsoon, M-Monsoon, RM-Retreating monsoon, W-Winter.

IV. Results

Table 1. Seasonal variation of the proximate composition of I MC

Proximate composition parameters	Catlacatla				Labeorohita				Cirrhinus mrigala			
	PM	M	RM	W	PM	M	RM	W	PM	M	RM	W
Moisture %	79.5±1.5	79.5±1.5	79±1.5	79.5±1.5	80.5±0.5	80.5±0.5	80±0.5	80.5±0.5	80.5±0.5	80.5±0.5	80±0.5	80.5±0.5
Protein	19.5±1.0	19.5±1.0	19±0.0	18.5±1.0	17.5±0.5	17.5±0.5	18±0.5	17.5±0.5	17.5±0.5	17.5±0.5	18.5±0.5	17.5±0.5
Ash %	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.7	0.5	0.5	0.5	0.5
Fat %	1.5±0.05	1.5±0.05	1.5±0.05	1.5±0.05	1.5±0.05	1.5±0.05	1.5±0.05	1.3±0.05	1.7±0.05	1.7±0.05	1.5±0.05	1.5±0.05
Carbohydrate %	1.87±1.5	1.89±1.5	1.86±1.5	1.89±1.5	1.82±0.5	1.85±0.5	1.82±0.5	1.82±0.5	1.95±0.5	1.97±0.5	1.93±0.5	1.95±0.5

Table 2. Total protein content in mg/g (wet weight tissue)

Organs	Catlacatla	Labeorohita	Cirrhinus mrigala
Muscle	36.56±0.67	71.9±0.01	156.2±0.02
Gill	8.72±0.44	60.76±0.021	99±0.02
Liver	30.0±0.49	101.83±0.03	160.6±0.03
Kidney	20.96±0.78	77.63±0.015	136.9±0.02

Table 3: Total Lipid Content in mg/100 mg (wet weight tissue)

Organs	Catlacatla	Labeorohita	C. mrigala
Muscle	10.08 ± 0.148	05.63 ± 0.124	9.75 ± 0.132
Gill	6.88 ± 0.0438	0.523 ± 0.124	6.54 ± 0.042
Liver	9.12 ± 0.0657	0.753 ± 0.249	8.82 ± 0.0515
Kidney	5.20 ± 0.0848	0.660 ± 0.163	5.0 ± 0.0759

Table. 4. Seasonal variation of free fatty acid % oleic acid

Species	Pre monsoon	Monsoon	Retreating monsoon	Winter
Catlacatla	5.0±0.5	5.0. ±0.5	5.0±0.5	5.0±0.5
Labeorohita	5.22±0.5	4.8±0.5	5.2±0.5	4.5±0.5
Cirrhinus mrigala	5.22±0.5	5.2±0.5	4.2±0.5	5.2±0.5

Table 5. Composition of total lipids (%w w b) and neutral lipids glycolipid and phospholipid (% of total lipids) in Indian Major carp.

FISH	TISSUE	TOTAL LIPID	NEUTRAL LIPID	GLYCOLIOLIPID	PHOSPHOLIPID
Labeo rohita	Meat	2.9±0.57	74.7±1.63	16.9±0.30	8.4±1.82
	Head	17.8±1.00	93.3±2.22	4.7±1.72	2.0±0.79
	Viscera	27.8±1.67	84.2±0.72	13.4±1.17	2.4±0.33
Catla catla	Meat	1.2±0.19	71.5±0.73	21.4±1.18	7.2±1.01
	Head	8.9±1.48	87.4±1.31	10.2±0.53	2.4±0.88
	Viscera	9.8±2.21	87.5±1.24	10.6±0.72	2.0±0.58
Cirrhinus mrigala	Meat	1.8±0.34	82.7±1.38	3.5±0.12	13.7±0.82
	Head	4.5±0.82	82.5±1.27	14.7±0.75	2.8±0.50
	Viscera	12.0±0.40	83.6±1.96	13.3±1.48	3.1±0.54

Table 6: Amount of fatty acids percent present in fish meat of the three species

Fish species	SFA	MUFA	PUFA
Labeorohita	34.1	37.2	24.9
Catlacatla	36.2	40.1	25.1
Cirrhinusmrigala	33.92	39.3	23.2

Table 7: Vitamin A content in terms of retinol, dehydroretinol in Major Indian carps

Parameter	Major Indian Carp					
	Catlacatla n=5		Labeorohita n=5		C. mrigala n=5	
	M	F	M	F	M	F
Retinol µg/g liver	23.5(±2.5)	20.5(±0.5)	22.6(±0.5)	22.5(±0.5)	19.2(±1.5)	17.0(±0.5)
Dehydroretinol µg/g liver	13.5(±1.0)	11.5(±0.5)	13.5(±0.5)	10.0(±0.5)	11.0(±1.5)	10.0(±0.5)

Acknowledgement

Authors are grateful to the department of zoology Gauhati University for allowing to perform the experiment in their laboratory.

V. Conclusion

It has been seen that the Indian major carps are comparatively of lower lipid content. The lipid content is comparatively low with lowest in Labeorohita but lipid subclasses vary in different parts of the body of same fish species. Protein content is seen maximum in Cirrhinusmrigal in comparison to the other two species.

Reference

- AcKman R.G. (1989) Fatty acids In Marine biogenic lipids, fat and oils, (Ackman, R.G. Ed.) Vol. 1. CRC Press Inc., Florida, 103-137
- AcKman R.G. and Takeuchi. R.F. (1986) Composition of Fatty Acid and lipids of smolting hatchery fed and wild Atlantic salmon (Salmosalar). Lipids 21:11
- Bieniarz. K, Koldras, M, Kaminski. J and Mejza. T, (2000) Fatty acid and cholesterol in some freshwater fish species in Poland Folia Univ. AgricStetin 27:2-4
- Bligh EG, Dyer WJ (1959). A rapid method of total lipid extraction and purification. Canadian J BiochemPhys 37:911-917
- Buckley.D.J, Gray.J, Ahsar.A, Price.J.F, Crackle.R.L, Booren.A.M, Pearson.A.M and Co.Inc.Princeton, New Jersey. 970
- Dubios, M., Gilles, K.A., Hamilton, J.K., Rebers P.A. and Smith (1956). Calorimetric method for determination of sugars and related substances. Analytical Chemistry, 28:350-356.
- Dyerberg, J. (1998) Effects of Omega-3 Fatty Acids on Cardiovascular Disease, 4 NHANES III Series 11, No 3A
- Edirisinghe, E.M. R.K.B., Perera, W.M.K., Tayasooriya, S.P. and Bamunuarachchi, A. (1998). Health related fatty acids in some pelagic fishes in Sri Lanka, Sri Lanka J. Aquat. Sci., 3: 97-10
- Fisher, M. (1971). Methods of estimation of moisture content of biological materials. Food Technol. 12(4):65-68.
- Folch, I. Lees, M. and Stanley, G.H.S. (1957). A simple method for extraction and purification of total lipids from animal tissues J. Biol Chem., 226, 497-509
- Goswami, U.C.; 1984. Metabolism of cryptoxanthin in freshwater fish. Brit J Nutr 52:575-582.
- Goswami, U.C.; Bhattacharya, S.; 1982. Bio-synthesis of 3-dehydroretinol: Metabolism of lutein in Clarias batrachus and Ompok pado. Biochem. Internet., 5(4) : 545-552
- Guha, B. C (1962) The role of fish in Human Nutrition In: Fish in Nutrition, published by Fishing News (Books) Ltd., Eland, pp : 93-42
- Halver, J.E (1980) Lipids and Fatty Acid in Fish Feed Technology United Nation Development Programs p 41, Food and Agriculture Organisation of the United Nations, Rome.
- I.S.I. (1982). Handbook of food analysis. vol. 6 Indian Standards Institute, New Delhi 60-64
- Javed, M.Y.; Saleem, A.; Khan M.N and Naeem .M. (1992). Weight-length and condition factor relationship of a freshwater wild Mahaseer (Tor putitora) from Islamabad (Pakistan). Proc. Pakistan Congr. Zool., 12:335-340.
- Kinsella J.E, Shimp J.L, Mai J and Weihrauch . J (1977) Fatty acid content and composition of freshwater fin fish J. Am. Oil Chem. Soc., 54 : 424-429
- Lavnigos, E. and D. Lopez-Cortes, 1997. Fatty acid composition and community structure of Plankton from the San Lorenzo Channel Gulf of California. Estuarine Coastal shelf Sci., 45:845-854.
- Lowry OH, Rosenborough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. J. Biol Chem 193: 265-275
- Mietadectsofiller .E.R (1989) Effect of antioxidants and oxidized oil on membrane lipid stability and pork product quality. J. Food Sci. 54:1193-1197
- Moore, T.; (1930). Vitamin A and carotene VI. The conversion of β-carotene to vitamin A in vivo. Biochem. J. 24 692-702
- Morris, B.J. (1959). The chemical analysis of foods and food products. D. Van Nostrand Nair, P.G.V. and Gopakumar, K. (1978) Fatty compositions of 15 species of fish from tropical waters, J. Food Sci., 43, 1162-1164.
- Planta, C.V.; Schwietter, U.; Choparddit Jean, L.; Reugg, R.; Kofler, M. and Isler, O.; 1962. Helv. Chim. Acta. 45, 548
- Stansby, M.E. (1985) Fish or Fish Oil in the diet and heart attack. Mar. Fish. Review, 46 (2): 60-63
- Suzuki . H, Okazaki . K, Hayakwa . S, Wadw S and Tamura .S (1986). Influence of commercial dietary Fatty Acid on PUFA of cultural freshwater fish and composition with those of wild fish of the same species J. Agric. Food Chem 34: 58-60.
- Viola, S, Mokady, S, Behar, D and Cogan, U (1988) Effects of PUFA in feeds of tilapia and carp. Body composition and Fatty Acid profiles at different environmental temperature Aquaculture 75 : 127-137.
- Vishwanathan, P.G and Gopakumar, K (1984) Lipid and Fatty Acid composition of fish and shell fish. J. Food Sci. Technol. 2 : 389