The Clarion Volume 5 Number 2 (2016) PP 24-29
The Clarion
International Multidisciplinary Journal
ISSN : 2277-1697

Fatty acids composition of the iced preserved *Wallogo attu* of river Brahmaputra and its tributaries.

Devajit Basumatari and **Tarali Kalita**
Department of Zoology, Cotton College, Guwahati, India

Abstract

Wallogo attu, an important catfish species found available in the rivers of Brahmaputra and its tributaries is having highly demand among the consumers. Though the species is found throughout the year in the markets but in the months from November up to March every year the catch reaches the peak with surplus amount. So there is a need for the preservation of the species with high food value. The flesh of *Wallogo attu* contains high amount of proteins along with fats. In the present study, an attempt has been made to study the changes of fatty acids during different conditions of ice preservation.

Keywords: Preservation, Fats, Fatty acids, *Wallogo attu*.

1. Introduction

The excellent standard quality of seafood in human nutrition lies not on their high quality protein for which there are many other alternatives, but in the high content of n-3 highly unsaturated fatty acids (n-3 HUFA), mainly the eicosapentaenoi acid (20:5n-3, EPA) and the docosahexaenoic acid (22:6n-3, DHA), which are associated for prevention of many humans diseases (Sargent and Tacon, 1999). The comprehension that the n-3 HUFA (22:5n-3; 22:6n-3) are essential for human bodily functions came much later than the comprehension that the arachidonic acid (22:4n-6, AA) was essential, by which diets in western societies contained high levels of 10:2n-6 derived from vegetable oils, low levels of 18:3n-3 derived from green leafy vegetables, and also low levels of n-3 HUFA derived more-or-less exclusively from fish (Sargent, 1997).

Today, there are enough amount of evidence about the importance of n-3 highly unsaturated fatty acids (n-3 HUFA; C>20), the eicosapentaenoic acid-EPA, as well as of the docosahexaenoic acid-DHA, for the prevention of several human diseases. An imbalance between n-6/n-3 ratio in favour of n-6 could contribute to increase the risk of coronary heart disease (Simopoulos, 1991).The lack of these fatty acids have been associated with the occurrence of other diseases, including hypertension, inflammatory and immune disorders, depression and neurological disorders. There are also some evidence that DHA carry out certain function in the brain and in the retina, that cannot be done by the n-6 fatty acid series (Neuringer *et al*., 1988).

It is well known that the vertebrate requires the EPA, DHA and AA for normal growth and development. These fatty acids as precursors of hormones called eicosanoids. The main precursor of eicosanoids is AA and those originated from EPA being less active. The eicosanoids are responsible and involved in many physiological functions and they are produced in response to stressful situations. High intakes of n-6 fatty acids, which are highly presented in modern Western food, lead to an increase of the eicosanoids production. However, it is known that the eicosanoids produced from EPA competes with the production of eicosanoids from AA. The appropriate balance of n-6 and n-3 fatty acids, is one of the most important nutritional aspect for human health, because of the capacity of n-3 fatty acid it inhibited the

Corresponding author : devajitbasu@yahoo.in ; taralikalita.tihu@gmail.com
DOI number: 10.5958/2277-937X.2016.00026.5

production of eicosanoids originated from n-6 fatty acids (Sargent *et al*., 1999).

While an appropriate EPA/AA ratio is very important to prevent high production of eicosanoids, the DHA plays important functions of neural and visual tissues of humans (Sargent *et al*., 1999). There are some concrete evidences that human beings, fed on deficient food of this fatty acid during early life development, can result in serious visual and cognitive sub-abnormalities (Sargent, 1997).

The importance of DHA in the neural development of the foetus and in post-natally has also focused the important role of this fatty acid series, in infants as well as for adult nutrition (Carlson *et al*., 1993; Salem and Pawlosky, 1994; Uauy et al., 1994). Actually, many formula feeds for premature and, on occasions, full-term infants are supplemented with the DHA fatty acid (International Society for the Study of Fatty Acids and Lipids, 1994). Furthermore, evidence is also emerging for a role of EPA and DHA in a considerable number of mental disorders, such as schizophrenia (Peet, 1997) and in some kinds of aggressive behavior (Okuyama *et al*., 1997). Add to that Crawford *et al*. (1976 a,b) reported the DHA was a limiting factor in the evolution of the brain (Broadhurst *et al*., 2002).

In modern society, there is a great concern about obesity problem. The obesity is directly related with the ingestion of certain types of fattiness food, rich in saturated fatty acids, such as the red meat. Consequently, there was a great stimulus for the production of low fat products and fat substitutes in the past years. Recent research suggests that there is a need to consider the quality and the quantity of fat in diets of western populations. Nowadays, there are a consensus that consumption of adequate levels n-3 HUFA acids are insufficient in most western diets, because the low consumption of seafood and its products, which are the main source of EPA and DHA (Willians, 2000).

Therefore, the aim of the present study was to investigate the proximate composition and the fatty acids content of the Brazilian indigenous mangrove oyster, *Crassostrea rhizophorae* along the year seasons, in order to evaluate its nutritional quality for human consumption.

The fish oil or lipids are prove to change their quality and quantity along with their affinity to prove to hydrolysis and oxidation. Lipids show considerable fluctuation during different seasons as well as corresponding to states of their life cycle and reproductive period. Growth, maturity stages, state of spawning and feeding habits also have pronounced effect on the muscle liver and visceral content. Different region of the body differ in their lipid content. The tail region contain high amount of lipids. The exterior part has a higher lipid content than the interior and in the ventral side lipid content is generally low than the dorsal side. This mode of storage of fat reduces the "drag" during swimming. The deposition of fat in the musculature energy reserve which are utilized during period of starvation. By and large most of the fish fall in low-fat high protein category. Lipid and water in fish muscle generally quantitatively complimentary to each other. As lipid increases water content decrease correspondingly, Lipids and water together form 80% in most fishes. Protein water relationships is less marked.

As fish lipids are prove to hydrolysis and oxidation during stage has been made to understand any changes of fatty acids of *Wallago attu* during different conditions of ice preservation. Fishes were preserved in -4^0C,-10^0C and -20^0 C) in refrigerators adjusted in the above mentioned freezing temperatures, and their fatty acids profiles containing saturated monoun-saturated and poly unsaturated are measured. Further, the attempt has been made to obtain same information under fatty acids profiles of ice-preserved fishes collected from different markets of Guwahati, isolation of lipids from the ice-preserved refrigerated fish samples are also followed.

2. Materials and methods

2.1 Fat

Fat content of moisture free sample was determined by extracting the fat with a suitable solvent (petroleum ether) by using soxhlet apparatus (AOAC, 1995). 10g of moisture free sample was taken in an extraction thimble and it was placed in the extractor with an attached receiving flask. The solvent was poured into the thimble through a glass funnel. The receiver containing petroleum ether was heated (40 to 60^0C) at such a rate that the ether drops from the condenser to the thimble at the rate of 5 to 6 per second. When sufficient solvent was transferred to the extracting tubes to fill the siphon arm, it siphoned back into the receiver. This process was continued until the extraction at 60 to 80^0C on a water bath. The residue was dried in an oven and cooled in a desiccators and weighed. The least weight of residue gives the weight of fat in the sample. The fat content of the sample was expressed on wet basis as percentage.

2.2 Determination of lipid quality – Free Fatty Acid (FFA)

The FFA content in sample was determined by the method recommended by Nambudiri (1985). A suitable amount of sample was blended with twice its weight of anhydrous Na_2SO_4. The blend was shaken thoroughly in distilled chloroform for 5 to 10 min and filtered. Fat content present in 10 ml of the extract was estimated by evaporating the chloroform. Another 10 ml of the extract was evaporated by using vacuum drier and 10 ml of neutral alcohol was added to it. It was titrated against 0.01 (N) NaOH using phenolphthalein as indicator. The result is expressed as % FFA as oleic acid.

2.3 Isolation of fatty acids

Crude lipids were gravimetrically determined after extraction by chloform method (2:1, v/v) according to the method of Folch *et al*, (1957). For isolation and analysis of different fatty acids, the made lipids were submitted to specification with potassium hydroxide (KOH-50%) and the fatty acid methyl ester (FAME) was prepared by esterification with boron-trifluoride in methanol (7%) (Metcalfe and Schmitz, 1961). The FAME was separated by gas-lipid chromatography on a Shimadzn GC-15A, equipped with a flame ionization detector (FID) and fitted with a fused silica capillary column (Omegawax 320x30mX0.32mm i.d., Supelco, Belefonte, USA). Hydrogen was used as a carrier gas with a flow rate of 40ml/min. Injection and detector temperatures were programmed to be isothermal (205^0C). The FAME were identified by reference to known standard (Supelco) and quantified by a Shimadzn C-R4 integrator.

3. Results and discussion

The samples were collected from different markets. Only *Wallago attu* fish was selected and randomly ice preserved fish of 40-50cm size were collected. As there is no proper record of the amount of ice applied and time of preservation, hence only the lipids i.e. fatty acids profiles were measured from the samples collected from five different markets. During collection, 3-5 samples from each market were collected on the same day and their lipids profiles were isolated separately and the values were later pooled together and mean, standard errors (SE) were shown.

Refrigeration of *W*allogo *attu*

The flesh of *Wallogo attu* was refrigerated at -4^0C, -10^0C and -20^0C in different refrigeration and preserved for 15 days. In each preservation 5 replicates (n=9) were stored and the analysis after 15 days of preservation.

In the present experiment, it has been seen that samples collected from the markets, which were not properly preserved last a considerable amount of fatty acids. There is wide variation of concentration and degradation. Further, it is difficult to understand the time-length of the contravention of ice-preservation of the concerned samples. There are no facilities to add more ice from time to time. Sometimes, during transportation ice is not used at all.

The experiment dealing with the refrigeration of *W*allago *attu* muscle for 15 days in different temperature viz -4^0C, -10^0C and -20^0C provides a clear picture that all the fatty acids corresponding to saturated, monounsaturated and polyunsaturated fats showed their loss during 15 days under found that -4^0C and -10^0C showed greater loss of different fatty acids than -20^0C. The samples refrigerated in -20^0C showed latter conditions, where almost all fatty acids could retain a considerable amount and the loss is very negligible.

The loss of different fatty acids is mainly due to hydrolysis and oxidation during the preservation period. In the present experiment no attempt has been made to measure the hydrolysis as well as oxidative deterioration of the samples. Emphasis has been laid to understand whether there in any effect in controlled refrigeration in -4^0C, -10^0C and -20^0C along with the status of fatty acids profiles in the ice-preserved samples available in the market. (Table 1 , 2).

Table:1 Fatty acids composition of *Wallago attu* during frozen conditioned on 15th day at different temperature. The values are mean value of 3 samples of same size and sex collected in the month of October.

Fatty acids	O.D. fresh condition	Samples preserved in different temperature		
		-4^0c	-10^0c	-20^0c
Saturated C12:0	4.5 ±0.5	3.0 ±1.0	4.0 ±0.5	4.5 ±1.0

C13:0	1.2 ±0.5	0.5 ±0.5	1.0 ±0.5	1.0 ±0.5
C14:0	4.5 ±1.0	3.0 ±0.5	3.2 ±0.5	3.5 ±0.5
C15:0	2.0 ±0.5	1.0 ±0.5	1.5 ±0.5	1.5 ±0.5
C16:0	23.5 ±7.2	20.5 ±5.5	21.5 ±7.5	22.0 ±5.5
C17:0	2.5 ±0.5	1.5 ±0.5	1.5 ±0.5	2.0 ±0.5
C18:0	10.2 ±2.5	8.0 ±1.5	8.5 ±1.5	9.0 ±1.5
C19:0	0.5	0.5	0.5	0.5
Total	*48.9	*37.5	*41.7	*44.0
Monounsaturated				
C16:1 n7	7.5 ±1.5	6 .0±0.5	6.5 ±0.5	7.0 ±2.5
C17:1 n7	0.5	0.5	0.2	0.5
C18:1 n9	23.5 ±5.5	20 ±5.5	20.5 ±3.5	22 ±5.5
C20:1 n9	1.2 ±0.5	0.5 ±0.5	1.0 ±0.5	1.0 ±0.5
C22:1 n9	6.5 ±1.5	0.5 ±0.5	0.5 ±0.5	1.0 ±0.5
Total	*39.2	27.0	28.5	*31.0
Polyunsaturated				
C18:2 n6	7.5 ±1.5	6.0 ±0.5	6.5 ±1.5	6.5 ±0.5
C18:3 n3	5.5 ±0.5	4.5 ±0.5	5.0 ±0.5	5.0 ±0.5
C18:4 n3	3.5 ±0.5	2.5 ±0.5	2.5 ±0.5	3.0 ±0.5
C20:2 n6	2.5 ±0.5	1.0 ±0.5	1.5 ±0.5	2.0 ±0.5
C20:3 n6	0.5	0.5 ±0.5	0.5 ±0.5	0.5 ±0.5
C20:4 n6	5.5 ±1.5	3.5 ±0.5	3.5 ±0.5	4.0 ±0.5
C20:5 n3	1.2 ±0.5	0.5 ±0.5	0.5 ±0.5	1.0 ±0.5
C22:4 n6	0.5	0.5	0.5	0.5
C22:5 n3	0.5	0.5	0.5	0.5 ±0.5
C22:6 n3	4.4±0.5	3.5 ±0.5	4.0 ±0.5	4.0 ±0.5
Total	*31.6	*23	*25	*27

* Significantly different (P≤0.5) at 5% level

Table : 2 Showing changes of fatty acids composition of *Wallago attu* during frozen conditioned at different temperatures. The % have been calculated taking the value of fresh fish and other values of different temperatures as shown in previous table.

Fatty acids	Fresh Fish	Samples preserved in different temperature		
		-4^{0}c	-10^{0}c	-20^{0}c
Saturated				
C12:0	4.5 ±0.5	66.6	88.8	100
C13:0	1.2 ±0.5	60.0	83.3	83.3
C14:0	4.5 ±1.0	66.6	71.1	77.7
C15:0	2.0 ±0.5	50.0	75.0	75.0
C16:0	23.5 ±7.2	87.2	91.4	93.6
C17:0	2.5 ±0.5	60.0	60.0	80.0
C18:0	10.2 ±2.5	78.4	83.3	88.2
C19:0	0.5	100	100	100
Monounsaturated				
C16:1 n7	7.5 ±1.5	80.0	86.6	93.3
C17:1 n7	0.5			
C18:1 n9	23.5 ±5.5	85.1	87.2	93.6
C20:1 n9	1.2 ±0.5	41.6	83.3	83.3
C22:1 n9	6.5 ±1.5	33.3	33.3	66.6
Polyunsaturated				
C18:2 n6	7.5 ±1.5	80.6	86.6	86.6
C18:3 n3	5.5 ±0.5	81.8	90.9	90.9
C18:4 n3	3.5 ±0.5	71.4	71.4	85.7
C20:2 n6	2.5 ±0.5	40.0	60.0	80.0
C20:3 n6	0.5	100	100	100
C20:4 n6	5.5 ±1.5	63.6	63.6	72.7
C20:5 n3	1.2 ±0.5	41.6	41.6	83.3
C22:4 n6	0.5	100	100	100
C22:5 n3	0.5	100	100	100
C22:6 n3	4.4±0.5	77.7	88.8	88.8

4. Summary

1. Ice-preserved samples available in markets showed high deterioration of fatty acids profiles in all samples. The status of fatty acids profiles indicates the poor quality fish as well as improper practice of ice-preservation.

2. Refrigerated samples preserved in -4^{0}C, -10^{0}C and -20^{0}C showed better conditions in - 20^{0}C preserved for 15 days than the samples refrigerated in -4ÚC,-10^{0}C different fatty acids could the preserved almost effectively in -20^{0}C for 15 days. Samples preserved in -4^{0}C showed maximum loss of all fatty acids

belonging to saturated monounsaturated and polyunsaturated fatty acids.

References

AOAC (Association of Official Analytical Chemists), 1995. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Brosdhurst, C. L.; Wang, Y.; Crawford, M. A.; Cunnane, S. C.; Parkington,J. E. and Schmidt, W. F. 2002, Brain-specific from marine lacustrine, or terrestrial food resources: potential impact on early African Homo sapiens . *Comp. Biochem. Physiol*., 131B, 653-678.

Carlson,C. J. 1969 . Superchilling fish- a review, In: Freezing and Irradiation of Fish, Ed R Kreuzer. Fishing News (Books) Ltd, London. 101-103.

Carlson,S. E.; Werkman, S. H.; Rhodes, P. G. and Tolly, E. A. 1993, Visual-acuity development in healthy preterm infants : effect marine-oil supplementation. American Journal Cl. Nutr., 58, 35-42.

Crawford, M. A.; Casperd, N. M. and Sinclair, A. J. 1976a, The long chain metabolites of linoleic and linolenic acids in liver brains of herbivores and carnivores. *Comp. Biochem. Physiol.*, 54 B, 395-401.

Crawford,M. A.; Hassan, A. G.; Willians, G. and Whitehouse, W. L.1976b, Essential fatty acids and fetal brain growth. Lancet 1 , 452-453.

Folch,J.;Lees, M. and Sloane-Stanley, G. H. 1957, A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226, 497-507.

International Society for the Study of Fatty Acids and Lipids 1994, Recommendations for the essential fatty acid requirements for infant formulae. International Society for the Study of Fatty Acids and Lipids. News Letter, 1, 4-5.

Metcalfe, A. P. and Schmitz,A. A. 1961. The rapid preparation of fatty acids for gas chromatographic analysis. *Anal. Chem*., 33, 363-364.

Nambudiri, D. D. 1985. Analytical Manual of Fish and Fishery Products. Directorate of Extension , Kerala Agri . University.

Neuringer, M.; Anderson,G. J. and Connor ,W. E. 1988,The essentiality of n-3 fatty acids for the development and function of the retina and brain. Ann. Rev. Nutr., 517-541.

Okuyama ,H. ; Kobayashi ,T. and Watanable, S. 1997, Dietary fatty acids the n-6/n-3 balance and chronic elderly diseases.Excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. Prog. Lipid Res., 35, 409-457.

Peet, M. 1997. Schizophrenia and omega-3 fatty acids. International Society for the study of Fatty Acids and Lipids. News Letter, 4, 2-5.

Sargent, J. R. 1997, Fish oils and human diet. British J. Nutr., 78, 5-13.

Sargent , J. R. and Tacon, A. G. J. 1999, Development of farmed fish : a nutritionally necessary alternative to meat. Proc. Nutr. Soc., 58, 377-383.

Sargent, J.; Bell, G.; McEvoy , L.; Tocher, D. and Estevez, A. 1999. Recent development in the essentially fatty acid nutrition. Aquaculture, 177, 191-199.

Simopoulos, A. P. 1991, Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr., 54, 438-463.

Salem Jr., N. and Pawlosky , R. J. 1994, Healthy policy aspects of lipid nutrition and early development. World .Rev. Nutr. Diet., 75 , 46 .

Uauy, R.; Hoffman, D. R.; Birch, D. G. ,Birh, D. G.; Kanseon, D. M. and Tyson, J. 1994, Safety and efficacy of omega-3 fatty acids in the nutrition of very low birth weight infants soy oil and marine oil supplementation of formula. J. Pediatr., 124, 612-620.

Willians, C. M. 2000, Dietary fatty acids and human health. Ann. Zootech. , 49, 165-180.

□□□