Fishery Technology
2005, Vol. 42(1) pp : 67 - 70

Biochemical and Microbiological Characteristics of Salt fermented Hilsa (*Tenualosa ilisha)*

Ranendra Kumar Majumdar*, Subrata Basu and R. Anandan*****
College of Fisheries (CAU), Lembucherra
Agartala, Tripura - 799 001, India

Lona ilish, is a salt fermented product from hilsa, is very popular in Bangladesh and Northeast part of India due to its typical flavour and aroma. Biochemical composition including amino acid profile of both raw hilsa fish and *Lona ilish* has been studied. A significant variation was observed in the amino acid profile of the product as compared to that of raw fish. The bacterial flora of the fermented product comprised of *Micrococcus* and *Bacillus* species.

Key words: Hilsa, salting, fermentation, biochemical composition, amino acids

Salting of fish is an ancient treatment still in use even in the developed countries, either because of economic reasons owing to its low production cost or in order to satisfy consumer's habits (Zugarramurdi & Lupin, 1980). There is usually a certain degree of fermentation involved in the salting of many fatty fishes (Hansen, 1980). *Lona ilish*, a salt fermented product from hilsa (*Tenualosa ilisha)* is very popular in Northeast part of India and Bangladesh. Subha Rao (1961) listed a number of fermented fish products but hilsa is missing in the list. The technology of *Lona ilish* preparation has originated in erstwhile East Pakistan about 100 years ago, in the villages on the bank of river Padma and Meghna under Noakhali District. The *Lona ilish* is very much popular for its typical flavour and aroma.

The present study was undertaken with a view to find out the biochemical composition including amino acid profile of both raw hilsa fish and its fermented product in order to assess the change during fermentation as well as to evaluate the nutritional value of the product after fermentation.

Traditionally fresh as well as 18-24 h ice preserved hilsa is used for *Lona ilish* preparation. Fish are first washed, descaled and beheaded without removing gut and cut diagonally into steaks of about 1.25 to 2.0 cm thickness. The fish steaks are then dry salted and kept 24-48 h in bamboo basket with a covering. During this time the fluid from the fish is allowed to drain. The salted fish steaks are then packed tightly in tin container usually of 18 L capacity. When the tin is almost filled, previously boiled and cooled saturated brine is poured slowly to fill the voids between the steaks and maintain a level of brine about 2 cm above the steaks. The tin is then closed with lid, which is tied with wire. The packed tins are stored for ripening for a period of 4 to 6 months before marketing.

Materials and Methods

Lona ilish of different grades and raw hilsa (ice preserved) fish were procured from the local market at different times for biochemical and microbial study. Moisture, ash and salt content were measured as per AOAC (1995). The pH of homogenate (10 g in 10 ml distilled water) was measured with a standard pH meter. Determination of total nitrogen (TN) and non-protein nitrogen (NPN) was done by using micro-kjeldahl method (AOAC, 1995) and total volatile basic nitrogen (TVB-N) by Conway's microdiffusion method (Conway, 1947). Alpha amino nitrogen (AAN) was estimated by the method of Pope & Stevens (1939). Total lipid was estimated by soxhlet method.

*Corresponding author : e-mail ID: rkmajumdar cife@rediffmail.com
** Central Institute of Fisheries Education, Versova, Mumbai - 400 061
*** Central Institute of Fisheries Technology, Matsyapuri P.O., Cochin - 682 029

Peroxide value (PV) and free fatty acid (FFA) were determined from chloroform extract by the method of Lima *et al.* (1981) and IS: 5734 (1970) respectively.

Amino acid composition of all the samples of raw hilsa and *Lona ilish* were determined by hydrolyzing the samples in 6 N HCl for 24 h at 110°C. The acid was removed by vacuum evaporation, made upto a known volume with 0.05 N HCl and then analysed by HPLC (Shimadzu, Japan) on an ion exchange column and a fluorescence detector after converting to o-phthalaldehyde derivatives (Chang *et al.*, 1991). Tryptophan content of the samples was determined after alkali hydrolysis (Sastry & Tummuru, 1985). An amount of 10 g of muscle from different sites of a sample was aseptically collected and macerated with 90 ml sterile saline (5% NaCl). After making serial dilution in the same diluant pour plating was done using nutrient agar fortified with 5% NaCl. Total viable count was made after 96 h of incubation at 37°C. Identification of the isolates upto generic level was made as per Le Chavallier *et al.* (1980)

Result and Discussion

The mean values for moisture, ash, protein and lipid content of raw hilsa are 60.5, 1.5, 17.01 and 20.5 respectively (Table 1). The mean value of pH (5.66) and moisture content of *Lona ilish* indicates it is a stable product. While hilsa was stored in saturated brine without pre dry salting phase, the moisture content was found to be 46% after 8 weeks of ripening (Rahman *et al.*, 1999). Salt content of *Lona ilish* is less than salt fermented anchovy and most of the other fermented fish products, which is significant from the view point that high dietary salt may pose a severe health risk.

Despite leaching out in brine, the contents of NPN (540 mg%), AAN (163 mg%) and TVBN (48 mg%) are still high in *Lona ilish* indicating degradation of tissue protein that may possibly be responsible for the generation of typical flavour and aroma of the final product. The role attributed to tissue enzymes versus microbial enzymes is

Table 1. Characteristics of raw hilsa fish and *Lona ilish*

Parameters	Value (mean ± S.D) Hilsa	*Lona ilish*
Moisture (%)	60.5±5.0	54.35±5.06
Ash (%)	1.5±0.27	16.73±1.13
pH	6.4±0.24	5.66±0.06
Total nitrogen (%)	3.03±0.11	3.35±0.42
Protein (%) (TN-NPN)x6.25	17.01	17.56
Non protein nitrogen (mg%)	308±25	540±60
Free alpha amino nitrogen (mg%)	49.0±9.0	163.5±32.4
Lipid (%)	20.5±5.5	9.41±0.74
Total volatile basic nitrogen (mg%)	16.7±1.35	48.0±6.08
Salt (%)	ND	15.75±1.16
Peroxide value (meq/kg lipid)	ND	40.0±4.5
Free fatty acid (% oleic acid)	ND	18.33±1.26

controversial. Microorganisms play an important part in the later stage of fermentation, and protein degradation by these organisms lead to production of volatile compounds from amino acids and small peptides (Lopetcharat & Park, 2002). Thongthai & Siriwongpairat (1990) suggested a significant role for bacteria and muscle bacterial proteases in the fermentation and flavour and aroma producing processes. The aroma in fermented fish product has been claimed to be derived from the activity of various types of halophilic bacteria (Van Veen, 1953).

The PV and FFA of *Lona ilish* have been found to be 40 meq/kg lipid and 18.22% as oleic acid, respectively. The high PV is due to oxidation of unsaturated fatty acids of lipid as sodium chloride has been reported to act as a pro-oxidant (Kanner & Kinsella, 1983; Connell, 1995). As reported by Srikar *et al.* (1993) the PV and FFA contents increased significantly throughout the period of storage of mackerel and pink perch at ambient temperature and reached 60 m mole O_2 per kg and 21.1% (as oleic acid), respectively, after 35 days of storage. Salt does not inhibit lipases responsible for liberation of free fatty acids (Roldan *et al.*, 1985; Perez-Villarreal & Pozo, 1992). Roldan *et al.* (1985) proposed that assessment of changes in FFA could provide an objective method for measuring the maturation of salted fish.

Table 2. Amino acid composition (g amino acid per 100 g protein) (n=5)

Amino Acids	Raw hilsa (mean±S.D)	*Lona ilish* (mean±S.D)	% loss during ripening
Aspartic acid	9.93 ± 0.014	7.27 ± 1.02	26.7
Threonine	4.11 ± 0.10	3.31 ± 0.42	19.4
Serine	3.37 ± 0.06	2.67 ± 0.30	20.7
Glutamic acid	16.59 ± 0.37	11.21 ± 1.76	32.4
Proline	0.99 ± 0.08	0.72 ± 0.17	27.2
Glycine	4.59 ± 0.80	4.51 ± 1.56	1.7
Alanine	6.34 ± 0.90	5.03 ± 1.21	20.6
Cysteine	0.68 ± 0.05	Not detected	100
Valine	4.65 ± 0.65	3.66 ± 0.60	21.3
Methionine	1.56 ± 0.08	1.44 ± 0.31	7.7
Isoleucine	4.04 ± 0.57	3.10 ± 0.53	23.2
Leucine	7.91 ± 0.18	5.70 ± 0.90	27.9
Tyrosine	1.58 ± 0.30	1.57 ± 0.17	0.63
Phenylalanine	4.09 ± 0.15	2.88 ± 0.37	29.6
Histidine	3.58 ± 1.05	1.86 ± 0.22	48.0
Lysine	11.52 ± 0.65	3.72 ± 0.26	67.7
Arginine	4.39 ± 0.86	3.49 ± 0.60	20.5
Tryptophan	1.17 ± 0.10	1.05 ± 0.10	10.2

Amino acid composition of a fish product contributes significantly to its taste and also decides the quality of the protein. Glycine, alanine, serine and threonine taste sweet while arginine, leucine, valine, phenylalanine, histidine and isoleucine give a bitter taste (Sikorski & Kolakowska, 1990). Table 2 shows the amino acid composition of raw hilsa and *Lona ilish*, the product, and also the percentage loss during ripening. Significant variation in the proportions of amino acids was noticed between product and raw fish. There is no significant change in the levels of amino acids such as glycine, methionine, tyrosine and tryptophan. But a significant reduction in the levels of all other amino acids has been observed in the product as compared to that of raw fish. This might be probably due to the formation of derivatives of amino acids such as amines and gluconeogenic substances. Lysine has been reduced to a greater extent in the product as compared to the raw fish. There was no significant or detectable quantity of cysteine present in the product. Meister (1965) reported disappearance of cysteine and found taurine in the fish sauce.

The total viable count (log cfu) of *Lona ilish* was found to be 2.3±0.05, comprised of *Micrococcus* (60%) and *Bacillus* (40%) species. The complex interaction of enzymatic activity and oxidation during the fermentation along with bacterial production of volatile fatty acids may be responsible for characteristic flavour and aroma of fermented fish products (Beddows *et al.*, 1980). Rose (1982) reported presence of *Bacillus* sp. in the early stages of *'patis'* fermentation. The author also stated that occurrence of *Micrococcus* spp. in one month old *'patis'* indicated the possible involvement of non-spore-forming microorganisms in the early stages of fish sauce fermentation. Perez-Villarreal & Pozo (1992) observed that during ripening of salted anchovy, the microflora was dominated by halophilic and halotolerant bacteria like *Micrococcaceae*, lactic acid bacteria and some moulds and yeasts.

References

AOAC, (1995) Association of Official Analytical Chemists, *Official Methods of Analysis*. 16th edn. Washington, DC, USA

Beddows, C.G., Ardeshir, A.G. & Daud, W.J. (1980) Development and origin of volatile fatty acids in Budu. *J. Sci. Food Agric.* **31**, pp. 86-92

Chang, C.M., Ohsima, T. & Koizumi, C. (1991) Lipid, free amino acid and organic acid composition of rice bran-fermented sardine. *Nippon Suisan Gakkaishi.* **57**, pp 1579-1585

Connell, J.J. (1995) *Control of Fish Quality*. 4th edn. Fishing News Books, London 245 p.

Conway, E.J. (1947) *Microdiffusion analysis and volumetric error*. 4th edn. Van Nostrad Co. Inc., Newyork

Hansen, P. (1980) Fish preservation methods. In *Advances in Fish Science and Technology*. (Connell, J.J. ed.) Fishing News Books Ltd., England, pp 28-33

Jerez, J.J. & Mor-Ventura, M.T. (1999) Total volatile basic nitrogen and other physicochemical and microbiological characteristics as related to ripening of salted anchovies. *J. Food Sci.* **64**, pp 344-347

IS: 5734 (1970) *Indian Standard specification for sardine oil.* Bureau of Indian Standards, New Delhi

Kanner, J. & Kinsella, J.E. (1983) Lipid deterioration initiated by phagocytic cells in Muscle foods: beta-carotene destruction by a myeloperodidase-hydrogen peroxide-halide system *J. Agril. Food Chem.* **31**, pp 370-378

Le Chavallier, M.W., Seider, R.K. & Evans, T.M. (1980) Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol.* **51**, pp 877-881

Lima, D.S., James, C.A.M.D. & Teutscher, F. (1981) Guidelines for chilled fish storage experiments. *FAO Fisheries Technical Paper* **210**, pp 17-19

Lopetcharat, K. & Park, J.W. (2002) Characteristics of fish sauce made from pacific whiting and surimi byproducts during fermentation stage. *J. Food Sci.* **67,** pp 511-516

Meister, A. (1965) *Biochemistry of amino acids.* Second Edition, Academic Press Newyork, 295 p

Perez-Villarreal, B. & Pozo, R. (1992) Ripening of the salted anchovy (*Engraulis encrasicholus)* Study of the sensory, biochemical and microbiological aspects. In: *Quality Assurance in the Fish Industry* (Huss, S.S., Jakobsen, M. and Liston, J. Eds.) Elsvier Science Publishers, Amsterdam, pp 157-167

Pope, C.G. & Stevens, M.F. (1939) The determination of amino nitrogen using a copper method. *J. Biochem.* **33**, pp 1070-1076

Rahman, M.A., Hossain, M.A. & Mansur, M.A. (1999) Effects of different salting methods on the nutritional and sensory characteristics of hilsa (*Hilsha ilish*) *Ind. J. Mar. Sci.* **29,** pp 171-175

Rao, J. (1967) *Fish processing in the Indopacific area.* Indopacific Fisheries Council Regional Studies No.4, FAO Regional Office For Asia and the Far East, Bangkok, Thailand

Roldan, H.A., Barassi, C.A. & Trucco, R.E. (1985) Increase in free fatty acids during Ripening of anchovies (*Engraulis anchoita*). *Food Technol.* **220**, pp 581-585

Rose, A.H.(1982) Fermented foods. In *Economic Microbiology* Vol. 7 (Rose, A.H. Ed) Academic Press, London, pp 1-13

Sastry, C.S.P. & Tummuru, M.K. (1985) Spectrophotometric determination of tryptophan in proteins. *J. Food Sci. Technol.* **22,** pp. 146-147

Sikorski, Z.E. & Kolakowska, A. (1990) Freezing of marine food. In *Seafood: Resources, Nutritional Composition and Preservation* (Sikorski, Z.E. Ed) CRC Press, Inc. Florida, pp. 112-124

Srikar, L.N., Khuntia, B.K., Reddy, G.V.S. & Srinivas, B.R. (1993) Influence of storage temperature on quality of salted Mackerel (*Rastrelliger kanagurta*) and Pink perch (*Nemipterus japonicus*) *J. Sci. Food Agric.* **63**, pp, 319-322

Subha Rao, G.N. (1961) *Fisheries Products Manual* (1st draft). FAO Regional Office for Asia and Far East, Bangkok, Thailand

Thongthai, C. & Siriwongpairat, M. (1990) The sequential quantitization of microorganisms in traditionally fermented fish sauce (Nam-pla). In *Postharvest-technology, Preservation and Quality of Fish in South East Asia* (Reillu, P.J.A., Parry, R.W.H. and Barile, LE. Eds.) International Foundation of Science Stockholm, pp 51-59

Van Veen, A.G. (1953) Fish preservation in South East Asia. In *Advances in Food Research.* **4**, pp 209-231

Zenitani, B. (1955) Studies on fermented fish products I. On the aerobic bacteria in 'shiokara'. *Bull. Jap. Soc. Sci. Fish.* **21,** pp 280-283

Zugarramurdi, A. & Lupin, H.M. (1980) A model to explain observed behaviour on Fish salting. *J. Food Sci.* **45**, pp. 1305-1311