Characterization of the traditional fermented fish product *Lona ilish* of Northeast India

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*Lona ilish* is a traditional salt fermented fish product widely consumed in Bangladesh and Northeast parts of India. It is prepared exclusively from *Hilsa, Tenualosa ilisha* (Ham-Buch, 1822), a high fat fish. Biochemical composition of market sample of *lona ilish* has been studied. The moisture, salt and pH of the product have been estimated as 54%, 15% and 5.66%, respectively. The product is stable when remains immersed in brine at ambient temperature. Lowering of water activity due to osmotic action of salt as well as effect of salt on spoilage bacteria are presumed to be the reasons behind this salt fermentation technique. High peroxide value is the indicative of lipid peroxidation but, however, did not impart any undesirable rancid taint to the product. The bacterial flora comprised of *Micrococcus* and *Bacillus* species. Lack of scientific study to standardize the methods, use of poor quality raw material fish, improper salting, inadequate fermentation period as well as adulteration in the process, etc. are some of the reasons for gradual deterioration of the quality of *lona ilish*.

**Keywords:** Fish fermentation, *Hilsa, Lona ilish*, Fermented food, Traditional fish product

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Fermented fish products are important dietary components in the protein deficient Far East especially in Southeast Asia, the near East and in Africa. Preservation of fish by salt is an age old technology. This method of preservation still enjoys popularity in many developing countries owing to its simplicity and low cost of processing. When fatty fishes are salted, there is usually a certain degree of fermentation involved. Fermentation of fish is brought about by autolytic enzymes from the fish and microorganisms in the presence of high salt concentrations. *Lona ilish* is a salt fermented product prepared from Indian shad (*Tenualosa ilisha*, Ham-Buch, 1822), a high-fat fish (fat content of adult *hilsa* ranges from 14-25%). This fish is popularly known as *hilsa* due to its earlier generic name *Hilsa*. *Lona ilish* is a very popular product and is widely consumed in Northeast India and Bangladesh mainly due to its typical flavour, aroma and texture. Unfortunately, this product has so far not found its due place in the literature among the fermented fish products of South East Asia mainly due to the lack of scientific work done on this product. The product is sliced *hilsa*, about 1.50 to 2.00 cm in thickness. A typical *lona ilish* has a uniform pink colour with a glossy appearance immediately after taking the product out of the brine. The texture remains firm and the flesh does not easily separate from its bone. It has a characteristic strong aroma mixed with some sweet, fruity and acidic notes along with some saltiness. The strong odour permeates the air during storage and gives the area a characteristic smell of *lona ilish*. It is kept immersed in saturated brine until consumption.

The technology of *lona ilish* actually originated in Bangladesh about 100 yrs ago on the bank of river Padma and Meghna under Noakhali district. It is assumed that the technology evolved during the glut period when there were no such preservation techniques except sun drying and salting was available. Sun drying was not suitable for *hilsa* like high fatty fishes due to rapid development of rancidity on being exposed to the sun. In addition, sun drying was difficult during continuous spell of rain in July-August that corresponds to the main glut period. This way of processing might have been started to quickly preserve large quantity of fish in an inexpensive way.

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The technology has not changed much since the earlier days and the practice is still one of the major means of preservation of *hilsa* by the fishermen community of Bangladesh. Although different technologies have come into being so far, no other preservation techniques except salt drying and to some extent canning are in use presently. The technology got entry into Northeast India through migrants. The large scale production and consumption of *lona ilish* is limited mostly in the Chandpur subdivision in Noakhali district of Bangladesh and Northeast India. One of the reasons of localized consumption in India may be due to the practical difficulties in its transport. However, there is a good demand for the product in the fish eating communities in other areas.

*Lona ilish* is traditionally prepared by dry salting the diagonally cut *hilsa* chunks followed by fermentation in saturated brine (previously boiled and cooled) in metal container till appearance of the characteristic flavour and texture (Figs 1-3). It is kept immersed in the fermenting medium (saturated brine) till consumption. The fermentation period is usually 4-6 months. The reports on biochemical composition of *hilsa* are scanty. The fat content of *hilsa* of different water bodies such as Mahanadi river (19.28%), Saurastra coast (16.73%), Padma river (14.40%), Brahmaputra river (7.17%) and Narmada river (5.40%) was investigated. The taste of the fish seems to be largely dependent on the fat content. The biochemical composition of the muscles of adult *hilsa* from the Calcutta market contain moisture (78.38%), dry matter (21.62%), protein (12.44%) and fat (18.01%)\(^6\). Adult *hilsa* is a fatty species with fat content varying from 11.8% (small fish) to 18.91% (large fish). The traditional methods of preparation of *lona ilish* and evaluation of some of its important biochemical and microbiological quality have been discussed (Fig. 4).

**Methodology**

**Biochemical analysis**

Samples of *lona ilish* were collected from local fish market at Agartala and brought to the laboratory for biochemical analysis. Moisture, \(pH\), ash, and salt contents were measured\(^8\). Ten gm of samples were homogenized with 10 ml of distilled water and the \(pH\) of the homogenate was then measured. Total titratable acidity (TTA) was measured\(^5\). The acidity was calculated as per cent lactic acid. Differences in weight were recorded after drying the sample in hot air oven at 100 ± 2°C overnight to determine the moisture content. Ashing was done by incineration in a muffle furnace at 550 ± 50°C until the ash was

![Fig. 1—Hilsa (*Tenualosa ilisha*)](image1)

![Fig. 2—*Lona ilish*](image2)

![Fig. 3—Traditional tin container used for fermentation](image3)

![Fig. 4—Preparation of *Lona ilish* by traditional method](image4)
obtained. The salt content was determined by titrating excess silver nitrate with ammonium thiocyanate using ferric alum as indicator. Total nitrogen was measured by using the micro-kjeldahl method. Ten per cent trichloroacetic acid (TCA) extract was used to estimate non-protein nitrogen (NPN), total volatile basic nitrogen (TVBN) and free α-amino nitrogen (FAN) by using micro-kjeldahl method, Conway’s micro-diffusion method and by copper method, respectively. Protein nitrogen was estimated by subtracting non-protein nitrogen from the total nitrogen. Total lipid was measured from dried sample by soxhlet extraction with petroleum ether. The peroxide value (PV) and the content of free fatty acids (FFA) were determined on the chloroform extracts of tissues.

**Microbiological analysis**

An amount of 10 gm of muscle from different locations of a sample was aseptically collected and macerated with 90 ml sterile saline (5% NaCl). After making serial dilution in the same diluents, pour plating was done using nutrient agar fortified with 5% NaCl. Total viable count (expressed as log cfu) was made after 96 hrs of incubation at 37°C and was expressed as log cfu (colony forming units). Bacterial identification was made up to the generic level using biochemical tests.

**Results and discussion**

The proximate composition of *lona ilish* is given (Table 1). The mean values (as % muscle) of moisture, salt and ash were found as 54.35, 15.75 and 16.73, respectively. The process however, includes diffusion of salt into the fish and elimination of water through the process of osmosis. Such transfer of moisture from the fish due to osmosis resulted in a decrease of moisture content with simultaneous increase of ash and salt content of the final product. The role of salt is highly significant to guarantee the quality and stability of the finished products in this category. The preservative action of salt lies in the reduction of water activity (a_w) of a system thus renders a condition less favourable for the microbial life. To achieve a_w of 0.90, which inhibits most bacteria, a salt solution of approx 15.5% is required. In general, food borne pathogenic bacteria are inhibited by a water activity of 0.92 or less that is equivalent to NaCl concentration of 13% (w/v). It was proposed to explain the preservative action of salt, that it exerts a poisonous action; makes moisture unavailable for the microorganisms; prevents bacterial growth by dehydrating the cells by plasmolysis, and destroys bacterial protoplasm. Salt content of *lona ilish* was found to be less than most of the other fermented fish products which is significant from the view point that high dietary salt pose a severe health risk. The pH and total titratable acidity (TTA) of *lona ilish* were found as 5.66% and 0.98%. The reason of low TTA value of the product may be attributed to the fact that samples were undergoing fermentation and not spoilage. In the same analogy, the high value of TTA seemed to be due to production of different organic acids. This implies that the fish underwent sufficient fermentation with endogenous and/or exogenous (microbial) enzyme systems.

A series of complex biochemical processes including proteolysis, lipolysis and lipid oxidation take place during fermentation (also known as ripening stage). The ripening stage renders a product with a firm consistency having characteristic pleasant aroma and taste. The physical and chemical changes that occur during ripening determine the overall sensory qualities of this salt fermented fish product. These changes are induced by enzymes, which break down both proteins and fats. Despite leaching out in brine during transfer of moisture and salt, the contents of non-protein nitrogen, free α-amino nitrogen and total volatile basic nitrogen of *lona ilish* were found as 540 mg, 163 mg and 48 mg, respectively. Accumulation of such low molecular weight

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Value (mean ± s.d.)</th>
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<tbody>
<tr>
<td>Moisture (%)</td>
<td>54.35 ± 5.06</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>16.73 ± 1.13</td>
</tr>
<tr>
<td>pH</td>
<td>5.66 ± 0.06</td>
</tr>
<tr>
<td>Total titratable acidity (TTA)</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>Total nitrogen (% muscle)</td>
<td>3.35 ± 0.42</td>
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<tr>
<td>Protein nitrogen (% muscle)</td>
<td>2.81 ± 0.29</td>
</tr>
<tr>
<td>Non protein nitrogen (% muscle)</td>
<td>0.540 ± 0.06</td>
</tr>
<tr>
<td>Free α-amino-nitrogen (mg %)</td>
<td>163.5 ± 32.4</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>9.41 ± 0.74</td>
</tr>
<tr>
<td>Total volatile basic nitrogen (mg %)</td>
<td>48.0 ± 6.08</td>
</tr>
<tr>
<td>Salt (%)</td>
<td>15.75 ± 1.16</td>
</tr>
<tr>
<td>Peroxide value (meq O_2/Kg lipid)</td>
<td>40.0 ± 4.5</td>
</tr>
<tr>
<td>Free fatty acid (% oleic acid)</td>
<td>18.22 ± 1.26</td>
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nitrigenous components might have resulted due to degradation of tissue protein and to some extent that may possibly be responsible for the generation of typical flavour and aroma of the final product, *lona ilish*. The role attributed to tissue enzymes versus microbial enzymes is controversial. Tissue enzymes rather than microbial enzymes are responsible for ripening of salted anchovy. Microorganisms play an important part on the later stage of fermentation and protein degradation by these microorganisms leading to the production of volatile compounds from amino acids and small peptides as raw materials. Bacterial proteases play a significant role 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. The total lipid content and their degraded products such as peroxides (indicated by peroxide value) and free fatty acids of *lona ilish* were found as 9.41%, 40% and 18.33%, respectively. Since, *lona ilish* is prepared from *hilsa* fish which comes under the high fatty fish group; it is not unlikely to find high fat content in *lona ilish*. The observed high PV is due to oxidation of unsaturated fatty acids of lipid. Sodium chloride acts as a pro-oxidant. A similar PV (41.3 meq O₂/Kg fat) and higher FFA (31.84%) have been reported in salted anchovy after 9 weeks of fermentation. The PV and FFA contents increased significantly throughout the period of storage of mackerel and pink perch at ambient temperature and reached 60 mmole O₂ 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. An assessment of changes in FFA could provide an objective method for measuring the maturation of salted fish. However, this increased PV did not impart any rancid taint to the product.

The total viable count (log cfu) of *lona ilish* was found to be 2.3 ± 0.05, comprised of *Micrococcus* (1.38 ± 0.03) and *Bacillus* (0.92 ± 0.06) species. Both *Micrococcus* and *Bacillus* constituted 60% and 40% of the total bacterial flora, respectively. It was opined that microbiological and other processes play an important role in the preparation of many fermented fish products. Halophilic *Bacillus* sp were reported from Thai fish sauce was found responsible for the production of volatile acids. 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. Microorganisms such as *Bacillus* and *Staphylococcus* which were isolated from *nam-pla*, *bakasang* and *patis* produced a significant amount of volatile acids. *Bacillus* sp in the early stages of *patis* fermentation was reported. Occurrence of *Micrococcus* sp in one month old *patis* indicated the possible involvement of non-spore-forming microorganisms in the early stages of some fish sauce fermentation. During ripening of salted anchovy, the microflora was found to be dominated by halophilic and halotolerant bacteria like *Micrococcaceae*, lactic acid bacteria and some moulds and yeasts.

Conclusion

The principle of preservation behind this salt fermentation technique has been presumed as lowering of water activity and effect of salt on spoilage bacteria. The process however, basically divided into two stages. The first includes diffusion of salt into the fish and elimination of water through the process of osmosis. The second slower stage of ripening which involves a series of complex biochemical processes including proteolysis, lipolysis and lipid oxidation. The ripening stage renders a product with a firm consistency and the characteristic pleasant aroma and taste. The physical and chemical changes that occur during ripening determine the overall sensory qualities of this salt fermented fish product. The *lona ilish* is different from other fermented fish products. Some distinctive features of *lona ilish* are: it does not come under the category of fish paste or fish sauce; exclusively *hilsa*, *Tenualosa ilisha* (Ham-Buch, 1822), a very high fatty fish is used for its preparation; whole fish is cut in to chunks and remains intact after maturation whereas, in the case of other salt fermented fish products whole fish is either crushed or dressed as whole and most of them do not retain original form after maturation; dry salting is followed by brining whereas either dry salting or simply brining is done in case of most of the similar type of products; once taken out from the brine and exposed to air, deterioration is very fast but this is not happened in case of most of the products. This is due to high fat content of the *lona ilish* that undergo autoxidation by atmospheric oxygen and the rate which is further accelerated due to presence of prooxidant, i.e. NaCl; unlike some of the fermented fish products, *lona ilish* must be cooked before
consumption; it is mainly consumed as main dish rather than condiment; and the typical flavour is mainly due to fat degraded products.

Traditionally, the fishermen themselves process the fish for *lona ilish*. Hygienic conditions are far from satisfactory in the manufacturing units. Firstly, the fish are selected irrespective of freshness and size. Sometimes, fish are not even washed before being cut. Secondly, the crude salt used is of cheap and unknown quality. The fish to salt ratio is also on a thumb rule basis only. Thirdly, dry salted fish chunks are often not covered properly, which sometimes results in infestation of flies in the fish. Insufficient salting often causes dilution of brine during ripening stage. Fourthly, due to over packing, the container sometimes bursts, the brine drains out and the top layers remain exposed and get spoiled. New and sound tin containers are seldom used. Lastly, the produce is often sold prior to proper maturation. Different types of adulterations have crept into the product. Quality of the product largely depends on the freshness of the raw fish, removal of water during dry salting, period of maturation, concentration of brine, etc. Since, there is no standard method of its preparation, different grades of *lona ilish* are available in the market. Some of them are of very poor quality especially due to improper salting, use of poor quality fish and lack of proper ripening period, etc.

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