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EVALUATION OF BIOCHEMICAL CHARACTERISTICS OF A TRADITIONAL SALT FERMENTED FISH PRODUCT OF NORTHEAST INDIA WITH SPECIAL REFERENCE TO ITS FLAVOUR COMPONENTS

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ABSTRACT

"Lona ilish", is a traditional salt fermented fish product, widely consumed and very popular in Northeast part of India and Bangladesh. It is prepared exclusively from a high fat fish, *Hilsa (Tenualosa) ilisha.* "Lona ilish" was prepared in the laboratory following traditional process. After 150 days of fermentation, a better quality "lona ilish" was obtained. Biochemical characteristics of market sample was estimated and compared with the laboratory prepared one. A variation in biochemical composition was observed. Sensory quality of the final product of laboratory prepared "lona ilish" was compared with the market sample and found that the laboratory prepared product scored better than the market sample. The moisture (49.89%) and salt (15.48%) of the final product was found to be satisfactory for stability of the 'lona ilish' at ambient temperature. Analysis of volatile compounds of "lona ilish" was done using GC-MS. It was concluded that, aldehydes, ketones and esters may possibly contribute characteristic aromas to the overall flavour of the salt fermented hilsa.

Key words: hilsa, traditional technology, fermentation, salting, biochemical composition, volatile components

INTRODUCTION

Salting is an age-old and most widespread technique of fish preservation practised by man (Wheaton and Lawson, 1985) to obtain a product with a tender consistency and specific pleasant aroma and taste as a result of enzymatic activity on the fish flesh (Filsinger *et al.,* 1987). Aroma perception is one of the foremost criteria for product evaluation and for its acceptance and preference. 'Lona ilish' is a salt fermented product prepared exclusively from a high fat fish, *Hilsa (Tenualosa) ilisha.* It is very popular and widely consumed in Northeast India and Bangladesh, has so far not found its due place in literature among the fermented fish products of Southeast Asia. This traditional fermented fish actually originated in the erstwhile undivided India (now Bangladesh) on the bank of river Padma and Meghna under Noakhali

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District. 'Lona ilish' is famous for its typical flavour and aroma.

A typical 'lona ilish' has a uniform pink colour with glossy appearance immediately after taking out from the brine. The texture remains firm and the flesh does not easily separate from its bone. It has a characteristics strong aroma of typical 'lona ilish' which is constituted chiefly by the typical aroma of hilsa fish mixed with some sweet, fruity and acidic note and saltiness. The strong odour permeates the air in and around the storage and gives the area a characteristics smell of 'lona fish'. It distinguishes itself from other fermented fish products by having a tough texture, very pleasant flavour and an attractive fresh fish flesh colour even after maturation.

The characteristic aroma and taste of salt fermented fish products are primarily due to protein and lipid degradation by autolytic and bacterial enzymes during fermentation (Beddows *et al.,* 1980; Saisithi *et al.,* 1966). Free amino acids, nucleotides and related compounds were found most important taste active compounds in case of fermented anchovy and yellow corvenia pastes (Cha and Lee, 1985), fermented shrimp and sardine pastes (Chung and Lee, 1976; Lee *et al.,* 1981). Dougan and Howard (1975) reported that lower fatty acids are responsible for cheesy odour of fish sauces. A total of 155 volatile compounds were detected by Cha and Cadwallader (1995) in salt fermented anchovy, big eyed herring, hair tail viscera and shrimp pastes and consisting mainly of aldehydes, ketones, alcohols, esters, aromatics, nitrogen and sulphur containing compounds. Flavour development during ripening of anchovy was studied by Triqui and Reineceius (1995) and volatiles of importance to anchovy flavour were characterised mainly in two groups, enzymatically generated C_8 alcohols and ketones alongwith (E,Z)-2, 6-non adienal, which contributed plant and cucumber like aromas and autoxidatively derived C_7 to C_{10} conjugated aldehydes which imparted fatty and fried fat-like aromas.

It is fact that the volatile flavour of fermented fish products has not been fully investigated, but to our knowledge no work related to the volatile components related to the flavour of salt fermented hilsa has been published.

Hilsa ilisha (Tenualosa ilisha) is exclusively used for its preparation during glut season (June to September). Traditionally, fish are washed, descaled and beheaded without removing gut and then cut diagonally into chunks (slanted steaks) which are rubbed with dry salt and heaped on the floor and covered with polythene sheet. After a period of 24-48th the fish chunks are filled compactly in steel container with tin coating. The container is finally closed with lid and stacked in a dark room for ripening.

During survey of traditional technology, it was revealed that there was no standard method of preparation particularly in respect of period of maturation and fish to salt ratio during dry salting. As a result, 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.

In order to standardize the procedure, especially the quality of raw material fish, fish to salt ratio, saturation of brine and period of fermentation etc. 'lona ilish' was prepared in the laboratory following traditional mechanism. The biochemical-characteristics of traditional 'lona ilish' was assessed and compared with the 'lona ilish' prepared in the laboratory. The present work was undertaken to assess the proximate composition of quality 'lona ilish', in addition to the detection and identification of volatile compounds, presumably responsible for its typical flavour.

MATERIALS AND METHODS

Laboratory preparation of 'lona ilish': The freshly landed fish *(Tenualosa ilisha)* was purchased from Chandpur market of Bangladesh and transported in ice packs within 20 h. The weights of the fishes ranged between 600 to 800g. The fishes were washed thoroughly with potable water, descaled, beheaded without removing gut and then cut diagonally into chunks (steaks) of about 1.25 to 2.0 cm thickness. The fish chunks were immediately dry salted (fish to salt ratio was 4:1) by thorough rubbing of salt and kept in a bamboo basket layer after layer with sprinkling of salt between each layer and covering the top layer with salt. The bamboo basket was well covered with black polythene sheet to prevent entry of light and allowed to stand. During this period the self-brine from the fish was allowed to drain. After 48h, the salted fish chunks were made free from adhering salt well and packed tightly in three tin coated steel container of 5L capacity. When the containers were almost filled, previously boiled and cooled saturated brine prepared from good quality salt was poured slowly to fill the voids between the chunks to a level about 2 cm above the fish. The containers were then closed with lid and stored in a room for maturation.

Chemical analysis : Five market samples of 'lona ilish' were collected for analysis. In case of laboratory prepared 'lona ilish', three chunks from different depths of each container were taken and allowed to drain the adherent brine for one minute giving little pressure by hand and macerated in a blender for about 10 sec. The mixtures were used as representative sample of each container.

Moisture, pH, ash and salt content were measured following standard method (AOAC, 1995). Ten-gm samples were homogenized with 10ml of distilled water and the pH of the homogenate was then measured using a standard pH meter (EXPO Hi-Tech). Differences in weight were recorded after drying the sample in hot air oven at 103 ± 2^0C overnight to determine the moisture content. Ashing was done by incineration in a muffle furnace at 550 ± 50^0C until the ash was obtained. The salt content was determined by titrating excess silver nitrate with ammonium thiocyanate using ferric alum as indicator (AOAC, 1995). Total nitrogen was measured by using the micro-kjeldahl method of AOAC (1995). Ten percent trichloroacetic acid (TCA) extract was used to estimate non-protein nitrogen (NPN), total volatile basic nitrogen (TVBN) and free alpha amino nitrogen (FAN) by using

micro-kjeldahl method (AOAC, 1995), Conway's micro-diffusion method (Conway, 1947) and by copper method (Pope and Stevens, 1939) respectively.

Total lipid was measured 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 according to the methods suggested by Jacobs (1958) and Takagi *et al.,* (1984) respectively. Thiobarbituric acid (TBA) values were determined by the titrimetric method of Tarladgis *et al.* (1960) using thiobarbituric acid standard in 90% glacial acetic acid.

Detection and identification of volatile compounds:

The volatile compounds of 'lona ilish' (laboratory prepared) was detected and identified according to the method given by Cha and Cadwallader (1995) and Triqui and Reineccius (1995) with little modification. The volatile compounds of 'lona ilish' collected from the market were not done due to unknown history of the product in respect of period of maturation, post fermentation handling and storage condition etc.

Isolation of volatiles : Fish (a representative sample of 50 g) was mixed with 150 ml of distilled water and blended in a warring type blender for 1 min. The slurry was then transferred to a 1L round bottom flask and 150 ml of distilled water was added. Distillation was continued for one hour using steam with a horizontal condenser. About 100 ml aqueous distillate was collected from the slurry. The distillate was then extracted three times with 50 ml peroxide free diethyl ether at each time. Anhydrous sodium sulphate was added to the extracts, which were then allowed to stand overnight before concentration to 0.5 ml. Concentration of extract was done at room temperature under a gentle stream of nitrogen.

Instrumentation: Gas chromatography was performed using DB-1 (30m x 0.25mm) capillary column. The following temperature programme was used. After 5 minutes at 40^0C, the temperature of the GC oven was raised at 5^0C/min to 50^0C, held 1 min isothermally, then raised at 6^0C/min to 250^0C and finally held isothermally for 5 minutes. The flow rate of the carrier gas (helium) was 1 ml/ min. At the end of the capillary, the effluent was split at split ratio 50. Injector temperature and interface temperature was maintained at 210^0C and 280^0C respectively. Mass analyses were performed using mass spectrometer. The scan range was 35-400 with scan interval of 0.5 sec and scan speed of 1000 amu/sec. Detector voltage was 1.1 kv.

A sensory scoring scheme for 'lona ilish' was developed after discussion with the traditional producers. The five factors: flesh, colour, flavour, texture and taste were rated separately and the sample mean was calculated to get the sensory scores.

RESULTS AND DISCUSSION

Salting of hilsa can be divided into two stages. The first includes diffusion of salt into the fish and elimination of water through the process of osmosis. The rate of salt penetration varies with the

thickness of muscle, temperature, freshness of the fish and fat content (Clucas, 1982). The second slower stage of ripening which involves a series of complex biochemical processes that broadly includes proteolysis, lipolysis and lipid oxidation. The ripening stage renders a product with tender consistency and the characteristic pleasant aroma and taste (Filsinger *et al.*, 1987). The physical and chemical changes that occur during ripening determine the overall sensory qualities of salted anchovy (Voskresensky, 1965). Hilsa is a high fatty fish (Pillay and Rosa, 1963; Jahan, 1993; Chonder, 1999; Majumdar and Basu, 2004). The taste of the fish seems largely dependent on its fat content. Khayat and Schwall (1983), Wheaton and Lawson (1985) and Triqui and Reineccius (1995) reported that oxidation of highly unsaturated fat of salted anchovy was responsible for the change of quality attributes like colour, texture and flavour.

A better quality 'lona ilish' was obtained after 150 days of fermentation. Sensory quality of the laboratory prepared 'lona ilish' was compared with products available in the market by a group of experts and presented in table 1. The criteria of selection were appearance, colour, odour, flavour, texture and overall acceptability of the 'lona ilish'. On organoleptic assessment it was seen that the 'lona ilish' prepared in the laboratory scored better than the average score of market products.

A comparative account of the biochemical characteristics of market sample of 'lona ilish' and laboratory prepared one is presented in Table 2. The average moisture content of market samples, was found about 54% which was higher than the laboratory prepared product. It is difficult to explain the differences especially when there is no record of the raw material used for market samples. However, few factors like improper dry salting, improper saturation of brine, fat content of fish or improper maturation etc. could be responsible for this. In case of average salt and ash content, no significant difference was observed. The pH of the laboratory product was found to be lower than the pH of the market sample. The total nitrogen and protein nitrogen content of the market sample was found to differ with the laboratory prepared product and the reason may be attributed to varying periods of maturation of the market sample and also

Table 1 : Sensory scores of 'lona ilish' (mean ± SD)

Products ('lona ilish'	Flesh colour	Odour	Flavour	Texture	Taste	Sensory scoure
Market sample	71.1 ± 6.1	65.6 ± 8.9	68.4 ± 5.9	70.4 ± 7.3	66.0. ± 4.2	68.3
Prepared in in the laboratory	88.0 ± 5.7	89.4 ± 3.0	90.0 ± 3.8	86.0 ± 3.1	91.0 ± 2.6	88.9

Table 2 : Comparative biochemical composition of 'lona ilish' collected from market and prepared in the laboratory by traditional process

Biochemical parameters	Values (mean ± SD)	
	Market product (n=5)	Laboratory prepared product (n=3)
Moisture (%)	54.35 ± 5.06	49.89 ± 2.19
Ash (%)	16.73 ± 1.13	16.37 ± 0.17
pH	5.66 ± 0.06	5.28 ± 0.05
Salt (%)	15.75 ± 1.16	15.48 ± 1.21
Total nitrogen (%, muscle)	3.35 ± 0.42	2.90 ± 0.17
Protein nitrogen (%, muscle)	2.81 ± .29	2.37 ± 0.15
Non-protein nitrogen (% muscle)	.54 ± .06	0.53 ± 0.02
Free alpha amino-nitrogen (mg %)	163.5 ± 32.4	140.0 ± 7.0
Total volatile basic nitrogen (mg %)	48.0 ± 6.08	23.38 ± 2.24
Lipid (%)	9.41 ± 0.74	16.90 ± 0.79
Peroxide value (meqO_2/Kg lipid)	40.0 ± 4.5	20.23 ± 1.11
Free fatty acid (% Oleic acid)	18.22 ± 1.26	14.66 ± 3.06
TBA (mg malonaldehyde/kg)	Not detected	18.23 ± 2.01

protein content of the raw material fish. No significant differences in non-protein nitrogen content was observed between the two products.

On the basis of organoleptic evaluation, the product prepared in the laboratory was of better quality than the commercially made ones. In addition to sensory quality, the lower values of pH, moisture, PV, FFA and TVBN in laboratory product indicated that proper care made a better product. However, as described earlier, the market samples of unknown history and improper handling during marketing could affect these values. Therefore, it was not tried to explain any such variation between market samples and laboratory product.

The chromatogram presented in figure 1, illustrates the profile of volatile compounds obtained for 'lona ilish' that was ripened for 5 months. Compound identification is reported in table 3. Sensory evaluation of hilsa chunks from this process showed that they exhibited the strong characteristic flavour of fully ripened 'lona ilish'. A total of 26 volatile compounds were detected. Amongst aldehydes, n-hexanal, pentanal and octadecanal were found. Levels of hexanal and pentanal were very low than that of octadecanal. Hexanal having odour quality of green grass-like was reported in high level in big-eyed herring paste (Cha and Cadwallader, 1995) and in fermented anchovy (Triqui and Reineccius, 1995). The

Table 3 : Volatile compounds detected by GC-MS

Peak No	Retention time	Area	Height	% Total	Name of the compounds	Groups
1	21.725	732165	81988	1.98	1,4-Benzene Diol	Alcohol
2	21.946	403311	75341	1.09	Indole	Heterocyclic
3	26.759	407121	126951	1.10	2-Hexen-4-Yen	Aliphatic
4	27.683	930660	380820	2.51	Pentadecane	"
5	28.638	62426	25942	0.17	Decanoic acid	Fatty acid
6	31.552	422877	167728	1.14	1-Hexadecane	Aliphatic
7	31.552	18251613	6652387	49.24	2,6,10,14-Tetramethyl-Pentadecane	"
8	32.706	2128358	351012	5.74	Myristic acid	Fatty acid
9	40.315	108619	45366	0.29	Pentanal	aldehyde
10	33.220	324414	104129	0.88	Ethyl Decanoate	Ester
11	33.317	224650	45622	0.61	*n*-Hexanal	aldehyde
12	35.044	69499	27695	0.19	Dodecenyl Acetate	Ester
13	35.426	2112452	782781	5.70	Heptadecane	Aliphatic
14	35.751	665674	189971	1.80	Butyl Phthalate	Ester
15	35.833	782379	218956	2.11	Oleic Acid	Fatty acid
16	36.255	2698481	548300	7.28	Palmitic Acid	"
17	36.352	316681	96734	0.85	9-Hexadecenoic Acid	"
18	36.760	196589	67319	0.53	Ethyl Palmitate	Ester
19	36.954	386836	94249	1.04	*n*-Tridecane	Aliphatic
20	37.164	351617	122238	0.95	Octadecanal	Aldehyde
21	37.810	185438	72612	0.50	Dodecatriene-1-ol	Alcohol
22	37.983	31279	15306	0.08	4-Octen-3-one	Ketone
23	39.612	593117	165399	1.60	Octadecane	Aliphatic
24	41.805	1078331	271743	2.91	Octacosane	Aliphatic
25	43.521	190974	51014	0.52	2,3,3-Trimethyl octane	"
26	44.072	1231495	276463	3.32	Tricosane	"

differences observed in the quantity of aldehydes between 'lona ilish' and fermented anchovy and herring paste, may be due to differences in their processing and fermentation. Most of the alkanals and enals are known to contribute fatty-oily, slightly rancid odours while the dienals contribute pleasant fried-fatty aromas (Vejaphan *et al.,* 1988).

Four esters were found of which butyl phthalate was found high followed by ethyl decanoate and ethyl palmitate. Esters may have arisen from esterification of various alcohols with carboxylic acids formed via microbial and enzymatic decomposition of lipids. Esters have been found in most fermented seafoods (Sanceda *et al.,* 1990, Josephson *et al.,* 1987).

Only two alcohols (1, 4-benzene-diol and dodecatriene-1-ol were found and corresponded to the low overall number and abundances of aldehydes detected. Alcohols might not have an impact on fermented fish flavour because of their high flavour thresholds (Health and Reineccius, 1986).

Fatty acids, namely, oleic acid, myristic acid, palmitic acid, 9-hexaenoic acid and decanoic acid were found of which levels of 9-hexaenoic and myristic acid were high. Cheesy odour of fish sauces was reported to be due to presence of lower fatty acids (Dougan and Howard, 1975).

As a first work of this kind, it may be concluded that aldehydes, ketones and esters may possibly contribute characteristic aromas to the overall flavour of the salt fermented hilsa. These compounds developed perhaps due to lipid oxidation and enzyme-mediated lipid and protein breakdown during fermentation. However, further research is needed to determine the effects of particular compounds in the flavour of fermented hilsa.

REFERENCES :

AOAC 1995. *Official Methods of Analysis.* Association of Official Analytical Chemists (16th edition). Washington D.C.

Beddows, C. G., Ardeshir, A. G. and daud, W. J., 1980. Development and origin of volatile fatty acids in Budu, *J. Sci. Food Agric.,* **31**; 86-92

Cha, Y. J. and Lee, E. H., 1985. Taste compounds of low salt-fermented anchovy and yellow corvenia, *Bull. Korean Fish. Soc.,* **18**: 325-332.

Cha, Y. J. and Cadwallader, K. R., 1995. Volatile components in salt fermented fish and shrimp pastes. *J. Food Sci.,* **60**: 19-24.

Chondar, S. L., 1999. *Biology of fin fish and shell fish.* SCSC Publishers (India), Howrah. pp. 10-27.

Chung, S. Y. and Lee, E. H., 1976. The taste compounds of fermented shrimp, *Acetes chinensis. Bull. Korean Fish., Soc.,* **9**: 79-110.

Clucas, I. J., 1982. Salting of fish; salt and methods. In: *Fish Handling, Preservation and Processing.* Part-2. Report of the Tropical Fishery Institute, London. p. 4-8.

Conway, E. J., 1947. *Microdiffusion analysis and volumetric error.* 4th edition. Van Nostrad Co.Inc., New York.

Dougan, J. and Howard, G., 1975. Some flavouring constituents of fermented fish sauces. *J. Sci. Food Agric.,* **26:** 887-894.

Filsinger, B., Sisti, and Bergamaschi, N. J., 1987. Chemical and sensory assessments in ripened anchovies. *Int. J. Food Sci. Technol.,* **22:** 73-76.

Heath, H. B. and Reineccius, G., 1986. Off-flavours in foods. In: Heath, H. B. and Reineccius, G. (ed) *Flavour Chemistry and Technology.* Macmillan Publishers Ltd. Basingstoke, England. pp. 121-127.

Jacob, M. B., 1958. *The Chemical Analysis of Foods and Food Products.* Kreiger Publishing Co. Inc. Newyork, USA. pp. 393-394.

Jahan, S. S., 1993. *Investigation of the changes of biochemical components of Hilsa fish (Tanualosa ilisha) under different storage conditions.* Ph.D. Thesis submitted to the Department of Biochemistry, The University of Dhaka, Bangladesh.

Josephson, D. B., Lindsay, R. C. and Stuiber, D. A., 1987. Influence of processing on the volatile compounds characterizing the flavour of pickled fish. *J. Food Sci.,* **52:** 10-14.

Khayat, A. and Schwall, D., 1983. Lipid oxidation in seafood. *Food Technol.,* **37:** 130-140.

Lee, E.H., Cho, S.Y., Cha, Y.J., Jeon, J. K. and Kim, S. K., 1981. The effect of antioxidants on the fermented sardine and taste compounds of product. *Bull. Korean Fish. Soc.,* **14:** 201-211.

Majumdar, R. K. and Basu, S., 2004. Studies on the biochemical composition of *Hilsa ilisa (Tenualosa ilisha)* and its fermented product 'lona ilish'. *Biochem. Cell. Arch.,* **4:** 29-32.

Pillay, S. R., Rosa, H., 1963. Synopsis of biological data on hilsa, *Hilsa ilisha* (Hamilton). *FAO Fish. Biol. Synopsis* No. **25,** FB/S 25.

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

Saisithi, P., Kasemsarn, B., Liston, J. and Dollar, A. M., 1966. Microbiology and chemistry of fermented fish. *J. Food Sci.,* **31:** 105-110.

Sanceda, N.G., Kurata, T. and Arakawa, N., 1990. Overall quality and sensory acceptance of a lysine fortified fish sauce. *J. Food. Sci.,* **55:** 983-988.

Takagi, T., Hayashi, K. and Itabashi, Y., 1984. Toxic effect of free unsaturated fatty acid in mouse assay of diarrhetic shellfish toxin by intraperitoneal injection. *Bull. Japan Sci. Fish.,* **50:** 1413-1418.

Tarladgis, B. G., Watts, B. M. and Younathen, M. T., 1960. A distillation method for the quantitative

determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.,* **37:** 44-48.

Triqui, R. and Reineccius, G. A., 1995. Flavour development in the ripening of anchovy *(Engraulis encrasicholus* L) *J.Agric. Food Chem.,* **43:** 453-458.

Vejaphan, W., Hsieh, T.C.Y. and Williams, S. S., 1988. Volatile flavour components from boiled crayfish *(Procambarus clarkii)* tail meat. *J. Food Sci.,* **53:** 1666-1670.

Voskresensky, N. A., 1965. Salting of herring. In: Borgstrom G, (ed). *Fish as food.* Vol.III. Processing: Part I. Academic Press, New York. London. pp 107-128.

Wheaton, F. W. and Lawson, T. B., 1985. Other preservation methods. In: Wheaton, F. W. and Lawson, T. B. (ed). *Processing Aquatic Food Products.* John Wiley and Sons. New York. pp 273-328.