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Biochemical Changes During Salt Fermentation of *Pangasius hypophthalmus* (Sauvage, 1878)

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Abstract

The suitability of *Pangasius hypophthalmus* as a raw material for salt fermentation and changes during 135 days was analysed. *Pangasius* steaks were fermented in food grade PVC jar and biochemical parameters such as pH, salt, thiobarbutric acid reactive substances (TBARS), free α-amino nitrogen (FAN), moisture and ash content were analysed. The moisture content of *Pangasius* had shown a slight increase during fermentation from 54.9% (0^{th} day) to 56.67 (135^{th} day). Ash content recorded an increase from 11.98% on 1^{st} day to 18.17% (135^{th} day). Salt content of *Pangasius* steaks has increased significantly ($p \leq 0.5$) during fermentation. TBARS content recorded an increase till 75^{th} day and decreased continuously till maturation. The FAN registered an increase from 44.71 mg% (0^{th} day) to 92.86 mg% (135^{th} day).The moisture, pH, ash, salt, TBARS, FAN in the final matured fermented product were 56.67%, 5.93%, 18.17%, 14.43% and 0.21 mg malonaldehyde kg^{-1} of fat and 92.86 mg% respectively. Therefore, it was concluded that *Pangasius* developed a firm consistency and characteristic flavour and taste over 135 days of maturation.

Keywords: *Pangasius*, salt fermentation, biochemical parameters

Introduction

For more than 6000 years, fermentation has been used as a means of improving the keeping quality of food. Fermentation served people in moderate and cooler regions to survive in winter and drought periods by improving shelf life and safety of food. It was assumed that, fermented beverages with an extended shelf life served as safe source of consumption in densely populated areas where safe water supplies were not available. Fermentation can improve the sensory quality and acceptability of foods in addition to imparting a preserving effect (Holzapfel, 1997). In Southeast Asia, the Near East and in Africa, fermented fish products are important dietary components (Adams et al., 1985). Salting is an old practice of preservation which is still popular due to its simplicity and low cost of processing. Takagi et al. (1984) suggested that some degree of fermentation occurs during salting of fatty fish. Salt fermentation is divided into two stages *i.e.* diffusion of salt into fish and removal of water by osmosis occurs in first stage which is faster. During the second stage, biochemical processes like proteolysis, lipid oxidation and lipolysis takes place. The whole procedure is complex and takes a long time to attain maturation. Ripened product will have firm consistency and also characteristic flavour and taste (Majumdar & Basu, 2010a).

Roy et al. (2015) evaluated the biochemical and microbiological changes during fermentation of *Setipinna phasa* fish locally called '*Phasa shidal',* a fermented product prepared in north eastern part of India. Majumdar & Basu (2010b) prepared *Lona ilish,* a traditional fermented fish product made from hilsa steaks and evaluated the changes in nitrogenous compounds during fermentation. Sarojnalini & Suchitra (2009) studied the biochemical composition, digestibility and microbial quality of fermented *Setipinna* spp. as a raw material for *Ngari,* a fermented fish product of north eastern part of India. Thapa et al. (2004) evaluated the microbial diversity in fermented *Puntius sophore* and *Hentak* (a ball like fish paste prepared by fermentation of mixture of sundried fish *Esomus danricus* powder and petioles of aroid plant *Alocasia macrorhiza*) and *Tungtap* (fermented fish paste from *Danio* spp.).

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Koffi-Nevry et al. (2011) evaluated the chemical composition and lactic microflora of *Adjuevan,* a traditional Ivorian fermented fish condiment. Christine et al. (2002) prepared *Plaa-som*, a Thai fermented fish product from snakehead fish and studied the microflora at different salt concentrations. Mah et al. (2002) analysed biogenic amines in 11 types of Jeotkals, Korean salted and fermented fish products *Ojingeo-jeot* (sliced squid), *Myeolchi-jeot* (anchovy), *Changran-jeot* (pollock entrails), *Myeongran-jeot* (Pollock roe), *Saeu-jeot* (shrimp), *Toha-jeot* (toha shrimp), *Jogae-jeot* (clam), *Baendaengi-jeot* (big eyed herring), *Eorigul-jeot* (oyster), *Kkolttugi-jeot* (small squid) and *Agami-jeot* (pacific cod gills). Skåra et al., 2015 has described the salt fermented products of Northern European countries. Hakarl is a traditional fermented product of Iceland prepared from Greenland shark. Rak fish is a fermented product prepared from lake trout or arctic charr in inland Norway. Surstromming is a fermented product in Baltic Sea region prepared from Baltic herring. Barrel-salted ripened herring and sprats are traditional fermented products from Norway.

Fermented products are a delicacy in north eastern region of India and South East Asian countries. The raw materials used for fermentation vary from small size and low cost puntius to snakehead. Pangasius is cultured in south-east Asia mostly in Vietnam, India, China etc and mainly exported in the form of fillets. There is a need to find alternative ways for the effective utilisation of Pangasius. There are no published works on salt fermentation of Pangasius. Hence, in the present study Pangasius was salt fermented to estimate the time taken for maturation and also to evaluate the changes in biochemical parameters during fermentation.

Materials and Methods

P. hypophthalmus of weight ranging from 800 to 1100 g was procured from local fish market (Four bungalows), Mumbai, India and brought to the laboratory in iced condition. Fish was subjected to the following steps of processing *viz.,* washing with chilled water to remove visible dirt and slime, deheaded, eviscerated and then finally steaks of 3 cm size were made. Steaks were washed thoroughly with chilled water to remove blood, viscera residue and were dry-salted for 48 h (fish to salt ratio 1:1, w/w). After dry salting, excess salt on surface of the steaks was removed by simple wiping and were packed in a polyvinyl chloride (PVC) can of 5 l capacity (dimensions 17.5x17.5x28 cm). Total of hundred steaks were packed in each can and filled with boiled, cooled and filtered saturated brine up to the top for fermentation. Packed cans were left undisturbed till the end of fermentation study period. The detailed procedure followed for salt fermentation of Pangasius is given in the flow chart in Fig. 1.

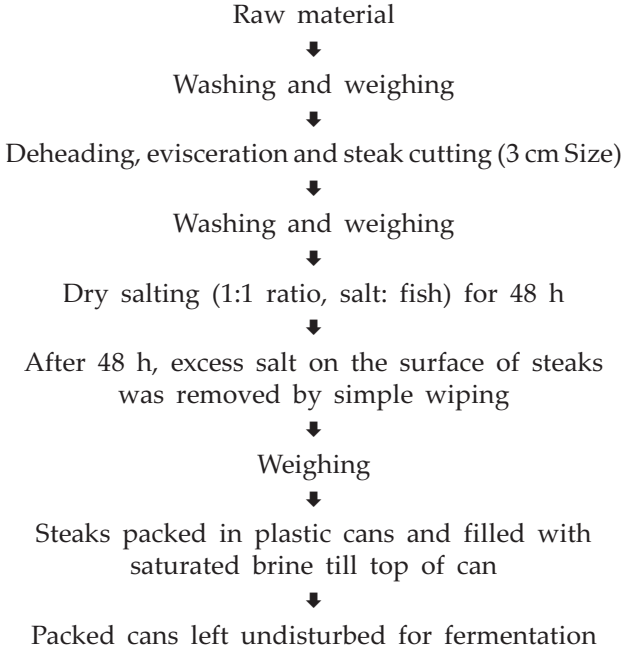

Fig. 1. Flow chart showing the processing steps followed for salt fermentation of *P. hypophthalmus*

The sampling was carried out on 0^{th}, 75^{th}, 105^{th} and 135^{th} days by taking 10 steaks from the can opened during the sampling (*i.e.* first can was opened on 75^{th} day, 2^{nd} can on 105^{th} day and 3^{rd} can on 135^{th} day) in order to avoid the disturbance to the anaerobic environment prevailing in the can. The pooled muscle tissues of all steaks were mixed, weighed, macerated in pestle and motor and used for analyses. The sampling was carried out till characteristic fruity flavour was achieved according to Majumdar & Basu, (2010a).

All the chemicals used in the present study were purchased from Merck, Mumbai, India. Moisture and ash content of the fish was analysed by the standard method as described in (AOAC, 2000). Differences in weight were recorded after drying the sample (10 g) in hot air oven at 100 ± 5°C overnight to determine the moisture content. Ashing was done by incineration in a muffle furnace (CEM Corpora-

tion, USA) at 550 ± 50ºC until white ash was obtained. TBARS was determined as described by the method of Tarladgis et al. (1960) and expressed as mg of malonaldehyde kg^{-1} of sample. Salt content was determined by Mohr's method and expressed as % NaCl. For pH 10 g of fish was homogenized in 50 ml distilled water in a homogenizer (Polytron system PT 2100, Kinematica, AG, Germany) for 30s and pH was measured using a digital pH meter (Eutech tutor pH/°C meter, Eutech Instruments, Singapore). Free alpha-amino nitrogen (FAN) was determined by the method of Pope & Stevens (1939).

All analyses were carried out in triplicates and analysis of variance was performed by one-way ANOVA with the application of Duncan's multiple range tests and descriptive statistics using SPSS 10 (SPSS10). The least significant difference (LSD) used to test for difference between means and significance was defined at ($p<0.05$). Results are reported as mean values of determinations ± standard deviations (SD).

Results and Discussion

Moisture content of Pangasius decreased from 77.87% (fresh) to 54.9% (0^{th} day) after 48 h of dry salting which may be due to diffusion of salt into the muscle and water out by ex-osmosis (Fig. 2). During salting, salt penetrates into muscle and moisture will be removed from muscle (Clucas, 1982). There was an increase in the moisture content of steaks packed in saturated brine on 75^{th} day (56.07%). A slight increase in moisture content during ripening may be due to absorption of water by muscle tissue as they swell when immersed in brine for prolonged period (Hernandaz-Herrero et al., 1999; Wheaton & Lawson, 1985). Moisture content decreased again on 105^{th} day and increased on 135^{th} day. According to Akiba (1955), the regular change of moisture and salt indicates continuous exchange of water and salt between fish and brine. The absorption of water from brine by fish tissue in final stage of ripening is due to formation of complex bodies formed from NaCl and protein. Similar trend was observed in fermented hilsa steaks (Majumdar & Basu, 2010b), Indian shad (Majumdar et al., 2006) and in anchovy (Hernandaz-Herrero et al., 2002).

Salt content increased in dry salted Pangasius from 0.38% (fresh) to 9.53% (0^{th} day) and to 14.98% (75^{th} day) (Fig. 3). The increase in salt content might be due to the diffusion of salt into the muscle with

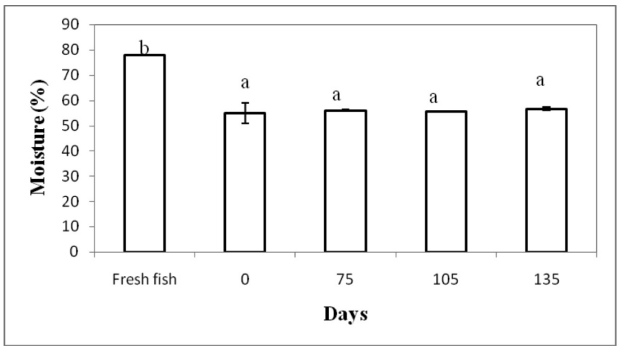

Fig. 2. Changes in moisture content during salt fermentation of Pangasius steaks

a,b letters indicate the significant difference ($p<0.05$); ± Standard error of mean (n=3)

removal of moisture. After 75^{th} day of fermentation there was very slight change in salt content. By 75^{th} day, the salt and moisture content in the muscle might have attained equilibrium and the increase or decrease in salt content during the later period may be due to the changes occurring in the muscle and simultaneously in salt concentration of surrounding brine. According to Shenderyuk & Bykowski (1990), the variations of chemical potentials of organic systems of fish and salt are due to continuous hydrolysis of protein. The salt content in fermented Pangasius was 14.43% on 135^{th} day. Similar results were observed during fermentation of salted mackerel and pink perch by Srikar et al. (1993).

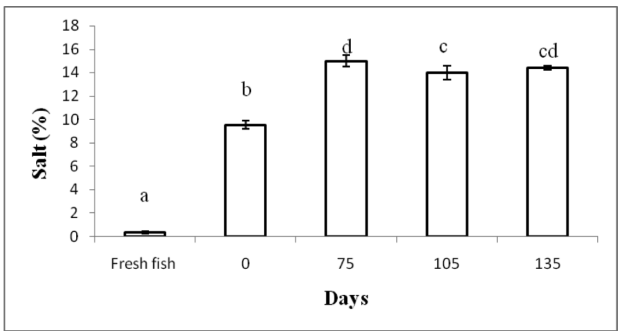

Fig. 3. Changes in salt content during salt fermentation of Pangasius steaks

a, b, c, d letters indicate the significant difference ($p<0.05$); ± Standard error of mean (n=3)

Ash content increased continuously from 11.98% (0^{th} day) to 18.17% (135^{th} day) during the period of fermentation (Fig. 4). It is a known phenomenon where a relationship exists between moisture, salt and ash content during salting i.e., as the moisture

content decreases salt content increases that causes ash content to increase. Though the salt content did not increase after 75th day, ash content increased continuously. From the results of free α-amino nitrogen, it's evident that protein degradation occurred continuously which may also be a probable reason for increase in ash content. So, increase in ash content may be due to combined effect of salt and degradation products in sample. Similar trend was observed by Majumdar & Basu (2010b) in fermented Hilsa steaks & Majumdar et al. (2006) in Indian shad.

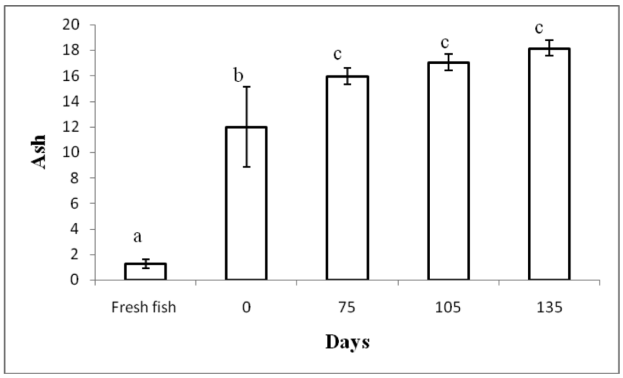

Fig. 4. Changes in ash content during salt fermentation of Pangasius steaks

a, b, c letters indicate the significant difference ($p<0.05$); ± Standard error of mean (n=3)

The pH of fish decreased till 105th day (5.83) but was found to increase on 135th day (5.93) of fermentation (Fig. 5). According to Lopetcharat & Park (2002), the initial decrease in pH after dry salting was due to dissociation of amino acids and small peptides in presence of salt. They observed that pH decreased from 7.0 to 6.8 after addition of salt. Further, increase in pH during ripening may be due to production of volatile basic compounds (Yatsunami & Takenaka, 1996). Similar trend was observed during fermentation of Indian shad where pH decreased till 120th day and then increased till 150th day (Majumdar et al., 2006). Similar trend was observed in fermented Hilsa steaks by Majumdar & Basu (2010b).

Thiobarbutric acid reactive substances (TBARS) content increased from 0.12 mg malonaldehyde kg^{-1} of fat (0th day) to 0.49 mg malonaldehyde kg^{-1} of fat (75th day) and then decreased to 0.21 mg malonaldehyde kg^{-1} of fat (135th day) (Fig. 6). Decrease in TBA values during ripening may be due

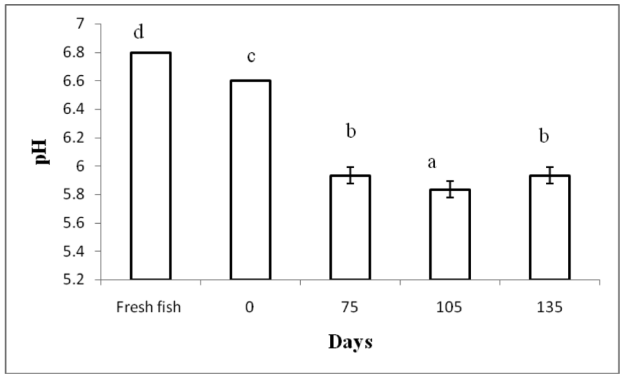

Fig. 5. Changes in pH during salt fermentation of Pangasius steaks

a, b, c, d letters indicate the significant difference ($p< 0.05$); ± Standard error of mean (n=3)

to interaction of protein degradation products with malonaldehyde to give tertiary products (Reddy & Setty, 1996). Mostafa & Salem (2015) observed an increase in TBA content in salt fermented mullet upto 60th day and then showed a decreasing trend till the end of fermentation.

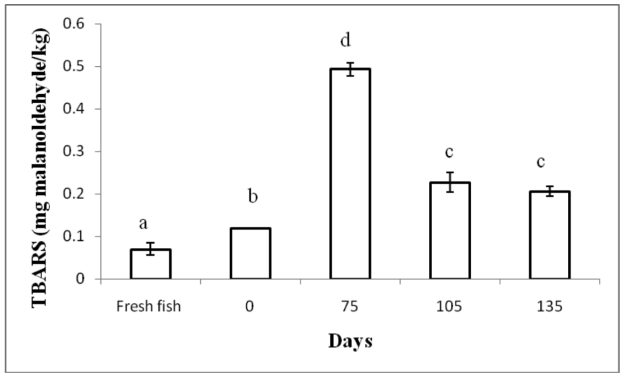

Fig. 6. Changes in TBA during salt fermentation of Pangasius steaks

a, b, c, d letters indicate the significant difference ($p<0.05$); ± Standard error of mean (n=3)

Free α-amino nitrogent content increased from 44.71 mg% (0th day) to 92.86 mg% (135th day) (Fig. 7). Combined effects of autolysis and microbial degradation of fish muscle are responsible for continuous increase in FAN (Ijong & Ohta, 1996). After dry salting, increase in FAN was due to degradation of protein (Voskresensky, 1965). Increase in FAN content may also be due to diffusion of a fraction of FAN to surrounding brine. Accumulation of FAN, TVBN and NPN indicates degradation of tissue

protein might be responsible for generation of typical flavour and aroma (Majumdar et al., 2006). Similar trend was observed during fermentation of Indian shad (Majumdar et al., 2006) and in fermented Hilsa steaks by Majumdar & Basu (2010b).

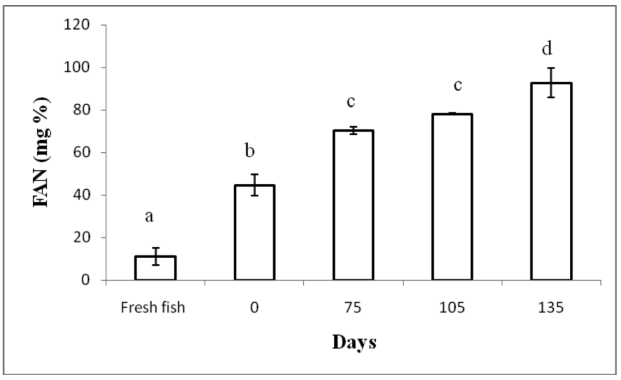

Fig. 7. Changes in Free α-amino nitrogen (FAN) during salt fermentation of Pangasius steaks

a, b, c, d letters indicate the significant difference ($p<0.05$); ± Standard error of mean (n=3)

From the study, it can be concluded that fermentation can be used as a suitable preservation method for Pangasius. This experiment can be used as a preliminary study to estimate the time taken to ferment Pangasius so that as a base for fermentation studies. Results of TBA indicate that the ripening has started on 75th day and it has attained its characteristic flavour on 135th day. From the above results it can be concluded that maturation of Pangasius occurs between 75th day to 135th day. Therefore, for complete maturation of salt fermented Pangasius it should be kept in cans for about 135 days. Further research can be focused on the isolation of predominant halophilic bacteria that are responsible for fermentation of Pangasius and adding the cultures into brine in-order to enhance the fermentation.

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References

Adams, M. R., Cooke, R.D. and Rattagool, P. (1985) Fermented fish products of Southeast Asia. Trop. Sci. 25: 61-73

Akiba, M. (1955) Discussion on the variation in the amount of bound water in fish meat during the processing of marine food products. Bull. Jap. Soc. Sci. Fish. 20: 937-940

AOAC (2000) Official Methods of Analysis of AOAC International, 17th edn., Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417 USA

Christine, P. M., Mette, M., Pairat, C., Lone, G. and Peter, L. M. (2002) Fermentation and microflora of *plaa-som*, a thai fermented fish product prepared with different salt concentration. Int. J. Food Microbiol. 73(1): 61-70

Clucas, I. J. (1982) Salting of fish: Salt and methods. In: Fish Handling, Preservation and Processing. Part-2. pp 4-8, Report of the Tropical Fishery Institute, London

Hernandaz-Herrero, M. M., Roig-Saugues, A. X., Lopez-Sabater, E. I., Rodriguez- Jerez, J. J. and Mora-Ventura, M. T. (1999) Total volatile basic nitrogen and other physico-chemical and microbiological characteristics as related to ripening of salted anchovies. J. Food Sci. 64: 344-347

Hernandaz-Herrero, M. M., Roig-Saugues, A. X., Lopez-Sabater, E. I., Rodriguez- Jerez, J. J. and Mora-Ventura, M. T. (2002) Influence of raw fish quality on some physico-chemical and microbial characteristics as related to ripening of salted anchovies (*Engraulis encrasicholus* L). J. Food Sci. 67: 2631-2640

Holzapfel, W. (1997) Use of starter cultures in fermentation on a household scale. Food Control 8(5): 241-258

Ijong, F. G. and Ohta, Y. (1996) Physicochemical and microbiological changes associated with *Bakasang* processing–a traditional Indonesian fermented fish sauce. J. Sci. Food Agric. 71: 69-74

Koffi-Nevry, R., Ouina,T. S. T., Koussemon, M. and Brou, K. (2011) Chemical composition and lactic microflora of A*djuevan*, a Traditional Ivorian Fermented Fish Condiment. Pak. J. Nutr. 10 (4): 332-337

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

Mah, J. H., Han, H. K., Oh, Y. J., Kim, M. G. and Hwang, H. J. (2002) Biogenic amines in *Jeotkals*, Korean salted and fermented fish products. Food Chem. 79(2): 239-243

Majumdar, R. K., Basu, S. and Nayak, B. B. (2006) Studies on the biochemical changes during fermentation of

salt-fermented Indian shad (*Tenualosa ilisha*). J. Aquat. Food Prod. Technol. 15(1) 53-69

Majumdar, R. K. and Basu, S. (2010a) Characterization of the traditional fermented fish product *Lona ilish* of Northeast India. Indian J. Tradit. Know. 9(3): 453-458

Majumdar, R. K. and Basu, S. (2010b) Changes in the nitrogenous compounds during fermented hilsa steaks. Fish. Technol. 47(2): 167-172

Mostafa, A. A. and Salem, R. H. (2015) Characterization of microbiological and nutritional variations in processed mullet (*Mugil cephalus*). Fish. Intl. J. 6(2): 108-122

Pope, C. G. and Stevens, M. F. (1939) The determination of amino nitrogen using copper method. Biochem. J. 33: 1070-1077

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

Reddy, K. P. and Setty, T. M. R. (1996) An intermediate moisture product from mackerel (*Rastrelliger kanagurta*) using salt curing, fermentation and drying. J. Aquat. Food Prod. Technol. 5: 65-82

Roy, D., Majumdar, R. K., Maurya, S. K. and Tripathi, H. H. (2015) Biochemical and Microbial changes Associated with Fermentation of *Setipinna phasa*. Fish. Technol. 52(1): 26-33

Sarojnalini, Ch. and Suchitra, T. (2009) Microbiological and Nutritional Evaluation of Fermented *Setipinna* Species. Fish. Technol. 46(2): 165-170

Shenderyuk, V. I. and Bykowski, P. J. (1990) Salting and marinating of fish. In: Seafood: Resources, Nutritional Composition and Preservation. (Sikorski, Z. E. Ed) pp 147-162 CRC Press Inc., Florida

SPSS (2010) SPSS for windows. Release 10. Chicago, 1:SPSS Inc

Srikar, L. N., Khuntia, B. K., Reddy, G. V. S., and 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: 319-322

Skåra, T., Axelsson, L., Stefánsson, G., Ekstrand, B. and Hagen, H. (2015) Fermented and ripened fish products in the northern European countries. J. Ethnic Foods 2(1): 18-24

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

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan Jr. L., (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. AM. Oil Chem. Soc. 37(1): 44-48

Thapa, N., Pal, J. and Tamang, J. P. (2004) Microbial diversity in *ngari, hentak* and *tungtap*, fermented fish products of North-East India. World J. Microbiol. Biotechnol. 20(6): 599-607

Voskresensky, N. A. (1965) Salting of herring. In: Fish as Food. Vol. III. Processing: Part 1. (Borgstrom, G. Ed). pp 107-128. Academic Press, New York. London

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

Yatsunami, K. and Takenaka, T. (1996) Changes in nitrogenous components and protease activity of fermented sardine with rice bran. Fish. Sci. 62: 790-795