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Research Note

Microbiological Quality of Myctophid Fish of the Arabian Sea

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Myctophids, which are popularly known as lantern fishes are important mesopelagic resources and are generally available in the twilight zone of sea at a depth ranging from 100 to 1000 m. Myctophids account for about 75 % of the total catches of small mesopelagic fishes (Shilat & Valinassab, 1998). Due to stagnation of catches from capture fisheries and over-exploitation of conventional fishery resources, it is important to pay enhanced attention to proper utilization of different non-conventional fishery resources including mesopelagic fish (Vivekanandan et al., 2005; Bhathal & Pauly, 2008; Worm et al., 2009) and myctophids are considered to be one such promising resource (Vipin et al., 2012). Stock sizes of mesopelagic fishes including myctophids have been estimated as 263 and 102 mt, in the Western Indian Ocean and Eastern Indian Ocean, respectively (Lam & Pauly, 2005). Studies by Boopendranath et al. (2009) and Pillai et al. (2009) showed that commercial exploitation of deep-sea prawns off Southwest coast of India yields a considerable amount of bycatch, in which myctophids very often contribute a major component. Gopakumar et al. (1983) established that myctophid fishes resemble most of the marine fish with regard to its biochemical composition and can be exploited for processing to various products, which can be used as food for both human and animals. Myctophids can also be utilized for preparation of various commercial products including fish meal, fish oil, fish silage, surimi, seasoning products, fish feed, poultry feed, crop fertilizers, lubricating oil, cosmetics and wax (Nair et al., 1983; Olsen et al., 2010; Rajamoorthy et al., 2013). The quality of the processed products largely depends on the quality of raw materials including microbial quality, which in-turn determines safety of the product and also predicts the risk factor of possible presence of various pathogenic organisms. The routine microbiological examination includes estimation of total aerobic bacteria, identification of spoilage organisms and enumeration of different indicator bacteria like total Enterobacteriaceae, total coliforms, coagulase-positive staphylococci and faecal streptococci. The presence of those indicator bacteria provides an overview of the hygienic status of samples as well as possible faecal contamination in the fish and its surrounding environment (Niemi & Taipalien, 1982).

However, in case of myctophid fish, very limited studies have been carried out so far to examine the microbiological characteristics of fresh fish and the products during storage. Hence, the present study was undertaken to examine the microbiological quality of myctophid fish caught by deep sea shrimp trawlers, operating off southwest coast of Kerala, India and to assess the microbiological changes in product prepared from myctophid fish during frozen storage.

Myctophid samples consisting of three species *Diaphus watasei*, *Myctophum obtusirostre* and *M. spinosum*, collected from bycatch of deep-sea shrimp trawlers engaged in multi-day fishing operation off southwest coast of India, during the period April–October 2009, were used in this study. The samples were stored in ice in the fishing vessel before

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landing and after landing, they were transported aseptically to the laboratory in the insulated container with crushed ice.

Ten grams of the sample of skin and muscle from a minimum of three fishes within same sample, was aseptically transferred into a sterile polythene pouch separately and blended in 90 ml sterile normal saline using a Stomacher blender (Seward, UK) for 30 sec at a speed of 230 rpm. The homogenate was further diluted by 10 fold serial dilution in sterile normal saline. The samples were analyzed for different microbiological parameters like aerobic mesophilic bacteria, total Enterobacteriaceae, coagulase-positive staphylococci, faecal streptococci and total coliforms. Analyses were performed according to the standard procedures for the enumeration and identification of microorganisms (FDA, 2005). All the studies were carried out in duplicate and the mean data were transformed into logarithms of the number of colony forming units per gram ($\log_{10}$ cfu g^{-1}). Coated product *viz.,* battered and breaded product was prepared out of myctophid fish meat sample (*D. watasei*), which was obtained off Cochin coast on 21-10-2009 (Table 1). The fishes were beheaded, de-finned and de-gutted with skin on and then washed with chilled water and it was followed by marination with salt and lemon juice. Then the marinated fishes were battered and coated with bread crumbs. The ingredients of batter were wheat flour, bengal gram flour, baking powder, milk powder, egg, salt, pepper, corn flour and water. The products were then packed in small styrofoam trays with the top being covered with transparent polyfilm. Then packed products were frozen at – 40ºC for 4 h and stored at – 20ºC for one year. Microbiological parameters were determined periodically under frozen storage following same procedure as mentioned previously (FDA, 2005). For enumeration of psychrophilic bacteria, different dilutions of sample were spread-plated onto pre-dried tryptic soya agar (TSA) and the plates were incubated at 7°C for 10 days.

The myctophid samples from landing centres had aerobic plate count ranging from 6.188 to 8.663 $\log_{10}$ cfu g^{-1}, while total Enterobacteriaceae count ranged from 2.949 to 4.851 $\log_{10}$ cfu g^{-1}. One sample (*M. obtusirostre*) was found positive for coagulase positive staphylococci and the presence of coliform was detected in one sample (*M. spinosum*). Faecal streptococci were not detected in any of the four myctophid samples screened for this indicator organism (Table 1). For coated product prepared from the *D. watasei*, the aerobic plate count at 37°C showed an increase from 0 day to 2 months and decrease thereafter during frozen storage up to 12 months. The total Enterobacteriaceae and faecal streptococci population decreased during frozen storage to 1.301 $\log_{10}$ cfu g^{-1} after 12 months of storage from their initial count (after freezing) of 2.748 and 2.531 $\log_{10}$ cfu g^{-1}, respectively. The coliforms were not detected from 4th month onwards. The decrease in total Enterobacteriaceae, total coliforms and fecal streptococci count during the frozen storage in the frozen myctophid product was due to damage of bacterial cell membrane because of formation of ice crystals. The initial count of total psychrophilic bacteria in the product prepared from *D. watasei* was more than that of total aerobic bacteria (mesophilic). However, at the end of storage, significant difference was not observed in both the counts (Table 2).

Table 1. Microbiological quality of myctophids landed in Kerala (Bacterial count, $\log_{10}$ cfu g^{-1})

Microbiological parameters	*Myctophum obtusirostre*	*Myctophum spinosum*	*Diaphus watasei*	*Diaphus watasei*	*Diaphus watasei*
Sample source	Kollam	Kollam	Kollam	Kollam	Cochin
Sample collection date	1-4-2009	1-4-2009	1-4-2009	1-10-2009	21-10-2009
Aerobic plate count	7.384	6.283	6.188	7.283	8.663
Total Enterobacteriaceae	2.949	4.762	3.869	4.851	-
Total coliforms	< 1.00	2.477	< 1.00	< 1.00	-
Coagulase positive staphylococci	3.924	< 1.00	< 1.00	< 1.00	-
Fecal streptococci	< 1.00	< 1.00	< 1.00	< 1.00	-

cfu: Colony forming unit

Table 2. Microbiological quality of coated product of *D. watasei* during frozen storage (Bacterial count, $\log_{10}$ cfu g^{-1})

Microbiological parameters	Storage time (months)						
	0	2	4	6	8	10	12
Aerobic plate count (at 37°C)	4.255	4.982	4.820	4.342	4.017	3.982	3.806
Total Enterobacteriaceae	2.748	2.505	2.477	1.903	1.602	1.602	1.301
Total coliforms	1.602	1.301	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
Coagulase positive staphylococci	< 1.00	< 1.00	-	-	-	-	-
Faecal streptococci	2.531	2.230	2.079	1.903	1.301	1.301	1.301
Psychrophilic bacteria	5.480	4.477	4.577	4.643	3.944	3.978	3.857

cfu: Colony forming unit

Gopakumar et al. (1983) reported that the total viable count of the frozen myctophid (*Benthosema pterotum*) was 2.9 x 10^3 cfu g^{-1}. Coliforms, faecal streptococci and coagulase positive staphylococci were not detected in the myctophid samples in that study. But, in the present study, a very high bacterial load in myctophid samples may be due to long storage of the samples on ice before reaching to harbour (Table 1). The increase in total bacterial flora during frozen storage is an unusual phenomenon. The increase in total aerobic microbial flora from 0 to 2 months is possibly because of multiplication of bacteria in some pockets where temperature might not have reached below 0°C due to possible delay in freezing. Duan et al. (2010) also observed an increase in aerobic plate count and psychrophilic count during frozen storage of lingcod fish fillets. The present study provided data on the microbiological quality of myctophid fish, which reached harbour after multi-day fishing as well as of coated product prepared from myctophid fish (*D. watasei*) during frozen storage study. However, more studies are required in future on microbiological quality of myctophid fishes especially by analyzing the freshly collected samples on-board to assess the actual level of different bacteria and microbial biodiversity in myctophid fishes and its surrounding environment.

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References

Bhathal, B. and Pauly, D. (2008) 'Fishing down marine food webs' and spatial expansion of coastal fisheries in India, 1950-2000. Fish. Res. 91: 26-34

Boopendranath, M. R., Remesan, M. P., Fernandez, T. J., Pradeep, K., Vipin, P. M. and Renju, R. (2009) Myctophids in the bycatch of deep sea shrimp trawlers. Fish. Tech. Newsletter 20: 1-2

Duan, J., Cherian, G. and Zhao, Y. (2010) Quality enhancement in fresh and frozen Lingcod (*Ophiodon elongatus*) fillets by employment of fish oil incorporated chitosan coating. Food Chem. 119: 524-532

FDA (2005) FDA Bacteriological Analytical Manual 18th edn., AOAC, Washington, DC

Gopakumar, K., Nair, K.G.R., Nair, P.G.V., Nair, A.L., Radhakrishnan, A.G. and Nair, P.R. (1983) Studies on lantern fish *Benthosema pterotum* biochemical and microbiological investigations. Fish. Technol. 20: 17-19

Lam, W. V. Y. and Pauly, D. (2005) Mapping the global biomass of mesopelagic fishes. Sea Around Us Project Newsletter, 30(4) accessed through http://www.seaaroundus.org/newsletter/Issue30.pdf on 25th June, 2015

Nair, A.L., James, M.A., Mathew, P.T. and Gopakumar, K. (1983) Studies on lantern fish *Benthosema pterotum* - nutrition evaluation, Fish. Technol. 20: 20-23

Nieni, M. and Taipalinen, I. (1982) Faecal indicator bacteria in fish farms. Hydrobiologia, 86: 171-175

Olsen, R. E., Waagbo, R., Melle, W., Ringo, E. and Lall, S. P. (2010) Alternative Marine Resources. In: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds (Giovanni, M., Turchini, G.M., Ng, W.K. and Tocher, D.R., Eds), Chapter 9, pp 268-324, CRC Press, Boca Raton, FL

Pillai, N. G. K., Bineesh, K. K., Sebastine, M. and Akhilesh, K. V. (2009) Lantern fishes (Myctophids): by-catch of deep sea shrimp trawlers operated off Kollam, south-west coast of India. Mar. Fish. Infor. Serv. T and E Ser. No. 202, pp 1-4

Rajamoorthy, K., Pradeep, K., Anandan, R., Libin Baby, Sankar, T. V. and Lakshmanan, P. T. (2013) Biochemical composition of myctophid species *Diaphus watasei* and *Myctophum obtusirostre* caught from Arabian Sea. Fish. Technol. 50: 41-44

Shilat, A.Z. and Valinassab, T. (1998) Trail fishing for lantern fishes (myctophids) in the Gulf of Oman (1989–1990) FAO fisheries circular No. 935, FAO, Rome Accessed through http://www.fao.org/docrep/w8239e/w8239e00.HTM on 25th June, 2015

Vipin, P. M., Renju R., Fernandez, T. J., Pradeep, K., Boopendranath, M. R. and Remesan, M. P. (2012) Distribution of myctophid resources in the Indian Ocean. Rev. Fish Biol. Fish. 22: 423-436

Vivekanandan, E., Srinath, M. and Kuriakose, S. (2005) Fishing the marine food web along the Indian coast. Fish. Res. 72: 241-252

Worm, B., Hilborn, R., Baum, J. K., Branch, T. A., Collie, J. S., Costello, C., Fogarty, M. J., Fulton, E. A., Hutchings, J. A., Jennings, S., Jensen, O. P., Lotze, H. K., Mace, P. A., McClanahan, T. R., Minto, C., Palumbi, S. R., Parma, A. M., Ricard, D., Rosenberg, A. A., Watson, R. and Zeller, D. (2009) Rebuilding global fisheries. Science 325: 578-585