

## Effect of low temperature on quality of Mola (*Amblypharyngodon mola*) fish pickle during storage and consumer preference towards

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**Abstract:** Mola (*Amblypharyngodon mola*) is one of the most important small indigenous species of Bangladesh having high vitamin A and calcium contents. To prepare ready-to-eat product from *A. mola* with extended shelf life, this study was carried out to determine the effects of refrigeration (5° to 8°C) and frozen (-18° to -20°C) temperatures on the quality parameters of *A. mola* pickle and consumer preference towards the product. *A. mola* pickle was prepared following standard methodology and stored in sealed and vacuum sealed packs at above mentioned temperatures. It was revealed that during refrigeration and frozen storage the percent moisture, protein and lipid contents decreased but ash content increased with the progress in storage period. At these temperatures, pH value of the pickle decreased very slowly but the TVB-N value and bacterial load increased slowly throughout the storage period. *A. mola* pickle may remain in acceptable condition until 120 days at refrigeration and about 300 days at frozen temperatures. Study on consumer's preference to fish pickle showed that, most of the respondents liked the product and scored as very good.

**Keywords:** Fish pickle, Mola (*Amblypharyngodon mola*), Low temperature storage

### Introduction

Fish is highly perishable, and unless correctly treated after harvesting, can soon become unfit for consumption and possibly dangerous to health caused by bacterial activity, chemical change and enzymatic breakdown. Proper handling, processing, preservation, packaging and storage measures are essential to improve shelf life, ensure safety and maintain quality. Consumer demand is high for quality processed foods with minimal changes in nutritional and sensory properties (Ahvenainen 2003). So the goal of food manufacturers is to develop and employ processing technologies that retain or create desirable sensory qualities or reduce undesirable changes in food due to processing (Belcher 2006). Many emerging technologies have the potential to extend the shelf life. There is evidence that human have been processing fish since the early Holocene (Nielsen 2003). The most common methods of fish processing are salting, smoking, drying, dehydration, pickling, cooking, canning and fermentation. Innovations and improvements in processing, storage, distribution, marketing and food science and technology have facilitated the trade and consumption of an expanded variety of species and product forms. Great technological development in fish processing and packaging is ongoing in many countries.

Among different processing methods pickling is safe and easy one of putting up fish for short term storage. Low moisture and reduced pH are the important major factors contributing to the self-stability of the pickles (Frazier and Westhoff 1984). Fish pickle prepared under most hygienic conditions with addition of required quantity of salt, preservatives and spices will have generally an average shelf life of one year. Most of the sea fish like prawn, tuna, pomfret, and mackerel are ideally suitable for making fish pickles. Frozen storage has been widely employed to retain fish properties before it is consumed or employed in other technological process (Ericson 1997). During freezing and frozen storage, fish muscle can undergo a number of changes, such as denaturation and aggregation of the myofibrillar proteins. These result in alteration of the functional properties of muscle proteins, loss of water-holding capacity and juiciness and unwanted changes in texture. Freezing and frozen storage have largely been employed to retain the sensory and nutritional properties of fish although enzymatic and non-enzymatic rancidity are known to develop strongly (Ericson 1997). Considering the mentioned points the attempt was taken to prepare pickle with a popular, round the year available, highly nutritive fish, (*Amblypharyngodon mola*) and to observe the changes in different quality parameters during extended storage period at low temperatures and also to check the consumer preference towards the product.

### Materials and Methods

**Sample collection:** Seven kg fresh mola (*A. mola*) fish were collected from local market of Mymensingh. The fishes were immediately transported to the Laboratory and kept at refrigeration (5°C to 8°C) and frozen temperature (18°C to -20°C).

**Preparation of fish pickle:** The fishes were gutted and washed with tap water and 1.5% salt water. The excess water was removed from fish with a fresh dry cotton cloth. The fish were marinated with required amount of turmeric, red chili, coriander powder and salt for at least 1hr in a refrigerator, fried in mustard oil, added other ingredients and finally heated till vinegar absorbed. During packing, care was taken to see that there was enough oil in the pickle packs. The recipe followed for the preparation of pickle is given in Table I.

**Table I. Ingredients used for fish pickle preparation**

Ingredients	Quantity
Mola fish	1kg
Chili powder	20g
Cumin powder	10g
Turmeric powder	5g
Garlic powder	10g
Clove powder	2g
Mustard oil	100ml
Salt	35g
Sugar	40g
Tamarind	50g
Pachforon	5g
Cinnamon	2g
Coriander powder	2g
Vinegar	20ml

**Sample storage:** A total of 15 packs of samples (50 g sample in each pack) were stored at refrigeration temperature (5° to 8°C) for quality analysis. Among them 12 packs were stored in sealed condition and 3 packs were stored in vacuum sealed condition for 120 days. Another 13 packs samples (same amount as kept for refrigeration temperature) were stored at frozen temperature (-18°C to -20°C). At this temperature 10 packs were stored in sealed condition and 3 packs were stored in vacuum condition for about 300 days. Biochemical analysis of the samples stored at refrigeration temperature in sealed packs was done at 15 days of interval and the samples in vacuum sealed packs at 50 days of interval. On the other hand biochemical analysis of the samples stored at frozen temperature in sealed packs was done at 30 days interval and at 120 days interval of the samples stored in vacuum sealed pack.

**Quality analysis:** Proximate composition (moisture, protein, lipid and ash) of fish pickle was tested monthly in triplicate according to the methods described in AOAC (2005). pH value was determined by homogenously mixing 5 g sample in 50 ml distilled water with an electronic pH meter (HANNA pH 211 Microprocessor pH Meter). Total Volatile Base Nitrogen (TVB-N) was determined according to the methods given in AOAC (1984).

The colony forming units (CFU) were counted under a Quebec dark field colony counter (Leica, Buffalo, NY, USA) equipped with a guide plate ruled in square centimeters. Plates containing 30-300 colonies were used to calculate bacterial load using following formula:

$$\text{APC (CFU/g)} = \frac{\text{No. of colonies on petridish} \times \text{Dilution factor} \times \text{Vol. of stock solution} \times 10}{\text{Wt. of pickle or condiment sample}}$$

A panel of 9 (nine) trained teachers and students of the Department of Fisheries Technology provided the sensory assessments of the products. Sensory evaluation of the fish pickle was conducted as following grades: 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely. Chewiness / Rubberiness was defined as the amount of effort the panelist had to exert in chewing to prepare the sample for swallowing. Color and flavor were evaluated organoleptically.

**Consumer preference towards fish pickle:** A survey was conducted for 2 months (July-August) in the year 2016. The data was collected from Faculty of Fisheries and local market using a questionnaire. The acceptability study was done people of on different classes.

**Statistical analysis:** The statistical analysis package SPSS 11.5 (SPSS Inc, Chicago, IL, USA) was used to calculate mean and standard deviation of the values. Relevant graphs were prepared using Microsoft Office Excel 2007.

## Results

**Biochemical composition of fresh fish and fish pickle on “0” day:** Four major constituents, viz., water, protein, lipid and ash of fresh mola (*A. mola*) were determined immediately after collection of the sample. The percent moisture content was obtained  $79.11 \pm 7.46$ , protein content  $11.51 \pm 1.51$ , lipid content  $6.51 \pm 0.58$  and ash content  $2.50 \pm 1.28$  (Table II). The values

obtained for percent moisture, protein, lipid and ash content in the mola pickles were  $37.00 \pm 0.02$ ,  $11.89 \pm 1.66$ ,  $21.47 \pm 0.01$  and  $5.71 \pm 0.88$ , respectively (Table II).

**Table II. Proximate compositions of fresh *A. mola* and mola fish pickle**

Component	Fresh Mola fish	Mola fish pickle
	Value (%)	Value (%)
Moisture	$79.11 \pm 7.46$	$37.00 \pm 0.02$
Protein	$11.51 \pm 1.51$	$11.89 \pm 1.66$
Lipid	$6.51 \pm 0.58$	$21.47 \pm 0.01$
Ash	$2.50 \pm 1.28$	$5.71 \pm 0.88$

\*mean value  $\pm$  standard deviation of 3 individual measurements.

**Changes in the moisture and ash content of fish pickle during storage:** In this experiment initial moisture content (%) of pickle was found 37.00% which was much lower than that of fresh fish. After 60 days of storage at refrigeration temperature moisture content of pickle in sealed pack increased to  $44.02 \pm 0.24\%$  and after 150 days of storage the value reduced to  $37.50 \pm 0.17\%$  (Table III). On the other hand, moisture content in pickle was obtained 37.69% after 100 days of storage in vacuum sealed pack which further decreased to 36.98% on 150<sup>th</sup> day of storage. In the case of ash content, the initial value was obtained 5.71 %. With lapse of storage period the percent ash content of pickle both in sealed and vacuum sealed packs increased. In sealed pack the percent ash content increased to 9.98% after 150 days of storage and 7.00% in vacuum sealed pack on 150<sup>th</sup> day of storage. In the case of frozen temperature, after 120 days of storage the moisture content of pickle in sealed pack increased to 62.34% while after 300 days of storage the value reduced to 54.09% (Table III). On the other hand moisture content in pickle was found 39.81% after 240 days of storage in vacuum sealed pack which further decreased to 36.77% on 360<sup>th</sup> day of storage. The initial value of ash content was obtained 5.71 % which increased to 8.03% after 300 days of storage in sealed pack and to 8.89% after 360 days of storage in vacuum sealed pack (Table III).

**Table III. Changes in moisture and ash content (%) of fish pickle at refrigeration (5°C to 8°C) and frozen (-18°C to -20°C) temperatures in sealed and vacuum sealed packs**

Days	Refrigeration temperature		Days	Frozen temperature	
	Moisture (%)	Ash (%)		Moisture (%)	Ash (%)
	Sealed Pack	Sealed Pack		Sealed Pack	Sealed Pack
0	$37.00 \pm 0.02$	$5.71 \pm 0.88$	0	$37.00 \pm 0.02$	$5.71 \pm 0.88$
15	$39.11 \pm 0.13$	$4.23 \pm 0.27$	30	$67.42 \pm 0.90$	$2.80 \pm 0.35$
30	$44.62 \pm 1.87$	$4.55 \pm 0.61$	60	$66.69 \pm 0.57$	$4.06 \pm 0.07$
45	$42.37 \pm 0.06$	$5.60 \pm 0.93$	90	$65.89 \pm 0.18$	$4.72 \pm 0.15$
60	$44.02 \pm 0.24$	$5.48 \pm 1.06$	120	$62.34 \pm 0.53$	$6.64 \pm 0.35$
75	$40.54 \pm 0.32$	$6.18 \pm 1.14$	150	$62.01 \pm 0.88$	$5.39 \pm 0.94$
90	$41.11 \pm 0.03$	$6.92 \pm 0.21$	180	$61.88 \pm 0.12$	$6.15 \pm 0.87$
105	$42.40 \pm 0.09$	$7.70 \pm 0.37$	210	$58.43 \pm 0.65$	$6.53 \pm 0.55$
120	$39.29 \pm 0.57$	$6.91 \pm 0.04$	240	$58.21 \pm 0.39$	$7.71 \pm 0.29$
135	$38.00 \pm 0.12$	$8.01 \pm 0.01$	270	$57.91 \pm 0.09$	$6.33 \pm 0.04$
150	$37.50 \pm 0.17$	$9.98 \pm 0.58$	300	$54.09 \pm 0.10$	$8.03 \pm 0.95$

Days	Vacuum Sealed Pack	Vacuum Sealed Pack	Days	Vacuum Sealed Pack	Vacuum Sealed Pack
0	37.00 $\pm$ 0.02	5.71 $\pm$ 0.88	0	37.00 $\pm$ 0.02	5.71 $\pm$ 0.88
50	41.08 $\pm$ 0.49	6.76 $\pm$ 0.95	120	36.27 $\pm$ 0.05	6.48 $\pm$ 1.07
100	37.69 $\pm$ 0.03	6.15 $\pm$ 0.24	240	39.81 $\pm$ 0.09	7.31 $\pm$ 0.05
150	36.98 $\pm$ 0.05	7.00 $\pm$ 0.16	360	36.77 $\pm$ 0.04	8.89 $\pm$ 0.56

\*mean value  $\pm$  standard deviation of 3 individual measurements.

**Changes in the protein and lipid contents of fish pickle during storage:** Here the initial protein content (%) of pickle was found 11.88% which was much lower than that of fresh fish. After 75 days of storage at refrigeration temperature protein content of pickle in sealed pack decreased to 9.021% and after 150 days of storage the value reduced to 7.12% (Table IV). On the other hand protein content in pickle was found 6.19% after 100 days of storage in vacuum sealed pack which further decreased to 6.10% on 150<sup>th</sup> day of storage. The initial lipid content of fish pickle was obtained 21.46%. With progress of storage period the percent lipid content of pickle both in sealed and vacuum sealed packs decreased 7.09% after 150 days of storage and 15.61% in vacuum sealed pack respectively. The protein content of pickle stored at frozen temperature in sealed pack increased to 13.012% after 120 days of storage while decreased to 10.10% after 300 days of storage (Table IV). On the other hand protein content in pickle was found 9.75 % after 240 days of storage in vacuum sealed pack which further decreased to 7.64 % on 360<sup>th</sup> day of storage. The initial lipid content was 21.46% which during storage both in sealed and vacuum sealed packs. In sealed pack the percent ash content increased to 9.03% after 300 days of storage and 14.0% in vacuum sealed pack on 360<sup>th</sup> day of storage of pickle sample (Table IV).

**Table IV. Changes in protein and lipid content (%) of fish pickle at refrigeration (5°C to 8°C) and frozen (-18°C to -20°C) temperatures in sealed and vacuum sealed packs**

Days	Refrigeration temperature		Days	Frozen temperature	
	Protein (%)	Lipid (%)		Protein (%)	Lipid (%)
	Sealed Pack	Sealed Pack		Sealed Pack	Sealed Pack
0	11.88 $\pm$ 1.66	21.46 $\pm$ 0.01	0	11.88 $\pm$ 1.66	21.46 $\pm$ 0.01
15	10.89 $\pm$ 0.07	13.36 $\pm$ 0.09	30	9.655 $\pm$ 1.29	21.67 $\pm$ 0.10
30	9.27 $\pm$ 0.09	13.30 $\pm$ 0.03	60	8.812 $\pm$ 0.87	20.76 $\pm$ 0.09
45	9.77 $\pm$ 0.09	12.01 $\pm$ 0.31	90	8.853 $\pm$ 0.27	16.27 $\pm$ 0.63
60	9.346 $\pm$ 0.02	13.94 $\pm$ 0.06	120	8.653 $\pm$ 0.35	16.85 $\pm$ 0.03
75	9.021 $\pm$ 0.13	13.04 $\pm$ 0.01	150	8.879 $\pm$ 0.85	15.37 $\pm$ 0.02
90	12.01 $\pm$ 0.35	11.81 $\pm$ 0.08	180	7.203 $\pm$ 0.19	16.653 $\pm$ 0.06
105	11.05 $\pm$ 1.26	9.44 $\pm$ 0.62	210	9.871 $\pm$ 0.33	13.01 $\pm$ 0.08
120	8.74 $\pm$ 0.02	11.00 $\pm$ 0.33	240	13.012 $\pm$ 0.67	13.95 $\pm$ 0.71
135	8.08 $\pm$ 0.09	9.37 $\pm$ 0.09	270	12.111 $\pm$ 0.11	11.03 $\pm$ 0.02
150	7.12 $\pm$ 0.05	7.09 $\pm$ 0.10	300	10.099 $\pm$ 0.89	9.03 $\pm$ 0.17
Days	Vacuum Sealed Pack	Vacuum Sealed Pack		Vacuum Sealed Pack	Vacuum Sealed Pack
0	11.89 $\pm$ 1.66	21.46 $\pm$ 0.01	0	11.88 $\pm$ 1.66	21.46 $\pm$ 0.01
50	7.112 $\pm$ 0.97	21.01 $\pm$ 0.01	120	10.17 $\pm$ 0.98	20.42 $\pm$ 0.53
100	6.193 $\pm$ 0.83	20.42 $\pm$ 0.20	240	9.75 $\pm$ 0.23	18.61 $\pm$ 0.03
150	6.095 $\pm$ 0.09	15.61 $\pm$ 0.07	360	7.64 $\pm$ 0.65	14.0 $\pm$ 0.07

\*mean value  $\pm$  standard deviation of 3 individual measurements.

**Changes in the pH and TVB-N values of fish pickle:** The initial pH value of fish pickle was  $5.53 \pm 0.00$ . After 90 days of storage at refrigeration temperature pH of pickle in sealed pack increased to  $5.83 \pm 0.06$  and then after 150 days of storage the value reduced to 4.43 (Table V). On the other hand pH in pickle was  $5.48 \pm 0.09$  after 100 days of storage in vacuum sealed pack which further decreased to  $4.87 \pm 0.03$  on 150<sup>th</sup> day of storage. In the case of TVB-N (mg/100g), the initial value was  $1.64 \pm 0.04$ . The TVB-N (mg/100g) content of pickle both in sealed and vacuum sealed packs increased to  $6.71 \pm 0.02$  after 150 days of storage and  $3.66 \pm 0.05$  in vacuum sealed pack after 150 days of storage. In the case of frozen temperature storage, after 180 days of storage the pH of pickle in sealed pack increased to  $5.80 \pm 0.04$  and then after 300 days of storage the value reduced to  $5.21 \pm 0.02$ . The pH value in pickle was  $5.01 \pm 0.08$  after 240 days of storage in vacuum sealed pack which decreased to  $4.82 \pm 0.02$  after 360 days of storage. The initial TVB-N (mg/100g) value was  $1.64 \pm 0.02$  which increased with the progress in storage both in sealed and vacuum sealed packs. In sealed pack the percent ash content increased to  $6.78 \pm 0.07$  after 300 days of storage and  $5.78 \pm 0.03$  in vacuum sealed pack after 360 days of storage of pickle sample (Table V).

**Table V. Changes in pH and TVB-N (mg/100g) value of fish pickle at refrigeration and frozen temperatures in sealed and vacuum sealed packs**

Days	Refrigeration temperature		Days	Frozen temperature	
	pH Value	TVB-N (mg/100g)		pH Value	TVB-N (mg/100g)
	Sealed Pack	Sealed Pack		Sealed Pack	Sealed Pack
0	$5.53 \pm 0.00$	$1.64 \pm 0.04$	0	$5.53 \pm 0.00$	$1.64 \pm 0.02$
15	$5.33 \pm 0.01$	$1.93 \pm 0.01$	30	$5.52 \pm 0.01$	$1.68 \pm 0.00$
30	$5.31 \pm 0.01$	$2.43 \pm 0.03$	60	$5.59 \pm 0.01$	$1.47 \pm 0.01$
45	$5.38 \pm 0.03$	$2.67 \pm 0.02$	90	$5.73 \pm 0.03$	$1.42 \pm 0.03$
60	$5.29 \pm 0.09$	$3.17 \pm 0.01$	120	$5.79 \pm 0.01$	$2.43 \pm 0.02$
75	$5.26 \pm 0.90$	$3.49 \pm 0.02$	150	$5.83 \pm 0.06$	$2.48 \pm 0.03$
90	$5.83 \pm 0.06$	$3.92 \pm 0.06$	180	$5.80 \pm 0.04$	$2.55 \pm 0.04$
105	$5.23 \pm 0.03$	$4.43 \pm 0.02$	210	$5.79 \pm 0.02$	$2.52 \pm 0.01$
120	$5.07 \pm 0.01$	$5.80 \pm 0.07$	240	$5.81 \pm 0.02$	$2.60 \pm 0.05$
135	$4.98 \pm 0.02$	$5.94 \pm 0.07$	270	$5.73 \pm 0.00$	$3.72 \pm 0.03$
150	$4.43 \pm 0.01$	$6.71 \pm 0.02$	300	$5.21 \pm 0.02$	$6.78 \pm 0.07$
Days	Vacuum Sealed Pack	Vacuum Sealed Pack		Vacuum Sealed Pack	Vacuum Sealed Pack
0	$5.53 \pm 0.00$	$1.64 \pm 0.04$	0	$5.53 \pm 0.00$	$1.64 \pm 0.03$
50	$5.51 \pm 0.01$	$1.69 \pm 0.01$	120	$5.17 \pm 0.02$	$2.97 \pm 0.02$
100	$5.48 \pm 0.09$	$1.52 \pm 0.03$	240	$5.01 \pm 0.08$	$4.56 \pm 0.09$
150	$4.87 \pm 0.03$	$3.66 \pm 0.05$	360	$4.82 \pm 0.02$	$5.78 \pm 0.03$

\*mean value  $\pm$  standard deviation of 3 individual measurements

**Changes in the bacterial load of fish pickle:** The bacterial load of fish pickle (Sealed Pack) increased during storage at refrigeration and frozen temperatures. Aerobic Plate Count (APC) for bacterial load showed inverse relation at frozen temperature storage. At refrigeration temperature bacterial load increased with the lapse of storage period. On the contrary bacterial load decreased at frozen temperature within 0 to 300 days storage. On "0" day of storage APC was  $2.1 \times 10^4$  CFU/g in room, refrigeration and frozen storage respectively. After 150 days of

storage at refrigeration temperature, APC was found  $6.6 \times 10^5$  CFU/g. On the other hand at frozen storage APC was found  $3.6 \times 10^2$  after 300 days of storage (Table VI).

**Table VI. Changes in bacterial load (CFU/g) of fish pickle at refrigeration and frozen temperatures in sealed packs**

Storage Temperature	Storage period (Day)	Bacterial load (CFU/g)
Refrigeration temperature (5°C to 8°C)	0	$2.1 \times 10^4$
	30	$7.1 \times 10^4$
	60	$9.1 \times 10^4$
	90	$2.7 \times 10^5$
	120	$4.2 \times 10^5$
	150	$6.6 \times 10^5$
Frozen Temperature (-18°C to -20°C)	0	$2.1 \times 10^4$
	60	$2.5 \times 10^3$
	120	$1.9 \times 10^3$
	180	$7.5 \times 10^2$
	240	$5.3 \times 10^2$
	300	$3.6 \times 10^2$

**Consumer preference towards fish pickle:** The categories and distribution of the selected characteristic of consumer response in this study are shown in Table VII. About 70% of the respondent in the study belonged to the middle age (from 31 to 50 years old). The maximum percentage of respondents (60%) was graduate. Occupation of most of the respondents (55%) was office service and the income level was medium (60%) that is 10,000 to 15,000 taka/month. All the respondents liked the product individually. Consumer responses were divided in five categories, such as very good, good, average, bad and very bad. Most of the respondents scored the product as very good in taste (63.22%), and good in color (60%) and flavor (60%). Overall taste (41.33%) also scored as very good. In case of texture 43.33% scored as good and 23.33% scored as average (Table VII). No respondents scored very bad or bad in respect of any parameter with exception of flavor. Among the respondents 5% scored bad in case of flavor parameter and the mean value of flavor was low (4.26%) among the sensory parameters.

## Discussion

The main constituents of whole fish are, water, protein, lipid and ash which ranges between 65-90%, 10-22%, 0.5-20 and 0.5-5%, respectively (Nowsad 2007). The percent moisture, protein, lipid, ash obtained in the present study for fresh fish was within this range. Shamim *et al.* (2012) revealed that, fresh Mola contained protein 17.95%, lipid 2.87%, ash 2.50% and moisture 76.86%. This values coincides with the values obtained in this study found for fresh Mola. Sen (2005) reported that the proximate composition of fish pickle was affected by moisture loss which concentrated the nutrient. Holma and Maalekuu (2013) reported reduced moisture content (%) in fish immediately after frying than that of fresh fish. In the present study, moisture content of fresh fish was found higher than fish pickle which was similar to the findings of other researchers.

**Table VII. Categories and distribution of the selected characteristics of consumer response**

Variable	Observed range	Possible range	Categories	Response	
				No.	Percent (%)
Age	16-55	Year	Young age (15-30)	20	60
			Adult age (31-50)	35	70
			Old age (> 50)	10	40
Education	5-18	Year of schooling	Illiterate	0	0.5
			Primary (1-5)	4	10
			Secondary (6-10)	10	23
			Higher secondary	12	55
			Service holder	35	60
Occupation	1-4	Type of occupation	Service	32	55
			Businessman	10	16
			Teacher	25	27
			Day labour	3	6
Annual income	7,000-23,000	Thousand	Low (5,000-10,000)	14	25
			Medium (10,000-15,000)	30	60
			High (15,000-25,000)	16	27

**Changes at refrigeration temperature (5°C to 8°C):** In the present study, moisture content of fresh fish was found higher than that of fish pickle. During storage at refrigeration temperature, the moisture content decreased throughout the storage period irrespective of packing conditions. Hossain *et al.* (2018) also reported decrease in moisture content in Thai pangus pickle during storage at refrigeration temperature. Here, moisture content reduced might be due to the dehydration occurred at refrigeration temperature. In this study, ash content increased in fish pickle compared to fresh fish. This is might be due to the loss of moisture during fish pickle preparation procedure. After that while fish pickle was stored at refrigeration temperature then also percent ash content gradually increased throughout the storage period for both packing conditions. This character of ash is related to reduction in moisture content during storage (Pawar *et al.* 2013). A similar trend in percent ash content increase during storage at refrigeration temperature for the pickle prepared with Thai pangus was reported by Hossain *et al.* (2018). Thus, it can be stated that there is an inverse relation between moisture content and ash content in fish pickle.

Devi and Sarojnalini (2012) reported an increasing trend in protein content of *A. mola* after cooking than that of fresh fish. In this experiment also percent protein content increased after pickle preparation. Then during storage at refrigeration temperature, protein content decreased



with the extension of storage period. Denaturation of fish protein and leaching out of water soluble protein might be associated with this decrease in protein in fishery products (Gandotra *et al.* 2012). On the other hand, percent lipid content of fish pickle also decreased during storage at this temperature though the rate of decrease was lower for the pickle stored in vacuum sealed pack might be removal of air from the vacuum sealed packs. The finding in this study coincides well with report of Hossain *et al.* (2018) for fish pickle prepared with Thai pangus.

The decrease in the pH might be due to the addition of vinegar, sodium benzoate and tamarind during processing and its gradual uptake by fish pickle. pH in prawn pickle reduced significantly by sodium benzoate (Abraham and Setty 1994). Similar decreasing trend in pH during storage of pickle was reported by many other authors (Tamilselvi *et al.* 2010, Dhanapala *et al.* 1994). Tanuja and Hameed (1998) recorded the pH of squilla pickle 4.46 which dropped gradually during storage. TVB- N value of fish pickle increased during storage at refrigeration temperature. At the initial stage, TVB-N value increased very slowly in both packing condition, after that a sharp increase in TVB-N value occurred. This increasing trend in TVB-N is similar to the smoked product of gilthead sea bream (*Sparus aurata* 1758), during storage at 4°C (Bilgin *et al.* 2008).

In the present study, bacterial load was found to increase with the progress in storage period while fish pickle stored at refrigeration temperature. The increase of bacterial load at this temperature might be due to the fact that- this temperature slows down bacterial action and multiplication but can't stop. Similar increment in total bacterial load in fish muscle at low temperature storage was reported by Obemeata *et al.* (2011).

**Changes at frozen temperature (-18°C to -20°C):** In this experiment, at frozen temperature, moisture content (%) of fish pickle decreased with the lapse of storage period. Alasalvar *et al.* (2002) reported a decrease in total moisture content in fish fillets during frozen storage. Ninan *et al.* (2008) reported a reduction in moisture content during frozen storage of the test samples might be due to deep frying of the samples at the time of sample preparation and also the dehydration occurred. These findings are similar to the findings of the present study. The percent ash content of fish pickle stored at frozen temperature increased gradually throughout the storage period might be due to reduction of moisture content. Pawar *et al.* (2013) reported this character of ash which has inverse relation to the reduction in moisture content during frozen storage. In the present study protein content (%) of fish pickle decreased slowly at frozen temperature. Gandotra *et al.* (2012) reported the similar trend for fishery products. Here, lipid content (%) also decreased but the rate was slower. McGill *et al.* (1974) reported that lipid content in fishery products started to decrease when oxidation occurs. In this present study partial lipid oxidation of fish pickle took place during frozen storage after eight months of storage.

In this study pH of fish pickle stored at frozen temperature decreased gradually. Low moisture and reduced pH are the important factors contributing to the self-stability of the pickles (Frazier and Westhoff 1984). Changes in the pH of fish muscle have been considered one of the causative factors in the denaturation of fish protein during frozen storage (Shimizu and Fujita, 1985). On the other hand, for TVB-N content a clear increasing trend was observed with the progress in storage but the rate of increment was very slow. Khanipour *et al.* (2014) reported a slight increase of TVB-N content in their sample at the 120 days of frozen storage. Dhar and Karthikeyan (2014) reported that, TVB-N content increased with the storage period of pickle

from small freshwater prawn *Macrobrachium dayanum* which coincides with the present study. The increase in TVB-N with the lapse of storage might be attributed by bacterial spoilage.

Total bacterial count found to show a gradual reduction during storage of fish pickle under frozen temperature. In the case of freezing, the idea is to stop bacterial action altogether. That is why aerobic plate count (APC) decreased in the present study. Abraham *et al.* (1996) reported that the bacterial population of fish pickles are salt and acid tolerant. They stated that freezing of fish at  $-18^{\circ}\text{C}$  is unfavorable for the growth and the survival of the microorganisms. Abraham and Setty (1994) found significantly reduced bacterial growth by using sodium benzoate (0.1%) in prawn pickle.

The changes in biochemical and microbiological parameters of fish pickle prepared with mola showed that at refrigeration and frozen temperatures pickle may retain an acceptable condition until 120 days and 300 days, respectively. The study on consumer preference to fish pickle revealed that, most of the respondents liked the product and scored as very good which indicated the prospect of mola fish pickle as a nutritive ready-to-eat product.

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