Storage Effects of Low Temperature on the Quality Parameters of Mince Based Fish Stick Prepared from Silver Carp (*Hypophthalmichthys molitrix*)

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Abstract
It is important to preserve the fish products in proper storage condition until further use. Keeping this point in view, fish stick prepared from silver carp (*Hypophthalmichthys molitrix*) mince by using different kinds of food additives were stored at refrigeration (5 to 8°C) and frozen temperatures (-18 to -20°C) temperature. The results showed that, on "0" day the sensory quality criteria score was 9.00±0.00[a] for general appearance which decreased to 8.70±0.39[a] after 3 days of storage at refrigeration temperature and to 8.49±0.41[a]at frozen temperature after 56 days of storage. The TVB-N values were found 22.54±0.17[a]and 16.04±0.39[a], respectively at refrigeration and frozen temperatures which was 7.84±0.95c on "0" day. The peroxide value of the product on "0" day was 6.19 ± 0.71[e]and the microbial load was 1.49 x10^4 CFU/g which reached to 19.94 ± 0.17[a], 21.58 ± 0.78a meq/kg and 2.13 x10^7 CFU/g, 1.12 ×10^2 CFU/g after storage at refrigeration and frozen temperatures. By observing these results present study could be concluded as-frozen temperature storage is comparatively better than refrigeration temperature for storing minced based 'ready to cook' products like fish stick and at this temperature the product can be stored for about 8 weeks.

Key words: Fish stick, Low temperature, Quality parameters, Silver carp, Storage effects

Introduction

In our diet, fish is a good source of animal protein. Undoubtedly fish is playing a significant role in overcoming the protein malnutrition in Bangladesh. The digestibility of fish protein is 85-95% and all dietary essential amino acids are present in fish flesh (Nelson, 1946). Fish protein generally tends to be higher in lysine content than that of meat. Besides protein, fish muscles also contains essential minerals like calcium, potassium, sodium, phosphorus, magnesium which are found in large quantities. Due to increasing awareness of the consumers on health issues, consumption of fish and fishery products are increasing day by day. Therefore, it is very important to develop new techniques of processing of underutilized protein resources to make them useful and palatable for human consumption. Considering these points, fish mince is a sort of fish product which has drawn much attention. Mince based value added products will bring immediate benefit to the existing fish processing industries of the country (Nowsad *et al.*, 1994). Institute of Food Science and Technology defines (1993) shelf life as "the period of time during which the food product will remain safe; be certain to retain its desired sensory, chemical, physical, microbiological and functional characteristics; where appropriate, comply with any label declaration of nutrition data, when stored under the recommended conditions". Determination of bacterial population of fish and fishery products are very important to assess the acceptable level and the extent of spoilage as well as contamination. Some public health authorities recommended that fish products must contain no more than a described number of bacteria of particular time in a unit weight area. Fish of good quality should have bacterial count less than 105 per gram (Pal *et al.*, 2016).

Frozen storage can be very useful for long time preservation of the products. In frozen storage rapid reduction in the microbial load can be explained initially due to the effect of freezing and antimicrobial properties of food additives used in products (eg. garlic). Garlic's ability to destroy microorganism is well documented in many studies. (Arora and Kaur, 1999; Beuchat *et al.*, 1994).By last few years several exotic carps are introduced in order to obtain more production at minimum cost and within the shortest possible period. They are now being cultured combinedly with the local carps. Among those, silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*); grass carp (*Ctenopharyn godonidella*), silver bard (*Puntius gonionotus*) are the most popular species. Among the Chinese carps, silver carp has got popularity due to its fast growth and unique food habit. They are mainly phytoplankton feeder and can control water body from obnoxious bloom formation. Moreover production of silver carp relatively more than other carps (i.e. rohu, catla, mrigal etc.) but market value is less than these carps.

As in recent years the preference of the consumers is directed towards the fast food consumption since there has been a rapid urbanization and an increase in working women, studies on the production and quality of the fishery products (fish cake, fish balls, fish stick and fish burgers etc. have been increased (Herborg, 1976; Sipos *et al.,* 1979; Siaw *et al.*, 1985; Choi *et al.*, 1988 and Lazos, 1996).

The technology for producing fish sticks is very simple and it requires less complex machinery. Therefore,

beside the commercial producer, coastal and rural people can also produce the products in homestead kitchen using kitchen utensils for domestic consumption as well as for marketing locally. The products can be stored at homestead refrigerator even. Therefore, the risk involvement is less in adopting the technology. Considering the above facts, the present study was, therefore, conducted to prepare fish sticks from silver carp mince and to observe the changed in sensory, chemical and microbiological parameters during storage at homestead refrigerator both at refrigeration and frozen temperatures for about 8 weeks.

Materials and Methods

Selection of fish species

Silver carp (*Hypophthalmichthys molitrix)* an exotic fish, was selected for the preparation of fish sticks. Silver carp was introduced in Bangladesh from China in 1969 (DoF, 2005).It is a fast growing dominant underutilized species and abundant throughout the year.

Collection of fish species

Fresh silver carp was collected from Kamal Ronjit (K.R) Market with crushed ice in an insulated box (Cosmos Ltd., Seoul, Korea, 20 kg capacity)and transported to the laboratory of the Faculty of Fisheries, Bangladesh Agricultural University. The average size of the fish was 32± 2.50 cm and 750± 0.35 gm.

Preservation of raw materials

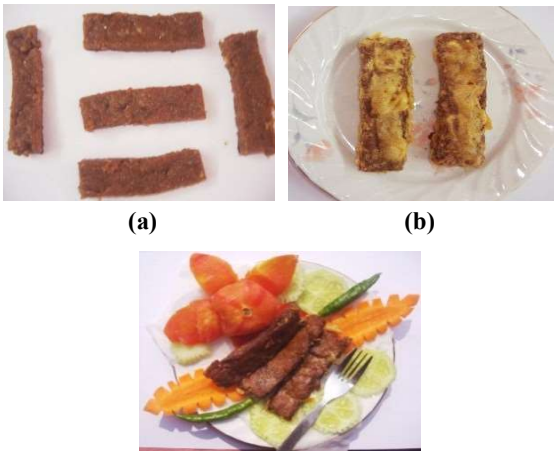
After bringing to the laboratory, the fishes were washed thoroughly, either minced immediately for use or packed in polyethylene pack and then frozen stored in a deep freezer (-18 to -20°C).

Preparation of fish mince

The fish stick was prepared in two steps. In the first step, mince was prepared from raw fish and then fish sticks were prepared from mince. The fishes were weighed and then washed with clean water, beheaded, eviscerated, skinned and washed with chilled water. The skinned fishes were filleted and deboned manually in iced condition. Then mince was prepared by a mechanical mincer through a 1mm orifice diameter so that all bones and connective tissues were removed from the muscles. All the utensils used in the experiment were cleaned with adequate washing and kept cool (5 to 6°C). Mince recovery from each fish was recorded. Huge amount of crushed ice was made available through an ice maker (Lab Tech Ice Macker, Series L cm-200m, R4044A, UK) to maintain adequate temperature throughout the product preparation. After mincing, the mince was kept in a small bowl that is fixed in a big plastic bowl around which huge amount of ice was kept.

Preparation of the fish stick from the mince

The ingredients used for the preparation of fish stick were: fish mince (55.0%); 10% of potato smash(during trial of fish stick preparation 0, 10, 15, 20% potato smash were used but 11 member's panelists team suggested to use 10% potato smash), wheat flour; NaCl (1.5%); MSG (1.0%); spices (onion + garlic + ginger + green chili paste = 5.0%); sugar (1.0%); and chilled water (15%). All these ingredients in appropriate quantities were thoroughly mixed together by hand wearing hand gloves and dough was made. The dough was formed into flat block in a frame and cut into small pieces of 3 inch × 1 inch × ½ inch (l×w×d). The fish stick was kept under chilled condition until used for further experiment. The battering solution was prepared by mixing egg white, spices, salt and MSG. The fish sticks were dipped into the battering solution and rolled in bread crumb. Battered and breaded fish sticks were then dip fried in soybean oil until the surface of the stick become golden brown color. Finally, prepared fish sticks were then kept on the paper towels in order to soak the extra oil form the surface of the fried fish sticks (Plate 1).



(a) **(b)**

(c)

Plate 1. Preparation of fish stick for serving (where, a- fish stick block; b- fish stick after dip frying in vegetable oil and c- ready to eat fish stick)

Quality analysis

Sensory evaluation

For the determination of sensory quality of the fish sticks scoring test was used (Paulus *et al*., 1979). All fish sticks were served to 11 panelists to evaluate the sensory attributes (color, odor, taste, texture, general acceptability) of the samples by using 9-points descriptive scale. According to the scoring table, scores between 7-9 indicated 'high quality', scores between 4-6 indicated moderate quality' and scores between 1-3 indicated the limit of 'unacceptability'.

Chemical evaluation

Total Volatile Base- Nitrogen (TVB-N) value determination-

Total Volatile Base Nitrogen (TVB-N) was determined according to the methods given in AOAC (1984) using the following formula:

$$Amount of TVB - N\ (mg/100g Sample = \frac{ml titrant \times\ 0.014\ \times normality of acid}{Sample wt.} \times 100$$

Peroxide value estimation

For the estimation of peroxide value, lipid was extracted from the mince and products according to Bligh and Dyer (1959).The peroxide value was calculated using the following formula:

Peroxide value = 2 (S-B)/W, meq./kg. of oil.

Where,

S = Sample titrate
B = Blank titrate
W = Weight of sample oil in g

Determination of microbial load

According to International Standard Organization (ISO, 1965) APC was calculated using the following formula:

APC/g = C × D × 10/S CFU/g

Where,

C = Number of colonies found
D = Dilution factor
S = Weight of sample in grams
CFU = Colony forming unit

Statistical analysis

One-way analysis of variance and the general linear model using Windows for SPSS 9.0 were used to analyze the data. The Duncan's New Multiple Range Test (DMRT) was used to find the significant differences between storage periods.

Results and Discussion

Changes in quality parameters of fish sticks at refrigeration (5 to 8°C) and frozen (-18 to -20°C) temperature

Sensory evaluation

One of the objectives of this study was to observe the changes in sensory quality parameters of fish stick during storage at refrigeration (5 to 8°C) and frozen (-18 to -20°C) temperature for a certain period. The changes in color, odor, taste, texture and general appearance of fish stick at these two temperatures are shown in Table 1. All the sensory attributes decreased with the progress of storage period ($p<0.05$) at both temperatures. At frozen temperature (-18 to -20°C) the product was more stable than at refrigeration temperature (5 to 8°C). At refrigeration temperature, the color, taste and general appearance did not changed markedly even after 72 hours ($p>0.05$) of storage, only a slight pungent odor was felt which was not considered as unacceptable. The textural quality changed significantly ($p<0.05$) on 2nd (6.70 ± 0.58^{b}) and 3rd days (4.90 ± 0.98^{c}) of storage but also considered as acceptable. During storage at frozen temperature the color, texture and general appearance of fish stick did not changed significantly during storage but at the 14th , 26thand 42nd days values for the odor and taste of fish stick showed little variation though these changes were not significant ($p>0.05$). The values for odor varied between $8.82\pm0.39^{ab}$to $8.57\pm0.31^{ab}$and $8.80\pm0.40^{ab}$to $8.68\pm0.10^{ab}$for taste, At the end of the storage period at frozen temperature, the sensory quality criteria scored quite near to those values obtained on "0" day. Ejaz *et al.* (2009) investigated the changes in quality of pangus burger during frozen storage at -18°C for over 3 months. He found that the sensory quality parameters decreased gradually throughout the storage period but none of the sensory quality parameters were beyond the acceptable limit. Pawar *et al.* (2013) studies the effect of frozen storage on biochemical and sensory quality changes of fish cutlets, made from fresh water fish catla (*Catla catla*). For sensory evaluation of cutlet kept in frozen storage he found, slight decrease in overall acceptability when storage period increased from 0 to 180 days. The cutlet kept in frozen stored was not in acceptable condition after 150 days. This may be due to formation of some volatile low molecular weight compounds, lipid oxidation and protein degradation during chilled and frozen storage (Undeland and Lingnert, 1999). In another study (Joseph *et al.*, 1984) on raw and flash fried cutlets prepared from lizard fish, threadfin bream, jew fish and miscellaneous fishes, lizard fish cutlet showed highest acceptability. The raw cutlet had storage life of 6 days, 11 weeks and 19 weeks at 4°C, -8°C and -20°C, respectively. The flash fried cutlets had shelf life of 22 weeks at -20°C. The flash fried cutlets were superior in organoleptic quality compared to raw cutlet during early stage of storage and at the end of storage both had almost same rating. For fish fingers prepared from croaker and perch and stored at frozen temperature were found acceptable by the panelists up to 22 weeks at -20°C, reported by Reddy *et al.*(1992).The trend in losing some points in different sensory quality parameters even at low temperature with the progress of storage period mentioned above are similar to the findings of the present study.

Table 1. Changes in sensory quality parameters of fish stick prepared from silver carp minceduring storage at refrigeration (5 to 8°C) and frozen (-18 to -20°C) temperatures

Storage temperature (°C)	Storage period (Days)	Color	Odor	Taste	Texture	General appearance
(5 to 8)	0	9.00±0.12^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.87^{a}	9.00±0.00^{a}
	1	8.60±0.34^{a}	8.85±0.45^{ab}	8.89±0.23^{a}	8.60±0.05^{a}	8.92±0.40^{a}
	2	8.50±0.15^{a}	8.80±0.11^{ab}	8.78±0.58^{a}	6.70±0.58^{b}	8.78±0.07^{a}
	3	8.40±0.46^{a}	8.30±0.50^{b}	8.41±0.13^{a}	4.90±0.98^{c}	8.70±0.39^{a}
(-18 to -20)	14	8.85±0.40^{a}	8.82±0.39^{ab}	8.80±0.40^{ab}	8.90±0.17^{a}	8.73±0.21^{a}
	28	8.79±0.21^{a}	8.70±0.12^{ab}	8.73±0.21^{ab}	8.89±0.21^{a}	8.61±0.76^{a}
	42	8.67±0.37^{a}	8.57±0.31^{ab}	8.68±0.10^{ab}	8.80±0.57^{a}	8.58±0.36^{a}
	56	8.60±0.15^{a}	8.42±0.21^{b}	8.53±0.20^{b}	8.75±0.31^{a}	8.49±0.41^{a}

**Each value is mean ± standard deviation of triplicate determinations.*
**Data bearing different superscripts in the same column differ significantly (p<0.05)*

Chemical evaluation

TVB-N value analysis

Total volatile base nitrogen (TVB-N) is an important compound provides a measure of the progress of spoilage. In seawater fish TVB-N comes from ammonia, trimethylamine, and dimethyl-amine, while in fresh water fishes and in the products prepared from fresh water fishes, most of the TVB-N comes from ammonia (Clucas, 1982). Connell (1980) suggested that 30 mg TVB-N/100g are usually regarded as the acceptable limit for fresh fish. The limit beyond this fresh fishes can be considered as too spoiled. He also suggested for a variety of salted and dried fish, TVB-N value should not exceed more than 100-120 mg/100g. The changes in TVB-N value of silver carp fish sticks are presented in Table 5. In this study at refrigeration temperature the values were found lower (22.54 mg/100g) than the acceptable limit even after 72 hours of storage. Ejaz *et al.* (2009) also found in a study, a gradual increase in TVB-N value in fish burger from 6.38 ± 0.13^{d} to 18.56 ± 0.59^{a}within 72 hours at refrigeration temperature which is quite nearer to the present study. During frozen storage also the TVB-N value increased with the progress of storage period. Within 56 days of frozen storage the TVB-n value of fish stick increased from 7.84±0.95^{c}to 16.04±0.39^{a}indicating a slower rate of increase in the value (Table-5). Siddaiah *et al.* (2001) studied the changes in lipids, proteins and kamaboko forming ability of silver carp (*Hypophthalmichthys molitrix*) mince during frozen storage and they observed that, the total volatile base nitrogen (TVB-N) contents increased significantly (P⩽ 0.05) throughout the storage period. Ravendra and Amjad (2005) carried out a study on the characteristics of pink perch (*Nemipterus japonicus*) surimi at frozen temperature. In their investigation they found that- Total volatile base nitrogen (TVBN) increased gradually from 3.5 ± 0.1 to 5.60 ± 0.17 mg% in 36 weeks. The increasing trend in the value of TVB-N observed in the above mentioned studies is in accordance with the present investigation.

Table 2.Changes in TVB-N (mg/100g) and peroxide (meq/kg) values of fish stick prepared from silver carp mince during storage at refrigeration (5 to 8°C) and frozen (-18to -20°C) temperatures

Storage temperature (°C)	Storage period (Days)	TVB-N values (mg/100g)	Peroxide value (meq/kg)
(5 to 8)	0	7.84±0.95^{c}	7.84±0.95^{c}
	1	8.42±0.31^{c}	7.84 ±0.31^{c}
	2	14.02±0.49^{b}	12.53 ±0.49^{b}
	3	22.54±0.17^{a}	19.94 ±0.17^{a}
(-18 to -20)	0	7.84±0.95^{c}	7.84±0.95^{c}
	14	7.60±0.27^{d}	11.00±0.03^{d}
	28	9.51±0.87^{c}	15.17±0.59^{c}
	42	13.71±0.95^{b}	19.02±0.21^{b}
	56	16.04±0.39^{a}	21.58±0.78^{a}

**Each value is mean ± standard deviation of triplicate determinations.*
**Data bearing different superscripts in the same column differ significantly (p<0.05)*

Peroxide value analysis

The changes of the peroxide value during refrigeration and frozen storage of fish sticks is shown in the Table 2. Oxidation of lipid is another important factor for the quality degradation of fish and fishery products which can be assumed by the changes in peroxide value. In the present study, according to Table2, the peroxide value increased significantly (p<0.05) throughout storage from day"0" (7.84±0.95^{c}) and reached to 19.94 ± 0.17^{a}meq/kg 3 days later at refrigeration temperature and 21.58 ± 0.78^{a}meq/kg at the end of storage period of 56 days at frozen temperature. For peroxide value (PV) in frozen stored cutlet Pagarkar *et al.* (2013)observed an increased from 2.6 to 5.2 meq of O_2/Kg respectively after 105 days of storage. Kamat (1999) observed that, peroxide value of mackerel mince cutlet prepared from bleached and unbleached surimi increased gradually from an initial

value of 2.8 and 3.4 to 30 and 40 meq of O_2/Kg at the end of 9 and 5 weeks period of storage at -14°C, respectively. In another study, Joseph *et al.* (1984) reported a decreasing trend of peroxide value in both flash fried and raw cutlets. The peroxide value of flash fried cutlets reduced from 8.16 to 5.81, 1.59 and 4.50 meq of O_2/Kg and in raw cutlets the value decreased from 9.5 to 6.23, 3.98 and 6.22 meq of O_2/Kg at 4°C, -8°C and -20°C, respectively. The cutlet was highly spiced and the anti-oxidant properties of spices might cause the further reduction of peroxide formation (Zain, 1980). Ninan *et al.* (2008) observed gradual increase in peroxide value of tilapia (*Oreocromismos sambicus*) fish cutlet up to 12-15 weeks in frozen storage and thereafter the value decreased. Battering and breading of the products can act as oxygen barrier, which might prevented the oxidation. During preparation of cutlets inclusion of spices which has strong anti-oxidant effect might have increased the frozen storage stability of the mince (Joseph *et al.*, 1992).

Aerobic plate count

Changes in the bacterial load of fish stick stored at refrigeration temperature and frozen temperature is shown in Table 3. In the rate of bacterial growth at refrigeration temperature during storage of 24 hours showed no big change, after that period the rate of bacterial growth gradually increased ($p<0.05$). After 72 hours/3 days of storage the APC reached to 2.13×10^7 CFU/g from 1.49 $x10^4$in fish stick. Bashar (2004) observed that the bacterial growth in fish sticks at refrigeration temperature was slower and even after 24 hours no appreciable change in APC was observed. Shammi (2005) found that bacterial growth in fish ball, fish stick and fish sausage gradually increased throughout the storage period, both at room and refrigeration temperature. She concluded that the shelf life of the products is very short and not more than 24 hours at room temperature and 48 hours at refrigeration temperature. Ejaz *et al.* (2008) also found that- bacterial growth in fish burger gradually increased throughout the storage period, both at room and refrigeration temperature. They concluded that, the shelf life of fish burger is very short, not more than 24 hours at room temperature and 72 hours at refrigeration temperature. On the other hand, during storage at frozen temperature the bacterial growth in fish stick was determined at every 2 weeks interval in order to monitor the changes in APC (Table 3). The initial bacterial count of the sticks was 1.49 $x10^4$CFU/g. After 14 days/2 weeks of storage APC decreased a little to 1.00×10^4. Throughout the frozen storage at -18 to -20°C, the initial microbial load reduced gradually ($p<0.05$) and at the end of storage period of 56 days, bacterial load of fish stick reduced to 1.12×10^2 CFU/g. Total viable count is an important criterion for quality evaluation. The maximum of 10^7 CFU/g is recommended for the acceptability of fresh and frozen fish by International Commission of Microbiological Standards for Foods (ICMSF, 1978).In the present study it was found that the bacterial load of the product was well below than the maximum recommended level by ICMSF (1978). The results of the present study showed the same phenomena, that the number of viable count of bacteria in the frozen fish sticks gradually decreased with the increase of storage period.

Table 3. Changes in bacterial load (CFU/g) of fish stick prepared from silver carp mince during storage at refrigeration (5 to 8°C) and frozen (-18 to -20°C) temperatures

Storage temperature (°C)	Storage time (Days)	Bacterial load	
		CFU/g	Log CFU/g
(5 to 8)	0	1.49 $x10^4$	4.17 ± 0.31^b
	1	6.00 $x10^4$	4.78 ± 0.78^b
	2	2.10 $x10^5$	5.32 ± 0.22^b
	3	2.13 $x10^7$	7.33 ± 0.83^a
(-18 to -20)	0	1.49 $x10^4$	4.17 ± 0.31^b
	14	1.00×10^4	4.00 ± 0.07^d
	28	3.79×10^3	3.58 ± 0.03^c
	42	5.10×10^2	2.70 ± 0.25^b
	56	1.12×10^2	2.05 ± 0.21^a

**Each value is mean ± standard deviation of triplicate determinations.*

**Data bearing different superscripts in the same column differ significantly ($p<0.05$)*

Conclusion

Therefore, from the obtained results, the present study could concluded that-the sensory, chemical and microbiological parameters of mince based fish stick prepared from silver carp (*Hypophthalmichthys molitrix*) remain acceptable comparatively longer period (about 8 weeks) at frozen temperature than stored at refrigeration temperature.

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