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Effect of storage temperatures on the quality parameters of fish condiment prepared from Thai pangus (*Pangasianodon hypophthalamus*)

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ABSTRACT

Quality parameters of a fish/food product changes with the storage temperature. Therefore, the present study was carried out to evaluate the effect of different storage temperatures on the changes in chemical and microbiological parameters of fish condiment prepared from Thai pangus (*Pangasianodon hypophthalmus*). The study was conducted in the Department of Fisheries Technology Laboratory from October 2015 to September 2016.Standard methodologies for product preparation and other analyses (chemical and microbiological) with some modifications were followed. The results of the experiment showed that- irrespective of storage temperature the TVB-N value increased progressively with the lapse of storage period. At room temperature (28°C to 32°C), the values increased very rapidly in compare to those of refrigeration (5°C to 8°C) and frozen temperature (-18°C to -20°C). The TVBN value increased from 1.63±0.01 to 3.31±0.06, 3.18±0.02 and 2.02±0.02 mg/100g on day 15th at room, 90th at refrigeration and 120th at frozen storage temperature, respectively. On the other hand, the peroxide values increased from 2.80±0.10 to 6.08±0.10, 6.97±0.20 and 5.40±0.20 meq/kg of oil, on 15th at room, 90th at refrigeration and 120th at frozen storage temperature, respectively. Throughout the storage period, the pH values of fish condiment also changed at different temperatures. The pH value decreased from 5.24±0.01 to 4.75±0.05, 4.51±0.11 and 4.49±0.90, respectively on day 15th at room, day 90th at refrigeration and day 120th at frozen storage temperature. The bacterial load (CFU/g) in condiment was found to increase at room temperatures (from 2.2 ×10^4 to 2.6×10^7). However, the growth of bacteria was slower at refrigeration temperature (from 2.2 ×10^4 to 2.5×10^7) and at frozen temperature bacterial growth found negative (from 2.2 ×10^4 to 3.6×10^2). So, from the observation on the changes in different quality parameters at different storage temperatures, it could be concluded that, the shelf life of Thai pangus fish condiment at room temperature was shorter but at refrigeration temperature fish condiment may remain in acceptable condition more than 90 days and more than 120 days at frozen temperature.

Introduction

As fish is highly perishable, proper processing, packaging and preservation helps in maintaining the quality of fish and fishery products. Consumers demand is high quality processed foods with minimal changes in nutritional and sensory properties (Ahvenainen, 2003). A 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). Proper storage conditions are essential to prevent the spoilage of fish and fishery products. Many emerging technologies have the potential to extend the shelf life (Nagarajarao, 2016).

Preservation of fish can be achieved by various methods, i.e., refrigeration, freezing, salting, canning, icing, smoking, glazing, drying, frying, etc. Therefore, the quality of fish processed by the various methods cannot be the same and hence its subsequent effect on the fish's shelf life also varies and nutritional compositions of fish (Al-Reza *et al*., 2015). Moreover, refrigeration is easy for application and storage conditions, neither adding nor removing any ingredients for fish. Refrigeration inhibits the activities of organism's in food as well as slows down the biochemical/enzymatic reactions. Freezing kills some but not all of the microorganisms present and growth will take place after thawing if time permits (Sayed *et al*., 2013).

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Quality parameters of fish condiment

Pangus (*Pangasianodon hypophthalmus*), also known basa catfish is one of world's fastest growing types of aquaculture species and having good demand in the international market. This species of fish is extensively cultured in Bangladesh because of suitable climatic condition. The growth and production rate of Pangas showed a significant amount 2,87,032 MT in the year 2013 (DoF, 2013). So, proper culture, processing and preservation of this fish is needed for high demand of protein obtain from fish. There are some products which have been developed from pangas fish like- fish burger, pickle, fillet, patties, fish ball etc. Fish condiment is also a new kind of product. For better utilization and new type of product development, fish condiment made from pangas has good prospect. There is no published literature yet on the fish condiment prepared with pangas fish and on it's quality changes. In recent year's value addition have received a wider attention because of increased urbanization. There is a growing demand for value-added products due to social and cultural changes (Pagarkar *et al*., 2011).

A study carried out by Shikha *et al.* (2019) on the changes in the nutritional composition of fish condiment prepared from Thai Pangus (*Pangasianodon hypophthalmus*) during storage at low temperature for longer period showed that- the moisture content (%) decreased at refrigeration and frozen temperature from 53.57 to 43.56 and 54.11 to 42.33, respectively from initial month of storage to final month of storage. Similarly, protein content (%) decreased from 24.05 to 18.31 and 23.96 to 16.97, respectively throughout the storage period. Lipid content (%) increased up to seven month of storage and then decreased gradually at refrigeration temperature while at freezing temperature it increased gradually during the whole storage period. Ash content (%) increased from 4.53 to 7.91 and 4.34 to 8.25 at refrigeration and frozen storage, respectively.

Therefore, considering these points the authors from the same research group designed the present study to prepare fish condiment with pangus fish muscle and to observe the changes in quality parameters of fish condiment at various storage temperatures.

Materials and Methods

Sample collection and experimental condition

Fresh Thai pangus (*Pangasianodon hypophthalmus*) fishes were collected from Kamal- Ranjit (KR) Market of Bangladesh Agricultural University (BAU), Mymensingh. Total 15 fishes were collected having weight from 1.2 to 1.5 kg. The experiments were carried out in the laboratories of the Department of Fisheries Technology, Faculty of Fisheries, Bangladesh Agricultural University (BAU) for a period of 12 months from October 2015 to September 2016.

Ingredients for fish condiment

The fish condiment was prepared from the collected fish according to the method described below. The standard recipe for the preparation of pickle and condiment are given in Table 1.

Table 1. Standard recipe for fish condiment preparation (according to Shikha *et. al.*, 2019)

Ingredient	Amount (g)	Ingredient	Amount (g)
Fish muscle	500 g	Vinegar	50 ml
Chili powder	30 g	Black pepper	2 g
Turmeric powder	5 g	Panch phoron*	5 g
Cumin	10 g	Sugar	50 g
Onion	30 g	Salt	30 g
Garlic	20 g	Tomato sauce	30 g
Ginger	10 g	Tamarind	30 g
Cloves	2 g	Sodium benzoate	1 g
Mustard oil	80 ml		

* Mixture of five spices such as cumin, brown mustard, fenugreek, nigella and fennel.

Fish condiment preparation procedure:

The fishes were thoroughly washed, cut into large pieces (2-3 pieces/fish) using sharp knife and repeatedly washed with tap water in the laboratory to remove bloods and contaminants. The pieces were marinated in a small amount of vinegar for 5-10 minutes. Then boiled in water and bones, skin were separated from the muscles. The muscles were blended and fried in mustard oil. The ingredients were fried and then mixed with the fried muscles. The mixture of fried fish muscle and ingredients were heated till vinegar was absorbed. The detail procedure is shown in following pictorial diagram (Plate 1).

Sample storage

Fish condiment was packed in plastic bottles. A total of 12 bottles of condiment were stored for quality analysis. Four bottle of condiment samples were stored at room (28° to 32°C) temperature, four bottles at refrigeration temperature and another four bottles at frozen temperature in a domestic refrigerator. In a domestic refrigerator the temperature usually varied between 5°C and 8°C, whereas frozen temperature varied from -18°C to -20°C.

Quality analysis

At room temperature storage, the quality parameters of fish condiment samples were analysis at each 3 days interval. On the other hand, the quality parameters of the condiment samples stored at refrigeration temperature were analyzed initially at 7 days interval.

Plate 1. Fresh fish; B- Fish cut into smaller pieces; C- Soaked in vineger for few minutes; D- Boiling in water; E- Boiled fish; F- Desking and deboning of fish; G- Deskined and deboned fish mucle; H- Frying of fish mucle; I – Fish muscle and other ingredients in frypan; J – Ready to serve or pack fish condiment; K and L – Packing of fish condiment; M and N – Fish condiment in sealed plastc packs; O – Fish condiment in plastic bottles

Then they were analyzed around 15 days interval whereas the analysis of quality parameters of the condiment samples stored at frozen temperature was done initially at 15 days interval and then 30 days interval. It was observed that at low temperatures the changes in quality parameters were slower than those of at room temperature, therefore it was decided to extend the time of interval to analyze the samples stored at low temperatures). Triplicate samples were taken to carry out the experiment.

Determination of total volatile base nitrogen (TVB-N)

To evaluate the changes in protein quality of fish condiment, TVB-N values were measured. The TVB-N value was determined according to the methods given in AOAC (1984) with certain modification as follows-

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

Determination of peroxide value

The peroxide value of fish condiment was determined according to the method described by Egan *et al.* (1981). One gm of sample oil was weighed accurately into a stoppered 250 ml conical flask and 20 ml of chloroform was then added to dissolve the lipid. The flask was then shaken for 30 seconds. A volume of 50 ml of a mixture of acetic acid and chloroform in the ratio of 3:2 was added. One ml of saturated aqueous potassium iodide was added and the flask was swirled for about 20 seconds and kept in the dark for 30 minutes. After that period, 100 ml of distilled water was added and liberated iodine was titrated against 0.002 M sodium thiosulphate ($Na_2S_2O_3.5H_2O$). Freshly prepared 1% starch solution was used as an indicator. The peroxide value was calculated as follows:

Peroxide value=2(S-B)/W meq/Kg of oil

where, S is sample titre; B is blank titre and W is weight of sample oil in gm.

Determination of pH value

The pH of fish condiment samples was measured at room temperature following the method described by AOAC (2005). At first accurately 5g sample was taken and mixed homogeneously in 50 ml distilled water. The pH was measured using an electronic pH meter (HANNA pH 211 Microprocessor pH Meter) with a glass electrode using an expandable scale.

Determination of aerobic plate count (APC)

The APC of fish condiments samples was calculated in terms of colony forming units (CFU) after counting the colonies of the agar plate 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 the following formula:

APC (CFU/g)=(No. of colonies on petridish × Dilution factor × Vol. of stock solution × 10)/(Wt. of pickle or condiment sample)

Statistical analysis

Data from different biochemical measurements were subjected to statistical analysis to know variance between different answers and the significant level of those results. Data were processed using Microsoft Office Excel 2007. Data are represented within the view of table form.

Results

Changes in TVB-N value

The TVB-N values of the fish condiment showed an increasing trend throughout the storage period irrespective of storage temperature (Table 2). The initial TVB-N value of condiment was 1.63±0.01 mg/100 g during storage at room temperature, which gradually increased with the lapse of storage period and reached to 3.31±0.06 mg/100g on the 15th day. In the case of storage at refrigeration temperature, TVB-N value increased to 3.18±0.02 mg/100 g on the 90th day whereas at the frozen storage temperature the value reached to 2.02±0.02 mg/100 g on the 120th day (Table 2).

Changes in peroxide value

The initial peroxide value of condiment found 2.80±0.10 meq/kg of oil during storage at room temperature, which increased gradually with the lapse of storage period. At the end of 15 days of storage at room temperature, the peroxide value increased to 6.08±0.10 meq/kg of oil. On the other hand, peroxide value of condiment stored at refrigeration temperature gradually increased to 6.97±0.20 meq/kg of oil on 90th day of storage from 0.27±0.08 meq/kg of oil (Table-2) whereas the value obtained 5.40±0.20 meq/kg of oil on 120th day at frozen storage temperature (Table 2).

Changes in pH value

The pH value of condiment was found 5.24±0.01, just after preparation. While fish condiment stored at room temperature the pH value decreased to 4.75±0.05 within 15 days. In case of refrigeration temperature, on the 90th day of storage, the value decreased to 4.51±0.11 whereas the pH value decreased to 4.49±0.90 at frozen temperature on 120th day of storage (Table 2).

Changes in APC (CFU/g)

The changes in bacterial loads of fish condiment during storage at room temperature shown are in Table-4. At this temperature, the initial bacterial load found 2.2 ×10^4 CFU/g which increased gradually within 15 days of storage to 2.6×10^7 CFU/g. In case of refrigeration temperature, bacterial load reached to 2.5×10^7 from its initial value 2.2 ×10^4 CFU/g within 90 days of storage. On the other hand, during frozen storage of 120 days, the bacterial load of fish condiment found 3.6×10^2 (Table 2).

Discussion

The total volatile base nitrogen (TVB-N) value

Conell (1980) and Pearson (1997) reported that samples could be considered consumable if the TVB-N level is less than 20mg/100 g fish and that a level of more than 30 mg determines the product is not consumable. In the present study, the TVB-N increased progressively throughout the storage period at room temperature (28°C to 32°C) which was similar to the changes of Total volatile base nitrogen (TVB-N) content below the range suggested by various researchers for fish and fish products (Kumar *et al*. 2013). Sarojnalini and Suchitra (2009) found total volatile base nitrogen (TVBN) were within the acceptable limit for Ngari (a fermented fish product of Manipur), prepared in large-scale industrial products at 30°C in laboratory conditions which was similar to the present study. Hossain *et al*. (2005) evaluated physicochemical changes in Thai Pangus muscle during ice storage in an insulated box which showed that the initial TVB-N value 1.37 mg/100g continuously increased with the lapse of storage period which was more similar to present finding. At refrigeration temperature (5°C to 8°C) the TVB-N value increased slowly up to 45 days after that the increasing rate was sharp which was similar to the smoked product of gilthead Seabream (*Sparus aurata* L., 1758), during storage at 4°C (Bilgin *et al*., 2008). In case of frozen storage (-18°C to -20°C) also a similar increasing trend in TVB-N value of fish condiment was observed. Khanipour *et al*. (2014) found that TVN-B slightly increased within the 120 days at frozen storage thereafter, it increased sharply for a new product from Kilka with tempura batter which is similar to the present study. Rathod and Pagarkar (2013) evaluated the shelf life of fish cutlet prepared from Pangasius (*Pangasianodon hypophthalmus*) fish on the basis its storage at frozen display unit (-15°C to -18ºC), resulted a rising trend in TVB-N which is quite similar to the present study. Dhar and Karthikeyan (2014) reported that- TVB-N increased with the lapse of storage period of pickle from small freshwater prawn, *Macrobrachium dayanam* which matches with the finding of the present study.

The peroxide value

Peroxide value (PV) is a measure of the degree of oxidation in the fat (Govindan, 1985; Gopakumar, 2002). According to Connell (1995) acceptable limit of peroxide value 10-20 meq per kg of fat. Fresh oil usually has PV well below 10 meq/kg and a rancid taste may be noticed when PV is between 20 and 40 meq/kg fat (Egan *et al*. 1981).

Table 2. Changes in TVB-N, peroxide value, pH and bacterial load of fish condiment during storage at three different temperatures

Storage temperature	Storage (Day)	TVB-N value (mg/100g)	Peroxide value (meq/Kg of oil)	pH	Bacterial load (CFU/g)
Room temperature (28^0C to 32^0C)	0	1.63±0.01	2.80±0.10	5.24±0.01	2.20×10^4
	3	1.71±0.02	3.60±0.10	4.97±0.01	2.80×10^4
	6	2.03±0.02	4.20±0.30	4.96±0.02	3.40×10^5
	9	2.12±0.03	5.80±0.10	4.84±0.05	4.10×10^5
	12	2.19±0.02	5.93±1.00	4.79±0.02	1.20×10^6
	15	3.31±0.06	6.08±0.10	4.75±0.05	2.60×10^7
Refrigeration temperature (5^0C to 8^0C)	0	1.63±0.01	2.80±0.10	5.24±0.01	2.20×10^4
	7	1.66±0.02	3.01±1.00	5.06±0.06	2.90×10^4
	15	1.68±0.01	3.90±0.26	5.03±0.03	3.40×10^5
	30	1.75±0.02	4.30±1.05	4.87±0.04	5.00×10^5
	45	1.77±0.02	5.03±0.80	4.81±0.02	3.10×10^6
	60	2.63±0.01	6.20±0.08	4.61±0.01	8.10×10^6
	75	3.07±0.02	6.70±0.11	4.59±0.13	1.70×10^7
	90	3.18±0.02	6.97±0.20	4.51±0.11	2.50×10^7
Frozen temperature (-18^0C to -20^0C)	0	1.63±0.01	2.80±0.10	5.24±0.01	2.20×10^4
	15	1.64±0.02	3.40±0.40	4.97±0.12	1.80×10^4
	30	1.65±0.10	4.07±0.40	4.93±0.18	1.10×10^4
	60	1.82±0.02	5.20±0.10	4.52±0.11	9.20×10^3
	90	2.05±0.04	5.33±0.10	4.49±0.04	7.30×10^3
	120	2.02±0.02	5.40±0.20	4.49±0.90	3.60×10^2

* mean value ± standard deviation of 3 individual measurements

In this study peroxide value increased comparatively faster at room temperature (28°C to 32°C) from 2.80±0.10 to 6.08±0.10 within 15 days which is quite similar to the finding of Dhar and Karthikeyan (2014) for pickle prepared from small freshwater prawn, *Macrobrachium dayanam*. In the case of refrigeration temperature (5° to 8°C) a similar increasing trend in peroxide value was observed. Praneetha *et al*. (2015) found that- the peroxide value increased from 10.73 to 17.8 meq/kg of oil of fish finger prepared from rohu (*Labeo rohita*) fish mince and stored at refrigeration temperature for 11 days. Nayak *et al*. (2015) stored two batches of fresh patties prepared from sutchi catfish (*Pangasius hypophthalmus*) under refrigerated condition (6±2ºC) without adding any preservatives and observed that- peroxide value increased significantly with the progress in storage period. Dhar and Karthikeyan (2014) reported similar finding of peroxide value increased significantly from 26.63 to 77.35 meq O2 kg-1 during storage confirming the rancidity of fat and a similar increase in peroxide value of calm pickle during storage was reported by Gupta and Basu (1985). All these findings are quite similar to the findings of present study. In case of frozen temperature (-18°C to -20°C) storage also, the peroxide value increased throughout the storage period. Khanipour *et al*. (2014) found that- the peroxide value of a new product from Kilka with tempura batter increased throughout the frozen storage of 4 months at -18°C which is similar to the trend observed for fish condiment in this study stored at frozen temperature for 120 days. Al-Bulushi *et al*. (2005) found that- the peroxide value in frozen stored fish burger increased significantly throughout the storage period of 3 months but did not reach to the detectable levels of rancidity. Rathod and Pagarkar (2013) found a rising trend in peroxide value of fish cutlet prepared from Pangasius (*Pangasianodon hypophthalmus*) during its storage at frozen display unit (-15°C to -18ºC).

The pH value

The pH value is a reliable indicator of the degree of freshness or spoilage to assess fish and fish product's qualities (Ruiz-Capillas and Moral, 2001). The increase in pH indicates the loss of quality (Erkan *et al*., 2011). Low pH inhibits most of the bacterial activity (Sugumarand Jayasekharan, 1994; Mukundan *et al*., 1981). Bacteria only grow between a minimum pH of 4.5 and a maximum of 8–9 with an optimum of 6.5–7.5 value. Sodium benzoate has an optimum pH range between 2.5 and 4.0. For preservation of a wide variety of foods, sodium benzoate or benzoic acid is used in amounts of less than 0.1 percent (Kyi, 2002). Fish condiment stored at room temperature (28°C to 32°C), pH decreased with the progress in storage period which coincides the study of Dhar and Karthikeyan (2014). In their study the found

a significant ($p<0.01$) reduction of pH from 4.63 to 4.00 of pickle prepared with small freshwater prawn *Macrobrachium dayanam*. Kolekar and Pagarkar (2013) observed that- the pH decreased from 6.38 to 6.07 of prepared fish ball curry within the storage period of 12 days which was more or similar to the present finding. In the present study pH of fish condiment stored at refrigeration and frozen temperature decreased slowly. The pH of fish condiment stored at these temperatures was within the acceptable limit stated by Huss (1988) and Erkan *et al*. (2011). Praneetha *et al*. (2015) found, the pH of fish finger decreased from 6.95 to 6.44 prepared with mince of rohu fish (*Labeo rohita*) stored in refrigerated condition (4°C ± 1°C) for 11 days which is similar to present study. Talab (2014) observed a decrease of pH value in different cooking methods (frying, microwave and halogen oven cooking) of carp fish cutlets during frozen storage at -18°C which is similar to present study.

Aerobic plate count (CFU/g)

In the present study, bacterial load of fish condiment increased with the lapse of storage period during storage at room temperature (28°C to 32°C) and refrigeration temperature (5°C to 8°C) though the level of increment in bacterial load was slower at refrigeration temperature. On the contrary, at frozen storage condition (-18°C to -20°C) APC of fish condiment decreased gradually during storage of 120 days. Sarojnalini and Suchitra (2009) found that- the total plate count of bacteria reached up to 10^8 cfug-1 for Ngari (a fermented fish product of Manipur) and the initiation of fermentation occurred at 30°C in laboratory condition. Sanni *et al*. (2002) obtained APC of fermented fish condiment 5.8×10^7 to 4.1×10^8 $CFUg^{-1}$ which was much higher than the present study. In the case of fish condiment stored at frozen temperature, bacterial load decreased gradually with increase of storage period which was similar to the findings of Ejaz *et al*. (2009) for frozen burger prepared with pangas. A significant decrease were observed in total plate count of cooked carp fish cutlets during frozen storage at -18°C by Talab (2014). Khanipour *et al*. (2014) observed that- the microbial count reduced during frying and frozen storage for a new product from Kilka with tempura batter which was similar to the present study. Rahman (2014) found an increasing trend in the APC count (4.84×10^4 to 4.5×10^7 CFU/g) of fish condiment prepared with pangus fish stored at refrigeration temperature (5°C to 8°C) and a gradual decreasing trend in bacterial count (4.5×104 to 6.9×102 CFU/g) at frozen temperature (-18°C to -20°C) which is quite similar to findings of the present study. The reduction in bacterial count at frozen temperature of the products might be due to the reduced activity of bacteria at this temperature.

Conclusion

This study could be concluded that, the shelf life of Thai pangas fish condiment at room temperature (28°C to 32°C) is shorter due to rapid bacterial growth. On the other hand, at refrigeration temperature (5°C to 8°C), the condiment may remain in acceptable condition more than 90 days and more than 120 days at frozen temperature (-18°C to -20°C).

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