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Changes in quality parameters of fish pickle prepared from Thai pangus (*Pangasianodon hypophthalmus*) at different storage temperatures

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Abstract. Pickle from Thai pangas ((*Pangasianodon hypophthalmus*) was prepared using fresh fish muscle, various hot spices, vinegar, sugar, salt, tomato sauce and tamarind were used as ingredients. Chemical and microbiological changes in fish pickle were determined at different storage temperatures. It was observed that, the TVB-N value increased progressively throughout the storage period irrespective of storage temperature. However, in case of room temperature (28°C to 32°C), the values increased rapidly compared to those of refrigeration(5°C to 8°C) and frozen temperature (-18°C to -20°C). The TVBN value increased from 1.18 ± 0.12 to 4.29 ± 1.05, 4.45 ± 0.51 and 2.05 ± 0.04 mg/100g on day 15th at room, 73rd at refrigeration and 90that frozen storage temperature, respectively. On the other hand, the peroxide values increased from 0.27 ± 0.08 to 2.43 ± 1.46, 2.96 ± 2.01 and 1.26 ± 1.93 meq/kg of oil, on day 15th at room, 73rdat refrigeration and 90that frozen storage temperature, respectively. The pH values of fish pickle also changed throughout the storage period at different temperatures. The pH value decreased from 4.41 ± 0.26 to 3.99 ± 0.21, 4.07 ± 0.19 and 3.97 ± 0.28, respectively on day 15th at room, day 73rdat refrigeration and day 90th at frozen storage temperature. The bacterial load (CFU/g)in pickle was found to increase at room temperatures (from 2.30×10^3 to 7.20×10^7). However, the growth of bacteria was slower at refrigeration temperature (from 2.30×10^3 to 9.10×10^7) and at frozen temperature bacterial growth found negative (from 2.30×10^3 to 0.27×10^2). 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 pangas fish pickle at room temperature was shorter, in fact not more than 15 daysbut at refrigeration temperature fish pickle may remain in acceptable condition more than 73 days and more than 90 days at frozen temperature.
Keywords: Fish pickle, *Pangasianodon hypophthalmus*, TVB-N value, Microbial load.

Introduction

There are two basic methods of pickling of fish. During pickling of fish, only high-grade distilled vinegar, course pickling salt, fresh spices, and earthen crock or glass jars should be used as metal containers may cause discoloration of “tinny” taste. Fish pickle when carefully prepared under most hygienic conditions with the addition of the required quantity of salt, preservatives and spices will have generally an average shelf life of one year. While products are not immediately consumed just after preparation, might necessary to store them at the low temperature. After preparation, refrigerated and frozen storage of pickle may extend its shelf-life and limit microbial and enzymatic

activity which causes deterioration. Frozen storage has been widely employed to retain fish properties before it is consumed or employed in other technological processes (Ericson 1997). Many studies have investigated the relationship between storage temperature, time and quality related changes in fish muscle. Several workers have studied pickles prepared from meat of different fish and other aquatic animals in India such as freshwater fish (Chattopadhyay *et al.* 1985), low cost marine fish (Vijayan *et al.* 1989) and edible oyster (Sugumar *et al.* 1994).

During last few years Thai pangus (*P. hypophthalmus*) had been cultured extensively in Bangladesh. Reasons identified for such less interest to this species were attributed to the characteristics nature of the flesh, like off- flavor, excessive lipid content and inedible belly meat. The problems related to its muscle (characteristics nature of the flesh, off flavor, excessive lipid content and inedible belly meat) can be minimized during pickle preparation through utilizing spices and additives. Considering the facts, the present study was conducted to develop pickle from Thai pangus and to know the changes in quality parameters at room (28°C to 32°C), refrigeration (5°C to 8°C) and frozen (-18°C to -20°C) storage temperature.

Materials and Methods

Sample Collection and Experimental Condition: Fresh Thai pangus (*P. hypophthalmus*) fishes were collected from Kamal- Ranjit (KR) Market of Bangladesh Agricultural University, Mymensingh. Total 10 fishes were collected having weight from 1.0 to 1.2 kg. The experiments were carried out in the laboratories of the Department of Fisheries Technology, Bangladesh Agricultural University for a period of 12 months from November 2015 to May 2016.

Ingredients for Fish Pickle: Fish pickle 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 I.

Table I. Standard recipe for fish pickle preparation

Ingredient name	Amount	Ingredient name	Amount
Fish muscle	500g	Vinegar	50ml
Chili powder	20g	Black pepper	2g
Turmeric powder	2g	Panchphoron (mixture of five spices such as Cumin, Brown Mustard, Fenugreek, Nigella and Fennel)	5g
Cumin	10g	Sugar	50g
Onion	20g	Salt	30g
Garlic	80g	Tomato sauce	30g
Ginger	10g	Tamarind	20g
Cloves	2g	Sodium benzoate	1g
Mustard oil	150ml		

Fish Pickle Preparation: The fishes were thoroughly washed with tap water to remove contaminants on the skin. Then the fishes were cut into small pieces (approx. 1 cm^3) using sharp knife and washed with tap water in the laboratory to remove bloods and other contaminants. The pieces were marinated, fried in mustard oil, added other ingredients and finally heated till vinegar absorbed. During packing care was taken to see that there was layer of oil over the contents in the bottles. The detail procedure is depicted in Fig. 1.

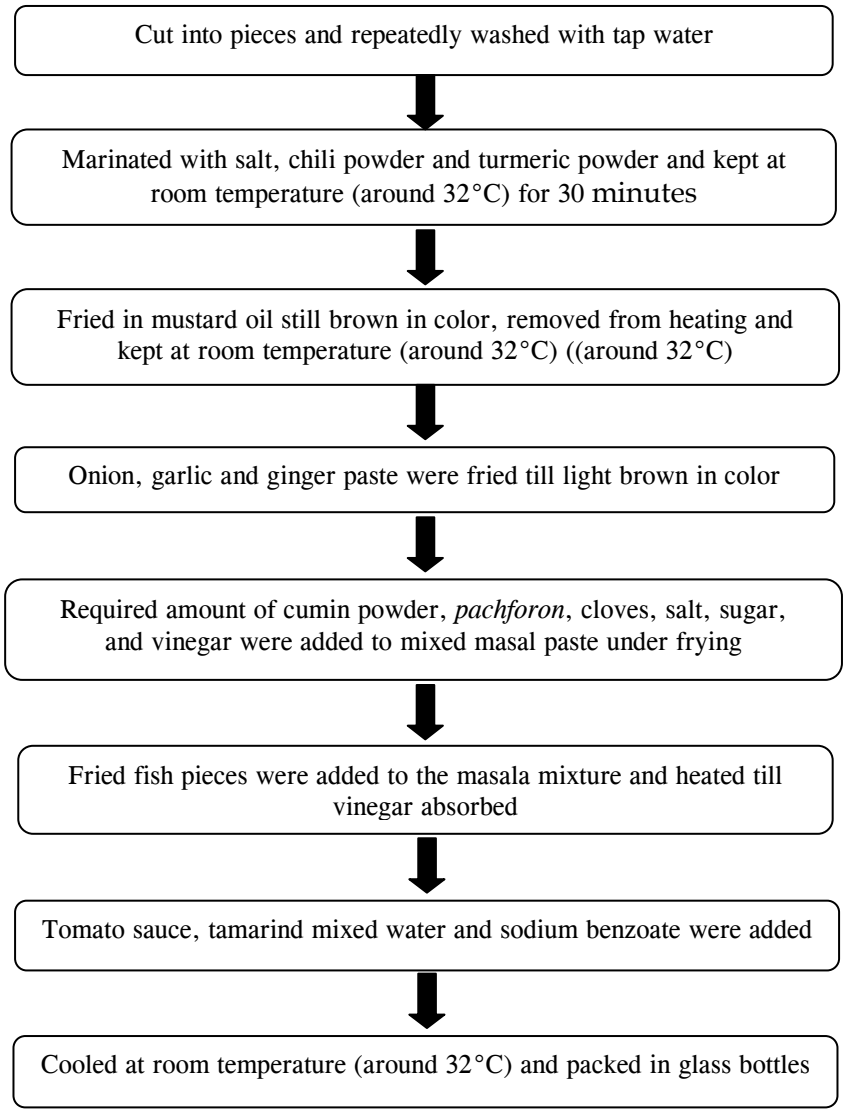

Fig. 1. Flow Diagram of fish pickle preparation from Thai pangas.

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

Quality Analysis: The quality parameters of fish pickle samples stored at room temperature were analyzed at every 3 days interval. On the other hand, the quality parameters of the pickle samples stored at refrigeration temperature were analyzed initially at 7 days interval and then analyzed around 15 days interval whereas the analysis of quality parameters of the pickle samples stored at frozen temperature was done initially at 15 days interval and then at 30 days interval. Triplicate samples were taken to carry out the experiment. Proximate composition (moisture, protein, lipid and ash) of fish pickle were tested according to the methods described by the Association of Official Analytical Chemists (AOAC 2005) with certain modifications.

Determination of total volatile base nitrogen (TVB-N) value: For evaluation of shelf-life of fish pickle, TVB-N values were measured. The TVB-N value was determined according to the methods given in AOAC (1980) 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 was determined according to the method described by Egan *et al.* (1981). One gram (g) of sample oil was weighed into a stoppered 250ml conical flask and 20 ml of chloroform was 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 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) with a glass electrode using an expandable scale.

Determination of aerobic plate count: The APC 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:

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

Statistical Analysis: The statistical analysis package SPSS 11.5 (SPSS Inc, Chicago, IL, USA) was used to calculate the mean and standard deviation of the values.

Results

Changes in TVB-N value of fish pickle during storage at different temperatures: TVB-N values of the fish pickle showed an increasing trend throughout the storage period (Table II). During storage at room temperature, the initial TVB-N value of pickle was 1.18±0.12 mg/100 g which gradually increased with the lapse of storage period and reached to 4.29±1.05 mg/100g on the 15th day. In case of storage at refrigeration temperature TVB-N value increased to 4.45±0.51 mg/100 g on the 73rd day whereas at the frozen storage temperature, the value reached to 2.05±0.04 mg/100 g on the 90th day (Table II).

Table II. Changes in TVB-N, peroxide value, pH and bacterial load of fish pickle 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 (28^0 to 32^0C)	0	1.18±0.12	0.27±0.08	4.41±0.26	2.30×10^3
	3	1.39±0.10	0.53±0.06	4.30±0.06	2.30×10^3
	6	1.86±0.01	1.11±0.19	4.33±0.08	3.81×10^5
	9	1.93±0.02	1.64±0.06	4.01±0.10	5.20×10^5
	12	2.48±0.38	2.17±0.20	3.93±0.06	5.35×10^6
	15	4.29±1.05	2.43±1.46	3.99±0.21	7.20×10^7
Refrigeration temperature (5^0 to 8^0C)	0	1.18±0.12	0.27±0.08	4.41±0.26	2.30×10^3
	7	1.45±0.38	0.46±1.95	4.28±0.06	2.93×10^3
	14	2.33±0.28	0.94±1.26	4.33±0.07	3.21×10^3
	27	2.58±0.15	1.55±1.07	4.31±0.66	5.05×10^4
	42	3.77±0.21	1.77±2.52	4.32±0.75	6.20×10^5
	56	4.12±0.84	2.03±2.38	4.22±0.87	6.50×10^6
	73	4.45±0.51	2.96±2.01	4.07±0.19	9.10×10^7
Frozen temperature (-18^0 to -20^0C)	0	1.18±0.12	0.27±0.08	4.41±0.26	2.30×10^3
	15	1.37±0.03	0.49±1.59	4.30±0.27	1.40×10^3
	30	1.66±0.03	0.77±1.30	4.17±0.20	0.62×10^3
	60	2.50±0.09	0.93±1.25	4.00±0.11	0.40×10^2
	90	2.05±0.04	1.26±1.93	3.97±0.28	0.27×10^2

Changes in peroxide value of fish pickle during storage at different temperatures: During storage at room temperature, the initial peroxide value of pickle was estimated 0.27 ± 0.08 meq/kg of oil 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 2.43 ± 1.46 meq/kg of oil. On the other hand, peroxide value of pickle stored at refrigeration temperature gradually increased to 2.96 ± 2.01 meq/kg of oil on 73rd day of storage from 0.27 ± 0.08 meq/kg of oil, whereas the value obtained 1.26 ± 1.93 meq/kg of oil on 90th day at frozen storage temperature (Table II).

Changes in pH value of fish pickle during storage at different temperatures: Just after preparation, the pH value of pickle was found 4.41 ± 0.26. While Fish Pickle stored at room temperature the pH value decreased to 3.99 ± 0.21 within 15 days. In case of refrigeration temperature, on the 73rd day of storage, the value decreased to 4.07 ± 0.19 whereas the pH value decreased to 3.97 ± 0.28 at frozen temperature on 90th day of storage (Table II).

Changes in APC of fish pickle during storage at different temperatures: The result of the changes in bacterial loads of Fish Pickle during storage at room temperature shown in Table-4. At this temperature, the initial bacterial load found 2.30×10^3 CFU/g which increased gradually within 15 days of storage to 7.20×10^7 CFU/g. In case of refrigeration temperature, bacterial load reached to $9.10 \times 10^7$from its initial value 2.30×10^3 CFU/g within 73 days of storage. On the other hand, during frozen storage of 90 days, the bacterial load of Fish Pickle found 0.27×10^2 (Table II).

Discussion

The Total volatile base nitrogen (TVB-N) value: In the present study, a clear trend to increase TVB-N content was observed with the lapse of storage period irrespective of storage temperature. During storage at room temperature TVB-N value reached to 4.29 ± 1.05 mg/100g on the 15th day of storage. The available report suggests that the upper limit of TVB-N is considered for finfish acceptability, 30 mg/100 mg (Connell 1975). The increases in TVB-N value with the lapse of storage may be attributed due to bacterial spoilage. However, the available information indicates that TVB-N mainly accumulated in fresh fish during the later phase of spoilage after the bacterial population has grown. In the present study, while pickle stored at refrigeration temperature the TVB-N value increased to 4.45 ± 0.51 mg/100 g on the 73rd day whereas at the frozen temperature the value reached to 2.05 ± 0.04 mg-100 g on 90th day. Simeonidou *et al*. (1997) studied on whole fish and fillets of horse mackerel (*Trachurus trachurus*) and Mediterranean hake (*Merluccius mediterraneus*), which were assessed for quality (physical, chemical and sensory attributes) changes throughout 12 months of frozen storage at -18°C. The pH, TVB-N, the thiobarbituric acid number (TBA), peroxide value increased, while TVB-N value increased very slowly during the frozen storage.

The peroxide value: According to Connell (1975) the recommended value of peroxide for fresh finfish is 10-20meq/kg of oil. At values above 20meq/Kg of oil, the fish were found to be emitting smell and tasted rancid. The peroxides were presumed to be eventually further oxidized to aldehydes and ketones which had a very disagreeable "fishy" or rancid odor and taste. However, depending on the fish species and storage condition a good correlation between peroxide value and organoleptic quality was found. In the present study, an increasing trend was observed in peroxide value with the lapse of storage period, though the increasing rate was slower at lower temperatures. The results obtained in the present study coincides with the findings of Patil *et al.* (2014) and Kumar and Basu (2001). Patil *et al.* (2014) reported an increasing trend of peroxide value throughout the storage study of 150 days from 0.29 to 1.16 meq/kg of oil for pangus pickle. The increase in the peroxide value might be due to the oxidation of fat during the storage period. Kumar and Basu (2001) also found an increase in peroxide value from 1.37 to 10.25 meq/kg of oil in prawn pickle.

The pH value: The detection of pH values is one of the most frequently used physical quality control for fish and fishery products, which is affected by the changes in the lipid hydrolysis, microorganisms or enzymes (Varlik *et al.* 2000). In the present study pH of fish pickle was stored at room temperature and sample tested every 3 days later. The value of pH at the initial stage decreased sharply might be due to the addition of vinegar, sodium benzoate and tamarind during processing and then the value decreased gradually with progress in storage time. A similar decreasing trend in pH during storage of pickle was reported by many authors (Tamilselvi *et al.* 2010; Collins *et al.* 1989; Dhanapal *et al.* 1994; Gupta and Basu 1985; Behanan *et al.* 1992). Tanuja and Hameed (1998) recorded the pH of squilla pickle 4.46 which dropped gradually during storage.

Aerobic plate count (CFU/g): In a live and healthy fish there is no effective penetration of tissues by the bacteria present on the body surfaces and in the intestine, and a balanced situation seems to exist whereby the numbers of theses bacterial remain at a rather level. Mukundan *et al.* (1981) reported that pickle contains very low bacterial counts due to the inhibitory action of low pH and high salt content of the pickles. Erichsen (1967) reported that pickled fish normally carry low level of bacteria in the range of 10^1 to 10^3 CFU/g. Chandrasekar (1979) reported total plate count in fish pickle within the range of 10^3 to 10^5 CFU/g. These values are quite similar to the present study. Karunasager *et al.* (1988) have reported a viable count of halophiles in the range of 10^6 to 10^7 CFU/g which is higher than the present study. In the present study microbial load (CFU/g) increased in fish pickle stored at refrigeration temperature. This is might be as because refrigeration slows down bacterial action and multiplication but can't stop. In a study, with the increase of storage time of fish pickle at ambient and refrigeration temperature total bacterial load increased through multiplication (Akter 2013). Similar increment on total bacterial load in fish muscle at low temperature storage was reported by Obemeata *et al.* (2011). The total bacterial

count found to show a gradual reduction during storage of fish pickle under the frozen temperature which is similar to the finding of Tanuja and Hameed (1998). 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°C was 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.

On the basis of the observation of the changes in different quality parameters at different storage temperatures, this study concludes that the shelf life of Thai Pangas fish pickle at room temperature (28°C to 32°C) is short, in fact not more than 15 days due to bacterial growth but at refrigeration temperature (5°C to 8°C), the pickle may remain in acceptable condition for more than 73 days and more than 90 days at frozen temperature (-18°C to -20°C).

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