Effects of packaging on the quality parameters of mustard ilish
(*Tanualosa ilisha*) at room temperature

F.H. SHIKHA, M.I. HOSSAIN* AND S. MAHMUDA
Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh2202, Bangladesh
*Email: ihossain.ft@bau.edu.bd

Abstract. The experiment was carried out to prepare mustard ilish at the laboratory and observe the effects of packaging on the quality parameters of the product at room temperature (28°C to 32°C). Biochemical and microbiological changes in mustard ilish prepared from Hilsa shad (*Tanualosa ilisha*) were determined. It was observed that percent moisture, protein, lipid, ash content and pH value in mustard ilish decreased after preparation of the product than those values obtained for raw fish. In quality parameters study, at room temperature (28°C to 32°C), percent moisture, and ash contents increased throughout the storage period, but protein and lipid contents decreased. The TVB-N, peroxide value and standard plate count (SPC) of bacteria increased with the progress of storage time but the rate of increment was comparatively slower in sealed and vacuum sealed packs than the rate observed for non-sealed pack. Therefore, on the basis of above mentioned points, the present study could be concluded as-though mustard ilish remain in acceptable condition for a short time at room temperature (28°C to 32°C) but packaging has some effect on the extension of shelf life of the product.

Key words: Mustard ilish, *Tanualosa ilisha*, Quality parameters, Packaging

Introduction

The world catch statistics of hilsa (T. ilisha) shows that a total of 90-95 per cent of the global harvest comes from three countries: Bangladesh (50-60 per cent), India 920-25 per cent) and Myanmar (15-20 per cent) (Rahman *et al.* 2011) In Bangladesh, hilsa occurs in inland, marine, and coastal waters and is harvested throughout the year. Hilsa has the highest contribution to Bangladesh fish production as a single species. It is considered the national fish of the country and contributes to the national economy, employment and export.

The fish is popular food amongst the people of South Asia and in the Middle East, but especially with Bengalis and Odias. Bengali fish curry is a popular dish made with mustard oil or seed. The Bengalis popularly call this dish Shorshe ilish. It is also popular in India, especially in West Bengal, Odisha, Tripura, Assam, Gujarat and Andhra Pradesh. It is also exported globally. In Bengal and Odisha, ilish can be smoked, fried, steamed or baked in young plantain leaves, prepared with mustard seed paste, curd, eggplant, different condiments like jira (cumin) and so on. It is said that people can cook ilish in more than 50 ways. Ilish roe is also popular as a side dish. Ilish can be cooked in very little oil since the fish itself is very oily. Ilish dish preparation though not that complicated but due to busy schedule of people, especially urban people they get less opportunity to taste this popular ilish item “shorshe ilish/mustard ilish” in their lunch or dinner menu. If mustard ilish is available in shops as “ready to eat” form, then people will be able to enjoy this popular item without any hassle and at the same time, ilish fishes caught in the peak season will be properly utilized. Considering all these facts, the present study was conducted to prepare mustard ilish in the laboratory and to study the keeping quality
of the product by observing the changes in its quality parameters at room temperature (28°C to 32°C) under three types of packaging conditions such as sealed, non-sealed and vacuum sealed.

**Materials and Method**

**Fish sample and ingredients collection**: Hilsa shad, commonly called ilish (*Tenualosa ilisha*), a high-priced fish, was used as a raw material for mustard ilish preparation for the shelf life study. A total of 5 kg fish was collected from Chandpur and immediately transported to the Fish Processing and Quality Control Laboratory, Department of Fisheries Technology, Bangladesh Agricultural University in icebox ensuing the exchange of ice at a certain interval. The fish were kept in a freezer (-18 to -20°C) until preparation of the mustard ilish sample. Before preparing the final product for different analysis 3 trials were done for standardizing the types and amounts of ingredients to be used to prepare the mustard ilish in the laboratory according to panelist’s taste. Table 1 shows the types and amounts of ingredients used to prepare mustard ilish. The experiment was carried out for a period of 2 months from October to December, 2016.

**Table 1. List of required ingredients for mustard ilish preparation**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount for mustard ilish preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish muscle</td>
<td>500 g</td>
</tr>
<tr>
<td>Chilli powder</td>
<td>30 g</td>
</tr>
<tr>
<td>Turmeric powder</td>
<td>5 g</td>
</tr>
<tr>
<td>Cumin powder</td>
<td>10 g</td>
</tr>
<tr>
<td>Sliced onion</td>
<td>150 g</td>
</tr>
<tr>
<td>Garlic powder</td>
<td>10 g</td>
</tr>
<tr>
<td>Ginger powder</td>
<td>10 g</td>
</tr>
<tr>
<td>Mustard seed</td>
<td>20 g</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>80 ml</td>
</tr>
<tr>
<td>Salt</td>
<td>30 g</td>
</tr>
</tbody>
</table>

**Preparation of mustard ilish**: The fishes were thoroughly washed, cut into pieces using a sharp knife and repeatedly (3 to 4 times) washed with tap water (24 to 27°C) to remove blood and contaminants and then kept in a tray. Fish slices were marinated (for about 12 to 15 minutes at around 28°C) with salt and turmeric powder. The mustard gravy was prepared by blending mustard seed with chilli and salt. Onion, garlic and ginger powder were fried in mustard oil till they turn to light brown in color in a fry pan. Cumin powder, turmeric powder, chili powder were added to spices mixture. A little amount of salt (about 30 g) and some water (about 25 ml) were added to the oil were added and cooked for another 1-2 minutes. The mustard paste was mixed with 1/2 cup water in a bowl and poured on the spices mixture on fire and cooked for another 2-3 minutes. Then marinated fish slices were added to mustard gravy and spices mixture, cooked for 6-8 minutes on low flame. After completion of cooking the cooked mustard ilish was taken out from fire and left for cooling at room temperature (around 28°C). After cooling down the mustard ilish samples were kept in different packs for storing. Figure 1 shows the procedure of mustard ilish preparation.

**Quality analysis**: Proximate composition of raw fish and mustard ilish was analyzed according to the methods described by AOAC (2005) and the total volatile base nitrogen (TVB-N) was
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determined according to the methods given in AOAC (1984). The method for the determination of peroxide value was followed as described by Egan et al. (1981). pH was measured following the method described in AOAC (2005). Determination of standard plate count (SPC) of bacteria was done according to the International Standard Organization (ISO 1965). Three samples (as replication) from each of the treatments were analyzed on 0, 1st and 2nd day of storage at room temperature.

Fig. 1. Preparation procedure of mustard ilish. (a) Weighing of ilish, (b) Pieces of ilish after gutting, scaling, cutting and washing, (c) Frying of onion and other spices, (d) Gravy for mustard ilish preparation, (e) Cooking of mustard ilish, (f) Packaging of cooled mustard ilish with sealer machine, (g) Packaging of cooled mustard ilish with vacuum sealer machine, (h) Packaged mustard ilish for storage.

Sensory evaluation: Mustard ilish was tested by an expert panel. The mustard ilish was served to 5 panelists to evaluate the sensory attributes (color, odor, taste, texture, general acceptability) by using 9-points descriptive scale (Paulus et al. 1979). 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.
Data analysis: Data from different biochemical and microbiological measurements were subjected to statistical analysis (one way ANOVA) and processed using Microsoft Office Excel 2010. Results are represented within the view of table form and column diagrams.

Results

Proximate composition of raw hilsa fish and mustard ilish: In this experiment, the moisture content in raw Hilsa was found 66.5±0.4%, protein 17.68±0.38 %, lipid 14.5±0.19 and ash content 1.86±0.20%; whereas after preparing mustard ilish, the values were obtained 63.29±0.01%, 17.80±0.40%, 13.06±0.40% and 4.09±0.40%, respectively (Table II).

Table II. Proximate composition of raw ilish and mustard ilish (on “0” day)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw ilish</td>
</tr>
<tr>
<td>Moisture</td>
<td>66.50±0.40</td>
</tr>
<tr>
<td>Protein</td>
<td>17.68±0.38</td>
</tr>
<tr>
<td>Lipid</td>
<td>14.5±0.19</td>
</tr>
<tr>
<td>Ash</td>
<td>1.86±0.20</td>
</tr>
</tbody>
</table>

Changes in the proximate composition

Moisture content (%): The initial moisture content of mustard ilish was obtained 63.29%, which was lower than the value of raw fish. After one day of storage, the moisture content of non-sealed mustard hilsa increased to 68%. On the 2nd day of storage, the moisture content reached to 74%. In the case of sealed and vacuum sealed pack, a similar trend of moisture increase was observed though the value was a little higher for non-packed sample (Fig. 2a).

Protein content (%): The initial protein content in raw fish was 17.68%. After preparing mustard ilish the value reached to 17.80%. With the increase of storage period protein content of mustard ilish decreased to 11.32% in non-sealed pack, 10.07% in sealed pack and 11.4% in vacuum sealed pack (Fig. 2b).

Lipid content (%): The initial lipid content of mustard ilish was found 13.06±0.4%, which was lower than that of raw fish (14.39%) (Fig. 2c). After one day of storage, the lipid content of non-sealed mustard ilish was found 10.02%, which decreased to 7.01% on the 2nd day. The lipid content of mustard ilish of the sealed pack was found 9.87% and 7.03%, respectively after 1 and 2 days of storage. In the case of vacuum sealed packs, the lipid content was obtained 8.78% after one day of storage, and then the value decreased on 2nd day to 7.40%.

Ash content (%): The initial ash content in raw fish was 1.86%, which increased to 4.9±0.4% after preparing mustard ilish (Fig. 2d). As time passed, the ash content in mustard ilish increased and on 2nd day of storage ash content increased to 7.48% in non-sealed pack, 7.83% in sealed pack and 6.81% in vacuum sealed pack.
Fig. 2. Changes in proximate compositions of mustard ilish in different packaging conditions at room temperature (28°C to 32°C). Here, (a) moisture (%), (b) protein (%), (c) lipid (% and (d) ash (%).

Changes in TVB-N (mg/100g) value: The TVB-N value of raw fish was found 2.01 mg/100g, and the initial value of mustard ilish at room temperature was found 2.84 mg/100g. After 2 days of storage this TVB-N values of mustard ilish reached to 8.91 mg/100g in non-sealed, 6.82±0.01mg/100g in sealed pack and 7.20±0.04 mg/100g in vacuum sealed pack (Fig. 3a).

Changes in peroxide value (mq/kg): The initial peroxide value of mustard ilish was 50.98 mq/kg in non-sealed pack which reached to 70 mq/kg after 2 days of storage. In the case of sealed and vacuum sealed packs, almost similar values were obtained for peroxide value (Fig. 3b).
Changes in pH value. In this study, the initial pH value of mustard ilish was found 7.22. After 2 days of storage in non-sealed pack, pH value decreased to 5.06 (Fig. 3c). The pH values of mustard ilish in other two packs also quite nearer to the value of non-packed samples. The decreased values of pH after 2 days of storage in sealed and vacuum sealed packs were obtained 5.08 and 5.12, respectively.

![Graphs showing changes in TVB-N and Peroxide Value over storage period.](a),(b)

![Graph showing changes in pH over storage period.](c)

Fig. 3. Changes in TVB-N, (a) PO value (b), and pH (c) of mustard ilish in different packaging conditions at room temperature (28°C to 32°C).

Changes in standard plate count (SPC) of bacteria. On the 0 day of storage, standard plate count (SPC) of bacteria in mustard ilish was found 0.35x10⁵ cfu/g at room temperature (28°C to 32°C) which increased to 57.0x10⁵ in non-sealed pack, 6.3x10⁵ in sealed pack and 5.8x10⁵(CFU/g) in vacuum pack on 2nd day of storage (Table III).
Table III. Changes in standard plate count (SPC) of bacteria of mustard ilish at room temperature (28°C to 32°C) during storage in different types of packages

<table>
<thead>
<tr>
<th>Storage temperature (28°C to 32°C)</th>
<th>Days</th>
<th>Value(CFU/g) Non-sealed pack</th>
<th>Sealed pack</th>
<th>Vacuum-pack</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>0</td>
<td>0.35x10^5</td>
<td>0.35x10^5</td>
<td>0.35x10^5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.61x10^5</td>
<td>0.59x10^5</td>
<td>0.53x10^5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>57.0x10^5</td>
<td>6.3x10^5</td>
<td>5.8x10^5</td>
</tr>
</tbody>
</table>

Changes in sensory quality: Sensory quality parameters of mustard ilish were tested by panelists. The changes in color, flavor, taste and texture of mustard ilish at room temperature (28°C to 32°C) are presented in Fig. 4 (a, b and c). A decreasing trend was observed in the sensory quality parameters with the progress of storage time, among which the deterioration rate was found faster in non-packed mustard sample.

Discussion

Proximate composition of raw hilsa fish and mustard ilish: The chemical composition of fish varies greatly from one individual to another depending on age, sex, environment and season with protein levels ranging from 16-21%, lipids 0.1-25%, ash 0.4-1.5%, moisture 60-81% with extremes of 96% having been reported (Huss 1995). Begum et al. (2016) reported that raw hilsa contains 69.51%, moisture, 19.44% protein, 6.78% lipid and 3.72% ash. Hossain et al. (2014) found moisture 60.37%, protein 18.16%, lipid 19.94% and ash 1.34% for hilsa fish of Bay-of-Bengal. The obtained result in the present study are more or less similar to the previous reports. Here, after product preparation, some decrease in moisture content and increase in ash content were observed. Alipour et al. (2010) observed that the moisture content decreased during cooking. The effect of cooking method on moisture and other contents of fish muscle had been studied by other scientists (Stephen et al. 2010, Weber et al. 2008, Kucukgulmez et al. 2006). This might be due to the release of water from the inner parts of muscle during heat processing, and the increase in percent ash content in mustard ilish might be due to the addition of different spices during product preparation.

Changes in the proximate composition, TVB-N, peroxide, pH values and standard plate count (SPC) of bacteria in mustard ilish: Irrespective of packaging condition, especially in non-sealed pack sample, the moisture content increased during storage of mustard ilish at room temperature (28°C to 32°C). Darmola et al. (2007) reported the reason behind such increase in moisture content for smoke-dried fish species stored at ambient temperature, might be due to the absorption of moisture from surrounding since there was no re-drying during the storage period. Sehgal et al. (2010) stated that cooking could be a possible reason for the reduction in the protein content. After a certain period of storage, the protein content (%) started to decrease.
Fig. 4. Changes in sensory quality parameters of mustard ilish in (a) non-sealed, (b) sealed and (c) vacuum sealed packs at room temperature (28°C to 32°C).

Hossain et al. (2019a) reported such decrease in percent protein content in sealed and vacuum sealed mola pickle during storage at room temperature (28°C to 32°C). They also found a slower decreasing rate in protein content in the sample stored at vacuum sealed pack than that of sealed pack. We have observed a decrease in protein content (%) in this experiment which might be attributed to denaturation of fish protein and leaching out of water soluble protein (Gandotra et al. 2012). In a study carried out on mola pickle at room temperature (28°C to 32°C) Hossain et al. (2019a) observed a decreasing trend in percent lipid content of the samples in both sealed and vacuum sealed packs though the decreasing rate was slower in vacuum sealed pack sample. In the present experiment also lipid content (%) started to decrease with the progress of storage period in all three packaging conditions with the lesser value for non-sealed pack sample. McGill et al. (1974) reported that lipid content (%) in fishery products started to decrease when oxidation occurs.
In this experiment, ash content (%) of mustard ilish increased in all three packaging conditions at room temperature (28°C to 32°C). Shikha et al. (2018a) observed a similar increasing trend in percent ash content for Thai pangas pickle stored at room temperature (30–35°C). The higher ash content in the cooked fish might be due to its higher bony consistency and high scaly nature. Pearson (1997) reported that samples could be considered consumable if the TVB-N level is less than 20 mg/100 g fish and that a level of more than 30 mg determines the product as not consumable. In the present study, the TVB-N values of mustard ilish increased throughout the storage period at room temperature (28°C to 32°C) which was similar to the changes of TVB-N value below the range reported by Kumar et al. (2013). Among samples stored in different packaging in the present study, the TVB-N value was found higher in non-sealed pack indicating the positive effect of air-tight and vacuum packaging of cooked fish products. Shikha et al. (2018b) reported a similar increasing trend in TVB-N value for pangas pickle stored at room temperature (28°C to 32°C). In a study, the effect of storage temperature on the chemical and sensorial quality of hot smoked Atlantic bonito (Sarda sarda) packed in aluminium foil was observed and found a similar increasing trend in TVB-N value (Koral et al. 2018).

Peroxide value (PV) is a measure of the degree of oxidation in the fat (Gopakumar 2002) and acceptable limit of peroxide value is 10-20 mq per kg of fat (Connell 1995). Fresh oil usually peroxide value well below 10 mq/kg, and a rancid taste may be noticed when peroxide value is between 20 and 40 mq/kg fat (Egan et al. 1981). In the present study, it was observed that with the lapse of storage period at room temperature (28°C to 32°C) peroxide value of mustard ilish increased in all three packaging conditions with lesser value for sample in vacuum sealed pack. Dhar and Karthikeyan (2014) found the peroxide value increased with the storage period for pickle from small freshwater prawn (Macrobrachium dayanam), which was similar to the present study. Hossain et al. (2019b) reported a similar increasing trend in peroxide value for pangas condiment at room temperature (28°C to 32°C) during 15 days of storage.

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). Shikha et al. (2018b) observed a decrease in pH from 4.41 to 3.99 in pangas fish pickle during 15 days of storage period at room temperature (28°C to 32°C). Kolekar and Pagarkar (2013) evaluated the quality of ready to eat fishball in curry. Prepared fish ball in the curry was packed in standup pouches and stored at 0 to -2°C. They found a decrease in pH from 6.38 to 6.07 during storage of 12 days. In the present study pH value of mustard hilsa also showed a decreasing trend throughout the storage period at room temperature (28°C to 32°C) which is quite similar to the previous findings.

Sachindra et al. (2005) studied that the shelf-life of tilapia could be extended to twelve and fifteen days when packed under vacuum packaging and recorded lower mesophilic bacterial counts. Manju et al. (2007) found that pearl spot kept under vacuum condition had an increase in shelf-life of ten days, compared to eight days in aerobic packaging. Etemadian et al. (2012) reported that kutum (Rutilus frisii) fillets packaged under vacuum packaging showed a reduction in aerobic plate count. Sarojnalini and Suchitra (2009) found that total plate count of bacteria reached up to 10⁸ cfu/g for Nga-pi (a fermented fish product of Manipur), prepared in large-scale industrial products and the initiation of fermentation at 30°C in laboratory conditions. An increase in total plate count of bacteria in mustard ilish at room temperature (28°C to 32°C)
during storage in this experiment is more or less similar in pattern with findings mentioned above.

Changes in sensory quality parameters of mustard ilish: A decreasing trend in sensory scores during storage of thermally processed product have been reported by Gopal et al. (2001) for traditional style mackerel curry and Mallick et al. (2006) for rohu curry. Ekran et al. (2007) studied the effects of modified atmosphere and vacuum packaging on quality of chub mackerel. Acceptability scores for odour, taste and texture of cooked air packaged, vacuum packed (VP) and modified atmosphere packaged (MAP) chub mackerel decreased with storage time. The sensory scores of chub mackerel stored in air, VP and MAP at +4°C were 10 and 12 days, respectively. The obtained result for sensory quality parameters of mustard ilish in the present study is in agreement with the above mentioned findings.

Conclusions

At room temperature (28°C to 32°C) though the effects of packaging on the nutritional parameters were not the prominent but clear effect of packaging was observed on values of total volatile base nitrogen (TVB-N), peroxide and standard plate count (SPC) of stored mustard ilish. The rate of increase in values of TVB-N, peroxide was comparatively slower and the standard plate count (SPC) of bacteria in samples stored in sealed and vacuum sealed packs was lower than those of the samples stored in non-sealed pack.

Acknowledgement: The financial support for this study was achieved from Ministry of Science and Technology, Peoples Republic of Bangladesh (Fiscal Year 2017-18).

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(Manuscript received 12 May 2020)