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Original Article

Changes in sensory, biochemical and microbiological parameters of smoked shrimps (both traditional and improved) in different packages at refrigeration temperature (5 to 8°C)

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A B S T R A C T

Smoking or smoke curing, like drying and salt curing, is an ancient method of preservation of shrimp. The present experiment was conducted to prepare improved smoked shrimp from four species of shrimps, namely Horina (*Metapenaeus monoceros*), Chali (*Metapenaeus brevicornis*), Chaka (*Penaeus indicus*) and Khogda (*Parapenaeopsis stylifera*) at laboratory condition to evaluate the changes in senseory, biochemical and microbiological parameters of the both laboratory and traditionally prepared (at Koyra, Khulna) smoked shrimps. For smoking, a locally made smoking kiln was used at laboratory. Then both improved and traditionally prepared smoked shrimps were stored in various packages at refrigeration temperature (5 to 8°C). The initial moisture level of improved smoked shrimps (Horina, Chali, Chaka and Khogda) ranged from 14.22 ± 0.02% to 16.15 ± 0.03% with the highest value in Khogda, but the moisture content of traditionally smoked Chali was 17.53 ± 0.11%. After long storage of 150 days at refrigeration temperature (5 to 8°C), the moisture content was found to be 10.02 ± 0.03% to 12.81 ± 0.03% for all the stored smoked shrimps. The initial protein content of improved smoked shrimp ranged from 62.15 ± 1.20 % to 64.05 ± 0.1.10 % with the highest value in Chali; whereas the protein content was lower in traditionally smoked Chali which was 61.18 ± 1.23 %. During the storage, protein content decreased with the lapse of time and ranged from 52.14 ± 1.12% to 54.47 ± 1.12%. Initial lipid content of improved smoked shrimp ranged from 9.73 ± 0.95% to 10.05 ± 0.05% and in traditional smoked Chali the value was 9.24 ± 0.09%. At the end of the storage period, the lipid content of smoked shrimp ranged from 7.65±0.08% to 8.75±0.08% and in traditional smoked Chali it was 7.69±0.09%. Ash contents of smoked shrimp increased and ranged from 11.06±0.14% to 23.00 ± 0.21%. The TVB-N value of improved smoked shrimp was significantly lower than that of traditionally prepared smoked shrimp. Initial TVB-N value of improved smoked shrimp ranged from 12.23 ± 0.12 mg/100g to 14.05 ± 0.09 mg/100g and in traditional smoked Chali the TVB-N value was 15.83 ± 0.12 mg/100g. With the progress of study period, TVB-N value also increased, which ranged from 25.42 ± 1.45 mg/100g to 31.14 ± 0.12 mg/100g. The initial APC of improved smoked shrimp was 4.3 x 10^2 CFU/g to 2.05 x 10^3 CFU/g and in traditionally prepared smoked Chali it was 3.6 x 10^3 CFU/g. After the storage period the total bacterial content ranged from 1.67 x 10^6 CFU/g to 6.17 x 10^6 CFU/g for all the stored products. The results obtained in the study showed that the steps followed for smoking of shrimps in the laboratory contributed to improve the quality of smoked shrimps, at the same time smoked shrimps were in better condition in air tight and vacuum packages than the samples stored in non-sealed packages.

Introduction

Shrimp smoking is a processing method of shrimp accomplished by a combination of drying and deposition of naturally produced chemicals resulting from thermal break down of wood or burning of wood. The property of smoked shrimp depends to a large extent on the type of wood from which it is produced. Thermal cracking of wood under reduced oxygen supply results in smoke containing several compounds. The gas phase contains several flavor-producing agents, aliphatic and aromatic, numbering over 200 (Horner, 1992). In modern world smoked fish is a delicacy. On the other hand importance in many countries of central Africa and Middle-eastern Asia are largely a technical necessity rather than delicacy due to dry and less humid climatic conditions and other reasons. In southeastern Asia, smoking is mostly practiced not necessarily to impart desirable color and flavor, but mainly to accelerate the drying of fish (Clucas and Ward, 1996). Various low cost fish processing methods are practiced in Bangladesh such as- drying, salting, smoking etc. For sun drying proper intensity of sunlight is required for producing quality dried products. Salting requires addition of extra capital and labor. Salting is also somewhat species specific and mainly used for fatty fish. But for small shrimps in rainy season smoking is a good technique for preservation and is being used from the time immemorial due to no other alternative methods available in rainy season (Hoq *et al*. 2006).

Smoking of small fish and shrimp is a profitable business in some coastal part of Bangladesh. (Source: low-cost processing of Fish in Coastal Bangladesh- FAO of the United Nations. BGD/97/017 Field Doc: 5/2005). In Koyra and Paikgacha, the smoked shrimp producers operate their activities all over the year except the months of November, December and January as the raw materials is not available during that time. The smoking activities are closely related with the lunar cycle as the lunar cycle determines the availability of shrimps. The smoked shrimp producer operates smoking process 12 days per month, 6 days during full moon and 6 days during new moon as the shrimps are captured in large amounts. Comparatively, shrimps are more abundant in full moon tide than new moon tide. About 20-30% of the shrimps caught in coastal region are used for smoke-drying purpose (Hoq *et al.,* 2003). The smoked shrimp producers in Koyra, Khulna uses three species of shrimps, locally called- Chali (*Metapenaeus brevicornis*), Chaka (*Penaeus indicus*) and Harina (*Metapenaeus monocerus*). Sometimes they process by mixing of all the three species. Shrimp naturally contain many parasites. These parasites are killed during the hot-smoking process, if the temperature reaches 60°C (Jahncke, 2008).

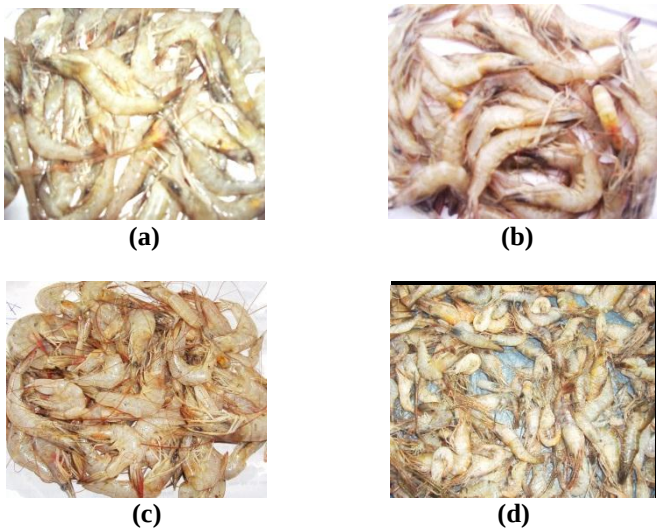
In recent years, smoked shrimp got much popularity in Bangladesh and have good market in Khulna, Chittagong region. Packaging extends the shelf life of food products by controlling the growth of microorganisms, pathogenic flora and it protects oxidative rancidity of the product. The technology for producing smoked shrimp and packaging of smoked product increase shelf life which require very simple machinery. Successful manufacture and marketing of smoked shrimp ensure the proper utilization of the small shrimp and finally raise the price of the products. Considering these points, the present study aimed to prepare improved smoked shrimp at laboratory condition and to compare the changes in biochemical and microbiological changes in both traditional and improved smoked shrimps at refrigeration temperature (5 to 8°C) under different packaging conditions.

Materials and Methods

The experiment was conducted in the laboratories of Department of Fisheries Technology, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh.

Shrimp species:

Four species of raw shrimps, namely Horina (*Metapenaeus monoceros*), Chali (*Metapenaeus brevicornis*), Chaka (*Penaeus indicus*) and Khogda (*Parapenaeopsis stylifera*) and traditionally smoked shrimps of same species were purchased from Koyra, Khulna and directly brought to the Fish Processing laboratory of the Department of Fisheries Technology, BAU (Plate-1).



(a) **(b)** **(c)** **(d)**

Plate 1. (a) Horina (*Metapenaeus monoceros*), (b) Chali (*Metapenaeus brevicornis*), (c) Chaka (*Penaeus indicus*) and (d) Khogda (*Parapenaeopsis stylifera*)

Production of improved smoked shrimp at laboratory condition

Smoking kiln

The smoking kiln was made with steel sheet as a rectangular box of 5.2 × 2.7 × 2.3 ft^3 size [Plate-2 (a)]. Horizontally, the box or chamber was divided into two equal parts by using a horizontal steel sheet and the bottom portion was used for burning saw dust as smoke source. The horizontal separator had a central circular hole of 8 inch diameter. The upper chamber had facilities of hanging 4-6mm iron rods supported from two sides as rack. Both the chambers had door which could be opened when needed. On the top, there was an outlet for smoke control. By controlling the lid of the outlet, the temperature inside the shrimp chamber i.e. the upper chamber could be controlled. Another small hole on the top was used to provide a sensitive thermometer to measure the temperature inside the chamber.

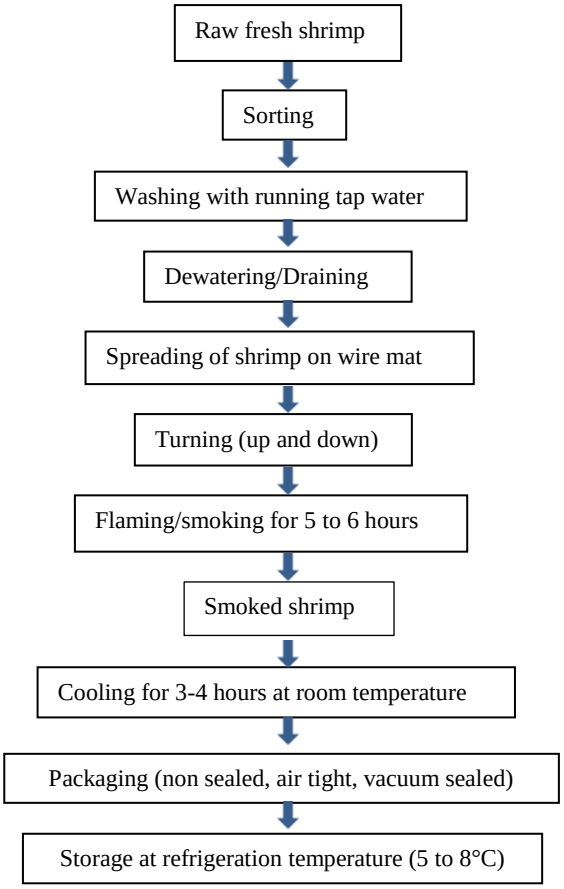
Preparation procedure of smoked shrimp (in laboratory)

Good quality fresh raw shrimps of 4 different specices were directly transported from Koyra, Khulna to BAU in an insulated box with ice (1:1). Fish larvae, small fish, other shrimps, sea snails and other mollusks were separated properly and discarded, then specific shrimp specices were selected to prepare inproved smoked shrimp. For smoking of shrimps, wood and saw dust were burnt to produce smoke in

smoking kiln. The smoke temperature varied between 60 to 70°C, which led to partially dry the shrimps within 4-5 hours. The saw dust used for smoking procedure were of black berry, mahogany, lebbeck, wood apple etc. tree and partially dried before using in the kiln. The saw dust was collected from a local saw-mill. The steps of smoked shrimp preparation is mentioned in Flow chart-1.

Storage of smoked shrimp

Certain amount of each improved smoked shrimp samples were kept in non-sealed, air tight and vacuum sealed packages [Plate-2 (c-e)]. The traditionally prepared smoked shrimp samples (purchased from Koyra, Khulna) were also packed as done with improved samples. Then all the packed samples were stored at refrigeration temperature (5 to 8°C) for further analysis.



Flow chart 1. Steps followed in the laboratory to prepare improved smoked shrimp

(a)

(b)

(c)

(d)

(e)

Plate 2. (a) Smoking kiln, (b) Smoking of shrims in smoking kiln, (c) Smoked shrimp in non sealed package, (d) Smoked shrimp in air tight package and (e) Smoked shrimp in vacuum package

Organoleptic quality assessment

For organoleptic quality assessment, sensory methods are considered to be the most useful. Since human beings are capable to detect defects from visual signs of deterioration such as loss of freshness and changes during storage. Sensory evaluation is considered as the most effective technique to assess fish freshness and quality deterioration (Martinsdóttir 2002; Alasalvar *et al*. 2011). Therefore, organoleptic characteristics (general appearance, taste, flavor, texture, and color) of the smoked shrimp were examined by this methods (through sight, touch, taste, smelling, etc.). The evaluation methods used in this study were based on the one that is currently in use in various institution of the world (Larmond, 1977). The organoleptic and physical characteristics like color, texture and odor when broken etc. were evaluated in the Department of Fisheries Technology, BAU by a five member's panel. The grading of smoked shrimps was done using the score on the characteristics that has been described by AOAC (1990) as freshness grade for fishery products with certain modification as described in Table-1 and Table-2.

Table 1. Grading of smoked shrimps

Grade	Points	Comments
A	<2	Excellent/ Acceptable
B	2 to <5	Good/ Acceptable
C	5	Bad/ Rejected

Table 2. Determination of defect points of smoked shrimp

Characteristics	Defect	Defect point	Grade
1. Color of smoked shrimp	a) Bright red color	1	Excellent
	b) Dark red color	2	Acceptable
	c) Brownish	3	Acceptable
	d) Whitish	5	Rejected
2. Odor	a) Strong smoky sweet odor	1	Excellent Acceptable
	b) Mild smoky odor	2	Acceptable
	c) Neutral odor	3	Rejected
	d) Rancid off odor	5	
3. Texture	a). Crisp, fragile texture	1	Excellent
	b). Fragile texture	2	Acceptable
	c). Less fragile and elastic texture	4	Acceptable
	d). Powdery structure with whitish fungal growth	5	Rejected

Biochemical analysis

Proximate composition analysis

Proximate composition analysis of moisture, crude protein, lipid and ash of smoked shrimp samples were done according to the methods as described in AOAC (1990) with certain modifications.

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

TVB-N was determined according to the methods given in AOAC (1984) with certain modification.

Determination of microbial load

Aerobic plate count was done by spread plate count method. Peptone diluent (0.2%) and plate count agar of commercial preparations or prepared in the laboratory as per method given in Cowan and Steel's Manual for the Identification of Medical Bacteria (edited by Barraw and Feltheam, 1993) were used in the shelf life study of smoked shrimp. Aerobic plate count was done by consecutive decimal dilution technique. According to International Standard Organization (ISO, 1965) APC was calculated by the following formula:

$$APC/g = C \times D \times 10/S \text{ CFU/g}$$

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

Statistical analysis

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

Results and Discussion

Organoleptic quality assessment of smoked shrimps

On the basis of color, odor, texture and overall other quality aspects, the organoleptic qualities of traditional and improved smoked shrimps were evaluated and the results are presented in Table-3. The smoked shrimp of bright red color, strong smoky odor and crisp, fragile texture is considered excellent and preferred by the consumers most. In the present study, the color of traditional smoked chali ranged from dark red to reddish color and the odor was mild smoky. The texture of traditional smoked chali was fragile to elastic which showed the medium quality of the product. The average defect point of the traditional smoked Chali was 2.33. On the basis of the organoleptic observation, traditional smoked chali was good/acceptable.

Table 3. Organoleptic quality of traditional smoked chali and improved smoked samples of four different shrimp species

Sample name	Characteristics	Defect Characteristics	Defect points	Average Defect Points	Grade/ Comments
Traditionally smoked Chali	Color	Reddish color	3	**2.33**	**B** Good/Acceptable
	Odor	Mild smoky odor	2		
	Texture	Fragile texture	2		
Improved smoked Horina	Color	Bright red color	1	**1.0**	**A** Excellent
	Odor	Strong smoky odor	1		
	Texture	Crisp fragile texture	1		
Improved smoked Chali	Color	Bright red color	1	**1.0**	**A** Excellent
	Odor	Strong Smoky odor	1		
	Texture	Crisp fragile texture	1		
Improved smoked Chaka	Color	Bright red color	1	**1.33**	**A** Excellent
	Odor	Strong Smoky odor	1		
	Texture	Fragile texture	2		
Improved smoked Khogda	Color	Dark red color	2	**1.33**	**A** Excellent
	Odor	Strong smoky odor	1		
	Texture	Crisp fragile texture	1		

Among the improved smoked shrimps, except Khogda, the other three shrimp species showed preferable bright red color after smoking. Khogda showed dark red color. The organoleptic characteristics of improved smoked Horina and Chali were better than those of traditionally smoked Chali. The texture of the improved smoked shrimp was crisp and fragile which was achieved through reduction of moisture properly. The average defect points of improved smoked Horina, Chali, Chaka, and Khogda were 1.0, 1.0, 1.33 and 1.33, respectively. Differences in the defect points of improved smoked shrimps might be due to the differences in the quality (size, freshness etc.) of the raw materials.

Changes in organoleptic characteristics of traditional and improved smoked chali during storage at refrigeration temperature

By sensory methods (through sight, touch, taste, smelling, etc.) the organoleptic characteristics (general appearance, taste, flavor, texture, and color) of the smoked shrimps were examined. On the basis of the method currently used by Fish Inspection and Quality Control (FIQC) of DoF, the present study was done with slight modifications, which are summarized in Table-4. In the case of non-sealed package, the sensory quality of traditional smoked shrimp was good up to 60 days at refrigeration temperature, whereas the quality of improved smoked shrimp were good till 90 days. In airtight package, the smoked shrimps were more stable than the samples stored in non-sealed package and the improved smoked chali was acceptable in this pack until 120 days and the traditional smoked chali up to 90 days. On the other hand, in vacuum sealed package the sensory quality of smoked shrimps remained very good even after storage of 120 days. Smoked shrimps stored in vacuum sealed package scored quite near to those of smoked shrimps on "0" days of storage. During the storage, the changes in color of different shrimp samples from whitish to brownish might occurred

due to the oxidation of lipid. Among the samples stored in three different packages, shrimps stored in non-sealed package were found more susceptible to lipid oxidation and the case of the oxidation might be due to the action of air over the samples.

Haq *et al*. (2008) carried out a comparative study on the shelf life of smoked shrimp products in different storage conditions. They used polythene bag, gunny bag, hogla made basket for storing both traditional and improved smoked shrimp at room temperature (28 to 30°C) for 4 months. According to that study, the traditional and improved smoked shrimps were bright red in color, odor was sweet and the texture was fragile-crispy. With the progress of storage time, the smoked shrimp absorbed atmospheric air, become rancid by hydrolysis. The product stored in polythene bag was comparatively in better condition than other two bags as polythene bag is less permeable of air than other two. These results are quite similar to the finding of the present study.

Nahid *et al*. (2017) conducted a comparative study on the shelf-life quality of salt and garlic treated smoke-dried *Xenentodon cancila* under different storage conditions. At the beginning of storage, all the sensory parameters of S+G (salt + garlic) treated smoke-dried fish samples were rated as good based on the grading scale. Moreover, no broken parts of the experimental fishes after smoke-drying process were found. The highest mean of general acceptability score was 8.90 (9-point ascending scale to evaluate changes in color, dor, texture, general appearance and mean of general acceptability score in freshly process S+G treated smoke-dried fish samples). The mean of general acceptability score decreased as storage-duration increased and the acceptability score <3.5 is considered as rejected or spoiled for S+G treated smoke-dried products during room and refrigeration storage. The mean of general acceptability score of the end product of S+G treated smoke dried *X. cancila* fish was 3.37 (14 months) and 3.36 (24 months) for room and refrigeration storage, respectively. The reduction in the sensory qualities with increase in storage period of processed fish could be attributed to higher activities of the spoilage agents which is in agreement with the findings of Olatunde *et al* (2013) and Daramola *et al*. (2007) who observed the changes during storage of crustaceans (oyster and shrimps) and revealed quality loss during storage both at ambient and chilling temperature. Similarly, Llobreda *et al*. (1986) and Reilly and Dela-cruz (1986) also studied the quality changes during storage of crustaceans (oyster and shrimps) and found quality losses both at ambient and chilling temperature. All these results are in an agreement with the present study.

Table 4. Changes in organoleptic characteristics of traditionally smoked chali and improved smoked chali during storage at refrigeration temperature (5 to 8°C) in different packages

Days of storage	Characteristics					
	Non-sealed pack		Air tight pack		Vacuum pack	
	Traditional smoked Chali	Improved smoked Chali	Traditional smoked Chali	Improved smoked Chali	Traditional smoked Chali	Improved smoked Chali
0	Bright red color Strong smoky sweet odor Crisp and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Crisp and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Crisp and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture
15	Bright red color Strong smoky sweet odor Crisp and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Crisp and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture
30	Mild red color Mild smoky odor Fragile texture	Dark red color Mild smoky odor Stringy and fragile texture	Dark red color Strong smoky sweet odor Crisp and fragile texture	Dark red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture
60	Brownish color Neutral odor Fragile texture with powder	Mild red color Mild smoky odor Crispa, fragile texture	Reddish color Mild smoky odor Fragile texture	Reddish color Mild smoky odor Fragile texture	Dark red color Strong smoky sweet odor Crisp and fragile texture	Bright red color Strong smoky sweet odor Stringy and fragile texture
90	* Whitish color Rancid, off odor Powdery structure with whitish fungal colony	Brownish color Neutral odor Less fragile and some elastic texture	Brownish color Neutral odor Fragile texture	Reddish color Mild smoky odor Fragile texture	Mild red color Mild smoky odor Elastic structure of muscle	Dark red color Strong smoky sweet odor Stringy and fragile texture
120	-	* Whitish color Rancid, off odor Powdery structure with whitish fungal growth -	* Whitish color Rancid, off odor Powdery structure with whitish fungal colony	Brownish color Neutral odor Less fragile and some elastic texture	Brownish color Neutral odor Crisp texture with powder	Reddish color Mild smoky odor Crispa, fragile texture

* indicates the stage of rejection in storage period and

– indicates the empty pack, after rejection of the samples before 120th day of storage

Changes in biochemical and microbiological parameters of smoked shrimps at refrigeration temperature

The quality parameters of a products are affected by the preparation procedure of the product, storage temperature, packing materials etc. The changes in the biochemical and microbiological parameters of smoked shrimps (both traditional and improved) during storage at refrigeration temperature (5 to 8°C) under different packing conditions are presented in Table-5.

Changes in biochemical parameters

Moisture

The moisture content of traditional smoked Chali was 17.53±0.11% at the initial stage of storage. In improved smoked Horina, Chali, Chaka and Khogda, the moisture contents were 15.36±0.05 %, 14.22±0.13 %, 16.02±0.02 % and 16.15±0.12 %, respectively. During storage in different packages, the moisture content (%) of all the species of smoked shrimps increased with the lapse of storage period. After 15 days of storage, the moisture contents (%) of traditional smoked Chali were 19.94±0.13, 18.95±0.13 and 17.55±0.12 in non-sealed, air tight and vacuum sealed packs, respectively which increased to 32.88±0.21, 22.75±0.12 and 18.83±0.21, respectively after storage period of 120 days at refrigeration temperature. In case of improved smoked Chali, Horina, Chaka and Khogda, similar increasing trend in moisture content (%) was followed (Table-5). The moisture contents (%) were 18.53±0.13, 16.45±0.14 and 14.29±0.12 in non-sealed, air tight and vacuum sealed improved smoked Chali after 30th day of storage, with the progress of storage period moisture content of these samples increased to 26.56±0.14, 18.09±0.11 and 14.78±0.15, respectively on 90th day of storage indicating the slower rate of moisture increase of the smoked shrimp samples both in air tight and vacuum sealed packages.

Protein

The protein content of traditional smoked Chali was 61.18±0.91% and in improved smoked Horina, Chali, Chaka and Khogda, the protein contents were 63.70±0.55%, 64.05±0.41%, 62.15±0.54 % and 63.11±0.41 %, respectively. Protein content (%) of all the species of smoked shrimps stored in different packages decreased with the progress of storage period. After 15 days of storage at refrigeration temperature, the protein contents (%) of traditional smoked Chali was 58.42±0.34, 59.39±0.66 and 60.21±0.45 in non-sealed, air tight and vacuum sealed packages, respectively which decreased to 44.78±0.22, 53.14±0.24 and 54.91±0.39, respectively after 90 days of storage period. For all other improved smoked samples similar decreasing trend in protein content (%) was observed (Table-5). The protein contents were 61.97±0.54%, 62.15±0.57% and 62.89±0.51% in non-sealed, air tight and vacuum sealed improved smoked Horina on 15th day of storage, with the lapse of storage time protein content of these samples decreased to 45.44±0.33 %, 53.11±0.55 % and 56.05±0.51 % on 120th day of storage indicating the faster rate of protein decrease in the smoked shrimp samples stored in non-sealed packages.

Lipid

At the beginning of storage, the lipid contents were 9.24±0.05%, 10.05±0.06%, 9.85±0.04%, 9.96±0.06% and 9.36±0.06% in traditional smoked Chali, improved smoked Horina, Chali, Chaka and Khogda, respectively. During storage, the lipid content (%) of all the species of smoked shrimps decreased with the progress in storage period. The lipid contents (%) of traditional smoked Chali were 8.75±0.04, 9.11±0.02 and 9.18±0.03 in non-sealed, air tight and vacuum sealed packages, respectively on 15th day of storage which decreased to 6.27±0.10, 6.79±0.08 and 6.79±0.08 after 120 days of storage at refrigeration temperature. A similar decreasing trend in lipid content (%) was found in all other improved smoked samples (Table-5). The lipid contents (%) were 9.73±0.05, 9.88±0.06 and 9.94±0.01 in non-sealed, air tight and vacuum sealed improved smoked Chaka on 15th day of storage. With the progress of storage period, lipid content of these samples decreased to 7.96±0.05, 9.01±0.07 and 9.33±0.05 after 90th day of storage indicating the slower rate of lipid oxidation of the smoked shrimp samples both in air tight and vacuum sealed packages.

Ash

The ash contents were 12.52±0.09%, 11.38±0.08%, 11.06±0.06 %, 11.79±0.07 % and 12.14±0.04 % in traditional smoked Chali, improved smoked Horina, Chali, Chaka and Khogda, respectively. Ash content (%) gradually increased with the progress of storage period in all species of smoked shrimps. The ash contents (%) of traditional smoked Chali were 14.52±0.05, 13.81±0.09 and 13.52±0.12 stored in non-sealed, air tight and vacuum sealed packages, respectively on 30th days of storage at refrigeration temperature, which increased to 19.11±0.07, 16.46±0.06 and 15.89±0.11 after storage period of 120 days. A similar increasing trend in ash content (%) was observed for all other improved smoked samples- (Table-5). The ash contents were 12.88±0.05%, 12.49±0.07% and 12.42±0.05% in non-sealed, air tight and vacuum sealed improved smoked khogda on 15th day of storage, with the lapse of storage time ash content of these samples decreased to 15.96±0.07 %, 14.52±0.09 % and 14.00±0.08 % on 90th day of storage indicating the faster rate of increase in ash content of the smoked shrimp samples in non-sealed packages.

TVB-N Value

Initially, the TVB-N values were 15.83±0.08, 12.86±0.07, 12.23±0.11, 14.05±0.13 and 13.14±0.10 (mg/100g) in traditional smoked Chali, improved smoked Horina, Chali, Chaka and Khogda, respectively. The TVB-N values of all the species of smoked shrimps increased with the progress of storage period. The TVB-N values of traditional smoked Chali were 18.69±0.09, 17.73±0.11 and 17.12±0.10 (mg/100g) in non-sealed, air tight and vacuum sealed packages, respectively on 15th day of storage which increased to 35.33±0.12, 30.15±0.10 and 25.72±0.11 (mg/100g) after 90 days of storage at refrigeration temperature. A similar increasing trend in TVB-N values was found for all other samples (Table-5). Among them, the TVB-N values were reported to be 18.78±0.12, 17.34±0.12 and 15.42±0.15 (mg/100g) in non-sealed, air tight and vacuum sealed improved smoked horina on 30th day of storage, with the progress of storage period the TVB-N values of these samples increased to 32.84±0.11, 29.89±0.12 and 25.94±0.14 (mg/100g) after 120 days of storage indicating that the rate of increase in TVB-N values in air tight and vacuum sealed packages is slower than in non-sealed package.

Changes in microbiological parameter

Aerobic plate count (APC) of bacteria

At the initial stage of storage, APC of bacteria were 3.6 0 x 10^3, 9.8 0 x 10^2, 4.30 x 10^2, 2.05x 10^3 and 1.12 x 10^3 CFU/g in traditional smoked Chali, improved smoked Horina, Chali, Chaka and Khogda, respectively at refrigeration temperature. During storage in different packages, APC of all the species of smoked shrimps increased with the progress of storage period. After storage period of 15 days, the APC of traditional smoked Chali were 4.40 x 10^4, 1.62 x 10^4 and 8.80 x 10^3 CFU/g in non-sealed, air tight and vacuum sealed packages, respectively which increased to 3.16 x 10^7, 2.07 x 10^6 and 3.10 x 10^5 CFU/g after storage period of 120 days at refrigeration temperature. In case of improved smoked Chali, Horina, Chaka and Khogda,a similar increasing trend in APC of bacteria was observed (Table-5). Among different smoked shrimp samples, APC of bacteria were 1.72 x 10^4, 8.50 x 10^3 and 3.10 x 10^3 CFU/g in non-sealed, air tight and vacuum sealed improved smoked Chaka on 15th day of storage. With the progress of storage period, APC of these samples increased to 1.18 x 10^7, 6.20 x 10^5 and 2.24 x 10^5 CFU/g after 90 days of storage indicating the slower rate of increase in APC count of the smoked shrimp samples both in air tight and vacuum sealed packages.

Hoq *et al* (2003) studied the nutritional qualities of smoked shrimp from the Sundarbans mangrove area, Bangladesh. They observed in their 90 days of storage quality study that, moisture level increased to 24% from initial 13% and the protein content of raw shrimp was 14.51%, after smoking the protein content was 68.08% due to loss of about 60% moisture. After one month storage, protein content reduced to 64.48%. The protein percentage further reduced to 57.17% after 60 days and at 90th day it stands at 52% due to gradual increase of moisture content. Lipid content gradually increased to 11.66% from 6.88%. Proximate composition and bacterial load study in market sample revealed increased moisture level (19.51%) and decreased protein content (60.83%) with a microbial load of 6.5x10^4 CFU/g. TVBN value increased with storage time from 22.75% at "0"day to 29.33% at 90th day. Total bacterial counts of the smoked product increased to 1.41x10^7 CFU/g from an initial 2.46x10^4 CFU/g over 90 days. These results are quite similar to the findings of present study.

A comparative study on the shelf life of smoked shrimp products in different storage conditions was carried out by Haq *et al*. (2008), which was in agreement with the present study. The study was performed at room temperature (28~30°C) and the smoked shrimp (Chali; *Metapeneous brevicornis*) was stored at polythene bag, gunny bag and hogla made basket. Different quality parameters of smoked shrimps were studied for 4 months at 15 days interval. In their 4 months study, moisture level increased from 11.87±0.51 to 25.48±0.28, 26.53±0.12 and 29.41±0.25 % in polythene bag, gunny bag and hogla made basket, respectively. The protein content reduced from 66.08±0.76 to 51.32 ±0.26, 51.89±0.42 and 46.80±0.50 in three above mentioned bags, respectively. Lipid content also gradually decreased from 9.43±0.65 to 9.35 ±0.41, 9.13±0.51 and 8.98±0.46, respectively. TVB-N value increased with the lapse of storage time from 13.42 to 28.60, 32.45 and 34.06, respectively for the samples stored in above mentioned bags.

Nahid *et al* (2017) conducted a study to determine of shelf life quality of three smoke-dried freshwater fishes in refrigerated storage. It was found that, moisture, protein, fat, ash and TVB-N value of freshly processed smoke-dried chapila, kaika and baim fish samples varied in the range of 8.22% to 12.36%, 45.25% to 74.85%, 5.25% to 32.05%, 8.31 to 10.83%, 6.39 to 11.08 mgN/100g respectively. During smoke-drying, the percentage of moisture content decreased whereas protein, fat and ash content increased due to water loss. Later during storage, it was observed that moisture and TVB-N value gradually increased whereas protein and fat content decreased at refrigeration storage condition. This increase in moisture content can be attributed to absorption of moisture from the surrounding since there was no re-drying during storage (Daramola *et al*., 2007). During storage, the protein content decreased significantly with the time due to water soluble protein diffused out to the surrounding for exosmosis (Hasan *et al*., 2013). This could be due to gradual degradation of initial crude protein to more volatile products such as total volatile bases, hydrogen sulphide and ammonia (Eyo, 2001). Similar drop in protein concentration was reported for *Heterobranchus longifilis* (Abolagba *et al*., 2008). At refrigeration temperature, reduction in lipid content over the storage period may be attributed to oxidation of poly-unsaturated fatty acids contained in the fish tissue to products such as peroxides, aldehydes, ketones and free fatty acids (Horner, 1992). Egbal *et al*. (2013) stated that decrease in fat during storage may also be due to the effect of packaging and storage condition. The increase in TVB-N value may be due to microbial activity, storage temperature, and absorption of moisture. Total Viable Counts (TVC) in terms of CFU/g of bacteria in smoke-dried chapila, kaika and baim fish samples were 3.3×10^4 CFU/g, 3.6×10^4 CFU/g and 3.7×10^3 cfu/g, respectively at the initial stage of storage of the fishes. TVC were found to be increased with storage at refrigeration temperature. As the duration of storage increased, the processed fish samples may absorb small amounts of moisture from surrounding atmosphere providing enabling environment for microbial growth (Eyo, 2006). All these literatures are in agreement with the findings in the present study accept the decreasing trend in lipid.

Conclusion

On the basis of the obtained results the study could be concluded as- improved smoked shrimps can be stored in air tight package or vacuum package at refrigeration temperature (5 to 8°C) for 150 days or more without significant quality loss. Improved smoked shrimp showed the longer shelf life compared to the traditional smoked shrimps. Among three different packages, non sealed is less effective than air tight or vacuum sealed packages for storing smoked shrimps.

Table 5. Changes in the biochemical and microbiological parameters of smoked shrimps during storage at refrigeration temperature (5 to 8°C) in different packs

Sample name	Packing condition	Days of storage	Changes in biochemical and microbiological parameters					
			Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	TVB-N (mg/100g)	APC (CFU/g)
Traditional smoked Chali	Non sealed pack	0	17.53±0.11	61.18±1.23	9.24±0.09	12.52±0.15	15.83±0.12	3.6 0 x 103
		15	16.68±0.03	59.52±1.14	9.05±0.12	13.71±0.11	17.82±0.13	2.87 x 104
		30	15.81±0.02	58.38±1.14	8.84±0.02	14.92±0.15	19.91±0.12	8.29 x 104
		60	14.59±0.07	56.05±1.20	8.48±0.13	17.38±0.19	23.76±0.13	2.93 x 105
		90	12.98±0.02	53.24±0.95	7.92±0.12	20.49±0.17	28.24±0.14	5.02 x 106
		120	12.10±0.02	51.76±0.78	6.89±0.12	22.27±0.12	32.21±0.13	-
		150	11.67±0.02	50.44±0.78	6.11±0.32	23.98±0.11	35.22±0.13	-
	Air tight pack	15	17.04±0.05	60.17±0.98	9.13±0.02	13.26±0.12	17.10±0.10	6.11 x 103
		30	16.30±0.02	59.15±1.15	8.96±0.09	14.15±0.14	18.76±0.11	2.42 x 104
		60	15.41±0.04	57.24±1.24	8.62±0.12	16.61±0.15	17.85±0.12	3.70 x 105
		90	14.05±0.06	54.91±0.88	8.28±0.13	18.90±0.17	23.92±0.10	9.21 x 105
		120	12.71±0.05	52.48±0.78	7.76±0.14	21.37±0.15	28.24±0.12	8.87 x 106
		150	11.11±0.02	51,37±0.68	6.79±0.12	23.32±0.14	31.98±0.13	-
	Vacuum sealed pack	15	17.04±0.05	60.56±0.91	9.15±0.05	13.11±0.09	16.78±0.11	4.42 x 103
		30	16.74±0.04	59.62±1.13	9.02±0.05	13.86±0.13	21.50±0.15	8.92 x 103
		60	15.83±0.05	58.00±1.10	8.78±0.10	15.13±0.21	20.10±0.14	1.89 x 104
		90	14.68±0.03	55.88±1.02	8.45±0.15	17.64±0.12	22.77±0.11	2.81 x 105
		120	13.44±0.04	53.57±1.12	8.08±0.09	19.88±0.18	25.56±0.12	9.05 x 105
		150	12.12±0.03	51.72±1.06	7.69±0.09	22.15±0.14	28.42±0.12	6.17 x 106
Improved Smoked Horina	Non sealed pack	0	15.36±0.05	63.70±1.13	10.05±0.15	11.38±0.12	12.86±0.11	9.81 x 102
		15	14.63±0.02	62.05±1.14	9.91±0.23	12.84±0.10	14.74±0.11	7.90 x 103
		30	14.08±0.03	60.74±0.98	9.71±0.09	14.19±0.14	16.72±0.14	3.70 x 104
		60	13.14±0.04	58.45±1.05	9.42±0.12	16.24±0.21	20.34±0.12	1.09 x 105
		90	11.95±0.01	55.62±1.02	8.88±0.09	18.98±0.16	24.55±0.20	8.02 x 105
		120	10.21±0.05	52.78±1.18	8.21±0.08	22.41±0.20	29.84±0.23	2.61 x 106
		150	10.40±0.03	50.23±1.15	801±0.08	24.09±0.17	32.06±0.13	-
	Air tight pack	15	15.02±0.03	62.62±1.15	9.98±0.02	12.27±0.10	13.89±0.05	2.37 x 103
		30	14.47±0.04	61.75±1.10	9.84±0.10	13.55±0.13	15.05±0.09	8.10 x 103
		60	13.68±0.02	59.98±0.98	9.68±0.05	15.05±0.15	18.38±0.14	1.05 x 104
		90	12.89±0.03	57.66±1.14	9.34±0.05	17.72±0.21	20.78±0.21	2.40 x 105
		120	11.65±0.01	55.23±1.17	8.95±0.07	19.88±0.21	24.65±0.24	9.21 x 105
		150	11.32±0.02	54.47±1.12	8.37±0.09	22.07±0.15	29.65±0.21	3.92 x 106
	Vacuum sealed pack	15	15.14±0.05	62.95±1.21	10.02±0.08	12.42±0.09	13.47±0.09	1.45 x 103
		30	14.75±0.02	62.11±1.12	9.95±0.06	13.37±0.11	14.34±0.12	5.25 x 103
		60	14.12±0.05	60.82±0.95	9.79±0.11	14.51±0.12	16.26±0.13	2.80 x 104
		90	13.60±0.05	58.95±1.09	9.51±0.04	16.17±0.23	18.14±0.12	1.51 x 105
		120	12.48±0.01	52.92±0.99	8.14±0.06	18.25±0.15	21.76±0.19	8.82 x 105
		150	15.36±0.05	63.70±1.13	8.68±0.08	20.89±0.17	25.96±0.20	3.81 x 106
Improved smoked Chali	Non sealed pack	0	14.22±0.02	64.05±1.10	9.85±0.09	11.06±0.14	12.23±0.12	4.30 x 102
		15	13.39±0.01	62.80±1.11	9.72±0.06	12.51±0.12	13.86±0.14	5.18 x 103
		30	12.84±0.03	61.24±1.13	9.51±0.06	13.89±0.15	16.38±0.10	9.77 x 103
		60	12.08±0.01	58.46±1.16	9.14±0.04	16.11±0.12	19.81±0.14	4.90 x 104
		90	10.93±0.06	60.56±1.14	8.72±0.03	20.47±0.15	24.16±1.24	1.74 x 105
		120	09.85±0.04	58.34±1.11	8.05±0.07	23.20±0.21	29.05±1.24	4.54 x 106
		150	10.02±0.03	51.84±1.13	7.67±0.08	24.89±0.13	33.41±1.22	-
	Air tight pack	15	13.88±0.04	63.28±1.12	9.79±0.07	12.12±0.13	12.98±0.17	3.78 x 103
		30	13.29±0.02	62.04±1.11	9.70±0.05	13.07±0.14	15.25±0.13	9.68 x 103
		60	12.50±0.05	59.75±1.15	9.52±0.01	14.72±0.11	17.77±0.12	1.56 x 104
		90	11.77±0.03	55.27±1.15	9.19±0.05	17.09±0.14	20.83±0.15	4.70 x 105
		120	10.89±0.01	54.86±0.94	8.64±0.03	19.81±0.20	24.65±1.10	9.17 x 105
		150	10.63±0.05	52.18±1.14	8.13±0.05	21.93±0.15	29.12±2.10	6.45 x 106
	Vacuum sealed pack	15	14.00±0.05	63.51±0.95	9.86±0.08	11.87±0.14	12.66±0.17	1.66 x 103
		30	13.56±0.04	62.72±0.90	9.78±0.04	12.65±0.15	14.78±0.15	8.35 x 103
		60	12.92±0.01	60.56±1.14	9.63±0.08	13.98±0.13	16.85±0.11	2.21 x 104
		90	12.28±0.02	57.19±1.10	9.35±0.06	16.16±0.16	19.53±0.12	1.31 x 105
		120	11.54±0.05	56.02±1.10	8.92±0.04	18.65±0.18	22.50±1.14	9.61 x 105
		150	11.10±0.07	53.24±1.10	8.48±0.03	20.76±0.19	25.42±1.25	3.79 x 106
Improved smoked Chaka	Non sealed pack	0	16.02±0.03	62.15±1.20	9.96±0.06	11.79±0.11	14.05±0.09	2.05 x 103
		15	15.51±0.02	60.92±1.10	9.82±0.05	12.86±0.09	15.96±0.08	1.71 x 104
		30	15.05±0.05	59.63±0.99	9.67±0.05	14.24±0.12	17.90±0.12	3.50 x 104
		60	14.17±0.02	54.94±1.16	9.33±0.05	16.75±0.09	21.56±0.10	1.78 x 104
		90	13.05±0.01	52.11±1.14	8.88±0.04	19.61±0.15	26.05±0.25	1.86 x 105
		120	11.71±0.05	52.08±1.10	8.26±0.06	22.57±0.12	31.06±0.18	3.60 x 105
		150	12.02± 0.03	50.11±1.10	7.54±0.05	23.87±0.08	34.45±0.13	-
	Air tight pack	15	15.83±0.04	61.58±1.14	9.89±0.09	12.51±0.08	15.08±0.05	8.10 x 103
		30	15.71±0.05	60.36±1.05	9.78±0.04	13.69±0.10	16.58±0.13	6.56 x 103
		60	14.98±0.02	58.92±1.12	9.61±0.06	15.11±0.05	18.71±0.15	3.10 x 104
		90	14.01±0.03	57.55±1.12	9.26±0.06	17.96±0.12	21.28±0.09	6.84 x 104
		120	13.12±0.05	54.87±1.12	8.84±0.05	20.18±0.12	25.64±0.21	9.91 x 105
		150	12.81±0.03	53.54±1.12	8.42±0.09	22.74±0.16	30.76±0.25	6.08 x 106
	Vacuum sealed pack	15	15.89±0.05	61.75±1.25	9.95±0.08	12.35±0.09	14.62±0.09	4.80 x 103
		30	15.68±0.05	60.84±1.02	9.88±0.06	13.12±0.12	15.75±0.12	7.70 x 104
		60	15.26±0.06	59.57±0.92	9.68±0.07	14.64±0.11	17.44±0.14	9.52 x 105
		90	14.55±0.04	57.84±1.14	9.39±0.04	16.49±0.15	19.85±0.15	3.02 x 106
		120	13.76±0.05	55.79±1.15	9.15±0.04	18.76±0.19	22.73±0.26	1.29 x 105
		150	14.17±0.02	53.54±1.12	8.75±0.08	21.18±0.14	26.84±0.19	1.67 x 106
Improved smoked Khogda	Non sealed pack	0	16.15±0.03	63.11±0.98	9.73±0.95	12.14±0.13	13.14±0.09	1.12 x 103
		15	15.26±0.05	61.88±1.13	9.48±0.99	13.66±0.16	15.62±0.21	3.40 x103
		30	14.52±0.01	60.36±1.10	9.04±0.12	14.95±0.14	17.15±0.13	9.79 x 103
		60	13.44±0.02	57.87±1.24	8.56±0.05	17.16±0.11	21.61±0.21	2.68 x 104
		90	12.16±0.02	55.08±1.12	8.04±0.07	20.05±0.14	25.40±0.21	1.50 x 105
		120	10.95±0.02	58.42±1.24	7.59±0.09	22.78±0.18	30.52±0.24	2.11 x 106
		150	11.23±0.04	55.93±1.14	7.02±0.13	24.23±0.16	-	-
	Air tight pack	15	15.65±0.02	62.51±1.14	9.62±1.03	13.21±0.15	14.50±0.12	2.86 x 103
		30	15.05±0.01	61.43±1.17	9.31±0.97	14.26±0.12	15.96±0.12	5.26 x 103
		60	14.21±0.03	59.64±1.15	9.08±0.15	16.15±0.10	19.02±0.10	2.82 x 104
		90	13.14±0.04	57.25±1.15	8.66±0.06	18.70±0.13	23.00±0.19	4.20 x 105
		120	12.35±0.01	51.57±1.10	8.25±0.08	20.96±0.15	26.75±0.20	9.89 x 105
		150	12.12±0.04	52.14±1.12	7.65±0.08	23.00±0.21	31.14±0.12	4.70 x 106
	Vacuum sealed pack	15	15.86±0.04	62.45±1.10	9.59±1.02	12.92±0.14	13.98±0.12	2.07 x 103
		30	15.38±0.07	61.74±1.15	9.42±0.98	13.87±0.12	15.45±0.08	7.81 x 103
		60	14.67±0.02	60.21±1.14	9.27±0.10	15.29±0.11	17.86±0.11	3.80 x 104
		90	13.78±0.03	58.42±1.24	8.92±0.05	17.57±0.12	20.96±0.18	9.24 x 104
		120	13.04±0.08	54.79±1.13	8.54±0.06	19.61±0.15	24.12±0.21	3.72 x 105
		150	16.15±0.03	53.58±0.99	8.05±0.07	21.87±0.19	27.48±0.15	2.94 x 106

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