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RESEARCH PAPER

Effects of different types and concentrations of salt on the quality aspects of salted hilsa (*Tenualosa ilisha*)

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ABSTRACT

A study was conducted to prepare salted hilsa using different monovalent (NaCl, KCl) and divalent salts ($CaCl_2$, $MgCl_2$) at different concentration (20%, 25%, 30% and 35%) and their effects on sensory, biochemical and microbial quality during maturing time. Hilsa fish were properly prepared and salts were spread and mixed on and in of hilsa fish. After salting, sensory quality (color, odor and texture) were observed carefully by a set of trained panelist. Proximate composition of fresh and salted hilsa was analyzed by standard AOAC method. Total microbial count of salted hilsa during the time of maturation was done by spread plate method. Based on sensory analysis, among four different concentrations (20%, 25%, 30% and 35% of total body weight), fish salted with 25% NaCl showed best result. Further, to find the appropriate salt for hilsa salting, different monovalent (NaCl, KCl) and divalent salts ($CaCl_2$, $MgCl_2$) were used at obtained 25% of fish body weight. Sensory quality characteristics of salted hilsa prepared with four different salts revealed that monovalent salts (NaCl, KCl) resulted better sensory characteristics than that of divalent salts ($MgCl_2$, $CaCl_2$). A significant decrease in moisture content (from 63.79% to 47.65%) and lipid content and, similar protein content was found between the fresh and salted hilsa. Microbiological study of salted hilsa showed microbial count was 9.28×10^5cfu/g for fresh hilsa at day 0 and salted ripened hilsa on day 6 had 1.35×10^8cfu/g. The study concluded that 25% of NaCl was suitable for good quality and maturing of salted hilsa.

Key words: Hilsa, microbial load, sensory characters, proximate composition

Introduction

Bangladesh is one of the leading fish producing country and it stands fourth in total inland fish production in the world. Hilsa (*Tenualosa ilishsa*) is one of the commercial important open water species in Bangladesh that available in the major river, estuaries and the sea. In Bangladesh about one tenth of total fish catch is the contribution of hilsa. In the financial year 2013-14 total hilsa catch was more than 3.85 lakh MT with the price of over 17,000 crore taka (DoF, 2015). Hilsa is the national fish of Bangladesh, due to its popularity and economic importance. Nowsad (2007) stated hilsa has more tastiness than most other fishes due to its typical biochemical composition especially fatty acid composition. Hilsa has a universal appeal to the consumer because of its excellent flavor and delicate taste (Rahman et al., 1999). The core flavor is enhanced by other taste active amino-acids, mainly taurine and arginine, and nudeotides and inorganic ions, peptides, organic bases, organic acids and sugars are also responsible for characteristic taste of some fish species. Flavor volatiles, lipids, fatty acids and glycogen also play important role in producing overall flavor. The unique taste of Hilsa has often been attributed to the presence of certain fatty acids like steareic acid, oleic acid and many poly unsaturated fatty acids (ω3, ω6) viz. linoleic, lenoleneic, arachidonic, eicosapentanoeic (EPA) and docosa-hexanoeic (DHA) acids. There is a close linkage between the poly unsaturation of fatty acids and the taste of the fish. Mouthwatering flavor and superb mouth feel made the fish "Macher Raja" means "the king of fish" of Bangladesh.

Traditionally no ice or small amount of ice was used for preservation of hilsa. However, a few examples of icing are found but not proper ratio and quality of ice are maintained for hilsa preservation. On the other hand, drying method is not used due to its high fat or lipid content. Salting is a one of the traditional methods used for preserving fatty fish due to its ability to create unfavorable environment for the growth of microorganisms by lowering the water activity of the

fish muscle. Salting is generally aimed to reduce water activity (a_w) which inhibits the growth of spoilage microorganisms as well as to inactive the autolytic enzymes (Horner *et al.,* 1997). Salting is not only important preservation method, but it also results in particular sensory properties of the product such as aroma and flavor (Harris & Tall, 1994). Quality of salted fish depends on types of salts, concentration of salt, quality of salt, quality of fish and salting method used. Length of salting period as well as salt concentration depends on the expected final product (Bellagha et al., 2007). Traditionally sodium chloride (NaCl) commonly known as table salt is widely used for salting of fish. Commercial salts vary widely in their composition; high quality salt may contain 99.9% sodium chloride, whereas low quality salt may contain 80% sodium chloride. The main chemical impurities of commercial salts are calcium and magnesium chlorides, sulphates, sodium sulphate and carbonate, and traces of copper and iron. Apart from chemical impurities different dust, sands and water caused contamination of salt also occurred. Solar salts tend to be less pure than mine-evaporated salts. Many commercial salts, particularly solar salts, contain large numbers of salt tolerant bacteria (halophiles) and counts of up to 10^5/g have been recorded. One group of halophiles, the red or pink bacteria, can be a problem in commercial fish curing operations as they cause a reddening of wet or partly dried salt fish.

Fine grain salt dissolves more rapidly in water and is preferred for making brines. Sodium chloride diffuses to muscles from the outside due to difference in osmotic pressure between the brine and fish muscle. Sodium and chlorine ions form a water binding complex with protein which itself exerts an osmotic pressure and eventually equilibrium is reached (Horner, 1997). In salted fish, where the salt concentration reaches about 20%, high ionic strength causes contraction of the myofibrils and dehydration of proteins. Also, pH of the medium and the type of salts used for salting can influence the degree of protein denaturation (Wheaton & Lawson, 1985). The aim of salting is not only to extend the shelf life of fresh fish but also to provide desirable sensorial changes (Andres et al., 2005). Microbial and biochemical quality assessment are necessary to ensure a safe and quality processed product (Azam et al., 2003). Among the microorganisms, moderate halophilic bacteria are the dominating floras in salted products. Moderately halophilic bacteria are a heterogeneous group of microorganisms characterized by growth over a wide range of salt concentrations (0.5-2.5 M NaCl). Salted Hilsa is usually spoiled by various microorganisms and by their metabolic activities that lead to the formation of gases and foul smelling compounds and eventually deteriorates fish quality (Shewan, 1976).

Both locally and internationally sodium chloride (NaCl) are commonly used for preparation of salted fishery product. Different studies has been conducted for preparation of salted hilsa (Rahman et al., 1999; Majumdar et al., 2010), microbial quality of salted hilsa (Sultana et al., 2008; Lawrence et al., 2010; Yam et al., 2015), biochemical composition of salted hilsa (Majumdar et al., 2010; Dewan et al., 2015; Hossain et al., 2014; Shamim et al., 2011; Rahman et al., 1999; Mustafa et al., 2012) and storage of salted hilsa (Sultana et al., 2008; Hossain et al., 2014). However, there were no studies conducted using different monovalent and divalent salts for preparation of salted fishery products and their effects on sensory quality, microbial quality and biochemical quality. Therefore, the objective of this study is to prepare salted fishery product using different monovalent and divalent salts at a concentration and their effects on the sensory, biochemical and microbial qualities during maturation time.

Materials and methods

Sample collection

For the preparation of salted hilsa, hilsa (*Tennualosa ilisha*) fish samples were collected from Alipur fish market of Kuakata and Pirtala fish market near the premises of Patuakhali Science and Technology University campus. Those samples were transported in iced condition to the Department of Fisheries Technology laboratory and kept in freezer at -20℃ until use.

Washing and weighing fish

The frozen stored hilsa fish were thawed by using running tap water. The fishes were then washed with flowing of tap water to wash away all the external elements especially the surface slime. After washing fishes were weighed carefully by a digital weight machine.

Scaling

The fishes were scaled by pushing the scales backward from caudal region to head region slowly by using blunt side of a knife. Scaling was followed by cutting the fins at the bases by a sharp knife.

Gutting

For removing guts, a cut was made from the ventral region near operculum up to lateral line mark. That cut made a mouth or opening for the guts to come out freely. Then using index finger all the gut materials were pulled out through that opening. After removing the gut, the fish was washed again to make sure that no blood was present there after gutting.

Cutting fish transversely

After gutting the fish samples were cut transversely using a sharp knife in such a way that the chunks remain attached at the ventral region. The thickness of the piece ranges from 0.75-1.0 cm from one part to another. Head portion was intact with the body.

Salting process

The prepared hilsa samples were brought to the procedure for salting. NaCl was added to separate fishes with a measure of 25% of body weight of the fish. The appropriate amount of salt was properly mixed into gills, mouths, eyes, abdomen and in between each chunks. The blank ventral canal and gill cavity were also rubbed with salt to ensure proper mixing. Along with different salt, a small amount of turmeric powder was used to develop a color in the experiment product. The salt remaining after mixing was spread over the fish. Fish mixed with salt placed on a porous polythene into a

plastic basket. This allows the exudates to come out and run away freely off the fish. After placing the fish was covered by a layer of plain nonporous polythene that protects the fish from attack of flies and insects. A cover was also placed over the plastic basket to protect the fish from rodent attack. After making protective measures the basket with fish was kept in a safe cool and dry condition inside the Department of Fisheries Technology Laboratory.

Sensory quality analysis

Sensory analysis of salted hilsa was evaluated by trained personnel using sensory method during the maturation or ripening time.

Color

Color of the salted hilsa samples was observed by a set of well-trained panelist every day after salting until the maturation occurs.

Odor

Odor of the salted hilsa samples was observed and analyzed by a set of well-trained panelists every day until maturation.

Texture

Texture or muscle consistency of the salted hilsa samples was observed and analyzed organoleptically by a set of well-trained panelists every day until maturation. A gentle pressure was applied by the index figure to determine the exact texture of the salted hilsa samples.

Biochemical study

Biochemical study for determining proximate composition of salted hilsa was conducted following the standard AOAC (2000) method. The composition was analyzed on wet basis (moisture and ash) and dry basis (protein and lipid) according to standard procedure of the Association of Official Analytical Chemists (AOAC, 2000) with certain modifications as described below.

Determination of moisture

Moisture content was determined using AOAC (2000) method. Around 1.0 g muscle tissue was collected from each salted hilsa samples and they were chopped by a sharp knife. Empty crucible was washed, dried, cooled and weighed. Then the tissue samples were transferred into a pre-weighed crucible. Crucibles with samples were then placed in a hot air oven (Kendro M110, Germany). The samples were dried in the oven for 24 hours at 105℃. After drying was done, the crucibles were cooled to the room temperature inside a desiccator and the tissue samples with crucible were weighed again. From the following formula moisture content was estimated for the salted hilsa samples.

$$\% \text{ Moisture} = \frac{w1-w2}{w} \times 100$$

Where, W_1= Initial wt. (sample + crucible) before dry
W_2 = Final weight (sample + crucible) after dry
W = Sample weight

Determination of crude protein content

AOAC (2000) method was followed to determine protein content of the samples. Different regent like Digestion mixture (100g Na_2SO_4 + 10g $CuSO_4.5H_2O$ + 1g selenium powder), 8% $Na_2S_2O_3$, 40% NaOH, H_2SO_4 (Conc.), 2% boric acid (H_3BO_3), Standard HCl (0.1 N), Mixed indicator (1g methylene blue + 2g methylene red in 100 ml ethanol), Methyl orange indicator were required and prepared for determination of crude protein. For the determination of crude protein by kjeldahl method first of all, the sample was taken and chopped into small pieces and was grinded by grinder (IKA A11 BS2, Germany). Approximately 1.0 g of sample was taken in a clean kjeldahl flask and 4 g of digestion mixture was added along with 25 ml of conc. H_2SO_4 by swirling the flask. Then the kjeldahl flask was placed in inclined position on heating device of kjeldahl apparatus (Buchi CH-9230, Switzerland) and was heated at 70℃ for about 1-1.5 hours. The end point of digestion was indicated by a completely clear and of light blue color solution.

The content of the flask was cooled at room temperature and 100 ml of distilled water and 25 ml of $Na_2S_2O_3$ were continuously added in each flask and were mixed and cooled. A few glass beads were added in each flask to prevent bumping. Then 100-120 ml of 40% NaOH was added in each flask to make the solution sufficiently alkaline. The flask was immediately connected to distilling bulb on condenser. A conical flask containing 50 ml of 2% boric acid (H_3BO_3) with 2 drops of mixed indicator was placed under the condenser against kjeldahl flask to collect the distillate. The end point of distillation step was indicated by greenish blue color solution.

After completion of distillation (about 100 ml distillate) the collected distillates were titrated with standard HCl. The conical flask was continuously shaken throughout the titration process. The end point was indicated by light pinkish color.

The total nitrogen was calculated by using following formula-

$$\% \text{ Nitrogen} = \frac{(T-B) \times 0.014 \times N \times 6.25}{weight\ of\ sample} \times 100$$

Where,

B = Reading of titrant of blank samples;
N = Normality of HCl;
0.014= Miliequivalent weight of nitrogen
6.25= Protein conversion factor for animal sources

Determination of crude lipid

AOAC (2000) method was followed to determine crude fat content of the samples. Soxhlet apparatus (Buchi CH-9230, Switzerland) was used for solvent extraction of lipid. 2 g dried sample was taken into extraction thimbles and placed into the Soxhlet apparatus and acetone was used as solvent. After evaporation of the solvent, filtration was allowed for 3 hours and solvent with lipid was transferred to pre-weighed empty beaker. The beaker with lipid was allowed to dry in the oven for 30 mins at 100° C. After cooling into the desiccator the beakers were weighed out. Percentage of lipid was calculated using the following formula:

$$\% \text{ Lipid} = \frac{weight\ of\ beaker\ with\ lipid - weight\ of\ empty\ beaker}{weight\ of\ sample} \times 100$$

Determination of ash

AOAC (2000) method was used to determine the total ash content. The pre-weighed crucible containing dried

sample from the moisture determination was then put into a muffle furnace (Cole-Parmer EW-33858-80, India) to burn at 550℃ for 6 hours. The crucible containing ash was cooled in a desiccator and was weighed in a sensitive electronic balance to find out the ash percentage as follows:

$$\% \text{ Ash} = \frac{w1 - w2}{w} \times 100$$

Where,

W_1= Initial weight (sample + crucible) before dry

W_2 = Final weight (sample + crucible) after dry

W = Sample weight

Bacteriological analysis

Bacteriological study was conducted for salted hilsa during the maturation time. Salted fish sample was collected every two days (Day 0, 2, 4, 6) and was used for Total Plate Count (TPC). To obtain the TPC the following microbiological procedure was maintained.

Media used

For bacteriological analysis, nutrient agar media were prepared by the following composition and procedure:

Plate count agar/ nutrient agar

It was used for total viable count. Plate count agar is a commercial preparation (HI Media: RM 093) having the composition Peptic digest of animal tissue 5.00 g/L, Beef extract1.50 g/L, Yeast extract1.50 g/L, Sodium chloride 5.00 g/L and Agar15.00 g/L.

For preparation of media, 28.0 g of plate count agar media was weighed and suspended into one liter of distilled water in a conical flask. The mixture was shaken for few minutes to make sure the media was suspended thoroughly. Then the mixture was boiled on electric heater to dissolve completely and sterilized.

Mixing the ingredients and boiling

The recommended quantities (28.0 g media powder in 1000 ml distilled water) of nutrient agar was weighed and then dissolved in the required amount of water. The mixture was then boiled on electric heater (Mars: HP-100A) to mix all the ingredients thoroughly.

Sterilization

Before using media were sterilized to kill any bacterial and fungal cells or spores present in the media or in the glasswares. Sterilization was accomplished by placing the media in an autoclave (Prestige Medical- 210134, England) for 20 minutes at a temperature of 121℃ under 15 lbs pressure. Then it was cooled down to around 50℃ and was poured into previously sterilized petridish.

Sample preparation and culture

Standard plate count expressed as colony forming units per gram (cfu/g) were determined by using consecutive decimal dilution technique using spread plate method. Stock solution was prepared for decimal dilution technique. For making stock solution around 8-10 g fish muscle was cut down from the whole sample fish. Skin and intramuscular bones were removed carefully. Then 5.0 g fish muscle sample was blended in 250 ml distilled water to make a homogenously mixed stock solution. For making decimal dilution physiological saline solution (0.8% NaCl solution) was used instead of distilled water. One milliliter stock solution was transferred with a micropipette to test tube containing 9 ml of physiological saline. The test tube was shaken thoroughly on a vortex mixture (VM-1000, Taiwan) in order to get 10^{-1} dilution of original sample solution. Using the similar process several dilutions of 10^{-2}-10^{-5} were made.

Aerobic plate count (APC)

To obtain APC triplicate sterile petridish were prepared using sterile nutrient agar media. From sample solution of different dilutions 0.1 ml samples were taken by a micropipette and transferred aseptically into the pre-prepared agar plates by raising the upper lid sufficient enough to enter the tips of the pipette. The samples were then spread homogenously and carefully by sterile flamed L-shaped glass rod throughout the surface of the media until the sample were dried out. All the inoculated plates were incubated at 37℃ for 24-48 hours. The colony forming units (cfu) were counted using appropriate counting measures. Plates containing 30-300 colonies were used to calculate bacterial load results, recorded as cfu/g of sample by using following formula:

$$\frac{cfu}{g} = \frac{No.of\ colonies\ on\ petridish \times 10 \times dilution\ factor \times volume\ of\ total\ stock\ solution}{weight\ of\ sample}$$

Statistical analysis

Experiments were run in triplicates. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test (DMRT). Analysis was performed using the SPSS package (SPSS 16.00 for windows, SPSS Inc., Chicago, IL, USA). Significance of differences was defined at $p<0.05$.

Result

Effects of different concentration of salt (NaCl) on the sensory quality of salted hilsa

To determine the appropriate salt concentration for hilsa (*Tenualosa ilisha*) salting, four separate hilsa fish samples were salted with different concentration (20, 25, 30 and 35%) of NaCl salt. Table salt (NaCl) was used due to its availability. Each of the samples was observed carefully of their organoleptic changes during maturing or ripening time. The different sensory changes (color, odor and texture) were observed by a set of skilled panelists throughout the maturing process or time.

Change in color of salted hilsa during maturation time effected by different salt concentration (20, 25, 30 and 35%) are presented in Table 1. In the first 2 days, whitish or silver color was observed for salted hilsa under any salt concentration used. However, slight brownish color were observed at day 3 for any salt concentration used, except for 20%. On the day 3, 20% salt concentration still remained the normal whitish or silver color. The slight brownish color for 20% and 25% salt concentration at day 4 was transformed into moderate brown color, when 30% and 35% salt used. On the other hand, moderate brown color was observed for 20 and 25% salted sample at day 5 and at the same time 30% and 35% showed dark brown color. Dark brown color was continued for all treatment at last day

(day 6). From the result it was clearly observed that different concentration of salt had different direct effects on the color of resulted salted fish. Greater the salt concentration was responsible for greater changes in color with increasing day. From the result it is clear that changes in color by different concentration of salt (Table 1) were remarkable where the higher concentration of salt, resulted more obvious changes in color. The salted samples showed moderate brown to dark brown color as concentration increased from 20% to 35% (Table 1). That's because of the excess amount of salts interact with each other and causes salt burn and makes the salted product undesirable among the buyers and consumers. Nowsad (2007) clearly stated that in dry-salted hilsa, any browning is undesirable which makes the product less desirable among the customers.

Table 1. Changes in color of salted hilsa during maturation period

Maturing Time	Salt Concentration (%)			
	20	25	30	35
Day 1	Normal whitish or silver color	Normal whitish or silver color	Normal whitish or silver color	Normal whitish or silver color
Day 2	Normal whitish or silver color	Normal whitish or silver color	Normal whitish or silver color	Normal whitish or silver color
Day 3	Normal whitish or silver color	Slight brownish color	Slight brownish color	Slight brownish color
Day 4	Slight brownish color	Slight brownish color	Moderate brown color	Moderate brown color
Day 5	Moderate brown color	Moderate brown color	Dark brown color	Dark brown color
Day 6	Dark brown color	Dark brown color	Dark brown color	Dark brown color

Table 2. Changes in odor of salted hilsa during maturation period

Maturing Time	Salt Concentration (%)			
	20	25	30	35
Day 1	Natural fishy odor	Natural fishy odor	Natural fishy odor	Natural fishy odor
Day 2	Natural fishy odor	Natural fishy odor	Natural fishy odor	Natural fishy odor.
Day 3	Natural fishy odor	Moderate maturation odor	Moderate maturation odor	Natural fishy odor
Day 4	Moderate maturation odor	Moderate maturation odor	Moderate maturation odor	Natural fishy odor; but decreased than before
Day 5	Faint decomposed odor	Moderate to strong maturation odor	Moderate to strong maturation odor	No odor
Day 6	Moderate to strong decomposed odor	Strong maturation odor	Strong maturation odor	No odor

Table 2 showed the changes in odor of salted hilsa during maturation time influenced by different salt concentration used. From the result, it was observed that natural fishy odor of fish was retained up to 2 days regardless of any salt concentration used. At day 3, moderate maturation odor was observed for salt concentration of 25% and 30%. However, 20% and 35% salt added fish still remain natural fishy odor. At day 4, all the salted samples except the sample with 35% salt showed strong maturing odor, and the fish with 35% salt still showed natural fishy odor but the strength of fishy odor was decreased a bit. On day 5, faint spoilt or decomposed odor was observed for the sample with 20% salt and 25% and 30% salt treated sample showed moderate to strong maturing odor. However, at the same day (day 5), neither natural fishy odor nor maturing odor was found, when 35% salt used. On the final day (day 6), the fish with 20% salt showed moderate decomposed odor while the sample with 35% salt was showing no odor. But the fish salted with 25% and 30% salt showed strong maturing odor. From the result it was noticed that, the odor of salted fish was moderate to largely effected by the concentration of salt.

Table 3 showed the changes in texture of salted hilsa during maturation time influenced by different salt concentration used. From the result, it was observed that

*Int J Inno Res*1(1):30–39, 2016

at day 1, the salted fish sample retained normal texture as fresh hilsa. At day 2, the texture of fish with 30% and 35% salt produced a slight hard texture while the fish with 20% and 25% salt retained the normal texture as day 1. The fish with 20% and 25% salt showed a slight soft texture on day 3, where on the same day, texture became hard and stiff for the fish with 30% and 35% salt. On day 4, the texture of the fish showed no distinguished difference for 20 and 25% with that of day 3. However, on the same day, hard and rough textures were observed for 30 and 35% salt used sample. The fish with 20% salt showed spoiled texture on day 5, while the texture of the fish with 25% was softer but not spoiled. But for the fish with 30% and 35% salt showed the texture with more hard and raptured respectively. On the final day (day 6), the sample salted with 20% salt showed decomposed texture and fish with 35% salt showed raptured texture. Slight harder texture was observed for the fish salted with 25% salt, while the fish salted with 30% salt retained its hard and rough texture till day 6. Upon the completion of maturing or ripening process, a fresh hilsa with high consistency muscle texture became soft to hard texture, where variation in texture was largely influenced by concentration of salt used.

From the result (Table 2 and 3) it was also observed that the color and texture of salted products were influenced by concentration of salt where changes in texture were greatly affected. Lower salt concentration causes less salt penetration in the fish muscle tissue had resulted less water removal from the muscle which leads to higher biochemical, enzymatic and microbial actions. These might be the potential cause behind the decomposed texture and odor of the sample with 20% salt. In case of higher salt concentration (30% and 35%), penetration of salt was higher in the muscle tissues which resulted higher water removal from the fish muscle. As most of the water was removed, thus the spoilage microorganisms could not grow and survive there. On the other hand, due to higher salt concentration inside tissue enzymatic reaction and autolysis was also reduced to some extent. Therefore, salted sample with 35% salt concentration on day 6 might have resulted odorless product. As days were being passed (day 4, 5, 6), when the moisture content was reduced to sufficient level the tension of the bonds that attaches the muscles with bones was decreased and the samples were resulted with harder texture. On day 6, with 35% salt, sufficient decrease in moisture (Table 7) content caused further decrease the tension of muscle tissue leads to shrinkage in the muscle that resulted in raptured texture in the experimental products. In this study, the result showed that the salt concentration of 25% of the fish body weight was suitable for salting (Table 1, 2, 3) which was similar with the result found by Nowsad (2007).

Table 3. Changes in texture of hilsa during maturation period

Maturing time	Salt Concentration (%)			
	20	**25**	**30**	**35**
Day 1	Texture normal	Texture normal	Texture normal	Texture normal
Day 2	Texture normal	Texture normal	Texture slight harder	Texture slight harder
Day 3	Slight soft texture	Slight soft texture	Texture hard and stiff	Texture hard and stiff
Day 4	More soft texture	Slight soft texture	Texture hard and rough	Texture hard and rough
Day 5	Spoiled texture	More soft texture	Texture hard and rough	Raptured texture
Day 6	Decomposed texture	Slight harder texture	Texture harder than before	Raptured texture

Table 4. Effects of different salts on change of color of salted hilsa during maturation period

Salt	Maturation Period					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
NaCl	Natural color	Natural color	Slight brownish yellow color	Slight brownish yellow color	Slight brownish yellow color	Moderate brown color
KCl	Natural color	Natural color	Slight brownish yellow color	Slight brownish yellow color	Slight brownish yellow color	Moderate brown color
$CaCl_2$	Natural color	Skin became a little bit fade	Pale and whitish brown color.	Pale and whitish color continues	Slight brown color appeared.	Dark brown color
$MgCl_2$	Natural color	Natural color	Brownish color	Pale brown color	Moderate brown color	Dark brown color

Effects of Different Types of Salts on sensory Quality of Salted Hilsa

To identify appropriate salt for hilsa salting, in this study four different salts (NaCl, KCl, $CaCl_2$, $MgCl_2$) were used at 25% of the fish body weight (selected from previous section). Then each of the salted hilsa samples was observed until maturation for organoleptic changes by a set of trained panelist.

Effect of different monovalent (NaCl, KCl) and divalent ($CaCl_2$, $MgCl_2$) salts on the changes in color of salted hilsa during maturation are presented in Table 4. From the result, it was clearly observed that in general both monovalent salts (NaCl and KCl) showed similar results during maturation time. On the other hand, divalent salts ($CaCl_2$ and $MgCl_2$) showed similar result which was distinguished different from monovalent salt results. Both monovalent salts (NaCl and KCl) showed normal color up to day 2. However, slight brownish to yellow color was observed from day 3 to 5 for both monovalent salt used. On the other hand, natural color was observed for $CaCl_2$ and $MgCl_2$ in day 1 and day 2. However, with the increasing of the day (day 3-4) during maturing time, pale and whitish brown color was observed for both divalent salts. On the last two days (day 5-6), color for both the fish salted with monovalent salts were moderate brown and for the fish salted with divalent salts showed dark brown color. The result also suggested that no changes in color were observed in the day 1 and day 2 regardless of the salt used. Moderate to strong changes in color was observed for salted hilsa during maturation time. In general, the effects of changes in color were more influenced by divalent salts than monovalent salts.

Effect of different monovalent (NaCl, KCl) and divalent ($CaCl_2$, $MgCl_2$) salts on the changes in odor of salted hilsa during maturation are presented in Table 5. Both monovalent salts (NaCl and KCl) and divalent divalent salts ($CaCl_2$ and $MgCl_2$) showed normal or fresh fish odor in the samples up to day 2. The fish salted with NaCl and KCl resulted faint maturation odor on day 3, where the fish salted with $CaCl_2$ and $MgCl_2$ showed a faint foul and spoiled odor on the same day. On day 4, the fish salted with monovalent salts showed moderate maturation odor while the fish salted with divalent salts produced a faint to strong spoiled odor. On day 5 and 6, the fish salted with monovalent salts observed moderate to strong maturation odor. But the samples salted with divalent salts were observed clearly identifiable spoiled odor at day 5 and on the last day (day 6), showed recognizable decomposed odor.

Table 6 is representing the effect of different monovalent (NaCl, KCl) and divalent ($CaCl_2$, $MgCl_2$) salts on the changes in texture of salted hilsa during maturation. From the result, it was clearly observed that in general both monovalent salts (NaCl and KCl) showed similar results during maturation time. On the other hand, divalent divalent salts ($CaCl_2$ and $MgCl_2$) showed similar result which was distinguished different from monovalent slat results. All the salts used showed same result up to day 2 with normal texture with high consistency of flesh. From day 3 to 5, the fish salted with monovalent NaCl and KCl remained slight soft textured.

On day 3, the fish salted with divalent $CaCl_2$ and $MgCl_2$ salt showed slight soft texture with slippery surface which was not present in the fish salted with monovalent slats. On day 4, the textural properties of the fish with divalent salts remain same as day 3 for both type of salt used. On day 5, the fish salted with $MgCl_2$ showed definite spoilt texture. The fish with $CaCl_2$ showed very soft texture and the muscle tissues were starting to get detached from the bones, the chunks were broken at the ventral point where all the chunks were attached.

At day 6, the samples with monovalent salts were still showing slight soft texture same as the 5th day. But for the sample with divalent $CaCl_2$ and $MgCl_2$ showed raptured texture and the muscle tissues were detached from the bones, which was clearly visible. From the result it was remarkable that, the texture of salted hilsa was greatly

Table 5. Effects of different salts on changes in odor of salted hilsa during maturation time

Salt	Maturation Period					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
NaCl	Normal or fresh fish odor	Normal or fresh fish odor	Faint mature odor	Moderate mature odor	Moderate to strong mature odor	Moderate to strong mature odor
KCl	Normal or fresh fish odor	Normal or fresh fish odor	Faint mature odor	Moderate mature odor	Moderate to strong mature odor	Moderate to strong mature odor
$CaCl_2$	Normal or fresh fish odor	Normal or fresh fish odor	Fishy odor reduced but no mature or foul odor	Faint spoiled odor	Clearly identifiable spoiled odor	Decomposed odor
$MgCl_2$	Normal or fresh fish odor	Fishy odor reduced but no mature or foul odor	Faint spoilt odor	Spoiled odor	Spoiled odor	Decomposed odor

influenced by the salt type used. Divalent salts had strong effects due to its strong chemical structure over the monovalent salts.

From the Table 4, 5 and 6; it was clearly understood that monovalent salts (NaCl, KCl) showed better quality in terms of texture, color and odor of salted hilsa and obtained maturation in better way than divalent salts ($CaCl_2$, $MgCl_2$). The fishes which were salted with $CaCl_2$ or $MgCl_2$ were found with higher moister at the final day of the experiment (day 6) than the fishes salted with NaCl or KCl. The study referred that divalent salts are hygroscopic that absorbs water and makes the fish more difficult to dry and keep it dry. This finding has matched the findings of a study conducted by Nowsad (2007). This study found that the colors of the salted fish with divalent salts were pale and whitish in comparison with the color of the fish salted with monovalent salts. This result supported with the findings of Nowsad (2007) who stated calcium and magnesium salts give a whitish appearance to the salted product.

Biochemical analysis

Based on the result from sensory quality analysis of salted hilsa, hilsa fish sample salted with 25% NaCl showed better sensory quality characteristics than other types and concentration of salt used. Therefore, hilsa fish sample salted with 25% NaCl treatment was selected for further biochemical and microbial quality analysis. Proximate composition (moisture, protein, lipid and ash) of fresh and salted hilsa (using 25% NaCl) was determined using the muscle tissue of the respective sample. For the proximate composition analysis, sample was collected on day 0 (before salting) and day 6 (after maturing of salted hilsa).

Table 6. Effects of different salt on changes in texture of salted hilsa during maturation period

Salt	Maturation Period					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
NaCl	Texture normal	Texture normal	Slight soft texture	Slight soft texture	Slight soft texture	Slight soft texture
KCl	Texture normal	Texture normal	Slight soft texture	Slight soft texture	Slight soft texture	Slight soft texture
$CaCl_2$	Texture normal	Texture normal	Texture slight soft and slippery	Texture slight soft and slippery	Muscle tissue started to get detached from the bones and the chunks are broken	Muscles got detached from bones
$MgCl_2$	Texture normal	Texture normal	Texture slight soft and slippery	Texture slight soft and slippery	Spoiled texture	Spoiled and raptured texture

Table 7. Biochemical (Proximate) composition of fresh and salted hilsa

Proximate composition (%)	Fresh hilsa (Day 0)	Ripened salted hilsa (Day 6)
Moisture	63.79 ± 0.56^{a}	47.65 ± 0.72^{b}
Protein	18.29 ± 0.62^{a}	17.52 ± 0.40^{a}
Lipid	14.94 ± 0.29^{a}	12.41 ± 0.70^{b}
Ash	0.81 ± 0.03^{b}	1.68 ± 0.22^{a}

Table 7 represented the results of proximate composition of fresh and salted hilsa where sample was collected for analysis on day 0 and day 6, respectively. Higher moisture content (63.79%) was found in fresh hilsa (at 0 day of salting). Moisture content was reduced to 47.65% at day 6 (after maturing the salted hilsa). In this study, significant difference was found in case of moisture content between fresh and ripened salted hilsa ($p<0.05$). Penetration of salt into fish muscle through endo-osmotic process and release of water from fish muscle might have caused this result. This result supported the findings of Dewan et al. (2015), who found moisture content in hilsa fish muscle of Chandpur region ranges between 64.45 to 71.78%. Hossain et al. (2014) found that the hilsa of Bay of Bengal have moisture content 60.37% and hilsa of Arabian Gulf have moisture content of 67.89%. On the other hand, non-significant difference in protein content was observed for fresh (18.29%) and salted (17.52%) hilsa ($p>0.05$). Denaturation of proteins during the time of maturation might be the cause for this decrease. Majumdar et al. (2009) found nitrogen percent in ripened dry salted hilsa 2.81% (crude protein 17.56%). Mustafa et al. (2012) stated that the protein percentage in hilsa is about 19.56% which is slightly higher than the result showed in table 10 for fresh and salted hilsa. In addition, there was significant difference found in this study in case of lipid content of fresh and salted ripened hilsa. Lipid content found in this study for fresh and unsalted (day 0) hilsa was 14.94% and for ripened salted hilsa was 12.41%. Due to oxidation of lipids during maturation time can be the probable cause of the decrease. Hossain et al. (2014) found 19.94% lipid in the muscle of hilsa from Bay of Bengal and 11.22% in muscle of hilsa from Arabian Gulf. The study conducted by Shamim et al. (2011) found that the fat content in fresh unsalted hilsa muscle ranges from

18.65% to 20.28% which is more than the result of the current study. However, ash content in salted hilsa was found to be slightly increased (1.68%) than fresh hilsa (0.81%). Gathering of decomposed material from denaturation of protein and oxidation of lipid might have caused this increase in ash content of salted hilsa. Majumdar et al. (2009) found the ash content in dry salted hilsa 16.73% which is distinguishably much more than the result of the current study. Mustafa et al. (2012) found 2.27% ash in fresh hilsa muscle. Hossain *et al.* (2014) found ash content 1.34% from the hilsa of Bay of Bengal and 1.50% from the hilsa of Arabian Gulf. Shamim et al. (2011) showed that the ash content in fresh unsalted hilsa ranges from 1.03% to 1.55%.

In general, a significant decrease in moisture and lipid content and non-significant decrease in protein content was found from the result (Table 7). Though, the ash content was significantly higher in salted hilsa than fresh one.

Microbial analysis

Bacteriological study of salted hilsa was conducted following standard method for total plate count. For the study muscle tissue samples of hilsa fish salted with 25% NaCl was collected in every two days (from 0-6 days). Spread plate technique was used for preparation of plate, sample and colony count.

Table 8: Microbial content (cfu/g) of salted hilsa during maturing time

Day	Microbial count (cfu/g)
0	9.28×10^5
2	1.1×10^7
4	1.37×10^8
6	1.35×10^8

From the result, the gradual continuous increase in the microbial content of the salted hilsa was observed up to day 4. However, slight decrease in the microbial content was found on day 6. The microbial contents were in a flow of continuous rise and the count was 9.28×10^5, 1.1×10^7 and 1.37×10^8cfu/g for day 0, 2 and 4, respectively. Microbial content at day 4 was 1.35×10^8cfu/g which was much higher than that of day 0 or initial microbial load of salted hilsa. Autolytic and enzymatic activity and their resulted products in fish muscle during maturing time might cause the initial increase in microbial content. On the other hand, penetration of salt and removal of moisture resulted lowering the water activity (a_w) which leads reduction of microbial content upon maturation (at day 6) of salted hilsa. In addition, upon maturing the salt content in fish muscle were increased initially which might have caused of unfavorable condition for growth of microorganisms other than halophilic or halotolerant bacteria. Sultana *et al.* (2008) showed in a study that the total viable bacterial count of salted Hilsa collected from different markets ranged from 2.2×10^5 cfu/g to 5.83×10^7cfu/g. Yam *et al.* (2015) reported that salted dried fish have a total bacterial count ranging from $2.2\times10^2$to 3.2×10^3cfu/g.

Conclusion

The study included organoleptic changes occurred during the maturing process thus to changes in proximate composition and microbial analysis of salted hilsa (*Tenualosa ilisha*). From the result it could be conclude that different concentration of salt had different direct effects on the color and odor of resulted salted fish. Greater salt concentration was responsible for greater changes in color and odor with increasing day. Moderate to strong changes in color and odor was observed for salted hilsa during maturating time and these changes in color and odor were influenced more by divalent salts than monovalent salts. Upon the completion of maturation or ripening process, a fresh hilsa with high consistency muscle texture became soft to hard texture, where variation in texture was largely influenced by concentration of salt used. The texture of salted hilsa was greatly influenced by different types of salts. Divalent salts had strong effects due to its strong chemical structure over the monovalent salts. Proximate composition analysis showed significant reduced moisture and slightly reduced lipid in ripened salted hilsa compared to fresh unsalted hilsa. Continuous increase in microbial load was observed in salted hilsa before maturating. However, the microbial count decreased upon maturating occurred. The findings of this study could be concluded that among the different types and concentration of salt, NaCl at 25% of the fish body weight was appropriate for preparation of quality salted hilsa.

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References

AOAC (2000) Official methods of analysis. Seventeenth edition. Association of Official Analytical Chemists. Washington, DC

Azam K, Basher MZ, Ali MY, Asaduzzaman M, Hossain MM (2003) Comparative Study of Organoleptic, Microbiological and Biochemical Qualities of Four Selected Dried Fish in summer and winter. *Pakistan Journal of Biological Science*, 6: 2030-2033.

Bellagha SA, Sahli A, Farhat N, Kechaou, Glenza A (2007) Studies on salting and drying of sardine (Sardinella aurita): Experimental kinetics and modeling. *Journal of Food Engineering*, 78: 947-952.

Dewan BK, Mia MS, Yeasmin F, Sarker SC, Siddiky MNSM, Kamal M (2015) Studies on the proximate composition of Hilsa of different size groups at Chandpur region. *International Journal of Natural and Social Sciences*, 2(5): 52-55.

DoF 2015; National Fish Week Compendium (In Bangla) (2015). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh. 148p.

Harris P, Tall J (1994) Rancidity in fish foods. London: Chapman and Hall

Horner WFA (1997) Preservation of Fish by Curing Drying, Salting and Smoking. In: Fish processing Technology, G.M. Hall, (Ed). 2nd, Blackie Academic and professional, London, 32-73.

Hossain MA, Almatar SM, Al-Hazza (2014) Proximate, fatty acid and mineral composition of hilsa, Tenualosa ilisha (Hamilton 1822) from the Bay of Bengal and Arabian Gulf., *Indian J. Fish.*, 61(2): 58-66.

Lawrance AR, Toms CJ, Nirmala T, Roswin J (2010) Studies on the microbial diversity of salted fishes under aerobic conditions. *Microbiology Research*, volume 1:e5

Majumdar RK, Basu S (2010) Traditional fermented fish product of northeast India; *Indian Journal of Traditional Knowledge*, 9 (3):453-458.

Majumdar RK and Basu S 2009: Studies on seasonal variation in the biochemical composition of the Indian shad, Tenualosa ilisha (Hamilton 1822). *Indian J. Fish*., 56(3): 205–209.

Mustafa MG, Begum SR, Khaleque MA, Jannat M and Ahsan DA (2012) Nutritional Quality of Hilsa and Sarpunti in Different Salt Curing Methods. *Dhaka Univ. J. Biol. Sci.*, 21(1): 97-104

Nowsad AKM (2007) Participatory Training of Trainers: A New Approach Applied in Fish Processing. Bangladesh Fisheries Research Forum. 329p.

Rahman MA, Hossain MA, Mansur MA (1999) Effects of different salting methods on the nutritional and sensory characteristics of hilsa (Hilsa ilisha); *lndian Journal of Marine Sciences*, 29(June): 171-175.

Shamim MAH, Ahmed MK, Abdullah ATM (2011) Proximate Composition of Different Portion of Hilsa, Tenualosa ilisha from two regions of the Bay of Bengal in Bangladesh. *Dhaka Univ. J. Biol. Sci.*, 20(2): 109-115

Shewan JM (1976) The bacteriology of fresh and spoilage fish and the biochemical changes induced by bacterial action. In Proceeding of Tropical Institute Conference on the Handling, Processing and Marketing of Tropical Fish, Tropical Products Institute, London. UK.139-157.

Sultana S, Abdullah AM, Chowdhury MAZ, Afroza T and Rashi H (2008) Microbial Load of salted Hilsa (Tenualosa ilisha) and its preservation by radiation. *Nuclear Science and Application*, 17: 96-101

Wheaton F and Lawson T (1985) Processing Aquatic Food Products. New York. 517 pp.

Yam BAZ, Morteza K, Sahar A and Maryam S (2015) Microbial Quality of Salted Dried Fish Sold Near Caspian Sea, Iran. *Scholarly Journal of Agricultural Science*, 5(6) : 215-219