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Nutritional Quality Analysis of Bangladeshi Fish Species, *M. Tengra* (Hamilton-Buchanan, 1822) Preserved with Different Salt Curing Methods in Laboratory Condition

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Abstract Salt-cured fishes are highly appreciated because of their characteristic taste, texture and storage stability. This piece of work was done in an attempt to evaluate the issue of the traditional fish salting by using NaCl which are easily available and cheaper cost wise and evaluate the difference between biochemical composition (moisture, protein, fat, ash, TVB-N, pH and FFA) of Dry-salted (DS) and pickle-salted (PS) *M. tengra* fish-products in laboratory condition using standard methods of analyses. In processed condition (after salting) the values of moisture (%), protein (%), fat (%), ash (%), TVB-N, pH and FFA were 41.41%, 22.05%,10.65%, 26.15%, 3.90 mg N/100 gm, 6.0 and 2.8% respectively in case of DS *M. tengra* fish and 45.88%, 20.43%, 9.40%, 24.62%, 4.92 mg N/100 g, 6.0 and 3.2% respectively in case of PS *M. tengra* fish-product. During storage period, moisture (%), TVB-N, pH and FFA value were increased significantly ($p<0.05$) whereas total protein, lipid and ash contents were significantly ($p<0.05$) decreased. The values of moisture (%) content were increased 44.82 (7 month) and 49.09 (6 month), in DS and PS *M. tengra* respectively. The values of protein (%), fat (%) and ash (%) content were decreased 20.99%, 9.59% and 25.00% respectively in case of DS (7 month) and 19.28%, 8.68% and 23.41% (6 month) respectively in case of PS *M. tengra* fish. There were no significant ($p<0.05$) different among the samples and between this two salted products, TVB-N, pH and FFA value rapidly increased in PS than DS *M. tengra* fish-products and at the end of 6 month, pickle salted (PS) *M. tengra* fish-product became spoiled whereas dry salted tengra fish-product still remained fresh. Experimentally it has been proved that the fishes preserved in Dry-salt (DS) has longer shelf life (7 month) and has found better way for preservation.

Keywords: *Dry-salting, pickle-salting, M. tengra, proximate-composition, chemical analysis*

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1. Introduction

There is a popular saying that "Fish and Rice makes a Bangali". This popular saying reminds us the importance of fish in our life. It is a good source of protein and lipid for the development of our body [1]. Fish also rich in vitamin and minerals for both young and old age consumers [2,3,4]. Fisheries items are the major protein source of Bangladesh which contributing 58% of the nation's animal protein demands [5]. Fish oil is not health hazardous rather it contain *Omega 3* which helps to reduce the cholesterol level, thereby, reducing the risk of cardiac diseases. The current fish consumption rate is 17.52 kg/people/year whereas the demand is 20.44 kg/people/year and is 29.74 MT per year [6,7]. Being a riverine country, Bangladesh is rich in both close and open water bodies with high potential of producing freshwater and marine fish. Each year several thousand metric tons of fishes are captures from these water bodies. Besides there are culture fisheries resources too. During post harvest period large amount of fish are spoiled and wasted due to lack of proper measure for processing and preservation because of the fact that neither we can consume all the fishes caught nor can we transport to other places wherever necessary due to our insufficient handling and transportation system. In other words, proper handling, processing and preservation during post harvest period are a prerequisite for minimizing the spoilage loss [8]. The

quality of fish in generally decreases after death due to chemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) and microbiological spoilage. Bangladesh is climatically a paradise for breeding and spreading of flora and fauna. Therefore, spoilage agents like microorganism, insects and other pests multiply tremendously and causes considerable damage to our fish resources during handling, transportation and storage. Preservation of a small quantity of loss will greatly improve the protein intake of our people. So to avoid fish spoilage and solve the fish deficit problem as well as uniform supply of fish in the market and even to the remote areas of the country throughout the season, development and utilization of proper scientific preservation techniques are very much essential for processors to produce a good quality of fish and fishery products. But it is not easy task to preserve fish scientifically as well as to maintain its nutritional value and flavor like the fresh one. The principal aim of preservation is to avoid spoilage of fish, avoid loss of protein component and lengthen shelf life. There are various types of preservation methods, such as icing, freezing, drying, salting, etc. in our country. Among them, salting process is considered as one of the oldest method of fish preservation. Salting is used to reduce water activity (aw) to obstruct or destroy the growth of microorganisms as well as inactive autolytic enzymes, where in this end the fish meat gets its way to durability [9,10]. Salted fish products are popular in many countries around the globe [11,12]. As these have been proven to be safe for millenniums, even in developed countries [13]. The aim of salting is not only to prolong the shell life of fresh fish but also to provide desirable sensorial changes [14,15]. In a developing country like Bangladesh, where freezing facilities are inadequate and there is an ever increasing energy crisis, energy intensive fish preservation procedures are not affordable. Furthermore, freezing does not eliminate pathogens which thus pose a health hazard.

Mystus tengra is one of the sole species of family Bagridae. This species very widely distributed in rivers, canals, khals, beels, ditches, inundated fields and other freshwater areas and is one of the most common catfish of the commercial catches of Bangladesh.

Proximate and biochemical analysis provides information on the nutritional value of a particular organism used as a source of food [16]. A number of studies on proximate composition of dried fishes were found in the literature [17-25]. But salting of freshwater fish species has a little in number compared to the dry fish.

The aim of this research is to carry on a comparative study on performance and quality assessment of 2 different type of salt curing method (Dry salting and Pickle salting) of *M. tengra* fish so that we can come to the conclusion, which one will be suitable and give better shelf life to create public awareness about the better process of salting.

2. Material and Methods

2.1. Collection of the Fishes and Location of the Experiment

Fresh experimental *Mystus tengra* fish had been collected from the river Meghna in the early hours of the day and the fishes were brought to the Fish Technology Section, IFST, BCSIR, Dhaka for conducting the research activities, starts in the month of June, 2013. The whole experimental period covered 7 months of duration started from June, 2013 to January, 2014.

2.2. Preparation of Fish

The fishes were carefully washed with cooled tap water. Fins, gills and viscera were removed and again washed with tap water to remove blood, slime and unnecessary flesh.

2.3. Fresh Sample

A few fresh tengra fish species was taken for quality analysis of fresh experimental fish and were chopped with head and bones and finally ground with an electric blender to make a homogenous sample before being sampled for analysis.

Therefore, the total cleaned fishes were grouped into 2 batches.

2.4. Method of Salting

Being a safe, antimicrobial and incidental food additive [26], toxic for some microorganisms [27], depressor of water activity (a^w) of the food [13], sodium chloride has been used as a seasoning and flavor enhancer as well as a preservative or curing agent.

2.4.1. Dry Salting (DS)

The raw fishes were enrolled by dry commercial salt (NaCl) of about 30% by weight of the dressed fish (fish weight: salt weight 3:1), stacked in containers and stored for a salting or curing period, at room temperature. In this method, the extracted water of the fish due to salt action had been removed from the container. Thus the fishes are always allowed to remain in dry condition for the production of dry salt cured fish.

2.4.2. Pickle Salting (PS)

The raw fishes were enrolled by dry commercial salt (NaCl) of about 30% by weight of the dressed fish (fish weight: salt weight 3:1), stacked in containers and stored for a salting or curing period, at room temperature. The salt reacts with the fish and water is extracted out from the fish-body and a salt-solution is formed. Thus in this method, the fishes are always, allowed to remain in such solution for the production of pickle-cured fish.

During dry-salting and pickle-salting process, moisture content decreased and salt content increased considerably during the first 6 to 7 days which is called ripening period. The ripening of the product was determined by observing the changes in sensory characteristics such as color, texture, flavor etc. and changes in moisture content and salt penetration rate.

2.5. Storage of the Product

At the end of ripening, 2 types of salted fish-product was packaging with plastic bag maintaining aseptic condition as far as possible and was stored at room

temperature (°C). The preservation period of product is linked to the amount of salt added; therefore a straight proportion is present between the Amount of salt used and the preservation period [28].

2.6. Sampling Procedures

Evaluation of the performance of 2 type's salted *M. tengra* fish-product was carried out 1 month interval in laboratory condition, until the fish become spoiling or inedible condition. The experiment was done for second time at regular intervals during salting period. Salt crystal was removed from the dry salted product using dry tissue paper before being sampled for analysis.

Analytical methods were applied for the determination of biochemical composition of the raw fish as well as of processed fish products on experimental basis. There are some bio-chemical parameters which determine the quality of salted fish during storage condition such as proximate composition (moisture, protein, fat, ash), Chemical composition (TVB-N value, pH, FFA) etc.

2.7. Estimation of Proximate Composition

Using conventional method of AOAC (Association of Official Analytical Chemicals), the proximate composition of fish was determined [29].

2.7.1. Estimation of Moisture

About 5 gram of previously prepared fairly minced samples were taken into each known weight basin and weighed in a digital balance (Toledo, Switzerland). The samples were allowed to dry into the oven (Memmet 854 Schwabach) at 105°C for 24 hours in order to remove the moisture until constant weight. After that, the basins are taken out of the oven, cooled in a desiccators and were weighed in a digital balance.

Calculation

$$\%\text{of Moisture} = \frac{\text{Weight Loss}}{\text{Original Weight of Sample Taken}} \times 100$$

2.7.2. Estimation of Protein

The protein content was estimated using conventional micro-kjeldahl method [30].

Calculation

$$\frac{\%\ \text{of } N_2 (*A) \times \text{strength of acid} \times 0.002 \times \frac{100}{5} \times 100}{\text{weight of sample taken}}$$

*A = titration reading - blank reading

For most routine purposes the percent of protein in the sample is then calculated by multiplying the % of N_2 with an empirical factor of 6.25 for fish.

$$\%\ \text{of protein} = \%\ \text{of total } N_2 \times 6.25$$

2.7.3. Estimation of Fat

About 5 g of the homogenous sample was taken into conical flasks and 10 ml of folch reagent (Chloroform: Methanol = 2:1) was added into the sample and homogenized properly and kept in air-tight condition for 24 hours. Fat contents of the fish muscle react with that solvent and remains in the solution. After 24 hours the solution of the flask was filtered in another weighed conical flask through a filter paper. Then these flasks were given in a hot water bath to dry up and removed the solvent. After that the flasks were kept into an oven for an hour to get the actual fat content. Then the flasks were weighed in an electronic balance to get the amount of fat content.

Calculation

$$\%\ \text{of Fat} = \frac{\text{Weight of the residue}}{\text{Weight of sample taken}} \times 100$$

2.7.4. Estimation of Ash

About 4-5 g fish sample was weighed into a pre-weighed crucible. The crucible with the contents was heated first over a long flame till all the material was completely churned. Then it was transferred in the Muffle Furnace held at dark red at a rate of 600°C for 5 hours until the residue become white. The crucible were cooled in desiccators and weighed. Finally the % of ash content was calculated.

Calculation

$$\%\ \text{of ash} = \frac{\text{Weight of fish}}{\text{Weight of sample taken}} \times 100$$

2.8. Estimation of Chemical Composition

To determine the quality of salted fishes during storage period some parameters, *viz.* TVB-N value, pH, FFA, etc. were analyzed.

2.8.1. Estimation of TVB-N (Total Volatile Base Nitrogen)

TVB-N has been used as an index for the determination of freshness of fish [31,32]. Volatile nitrogenous bases increase in concentration during the spoilage of fish [33]. The TVB-N measurement can be used as a parameter for the determination of microbiological and enzymatic spoilage of fish product. The TVB-N value that helps for the determination of level of fish spoilage has an inverse relationship with the sensory score of salted fishes. When the sensory score decreased then the TVB-N value increases and vice versa.

TVB-N value was determined by using Conway modified micro-diffusion technique [34]. Samples that were in the different levels of acceptability from highly acceptable condition to unacceptable condition had been selected for TVB-N analysis. 25 ml of 10% Trichloro Acetic Acid (TCA) was added to 2 gm of ground fish sample and kept overnight and then filtrated with known volume. 2% boric acid, TCA, K_2CO_3 and the solutions made from the fish samples were taken into the Conway dishes.

After the addition of Potassium Carbonate (K_2CO_3), each dish was covered by a piece of glass that was stacked with glue (Paraffin soft white) initially. Then it was kept for 24 hours. The samples and Potassium Carbonate (K_2CO_3) reacts to form NH_3 which was absorbed by the boric acid and then the solution of each Conway dish had been titrated by N/70 H_2SO_4 with the help of a micro-burette.

Finally TVB-N was calculated.

Calculation

$$\text{TVB-N} = (*A) \times \text{Strength of acid} \times 0.2 \times \frac{\text{Volume of extract}}{\text{Volume of extract taken}} \times \frac{100}{\text{Weight of sample taken}}$$

(*A = titration reading - blank reading)

2.8.2. Estimation of pH

pH value of the sample was determined with the help of a pH meter (Mettler Toledo 320-s, Shanghi, China) following standard method [35].

2.8.3. Free Fatty Acid (FFA) Estimation

Oil sample used throughout the work was prepared by extracting the salted fish by Folch reagent (chloroform and methanol in the ratio of 2:1 v/v).The salted fish was first cut into small pieces and then ground. The ground material was then mixed with folch reagent in a large wide mouthed stopper glass bottle for 24 hours at room temperature after 15 minutes stirring with glass rod.

Extraction was facilitated by occasional shaking. The mixture was filtered through a Buchner funnel and the filtrate was evaporated in batches under heater or oven at 60°C. Seven gram of well-mixed oil was taken into 250 ml flask and 50 ml ethanol was added, previously neutralized by adding 2 ml phenolphthalein solution. Tritation was done by 0.25 N sodium hydroxide with vigorous shaking until permanent final faint pink color appeared and persisted for at least one minute. The value was reported as percentage (%) of free fatty acid expressed as oleic acid. Milli litre of 0.25 N NaOH required for titration corresponds to the percentage of free fatty acid.

To calculate significance at $p < 0.05$ level all data was analyzed with the help of SPSS for windows, version 20 statistical software [36].

3. Results and Discussion

Determination of the biochemical-composition of experimental Tengra (*M. tengra*) fish in fresh condition as well as dry-salted (DS) and pickle-salted (PS) condition (storage at room temperature) were done. It has been established that the proximate composition of fish may vary in different species and even within the same species from one individual to another is mainly due to age, sex, season, size, species, starvation of the day, energy spending procedure and so on [37,38,39,40].

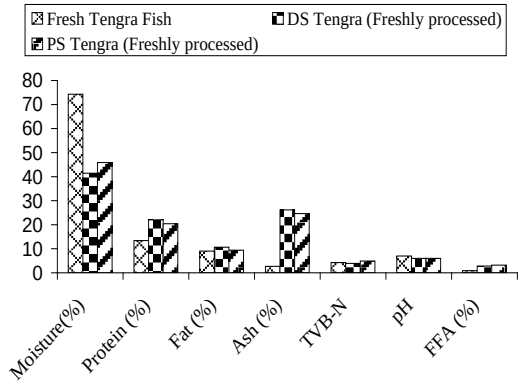

Figure 1. Bio-chemical composition of the fresh experimental fish, freshly-processed dry-salted (DS) and pickle-salted (PS) *M. tengra* fish-products

In case of fresh *M. tengra* fish the percentage of moisture, protein, fat, ash (Proximate composition) was 74.27%, 13.43%, 9.04% and 2.67% and chemical composition (TVB-N, pH, FFA) was 4.27 mgN/100 g, 7 and 0.9% respectively (Figure 1).

In present experiment, fresh *M. tengra* fish recorded a high moisture and low protein content, similar to previous report [41]. Generally, lipid content varies within species (1.46 to 5.77%) and is affected by the catching season (1.2 to 18.4%) [42,43]. The moderate lipid level in this small indigenous fish are similar than those found in other species. [42,44,45,46]. Ash content of fresh *M. tengra* fish was higher than those found in other species [47,48]. The higher ash content could be explained by the presence of the bones in the samples.

3.1. Proximate Analysis

3.1.1. Moisture (%)

Moisture content varies from species to species and temperature to temperature and at different time duration of its preservation. In present experiment, moisture content was found 41.41% and 45.88% in fresh processed DS and PS *M. tengra* fish and after completing the duration of storage period moisture content was increased 44.82% (7 month) and 49.09% (6 month) in DS and PS *M. tengra* fish respectively (Figure 2 and Figure 3). The moisture uptake during the storage period was significant in the products stored in room-temperature. Moisture absorption in such products is obvious during monsoon due to high relative humidity difference.

3.1.2. Protein (%)

Protein content was found 22.05% and 20.43% in fresh process DS and PS *M. tengra* fish and after completing the duration of storage period it was decreased into 20.99% (7 month) and 19.28% (6 month) in DS and PS *M. tengra* respectively (Figure 2 & Figure 3). The decrease of protein level was found to be significantly proportional ($P<0.05$). Protein decreased with storage of cured meat was attributed to some changes during storage that caused by 'maillard reaction and changes in pH [49]. Salt causes the proteins in fish muscle to swell and salt lead the protein become denatured if increases in the muscle [50].

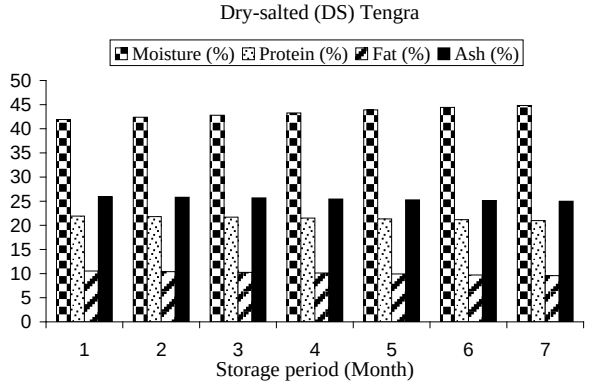

Figure 2. Changes in proximate composition of dry-salted (DS) *M. tengra* fish during storage at room temperature

3.1.3. Fat (%)

Fat content is found to be influenced by season and geographic location [51]. In DS and PS *M. tengra* fish-

products fat content was found 10.65% and 9.40% in fresh process condition and after completing the duration of storage period it was decreased into 9.59 % (7 month) and 8.68 % (6 month) respectively (Figure 2 & Figure 3). It is clear from the present results that fat content was decreased significantly ($p<0.05$). This might be due to oxidative deterioration, thereby affecting lipid extraction [52]. Decrease in the level of crude protein and fat contents of small and large salted Bouri fish muscle (*Mugil cephalus*) were reported [53].

3.1.4. Ash (%)

In DS and PS *M. tengra* fish products ash content was found 26.15% and 24.62% in fresh process condition and after completing the duration of storage period it was found 25.0% (7 month) and 23.41% (6 month) respectively (Figure 2 & Figure 3). The higher value of total ash content in freshly processed DS and PS *M. tengra* fish than fresh fish was attributed to high salt content. Similar levels of ash content in salted fish were noticed by several workers [54].

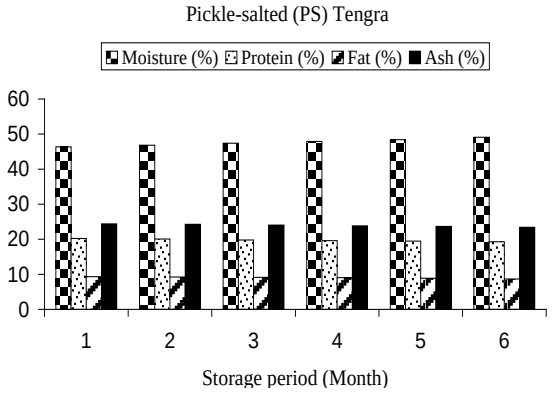

Figure 3. Changes in proximate composition of pickle-salted (PS) *M.tengra* fish during storage at room temperature

3.2. Chemical Analysis

3.2.1. Changes in TVB-N

TVB-N values were found to vary from 3.9 (0 day) to 30.22 mg N/100 g (7 month) for DS *M. tengra* and 4.92 (0 day) to 31.04 mg N/100 g (6 month) for PS *M tengra* fish-products. Significant statistical differences were found between the initial product and end product ($P < 0.05$) during storage period (Figure 4).

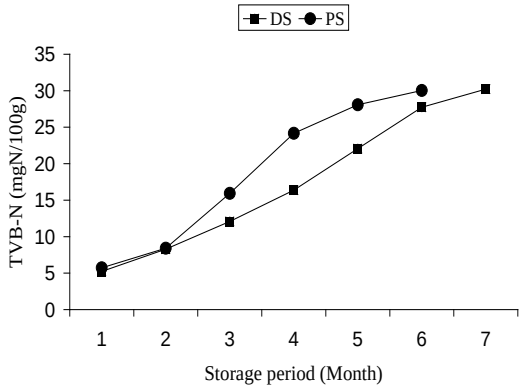

Figure 4. Changes in TVB-N contents of dry-salted (DS) and pickle-salted (PS) *M.tengra* during storage at room-temperature

TVB-N values of the products stored at room temperature showed linearly increasing pattern throughout storage period but neither of the value exceeded the recommended value set for fish regarded as acceptable condition. According to Connell the limiting level for rejection of TVB-N is 30-40 mgN/100 g for storage at ambient temperature. The present findings are in close association with him [55].

Total volatile base nitrogen (TVB-N) is important compound provide a measure of the progress of spoilage that is dependent of sensory assessment. The level of TVB-N in fish & fish products are mostly used as spoilage indicator through bacterial activity [56]. The same result has been evident in the present study. The TVB-N of fish is an indicator of the freshness of the raw material [57]. High TVB-N values are unacceptable and are associated with unpleasant smell in the meat [58]. Assumably, this is because of the impact of the various treatments of TVB-N, which primarily includes nitrogen from ammonia, TMA, and dim ethylamine which reflects the extent of degradation of proteins and non protein nitrogenous compounds which can be explained by proteolysis, due to enzymatic and microbial activities in the samples on storage [59]. In early storage, spoilage rate become slower than later storage time, it would appear from the Figure. This is also supported by other researchers [60,61]. The stage perhaps autolysis is mainly to the tissue as a result TVB-N does not increase highly [62].

3.2.2. Changes in pH

pH is an indicator of the Extent of microbial spoilage in fish and some proteolytic microbes produce acid after decomposition of carbohydrate, thereby increasing the acid level of the medium [63]. pH value is a reliable indicator of the degree of freshness or spoilage.

The pH in fresh condition fresh- water fish flesh is almost neutral. [64]. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh [65]. The increase in pH indicates the loss of quality. The pH value of Dry-salted (DS) and Pickle-salted (PS) *M. tengra* fish-product was increased significantly ($P<0.05$) with storage period. pH value of fresh Shoal fish was 6.9 in our study. But when salt is added with the fish, pH value decrease due to increase of acidic compound and after that among shelf life study pH value increases in the time interval due to increase of basic compounds. In the present study pH value were found to vary from 6.0 (0 day) to 7.9 (7 month) for DS *M. tengra* and 6.0 (0 day) to 8.0 (6 month) for PS *M. tengra* fish-products (Figure 5).

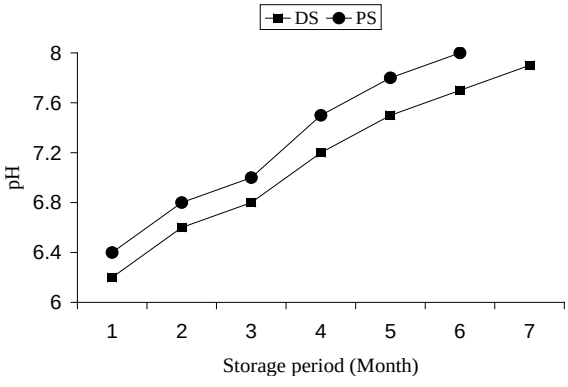

Figure 5. Changes in pH value of dry-salted (DS) and pickle-salted (PS) *M.tengra* during storage at room-temperature

The fish products are acceptable up to a pH of 6.8 but are considered to be spoiled above a pH of 7.0 [32]. The limit of acceptability is usually 6.8 to 7.0 [66]. While the initial pH values in the samples were similar to findings of other researchers; the increase in pH values during the storage of room temperature (27-31°C)was higher than others. The probable reason of these differences is differences in fish species and different methods of salting.

3.2.3. Changes in FFA (Free Fatty Acid)

Among the various parameters to assess the extent of deterioration in fish, determination of free fatty acid (FFA) content has been widely used.

Free fatty acid (FFA), a tertiary product of rancidity, increased during storage. The FFA is a measure of hydrolytic rancidity-the extent of lipid hydrolysis by lipase action. In most fish oils, rancidity is noticeable when the FFA (calculated as oleic acid) is in between 0.5%-1.5% [63]. It produced as a result of fat oxidation (rancidity). FFA value is a measure of the extent of oxidative deterioration in oily fish, but it can fall further at latter stages of fish spoilage [67]. A high level of FFA is characteristics of product that have undergone both microbial and biochemical spoilage [10,68]. This may indicate that greater proportions of unsaturated fatty acids were liberated and were subjected to oxidative splitting at the double bonds. The resulting substances, mostly ketones and aldehydes, appear to be largely responsible for flavor, odor and taste of the salted fish product [53]. The same result was found in the present study.

The FFA value of dry and pickle salted *M. tengra* increased gradually with the passing of storage period (Figure 6). Significant statistical differences were found between the initial product and end product ($P < 0.05$) after storage period. It was vary from 2.8% (o day) to 12.1% (7 month) for DS and 3.2 % (o day) to 12.7% (6 month) for PS *M. tengra* respectively.

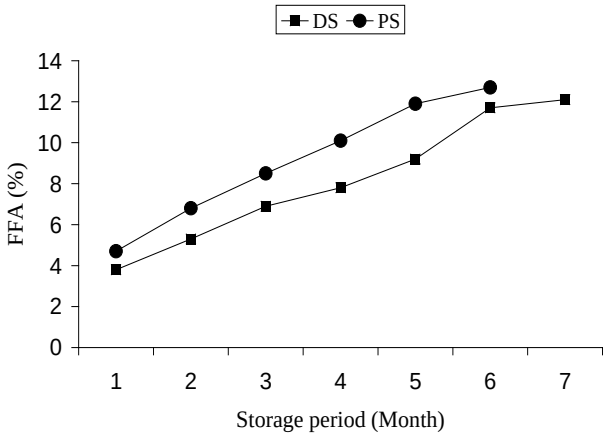

Figure 6. Changes in FFA contents of dry-salted (DS) and pickle-salted (PS) *M.tengra* during storage at room-temperature

Free fatty acid (FFA) value ranged from 2.9% of total lipid on the start day to 7.58, 7.15 and 8.56% on the 60 days of observation in dry salting, wet salting and sundry salted fishes respectively [69]. While the initial value of raw pre-spawning and post-spawning hilsa was 1.15% and 1.21% respectively which after dry salting increased to 7.86% and 8.35% respectively on 18th days of storage at 28°C to 32°C [70].

The present study denoted that the contents of free fatty acid values are similar with the above mentioned studies. The FFA value increased in a characteristics pattern to a certain level of storage period.

High level of FFA is an indication of microbial spoilage activity [32].Most fat acidity begins to be noticeable to the palate when the FFA value calculated as Oleic acid is about 0.5 -1.5 % [71].The result of free fatty acids (FFA) indicated that the salting conditions accelerate lipid oxidation and this is in agreement with the results as shown by other researchers [72,73].

4. Conclusion

It is evident from different test that, fish kept in dry salt maintains the longer period of shelf life and also maintains best quality in protein and fat content than other keeping condition.

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