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Comparative study on the ripening process of dry-salted and brine-salted small catfish tengra (*Mystus tengra*; Hamilton-Buchanan, 1822) and their shelf-life quality during Refrigeration storage (4°C)

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Abstract
A study was conducted on the effect of ripening process and shelf-life quality of dry-salted and brine-salted tengra (*M. tengra*) fish-products by analyzed their sensory-evaluation (flavor, color, texture, sensory-score), chemical-composition (TVB-N, pH, FFA) and bacterial load (SPC, HBC) during storage at refrigeration temperature (4°C). There was a general decline in sensory score of these two types of salted fish-products during storage. With the laps of storage period, values of chemical-composition and bacteriological load increased significantly but the values did not exceed the rejection limit. Dry-salted and brine-salted tengra fish-products became spoiled at the end of 32 and 24 months. No yeast or mould was detected in these salted fish products. Therefore, it can be inferred that dry-salted and brine-salted tengra fish-products have long shelf-life when they are stored at refrigeration temperature (4°C) and among these two salted fish-products, dry-salted tengra fish-product has highest shelf life.

Keywords: Dry-salting, brine-salting, ripening-period, shelf-life study, refrigeration-storage

1. Introduction
The freshwater small indigenous fish species are a valuable source of macro and micro-nutrients and play an important role to provide essential nutrients for the people of Bangladesh. Analysis of small fish showed that they contain a large amount of calcium and most likely also iron and zink. Many small indigenous fish are eaten whole, contributing calcium, phosphorus and vitamin to the human body. In small fish, vitamin-A is present as retinal which is already absorbed and utilized by humans, while in vegetables vitamin A is found as B-carotene which is not readily absorbed as retinal but destroyed to some extent by cooking. Small indigenous fish species defined as species attaining a maximum length of 25 cm [1]. In low income countries like Bangladesh, small fish are only consumed protein sources, as they are accessible, less expensive, affordable well liked, culturally acceptable and can be purchased in small quantities [1]. Small fish plays an important role in the everyday diet of Bangladesh. Popular small indigenous catfish tengra (*Mystus tengra*) is selected for the present study. Catfish are a very diverse group of bony fish which have inhabited all continents at one time or another, and they have been widely caught and farmed for food for hundreds of years in Asia, Europe, Africa and North America [2]. In our country, small indigenous fish like tengra (*Mystus tengra*) in a fresh condition is not always available. Major fishing grounds are far away from the cities and the consuming centers which are not easily accessible. Fishing is also seasonal. Seasonal abundance in certain places and a dearth of fish in others stimulates fisherman to preserve their catch.
Immediately the fish dies, a number of physiological and microbial deterioration set in and there by degrade the fish [3]. Microbial action has been known to play a large part in the spoilage of fish [4]. Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odors [5]. Fish is a perishable food which needs processing and preservation [6, 7].
Salting has been used for centuries as a method of fish preservation [8]. Spoilage of fish is slowly when water is drawn from fish are achieved by salting. Performance of a salt curing method is judged by the capacity of the salting process to keep the fish fresh with a longer duration. Longer the existence of fish freshness, more acceptable the salt curing method for that kind of fish, as it helps to preserve the fish to be marketed in the off-seasons.
The objective of the present work was to study ripening process of dry and brine salted tengra (*Mystus tengra*) fish and their shelf-life quality during refrigeration storage as assessed by sensory analysis, determination of chemical indices and bacteriological study.

2. Materials and methods
2.1 Collection and handling of experimental fishes
Fresh fishes (*M. tengra*) had been collected from the river Meghna in the early hours of the day. They were carried in clean, good quality polythene bag with ice in order to keep the fish fresh.

2.2 Preparation of fishes
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 adherents.

2.3 Place of the experiment
Chemical analysis and microbial analysis were carried out at the 'Fish Technology Section' and 'Food Microbiology

Section' of the Institute of Food Science and Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR) Dhaka, Bangladesh.

2.4 Method of salting

Being a safe, antimicrobial and incidental food additive, toxic for some microorganisms, depressor of water activity (aw) of the food sodium chloride has been used as a seasoning and flavor enhancer as well as a preservative or curing agent [9-11].

2.4.1 Dry salting

Tengra 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 Brine salting

During this experiment A 30% salt solution is prepared (30 gm salts in 100 ml water) which is called brine. Tengra fishes are kept at this saturated brine solution stacked in containers and stored for a salting or curing period, at room temperature for the production of brine-salted fish. The fish in brine were kept immersed by putting a glass weight on it.

2.5 Storage of the product

At the end of salting, dry and brine salted tengra fishes were packaging with plastic bag maintaining aseptic condition as far as possible and were stored at refrigeration temperature (4°C).

2.6 Sampling procedures

Evaluation of quality changes of dry and brine salted fishes were carried out 4 months interval until the fish became inedible for consumption. Fishes were chopped with skin and bone and finally ground with an electric blender to make a homogenous sample before being sampled for analysis.

2.7 Ripening period

During salting process, the changes in chemical and physico-chemical characteristics takes place and in certain stage, the original characteristics of the fresh fish is found virtually absent. This stage is regarded as 'salt ripening of fish'. According to Vokresensky the ripening of the fish was observed after 7 to 10 days of salting [12]. Salt-ripening process starts when the surface of fish goes in contact with salt and is completed when all the fish reach the appropriate salinity, taste, consistency and odor. During ripening process moisture content decreased and salt content increased considerably during the first 6 to 7 days. The physical and chemical changes that occur during ripening, determine the overall sensory qualities of salted fish-products [13].

2.8 Parameters of ripening process and shelf-life quality assessment

The analytical methods used in this experiment are given below:

- Moisture and salt contents of the fishes were determined by AOAC method [14].
- Sensory score evaluation has been done by using 9-point hedonic scales (9. Like extremely; 8. Like very much; 7. Like moderately; 6. Like slightly ;< 5. Bad) [15].
- TVB-N was determined by Conway modified micro-diffusion technique as described by Conway and Byrne [16].
- pH was determined using a pH meter [17].
- FFA of the fishes were determined by AOAC method [18].
- Bacteriological study (SPC and HBC) was done according to the standard methods of AOAC and FDA BAM [19, 20].

3. Results and Discussion

3.1 Study of ripening period

In this experiment, two parameters were used to determine the ripening process of dry and brine salted tengra fishes; changes in salt penetration rate and its effect on moisture content and their percent weight loss during ripening period.

3.1.1 Changes in salt penetration rate and its effect on moisture content

Changes in salt penetration rate and its effect on moisture content of dry and brine salted tengra (*M. tengra*) fishes during 7 days of ripening period is presented in Table 1.

Table 1: Changes in salt penetration rate and its effect on moisture content of dry and brine salted tengra (*M. tengra*) fishes during 7 days of ripening period

Days of ripening period	Dry-salted tengra		Brine-salted tengra	
	Moisture (%)	Salt (%)	Moisture (%)	Salt (%)
0*	74.27	0	74.27	0
1	60.73	7.2	69.95	6.9
2	54.23	10.9	66.12	9.96
3	49.70	12.5	64.01	12.94
4	45.68	14.08	62.20	14.6
5	42.22	15.33	60.05	15.43
6	41.81	16.60	58.44	16.2
7	41.41	16.80	57.35	16.5

*= Initial (Fresh fish)

The moisture content of dry-salted and brine-salted tengra (*M. tengra*) was decreased from 74.27 to 41.41% and 74.27 to 57.35% respectively during ripening period of 7 days. On the other hand, salt content gradually increased in dry-salted and brine-salted tengra (*M. tengra*) and was observed in 16.80 and 16.5 respectively at the end of 7 days of ripening period. Moisture content and salt percentage play an important role in the keeping quality of salt-cured fish products [21]. During salting, the mass transfer occurs basically between salt and water: the fish-muscle takes up salt and loses water [22, 23]. Many workers agree that maximum salt uptake takes place within 6-7 days of salting without further uptake during subsequent storage [24-26]. Similar results also obtained in the present study where the fish contained maximum salt content in 7 days of dry and brine salting process. The rates of water diffusion are positively correlated with increasing of salt concentration which is very important with regard to weight change and quality of the final products [27, 28].

3.1.2 Percent weight loss

The comparative features of changing pattern of weight loss (%) of dry-salted and brine-salted tengra (*M. tengra*) during

ripening period are shown in Figure 1. It is clear here that the weight of dry-salted and brine-salted tengra (*M. tengra*) gradually decreased throughout the ripening period and this may occur due to maximum water was coming out from the fish-flesh because of osmosis and diffusion. This type of weight loss was observed as reported by several researchers during salting of fish [29-33]. The relationship between changes in weight, salt and water contents is almost linear. From the Figure it is shown that, significantly higher amount of weight loss observed in dry-salted tengra (*M. tengra*) whereas, the least percent weight loss was found in brine-salted tengra (*M. tengra*) fish-products.

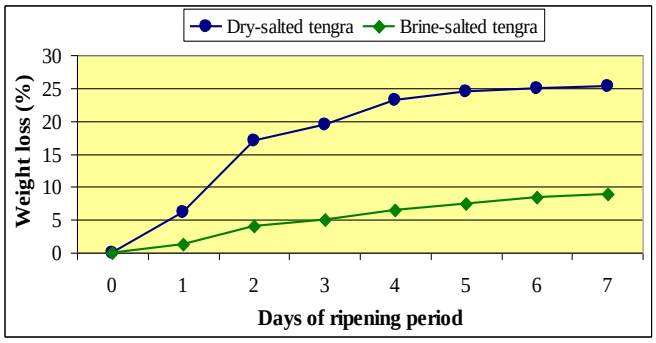

Fig 1: Weight loss (%) of dry-salted and brine-salted tengra (*M. tengra*) fish during ripening period

3.2 Shelf life study

The shelf-life of a food is the period for which it remains safe and suitable for consumption. On the other hand shelf-life is the length of time that corresponds to a tolerable loss in quality of a processed food and other perishable items. In present research work, shelf-life study of dry and brine salted tengra fish-products was done by assessing their sensory characteristics and score, chemical composition (TVB-N, pH, FFA value) and bacteriological study.

3.2.1 Sensory characteristics and score

Changes in sensory characteristics and scores of dry and brine salted tengra (*M. tengra*) during observation at refrigeration (4°C) storage is presented in Table 2. During this study, high quality dry-salted and brine-salted tengra (*M. tengra*) fish products with excellent sensory and physical properties were obtained through dry and brine salting process. Now it is of interest to see how long these salted fish-products could be kept in acceptable condition during storage at refrigeration temperature (4°C). There was significant change in flavor, color, texture in this fish species subjected to dry and brine-salting methods. The table shows that with the lapse of storage time both salted fish products produce a salty taste with different degree of smell, color and texture. The changes of color from whitish to faded may be due to slightly lipid oxidation during storage period. In the present study, there was no fungal attack in these two types of salted tengra fish-products. According to the panel's evaluation, the sensory properties of dry and brine salted tengra (*M. tengra*) fish-products were in acceptable condition throughout storage period though, statistically there was significant difference ($p<0.05$) in the sensory evaluation during storage period based on the panel's score. The initial score of the sensory evaluation of dry and brine salted tengra was 9. But during storage at refrigeration temperature (4°C), this score decreased slowly and at the end of the storage period, the score was 5 in case of dry-salted tengra (32 months) and brine-salted tengra (24 months) (Table 2). Yu applied this hedonic rating scale to evaluate the acceptability of sun dried fishes by their external morphological and quality changes [34]. Morshed applied the hedonic rating scale by using 9-points for the sensory evaluation of the dried and dehydrated fish [35]. The sensory analysis of dry and brine salted tengra (*M. tengra*) fish-products were done and observed that the quality of dry salted tengra (*M. tengra*) fish-products was much better.

Table 2: Changes in sensory characteristics and scores of dry and brine salted tengra (*M. tengra*) during observation at refrigeration (4°C) storage (Peryam and Pilgrim, 1957)

Months of storage	Product	Flavor(Smell/Odor)	Color	Texture	Remarks	Hedonic scale score (0-9)
0	Dry-salted tengra	Attractive salty odor	Whitish	Slightly tough	Excellent	9
	Brine-salted tengra	Attractive salty odor	Whitish	Elastic	Excellent	9
4	Dry-salted tengra	Attractive salty odor dominant	Whitish	Tough & shrunken	Excellent	8.7
	Brine-salted tengra	Attractive salty odor dominant	Whitish	Elastic	Very good	8.5
8	Dry-salted tengra	Stimulating fishy & salty odor	Whitish	Tough & shrunken	Good	8.5
	Brine-salted tengra	Characteristic salty odor	Whitish	Elastic	Slightly good	7.5
12	Dry-salted tengra	Characteristic fishy odor	Whitish	Slightly firm	Moderately good	8.2
	Brine-salted tengra	Stimulating fishy & salty odor	Whitish	Slightly elastic	Slightly accepted	6.5
16	Dry-salted tengra	Stimulating fishy odor	Whitish	Slightly firm	Moderately accepted	7.2
	Brine-salted tengra	Fishy odor dominant & slightly salty odor	Fade whitish	Slightly soft	Just accepted	6
20	Dry-salted tengra	Slightly salty and fishy odor	Fade	Slightly soft	Slightly accepted	6.5
	Brine-salted tengra	Salty odor absent	Fade	Comparatively soft & little slimy	Not so good	5.5
24	Dry-salted tengra	Fishy odor dominant and slightly salty odor	Fade white	Slightly soft	Just accepted	6.0
	Brine-salted tengra	Slightly faded fishy odor	Fade	Slightly soft	Neither like or dislike	5
28	Dry-salted tengra	Salty odor absent	Fade white	Comparatively soft	Not so good	5.5
	Brine-salted tengra	Slightly putrid odor	Fade	soft	rejected	4.5
32	Dry-salted tengra	Fishy odor dominant	Fade	Comparatively soft	Neither like or dislike	5
	Brine-salted tengra	*	*	*	*	*
36	Dry-salted tengra	Slightly rancid odor	Fade	Comparatively soft	rejected	4.5
	Brine-salted tengra	*	*	*	*	*

*= Rejected due to sensory evaluation

3.2.2 Chemical analysis

Changes in chemical composition (TVB-N, pH and FFA) of dry and brine salted tengra (*M. tengra*) fish-products during different duration of storage at refrigeration temperature (4°C) are presented in Table 3.

The range of TVB-N value of dry and brine salted tengra fish-products during storage at refrigeration temperature were 3.90 to 20.23 mgN/100g of fish (32 months) and 2.59 to 20.98 mgN/100g of fish (24 months). The TVB-N measurement can be used as a parameter for the determination of microbiological and enzymatic spoilage of fish product. The limiting level for rejection of TVB-N is 20mgN/100g for storage at refrigerator temperature [36]. In present investigation, TVB-N values of the products stored at refrigeration temperature showed linearly increasing pattern throughout storage period but neither of the value exceeded the recommended value set by different researchers for various fish and fish products as acceptable condition. In the present study, increase in TVB-N throughout the storage period may be due to microbial activity, absorption of moisture. Increase in TVB-N value is related to bacterial spoilage.

In case of dry and brine salted tengra fish, during storage at refrigeration temperature pH value was varied in the range of 6.0 to 6.9 (32 months) and 5.9 to 6.9 (24 months) respectively. The increase in pH indicates the loss of quality. The pH value is a reliable indicator of the degree of freshness or spoilage. Changes in pH value of 20% brine salted red mullet stored in 4°C (refrigerator temperature) was found slightly increased in a range of 6.51 to 6.59 during 11 days of storage, which indicate that pH value of salted fish also increased during refrigeration storage [37]. The pH is an important factor that affects microbial growth and spoilage of foods. A good relationship between changes in pH and organoleptic qualities of fish was observed, where the quality greatly decreased along with the increase of pH [38]. This finding is similar with present study.

During storage at refrigeration temperature, FFA values were found to vary from 2.8 to 11.0% (32 months) in dry-salted and 1.0 to 11.6% (24 months) in brine-salted tengra respectively. The FFA value which indicates the rancidity of fat, increased gradually with the passing of storage period. A high level of FFA is characteristics of product that have undergone both microbial and biochemical spoilage [39].

Table 3: Changes in chemical composition (TNB-N, pH and FFA) of dry and brine salted tengra (*M. tengra*) during different duration of storage at refrigeration temperature (4°C)

Storage period (months)	TVB-N (mgN/ 100g)		pH		FFA (%)	
	Dry-salted tengra	Brine-salted tengra	Dry-salted tengra	Brine-salted tengra	Dry-salted tengra	Brine-salted tengra
0*	3.90	2.59	6.0	5.9	2.8	1.0
4	4.95	6.27	6.1	6.2	3.5	2.9
8	7.57	8.25	6.1	6.3	4.8	3.8
12	10.13	11.42	6.2	6.4	5.7	5.3
16	12.21	14.53	6.3	6.5	7.1	7.5
20	15.31	17.86	6.4	6.7	8.2	9.1
24	16.76	20.98	6.6	6.9	9.5	11.6
28	19.87	-	6.7	-	10.3	-
32	20.23	-	6.9	-	11.0	-

*Just after completion of ripening period

3.2.3 Bacteriological study

Bacteriological study (SPC and HBC) of dry and brine salted tengra (*M. tengra*) during different duration of storage at refrigeration temperature (4°C) are presented in Table 4.

In this study, the range of standard plate count (SPC) of dry and brine salted tengra fish-products were 3.8×10^3 to 2.1×10^6 cfu/g (32 months) and 1.5×10^4 to 3.0×10^6 cfu/g of fish respectively during storage at refrigeration temperature. Determination of the microbial load of fishes and their products considered as the most important tests to determine the flesh quality and storage period. Gradual increase of SPC count of dry and brine salted tengra fish-products were observed during storage at refrigeration temperature due to increase in moisture content of the product. In this study, the standard plate count (SPC) of salted samples was varied during storage time but evaluation of these salted products were within the limits of 10^7cfu/g specified for quality grading of fish by the International Commission of Microbiological Standards for Foods [40]. There is a positive relationship between moisture content and bacterial growth in fish. A correlation was also found between bacterial count and total volatile base nitrogen. The sample with high total volatile base nitrogen showed maximum bacterial count [41].

Although salt prevents the growth of spoilage bacteria, but other microorganisms such as high salt tolerant and halophiles are not affected by the presence of salt. In case of dry and brine salted tengra fish-products, halophilic bacterial count (HBC) were varied in the range of 3.2×10^2 to 3.2×10^5 cfu/g (32 months) and 1.8×10^3 to 5.0×10^5 cfu/g of fish respectively during storage at refrigeration temperature. Halophilic bacterial populations increased in 3.2 to 7.12 log cfu/g in dry salted whole sardine and 3.59 to 6.11 log cfu/g in dry salted fillet sardine were found when 5 months stored in 4°C (refrigeration temperature**)** [42]. This finding is similar with present results.

Table 4: Changes in standard plate count (SPC) and halophilic bacterial count (HBC) of dry and brine salted tengra (*M. tengra*) during different duration of storage at refrigeration temperature (4°C)

Storage period (months)	SPC (cfu/g)		HBC (cfu/g)	
	Dry-salted tengra	Brine-salted tengra	Dry-salted tengra	Brine-salted tengra
0*	3.8×10^3	1.5×10^4	3.2×10^2	1.8×10^3
4	6.2×10^3	4.1×10^4	4.2×10^2	4.1×10^3
8	8.2×10^3	7.2×10^4	7.0×10^2	6.6×10^3
12	1.3×10^4	2.4×10^5	2.5×10^3	2.5×10^4
16	2.6×10^4	3.8×10^5	4.1×10^3	5.1×10^4
20	4.6×10^4	7.5×10^5	8.8×10^3	3.2×10^5
24	2.5×10^5	3.0×10^6	1.9×10^4	5.0×10^5
28	7.7×10^5	-	3.9×10^4	-
32	2.1×10^6	-	3.2×10^5	-

*Just after completion of ripening period

4. Conclusions

In summary, this study demonstrated the effectiveness of preserving the qualities of tengra fish by combining two different salting treatments and storage these salted fish-products at low temperature (4°C) over a long period of time. The present study also reveals that ripening of dry-salting and brine-salting process has a positive significant role on the bio-chemical composition of tengra fish and reduces moisture content as well as makes them suitable for all. The main hypothesis of this study is to understand the effect of different treatments used for salting and to find out the best method of salting in refrigeration storage (4°C) for the consumer's safety and economic benefit of the fishermen. Commercial traders those who produce market salted-fish in our country may be asked to follow the suggestions made over here on the basis of the findings of the present study.

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