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Effectiveness the application of pickle and brine salting prolonging the shelf life of Shol (*Channa striatus* Bloch, 1801) fish-product stored at Ambient Temperature (26-32˚C)

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Abstract
Processing and preservation method like salting have been practiced throughout the world including Bangladesh to minimize the loss of fish-spoilage. This experiment was conducted to evaluate the effect of pickle and brine-salting on the shelf lives extension of shol fish stored at ambient (26-32°C) temperature by analyzing their proximate (moisture, protein, fat, ash) composition, chemical (TVB-N, FFA, pH) composition and bacteriological (SPC, HBC) study. During storage period protein, fat and ash content decreased whereas moisture and bacteriological load increased significantly but the values did not exceed the rejection limit. No yeast or mould was detected in these salted fish products. According to quality assessment, the pickle salted shol fish-products kept at ambient temperature showed a higher shelf-life of 150 days than brine-salted shol fish-products (135 days). Experimentally it has been proved that the fishes preserved in pickle-salting process has longer shelf life and has found better way for preservation.

Keywords: Shol-fish, ripening-process, pickle-salting, brine-salting, shelf-life, Ambient-temperature

1. Introduction
Fish is known to be one of the cheapest sources of animal protein and other essential nutrients required in human diets [1, 2]. Fish also contain many vitamins and minerals. It is an extremely perishable food and required preservation for future uses. The ambient temperature in Bangladesh for most parts of the year is more than 25°C, which would encourage growth of spoilage microorganisms. Quality loss can also occur very rapidly after catch [3]. Much attention is being directed at fresh water fish because of its health benefits, as a result of the presence of omega-3-fatty acids in the fish oil [4, 5]. So, processing or storage method of fisheries product is a vital factor in fish consumption. In spite of adopting developed techniques for preservation we cannot expect the nutritional value as we get in fresh condition because the nutritional value changes with the passage of time and various storage or preservation methods.

Fish curing is the cheapest and simplest way of fish preservation. Preservation techniques are needed to prevent fish spoilage and lengthen shelf life. Several methods are followed over the world for preserving fish to extend its shelf-life, including drying, salting and smoking [6, 7]. Fish in any of these forms give rise to products of great economic importance and the demand for such products has been increasing. Among them, salting process is considered as one of the oldest method of fish preservation. Salting is generally aimed at reducing water activity (a^w) to obstruct or destroy the growth of the microorganism as well as inactive autolytic enzymes, where in this end the fish meat gets its way to durability [8, 9]. Salted fish products are popular in many countries around the globe [10, 11]. As these have been proven to be safe for millenniums, even in developed countries [12]. The aim of salting is not only to prolong the shell life of fresh fish but also to provide desirable sensorial changes [13, 14] A straight proportion is present between the amount of salt used and the preservation period [15].

Shol fish belonging to the family Channidae is an obligate air breathing fresh-water fish which inhabits all types of water bodies from small ditches to rice fields, rivers and lakes across tropical and subtropical Asian countries. It is a good source of health food amongst Asians because it contains high levels of amino acids and fatty acids [16]. In South East Asia, consumers believe that shol fish contains all essential elements to maintain good health and to recover the lost energy after prolonged illness. In Bangladesh, shol-fish is popular to the consumers as a delicious and nutritious fish as well as bear high market price.

Usually fatty fishes are used for salt-curing in Bangladesh. But in the present research work, shol fish is selected because this fish considered widely accepted and preferred by the peoples and they need this fish when this are not available. To make this fish available in off seasons, attempts have been made to cure them using commercial salt. Besides, this highly accepted fish is not yet tried to preserve using salting or in any other forms.

Many scientific studies have unraveled the biomedical potential of fresh shol fish. But, there is very little scientific information about chemical and nutritional quality of salt treated preserved shol fish products.

Therefore, the present communication was designed to

document the effectiveness of two different types of traditional salting process (pickle-salting and brine-salting) in preserving popular fresh water shol fish at ambient temperature (26-32°C) 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. Materials and methods

2.1 Collection and handling of experimental fishes

Fresh shol fishes had been collected from the river Meghna in the early hours of the day. Being air breathing fish, shol fish were transported to the research laboratory in dram full with water.

2.2 Place of the experiment

Biochemical 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.

2.3 Preparation of fishes

The fishes were carefully washed with cooled tap water. Scales, fins, gills and viscera were removed and again washed with tap water to remove blood, slime and unnecessary flesh. Due to the presence of hard shield like bony elements, bones and head of shol fish are discarded as the waste.

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 [17, 18, 12].

2.4.1 Pickle-salting

During this experiment, the fresh shol fishes were salted by dry commercial salt (NaCl) of about 30% by weight of the dressed fish stacked in containers and stored at room temperature. The salt entered the fish and water 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.

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. Shol 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, pickle and brine salted shol fishes were packaging with plastic bag maintaining aseptic condition as far as possible and were stored at ambient temperature (26-32°C).

2.6 Sampling procedures

Evaluation of quality changes of pickle and brine salted shol fishes were carried out 15 days interval for biochemical analysis and 1 month interval for bacteriological study until the fish became inedible for consumption. Salted 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 process

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 [19]. Salt-ripening process starts when the surface of shol 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 [20].

2.8 Parameters of quality assessment

The analytical methods used in this experiment are given below:

- The moisture, fat, ash and salt contents of the fishes were determined by AOAC method [21].
- The crude protein of the fishes was determined by Micro-Kjeldhal method as described by Pearson [22].
- TVB-N was determined by Conway modified micro-diffusion technique as described by Conway and Byrne [23].
- FFA of the fishes was determined by AOAC method [24].
- pH was determined using a pH meter [25].
- Bacteriological study (SPC and HBC) was done according to the standard methods of AOAC and FDA BAM [26, 27].

3. Results & Discussion

3.1 Study of ripening process of pickle and brine-salting

In this experiment, changes in salt penetration rate and its effect on moisture content of shol fishes were analyzed to determine the ripening process of pickle and brine salted shol. An inverse relationship was found between salt penetration rate and the moisture content of pickle-salted and brine-salted shol fish during 7 days of ripening period which is given in Figure 1.

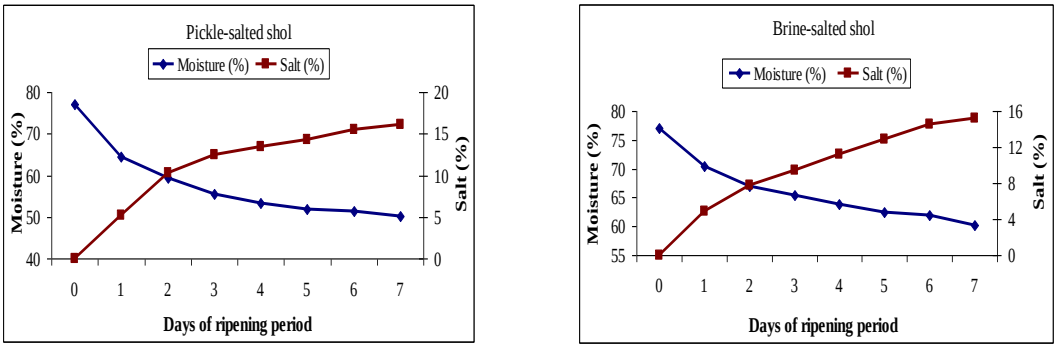

Fig 1: Relationship of changing pattern of moisture (%) and salt (%) content during 7 days of ripening period of pickle and brine salted shol fish products.

According to Beraquet and Barrera, moisture content was inversely proportional to NaCl content of the fish [28]. As the salt concentration increased, the amount of moisture content decreased almost linearly. This can be explained by the fact that since salt is hygroscopic; increase in salt concentration increases the amount of salt particles for absorbing water molecules from the fish samples [29]. Moisture content and salt percentage play an important role in the keeping quality of salt-cured fish products [30]. During salting, the mass transfer occurs basically between salt and water: the fish-muscle takes up salt and loses water [31, 32]. Many workers agree that maximum salt uptake takes place within 6-7 days of salting without further uptake during subsequent storage [34, 34, 35]. Similar results also obtained in the present study where the shol fish contained maximum salt content in 7 days of pickle and brine salting. 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 product [36, 37].

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 pickle and brine salted shol fish-products was done by assessing their proximate composition (moisture, protein, fat and ash), chemical composition (TVB-N, FFA and pH value) and bacteriological study.

3.2.1 Proximate composition

Proximate composition of pickle-salted and brine-salted shol fish-products during different duration of storage at ambient temperature (26-32°C) are shown in Table 1.

Table 1: Changes in Proximate composition (moisture, protein, fat and ash) of pickle-salted and brine-salted shol fish-products during different duration of storage at ambient temperature (26-32°C)

Storage period (Days)	Moisture (%)		Protein (%)		Fat (%)		Ash (%)	
	Pickle salted shol	Brine salted shol	Pickle salted shol	Brine salted shol	Pickle salted shol	Brine salted shol	Pickle salted shol	Brine salted shol
0*	50.29	60.17	27.64	20.72	3.79	3.20	18.69	16.06
15	51.08	60.72	27.13	20.65	3.73	3.17	18.62	15.94
30	51.65	61.44	26.79	20.07	3.68	3.11	18.51	15.83
45	52.68	61.78	25.97	19.80	3.63	3.08	18.42	15.72
60	52.95	62.46	25.72	19.51	3.57	3.01	18.33	15.60
75	53.02	62.98	25.65	19.22	3.52	2.98	18.21	15.54
90	53.06	63.32	25.57	18.80	3.48	2.93	18.17	15.45
105	53.80	63.91	25.33	18.25	3.41	2.86	18.00	15.33
120	54.00	64.85	25.28	17.53	3.36	2.80	17.84	15.28
135	54.60	65.97	24.99	17.06	3.20	2.72	17.59	15.14
150	54.94	-	24.82	-	3.11	-	17.45	-

*Just after completion of ripening period

In case of pickle and brine-salted shol fish-products, during storage at room temperature moisture (%) content was varied in the range of 50.29 to 54.94 and 60.17 to 65.97%; protein (%) content was varied in the range of 27.64 to 24.82 and 20.72 to 17.06%; fat (%) content was varied in the range of 3.79 to 3.11 and 3.20 to 2.72%; ash (%) content was varied in the range of 18.69 to 17.45 and 16.06 to 15.14% respectively. During the storage period, moisture content significantly increased whereas protein, fat and ash content significantly decreased. The phenomenon of increasing moisture content in room temperature is due to absorption of moisture from surrounding atmosphere. Increase in moisture content could be attributed to the difference in the moisture of the processed fish relative to the surroundings [38]. The increase in the moisture content of salted fish-products could be as a result of moisture reabsorption during entire storage period [39]. In storage condition the protein content decreased significantly with the time due to water soluble protein diffused out to the surrounding for exosmosis [40]. Protein decomposes with passing time [41, 42]. Decrease in the level of fat contents of small and large salted Bouri fish muscle (*Mugil cephalus*) was reported by El-Sebahy and Metwali [43]. Likewise,

Rahman reported that lipid content of dry salted hilsa was 26.93% on '0' day and decreased in 15.67% on 42nd day of storage respectively [44]. Similarly, Sayed reported that, during 18 days of observation of hilsa salting, lipid content decreased from 24.87% to 15.46% and 16.15% to 12.49% respectively [45]. Hassan *et al.* stated that, the ash content changes with the time of storage due to absorbance of moisture and loss of protein [46].

3.2.2. Chemical composition

Chemical methods are reliable measures of freshness or state of deterioration of products, because the concentrations of chemicals are dependent on storage time and temperature. Knowledge on chemical composition of fish will help the processors to define the optimum processing and storage conditions for premium quality products. Abu Gideiri *et al.* found a significant change in some chemical constituents of salted fish (*Oreocheomis niloticus*) [47]. Chemical composition of pickle-salted and brine-salted shol fish-products during different duration of storage at ambient temperature (26-32°C) are shown in Table 2.

Table 2: Changes in chemical composition (TNB-N, FFA and pH) of pickle-salted and brine-salted shol fish-products during different duration of storage at ambient temperature (26-32°C)

Storage period (days)	TVB-N (mgN/ 100g)		FFA (%)		pH	
	Pickle salted shol	Brine salted shol	Pickle salted shol	Brine salted shol	Pickle salted shol	Brine salted shol
0*	5.28	3.64	1.3	1.8	6.4	6.5
15	7.27	5.27	2.4	2.9	6.5	6.7
30	8.81	9.18	2.8	3.5	6.6	6.9
45	10.13	12.75	4.4	6.7	6.8	7.2
60	14.98	14.04	6.1	9.2	6.9	7.5
75	18.15	16.27	7.4	10.5	7.1	7.7
90	21.55	20.84	8.5	11.2	7.3	7.9
105	26.48	27.63	9.2	12.7	7.5	8.1
120	30.47	30.33	10.5	13.3	7.7	8.2
135	32.92	34.52	12.2	14	8.0	8.3
150	34.99	-	13	-	8.3	-

*Just after completion of ripening period

In case of pickle and brine-salted shol fish-products, during storage at room temperature TVB-N (mgN/100g) value varies in the range of 5.28 to 34.99 and 3.64 to 34.52 mgN/100g; FFA(%) value varies in the range of 1.3 to 13 and 1.8 to 14%; pH value varies in the range of 6.4to 8.3 and 6.5 to 8.3 respectively. A significant increase in TVB-N, FFA and pH values was observed in these two types of salted shol fish-products. The limiting level for rejection of TVB-N is 30-40 mgN/100g for storage at ambient temperature [48].

A comparable pattern of the increase in TVB-N has been reported in brined anchovies and brined chub mackerel [49, 50]. Rahman reported that, the FFA value of dry salted hilsa products increased from 1.16% to 11.7% during 8 weeks storage at room temperature (26-30°C) [51]. Gümüş *et al.* investigated that, pH value of 20% brine salted red mullet increases from 6.51 to 6.59 during 11days of storage at 4°C (refrigerator temperature) [52].

3.2.3 Bacteriological study

The bacteriological examination of fish products was to evaluate the possible presence of bacteria in terms of quantity of public health significance and to give an impression of the hygienic quality including temperature abuse and hygiene during handling and processing [53]. Standard plate count (SPC) (cfu/g) and halophilic bacterial count (HBC) (cfu/g) of pickle-salted and brine-salted shol fish-products during different duration of storage at ambient temperature (26-3211/7/2016C) are given in Table 3.

Table 3: Standard plate count (SPC) (cfu/g) and Halophilic bacterial count (HBC) (cfu/g) of pickle-salted and brine-salted shol fish-products during different duration of storage at ambient temperature (26-32°C)

Storage period (months)	SPC (cfu/g)		HBC (cfu/g)	
	Pickle salted shol	Brine salted shol	Pickle salted shol	Brine salted shol
0*	3.6×10^3	2.1×10^4	4.1×10^2	4.0×10^3
1	0.9×10^4	8.2×10^4	8.1×10^2	2.5×10^4
2	3.3×10^4	3.4×10^5	2.0×10^3	7.8×10^4
3	6.7×10^4	9.0×10^5	2.6×10^4	2.6×10^5
4	5.5×10^5	3.7×10^6	8.8×10^4	3.4×10^5
5	2.0×10^6	-	2.4×10^5	-

*Just after completion of ripening period

In present study, the range of SPC of pickle and brine-salted shol fish-products during storage at room temperature were 3.6×10^3 to 2.0×10^6 and 2.1×10^4 to 3.7×10^6 whereas HBC of pickle and brine-salted shol fish-products during storage at room temperature were 4.1×10^2 to 2.4×10^5 and 4.0×10^3 to 3.4×10^5 respectively. In Bangladesh, DOF and BSTI recommended the SPC of processed fish to be not more than 10^6 cfu/g. If any sample contains more than 10^8 cfu/g bacterial counts then these microbes can cause spoilage of that product [54]. In this study, SPC of these two salted fish-products increases during the storage period but were within the limits of 10^7cfu/g specified for quality grading of fish by the International Commission of Microbiological Standards for Foods [55]. 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. Can observed that, halophilic bacterial populations increased in 3.59 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 when 5 months stored in 4°C (refrigeration temperature) [56].

4. Conclusions

The present study reveals that, ripening process have a positive significant role on the proximate and chemical composition of pickle-salted and brine-salted shol and reduces bacterial load as well as makes them nutritionally suitable for all. This research also provides basic nutritional information on pickle-salted and brine-salted fish-products, which is necessary to formulate guideline for common people to help them to plan better nutritional diet for good health especially in developing countries where all the required sophisticated storage equipment is not available. Pickle-salting and brine-salting as preservation-methods, are efficient in the post-harvest management of fishery-products which could be improved the preservative strategies of fish and thus prolong the shelf life of one of the commercially important food commodities in the tropics. From this research, it can be concluded that salted fish-products can provide satisfactory nutrition for the nation. Commercial traders those who produce market salted fishes 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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