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RESEARCH ARTICLE

Effects of Dry, Pickle and Brine Salting on biochemical and mineral composition and bacterial load of freshwater snakehead fish Taki (*Channa punctatus*)

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Abstract

Salt-cured fishes are highly appreciated because of their characteristic taste, texture and storage stability. In present study, effects of dry, pickle and brine salting on the production of high quality salted fish-products from taki fish (*Channa punctatus*) and their nutritive value was investigated. In fresh, dry salted (DS), pickle salted (PS) and brine salted (BS) taki fish-products, moisture (%) content were 78.65±.07%, 46.21±.04%, 52.71±.06% and 62.28±.02%; protein (%) content were 16.89±.10%, 23.58±.01%, 21.39±.02% and 18.02±.01%; fat (%) content were 2.50±.06%, 3.93±.01%, 3.40±.01% and 2.76±.01%; ash (%) content were 1.36±.11%, 26.37±.02%, 22.96±.01% and 17.24±.01%; salt (%) content were -, 16.06±.06%, 16.22±.04% and 15.50±.04%; TVB-N content were 3.43±.02, 4.18±.00, 3.79±.02 and 5.70±.01 mgN/100g of fish; FFA (%) value were 0.5±.1%, 1.9±.15%, 2.4±.21% and 2.6±.15%; pH value were 7.0±.06, 6.5±.06, 6.6±.15 and 6.8±.10; Ca content were 16.35, 600, 497.5 and 123 mg/100g of fish; Mg content of 9.425, 147.75, 65.5 and 52.5 mg/100g of fish; Fe content were 1.275, 3.75, 2.95 and 2.07 mg/100g of fish; Cu content were 0.65, 0.425, 0.25 and 0.2 mg/100g of fish; Zn content were 0.425, 1.525, 1.43 and 1.175 mg/100g of fish and Mn content were 0.05, 0.625, 0.60 and 0.55 mg/100g of fish respectively. The bacteriological examination revealed that fresh and dry, pickle and brine salted taki fish-products are within the acceptable levels of the specified bacteriological limits for fish and fishery-products.

Introduction:-

Fish is a highly proteinous food consumed by a larger percentage of populace because of its availability and palatability, while fewer percentages do so because of its nutritional value **(Foran *et al.*, 2005)**. Fish is especially important in the developing world. In some countries of Asia (Bangladesh and Cambodia) obtained as much as 75% of their daily protein from fish. Often fish provides essential nourishment, especially high quality proteins and fats (omega 3 and 6 fatty acids), vitamins and minerals. In the economy of Bangladesh fishery products play a vital role.

Fish processing has been practiced in Bangladesh for a long time. The methods commonly used for preservation, are the traditional techniques such as salting or brining, sun-drying and smoking, which also increase fish availability to the consumers **(Abolagba *et al.*, 1996).** Traditional preserving methods of fish products have still wide acceptance around the world due to their accustomed taste and aroma **(Kose, 2010).**

Salting is a popular procedure for preserving fish. Salted fish products are popular in many countries around the globe **(Basti *et al.*, 2006; Lakshmanan *et al.*, 2002).** Sodium chloride (NaCl), also called salt, is generally recognized as a safe, antimicrobial and incidental food additive **(Klaassen, 1986).** Salt has been used as a seasoning and flavor enhancer as well as a preservative or curing agent and is a powerful depressor of water activity (a^w) of the food **(Turan *et al.*, 2007).** Moreover, it is known that chloride ions are toxic for some microorganisms **(Leroi *et al.*, 2000).**

The presence of sufficient salt in fish prevents or drastically reduces the action of bacteria. Salting involves the application of salt to fish either directly or as brine. It removes moisture by the process of osmosis, creating medium unsuitable for microbial growth. The rate of salt uptake and moisture loss is influenced by such factors as temperature, thickness of the flesh, fattiness of the flesh, freshness of the fish and the chemical purity of the salt used for curing. Length of salting period as well as salt concentration depends on the expected final product **(Bellagha et *al.*, 2007).** Salting fish is likely to remain in good demand by those who value tradition and taste but it has also gained acceptance in innovative products that provide convenience. The principle of salting and storage is also to control or minimize the fish deterioration by killing or reducing the growth of microorganisms in the fish. The high concentration of salt has been shown to prevent microbial spoilage in similar products **(Andersen *et al.*, 2007).**

Biochemical analysis provides information on the nutritional value of a particular organism used as a source of food. The measurement of some proximate profiles such as protein contents, carbohydrates, lipids, moisture contents and ash percentage is often necessary to ensure that they meet the requirements of food regulations and commercial specifications **(Watermann, 2000).** In the developed world, people are more concern about the risk and health issues **(Redmond and Griffith, 2005).** On the other hand, in the developing countries due to social inequality some consumers have higher purchasing power also conscious about health issues regarding intake of food **(Petrovici *et al.*, 2004).** Nutrition is an important influencing factor of fish product consumption **(Olsen, 2004 and Ahamed, 2009).** At present, people are aware about health and nutritional issues **(Hossain *et al.*, 2008)** and they concern about the nutritional value of the food items when they buy food items for their household. A number of studies found that higher income people are more concern about harmful and health hazardous food intake in Bangladesh **(Siddique, 2011).**

A number of study on biochemical composition of dried fishes were found in the literature but salting of freshwater fish species has a little in number compared to the dry fish.

Among the freshwater fish species, Taki (*C. punctatus*) fish is delicious, nutritious and popular to the consumers as well as bear high market price. So it is necessary to take some steps for their proper preservation and marketing and during this period maintain proper quality.

The purpose of the present study is to develop an efficient and effective model for salt-curing of freshwater fish species Taki (*C. punctatus*) for the production of high quality Dry-salted, Pickle-salted and Brine-salted products and transfer the technology to the rural small-scale fisher folks allover Bangladesh.

Materials and Methods:-

Collection of the fishes:

Fresh taki fish (*C. punctatus*) had been collected from the river Meghna in the early hours of the day.

Handling of experimental fish in laboratory:

Being air breathing fish, Taki fish was transported to the research laboratory in dram full with water.

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), and from center for Advanced Research in Sciences (CARS) Dhaka, Bangladesh for Minerals.

Preparation of fish:
The fishes were carefully washed with cooled tap water. Head, scales, fins, gills and viscera were removed and again washed with tap water to remove blood, slime and unnecessary flesh. Because of hard and large bones of the head, the head and bones of taki were included as the waste.

For salt curing, total cleaned fishes were grouped into 3 batches.

Methods of salting:-

Dry salting (DS) method: Fresh taki 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.

Pickle-salting (PS) method: In this method, fresh taki 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-salted fish.

Brine salting (BS) method: During this experiment, 30% salt solution is prepared (30 gm salt in 100 ml water) which is called brine. Fresh taki fishes were kept at this saturated brine solution stacked in containers and stored for a salting or curing period, at room temperature (26^0C-30^0C) for the production of brine salted fish. The brine solution was changed with new solution once every week for keeping nearly constant saturation outside the fish. The fish in brine were kept immersed by putting a glass weight on it.

Sampling procedures:
6 or 7 slice was taken randomly which represented the parts from whole body of the taki fish. Salt crystals (if any) were removed from salted fishes by using tissue paper. Then the slices were chopped and finally ground with an electric blender to make a homogenous sample before being sampled for analysis.

Parameters of quality assessment:
The analytical methods used in this experiment are given below**:**

- The moisture, fat, ash and salt contents of the fish were determined by AOAC method **(AOAC, 1990).**
- The crude protein of the fish was determined by Micro-Kjeldhal method as described by **Pearson (1999).**
- TVB-N was determined by Conway modified micro-diffusion technique as described by **Conway (1933).**
- pH was determined using a pH meter **(Vyncke, 1981).**
- FFA of the fish was determined by AOAC method **(AOAC, 2000).**
- Bacteriological study (SPC and HBC) was done according to the standard methods of **AOAC (1995)** and **FDA BAM (2001).**
- Samples for mineral analysis were prepared according to recommendations of **Perkin Elmer's** procedures of Atomic Absorption Spectrometer **(1996).**

Statistical analysis: Data were analyzed by using SPSS for windows-20 statistical programme. Significance was established at $p< 0.05$.

Results & Discussion:-

Bio-chemical composition:
Bio-chemical composition of fresh taki fish and freshly processed Dry salted (DS), Pickle salted (PS) and Brine salted (BS) Taki fish-products are shown in **Table 1.**

In present study, fresh, freshly processed dry salted (DS), pickle salted (PS) and brine salted (BS) **taki** fish-products, **moisture (%)** content were 78.65±.07%, 46.21±.04%, 52.71±.06% and 62.28±.02%**; protein (%)** content were 16.89±.10%, 23.58±.01%, 21.39±.02% and 18.02±.01%**; fat (%)** content were 2.50±.06%, 3.93±.01%, 3.40±.01% and 2.76±.01%**; ash (%)** content were 1.36±.11%, 26.37±.02%, 22.96±.01% and 17.24±.01%**; salt (%)** content were -, 16.06±.06%, 16.22±.04% and 15.50±.04%; **TVB-N** content were 3.43±.02, 4.18±.00, 3.79±.02 and 5.70±.01

mgN/100g of fish; **FFA (%**) value were 0.5±.1%, 1.9±.15%, 2.4±.21% and 2.6±.15%**; pH** value were 7.0±.06, 6.5±.06, 6.6±.15 and 6.8±.10 respectively.

In comparison of fresh fish, the salting process resulted in a significant decrease ($P < 0.05$) in moisture content and a significant increase ($P < 0.05$) in protein, fat and ash content of Dry-salted, pickle-salted and brine-salted Taki fish samples. This is consistent with the observations by **Abraham-Olukayode *et al.* (2012).** Nutritional components, such as protein, lipid, and ash, were increased due to the loss of water in fish muscle in the salting process **(Bras & Costa, 2010; Chaijan, 2011).**

Significant increased in protein levels ($p<0.05$) in all salted fish-products when compared with the fresh fish, suggested that protein nitrogen was not lost during salting **(Nahid *et al.*, 2014).** The findings in the protein and fat contents were also in agreement with related studies by **(Thorarinsdottir *et al.*, 2004)** where quite related trends were observed with indications that the amount of protein was a function of salt concentration and water content.

The higher value of total ash content in freshly processed Dry-salted, pickle-salted and brine-salted Taki fish than fresh fish was attributed to high salt content which added more ash components to the products. These results were in accordance with **El-Bassir *et al.* (2015).**

The FFA (%) value recorded in this work is low and hence there is no fear of rancidity. This agrees with the work of **Frazier and Westhoff (1998).**

The salting process caused a decrease in pH values in all salted fish samples. **Goulas (2005)** observed that pH values of mackerel (*Scomber japonicus*) decreased after salting.

According to **Hernandez-Herrero *et al.* (1999),** after salting of Anchovies, salt content increased from 0.32 to 19.3%, ash content increased from 1.6% to 21% and pH decreased from 6.13 to 5.72 which are in harmony with present findings.

Monsur (2007) observed that, 30% dry salted, pickle salted and brine salted punti fish had salt content of 24.21%, 24.68% and 17.28% respectively and chapila fish had salt content of 25.11%, 21.43% and 15.94% respectively which is higher from present study.

Mineral contents:-

The mineral composition of fresh taki fish and freshly processed Dry salted (DS), Pickle salted (PS) and Brine salted (BS) taki fish-products are shown in **Table 2.**

In case of fresh, dry salted (DS), pickle salted (PS) and brine salted (BS) **taki** fish-products, **Ca** (calcium) content were 16.35, 600, 497.5 and 123 mg/100g of fish; **Mg** (magnesium) content were 9.425, 147.75, 65.5 and 52.5 mg/100g of fish; **Fe** (iron) content were 1.275, 3.75, 2.95 and 2.07 mg/100g of fish; **Cu** (Cooper) content were 0.65, 0.425, 0.25 and 0.2 mg/100g of fish; **Zn** (zinc) content were 0.425, 1.525, 1.43 and 1.175 mg/100g of fish and **Mn** (manganese) content were 0.05, 0.625, 0.60 and 0.55 mg/100g of fish respectively.

From **table 2.** it is clear that three types of salting methods raised the mineral composition of dry salted, pickle salted and brine salted taki fish products.

There is very little information on mineral contents of salted fish-products. However, the present values are comparable with the results reported for some dry freshwater fish (**Ahmed *et al.*, 2011; Mohammed, 2013).** According to **Begum *et al.* (2012),** freshly processed salted-dried punti (*Puintius sophore*) fish have calcium (Ca) and Iron (Fe) was in a range of 320-330 mg/100g and 3.1-3.9mg/100g of fish respectively.

Bacteriological study:-

Standard plate count (SPC) (CFU/g) or total bacterial count (TVC) and halophilic bacterial count (HBC) (CFU/g) of fresh, dry salted (DS), pickle salted (PS) and brine salted (BS) **taki** fish-products are given in **Table 3.**

The Standard plate count (SPC) in fresh, dry salted (DS), pickle salted (PS) and brine salted (BS) **taki** fish-products were recorded in 1.1×10^5, 4.0×10^3, 3.0×10^4 and 4.3×10^4 CFU/g whereas halophilic bacterial count was recorded in -, 3.0×10^2, 3.3×10^3 and 4.1×10^3 CFU/g respectively.

According to **Surendran (2006)** the acceptable limit for bacterial count is 5×10^5/g for fresh fish which is in accordance with present findings of fresh taki fish. Freshly process dry salted (DS), pickle salted (PS) and brine salted (BS) **taki** fish-products also indicated an acceptable microbial load ($<10^5$ CFU/g^{-1}) **(ICMSF, 1988).**

From **Table 3.** it was observed that in comparison with the fresh fish, there was a decrease in total bacterial count which may be due to the presence of high salt concentration, so the pathogenic microorganism growth is controlled. This result is in agreement with the findings of **Abu Giddeire (2001).**

According to **Abbas Bakhiet and Khogalie (2011),** salting process reduces total bacterial count of *Hydrocynus spp.* fish and found that TVC of fresh fish was 58.1×10^3 and it reduces in freshly processed 15%, 20% and 25% salted fish-products in 10. 0×10^3, 7.8×10^3 and 4×10^3 respectively which is in agreement with the present study.

Ames *et al.* (1991) reported that when the water activity is considerably reduced, most microorganisms become inactive but haploidic microorganisms become the major causes of microbial spoilage. The high salt concentration leaves only salt tolerant microorganisms to survive **(Horner, 1997).**

Table 1: Biochemical composition of fresh, Dry-salted (DS), Pickle-salted (PS) and Brine-salted (BS) Taki fish:

Samples	**Moisture** (%)	**Protein** (%)	**Fat** (%)	**Ash** (%)	**Salt** (%)	**TVB-N** mgN/100g	**FFA** (%)	**pH**
Fresh Taki	78.65±.07	16.89±.10	2.50±.06	1.36±.11	-	3.43±.02	0.5±.1	7.0±.06
DS Taki	46.21±.04	23.58±.01	3.93±.01	26.37±.02	16.06±.06	4.18±.00	1.9±.15	6.5±.06
PS Taki	52.71±.06	21.39±.02	3.40±.01	22.96±.01	16.22±.04	3.79±.02	2.4±.21	6.6±.15
BS Taki	62.28±.02	18.02±.01	2.76±.01	17.24±.01	15.50±.04	5.70±.01	2.6±.15	6.8±.10

Table 2: Mineral composition of fresh, Dry-salted (DS), Pickle-salted (PS) and Brine-salted (BS) Taki fish:

Samples	**Macro elements**		**Micro / Trace elements**			
	Ca Mg/100g	**Mg** Mg/100g	**Fe** Mg/100g	**Cu** Mg/100g	**Zn** Mg/100g	**Mn** Mg/100g
Fresh Taki	16.35	9.425	1.275	0.65	0.425	0.05
Dry-salted Taki	600	147.75	3.75	0.425	1.525	0.625
Pickle-salted Taki	497.5	65.5	2.95	0.25	1.43	0.60
Brine-salted Taki	123	52.5	2.07	0.2	1.175	0.55

Table 3: Standard plate count (CFU/g) and halophilic bacterial count (CFU/g) in fresh, Dry-salted (DS), Pickle-salted (PS) and Brine-salted (BS) Taki fish

Samples	**SPC (CFU/g)**	**HBC (CFU/g)**
Fresh Taki	1.1×10^5	-
DS Taki	4.0×10^3	3.0×10^2
PS Taki	3.0×10^4	3.3×10^3
BS Taki	4.3×10^4	4.1×10^3

Conclusion:-

The present study reveals that different fish salting methods have a positive significant role on the biochemical and mineral composition of freshwater Taki (*C. punctatus*) fish and reduces bacterial load as well as makes them nutritionally suitable for all.

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