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Performance and quality assessment of sun-dried salted *Channa punctatus* (Bloch, 1793) and *Mystus tengra* (Hamilton-Buchanan, 1822) during refrigeration (4 °C) storage

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Abstract
The present study was performed to investigate the influence of sun-drying treated with salt (30%) and storage time under refrigeration (4 °C) temperature on the nutritive value (moisture, protein, fat, ash), chemical composition (TVB-N, FFA, pH) and bacteriological analysis (SPC, HBC) of two different size fresh water fish-species (*Channa punctatus* and *Mystus tengra*). Values of moisture, TVB-N, FFA, pH and bacteriological-load increased significantly with the laps of storage period (months), but the values did not exceed the rejection limit. These values increased rapidly in sun-dried salted *C. punctatus* than *M. tengra* and at the end of 24 months of refrigeration-storage, sun-dried salted *C. punctatus* became spoiled whereas sun-dried salted *M. tengra* still remained fresh. No yeast and mould were detected during the storage period. The result of this study indicated that, small sun-dried salted fish like *M. tengra* had longer shelf-life (32 months) than medium-size sun-dried salted fish like *C. punctatus*.

Keywords: sun-dried salting, freshwater-fish, nutritional-composition, chemical-composition, bacterial-count

1. Introduction
Fish is a highly proteinous food consumed by the populace. Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body [1]. A larger percentage of consumers do eat fish because of its availability, flavors, palatability while fewer percentages do so because of its nutritional value. The less developed countries capture 50% of the world fish harvest and a large proportion of the catch are consumed internally [2]. In many Asian countries over 50% of the animal protein intakes come from fish. Consumption of fish provides an important nutrient to a large number of people worldwide and thus makes a very significant contribution to nutrition. Fish has edge over meat because it is cheaper and relatively more abundant.

Fishes normally spoil within 12-20 hours depending on the species and the methods of capture. If the fishes are not processed immediately after they are captured, certain irreversible spoilage and deterioration reactions begin to take place [3]. Most of the processing or preservation operations are intended to reduce the rate of spoilage by reducing water activity of the fish [4].

Drying reduces or completely eliminates physiological, microbial and enzymatic degradation of biological materials such as fish [5]. Dried processed fishery products created a position in our exported fishery items. In Bangladesh, the process of drying fish is mainly performed by the household of local fisherman who are mostly illiterate using traditional methods. So, a large quantity of dried fish is spoiled each year due to lack of proper drying, preservation and storage facilities particularly during the glut season [6]. The major problem associated during the storage of dried products is the fly infestation and contamination [7]. Through in traditional fish drying method, the fish is contaminated by dust, dirt or sand and pathogens. Moreover, it is very slow process which makes the product unhygienic by contributing the partial destruction of protein contents of the fish through oxidation and bacterial or enzymatic degradation. The physical and organoleptic qualities of most of the traditional sun-dried products available in the market are not satisfactory for human consumption [8, 9, 10, 11, 12]. Their products do not meet quality standards and thus shortens shelf life of dried product. Moreover, the problem markedly evident with dried products is the contamination during different stages of handling and due to lack of proper packaging. During long storage time, lack of proper packaging increase moisture and for this reason the dried fishery product become brownish, yellow or brown which indicates varying stages of spoilage [13]. As a result, in tropical country like Bangladesh, where relative humidity is high almost throughout the year, the dried products absorb moisture from the environment. Determination of microbiological quality of such processed fishes is very important for guarding consumer's health and hygiene [14].

Therefore, alternative affordable, safe, hygienic and environmental friendly methods must be developed and adopted for fish drying. In this study, salt is used with sun-drying as a natural preservative. Sodium chloride (NaCl), also called common salt, and table salt, is generally recognized as a safe, antimicrobial and incidental food additive [15]. Salt has been used as a seasoning and flavor enhancer as well as a preservative or curing agent, had been purchased from the local market. Salt is a powerful depressor of water activity (aw) of the food [16]. Moreover, it is known that chloride ions are toxic for some microorganisms [17].

In this research works it was considered two different size freshwater fish species viz; medium size *Channa punctatus*, and small size *Mystus tengra* for sun-dried salting. The size variation of selected fish species for present experiments bears significant consideration. Rahman stated that, soft textured fish tend to absorb salt faster than tough or firm-textured fish [18]. He also stated that high fat content fish absorb salt slower than low-fat fish. The small indigenous species of fishes in Bangladesh are generally considered to be those which grow to a length of approximately 5-15 cm at maturity [19]. It is assumed that the size of fishes considered to be selected for preparing any processed product has got some significance on the quality of the product. Therefore, consideration of different fat content and different sizes of experimental fish-species in this research work may be considered as justifiable.

The initial quality of fresh fish material strongly influences subsequent performance in sun-dried salting. Degree of excellence of any product is 'Quality'. Quality assessments are necessary to ensure the food safety of any processed products. Very little scientific information on the quality changes is available about refrigeration stored sun-dried salted fish-products.

In view of above facts, the present study was therefore initiated to produce high quality sun-dried salted fish-products and to assess their nutritional compositions, chemical-compositions and bacterial-load for guarantee its optimal use as a good source of healthy food.

2. Materials and methods

2.1 Collection of the fishes

Two different sizes freshwater fish species; medium size *Channa punctatus* (Taki fish) and small *Mystus tengra* (Tengra) fish had been collected from the river Meghna in the early hours of the day.

2.2 Handling of experimental fishes in laboratory

Being air breathing fish, *C. punctatus* fish were transported to the research laboratory in dram full with water. In case of *M. tengra* fish they were carried in clean, good quality sterile polythene bag with ice in order to keep the fish fresh and avoid any type of microbial contamination.

2.3 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).

2.4 Preparation of fishes

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 *C. punctatus* are discarded as the waste.

2.5 Sun-dried-salting method

During this experiment, fresh fishes were well rubbed with dry common table salt (NaCl) of about 30% by weight of the dressed fish. They were then kept on a plastic made basket in the sun. They were kept in sun regularly during day time (9:00 a.m. to 3 p.m.) for 2-7 days as sometimes the sky was cloudy and until the required drying period was over. During sun-drying, they were kept covered by small meshed nylon or mosquito net to avoid external contamination and to prevent bird attack and fly infestation. The process and principle of sun dried salting is shown in Figure 1. This model is adapted from Nowsad [20].

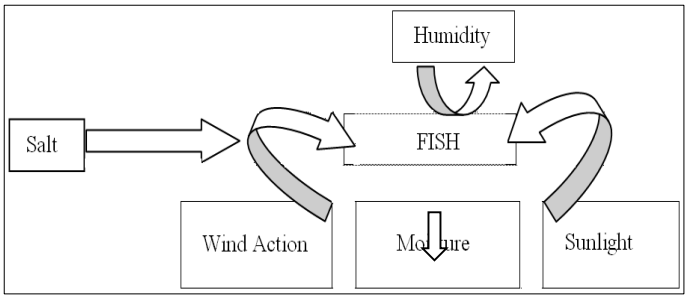

Fig 1: Process of sun-dried salting of fish

2.6 Storage of the sun-dried salted fish-products

At the end of sun-dried salting process, dried *C. punctatus* and *M. tengra* fish-products was packaged in plastic bag maintaining aseptic condition as far as possible. The bags were sealed by using an electrical sealing machine. After that, dried fish-products were stored at refrigeration temperature (4 °C) for shelf life study.

2.7 Sampling procedure

Few sun-dried salted *C. punctatus* and *M. tengra* fish-products was randomly taken. Then they were chopped with skin and finally ground with an electric blender to make a homogenous sample before being sampled for quality analysis. Salt crystals (if any) were removed from sun-dried salted fish-samples by using dry tissue paper. Analysis was done at 4 months interval until the fish became inedible.

2.8 Parameters of quality assessment

The analytical methods used in this experiment are given below**:**

- Moisture, fat and ash contents of the sun-dried salted fishes were determined by AOAC method [21].
- The crude protein of the fish was determined by Micro-Kjeldahl method [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 Nutritional composition

Nutritional composition of sun-dried salted *C. punctatus* and *M. tengra* during different duration of storage at refrigeration temperature (4°C) is shown in Table 1. In case of refrigeration-temperature stored sun-dried salted *C. punctatus* and *M. tengra* fish-products, moisture (%) content was varied in the range of 9.77 to 13.22 and 4.9 to 6.74%; protein (%) content was varied in the range of 47.69 to 46.06 and 43.00 to 42.40%; fat (%) content was varied in the range of 7.47 to 6.53 and 15.99 to 15.09%**;** ash (%) content was varied in the range of 35.16 to 34.58 and 36.20 to 35.50% respectively.

Table 1: Changes in nutritional composition (moisture, protein, fat and ash) of sun-dried salted *C. punctatus* and *M. tengra* during different duration of storage at refrigeration temperature (4 °C)

Storage period (months)	Moisture		Protein		Fat		Ash	
	C. punctatus	*M. tengra*	*C. punctatus*	*M. tengra*	*C. punctatus*	*M. tengra*	*C. punctatus*	*M. tengra*
0*	9.77	4.9	47.69	43.00	7.47	15.99	35.16	36.20
4	10.09	5.14	47.53	42.93	7.21	15.90	35.03	36.13
8	10.79	5.39	47.32	42.86	7.08	15.82	34.89	36.01
12	11.53	5.55	47.09	42.79	6.86	15.74	34.76	35.95
16	12.11	5.73	46.73	42.70	6.71	15.67	34.81	35.90
20	12.82	5.95	46.37	42.62	6.62	15.54	34.67	35.83
24	13.22	6.19	46.06	42.53	6.53	15.39	34.58	35.71
28	-	6.40	-	42.47	-	15.20	-	35.67
32	-	6.74	-	42.40	-	15.09	-	35.50

*= Immediate after sun-dried salting

The relative lower percentage moisture of '0' day (freshly processed) salt and turmeric treated sun-dried fish-products could be as a result of the combined effects of the salt and heat of sun, which enhanced greater dehydration. The lowest moisture content of these sun-dried salted fish-products indicated that it was more resistant to the enzymatic and microbial activities and these qualities might have lengthened their shelf life. According to BSTI, the increased moisture content of dried fish above 15% favor the growth of mould and insect infestations which in turn would accelerate the spoilage of fish product [28]. According to Waterman, bacterial action stops at 25% water content and moulds action increased at 15% water content [29]. During the storage period, moisture content slightly increased. The phenomenon of increasing moisture content during storage 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 [30]. Siddique and Akter observed percentage of moisture content increased in ranged 13.81-20.50%, 22.22-34.99% and 20.76-32.65% whereas protein level decreased from 58.33-51.98%, 64.39-56.46% and 71.90-67.22% in dried marine fishes (*Lepturacanthus savala*, *Harpodon nehereus* and *Johnius dussumieri*) stored at room temperature for 2 year [31]. Bhuiyan observed 6.9% - 14.2% moisture in dried marine fishes [32]. Akter observed that, percentage of protein decreased from 58.51% to 57.73% and 59.51% to 58.28% in case of 8 months refrigeration-stored sun-dried salted and turmeric treated sun-dried salted *M. vittatus* fish-products [33]. Decrease in protein during refrigeration storage is in agreement with the findings of Bhat *et al.* and Arekemase *et al.* [34, 35]. Akter observed, moisture content of salted-dried and salt and turmeric treated sun-dried kachki (*C. soborna*) gradually increased whereas protein content gradually decreased through entire period of storage both room and refrigerator temperature which is in harmony with present findings [36]. According to Kumar *et al.*, moisture content increased from 7.14 to 15.07% and protein and fat content decreased from 51.32 to 46.33% and 9.48 to 5.78% respectively in case of 6 month stored 30% salted sun-dried *L. gonius* fish at room temperature [37].

3.2 Chemical composition

Chemical compositions of sun-dried salted *C. punctatus* and *M. tengra* during different duration of storage at refrigeration temperature (4 °C) are shown in Table 2.

Table 2: Changes in chemical composition (TVB-N, FFA and pH) of sun-dried salted *C. punctatus* and *M. tengra* during different duration of storage at refrigeration temperature (4 °C)

Storage period (months)	TVB-N (mg/ 100g)		FFA (%)		pH	
	C. punctatus	*M. tengra*	*C. punctatus*	*M. tengra*	*C. punctatus*	*M. tengra*
0*	2.27	1.90	1.8	1.8	5.9	6.3
4	4.21	5.02	2.8	2.8	6.0	6.3
8	7.03	7.63	4.2	3.7	6.3	6.4
12	10.17	9.26	5.4	5.1	6.6	6.5
16	15.74	11.74	7.3	6.6	6.9	6.6
20	17.19	14.01	9.5	7.9	7.3	6.7
24	19.96	15.99	11.3	9.1	7.5	6.8
28	-	18.04	-	10.2	-	6.9
32	-	20.75	-	11.1	-	7.0

*= Immediate after sun-dried salting

In case of refrigeration-temperature stored sun-dried salted *C. punctatus* and *M. tengra* fish-products, TVB-N (mg/100g) value varies in the range of 2.27 to 19.96 and 1.90 to 20.75 mg/100g; FFA (%) value varies in the range of 1.8 to 11.3 and 1.8 to 11.1%; pH value varies in the range of 5.9 to 7.5 and 6.3 to 7.0 respectively. Significantly higher increase in TVB-N, FFA and pH values was observed in refrigeration stored *C. punctatus* fish-products and after 24 months of storage period, this fish-product became spoiled whereas refrigeration stored *M. tengra* fish-product remain in good condition. TVB-N measurement can be used as a parameter for the determination of microbiological and enzymatic spoilage of fish product [38]. According to Connell, the limiting level for rejection of TVB-N is 20 mg/100g for refrigeration temperature stored fish-products [39]. Chakraborty *et al.* found that, free fatty acid (FFA) value ranged from 2.9% to 8.56% on the 60 days of observation in sun-dry salted fishes respectively [40]. Sharma *et al.* also found an increasing trend of FFA with a value of 5.24% to 28.34% in 6 months stored sun-dried salted *G. chapra* fish-products [41]. Increase of pH value in refrigeration stored sun-dried salted *C. punctatus* and *M. tengra*

fish-samples agrees with the findings of Yatsunami and Takenaka and Erkan and Ozden that an increase in pH value during storage period is due to the increase in volatile bases from decomposition of nitrogenous compounds by endogenous or microbial enzymes [42, 43].

3.3 Bacteriological study

Standard plate count (SPC) (cfu/g) and halophilic bacterial count (HBC) (cfu/g) of sun-dried salted *C. punctatus* and *M. tengra* during different duration of storage at refrigeration temperature (4°C) are shown in Table 3. In case of refrigeration-temperature stored sun-dried salted *C. punctatus* and *M. tengra* fish-products, the range of SPC were 3.9×10^3 to 6.1×10^5 (24 months) and 2.8×10^3 to 1.8×10^6 cfu/g (32 months) whereas HBC were 2.4×10^2 to 7.1×10^4 (24 months) and 1.5×10^2 to 2.8×10^5 cfu/g (32 months) respectively. In Bangladesh, BSTI recommended the SPC of processed fish to be not more than 10^6 cfu/g [28]. If any sample contains more than 10^8 cfu/g bacterial counts then these microbes can cause spoilage of that product [44]. Spoilage of fish not only occurs at room temperature but also at the refrigeration temperature [45]. In this study, SPC of this two dried fish-products increases during refrigeration storage period but were within the limits of 10^7 cfu/g specified for quality grading of fish by the International Commission of Microbiological Standards for Foods [46]. No microorganisms (Yeast, Mold & Bacteria) can grow in a fish product of moisture content less than 14% [47].

Table 3: Changes in standard plate count (SPC) and halophilic bacterial count (HBC) of sun-dried salted *C. punctatus* and *M. tengra* during different duration of storage at refrigeration temperature (4 °C)

Storage period (months)	SPC		HBC	
	Taki	Tengra	Taki	Tengra
0*	3.9×10^3	2.8×10^3	2.4×10^2	1.5×10^2
4	6.1×10^3	4.1×10^3	3.5×10^2	3.5×10^2
8	1.3×10^4	1.8×10^4	1.6×10^3	2.5×10^3
12	2.8×10^4	3.1×10^4	3.9×10^3	4.1×10^3
16	4.6×10^4	5.1×10^4	6.1×10^3	6.5×10^3
20	1.6×10^5	2.3×10^5	1.8×10^4	3.3×10^4
24	6.1×10^5	4.2×10^5	7.1×10^4	5.5×10^4
28	-	8.0×10^5	-	8.1×10^4
32	-	1.8×10^6	-	2.8×10^5

*= Immediate after sun-dried salting

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) [48].

4. Conclusions

To the best of our knowledge this is the first study of the shelf life of salt treated sun-dried *Channa punctatus* and *Mystus tengra* fish stored at refrigeration temperature. Based on the presented data (TVB-N, FFA, pH values, and microbial load counts) the optimal shelf life of sun-dried salted fishes is approximately 24 to 32 months for refrigeration (4 °C) storage. The results also indicated that dried fish have greater nutritive value in terms of percentage crude protein for maintaining human health. Also the effect of heat and dryness associated with open sun-drying reduces the water activity of the fish thereby limiting microorganisms, a prerequisite for spoilage. Fish processing by the combination of salting and drying recommended for use because it gives relatively greater percentage protein and fat. Hence, it is suggested that low storage temperature (4 °C) and the traditional preservatives like NaCl (table salt) which are easily available and cheaper cost wise, along with traditional sun-drying process can be used by the fisher folk to arrest the growth of bacteria in fishes, thereby avoiding fish poisoning. Moreover, the information in the present paper will be useful for all the producers of dried fish and of practical value from a health perspective for populations who consume these fish, as well as scholars interested in fishery and aquaculture.

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