



Physico-Chemical, Microbiological and Sensory Changes in Sun-dried *Trichogaster fasciata* During Storage

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 07/04/2019 Accepted : 16/08/2019</p> <p>Keywords: <i>Trichogaster fasciata</i> Chemical composition Lipid oxidation Microbial load Storage time</p>	<p>Sun dried (<i>T. fasciata</i>) was stored with airtight polyethylene bags at room temperature to investigate the changes in physical, chemical, microbiological and sensory characteristics for 90 days. Sensory and physical (water reconstitution, pH) characteristics of dried <i>T. fasciata</i> showed that the product was acceptable up to 60 days of storage. Moisture content of <i>T. fasciata</i> was significantly increased from 15.06% to 17.80% during the storage period. No significant difference was observed in protein, lipid and ash content on dry matter basis during storage. However, amount of lipids of the dried fish was slightly decreased with the increasing of storage time. The pH value of dried <i>T. fasciata</i> was decreased significantly from 6.51 to 5.94 during the storage period. The peroxide value was increased from 13.84 to 27.87 meq/Kg of lipid. Similarly, acid value and conjugated diene of the lipids were increased significantly, and this result suggested that lipid oxidation occurred over this period of time. Microbial load was also increased from 1.13 to 8.37 log CFU/g with the increasing of storage time. Results of this study showed that the product was oxidized marginally during the storage period and suitable for human consumption up to 60 days.</p>

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Introduction

Fish is one of the most important sources of animal protein and other essential nutrients for the maintenance of a healthy body (Arannilewa et al., 2005; Öz, 2016; Öz et al., 2017) of which a significant part comes from dried fishes. Dried fish is an exceptionally favourite food item among Bangladeshi people. Moreover, the product is easily storable, transportable and marketable, and has a high consumer acceptance (Majumdar et al., 2017). The dried fish products are also highly preferred by the consumers due to their traditionally desirable taste, flavour and high level of ω -3 unsaturated fatty acids that have numerous health benefits for human (Li et al., 2003). Nowadays, people are more aware about their health and nutritional quality of food when purchasing it for their consumption (Hossain et al., 2008; Öz, 2018). Many studies have already been conducted on the chemical composition of

various dried marine fishes (Tawfik, 2009; Sutharshiny and Sivashanthini, 2011). However, chemical changes of dried freshwater fish during storage have not been studied so far.

Production of dried fish in Bangladesh is usually carried out by traditional sun drying in the open air. However, traditionally produced dried fish are not safe for human due to random use of different kinds of pesticides and extreme level of insect infestation (Bhuiyan et al., 2008). Netting and *neem* (*Azadirachta indica*) leaves are generally used to minimize this problem. Another major problem of dried fish product is microbial and fungal growth, which can be reduced by maintaining proper drying and packaging of the product. On the other hand, the storage time is shortened due to lipid oxidation of dried fish product as well as the product quality is reduced.

Previous result suggests that the degree of lipid oxidation is very high in the dried fish products available in Bangladesh (Majumdar et al., 2017). It has been reported that consumption of lipid oxidized products may cause adverse effects (aging, heart disease, brain dysfunction and cancer) on human body (Kinsella, 1987).

About 240 freshwater fish species are found in Bangladesh and most of them are small indigenous fish. Kholisa, *Trichogaster fasciata* is an important and widely accepted dried fish product in Bangladesh for its characteristic taste, flavour and low price. Moreover, dried *T. fasciata* contains comparatively higher amount of lipid among the other species, which is susceptible to oxidation. However, changes in quality especially oxidation of lipids during storage of this product has not been studied so far. Therefore, this study was conducted to evaluate the changes in physico-chemical, microbiological and sensory characteristics of sun dried *T. fasciata* during storage.

Materials and Methods

Collection of Fish Samples

The experiment was conducted in the Department of Fisheries Technology of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. Freshwater Kholisa (*T. fasciata*) (length 8.76±0.74 cm, and weight 13.36±2.81 g) were caught early in the morning and purchased from a fish market in Gazipur and were transported to the laboratory in an insulated ice box. Fish were preserved in ice before processing.

Sun Drying Method of Fish

In traditional sun drying method, collected fishes were gutted, washed and spread on the bamboo mats without any pre-treatment such as icing, salting etc. The fish was then dried under the sun for 5-6 days until the moisture content reduced to approximately 15-20%. The dried fish was packed by airtight polythene bag and stored at room temperature (24°C to 29°C). During the storage period, quality of the dried fish was investigated after every 30 days interval by determining the physical, chemical, microbiological and sensory aspects up to acceptable limit for human consumption.

Determination of Sensory Properties

Color, odor, texture, broken pieces and insect infestation of the traditionally produced sun-dried fishes were examined by sensory analysis according to the method described by Rasul et al. (2018). Sensory evaluation was performed in the separated sensory booths. The panel was composed of seven trained assessors (ages between 22 and 35 years) from the Department of Fisheries Technology of BSMRAU.

Evaluation of Water Reconstitution Behaviour

Water reconstitution behavior was investigated following the method described by Hasan et al. (2016) with slight modification. Briefly, about 8-10 g of sample was weighed and immersed into water at room temperature (24 to 29°C), 40°C and 60°C for 60 minutes. The soaked dried fish was taken out from water at every 15 minutes interval and reweighed. Per cent water reconstitution was calculated using the following formula;

$$\text{Water reconstitution (\%)} = \frac{W_r - W_i}{W_i} \times 100$$

Where,

W_i = Initial weight of dried fish

W_r = Final weight of rehydrated dried fish

Determination of pH

The dried fish (10 g) was homogenized with 100 ml of distilled water and the pH was directly determined using a pH meter (MeterLab PHM 310, China).

Analysis of Proximate Composition

The proximate composition such as moisture, crude protein, crude lipid and ash content were analyzed according to standard procedure given in AOAC (2006) methods.

Extraction of Lipid

Total lipid was extracted from dried fish by the Bligh and Dyer (1959) method. The extracted lipid was stored in chloroform at -20°C until further analysis.

Determination of Peroxide Value

Peroxide value was measured according to AOAC (2006) methods. About 0.5 g of total lipids was added to glacial acetic acid and chloroform solution (3:2, v/v) in a conical flask. Saturated potassium iodide (0.5 mL) was added to the mixture and kept in the dark for 10 minutes. Then distilled water (30 mL) and freshly prepared 1% starch (0.5 mL) were added to it. The solution was shaken and titrated with 0.01N sodium thiosulfate. The result was expressed as meq/Kg of lipid.

Determination of Acid Value

To measure acid value, 0.5 g of total lipid was mixed with ethanol and diethyl ether solution (1:1, v/v) and phenolphthalein was added to it. The mixture was then titrated with 0.01N potassium hydroxide. The result was expressed as mg KOH/g lipid (AOAC, 2006).

Determination of Conjugated Diene

To determine conjugated diene (CD), 0.1 g of total lipid was mixed with isoctane (5.0 mL). A spectrophotometer (UV-1601, Shimadzu, Japan) was used to measure the CD at 234 nm (Frankel et al., 1996).

Estimation of Microbial Load

Microbial load of the dried fish was estimated using aerobic plate count method. Physiological saline solution (0.85% NaCl) and plate count agar (Hi media, India) were prepared according to the methods given in Cowan and Steel's manual (Barraw and Feltheam, 1993).

Statistical Analysis

All the analyses were carried out by one-way ANOVA. All data are expressed as mean ± standard deviation (SD). Duncan's multiple range test (DMRT) was done to compare the means at 5% level of significance. All analyses were performed using statistical software, Statgraphics (StatPoint Inc., Virginia, USA) version 7.0.

Results and Discussion

Changes in Physical and Sensory Characteristics of *T. fasciata*

Changes in physical and sensory characteristics during storage of dried *T. fasciata* are presented in Table 1. Characteristic color and odor were observed in the dried fish up to 30 days of storage period. Texture of the dried fish was firm and flexible during this period. On the other hand, color was turned into slightly yellowish in the 60 days then it was turned into slightly brownish in the 90 days of storage period. Moreover, sensory scores also revealed that the dried fish products were in excellent quality up to 60 days of storage period (Table 2). In the dried fish, non-enzymatic browning reaction might be attributed to the changes in color, which progressed continuously during the storage period (Koizumi et al., 1992). Firmness of texture and odor of dried fish was lost in the 60 days of storage period. However, slightly rancid and dried fish odor was decreased in the 90 days of storage period. Infestation of insects and disrupted muscles of dried fish were not found in the sample over the storage time.

Water reconstitution Behaviour of Dried Fish

The water holding capacity of dried fish products were shown to increase with the increase of reconstitution temperature and time and the rehydration ability decreases with the increase of storage time (Figure 1). It was observed that 0 day dried *T. fasciata* were shown to hold 61.72% water after 60 minutes at room temperature, whereas 30 days, 60 days and 90 days dried products were shown to have 48.87%, 42.94% and 40.94%, respectively.

Similar trend was also observed when dried fish were soaked at 40 °C and 60 °C for 60 min. Sikorski et al. (1995), found that water uptake of dried fish is completed within 3 to 15 minutes. Dried fish with poor texture (tough, rubbery and compact structure of muscle) had poor water reconstitution ability (Connell, 1957; Lahiry et al., 1961).

Moreover, rehydration ability was proportionate to physical properties of dried fish (Reza et al., 2005, Rahman et al., 2017). The muscle structure of a species, reconstitution time and temperature were also influenced the rehydration ability of food products (Brennan et al., 1990; Nurullah, 2005; Akintunde, 2008).

Changes in pH Value of Dried Fish

The pH value of dried *T. fasciata* was significantly ($P < 0.05$) decreased from 6.51 to 5.94 during the storage period (Figure 2). The decreasing trend of pH in the dried fish is almost similar to that reported by Shah et al. (2009). They found that the pH value was decreased from 6.79 to 6.42 during drying of herring fillets and they also suggested that the decrease in pH might be due to the formation of free fatty acids produced by lipolysis as well as glycogen was decomposed to lactic acid during drying. Similar results were also observed by Nakagawa et al. (2007), whom found that pH was decreased from 6.50 to 6.35 during industrial soft drying of herring fillets.

Changes in Chemical Composition of *T. fasciata*

The moisture content of *T. fasciata* was significantly increased from 15.06% to 17.80% during the storage period (Table 3). The highest moisture content was found in the 90th day which might be due to moisture was absorbed during the storage period. The highest (63.57%) crude protein content was found in the dried fish stored for 0 day, while the lowest (62.97%) was observed at 90 days. However, no significant difference was found in the protein content during the storage period. Bhuiyan (1992) reported that the crude protein of dried *Harpodon nehereus* and *Johnius dussumieri* varied from 55.8% to 75.9%. Haque et al. (2013) found that the protein content of Silver pomfret and Bombay duck ranged from 41.16 to 54.32%, and 50.66 to 54.58%, respectively in traditional sun dried samples. They also reported that the solar dried fish contains comparatively higher amount of protein than the traditional sun dried samples.

Table 1 Changes in physical characteristics during storage of dried *T. fasciata*.

ST	Colour	Odour	Texture	II	BP	OQ
0	Slightly blackish and silvery (Characteristics colour)	Characteristic	Firm and flexible	Nil	Nil	Excellent
30	Slightly blackish and yellowish	Characteristic	Firm and flexible	Nil	Nil	Excellent
60	Slightly yellowish	Slightly decrease of dried fish odour	Some loss of firmness	Nil	Nil	Good
90	Slightly brownish	Slightly rancid and decrease of dried fish odour	Tough and some loss of elasticity	Nil	Nil	Average

ST: Storage time (days), II: Insect infestation, BP: Broken pieces, OQ: Overall quality

Table 2 Sensory score of *T. fasciata*.

ST	Colour	Odour	Texture	Insect Infestation	Overall Quality
0	1.38±0.48	1.60±0.18	1.63±0.29	0.00±0.00	Excellent
30	3.21±0.21	1.95±0.84	1.93±0.38	1.00±0.00	Excellent
60	4.30±0.17	3.85±0.31	3.63±0.32	1.00±0.00	Good
90	5.84±0.12	6.60±0.26	4.76±0.13	1.60±0.41	Average

ST: Storage time (days)

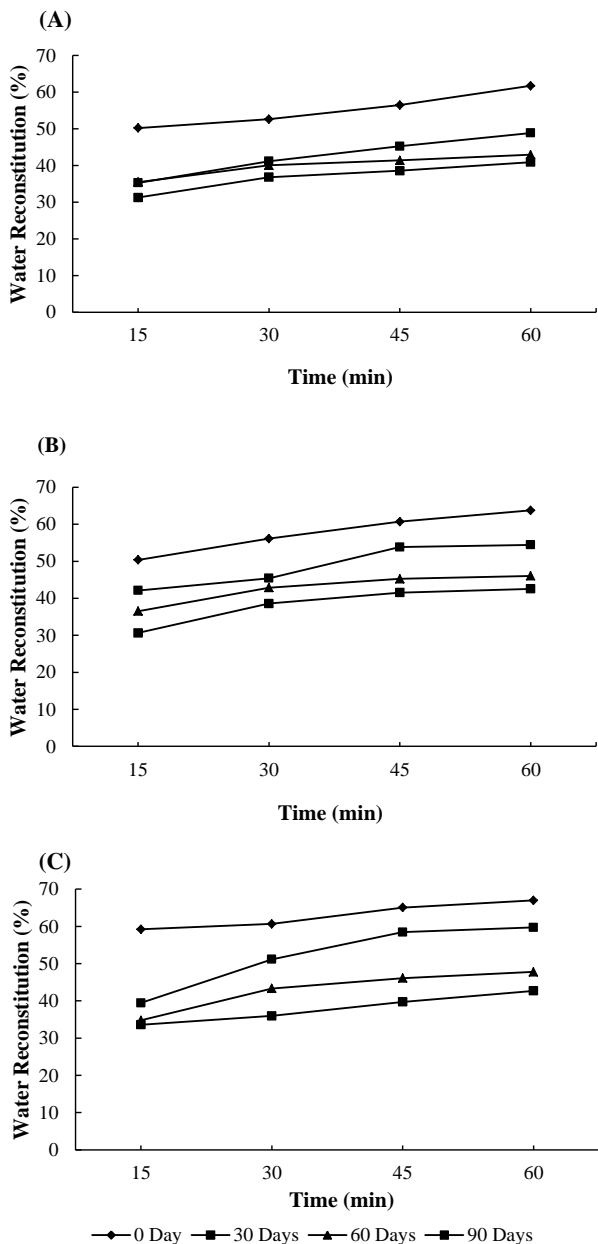


Figure 1 Water reconstitution of sun dried *T. fasciata* at (A) room temperature, (B) 40°C, and (C) 60°C during storage

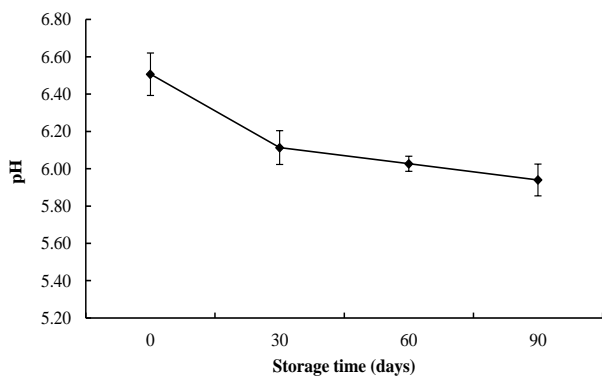


Figure 2 Changes in pH values during storage of dried *T. fasciata*

Crude lipid content of *T. fasciata* was decreased gradually from 12.97% to 12.07% by the 60 day of storage period and then it was slightly increased to 12.13% by the 90 day of storage period on dry matter basis. The lipid content was decreased from 13.80% to 11.50% in the pickled herring during 1 year of storage (Aro et al., 2005). Ash content of *T. fasciata* was varied between 22.73% and 23.17% during the storage period. Overall, there was an inverse relationship between moisture content and other chemical composition (crude protein, crude lipid, and ash content on fresh matter basis) of dried fish stored for 90 days.

Oxidation of Lipid During Storage of Dried Fish

Changes in lipid oxidation (peroxide value, acid value and CD) of dried *T. fasciata* during storage are shown in Table 4. Oxidation of lipid in fish muscle usually develops off-flavor and off-odor that ultimately affects the storage time (Ramanathan and Das, 1992). Peroxide value of *T. fasciata* was significantly increased from 13.84 to 27.87 meq/Kg of lipid during the storage period. The highest peroxide value was found at 90 days of storage period and the value was higher than the acceptable limit ($PV \leq 20$ meq/Kg fish lipid) for human consumption (Connell, 1995). Shah et al. (2009) reported that peroxide value of herring (*Clupea pallasii*) lipids (5.52-11.86 meq/Kg) significantly increased during drying. Moreover, lipids were degraded during the storage period, which had effect on fish flavor due to the presence of unsaturated fatty acids (Kawai and Sakaguchi, 1996; Alasalvar et al., 2005). Pacheco-Aguilar et al. (2000) also observed an increase in peroxide value of Monterey sardine muscle during storage at 0°C for 15 days. Moreover, lipid hydroperoxides are readily decomposed into a wide range of carbonyl compounds, hydrocarbons, ketones, and other materials that contribute to off flavor of food (Frankel, 1991).

In addition to peroxide value, acid value was determined to measure the degree of lipid hydrolysis in dried fish. Generally, fish muscle lipids are hydrolyzed by the activity of lipases and phospholipases, which releases free fatty acids (Pacheco-Aguilar et al., 2000). In this study, acid value of *T. fasciata* lipid was increased slightly from 12.05 to 13.75 mg KOH/g of lipid within 30 days of storage and then increased gradually up to 90 days (22.25 mg KOH/g of lipid) of storage period (Table 4). Since the amount of FFAs in fish muscle increases during storage, it is known that there is a close relation between the amount of FFAs and loss of fish freshness (Barassi et al., 1987). This result suggests that the formation of free fatty acid is one of the important factors to increase acid value in the dried fish product. In addition, these free fatty acids were further oxidized and produced secondary oxidation products, which generates characteristic taste and off-odor of fish and fishery products (Toyomizu et al., 1981).

A progressive formation of oxidized products can also be determined by measuring conjugated diene (CD) of lipid. Generally, after per-oxidation of lipids, polyunsaturated fatty acids are changed to conjugated double bonds ($C=C-C=C$) from the non-conjugated double bonds ($C=C-C-C=C$) (Gunstone and Norris, 1983). As a result, the optical density of 234 nm is increased (Zuta et al., 2007), which is an indicator of auto-oxidation in lipids (Weber et al., 2008). In this study, the CD value of dried *T. fasciata* lipid was increased with the increasing of storage time (Table 4). Significantly the highest

CD value was observed at 90 days of storage period. Changes in CD value of *T. fasciata* lipid might be due to the formation and decomposition rates of conjugated double bonds in lipids (Chaijan et al., 2006). A gradual increase of CD value was also observed in dorsal muscle of Atlantic pomfret stored in chilled condition for a prolonged period (Perez-Alonso et al., 2003).

Microbial Changes in *T. fasciata* During Storage

Changes in microbial load of dried *T. fasciata* during storage are depicted in Table 5. Total plate count of *T. fasciata* were 1.13 log CFU/g, 5.44 log CFU/g, 6.02 log CFU/g and 8.37 log CFU/g at 0, 30, 60 and 90 days, respectively. It was observed that the microbial load of *T. fasciata* increased with the increasing of storage period. It has been reported that acceptable limit of total plate count for fresh fish is 7 log CFU/g (Ojagh et al., 2010). Mansur et al. (2013) reported that the total bacterial count of some

sun-dried fishes was ranged between 1.84×10^4 and 5.3×10^6 CFU/g. The aerobic plate count of some marine dried fish products was ranged between 3.27 and 4.49 log CFU/g (Reza et al., 2009).

Conclusion

The physical and sensory characteristics of *T. fasciata* revealed that the product was excellent quality up to 30 days but acceptable up to 90 days of storage period. Moreover, the dried fish causes some chemical changes and at the same time lipid oxidation was progressed during storage. Microbial load also showed that the product was acceptable for human consumption up to 90 days of storage. Results of this study demonstrated that the dried fish was oxidized gradually over the storage time and safe for human consumption up to two or less than three months of storage.

Table 3 Changes in chemical composition (% fresh matter) of dried *T. fasciata* during storage¹.

ST	Moisture	Protein	Lipid	Ash
0	15.06±0.13 ^a	53.98±0.57 ^{a1} (63.55±0.67 ^d)	11.02±0.09 ^a (12.97±0.10 ^d)	19.68±0.38 ^a (23.17±0.44 ^d)
30	16.32±0.08 ^b	53.19±1.27 ^{ab} (63.57±1.52 ^d)	10.69±0.98 ^a (12.77±1.17 ^d)	19.02±0.34 ^a (22.73±0.41 ^d)
60	17.09±0.15 ^{bc}	52.31±0.53 ^b (63.09±0.64 ^d)	10.01±0.38 ^a (12.07±0.45 ^d)	18.95±0.34 ^a (22.86±0.40 ^d)
90	17.80±0.92 ^c	51.76±0.92 ^b (62.97±1.11 ^d)	9.97±0.91 ^a (12.13±1.10 ^d)	18.93±0.83 ^a (23.03±1.01 ^d)

¹Means with different characters (a, b, c denote fresh matter; d denotes dry matter) within a column indicates significant differences (P<0.05), ST: Storage time (days)

Table 4 Changes in peroxide value, acid value and CD of dried *T. fasciata* during the storage period¹.

ST	Peroxide Value (meq/Kg of lipid)	Acid value (mg KOH/g of lipid)	CD (OD 234 nm)
0	13.84±0.71 ^a	12.05±0.40 ^a	3.369±0.022 ^a
30	16.23±0.64 ^b	13.75±0.33 ^b	3.394±0.065 ^a
60	19.17±0.76 ^c	16.24±0.64 ^c	3.437±0.031 ^a
90	27.87±0.97 ^d	22.25±0.75 ^d	3.542±0.040 ^b

¹Means with different characters within a column indicates significant differences (P<0.05), ST: Storage time (days)

Table 5 Changes in microbial load (CFU/g) of dried *T. fasciata* during storage.

Storage time (days)	Microbial load (log CFU/g)
0	1.13
30	5.44
60	6.02
90	8.37

Conflicts of interest

The authors declare that they have no conflict of interest.

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