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Effects of frozen storage on the proximate composition and formaldehyde content in some selected fish from three different sources of southern Bangladesh

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Abstract. The study was conducted in aims to investigate the effects of frozen storage and cooking conditions on proximate compositions and formaldehyde content (FA) in some selected fish from three different sources in Bangladesh. Proximate composition in fresh and final frozen samples was determined by standard AOAC method and FA content in fresh, frozen stored, and cooked samples was determined by spectrophotometric method. Among the studied fishes, marine fish contained higher protein (except Rita), lipid, and ash followed by estuarine and culture fish samples. Protein, moisture and ash content decreased and lipid content increased significantly ($p<0.05$) during frozen storage for all samples and sources. The FA was lower in cultured fish samples compared to that of the river and marine fish samples, both at fresh and end of frozen storage. At fresh condition, FA content in all samples ranged from 0.41 to 0.71μg/g, 0.51 to 0.89μg/g, and 0.73 to 1.69μg/g which increased to 0.95 to 2.11μg/g, 1.74 to 1.95μg/g, and 3.22 to 5.20μg/g at end of the storage period, respectively ($p<0.05$). Further, FA content significantly decreased after cooking in all the fish samples ($p<0.05$). However, irrespective of fish species and sources, the FA content was higher than WHO recommended value (0.2 μg/g). The study findings revealed that longer frozen storage of fish could be a public health concern to the consumers.

Keywords: Frozen storage, Proximate composition, Formaldehyde content, Spectrophotometric method

Introduction

Recent trends in global food production, preparation, processing and distribution are creating an increasing demand for food safety research to ensure safe food supply globally (Bianchi *et al.* 2007, Norliana *et al.* 2009, Hoque *et al.* 2016, Hoque *et al.* 2018). The supply of quality foods mainly interrupted by the different types of physical, chemical, and biological hazards present in food (Erondu and Anyanwu 2005, Bianchi *et al.* 2007, Ahmed *et al.* 2012, Chiou *et al.* 2015, Handford *et al.* 2016). Among the different chemical contaminants in food, great attention has been paid towards volatile toxic aldehydes like formaldehyde (FA) (Bianchi *et al.* 2007; Norliana *et al.* 2009, Zhang *et al.* 2015). In fish, FA could be accumulated naturally upon post-mortem changes and during frozen storage from the enzymatic reduction of trimethylamine-N-oxide (TMAO) to dimethylamine and FA (Sotelo 1995, Bianchi *et al.* 2007) as shown in Fig. 1.

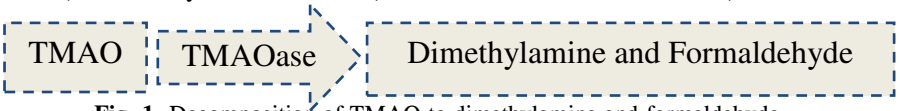

Fig. 1. Decomposition of TMAO to dimethylamine and formaldehyde.

FA contents in fish vary from species to species, location, and other factors (Hoque *et al.* 2018). Marine fish contained higher TMAO contents indicating higher FA (Tsuda *et al.* 1988, Jiang *et al.* 2006) than freshwater fish. Fish frozen is a common scenario as it inhibits the microbial, enzymatic, and autolytic activity in fish lowering the body temperature thus allowing the fish to preserve for a period. But, during frozen storage leaching, drip loss, denaturation, and degradation of protein and other biochemical components caused lower food value (Benjakul *et al.* 2003, Emire and Gebremariam 2010). Also, the duration of frozen storage has a great impact to increase muscle FA content to a great extent (Sotelo *et al.* 1995, Bianchi *et al.* 2007, Zhang *et al.* 2015). Allow dose of FA can cause pain, vomiting, lethargy whereas a large doses a source of death for consumers (Zhang *et al.* 2015). Consumption of fish containing FA could increase the risk of cancer or uncontrolled cell growth on different parts of consumers like the stomach, lung, and also on the respiratory system through inhalation (Hoque *et al.* 2016).

Frozen storage condition and duration affects its proximate composition and FA formation, content, and food value in consequences on the health of the consumers. In Bangladesh, limited study has been conducted to see the effects of frozen storage on the proximate composition (Mazrouh 2015, Mahboob *et al.* 2019, Adel *et al.* 2019). However, no study conducted on the effects of frozen storage on the FA content in fish from different sources. Thus, addressing this problem, present study has been directed based on proximate composition and FA content in different fish from three sources of water bodies in the Southern Bangladesh and to reveal the changes of nutritional properties and FA content both in fresh, cooked and frozen storage condition.

Materials and Methods

***Fish samples collection*:** Fish samples from three different sources i.e., PSTU fish culture pond (Pangas, *Pangasius pangasius*; Koi, *Anabas testudineus* and Catla, *Gibelion catla*; Paira river (Ramchos, *Thryssa purava*; Lalpoa, *Otolithoides pama* and Tulardadi, *Sillaginopsis panijus*) and Kuakata marine water (Rita, *Rita rita;* Sardine, *Sardinella longiceps* and Tuna, *Sarda orientalis*) were collected for proximate composition and FA determination in fresh and frozen storage (Walton, W2D-1H5, Bangladesh) condition.

***Determination of proximate composition*:** Nutritional properties of fishes under the designation of moisture, protein, lipid, and ash content were determined by AOAC (2000). Moisture, protein, lipid, and ash were determined by using a hot air oven (Kendro M110, Germany), Kjeldahl apparatus (Buchi CH-9230, Switzerland), soxhlet apparatus (SRICO SMX 100) and muffle furnace (Cole-Parmer EW-33858-80, India), respectively.

***Determination of FA content*: *TCA and Nash's Reagent preparation*:** 6% w/w TCA (Trichloroacetic acid) and Nash's reagent was prepared followed by Jaman *et al.* (2015).The verified fish samples are subjected to adjust the pH within a range from 6.0 to 6.5. For that, a 0.1 N KOH and 0.1 N HCl have been prepared. All reagents were stored in the dark-glass reagent bottle for all time to maintain its quality.

***Fish sample preparation for determination of FA*:** FA content was determined by the spectrophotometric method. Fish samples were cut into small pieces under verification. Each of

the fish samples weighted 30g by electronic balance. Then fish flesh was taken into a blender and for homogenization, blended for 10 min. Then a 60ml of 6% w/w TCA was added for extraction of FA from the fish flesh. Whatman number 1 filter paper was used to filter the extract solution to remove unwanted particles. Though the addition of TCA reduced the pH value of the sample, thus the pH was adjusted from 6.00 to 6.50 using 0.1 N KOH and 0.1 N HCl. From the pH adjusted extract, 5 ml sample was taken in a 50 ml volumetric flask and kept in a freeze at a normal temperature for 1hr. After the stipulated time, the sample taking out, and 2 ml Nash's reagent was added as an indicator. Then, the sample was heated at 60 ^{0}C using a water bath for 30 mins. The absorbance of the sample in the cuvette was measured at 415 nm immediately by UV/Vis spectrophotometer. Triplicate absorbance was made for each sample and recorded for further calculation. Then the sample reading was placed in the standard curve for the calculation of the FA content of the sample. To study the effect of cooking on FA content, the frozen stored samples were further cooked at 100^{0}C for 15 minute and determined the FA content following the same method.

***Data analysis*:** Experiments were run in triplicates. Data were subjected to analysis of variance (ANOVA) and mean comparisons (T-test) were carried out by Duncan's multiple range test. The analysis was performed using the SPSS package (SPSS 16.00 for windows, SPSS Inc., Chicago, IL, USA) (Gomez and Gomez, 1984).

Results and Discussion

***Proximate composition*:** The result of moisture, protein, lipid, and ash content of fresh and frozen stored samples from three different sources are shown in Table I. In fish samples from cultured source, fresh *P. pangasius* showed higher moisture content (78.20%) which was reduced to 75.11% in the frozen stored sample. Similar moisture content was found between *G. catla* and *P. pangasius* ($p>0.05$). *A. testudineus* showed lower moisture content in both fresh (75.81%) and frozen stored (74.52%) conditions compared to other fish from cultured pond source. Moisture content in river fishes, *T. purava*, *S. panijus* and *O. pama* were 75.69, 79.32, and 74.26% which significantly ($p<0.05$) decreased to 73.72, 77.26 and 73.29%, respectively at the end of the frozen storage period. Among the marine fish samples, fresh *R. rita* represents maximum moisture content (75.87%) followed by *S. longiceps* (73.78%) and *S. orientalis* (71.65%). After the frozen storage period, moisture content was increased for *R. rita*, (76.18%) while decreased for *S. orientalis* (70.69%) and *S. longiceps* (72.76%). Among all the species used in this experiment, the higher moisture content was found for *S. panijus* both fresh (79.32%) and frozen storage (77.26%) while the lower value for marine *S. orientalis* (from 71.65 to 70.69%). Moisture content was found low for all species (except *R. rita*). The decrease in moisture content might due to sublimation of surface water of meat in the freezer (Mazrouh 2015).

Protein content in culture fish was ranged from 16.70 to 18.23% and 15.44 to 16.51% in fresh and frozen stored samples, respectively. Compare to other fish species from cultured source, fresh *A. testudineus* fish had significantly higher protein content (18.23%) which was significantly decreased (16.51%) after the frozen storage ($p<0.05$). On the other hand, all three fish from the estuarine river source showed similar protein content ($p>0.05$). In fresh

condition, the protein content was 18.89, 18.17, and 18.07% for *T. purava*, *S. panijus* and *O. pama* and which was decreased to 17.82, 16.99 and 16.91%, respectively ($p>0.05$).

Among the marine fish, protein content was found higher in *S. orientalis* (24.71%) followed by *S. longiceps* (20.80%) and *R. rita* (16.75%) which decreased to 22.53, 20.06, and 16.32%, respectively, after the frozen storage period. When protein content was compared among all the species, *S. orientalis* showed significantly higher protein content (24.71%) at fresh fish which significantly reduced to 22.53% at the end of frozen storage ($p<0.05$). In general, lower protein content was observed in the frozen stored sample than the fresh sample. Irrespective sources of all fish samples resulted in lower protein content in frozen stored fish sample than fresh fish sample might due to leaching, drip loss, degradation and denaturation of protein during frozen storage. Temperature abuse during the frozen period of fish could affect the migration of water vapor from the product to the surface of the container (Emire and Gebremariam 2010) thus allow the lowering of protein content. Leaching of protein was a common phenomenon during the cold storage period (Gandotra *et al.* 2012, Aberoumand 2013). Slow freezing and freezing temperature were reported as two major factors for protein denaturation during the frozen time (Emire and Gebremariam 2010). The post rigor stage, fish undergo rapid protein degradationas a result of endogenous bacterial enzymatic activities. Protein degradation is correlated with the destruction of its secondary, tertiary and quaternary structures which ultimately convert the proteins to the simple polypeptide chains (Careche and Li-Chan 1997).

In the culture pond, the higher lipid content (3.04%) was found for *P. pangasius* followed by *A. testudineus* (2.24%), and a lower-valued was recorded for *G. catla* (1.36%). After frozen storage, a significant increase in lipid content was found for *P. pangasius* (from 3.04% to 7.19%), *A. testudineus* (from 2.24 to 3.22%), and *G. catla* (1.36 to 2.06%) ($p<0.05$). Estuarine river fish showed a similar result of lipid content both at fresh and frozen storage conditions ($p>0.05$). Fish from marine sources showed that fresh *S. orientalis* fish had higher lipid content (7.48%) than that of *S. longiceps* (6.92%) and *R. rita* (4.81%). After frozen storage, lipid content increased to 7.81%, 8.63% and 6.16% for *S. orientalis*, *S. longiceps* and *R. rita*, respectively ($p<0.05$). Among all the species from different sources, fresh marine *S. orientalis* had higher lipid content (7.48%) while marine *S. longiceps* showed higher lipid (8.63%) at the end of frozen storage ($p<0.05$). Variation in lipid between fresh and frozen stored different fish samples might due to different biochemical changes occurred during frozen storage.

In the cultured species, higher ash content was found for *P. pangasius* (1.81%) and lowered value for *A. testudineus* (0.96%). A significant difference in ash content was found for all cultured samples ($p<0.05$). Further, the ash content of estuarine species was 1.44%, 1.74%, and 0.99% for *T. purava*, *S. panijus* and *O. pama*, respectively ($p<0.05$). A slight decreased in ash content was observed for estuarine species from fresh to frozen storage. On the contrary, the average ash content of fresh marine fish was from 1.14 to 1.85% and frozen stored was ranged from 1.03 to 1.18%, respectively (Table I). Fresh *R. rita* had higher ash content (1.85%) compared to other species from three separate water bodies while frozen *S. panijus* showed higher ash content (1.57%) among all the frozen samples. Temperature in fluctuation during frozen storage resulting fluctuation of fish body temperature could cause moisture and ash content reduction during frozen storage. Gandotra *et al.* (2012) reported that during frozen

Table I. Proximate composition (% of wet basis) of fish from different sources under fresh and frozen stored condition

Sources of fish	Species	Fresh				Frozen stored (120 days)			
		Moisture	Protein	Lipid	Ash	Moisture	Protein	Lipid	Ash
Culture pond	*Gibelion catla*	78.01 ± 2.08^{aAtu}	16.70 ± 0.70^{bAx}	1.36 ± 0.12^{cAy}	1.13 ± 0.11^{bAvw}	75.12 ± 1.06^{aAv}	15.44 ± 0.69^{aAxw}	2.06 ± 0.29^{cBz}	0.97 ± 0.05^{aAv}
	Pangasius pangasius	78.20 ± 0.6^{baAt}	16.99 ± 0.30^{bAx}	3.04 ± 0.25^{aAw}	1.81 ± 0.06^{aAt}	75.11 ± 0.34^{aBv}	15.48 ± 0.54^{aAxw}	7.19 ± 0.28^{aBv}	1.01 ± 0.03^{aBu}
	Anabas testudineus	75.81 ± 0.27^{caAut}	18.23 ± 0.47^{aAvw}	2.24 ± 0.30^{bAx}	0.96 ± 0.04^{cAxw}	74.52 ± 0.53^{aBvw}	16.51 ± 0.50^{aBwx}	3.22 ± 0.67^{bBxy}	1.15 ± 0.12^{aBu}
Estuarine river	*Thryssa purava*	75.69 ± 0.47^{bAut}	18.89 ± 0.18^{aAv}	2.86 ± 0.11^{aAw}	1.44 ± 0.03^{bAu}	73.72 ± 0.27^{bBwv}	17.82 ± 0.43^{aBv}	2.99 ± 0.20^{bAxy}	1.15 ± 0.05^{bBu}
	Sillaginopsis panijus	79.32 ± 0.68^{aAt}	18.17 ± 0.67^{aAvw}	2.36 ± 0.24^{cAx}	1.74 ± 0.08^{aAt}	77.26 ± 0.46^{aBt}	16.99 ± 0.20^{bBw}	2.61 ± 0.18^{bAyx}	1.57 ± 0.10^{aAt}
	Otolithoides pama	74.26 ± 0.45^{cAv}	18.07 ± 0.14^{bAwv}	3.32 ± 0.11^{aAw}	0.99 ± 0.04^{cAwvx}	73.29 ± 0.31^{bBw}	16.91 ± 0.21^{bBw}	3.09 ± 0.21^{aAx}	0.95 ± 0.03^{cBv}
Marine	*Sarda orientalis*	71.65 ± 0.36^{cAw}	24.71 ± 0.06^{aAt}	7.48 ± 0.27^{aAt}	1.14 ± 0.03^{bAv}	70.69 ± 0.07^{cBx}	22.53 ± 0.10^{aBt}	7.81 ± 0.12^{bAu}	1.03 ± 0.06^{aBu}
	Rita rita	75.87 ± 1.04^{aAut}	16.75 ± 0.75^{cAx}	4.81 ± 0.63^{cAv}	1.85 ± 0.05^{aAt}	76.18 ± 0.60^{aAu}	16.32 ± 0.61^{cAxw}	6.16 ± 0.42^{cBw}	1.26 ± 0.17^{aBu}
	Sardinella longiceps	73.78 ± 0.27^{bAv}	20.80 ± 0.51^{bAu}	6.92 ± 0.20^{bAu}	1.22 ± 0.13^{bAv}	72.76 ± 0.83^{bBw}	20.06 ± 0.12^{bBu}	8.63 ± 0.18^{aBt}	1.18 ± 0.13^{aAu}

Means $\pm$ standard deviation (n=3).

Different small alphabet (a-c) as superscript within the same column indicate a significant difference ($p<0.05$) among the 3 species from the same source; different capital alphabet (A-B) as superscript within the same row indicate a significant difference ($p<0.05$) between same constituent and same species; different small alphabet (t-z) as superscript within the same column indicate a significant difference ($p<0.05$) among the 9 species from the 3 different sources.

storage ash and moisture content decreased significantly. In general, during the frozen storage period, decreased moisture content upon frozen storage in all fish samples might due to dehydration and drip loss occurred in the fish sample. Further, an inverse relationship was found between the moisture, and lipid content. Thus, during frozen storage lipid content significantly increased with decreasing moisture, protein, and ash content. Similar results were also available in the literature (Emire and Gebremariam 2010, Beklevik *et al.* 2005). In general, the condition of fish at the time of frozen storage relates to the nutritional status or the stage of spawning that could affect both chemical compositions of frozen fish. The present result indicated that fish of marine sources represented higher protein (except *R. rita*), lipid and ash content followed by estuarine river, and culture pond fish species. Thus, the present findings indicated that proximate compositions of fish were varied widely based on species and location.

***FA content*:** In fresh *G. catla*, the FA concentration was 0.63μg/g and similar FA content was observed during 14 days frozen storage ($p>0.05$). With increased frozen storage time, a significant higher FA content was 0.80 and 0.95μg/g at 30 and 120 days storage time, respectively. In the case of *P. pangasius*, with increasing storage time significant increase in FA concentration was observed during the whole storage period ($p<0.05$) (Table II). Furthermore, *A. testudineus* showed non-significant differences in FA content (0.71-0.76μg/g) from 0 to 14 days of frozen storage time ($p>0.05$). After 30 and 120 days of frozen storage period, the FA were 0.99 and 1.53μg/g, respectively ($p<0.05$). Among the three species, in fresh condition, *P. pangasius* had the lowest and *A. testudineus* had the highest FA content ($p<0.05$). But, with increased frozen storage time from 7 to 120 days, *P. pangasius* revealed the highest (2.11μg/g) and *G. catla* lowest (0.95μg/g) FA content ($p<0.05$). Among the three species, *G. catla* represented lower FA content compared to other fish species at any day storage time (Table II).

Table II. FA content in fish from different sources during frozen storage conditions

Sources of fish	Fish species	FA (μg/g)				
		0 day	7 days	14 days	30 days	120 days
Culture pond	*Gibelion catla*	0.63 ± 0.02^{cBx}	0.66 ± 0.05^{cBx}	0.64 ± 0.05^{cBy}	0.80 ± 0.08^{bCx}	0.95 ± 0.02^{aCy}
	Pangasius pangasius	0.41 ± 0.02^{eCz}	0.47 ± 0.03^{dCy}	0.61 ± 0.04^{cBy}	1.95 ± 0.06^{bAv}	2.11 ± 0.15^{aAw}
	Anabas testudineus	0.71 ± 0.01^{cAw}	0.74 ± 0.03^{cAw}	0.76 ± 0.04^{cAx}	0.99 ± 0.05^{bBw}	1.53 ± 0.12^{aBx}
Estuarine river	*Thryssa purava*	0.51 ± 0.03^{cBy}	0.67 ± 0.06^{bBxw}	0.66 ± 0.06^{bBy}	0.76 ± 0.10^{bCx}	1.74 ± 0.05^{aAw}
	Sillaginopsis panijus	0.89 ± 0.01^{cAv}	0.96 ± 0.05^{bAv}	1.15 ± 0.13^{bAw}	1.07 ± 0.09^{bBw}	1.94 ± 0.13^{aAw}
	Otolithoides pama	0.53 ± 0.01^{dBy}	0.76 ± 0.06^{cBxw}	1.12 ± 0.11^{bAw}	1.83 ± 0.10^{aAv}	1.95 ± 0.11^{aAw}
Marine	*Sarda orientalis*	1.69 ± 0.01^{eAt}	1.99 ± 0.07^{dAt}	2.36 ± 0.08^{cCv}	3.27 ± 0.08^{bAt}	5.20 ± 0.09^{aAt}
	Rita rita	0.73 ± 0.02^{eCw}	0.95 ± 0.02^{dCv}	2.62 ± 0.06^{cAt}	2.93 ± 0.05^{bBu}	3.22 ± 0.08^{aCv}
	Sardinella longiceps	1.65 ± 0.02^{dBu}	1.72 ± 0.05^{dBu}	2.49 ± 0.05^{cBu}	3.02 ± 0.05^{bBu}	3.96 ± 0.11^{aBu}

Means $\pm$ standard deviation (n=3); Different small alphabet (a-c) in the same row represent the significant difference ($p<0.05$) in FA content in same species; different capital alphabet in the same column represent the significant difference ($p<0.05$) in FA content in 3 different fish species from the same source; different small alphabet (t-z) as superscript within the same column indicate significant differences among the 9 species from the 3 different sources ($p<0.05$). 0, 7, 14, 30, and 120 are indicating freezing day (s).

Hoque *et al.* (2016) and Jaman *et al.* (2015) reported that cultured samples had a small quantity of naturally occurring FA in their muscle having values ranging from 1.45 to 2.60μg/g.

In *T. purava*, FA content was 0.57, 0.67, 0.66, 0.76 and 1.74μg/g at 0, 7, 14, 30 and 120 days frozen storage time, respectively. Lower and higher FA content was found in fresh and 120 days of storage fish, respectively. During frozen storage time at 7, 14, and 30 days non-significant differences in FA contents was found for *T. purava* ($p>0.05$). On the other hand, the FA content was 0.89μg/g in fresh *S. panijus*. FA contents were found as increasing trends from days 7 to days 120 which ranged from 0.96 to 1.94μg/g, respectively. Non-significant difference in FA contents were observed at 7, 14 and 30 days storage time ($p>0.05$). On the other hand, the lower and higher values for FA content were 0.53 and 1.95μg/g in *O. pama*, which was calculated during fresh (0 day) and 120 days of storage conditions, respectively. With increasing frozen storage time from days 7 to 120 days, FA content was increased ($p<0.05$). Non-significant differences in FA contents were found at 30 and 120 days storage time ($p>0.05$). Among the three estuarine river fish samples, *T. purava* had lower FA content followed by *O. pama* and *S. panijus*. At the end of the storage period (120 days), the non-significant difference in FA content was found among fish samples ($p>0.05$) which was significantly higher than the value at 0 day ($p<0.05$) (Table II). Xu and Rogers (1995) found 0.563μg/g of FA for river catfish at the fresh conditions which also a similar result of the present study. FA content in three marine water fish species under fresh and frozen storage conditions is also determined (Table II). The result revealed that with the increasing storage time from 0 to 120 days, asignificant increase of FA content was found for both *S. orientalis* and *R. rita* ($p<0.05$). Compared among three marine fish species, *S. orientalis* had higher FA content both at fresh (0 day) and after frozen storage time (120 days) which valued 1.69μg/g and 5.20μg/g, respectively. However, at the same storage time (0 day and 120 days) *R. rita* had the lowest FA content (0.73 and 3.22 μg/g) than other marine fish species. Another marine sample like *R.rita* had FA content 0.73μg/g at initial period. *S. longiceps* had a FA content 1.65, 1.72, 2.49, 3.02, and 3.96μg/g at 0, 7, 14, 30, and 120 days frozen storage period, respectively. *S. longiceps* showed a non-significant difference in FA content between 0 day (fresh) and 7 days frozen storage ($p>0.05$). In general, *S. orientalis* had higher FA content both in fresh and end of frozen storage period followed by *S. longiceps* and *R.rita*. Frozen marine fish had higher natural FA in their muscle (ranged from 1.55 to 3.90μg/g) (Jaman *et al.* 2015) which supports the present results.

In general, the present result revealed that FA content in fish varies from species to species, location to location, under storage condition, and most importantly based on red muscle content as well (Hoque *et al.* 2018). The variation of FA contents observed in present study among the fish samples could be described based on TMAO content. TMAO content has a direct link with red muscle i.e., higher red muscle fish have higher TMAO content. Marine fish have a higher level of TMAO content due to the presence of red muscle (Tsuda *et al.* 1988, Jiang *et al.* 2006, Jaman *et al.* 2015) which is a similar result of the present study (Fig. 1). During frozen storage of fish at low temperatures (below 0), TMAO content is increased with increasing frozen storage time. Bianchi *et al.* (2007) reported that the FA content increased to134% after 6 days of icing, thus confirm the production of FA compound at temperatures around 0^0C. After 3 months of home-frozen, the FA content of Mullet was 3.38μg/g and after 4 months, the FA of Mackerel was 2.6 μg/g (Bianchi *et al.* 2007) which support the present results. Xu and Rogers (1995)

documented that the endogenous FA of several fish species was ranged from 01-31.8μg/g. FA content in fresh marine samples was in the range of 0.38 to 15.75μg/g (Noordiana *et al.* 2011).

Effects of cooking on FA content: In Bangladesh, fish are generally eaten after cooking, thus all the samples were also analyzed just after boiling to evaluate the effect of cooking on the FA content of frozen fish (120 days frozen stored samples). The result revealed that after cooking, FA content was decreased for all fish samples to a great extent irrespective of sources. Among the culture pond fish samples, after cooking *P. pangasius* had a significant reduction in FA (from 2.11 to 0.03μg/g) (Fig. 2). *A. testudineus* had higher FA (0.38μg/g) under cooking conditions compare to other species from the same sources. On the other hand, FA content of estuarine river water fish samples showed a significant reduction when comparing 120 days of frozen samples before and after cooking. At 120 days, FA content in frozen *T. purava*, *S. panijus* and *O. pama* were 1.74, 1.94, and 1.95μg/g which reduced to 0.59, 0.49 and 0.52μg/g, respectively after cooking ($p<0.05$). Further, marine water fish samples also had a similar trend to reduce FA content under cooking conditions.

After frozen cooked, *R. rita* showed a lower value of FA content (0.22μg/g) in comparison with other species from the same source (Fig. 2).

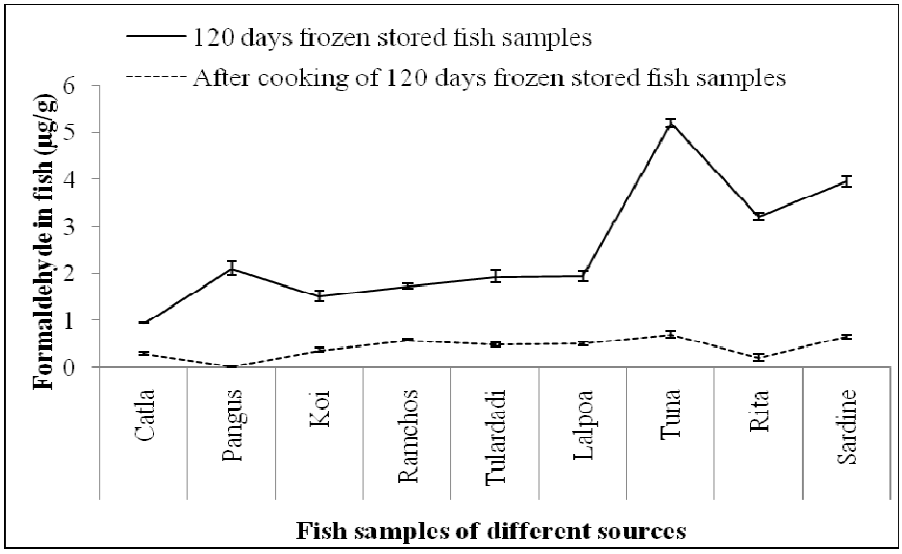

Fig. 2. Comparison of FA content in frozen and cooked fish samples after 120 days storage period (straight line indicates frozen fishes and dotted line indicates cooked samples after 120 days of storage time).

In general, frozen storage of fish caused for an increase in FA content significantly (Table II) which further decreased due to cooking. The present findings were similar to the study of Hoque *et al.* (2016). Bianchi *et al.* (2007) also reported a decrease in the FA content from 10.3 μg/g to 7.1 μg/g for roasting of *S. longiceps* fish. The same study also found reduced FA content in cod and hakes due to boiling and roasting. All frozen stored fish samples in this experiment contained much higher FA content compared with the recommended value set by the United States Environmental Protection Agency (0.2μg/g) and WHO (0.15μg/g) (Zhang *et al.*

2015). However, fresh and frozen stored cooked fish samples had slightly higher FA content than the recommended value. These elevated concentrations of FA in fishes may be antigenic and toxic or fatal to consumers (Wooster *et al.* 2005). Therefore, the study recommended that fresh i.e., minimum frozen storage duration and properly cooked fish could minimize the public health risk caused by hazardous chemicals like FA formed in fish.

The quality aspects (proximate composition and FA content) of fish from three different sources were analyzed under fresh and frozen stored condition. Losses of protein and moisture content during frozen storage were common scenarios for all the fish species studied here due to dehydration, drip loss, and denaturation of the protein. In fresh samples, marine fish showed higher FA content compare to estuarine river and cultured samples. The experiment also proved that among the frozen samples, a significant increase in FA content was found for all samples and the evidence was most prominent in marine fish species. However, the cooking process could decrease the FA content to some extent irrespective of fish species and sources. In conclusion, frozen storage of fish could alter the nutritional properties and increased health hazardous chemicals especially FA which could be highly concern in terms of food safety aspects.

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