

Research Article

Changes in Quality Attributes and Sensorial Properties of Mullet Fish (*Mugil cephalus*) During Freezing and Their Cold Smoked Products

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Abstract

Changes in quality criteria of frozen mullet fish obtained from two different farms, A and B located in Fayoum governorate, Egypt during August 2015 and their cold smoked products at 0, 60, 120 and 180 days of frozen storage at -18°C were determined in this work. pH values of fresh fish samples were 6.4 and 6.22 for farm A and B decreased to 6.29 and 6.12 after 6 months of frozen storage respectively. While after cold smoking pH values were increased in all samples. TVB-N values of fresh fish were 8.68 and 8.4 mg/100g increased to 10.92 and 10.36 mg/100g after 180 days of frozen storage at -18°C for farm A and B respectively. TBA values of fresh fish were 0.10 and 0.17 mg/kg increased to 1.38 and 1.43 mg/kg after 180 days of frozen storage for farm A and B samples. These values were increased after smoking in all samples. Overall acceptability scores were 8.56 and 8.87 for cold smoked samples prepared from raw samples after 180 days of frozen storage.

Keywords: Smoking; TVB-N; TBA; Freezing

Introduction

Fish is one of the most highly perishable commodities and the public has always required continuous reassurance about its quality.

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The spoilage process begins immediately after capture. Harmless, natural spoilage bacteria on the skin and in the slime of the fish quickly invade the muscle blocks. In addition, at high ambient temperature, fish quality deteriorates very rapidly while low temperature storage is the method of preservation recommended to retard microbial spoilage of fish [1,2]. Global fish production has grown steadily in the last five decades with food fish supply increasing at an average annual rate of 3.2 percent, outpacing world population growth at 1.6 percent. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 20.1 kg in 2014. This impressive development has been driven by a combination of population growth, rising incomes and urbanization, and facilitated by the strong expansion of fish production and more efficient distribution channels [3].

Fresh fish is a highly perishable product due to its biological composition. This condition is favorable for the growth of microorganisms, which leads to eventual spoilage. Provoking loss of essentially fatty acids, fat-soluble vitamins, protein functionality and production of biogenic amines and formation of off-odors should be considered. Therefore, fishes need to be preserved, because they get spoiled very quickly even in temperate regions [4,5,6,7,8]. The concept of frozen storage relies on the lowering of the product temperature to slow down spoilage so that the thawed fish can retain the freshness for longer time [9]. It is a usual method to preserve commercial fish since it stops chemical and microbiological degradation, and is an excellent method of preserving the organoleptic attributes of fish flesh during prolonged periods of time [10]. Freezing and frozen storage have largely been employed to retain the sensory and nutritional properties of fish although enzymatic and non-enzymatic rancidity is known to develop strongly under such conditions [11]. Smoking is one of the oldest methods that used in fish preservation; which inhibited fat oxidation, bacterial growth and may extend the shelf life of the final product. The technology of fish smoking includes salting and the treatment with smoke. Salting is used to provide a salty flavor and to impart storage stability, preservation properties of smoking treatment are mainly due to the partial drying trend and the precipitation of aliphatic and aromatic vapors on fish surface [12]. The quality of smoked fish is affected by raw material [13,14], salting method, brining concentration [15,16,17], processing conditions [18], composition of smoke [19] smoking method [20], smoke agents [21] and storage conditions.

The main objectives of the current study were Investigate the effect of irrigation resource; Al-Battsdrain and El-Wadi drain of fish farms located at Fayoum Governorate, frozen storage conditions at -18°C for 180 days and cold smoking on the biochemical quality criteria and sensorial attributes of fresh mullet (*Mugil cephalus*) stored for 0,60,120 and 180days.

Materials and Methods

Fish samples

Fresh mullet fish samples (*Mugil cephalus*) about 20 kg were purchased from two fish farms (A and B). The main resources of irrigation

water were agricultural discharge for A (Al-Batts drain) and B (El Wadi drain) during August 2015 at El-Fayoum governorate, Egypt. They were transported immediately to Fish Processing and Technology Lab, Shakshouk Station for Water Resource, National Institute of Oceanography and Fisheries (NIOF), Egypt. Average of weight $525 \text{ gm} \pm 25 \text{ gm}$ and length $36 \pm 1 \text{ cm}$ for raw samples from Farm A (Al-Battsdrain) and $526.6 \pm 25.1 \text{ g}$ and $38 \pm 1 \text{ cm}$, respectively for raw samples from Farm B (Agricultural discharge). After that, fish samples were carefully washed with tap water, glazed, packed in polyethylene bags and stored at -18°C for 180 days.

Smoking process

After 180 days of raw mullet fish frozen storage, Fish samples (from A and B farm) were thawed at 4°C then soaked in 10% brined solution (Sodium chloride) for two hrs., rinsed with tap water for 1 min and semi-dried at 25°C for two hrs. The smokehouse had inside dimensions of $1.20 \times 1.0 \times 3.5 \text{ m}$ with pours-metal plates localized above the smoke source by 75 cm. the Semi-dried fish samples were hooked at distance about 250 cm in smoking house. Traditional cold smoking was carried out at $28\text{-}32^\circ\text{C}$ for 8-10 h. using sawdust as smoke source. After smoking the fish samples were cooled under ambient temperature.

Analytical methods

Raw, frozen and cold smoked mullet fish samples were analyzed at intervals of 0, 2, 4 and 6 months of storage. All the results were triplicates and expressed as mean \pm SD.

pH value: 5 g of raw and processed fish samples were homogenized with 50 ml of distilled water and filtered using filter paper. The pH value of the filtrate was measured using digital pH meter (Adwa AD 1030) according to the method of Pearson (1976).

Total Volatile Basic Nitrogen (TVB-N) content: TVB-N content was determined by the method described by Pearson (1976) using Macro-Kjeldahl distillation apparatus as follow: 10g of minced fish sample were mixed with 100 ml of distilled water, 200 ml distilled water, 2 g of MgO and antifoaming agent was added. 25 ml of 2% boric acid solution were added into 500 ml receiving flask and a few drops of mixed indicator (0.1g of methyl red and 0.1g of methylene blue to 100 ml of ethanol) where the condenser terminal must be dipped in boric acid solution. After boiling by heating, the condenser was washed with distilled water and the distillate was titrated with sulfuric acid (0.1 N). Multiply the titration (minus blank) by 14 to obtain the TVB-N as mg N per 100g sample.

Thiobarbituric Acid (TBA) value: TBA was determined colorimetrically in minced fish flesh samples as described by [22]. 10 grams of minced fish flesh were macerated with 50 ml of distilled water for 2 min, washed into a distillation flask with 47.5 ml distilled water, and 2.5 ml of hydrochloric acid (4 N) were added. A volume of 50 ml distillate was collected, and from which 5 ml were pipette into glass copered tube and mixed with 5 ml of TBA reagent. The mixture was heated in boiling water bath for 35 min. after cooling; the optical density was measured against the blank at wavelength of 538 nm. The method based on the spectrometric quotation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA) with two molecules of TBA. The TBA value was expressed as mg Malonaldehyde per kg sample (mg MA/kg).

Sensory evaluation: Sensory evaluation of raw, smoked and Mullet fish was performed by ten panelists chosen from the staff members of Shakshouk Research Station (NIOF). The organoleptic properties of raw, processed products were tested according the scale described by [23] as follows:

1-2	rejected;	3-4	accepted;
5-6	good;	7-8	very good;
9-10	excellent.		

The characteristics of color, odor, taste, texture and overall acceptability were tested.

Statistical analysis: The statistical analysis of the results obtained was carried out according to SPSS version 16 software program 2007. Means and standard deviation (SD) measure by L.S.D at 5% level of significant.

Results

Sensory properties of fresh mullet fish

It is well known that there are two types of sensory methods, subjective and objective. Subjective assessments of fish are often estimated generally using adjectives such as like/dislike or good/bad, which require subjective decisions [24]. Objective scoring schemes require trained, expert judges, but the advantage is that panels can be small [25].

Objective scores of sensorial properties (i.e., appearance, texture and odor) for raw mullet fish samples are summarized in table 1. Results of the subjective assessments for raw mullet samples obtained from both A and B farms showed similar observations in all the parameters; appearance, texture, odor, gills, eyes, pupil, and acceptability. And, no differences were found. So, the objective scoring was applied to know the little changes in these parameters studied. The scores of appearance, texture and odor were 9.2, 9.1 and 9.5 for fish obtained from farm A (Al-Batts Drain) and 9.5, 9.2, 9.6 for that from farm B (El-Wadi Drain), respectively. Also, the color scores of gills and eyes were 9.7 and 9.4, for fish A and 10, 9.7 for fish B, respectively. Transparent scores were 9.6 and 9.7 for fish samples A and B, respectively. Therefore, the overall acceptability scores were high; 9.4 and 9.7 for fish A and B, respectively. Our data indicated that any physical damage signs or foreign objects were not found in mullet fish samples either A or B.

The obtained data are in accordance to those findings by [26] who reported that sensory properties (appearance, flavor and texture) are considerable very important parameter to control fish quality as raw and processed products. In addition, in our study we used objective assessments to confirm the data recorded to limit the freshness degree for fish investigated.

pH value

PH is the most critical factor affecting microbial growth and spoilage of foods. PH is commonly used to measure fish deterioration; it has been common to measure the pH of the muscle tissue [27,28]. Table 2 shows the values of pH of mullet fish samples from the two sources (A and B farms) during frozen storage and smoking. From the table, the values of pH were 6.4 and 6.22 for fresh samples from farm A and B respectively. These results agreed with [29,30] who reported that the fresh fish muscle pH is most frequently in the 6.0 to

6.5 range. The variability of different pH changes depends on species, harvesting procedures, biological condition, variation of season, and methods of killing [28,31]. After smoking the pH values increased from 6.4 and 6.22 to 6.56 and 6.35 for samples from farm A and B, respectively. The pH value of smoked silver catfish samples ranged from 6.27-6.86 [27].

Quality attribute	Subjective evaluation	Objective scores values (10 degrees)	
		Farm A	Farm B
Control	3 rd day	7 th day	13 th day
Appearance	Bright	9.2 ± 0.07	9.5 ± 0.11
Texture	Rigor stage (firm)	9.1 ± 0.11	9.2 ± 0.15
Odour	Fresh fishy odor	9.5 ± 0.19	9.6 ± 0.01
Gills	Slight dull red	9.7 ± 0.12	10 ± 0.00
Eyes	Slight dull	9.4 ± 0.09	9.7 ± 0.07
Pupil	Transparent	9.6 ± 0.15	9.7 ± 0.29
Acceptability	Excellent	9.4 ± 0.04	9.7 ± 0.12

Table 1: The subjective evaluation and objective scores values (M ± SD) of raw Mullet fish samples obtained from A and B farms.

Note: Farm A: Al-Batts Drain. Farm B: El-Wadi Drain.
Scores: 1-2 = rejected, 3-4 = accepted, 5-6 = good, 7-8 = very good and 9-10 = excellent.

Period of storage (days)	Farm A		Farm B	
	(Fresh 6.41)		(Fresh 6.2)	
	Frozen fish	Smoked fish	Frozen fish	Smoked fish
0	6.4 ± 0.02	6.56 ± 0.03	6.22 ± 0.01	6.35 ± 0.04
60	6.51 ± 0.04	6.96 ± 0.31	6.34 ± 0.04	6.73 ± 0.01
120	6.42 ± 0.02	6.55 ± 0.04	6.26 ± 0.02	6.38 ± 0.04
180	6.29 ± 0.01	6.61 ± 0.02	6.12 ± 0.04	6.45 ± 0.02

Table 2: Effect of frozen storage (at -18°C) and cold smoking on pH values (M ± SD) of mullet fish samples.

Note: Farm A = Al-Batts Drain Farm B = El-Wadi Drain
M: mean **SD:** Standard Deviation

After 60 days the values of pH were increased to 6.51 and 6.34 for frozen samples and 6.96 and 6.73 for smoked samples obtained from farms A and B, respectively [32] reported that the pH value was increased after 2 months of frozen storage at -18°C. Increase in pH value during frozen storage could be attributed to proteolysis and breakdown of protein fraction and enzymatic activity resulting in some ammonia and other basic products [33,29,34]. After 120 days the pH values decreased to 6.42 and 6.26 for frozen samples from farm A and B, respectively, while after smoking pH values increased to 6.55 and 6.38 in smoked samples from farms A and farm B, respectively. As the storage period extended to 180 days pH values decreased in frozen samples from farms A and B to 6.29 and 6.12, respectively while after smoking the values of pH increased up to 6.61 and 6.45 for farms A and B, respectively.

Total volatile nitrogen (TVB-N) value

The determination of TVBN by direct distillation of fish portions is suitable as a standard method to assess the marketability of fish because it is simple, quick, and economical. The TVBN concentration in unfrozen seafood consists primarily of ammonia and trimethylamine,

whereas the TVBN in frozen seafood consists primarily of ammonia, Trimethylamine, and dimethylamine [35]. TVBN determinations are used as a standard method to determine if chilled, frozen, dried, and canned seafood is spoiled [25]. Table 3 illustrate the changes in TVB-N values in mullet fish mussels during frozen storage at -18°C for 180 days and their pre frozen smoked products. From the table the TVB-N values of fresh mullet fish from the two sources were 8.68 and 8.4 mg/100g sample for fish samples from farms A and B, respectively. After smoking the values of TVB-N increased for samples from the two sources to 13.16 and 17.36 mg/100g for farms A and B, respectively. According to [36] these results showed that the TVB-N of mullet fish < 15 mg/100g for the two fish courses, which indicates the good freshness of the fish. These results agree with [37] who reported that the initial TVB-N concentration in freshwater *M. cephalus* muscle samples was 8.2 ± 0.53 mg /100 g.

This difference may be attributed to the origin of the fish as marine fish muscles contain higher amount of non protein nitrogen precursor of post-mortem TVB-N formation [38]. The limits the TVB-N in smoked fish were 10 mg/100g in Egyptian standards specification (288, 2005). After 60 days TVB-N values increased in frozen samples from two farms and recorded 9.52 and 16.8 for farms A and B, respectively. After smoking the TVB-N values increased to 18.48 and 19.6 for farms A and B, respectively.

After 120 days TVB-N value increased in frozen samples from farm A reached to 14.28 mg/100g while decreased in the frozen sample from farm B, to 14.00 mg/100g. After smoking the values of TVBN increased to 22.96 and 24.64 mg/100g for farms A and B, respectively. The same trend was found by [17] who reported that smoking processes influenced the TVB-N level of smoked rainbow trout where the TVB-N increased after smoking process. At the end of storage time (180 days) the values of TVB-N decreased in both farms. The values were 10.92 and 10.36 mg/100g for frozen samples from farms A and B, respectively. After smoking these values increased to 18.76 and 18.2 mg/100g for farms A and B, respectively. Therefore it is clear that fish freezing is a reliable preservation method to prevent the changes in fish meat caused by proteolysis. From these results it could be concluded that the TVB-N remained significantly lower than the range of values (30-45 mg per 100 g) that are commonly found in good-quality smoked products [39].

Period of storage (days)	TVB-N (mg/100g)			
	Farm A		Farm B	
	Fresh 8.6		Fresh 8.40	
	Frozen fish	Smoked fish	Frozen fish	Smoked fish
0	8.68 ± 0.43	13.16 ± 0.43	8.4 ± 0.42	17.36 ± 0.57
60	9.52 ± 0.62	18.48 ± 0.70	16.8 ± 0.46	19.6 ± 0.57
120	14.28 ± 0.84	22.96 ± 1.10	14.00 ± 1.25	24.64 ± 1.42
180	10.92 ± 0.11	18.76 ± 1.49	10.36 ± 0.19	18.2 ± 0.28

Table 3: Effect of frozen storage (at -18°C) and cold smoking on TVB-N values (M ± SD) of mullet fish samples.

Note: Farm A = Al-Batts Drain farm B = El-Wadi Drain
M: mean **SD:** Standard Deviation

Thiobarbituric acid (TBA) value

A good technique for the monitoring of oxidation processes in meat is using the Thiobarbituric acid assay (TBA) after conversion to

malondialdehyde equivalents [29]. Malonaldehyde (MA) as a carbonyl compound formed during oxidation of polyunsaturated fatty acids was evaluated at any given time of frozen storage at -18°C followed by smoking processes of mullet fish. It has been suggested that a maximum TBA value, indicating a good quality of the fish, is 5 mg malonaldehyde (MA/kg), while fish may be consumed up to a TBA value of 8 mg MA/kg [40]. Table 4 shows the changes in TBA values during frozen storage (-18°C for 6 months) followed by smoking of mullet fish samples from the two farms A and B. The TBA values of fresh fish from both farms A and B were 0.10 and 0.17 mg/kg, respectively. After cold smoking these values increased to 0.36 and 1.57 mg/kg for farm A and B, respectively.

After 60 days of storage the TBA values were up to 0.69 and 0.79 mg/kg for farms A and B samples, respectively. Also after smoking these values increased to 1.55 and 2.26 mg/kg samples from farms A and B, respectively. The increased TBA values in the smoked fish probably originated from the breakdown of oxidation products, mainly malonaldehyde, during smoking due to the high temperature [41]. After 120 days the TBA increased also in frozen mullet samples from both farms and reached to 1.15 and 1.38 mg/kg for farm A and B, respectively. After smoking the TBA values were 1.28 and 2.21 mg/kg for farms A and B, respectively. These increases in TBA value might be due to the ice crystals formed which injure the cell and cause the release of pro-oxidants for lipid oxidation, especially free irons [42]. After 180 days the TBA values were increased in all samples, in frozen samples, to 1.38 and 1.43 mg/kg for samples from farms A and B, respectively. These values increased after smoking to 2.26 and 3.83 mg/kg for samples from farms A and B, respectively.

Organoleptic evaluation of smoked mullet fish

Sensory characteristics are the main criteria that affect the consumer acceptability of food products. Nowadays, shifting for high sensory quality product is the main purpose of smoking. The smoked products have higher moisture and lower salt content than in the past [43]. Table 5 shows the organoleptic properties of pre frozen cold smoked mullet fish at periods of 0, 60, 120, and 180 days during storage at -18°C for raw mullet samples then cold smoking after each storage period. From the table the scores of texture, taste, odor, color and overall acceptability were 8, 7.75, 8.25, 8.25 and 8.06 for smoked samples of farm A. And 7.9, 8.7, 8, 8.1 and 8.17 for farm B respectively. Smoking of mullet fish imparts a mild flavor, color and all other sensory attributes were judged by panelists to be extremely very good. After 60 days of raw samples frozen storage, the samples were cold smoked and the scores were 8.6, 8, 8.6, 8.4 and 8.4 for texture, taste, odor, color and over all acceptability for samples from farm A, respectively. While for samples from farm B, scores were 8, 9.3, 7.3, 8 and 8.15 for texture, taste, odor, color and over all acceptability, respectively.

After 120 days the scores of texture, taste, color and overall acceptability increased to 8.7, 9 and 8.75, while score of odor decreased as compared with previous period for samples from farm A respectively. On other hand the scores of texture, taste and over all acceptability decreased to 7, 8.75 and 8.06, while odor and color scores increased to 7.5 and 9 for farm B, respectively. [15] reported that during the cold-smoking of fish, which normally occurs the temperature below 30°C, texture of smoked salmon was not affected by the smoking temperature (20°C and 30°C) when the shear force was used as a textural properties indicator. At the end of storage, (180 days) the organoleptic of pre frozen smoked mullet fish increased with texture 9, odor 8 and overall acceptability 8.56, while decreased with taste 8.5 and not change with color for farm A samples. On other hand the scores of texture, taste, odor and overall acceptability increased to 9, 9.25, 8.25 and 8.87 respectively, while color score, 9 still no change for farm B samples. Cold smoking is being used as a flavor enhancer for items such as cod, beef, pork chops, salmon, scallops and steak [44].

Period of storage (days)	TBA (mg MA/kg)			
	Farm A		Farm B	
	Fresh 0.1		Fresh 0.16	
	Frozen fish	Smoked fish	Frozen fish	Smoked fish
0	0.10 ± 0.03	0.36 ± 0.02	0.17 ± 0.01	1.57 ± 0.02
60	0.69 ± 0.12	1.55 ± 0.03	0.79 ± 0.04	2.26 ± 0.05
120	1.15 ± 0.07	1.28 ± 0.01	1.38 ± 0.02	2.21 ± 0.04
180	1.38 ± 0.02	2.26 ± 0.01	1.43 ± 0.02	3.83 ± 0.01

Table 4: Effect of frozen storage (at -18°C) and cold smoking on TBA values (M ± SD) of mullet fish samples.

Note: Farm A = Al-Batts Drain farm B = El-Wadi Drain
M: mean **SD:** Standard Deviation

Storage period (day)	Farm A					Farm B				
	Texture	Taste	Odor	Color	Overall accept.	Texture	Taste	Odor	Color	Overall accept.
0	8 ± 0.25	7.75 ± 0.11	8.25 ± 0.36	8.25 ± 0.71	8.06 ± 0.41	7.9 ± 0.77	8.7 ± 0.06	8 ± 0.41	8.1 ± 0.21	8.17 ± 0.31
60	8.6 ± 0.15	8 ± 0.26	8.6 ± 0.45	8.4 ± 0.39	8.4 ± 0.28	8 ± 0.68	9.3 ± 0.31	7.3 ± 0.63	8 ± 0.09	8.15 ± 0.22
120	8.75 ± 0.32	9 ± 0.05	7.5 ± 0.38	8.75 ± 0.20	8.5 ± 0.33	7 ± 0.37	8.75 ± 0.51	7.5 ± 0.17	8.06 ± 0.14	8.06 ± 0.14
180	9 ± 0.17	8.5 ± 0.10	8 ± 0.31	8.75 ± 0.04	8.56 ± 0.06	9 ± 0.02	9.25 ± 0.62	8.25 ± 0.24	9 ± 0.13	8.87 ± 0.02

Table 5: Organoleptic scores of pre frozen mullet fish stored for 180 days followed by cold smoking of mullet fish samples (M ± SD).

Note: Farm A = Al-Batts Drain farm B = El-Wadi Drain
M: mean **SD:** Standard Deviation
Scores:
 1-2 = rejected 3-4 = accepted 5-6 = good
 7-8 = very good 9-10 = excellent

Conclusion

Frozen storage and cold smoking methods affected on physicochemical quality parameters and organoleptic scores of pre frozen mullet fish stored for 180 days. TVB-N, TBA and pH parameters of smoked Mullet fish increased gradually during frozen storage period. At the end of 180 days storage period of Mullet fish had good parameters and maintained on their quality.

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