Changes in quality attributes of sand smelt (*Atherina hepsetus*) fish burger and finger during frozen storage.

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Abstract

The present investigation has been carried out with the objectives study the effect of frozen storage on quality attributes of fish burger and fingers were made from sand smelt (bassaria) (*Atherina hepsetus*) with substitution levels from soybean flour and minced potato. Sand smelt has found in Qarun Lake by unaccepted from consumers. The physicochemical and microbiological analysis was carried on raw products immediately after processing and during frozen storage. Total Volatile Basic Nitrogen (TVBN), Trimethylamine (TMA), Thiobarbituric Acid (TBA) and pH values of sand smelt fish products showed significant increase during frozen storage for 3 months except pH value. Quality attributes parameters values of control samples were higher than those with substitution levels by soybean flour and minced potato. Sand smelt fish burger and finger made from sand smelt fish maintained on their good quality parameters until end of storage period.

Keywords: Fish products, Preservation technique, Low temperature storage, Processed fish.

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Introduction

Fish and fishery products have been recognized as very important sources for human nutrition. Fishes are characterized by their high contents of high quality and easily digestible protein and essential amino acids. Moreover, they are low in the saturated fatty acids and contain considerable amounts of the unsaturated fatty acids, especially omega-3 fatty acids, which are regarded as oxidation preventive compounds. The oil–soluble vitamins are known to be rich in fish. Also, fish is a good source of several minerals particularly fluorine and iodine, which are needed for the development of strong teeth and the prevention of goiter in man [1,2]. In 2012, aquaculture provided around half (50.3%) of all fish supplies destined for direct human food consumption. However, fish has been estimated to account for only 6.5% of the global protein consumption (and about 14% of total fish and animal protein supply). Fish provides about 60% of palatable fish is utilized by end-users while the remaining is either converted or lost. Post-harvest loss due to decay is estimated to be between 10-12 million tons annually which accounts for about 10% of global capture and culture fish [3-6]. Sand smelt (*Atherina boyeri*) is a common species in the Mediterranean Sea and has shown distribution from northeast Atlantic to northwest coast of Scotland. Also, this species lives in the Black Sea, Aegean Sea, Marmara Sea, Aral and Caspian Sea [7]. In recent years, increases of the civilization and changes in the socioeconomic factors such as more women in the work force, have resulted in increased consumers' preference for ready-to-eat foods (heat and eat). Thus, efforts have been made to improve the quality and stability of these foods and those made from fish are becoming very popular, such as fish minced, burgers, fingers, marinated products, etc. [8-10]. Total Volatile Basic Nitrogen (TVBN), Trimethylamin (TMAN) and Thiobarbeturic Acid (TBA) are good index to determine the freshness and quality of fishes and their products. The objective of the investigation are summarized in following up the storage changes occurred in chemical quality parameters to assess the storage stability of sand smelt fish products during frozen storage.

Materials and Methods

Fish

Fresh sand smelt (bassaria) fish (*Atherina hepsstia*) was obtained from Qarun Lake at Fayom Governorate, Egypt during April, 2017. About 25 kg of fish were transported in ice to the laboratory of Fish Research Station (at Shakshouk, Fayoum), National Institute of Oceanography and Fisheries.

Filling materials

Soybean Flour (SF) and Minced Boiled Potatoes (MBP) were used as filling materials in the processing of fish products. SF was obtained from Food Technology Research Institute, Agriculture Research Center at Giza Governorate, Egypt. MBP was prepared by boiling potatoes for 15 min, then peeled and minced.

Ingredients

The ingredients included sunflower oil, starch, wheat flour, sugar, salt, onion, garlic, spices, and other additives were obtained from the local market.

Preparation of fish

On arrival at the laboratory, fish was beheaded, gutted and washed gently with tap water. The edible portion of fish was soaked in 1% salt solution contained 0.5% acetic acid for 5 min to remove the fishery odor and taste, then drained off and minced using an electric meat mincer (Braun plus 1300). A portion of the minced fish was used in the chemical analysis and the microbial examination of the raw material (sand smelt fish). The remaining quantity was divided into two portions, kept to process fish products of burger and finger.

Fish burger formulation and processing

Control fish burger (no filling material) was composed of 75 g of minced fish and 25 g of the ingredients mixture for 100 g batter as shown in Table 2 which indicates the recipe of sand smelt burger as mentioned by [11]. The experimental burgers were formulated by using SF and MBP as filling materials. Each of the two filling materials was used at levels of 10, 15 and 20% of weight of the minced fish used. The experimental formulated samples were made by replacing the minced fish with the filling material at the desired level as illustrated in Table 1. All the formulations were needed by hand until homogenous dough was obtained. Portions of 50 g were shaped (8.5 cm diameter and 1.0 cm thickness) by manually operated forming machine (NOAW-Affetacrane, Italy). Burger samples were packaged in polyethylene bags and stored at -18℃ until required for analysis.

Fish fingers formulation and processing

Fish fingers were prepared as described by [8] and [12]. Control fish fingers (without filling material) were composed of 93.5 g of minced sand smelt fish and 6.5 g of the ingredients mixture as shown in Table 1. The experimental fingers were formulated by using the filling materials i.e. SF and MBP at levels of 10, 15 and 20% of the minced fish as described in burger formulation. The formulated samples were made by replacing the minced fish with the filling materials at the tested levels (Table 2). The minced fish was mixed with the ingredients and the mixture was homogenized until smooth dough was obtained. The dough was shaped into fingers and frozen at -18°C for 2 h before battering. The frozen fish fingers were rapidly coated with batter solution (3 parts of water plus 2 parts contained 94% maize flour, 2% skim milk, 2% egg yolk and 2% salt) and then they were rubbed with ground crumb.

Table 1. *Substitution levels of the filling materials (SF and MBP) used in the preparation of sand smelt fish burger and fingers*
Soybean flour, MBP: Minced boiled potato

Fish burger		Fish fingers		Substitution Level (%)
Minced fish (g)	(SF) or (MBP)(g)	Minced fish (g)	(SF) or (MBP) (g)	
75.00	-	93.50	-	0
67.50	7.50	84.15	9.35	10
63.75	11.25	79.47	14.03	15
60.00	15.00	74.80	18.70	20

Table 2. *Recipes of sand smelt fish burger and fingers.*
**Spices mixture composed of 32% black pepper, 22.5% coriander, 15% cumin, 10% cardamom, 9% red pepper, 7.5% cubeb and 4% clove*

Fish burger		Fish fingers	
Ingredients	%	Ingredients	%
Fish mince	75.00	Fish mince	93.50
Vegetable oil	9.00	Salt	1.50
Starch	8.00	Sugar	1.00
Salt	2.30	Wheat flour	3.00
Sodium bicarbonate	0.40	Cumin	0.24
Onion	2.50	Onion	0.24
Garlic	0.50	Garlic powder	0.24
Polyphosphate	0.30	Black pepper	0.24
*Spices mixture	2.00	Thyme	0.04

Storage stability

Storage studies were made to investigate the storage stability of sand smelt fish products (burgers and fingers) during frozen storage. Based on the data collected from the sensory evaluation for the different formulation of burgers and fingers, it was selected the more suitable levels of filling materials (SF and MBP) to carryout storage studies. The selected formulated samples along with the control samples of burgers and fingers were packed in polyethylene bags and stored in deep freezer at -18℃ for three months. Samples were withdrawn periodically at intervals of 15 days for analysis.

Analytical methods

Total volatile basic-nitrogen was determined by macro-distillation method as described by [13] and the results obtained were expressed as mg TVB-N/100 g fresh sample. TMA-N was determined as described in [14] and the results were expressed as mg TMA/100 g dry sample. Malonaldehyde content was calorimetrically determined as described by [13] and the results were expressed as mg malonaldhye/kg of sample. The pH value was measured according to [14] as the follows: 10 g of the minced sample were homogenized with 100 ml of distilled water and the mixture was filtered by using filter paper. The pH value of the filtrate was measured using pH meter with combined electrode.

Statistical analysis

The statistical analysis of the results obtained was carried out according to SPSS version 16 software program 2007. Means and Standard Error (SE) measure at 5% level of significant.

Results and Discussion

Chemical quality parameters and pH

Total volatile basic nitrogen, trimethylamine nitrogen, thiobarbeturic acid as well as pH values are widely used as chemical quality parameters to assess the quality and storage stability of fish and fishery products.

Total volatile basic nitrogen (TVB-N)

TVB-N contents of sands melt fish products (burgers and fingers) were determined periodically at intervals of 15 days during frozen storage for 90 days. The results obtained are shown in Table 3. It was observed that the values of TVB-N showed wavy progress for both fish burgers and fingers samples. The initial values of TVB-N for control burger sample and those formulated by the incorporation of 15% SF and 15% MBP were determined by 17.11, 14.52 and 12.00 mg/100 g respectively. After 30 days of frozen storage, these values increased up to 18.20, 16.50 and 13.53 mg/100 g (dry weight basis) respectively then decreased down to 17.85, 14.17 and 13.18 mg/100 g respectively. At the end of 90 days of frozen storage, TVB-N

Table 3. *Changes in TVB-N content (mg/100 g) of sand smelt fish products during frozen storage at -18 ℃.*
Data are presented as mean ± SE of 3 replicates. SE: standard error. Significant difference at P<0.05. SF: Soybean flour. MBP: Minced boiled potato

Storage period (day)	Fish Burger			Fish Finger		
	Control	SF (15%)	MBP (15%)	Control	SF (20%)	MBP (15%)
0	17.11 ± 0.063	14.52 ± 0.184	12.60 ± 0.202	18.55 ± 0.144	15.81 ± 0.121	13.06 ± 0.034
15	18.00 ± 0.577	15.60 ± 0.785	13.02 ± 0.242	19.45 ± 0.259	16.01 ± 0.118	13.46 ± 0.265
30	18.20 ± 0.115	16.50 ± 0.288	13.53 ± 0.115	19.67 ± 0.057	16.69 ± 0.178	13.68 ± 0.184
45	18.05 ± 0.202	14.50 ± 0.230	13.23 ± 0.132	19.06 ± 0.034	16.17 ± 0.098	13.09 ± 0.051
60	17.85 ± 0.144	14.17 ± 0.098	13.18 ± 0.103	18.95 ± 0.144	16.03 ± 0.240	12.36 ± 0.207
75	18.32 ± 0.184	15.22 ± 0.127	14.52 ± 0.300	20.46 ± 0.265	17.59 ± 0.236	13.69 ± 0.178
90	19.50 ± 0.288	16.63 ± 0.213	14.72 ± 0.127	21.87 ± 0.213	17.80 ± 0.173	15.22 ± 0.127
Sig.	.002	.001	.000	.000	.000	.000

Table 4. *Changes in TMA-N content (mg/100 g) of sand smelt fish products during frozen storage at -18 ˚C.*
Data are presented as mean ± SE of 3 replicates. SE: standard error. Significant difference at P<0.05. SF: Soybean flour. MBP: Minced boiled potato

Storage period (day)	Fish Burger			Fish Finger		
	Control	SF (15%)	MBP (15%)	Control	SF (20%)	MBP (15%)
0	1.30 ±.028	1.05 ± 0.034	0.81 ± 0.034	2.23 ± 0.132	2.10 ± 0.057	0.90 ± 0.057
15	1.53 ±.075	1.2 ± 8.161	0.83 ± 0.075	2.48 ± 0.161	2.18 ± 0.103	1.10 ± 0.051
30	1.70 ±.173	1.51 ± 0.063	0.89 ± 0.051	2.60 ± 0.115	2.25 ± 0.144	1.23 ± 0.132
45	1.86 ±.092	1.76 ± 0.150	0.94 ± 0.080	2.66 ± 0.086	2.36 ± 0.115	1.45 ± 0.028
60	2.00 ±.115	1.77 ± 0.155	1.12 ± 0.069	2.75 ± 0.086	2.32 ± 0.184	1.50 ± 0.144
75	2.10 ±.057	1.83 ± 0.190	1.35 ± 0.202	2.80 ± 0.144	2.55 ± 0.086	1.67 ± 0.118
90	2.17 ±.086	1.89 ± 0.051	1.62 ± 0.069	2.87 ± 0.098	2.76 ± 0.034	1.95 ± 0.086
Sig.	.000	.003	.000	.033	.016	.0000

values in control, 15% SF and 15% MBP burger samples became 19.50, 16.63 and 14.72 mg/100 g (wet weight basis) respectively.

Fish finger samples showed the same behavior regarding the changes in their contents of TVB-N during frozen storage (Table 3). The initial values of TVB-N in control finger sample and those formulated with adding 20% SF and 15% MBP were 18.55, 15.81 and 13.06 mg/100 g (wet weight basis) respectively. These values irregularly changed during frozen storage reaching to 21.87, 17.80 and 15.22 mg/100 g at the end of 90 days storage respectively.

The fluctuation of TVBN levels found in sands melt fish products had been reported for some fish products during storage under similar conditions [15,16]. Increasing TVB-N values during storage was attributed to the activity of microbial and endogenous protolytic enzymes which breakdown proteins to volatile nitrogenous compounds [17,18]. Meanwhile, the elimination of dissolved volatile constituents through drip could be caused decreasing of TVB-N values during frozen storage [19,20].

TVB-N is one of spoilage parameters in fish and fish products. In the study made by [21] it was suggested the limit values of TVBN as very good until 25 mg/100 g, good until 30 mg/100 g, marketable until 35 mg/100 mg and spoilt more than 35 mg/100 g. In the present study, TVBN contents of sands melt fish products ranged between the lowest values determined by 12.60 mg/100 g in the 15% MBP burger sample at zero day storage to the highest value of 21.87 mg/100 g for control fingers sample stored for 90 days. These findings indicated the good quality of sands melt fish products even after 90 days of frozen storage.

Trimethylamin nitrogen (TMA-N)

Data presented in Table 4 show the changes in TMA-N contents of sand smelt fish products during frozen storage at -18 ℃ for 90 days. The results indicated that burger and finger samples contents of TMA-N gradually increased as the storage period extended. The initial values of TMA-N contents of control burger sample and 15% SF and 15% MBP formulated samples were determined by 1.30, 1.05 and 0.81 mg/100 g respectively. These initial values gradually increased up to 2.17, 1.89 and 1.62 mg/100 g respectively at the end of 90 days of frozen storage. Similarly, changed in TMA-N contents of sand smelt fingers followed the sample trend. The initial levels of TMA-N in control finger sample and the 20% SF and 15% MBP formulated samples were 2.23, 2.10 and 0.90 mg/100 g respectively. These values indicated that TMAN contents of finger samples were slightly higher than their levels in burger samples. During storage, these values gradually increased up to 2.87, 2.76 and 1.95 mg/100 g after 90 days storage at -18°C respectively. Such increments in TMA-N during storage may be due to the conversion of TMAO into TMA by the endogenous and/or bacterial enzyme TMAase. TMA is used as an index of freshness and quality of fish. It had been reported [22] that perfectly fresh fish had 3.37 mg/100 g TMA-N; good grade fish showed a level of 3.79-5.90 mg/100 g and fair fish had 12.56-16.02 mg/100 g. Accordingly, values of TMA-N found in the present study indicated the good quality of sand smelt fish burgers and fingers even after 90 days of frozen storage.

Thiobarbituric acid (TBA)

TBA is widely used as an indicator of the degree of lipid oxidation. In the present study, TBA values for sand smelt fish products during frozen storage were determined as malonaldhyde (mg/kg) and data obtained are presented in Table 5. Initially, TBA values of control, 15% SF and 15% MBP burger samples were 0.70, 0.65 and 0.61 mg MDA/kg respectively. During frozen storage of burger samples, TBA values showed a gradual increase. At the end of storage period (90 days), TBA values of control, 15% SF and 15% MBP increased up to 1.42, 1.30 and 0.96 mg MDA/kg respectively. Similar observations were also found in the changes of TBA values of sand smelt fish finger samples during frozen storage. The results given in Table 5 indicated that the initial values of TBA in control, 20% SF and 15% MBP increased from 0.59, 0.53 and 0.36 mg MDA/kg respectively before storage to 0.77, 0.69 and 0.56 mg MDA/kg after 90 days storage respectively. The increasing of TBA value during frozen storage of sand smelt fish products has been demonstrated for similar products made from other fish types [12,23,24].

TBA determination is an important quality index, indicating fat oxidation .The TBA value should be less than 3 mg MDA/kg in perfect quality material and should not be more than 5 mg MDA/kg in good quality material and consumption limits were from 7 to 8 mg MDA/kg [25]. In the present study, the highest value of TBA was determined as 1.42 mg MDA/kg for control burger sample indicating the good quality of the different samples of sand smelt fish products even after 3 months of frozen storage.

pH value

pH value was measured in fish products made from sand smelt fish during frozen storage and results obtained are presented in Table 6. Initially, it was observed that pH values of finger samples were higher than those of burger samples. As shown in Table 6, the initial pH values for burger and fingers samples ranged between 6.45-6.58 and 6.64-6.70 respectively. These variations may be due to the differences between the ingredients used in the preparation of fish burgers and fingers [26] reported similar observations. The changes in pH value during frozen storage of sand smelt fish products showed gradual increase as the storage period extended. The initial pH values of control, 15% SF and 15% MBP burger samples increased from 6.48, 6.58 and 6.45 to 6.80, 6.68 and 6.60 after 3 months storage at -18 ℃. Similarly, pH values of control, 20% SF and 15% MBP fingers samples gradually increased from 6.70, 6.69 and 6.79 to 7.03, 6.95 and 6.79 at the end of 90 days storage. Several studies showed inconsistency results regarding increasing or decreasing pH value in various fish species during storage [20, 25, 27-29]. The increase in the pH value during storage could be due to the enzymatic degradation of fish muscle components [30]. Rising pH during storage also may be due to formation of dimethyl amine from trimethylamine oxide [31]. pH values between 6.8-7.0 were proposed as acceptance limit of fish, and values above 7 were considered to be spoiled [32,33].

Conclusion

Sand smelt fish products (Burger and Fingers) maintained a good quality during frozen storage at -18°C for 3 months. Utilization of substitution levels from soybean flour and mince potato improved the quality attributes of fish burger and fish finger made from sand smelt fish. Sand smelt fish can be used to produce high quality fish products on as large scale manufacturing.

Table 5. *Changes in TBA value (mg MDA/Kg) of sand smelt fish products during frozen storage at -18 ˚C.*
Data are presented as mean ± SE of 3 replicates. SE: standard error. Significant difference at P<0.05.SF: Soybean flour. MBP: Minced boiled potato

Storage period (day)	Fish Burger			Fish Finger		
	Control	SF (15%)	MBP (15%)	Control	SF (20%)	MBP (15%)
0	0.70 ± 0.034	0.65 ± 0.028	0.61 ± 0.023	0.59 ± 0.011	0.53 ± 0.017	0.36 ± 0.034
15	0.81 ± 0.023	0.71 ± 0.023	0.67 ± 0.028	0.61 ± 0.017	0.55 ± 0.028	0.40 ± 0.011
30	0.90 ± 0.028	0.77 ± 0.040	0.68 ± 0.023	0.63 ± 0.017	0.59 ± 0.023	0.40 ± 0.017
45	1.15 ± 0.086	1.02 ± 0.023	0.71 ± 0.028	0.70 ± 0.046	0.59 ± 0.034	0.43 ± 0.023
60	1.16 ± 0.080	1.16 ± 0.092	0.75 ± 0.028	0.70 ± 0.057	0.62 ± 0.040	0.48 ± 0.034
75	1.25 ± 0.115	1.24 ± 0.057	0.92 ± 0.069	0.73 ± 0.017	0.67 ± 0.023	0.51 ± 0.017
90	1.42 ± 0.069	1.30 ± 0.132	0.96 ± 0.034	0.77 ± 0.040	0.69 ± 0.040	0.56 ± 0.034
Sig.	.000	.000	.000	.018	.022	.001

Table 6*. Changes in pH value (mg MDA/Kg) of sand smelt fish products during frozen storage at -18 ˚C.*
Data are presented as mean ± SE of 3 replicates. SE: standard error. Significant difference at P<0.05. SF: Soybean flour. MBP: Minced boiled potato

Storage period (day)	Fish Burger			Fish Finger		
	Control	SF (15%)	MBP (15%)	Control	SF (20%)	MBP (15%)
0	6.48 ± 0.173	6.58 ± 0.046	6.45 ± 0.028	6.70 ± 0.115	6.69 ± 0.051	6.64 ± 0.011
15	6.57 ± 0.057	6.58 ± 0.046	6.48 ± 0.034	6.79 ± 0.109	6.77 ± 0.115	6.65 ± 0.086
30	6.60 ± 0.173	6.60 ± 0.115	6.49 ± 0.167	6.8 ± 0.144	6.79 ± 0.051	6.70 ± 0.115
45	6.62 ± 0.098	6.60 ± 0.086	6.55 ± 0.086	6.86 ± 0.057	6.79 ± 0.115	6.70 ± 0.144
60	6.65 ± 0.115	6.60 ± 0.069	6.56 ± 0.178	6.86 ± 0.173	6.80 ± 0.069	6.72 ± 0.069
75	6.68 ± 0.046	6.63 ± 0.103	6.58 ± 0.057	6.86 ± 0.092	6.83 ± 0.106	6.75 ± 0.086
90	6.80 ± 0.115	6.68 ± 0.057	6.60 ± 0.086	7.03 ± 0.075	6.95 ± 0.086	6.79 ± 0.051
Sig.	.672	.973	.983	.614	.607	.902

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