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Changes in Microbial Quality of Tilapia Fish during Frozen Storage and Their Fried Products

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Abstract

Microbial quality of Tilapia Fish Samples during frozen storage at -18°C for 180 days and their fried products were determined. Total viable counts of bacteria were 2.25×10^4 and 1.7×10^4 cfu/g for farm A and B respectively. Yeast and Mold counts (Y&M) were 9.5×10^2 and 4.66×10^2 cfu/ g for farms A and B samples, respectively. Total viable counts of frozen tilapia fish samples increased to 3.13 and 3.93 $\times10^4$ cfu/g after 60 days of storage for farms A and B, respectively, then decreased to 0.7 and 0.66×10^4 cfu/g after 120 days of storage for farms A and B, respectively, then increased to 4.26 and 2.43×10^4 cfu/g for frozen stored samples from farms A and B, respectively at the end of storage period. After frying the total viable counts increased in samples at zero time, and then decreased with storage periods of raw frozen samples until the end of frozen storage. After the frying process the counts of Y&M increased in all samples at the storage periods, while the fried samples from farm B decreased to 14.66×10^2 cfu/g after 60 days raw storage. At the end of storage the counts of thermophilic bacteria increased to 17 and 12 ($\times10^3$ cfu/g) for fried samples from farms A and B, respectively..

Keywords:

Microbial examination; Tilapia; Practice; Frozen storage; Frying

Introduction

The total quantity of catch fish recorded 1,706,273 tones, while the total quantity of Exports and Imports of fish were 47812 and 311068 tones, respectively. Annual average share per capita of fish was 21.64 kg [1]. Global changes in consumer lifestyle marked by increasing demand for nutritional and healthy food products have spurred the continuing rise in demand for fresh and ready-to-cook fish and fishery products. This rising demand for ready to eat fresh instead of frozen fish requires the use of preservation procedures that can add value and reduce postharvest losses [2-5]. Freshly caught fish is variable and delicate food due to soft and moist texture, sweat flavor and great nutrition and health components. However because of its high moisture (high water activity (aw ≥ 0.95), post mortem pH of about 5.2 and free amino acids combined with lower level of connective tissue compared with other muscle foods, seafood is more susceptible to spoilage and therefore have short shelf-life. Hence it's essential to take immediate steps to preserve the fresh quality and safety of fish products. Seafood quality deterioration occurs from wide range of causes, such as poor catching practice; poor handling and keeping of raw materials and inefficient refrigeration and storage. To ensure that the fresh quality is well preserved, and to establish the most efficient methods for preservation of seafood quality, both objective and subjective methods of quality analysis should be performed. Chemical, biochemical, physical and microbiological methods have been used as objective methods to evaluate the quality of fish during chilled storage [6]. Fresh fish is a highly perishable product due to its biological composition. This condition is favorable for the growth of micro-organisms, 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 spoilt very quickly even in temperate regions [7,8]. Freezing preservation of food has been used for thousands of years because of high product quality. 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 [9,10]. Frying is one of the oldest methods of food preparation. It improves the sensory quality of food by formation of aroma compounds, attractive color, crust and texture. Deep fat frying is a process where in a food is allowed to be immersed and held in hot fat for cooking. Fried foods have a distinct position, attributed to their characteristic color, flavor and aroma. During this process, complex operations could occur involving numerous chemical and physical changes such as starch gelatinization, protein denaturation, moisture evaporates and forming coating [11]. The main objective of the current study could be summarized in Determine the microbial quality of fried Tilapia fish during frozen storage.

Material and Methods

Fish samples

About 20 kg of Tilapia (*O. niloticus*) fish samples were obtained from two farms (A and B) during, June 2016 at Fayoum Governorate. Farm A located in eastern Fayoum and farm B located in western Fayoum governorate. The main source of farm A (Al-Batts Drain) and farm B (El-Wadi Drain) is irrigation water. Averages of weight and length were 303 ± 31.5 g and 25.9 ± 0.22 cm for raw samples obtained farm A (Al-Batts Drain), while, farm B (El-Wadi Drain) they were 327 ± 93.8 g and 26 ± 2.8 cm, respectively. Fish samples were transported in ice box to Fish Processing and Technology Laboratory, Shakshouk research Station for

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Aquatic Resources, National Institute of Oceanography and Fisheries (NIOF). Fish samples were carefully washed with tap water, glazed, packed in polyethylene bags and stored at -18°C for 180 days. Raw and frozen fish samples were deep-fried in oil at intervals of 60 days during storage.

Sunflower oil

Sunflower oil was purchased from the local market at Fayoum governorate, it contained: Saturated fat (12.85%); Polyunsaturated fatty acid (60%); Monounsaturated fatty acid (27.14%) and produced by Arma for food Industries Company.

Frying process

The prepared fish was rubbed with flour left for 3-4 min and fried in pre-heated deep fried oil at 170-180°C for 10-15 min using Electrical Fryer pan (Moulinex brand). Fried fish samples were removed when a golden brown color was appeared on their surfaces. They were placed in the frying basket to drain out the excess amounts of cooking oil then cooled and kept for analysis of quality properties.

Analytical methods

Raw, frozen and processed Tilapia fish samples were analyzed at intervals of 0, 60, 120 and 180 days of storage. All the results were triplicates and expressed as mean ± SD.

Microbiological analysis

10 grams of fish sample were aseptically weighted and homogenized with 90 ml of sterile saline water for 1 min from each treatment. The homogenized samples were serially diluted using 9 ml sterile saline for bacteriological analysis. Total Viable Count (TVC), Thermophilic Bacteria (TBC), Yeasts and Molds count were examined during storage periods.

Total Viable Count (TVC)

TVC was determined by using nutrient agar medium [12].

Standard agar medium:

Yeast extracts 2.5 g; Blood pressure 1.0 g; Tryptone 5.0 g; Agar 15.0 g; Distilled water 1.0 L; pH value 7.0.

The media was autoclaved at 121°C for 15 min. the results were expressed as cfu/g.

Thermophilic Bacterial Count (TBC)

The thermophilic bacterial counts were determined using nutrient agar medium, and the results were expressed as cfu/g sample.

Yeasts and molds count

Yeasts and molds counts were enumerated on malt agar as mentioned by APHA [13].

Malt agar medium

Malt extracts 30 g, Distilled water 1.0 L; Agar 15.0 g

The media was autoclaved at 121°C for 15 min. the results were expressed as cfu/g sample.

Antibiotic solution

500 mg of each of chlortetracycline HCL and chloramphenicol were added to 100 ml sterile phosphate buffered distilled water and mixed. 2 ml of this solution was added per 100 ml of malt agar to give a final concentration in the medium of 100 g/L of each of the antibiotics. After that, 1ml was pipette of each dilution and poured into each of appropriately marked duplicated Petri dishes, tempered to 45°C and mixed thoroughly and allow to solidity. The plates were incubated at 20-25°C for 3-5 days.

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 and Discussions

Microbial quality of fresh Tilapia fish

The Table 1 shows the results of microbial quality criteria of fresh tilapia fish samples obtained from two farms A and B. The Total Viable Count (TVC) of tilapia samples from the two farms were 2.25×10^4 and 1.7×10^4 cfu/ g respectively. While the Yeast and Mold Counts (YMC) were 9.5×10^2 and 4.66×10^2 cfu/ g for farms A and B respectively. The bacterial counts of fresh sardine were 3.5×10^4 cfu/g flesh fish [14-16]. The TPC of tilapia fish was 2.35 (log cfu/g). It has been found in the present studies that the TVC within the permissible limit of 6 log cfu/g [17,18].

Microbiological changes of tilapia fish samples during frozen storage at -18°C for 180 days followed by deep frying

Total Plate Count (TPC)

Changes in total plate count of frozen tilapia fish during frozen storage at -18°C for 180 days followed by deep frying at 170°C for 10 min. are expressed as count $\times10^4$cfu/g are presented in Table 2. Total viable count of bacteria was 2.25 and 1.63×10^4 cfu/g for fresh tilapia fish samples obtained from farms A and B, respectively. These values increased after frying to 3.5 and 1.9×10^4 cfu/g for samples from farms A and B, respectively.

Similar results were obtained that the TVC of sardine fillets were 4.22 log cfu/g [19].

After 60 days of frozen storage the TPC counts increased to 3.13 and 3.93×10^4 cfu/g for for samples from farms A and B, respectively. After

Microbial aspects	Farm A	Farm B
TVC (cfu/g)	2.25×104 ± 0.35	1.7×104 ± 0.15
YMC (cfu/g)	9.5×102 ± 0.57	4.66×102 ± 0.51
TVC: Total Viable Count; cfu: Colony Forming Units; YMC: Yeast and Mold Counts.		

Table 1: Microbial counts (M ± SD) of fresh Tilapia Fish samples from two farms.

Period of storage (days)	TVC counts ($\times10^4$ cfu/g)			
	Farm A		Farm B	
	Frozen fish	Fried fish	Frozen fish	Fried fish
0	2.25 ± 0.35	3.5 ± 0.70	1.63 ± 0.15	1.9 ± 0.65
60	3.13 ± 0.32	2.73 ± 0.50	3.93 ± 0.30	2.95 ± 0.63
120	0.7 ± 0.26	0.63 ± 0.32	0.66 ± 0.23	1.5 ± 0.14
180	4.26 ± 0.64	1.3 ± 0.28	2.43 ± 0.92	2.2 ± 0.52
Farm A: Al-Batts Drain; Farm B: El-Wadi Drain; M: Mean; SD: Standard Deviation.				

Table 2: Effect of frozen storage (at -18°C for 180 days) followed by deep frying on TVC count ($\times10^4$ cfu/g) (M ± SD) of tilapia fish samples.

frying these counts reduced to 2.73 and 2.95×10^4 cfu/g for samples from farms A and B, respectively. Similar results were found that the TVC of frozen tilapia increased after 2 months of frozen storage at -18°C to 5.75 log cfu/g then reduced to 3.55 log cfu/g after deep frying [17,20].

In contrast, after 120 days the bacterial counts were reduced to 0.7 and 0.66×10^4 cfu/g in frozen tilapia fish samples from farms A and B, respectively. After deep frying these counts of TVC decreased to 0.63×10^4 cfu/g for farm A, while increased to 1.5 for farm B. El Sherif et al. reported a decrease in TVC of frozen tilapia fish after 4 month of storage and after frying the bacterial counts decreased. El-Lahamy et al. [21] investigated that Mullet fish samples are expressed as log10 cfu/g. The results showed a decreasing trend after cooking methods. Total Plate Count (TBC) of raw, fried, grilled samples of Mullet fish recorded 3.3 ± 0.144, 2.95 ± 0.040 and 3.04 ± 0.023 (log cfu/g), respectively. Similar observations were also found in the changes of yeast and mould counts of Mullet fish samples after cooking process.

At the end of storage the TVC counts increased to 4.26 and 2.43×10^4 cfu/g in frozen samples from farms A and B, respectively. These counts were decreased to 1.3 and 2.2×10^4 cfu/g after deep frying of tilapia samples from farms A and B, respectively. Obemeata et al. [22] stated that freezing of fish at -18°C created an unfavorable environmental condition for the growth and the survival of the micro-organisms.

From these results it could be concluded that the fresh, frozen and fried samples TVC were within the permissible limits of aerobic pate counts of fish (105-108) as reported by ICMSF [18] and 104-106 cfu/g as reported by Australian meat standards committee [23].

Yeast and mold counts

Dated presented in Table 3 show the count of Yeast and Mold (Y&M) ($\times10^2$ cfu/g) of tilapia fish samples during frozen storage (-18°C) for 180 days followed by deep frying (170°C for 10 min) for two different farms A and B. from the table the counts of yeast and molds were 10.6 and 4.66×10^2 cfu/g of fresh tilapia fish from farms A and B, respectively. These values increased after frying to 12.5 and 5.33×10^2 cfu/g for samples from farms A and B, respectively.

After 60 days of storage the counts of Y&M decreased to 5 in farm A frozen samples, while increased in farm B frozen samples. After frying the counts of Y&M increased with farm A samples, on other hand decreased in fried samples from farm B. Yeast and mold counts decreased in frozen samples from farms A and B after 120 days, reached 3.33 and 5.33×10^2 cfu/g for samples from farms A and B, respectively. In contrast these values were increased after deep frying at 170°C for 10 min. the counts were 12.33 and 10.66×10^2 cfu/g from farms A and B, respectively.

At the end of storage the counts of yeast and mold increased in the farms A and B frozen stored samples. The counts were 9 and 5.66×10^2 cfu/g and increased to 13 and 19×10^2 cfu/g for samples from farms A and B, respectively.

Period of storage (days)	Yeasts and molds counts ($\times10^2$ cfu/g)			
	Farm A		Farm B	
	Frozen fish	Fried fish	Frozen fish	Fried fish
0	10.6 ± 0.57	12.5 ± 0.70	4.66 ± 0.51	5.33 ± 0.57
60	5.00 ± 0.88	34.00 ± 0.82	22.00 ± 0.84	14.66 ± 0.79
120	3.33 ± 0.76	12.33 ± 0.76	5.33 ± 0.57	10.66 ± 0.35
180	9.00 ± 0.86	13.00 ± 0.98	5.66 ± 0.65	19.00 ± 0.84
Farm A: Al-Batts Drain; Farm B: El-Wadi Drain; M: Mean; SD: Standard Deviation.				

Table 3: Effect of frozen storage (at -18°C for 180 days) followed by frying on yeasts and molds count ($\times10^2$ cfu/g) (M ± SD) of tilapia fish.

Period of storage (days)	Thermophilic bacterial count ($\times10^3$ cfu/g)	
	Farm A	Farm B
	Fried fish	Fried fish
0	6 ± 0.70	4.33 ± 0.57
60	7.5 ± 0.98	11 ± 0.42
120	UD	9 ± 0.16
180	17 ± 0.32	12 ± 0.28
Farm A: Al-Batts Drain; Farm B: El-Wadi Drain; M: Mean; SD: Standard Deviation.		

Table 4: Thermophilic bacterial count ($\times10^3$ cfu/g) (M ± SD) of pre frozen deep fried tilapia fish from two farms.

Thermophilic bacterial count

The Table 4 shows the effect of deep frying at 170°C for 10 min of tilapia fish samples from farms A and B on the counts of thermophilic bacteria ($\times10^3$ cfu/g). Thermophilic bacterial counts of fried fish at zero time were 6 and (4.33×10^3 cfu/g) for farms A and B, respectively. After 60 days of frozen store of raw samples, the tilapia samples fried at 170°C for 10min and the counts of thermophilic bacteria were 7.5 and 11×10^3 cfu/g for samples from farms A and B, respectively. From the table it could be noticed that the thermophilic bacterial counts increased when compared with zero time of frozen storage.

After 120 days of frozen storage of raw samples thermophilic bacteria not detected in fried tilapia samples for farm A, while the counts were 9 ($\times10^3$ cfu/g) for farm B sample. At the end of frozen storage of raw samples, tilapia fish fried and the counts of thermophilic bacteria were 17 and 12 ($\times10^3$ cfu/g), for samples from farms A and B, respectively.

Conclusion

Based on our results, it could be concluded that fresh, frozen and fried Tilapia fish samples were not exceed than the permissible limits of microbial quality criteria, and they were good accepted until the end of 180 days storage period.

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