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Effect of Smoking Methods and Refrigeration Storage on Microbiological Quality of Catfish Fillets (*Clarias gariepinus*)

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Abstract

The current investigation aimed to study the impact of smoking process and refrigeration storage on microbiological load for smoked fillets of catfish. Total bacterial counts (TBC) and yeast and moulds (Y and M) of fresh fillets of Catfish products decreased after smoking process. The decline of microbiological load was greater in hot smoked than cold. Microbiological aspects of smoked catfish product gradually increased ($p<0.05$) during storage period. Appearance of yeast and moulds growths on the surface of smoked catfish products were firstly on hot products followed by cold smoked product after 35 and 40 days respectively.. Microbiological load of smoked products were within the permissible limits until end of storage period.

Keywords: Catfish; Refrigeration storage; Total bacterial count; Yeast and moulds; Smoking process

Introduction

The newly caught fish is highly perishable foods due to the action of the microorganism's activity that occurs on the surface. In fish technology, the microbiological control of raw fish and other ingredients in order to produce fishery products with good quality and safe for the consumers [1].

Total bacterial count (TBC) is an important criterion for quality evaluation of processed fish products. The maximum recommended bacterial count for good quality products is 5.7 log10 cfu/g, while the maximum recommended bacterial count for marginally acceptable quality products is 7.0 log10 cfu/g [2].

Shen [3] found that for fresh fish the total plate count was determined by <104 cells/g, for sub fresh it was 104-106 cells/g, while for the deteriorated fish, total plate count was as high as >106 cells/g. Fish is usually cooked in different methods such as boiling, smoking, roasting, frying and grilling. The processing methods improve hygienic quality by eliminating pathogenic microorganisms [4].

Materials and Methods

Fish samples

Catfish (*Clarias gariepinus*): Samples of Catfish (*Clarias gariepinus*) were obtained from Wadi EI- Rayan Lake - Fayoum Governorate - Egypt in August 2015. Length and weight Averages of fresh fish ranged between 56-60 cm and 1.8-2.3 kg, respectively. The fresh fish were transported in icebox to the laboratory of fish processing technology, Shakshouk station for fish research (NIOF), Fayoum governorate, Egypt. Samples were beheaded and gutted then washed gently with tap water and skinned then filleted manually.

Smoking process

Smoked fillets products were produced by the traditional smoking methods of Cold and Hot smoking using smoking oven at Shakshouk fish research station (NIOF). The conditions of smoking process are showed in Table 1 as found by Abd El-Mageed (Table 1) [5].

Smoked fillets products

Hot and cold smoked product samples were refrigerated at 4˚C for 40 days. During storage for analysis, Samples were taken periodically at intervals of 5 days for analysis.

Microbiological analysis

Ten grams of sample were taken aseptically from different places of fish samples and homogenized in 90 mL sterile distilled water (9.0 g NaCl/1000 mL distilled water) as described in ICMSF, [6]. The homogenized samples were used for microbial determination.

Total bacterial count (TBC): Total bacterial count (TBC) was determined by using nutrient agar medium [7]. Sterilization of media was processed by autoclave at 121°C for 15 min. 1.0 mL from the final dilution was placed on the above medium (15-20 ml) in three replication and incubated at 37 °C for 2 days. The bacterial count was calculated per one gram of sample and expressed as mean log cfu/g sample.

Yeasts and molds count: Yeasts and molds count were enumerated on malt agar as mentioned by Refai [8].

Statistical Analysis

Statistically analysis

The statistical interferences were evaluated using the following statistical tools as following: means, standard Error (SE) and One-way

Smoking parameters	Cold	Hot
Brine solution (%NaCl)	10%	10%
Brining time (h)	1 (h)	1(h)
Air drying time (h)	3 (h)	3(h)
Temperature degree (˚C)	30 - 40˚C	50 - 90˚C
Smoking time (h)	11-12 (h)	5 - 6 (h)
The fuel	Saw-dust	Saw-dust

Table 1: Conditions of smoking.

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ANOVA for comparing more than two groups which calculated using SPSS 16.0 for windows.

Results and Discussion

Microbiological load of catfish fillets

The producers of smoking process may affect the microbial gross of smoked fish products. Therefore, to assess the safety of smoked catfish fillets, the smoked fillets were analyzed for microbiological indicators as total bacterial count and yeast and mold count and the data obtained are given in Table 2.

Total bacterial count (TBC): The results showed that smoking process resulted in reduction the total bacterial count. Data showed that TBC of fresh raw samples decreased from 4.49 ± 0.051 to 3.07 ± 0.040 and 3.23 ± 0.161 log10cfu/g for hot smoked fillets and cold fillets which clearly showed that hot smoking method caused marked decline in the total bacterial count. These findings are in agreement with these reported by Yanar [9], Omojowo et al., [10] and Abd El-Mageed [5]. The reduction in total bacterial load of the smoked catfish products may be due to the actions of several factors. The antimicrobial effect of smoke constitutes, heating during smoking and partially dehydration in addition to the effect of sodium chloride in lowering the water activity of fish muscles and the harmful action of chloride ion of sodium chloride on microorganisms [11]. Meanwhile, it was showed that microbial load of cold smoked fillets was greater than that found in hot smoked fillets samples which may be due to the high temperature of hot smoking method (≥ 70°C) than in cold smoking method which was carried at 30-40°C.

Yeast and molds (Y&M): After smoking process the yeast and mold count decreased from 2.30 ± 0.069 to 1.0 ± 0.040 and 1.11 ± 0.057 for hot smoked fillets and cold smoked fillets, respectively. This observation may be attributed to the effect of concentration of salt and smoke during smoking process. The hot smoking was effective more than cold smoking in the reduction of vegetative molds and yeasts growth. Similar results were found by Idris et al., [10], Omojowo et al. [12]. Meanwhile, Abd El-Mageed [5] observed that the yeast and mould count did not detected in hot and cold smoked Silver Carp fillets immediately after smoking process.

Changes of microbiological quality during refrigeration storage

Total Bacterial Count (TBC): Microorganism's activity is the important factor limiting the shelf life and storage period of fish and fish products. Hot smoked and cold smoked fillets of Catfish were analyzed microbiologically for TBC as well as Y and M counts at zero time of storage period and during the refrigeration storage for 40 days at 4 ± 1°C. As given in Table 3 total bacterial counts for hot smoked and cold smoked fillets of Catfish at zero time were 3.07 ± 0.040 and 3.38 ± 0.161 log10 cfu/g, respectively. These initial values gradually increased ($p<0.05$) up to 5.60 ± 0.115 and 5.84 ± 0.057 log10 cfu/g, respectively after 40 days of refrigeration storage. TBC is an important criterion for quality evaluation; the maximum recommended bacterial count for good quality products is 5.70 log10 cfu/g, and the maximum recommended bacterial count for marginally acceptable quality products is 7.00 log10 cfu/g [2]. From these data, it is possible to say that total bacterial number of hot and cold smoked catfish products were not exceeded the permissible limits of acceptability during refrigeration storage even after 40 days. El-Akeel [13] explained the increasing of total bacterial number of hot and cold smoked catfish fillets during refrigeration storage period may due to the loss of both phenolic and carbonylic compounds which acted as antimicrobial agents. Similar results were found by Abd El-Mageed [5], Kolodziejska et al. [14] Yanar [9], and Frank et al., [15] who mentioned that total bacterial count of smoked fish increased during refrigeration storage (Table 3).

Yeast and molds (Y&M): The data given in Table 4 show the microbiological changes of moulds and yeasts counts of hot and cold smoked Catfish fillets during refrigeration storage. The initial counts of yeast and mold in the hot smoked and cold smoked fillets were 1.00 ± 0.040 and 1.11 ± 0.057 log10 cfu/g, respectively. These initial values increased ($p<0.05$) up to 4.75 ± 0.040 and 4.5 ± 0.028 log10 cfu/g, respectively at the end of 40 days of refrigeration storage.

Mold rather than bacterial growth is the major problem (smoked fish) because of its low water activity [16]. During storage, the low water activity of hot smoked Catfish fillets was more suitable for yeast and mold growth compared with cold smoked Catfish fillets, these led

Parameters	Fresh fillets	Catfish fillets	Cold smoked fillets	Sig.	L.S.D
		Hot smoked fillets			
TBC (log cfu/g)	4.49 ± 0.051	3.07 ± 0.040	3.23 ± 0.161	0.000	0.282
Y&M (log cfu/g)	2.30 ± 0.069	1.0 ± 0.040	1.11 ± 0.057	0.000	0.154

Data are presented as mean ± SE of 3 replicates. SE: Standard Error -Significant difference at $P<0.05$.
TBC: Total Bacterial Count (Colony Forming Unit/g); Y&M: Yeast and Moulds.

Table 2: Microbiological load of smoked Catfish fillets.

Storage time (days)	(TBC) log cfu/g		Sig.	LSD
	Hot smoked fillets	Cold smoked fillets		
0	3.07 ± 0.040	3.38 ± 0.161	0.136	0.334
5	3.84 ± 0.080	4 ± 0.086	0.248	0.236
10	4.04 ± 0.173	4.17 ± 0.098	0.549	0.396
15	4.23 ± 0.132	4.47 ± 0.144	0.288	0.339
20	4.69 ± 0.178	4.95 ± 0.202	0.390	0.539
25	5.14 ± 0.080	5.49 ± 0.121	0.074	0.292
30	5.43 ± 0.248	5.6 ± 0.080	0.550	0.521
35	5.47 ± 0.202	5.7 ± 0.161	0.424	0.516
40	5.6 ± 0.115	5.84 ± 0.057	0.137	0.258
Sig.	0.000	0.000	-	-
L.S.D	0.432	0.372	-	-

Data are presented as mean ± SE of 3 replicates. SE: Standard Error - Significant difference at $P<0.05$.

Table 3: Changes in total bacterial count (TBC) log cfu/g of smoked Catfish fillets during refrigeration storage at 4.0 ± 1°C.

Storage time (Days)	Yeast and mold count (log cfu/g)		Sig.	LSD
	Hot smoked fillets	Cold smoked fillets		
0	1.00 ± 0.040	1.11 ± 0.057	0.194	0.136
5	1.84 ± 0.069	1.00 ± 0.175	0.022	0.375
10	2.69 ± 0.190	2.46 ± 0.034	0.301	0.386
15	3.17 ± 0.051	2.6 ± 0.057	0.002	0.154
20	3.4 ± 0.190	2.77 ± 0.034	0.022	0.386
25	3.95 ± 0.127	3.23 ± 0.132	0.017	0.368
30	4.3 ± 0.063	3.9 ± 0.075	0.015	0.200
35	4.69 ± 0.109	4.27 ± 0.046	0.024	0.236
40	4.75 ± 0.040	4.5 ± 0.028	0.007	0.103
Sig.	0.000	0.000	-	-
L.S.D	0.322	0.242	-	-

Data are presented as mean ± SE of 3 replicates; SE: Standard Error - Significant difference at P<0.05.

Table 4: Changes in yeast and mold (Y&M) log cfu /g of smoked Catfish fillets during refrigeration storage at 4.0 ± 1˚C.

to appearance of yeast and mold growths on the surface of hot and cold smoked fillets after 35 and 40 days respectively. So, smoked Catfish fillets were rejected after 35 and 40 day for hot and cold smoked fillets, respectively. Daramola et al. [17] showed that the control sample were high throughout the period of storage and were even completely covered by mold at the end of the 5-week storage. In spite of the slightly reduction in moisture contents. These results coincided with those by Idris et al. [10] and Daramola et al. [17].

Fungal growth in smoke dried and sundried chela (*Laubuka dadiburjori*) after 30 and 60 days of storage was higher than frozen and canned chela this may be attributed to increase in aW of products [18]. Gandotra et al. [19] observed that growth of molds causes products deteriorate when moisture content is about 15%. This finding supports our current study. The reduction in water activity (aw<0.75) is a controlling factor of mold growth during storage (Table 4).

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