

## CHEMICAL AND MICROBIOLOGICAL EVALUATION OF SOME FISH PRODUCTS SAMPLES

El-Dengawy, R. A.<sup>1</sup>; S. M. El-Shehawy<sup>2</sup>; A. E. M. Kassem<sup>2</sup>; S. M. El-Kadi<sup>3</sup> and Zeinab S. Farag<sup>1</sup>

<sup>1</sup> Food Industries Dept., Fac. of Agric., Damietta University, Damietta, Egypt

<sup>2</sup> Food Industries Dept., Fac. of Agric., Mansoura University, Mansoura, Egypt

<sup>3</sup> Microbiology Dept., Fac. of Agric., Damietta University, Damietta, Egypt

### ABSTRACT

Different kinds of fish products namely canned, salted, smoked, and frozen fish were collected from local markets in Damietta Governorate. The aim of this study was to report if these fish products were in compatible with Egyptian standard specifications or not. Subsequently, be ensure that these fish products highly consumed in Damietta Governorate were safe foods for human or not. Total viable bacterial count, aerobic spore forming bacteria, *Staphylococcus aureus*, coliform group, *Clostridium* spp. and anaerobic spore formers producing H<sub>2</sub>S were studied. Finally, from the obtained results it could be reported that all studied fish products were compatible with their standard specifications from chemical and microbiological view except salted fish products (salted sardine and salted mullet named Feseikh) did not agree with their standard specification from microbiological aspects and may be harmful for consumers.

**Keywords:** Microbiological examinations, canned fish, salted fish, frozen fish, packaged and unpackaged smoked herring.

### INTRODUCTION

Fish are source of protein rich in essential amino acids, micro and macro elements (calcium, phosphorus, fluorine, iodine), fats that are valuable sources of energy, fat-soluble vitamins, and unsaturated fatty acids that, among other benefits, have a hypocholesterolic effect (anti-arteriosclerosis) (Usyus *et al.*, 2008). Fish is one of the most highly perishable food products and the shelf life of such products is limited in the presence of normal air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms. The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colour, odor and texture (Özogul *et al.* 2004). Spoilage of fresh and lightly preserved fish products is caused by microbial action. Microbiological spoilage of foods may take diverse forms, but all of them are a consequence of microbial growth and/or activity, which manifests itself as changes in the sensory characteristics. Fish products with high salt contents (salted fish) may spoil due to growth of halophilic bacteria or growth of anaerobic bacteria and yeasts (Gram and Huss, 1996).

The Egyptian Organization of Standardizations (EOS 2005<sup>a, b, c, d, e</sup> and EOS 2009) for microbiological aspects of fish products (canned Tuna, canned Sardines, El-Feseekh, salted Sardine, smoked fish and frozen fish), stated that these fish products shouldn't have *Clostridium*. The same author

also reported the following microbiological standards: Anaerobic spore formers producing  $H_2S$  should not exceed  $10^2$  CFU/g in smoked and salted fish, but canned fish shouldn't have it. Salted, smoked and frozen fish shouldn't have *E. coli*. Coliform group should be less than  $10^3$ ,  $10^1$  CFU/g in frozen and smoked fish, respectively. Total viable bacterial count should not exceed  $10^5$  and  $10^6$  CFU/g in smoked and frozen fish products, respectively. *Saphylococcus* should be less than  $10^3$ ,  $10^2$  and zero CFU/g in frozen, salted and smoked fish, respectively.

Sallam *et al.* (2007) reported that total volatile bases nitrogen (TVB-N) is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile basic nitrogenous compounds associated with seafood spoilage. The same author stated that (NaCl) is added to foods for its effects on sensory, functional and preservation properties. NaCl inhibits microbial growth by restriction of the available water (i.e. lowers  $a_w$ ) in the meat and fish products.

Consumption of these types of fish increasing in spring season special (Spring Festival) and some cases of poisoning occurred in the absence of adequate control on it. So, the aim of this work was to (1) evaluate the collected fish products samples (canned, salted, smoked, and frozen fish) from microbiological view, (2) answer the question concerning with the human consumption validity of such products and (3) stated a fact related to the compatibility of these fish products with EOS.

## MATERIALS AND METHODS

### Materials:

#### Fish products collected:

Four different kinds of fish products used in this study were obtained from different local markets in Damietta Governorate. The samples were collected from six different cities in Damietta Governorate. Sixteen samples of fish products were examined namely, imported canned fish (two samples of canned tuna (CT), two samples of canned sardine, (CS) and two samples of canned Mackerel, (CM)) were collected in September 2011. CT1, CS1 and CM1 were from the same factory. Salted fish (two samples of salted sardine, (SS) and two samples of salted mullet (SM)) were collected in October 2011, smoked Herring (two vacuum packed samples of smoked herring, (VPSH) and two unpacked samples of smoked herring, (UPSH)) were collected in November 2011 and two samples of imported frozen mackerel were collected in December 2011. All of samples were collected during their shelf life and were placed in cold portable insulated boxes and transported to the laboratory for microbiological and chemical examinations.

### Methods:

#### Chemical analyses:

Percentage of moisture and sodium chloride were determined using methods in AOAC (2005)

Water activity ( $a_w$ ) was theoretically calculated from the determined moisture and salt content using the following equation according to Demeyer, (1979).

If  $X < 0.1775$ ,  $a_w = 1.0014 - 0.6039x$ .

If  $X > 0.1775$ ,  $a_w = 1.0288 - 0.7614x$ .

Where:  $X = \text{NaCl \%} / \text{Moisture \%}$ .

Total volatile nitrogen (TVN) was determined according to the method mentioned by Pearson (1968). Results were expressed as mg nitrogen per 100g sample.

Acid value (AV) of fish oil extracted from dried minced fish samples was carried out according to the method of AOAC (2005) and was expressed as mg KOH/g oil.

#### **Microbiological examinations:**

The tested samples of fish products sample were aseptically opened and 10g of each sample was transferred to 90 ml of sterile water. The suspension was handily shaken for 5 minutes to prepare a 1:10 dilution. Further dilutions were prepared as needed appropriate dilution was and plated in triplicate (Özogul, *et al.*, 2006).

#### **1. Total viable bacterial count (TVBC):**

Poured plate method of Anon, (1992) was used. After preparing ten serial dilutions of fish samples, the test tubes were aseptically inoculated and vigorously agitated then 1 ml was transferred into sterile glass petri dishes in triplicates. Approximately ten ml of melted nutrient agar medium (45-50°C) was poured in each plate, then thoroughly mixed and left 10 min for solidification. The plates were incubated at 30°C for 48 hours. After the incubation period, developed colonies were counted per each plate of the same dilution. The total colonies count per gram of samples was calculated as follows: Total viable bacterial count = average number of triplicate plates of the same dilution x reciprocal of the dilution used colony forming unit (CFU)/g sample.

#### **2. Detection of coliform group:**

This test was done in two stages, where in the first step to detect the presence of acid and gas "presumptive test", and to ascertain the presence of coliform bacteria in the second step "confirmed test".

##### **2.1. Presumptive test .**

Coliform counts were estimated based on most probable number (MPN) technique using Mc crady's tables for calculating the presumptive number. Three decimal dilutions for each sample in three replicate tubes were employed, and then incubated at 37°C for 48 hours. The number of positive tubes showing acid and gas were recorded. The MPN of coliform bacteria per gram of sample was calculated from standard table according to FDA, (1992).

##### **2.2. Confirmed test (APHA, 1998).**

From positive presumptive tubes, inoculation was made onto Eosin Methylene Blue (EMB) agar plates. The metallic sheen colonies as well as typical coliform colonies were recorded as positive confirmed test. Confirmed organisms (typical coliform colonies) were transferred into MacConkey broth and onto agar slants. After incubation at 37°C for 24-48 hours, the production of acid and gas in the broth medium and the presence of Gram negative short rods in smears prepared from slants were considered as a positive test.

### **3. Detection of *Staphylococcus aureus*:**

*Staphylococci* was determined as described in APHA (1998) by inoculation of 1.0 ml sample on the surface of Staph medium No. 110 (Oxoid, 2006) plates, then incubated at 37°C for 24 hours. The growing colonies had yellow zones, flat and 1.2 mm diameters.

#### **Identification tests of *Staphylococci***

Representative colonies were picked up and transferred to brain heart infusion broth tubes and incubated at 37°C for 48 hours. Two slants were made, one as a storing culture and the other for catalase test and other tests on nutrient agar. Isolates were microscopically examined after Gram staining to ensure purity. Catalase, coagulase, gelatinase and sugar fermentation tests were performed (Robert and Noel, 1981).

### **4. Detection of aerobic spore forming bacteria.**

The dilutions were pasteurized at 80°C for 20 min. in water bath, then the dilutions from  $10^{-3}$  to  $10^{-6}$  were plated on nutrient agar medium and incubated at 30°C for 3 days (Kilinc and Cakli, 2004).

### **5. Detection of anaerobic spore forming bacteria.**

Dilution frequency technique was adopted to determine the densities of anaerobic spore forming clostridia, using Cooked Meat Medium (CMM), in 5 tubes for each dilution. The inoculated tubes were sealed with sterile mixture of Vaseline and Paraffin oil in 1:1 ratio and incubated at  $35\pm 2^{\circ}\text{C}$  for up to 7 days. The presence of clostridia was detected at the end of the incubation period by accumulation of gases pushing the vaspar layer up (Difco, 1974).

### **6. Detection of anaerobic spore formers producing $\text{H}_2\text{S}$ :**

Dilution frequency technique was adopted to determine the densities of anaerobic spore forming bacteria producing  $\text{H}_2\text{S}$ , using peptone iron agar (PIA), in 5 tubes for each dilution. The inoculated tubes were sealed with sterile mixture of Vaseline and Paraffin oil in (1:1) ratio and incubated at 55 °C for 3-5 days. After incubation period the number of black tubes was counted (Oxoid, 2006).

## **RESULTS AND DISCUSSION**

### **Chemical evaluation of canned fish samples:**

Data in Table 1 show that moisture percentages in all canned fish samples ranged between  $52.41\pm 0.035$  to  $78.53\pm 0.142$  %. It could be observed that all canned fish samples had high values of water activity (0.990-0.999). Meanwhile, NaCl content ranged between  $0.13\pm 0.000$  to  $1.20\pm 0.042$  %. TVN values in canned fish samples ranged between  $7.01\pm 0.254$  mg N/100g sample to  $18.04\pm 0.593$  mg N/100g sample. In addition, acid value of canned fish samples had the highest value of AV being  $20.39\pm 0.000$  mg KOH/g oil in  $\text{CM}_1$ .

**Table 1: Chemical characteristics of studied canned fish samples**

Canned fish samples	Moisture %	Water activity	NaCl % (W.W.)	TVN mg/100g	AV mg KOH/g oil
CT <sub>1</sub>	69.34±0.802	0.992±0.000	1.03±0.000	13.65±0.503	3.40±0.090
CT <sub>2</sub>	74.55±0.458	0.992±0.0003	1.12±0.052	10.58±0.277	5.01±0.170
<b>T test</b>	-5.647**	-1.000 <sup>N.S</sup>	-1.735 <sup>N.S</sup>	5.344**	-8.370**
CS <sub>1</sub>	52.41±0.035	0.988±0.001	1.13±0.013	17.69±0.597	2.78±0.085
CS <sub>2</sub>	53.07±0.992	0.999±0.000	0.13±0.000	18.04±0.593	1.52±0.095
<b>T test</b>	-0.658 <sup>N.S</sup>	-8.875**	75.25**	-0.416 <sup>N.S</sup>	9.963**
CM <sub>1</sub>	78.53±0.142	0.993±0.000	1.08±0.026	7.01±0.254	20.39±0.000
CM <sub>2</sub>	67.53±0.361	0.990±0.0003	1.20±0.042	8.38±0.207	9.78±0.065
<b>T test</b>	28.368**	8.000**	-2.609*	-4.194**	163.308**

Mean values ± standard error (n=3). \* means significant at P> 0.05, \*\* means significant at P> 0.01, N.S means there are no significant differences.

From results, it was clear that the main preservative factors in such canned products was thermal process and anaerobic conditions used during fish processing, so NaCl did not play any role in canned fish preservation. As for statistical analysis, there were highly significant differences between each couple of canned fish in acid value. In case of canned mackerel, there were significant differences between CM<sub>1</sub> and CM<sub>2</sub> in all chemical characteristics. These results are in disagreement with those obtained by USDA, (2011), while TVN values were in permissible limits stated by EOS (2005a and b).

**Microbiological evaluation of canned fish samples:**

Values of microbiological examinations of canned fish samples are presented in Table 2. Listed results showed that CT and CS samples had the lowest total viable bacterial count, but CM sample had the highest count being 2.5±0.088 ×10<sup>2</sup> and 4.6±0.208 ×10<sup>3</sup> CFU/g in CM<sub>1</sub> and CM<sub>2</sub>, respectively. Similarly, CM samples had the highest value of aerobic spore forming bacteria being 0.23±0.033 ×10<sup>2</sup> and 0.26±0.033 ×10<sup>2</sup> CFU/g in CM<sub>1</sub> and CM<sub>2</sub>, respectively. Aerobic spore forming bacteria is not detected in CT samples. *Staphylococcus aureus*, coliform group, *Clostridium sp.* and anaerobic spore formers producing H<sub>2</sub>S were not detected in all samples. These results are in agreement with EOS (2005<sub>a and b</sub>). It was observed that, there was a relationship between the TBC and TVN; all canned samples had low values of TVN, this maybe due to low values of TBC. EOS (2005<sub>a and b</sub>) stated that CT and CS shouldn't have *Clostridium* or anaerobic spore forming bacteria producing H<sub>2</sub>S. It was obvious that total viable count and aerobic spore formers had no significant differences for all samples, except total viable count for canned mackerel samples which had highly significant differences between the studied two samples.

**Table 2: Microbiological evaluation of canned fish samples.**

Tested bacterial groups	Bacterial count (CFU×10 <sup>2</sup> /g samples)									EOS, 2005
	Canned tuna (CT)		T test	Canned sardine (CS)		T test	Canned mackerel (CM)		T test	
	CT <sub>1</sub>	CT <sub>2</sub>		CS <sub>1</sub>	CS <sub>2</sub>		CM <sub>1</sub>	CM <sub>2</sub>		
Total viable count	(0.03±0.033)	ND	1.00 <sup>N.S</sup>	0.06±0.033	0.3±0.100	-2.214 <sup>N.S</sup>	2.5±0.088	4.6±0.208	-8.994 <sup>**</sup>	--
Aerobic spore formers	ND	ND	--	0.03±0.033	0.03±0.033	0.000 <sup>N.S</sup>	0.23±0.033	0.26±0.033	-0.707 <sup>N.S</sup>	--
Anaerobic spore formers producing H <sub>2</sub> S	ND	ND	--	ND	ND	--	ND	ND	--	Free
<i>Staphylococcus aureus</i>	ND	ND	--	ND	ND	--	ND	ND	--	--
<i>Coliform group</i>	ND	ND	--	ND	ND	--	ND	ND	--	--
<i>Clostridium</i> spp.	ND	ND	--	ND	ND	--	ND	ND	--	Free

: ND = Not detected.

Mean values ± standard error (n=3). \* means significant at P> 0.05, while \*\* means significant at P> 0.01, N.S means there are no significant differences.

**Chemical evaluation of salted fish samples:**

Moisture content of different salted fish samples ranged from 45.62±1.444 to 57.92±0.553 % as given in Table 3. It was clear relationship between moisture content, NaCl % and consequently water activity, where increasing of NaCl content of fish flesh, decreasing of moisture content and water activity. So, it was clear that SM<sub>2</sub> had the highest content of moisture, the least value of salt (5.64%) and directly the highest value of water activity (0.9415). SM<sub>1</sub> had the highest value of TVN (40.79mg/100g). All salted samples had high value of AV except SM<sub>2</sub> sample which had a value of 3.82±0.122 mg KOH/g oil.

As for statistical analysis, there were highly significant differences between each couple of salted fish in moisture content, water activity, NaCl%, TVN and AV. These results mean that there were no homogeneity or standard rules in these salted fish manufacturing.

**Table 3: Chemical characteristics of salted fish samples**

Salted fish samples	Moisture %	Water activity	NaCl % (W.W.)	TVN mg/100g	AV mg KOH/g oil
SS <sub>1</sub>	45.62±1.444	0.7879±0.000	14.43±0.000	24.82±0.000	15.37±0.232
SS <sub>2</sub>	51.06±1.055	0.8317±0.002	13.75±0.107	21.41±0.000	45.59±1.367
T test	-4.183 <sup>**</sup>	-28.543 <sup>**</sup>	6.406 <sup>**</sup>	34.048 <sup>**</sup>	-21.787 <sup>**</sup>
SM <sub>1</sub>	50.88±0.471	0.7939±0.002	15.48±0.154	40.79±0.613	36.81±0.737
SM <sub>2</sub>	57.92±0.553	0.9415±0.0002	5.64±0.026	21.42±0.443	3.82±0.122
T test	-10.663 <sup>**</sup>	-63.071 <sup>**</sup>	62.923 <sup>**</sup>	25.595 <sup>**</sup>	44.125 <sup>**</sup>

Mean values ± standard error (n=3). \* means significant at P> 0.05, while \*\* means significant at P> 0.01, N.S means there are no significant differences.

These results are in good agreement with those obtained by Chouliara et al., (2004) who found that a much higher TVBN level (60.5 mg N/ 100 g) was reported by day 42 in vacuum-packaged, salted sea bream stored under refrigeration at 4°C.

**Microbiological evaluation of salted fish samples:**

Data in Table (4) showed that the values of total viable bacterial count were  $57.33 \pm 5.897 \times 10^4$ ,  $17.3 \pm 9.279 \times 10^4$ ,  $0.216 \pm 0.006 \times 10^4$  and  $0.49 \pm 0.058 \times 10^4$  CFU/g in SM<sub>1</sub>, SM<sub>2</sub>, SS<sub>1</sub> and SS<sub>2</sub>, respectively. SM<sub>1</sub> had the highest value of TVN (Table (3)); this may be due to the activities of proteolytic bacteria. The high content of NaCl (in the case of SM<sub>1</sub> being 15.48) in salted fish samples may induce halophilic bacteria, where the total bacterial count reached the maximum value in this sample being  $57.33 \times 10^4$  CFU/g. It was observed that the highest acid value (AV) in the case of SS<sub>1</sub>, SS<sub>2</sub> and SM<sub>1</sub> may be due to the lipolytic bacteria which hydrolyze fish oil and liberate free fatty acids. Salted mullet had the highest value of aerobic spore formers being  $7.33 \pm 2.186 \times 10^4$  and  $0.1 \pm 0.100 \times 10^4$  CFU/g in SM<sub>1</sub> and SM<sub>2</sub>, respectively. While SS<sub>1</sub> and SS<sub>2</sub> contained  $0.0066 \pm 0.006 \times 10^4$  and  $0.17 \pm 0.058 \times 10^4$  CFU of aerobic spore forming bacteria per gram, respectively. All samples were free from *Staphylococcus aureus* and *E. coli*. These results are in agreement with EOS, (2005<sub>c and d</sub>). *Clostridium* sp. count were  $0.360 \pm 0.000 \times 10^4$ ,  $9.300 \pm 0.000 \times 10^4$ ,  $9.300 \pm 0.000 \times 10^4$  and  $4.300 \pm 0.000 \times 10^4$  CFU in SS<sub>1</sub>, SS<sub>2</sub>, SM<sub>1</sub> and SM<sub>2</sub>, respectively. This may be due to the conditions of production or storage which was anaerobic. Anaerobic spore forming bacteria producing H<sub>2</sub>S was  $1.500 \pm 0.000 \times 10^4$  CFU in SS<sub>2</sub>, while it was not detected in other samples. These results are in agreement with those obtained by Nassar (2001) who found that TVBC reached to  $0.8 \times 10^6$  CFU of pre-fermented mullet with 25% dry salting at the end of storage period.

**Table (4): Microbiological evaluation of salted fish samples.**

Tested bacterial groups	Bacterial count, CFU×10 <sup>4</sup> /g samples						EOS, 2005
	Salted Sardine (SS)		T test	Salted mullet (SM)		T test	
	SS <sub>1</sub>	SS <sub>2</sub>		SM <sub>1</sub>	SM <sub>2</sub>		
Total viable bacterial count	0.216±0.006	0.49±0.058	-4.635**	57.33±5.897	17.3±9.279	6.700**	---
Aerobic spore formers	0.0066±0.006	0.17±0.058	-2.808*	7.33±2.186	0.1±0.100	2.635*	---
Anaerobic spore formers producing H <sub>2</sub> S	ND	1.500±0.000	--	ND	ND	--	10 <sup>2</sup>
<i>Staphylococcus aureus</i>	ND	ND	--	ND	ND	--	Free
<i>E. coli</i>	ND	ND	--	ND	ND	--	Free
<i>Clostridium</i> spp.	0.360±0.000	9.300±0.000	--	9.300±0.000	4.300±0.000	--	Free

: ND = Not detected. Mean values ± standard error (n=3). \* means significant at P> 0.05, while \*\* means significant at P> 0.01, N.S means there are no significant differences.

As for statistical analysis, there were highly significant differences (P>0.01) between each couple of salted fish in case of total viable bacterial count, but there were significant differences (P>0.05) between each couple of salted fish in case of aerobic spore formers. EOS, (2005<sub>c and d</sub>) stated that salted sardine and salted mullet shouldn't have *Clostridium* and *E. coli*, while

both of anaerobic spore-forming bacteria producing H<sub>2</sub>S and staphylococcus shouldn't exceed 10<sup>2</sup> CFU/g.

**Chemical evaluation of smoked fish samples:**

Data illustrated in Table (5) showed that moisture content, water activity, NaCl%, TVN and AV in smoked fish samples. Moisture content and water activity ranged from 57.03±0.224 to 63.32±0.845 and from 0.923±0.001 to 0.945±0.001, respectively. VPSH<sub>1</sub> had the highest value of TVN (42.11±0.000 mg/100g). All of herring samples ranged from 20.92±0.517 to 26.24±0.512 mg KOH/g oil for AV. As for statistical analysis, there were significant differences (*P*>0.05) between all smoked fish in all studied chemical parameters except in case of TVN. These results are in good agreement with those obtained by El-Sherbieny (2003) who found that water activity (a<sub>w</sub>) decreased from 0.989 to 0.882 in smoked herring.

**Table 5: Chemical evaluation of smoked fish samples**

Smoked fish samples	Moisture (%)	Water activity	NaCl (%)		TVN mg/100g	AV mg KOH/g oil
			As wet weight			
UPSH <sub>1</sub>	57.04 <sup>b</sup> ±1.059	0.929 <sup>c</sup> ±0.001	6.82 <sup>b</sup> ±0.050		26.22±0.000	26.24 <sup>a</sup> ±0.512
UPSH <sub>2</sub>	63.32 <sup>a</sup> ±0.845	0.945 <sup>a</sup> ±0.001	5.96 <sup>c</sup> ±0.043		24.65±0.000	22.54 <sup>b</sup> ±0.519
VPSH <sub>1</sub>	57.03 <sup>b</sup> ±0.224	0.923 <sup>d</sup> ±0.001	7.43 <sup>a</sup> ±0.072		42.11±0.000	26.11 <sup>a</sup> ±0.475
VPSH <sub>2</sub>	62.07 <sup>a</sup> ±0.838	0.934 <sup>b</sup> ±0.001	6.88 <sup>b</sup> ±0.023		25.49±0.000	20.92 <sup>c</sup> ±0.517

Means of treatments having the same letter(s) within a column are not significantly different (*P*> 0.05).

**Microbiological evaluation of smoked fish samples:**

From tabulated data in Table 6, Unpackaged smoked herring samples had higher total viable bacterial count more than vacuum packaged smoked herring samples. (UPSH) samples 1 and 2 had the highest total count being 246±8.800x10<sup>4</sup> and 109±7.40x10<sup>4</sup> CFU/g, respectively. Meanwhile, VPSH<sub>1</sub> and VPSH<sub>2</sub> samples had 128±11.400x10<sup>4</sup> and 2.45±0.029x10<sup>4</sup> CFU/g, respectively. It was observed that, VPSH<sub>1</sub> sample had the highest value of aerobic spore forming and TVN (Table 5) being 4.3±2.404x10<sup>4</sup> CFU/g and 42.11mg%, respectively. This may be due to the proteolytic bacteria.

**Table 6: Microbiological evaluation of smoked fish samples.**

Tested bacterial groups	Bacterial count, CFU x10 <sup>4</sup> /g samples				EOS, 2005
	Unpackaged smoked herring (UPSH)		Vacuum packaged smoked herring (VPSH)		
	UPSH <sub>1</sub>	UPSH <sub>2</sub>	VPSH <sub>1</sub>	VPSH <sub>2</sub>	
Total viable bacterial count	246 <sup>a</sup> ±8.800	109 <sup>b</sup> ±7.400	128 <sup>b</sup> ±11.400	2.45 <sup>c</sup> ±0.029	<10 <sup>5</sup>
Aerobic spore forming	0.0166 <sup>b</sup> ±0.006	0.040 <sup>b</sup> ±0.031	4.3 <sup>a</sup> ±2.404	0.0033 <sup>b</sup> ±0.003	---
Anaerobic spore formers producing H <sub>2</sub> S	ND	ND	ND	ND	<10 <sup>2</sup>
<i>Staphylococcus aureus</i>	ND	ND	0.010±0.006	ND	Free
<i>E. coli</i>	ND	ND	ND	ND	Free
<i>Clostridium</i> sp.	ND	ND	24.0±0.000	ND	Free

: ND = Not detected.

Means of treatments having the same letter(s) within a column are not significantly different (*P*> 0.05).

The count of *Staphylococcus aureus* and *Clostridium* sp. for VPSH<sub>1</sub> sample were 0.010±0.006x10<sup>4</sup> and 24.0±0.000x10<sup>4</sup> CFU/g, respectively, this maybe due to the anaerobic conditions of VPSH samples. Other samples were free

from *Staphylococcus aureus* and *Clostridium* sp. Also, *E. coli* and anaerobic spore forming produce H<sub>2</sub>S count were not detected in all samples. These results are in agreement with those obtained by Dondero *et al.*, (2004).

As for statistical analysis, there were significant differences ( $P > 0.05$ ) between all smoked fish in case of total viable bacterial count and aerobic spore formers. EOS, (2005e) stated that smoked fish shouldn't have *Clostridium*, *staphylococcus* and *E. coli*, while aerobic bacteria and anaerobic spore forming produce H<sub>2</sub>S shouldn't exceed 10<sup>5</sup> and 10<sup>2</sup> CFU/g, respectively.

**Chemical evaluation of frozen fish samples:**

Moisture content, water activity and NaCl% of collected frozen mackerel were shown in Table 7. From tabulated data, it could be noticed that moisture content was 69.45±0.437 and 68.66±0.905 in FM<sub>1</sub> and FM<sub>2</sub>, respectively. Frozen mackerel samples had low content (%) of NaCl (0.23±0.000) in FM<sub>1</sub> and (0.31±0.0000) in FM<sub>2</sub> as wet weight. TVN values of all frozen samples did not exceed the permissible limit of the EOS (2009) which reported that, total volatile nitrogen of frozen fish must not be more than 25 mgN/100g sample. Acid value for frozen mackerel samples were 14.87±0.434 of FM<sub>1</sub> and 20.28±0.195 of FM<sub>2</sub>. There were significant differences between the two frozen sample in case of TVN and AV. These results are in agreement with EOS (2009), but in disagreement with those obtained by Sahari *et al.*, (2009).

**Table (7): Chemical evaluation of frozen fish samples:**

Frozen Mackerel samples	Moisture %	Water activity	NaCl % As wet weight	TVN mg/100g	AV mg KOH/g oil
FM <sub>1</sub>	69.45±0.437	0.999±0.000	0.23±0.000	13.34±0.000	14.87±0.434
FM <sub>2</sub>	68.66±0.905	0.998±0.000	0.31±0.000	16.23±0.000	20.28±0.195
T test	0.783 <sup>N.S</sup>	---	---	-111.929	-11.358
EOS, (2009)				25	

Mean values ± standard error (n=3). \* means significant at  $P > 0.05$ , while \*\* means significant at  $P > 0.01$ , N.S means there are no significant differences.

**Microbiological evaluation of frozen fish samples:**

Table 8 show that, frozen mackerel samples had total viable bacterial count less than the permissible limit (10<sup>6</sup> CFU/g), these values were 4.7±6.250x10<sup>3</sup> and 99±1.800 x10<sup>3</sup> CFU/g FM<sub>1</sub> and FM<sub>2</sub> samples, respectively. These results are in agreement with EOS (2009). Aerobic spore forming bacteria were 0.044±0.044x10<sup>3</sup> and 1.30±0.760x10<sup>3</sup> CFU/g for FM<sub>1</sub> and FM<sub>2</sub> samples, respectively. There was a relationship between the high content of TVBC and aerobic spore forming bacteria and the high value of AV, this may be due to the activities of lipolytic bacteria. *Staphylococcus aureus*, *E. coli* and *Clostridium* sp. were not detected in all samples. Anaerobic spore forming bacteria producing H<sub>2</sub>S reached the highest count being 1.380±0.000x10<sup>3</sup> CFU/g in FM<sub>2</sub>. These results were in agreement with those obtained by Kilinc and Cakli (2004) and Özogul *et al.*, (2004).

**Table 8: Microbiological evaluation of frozen fish samples.**

Tested bacterial groups	Bacterial count, CFU x10 <sup>3</sup> /g samples			
	Frozen mackerel (FM)		T test	EOS, 2009
	FM <sub>1</sub>	FM <sub>2</sub>		
Total viable bacterial count	4.7±6.250	99±1.800	-7.056**	<10 <sup>5</sup>
Aerobic spore formers	0.044±0.044	1.30±0.760	-1.000 <sup>N.S</sup>	No limit
Anaerobic spore formers producing H <sub>2</sub> S	ND	1.380±0.000	--	No limit
<i>Staphylococcus aureus</i>	ND	ND	--	10 <sup>3</sup>
<i>E. coli</i>	ND	ND	--	Free
<i>Clostridium</i> sp	ND	ND	--	Free

: ND = Not detected.

Mean values ± standard error (n=3). \* means significant at P> 0.05, while \*\* means significant at P> 0.01, N.S means there are no significant differences.

Statistical analysis showed that there were significant differences between the two frozen sample in total viable count. **(EOS) 2009** stated that frozen fish shouldn't have *Clostridium* and *E. coli*, while aerobic bacteria and *staphylococcus aureus* shouldn't exceed 10<sup>6</sup> and 10<sup>3</sup> CFU/g, respectively.

**Staphylococci identification from studied fish product samples:**

Biochemically identified as shown in Table (9). Typical colonies representing staphylococci growth, white, yellow or orange colored colonies, were picked up and streaked onto nutrient agar slant. After growth, Gram positive, spherical cells, arranged in irregular clusters, catalase producer growth were considered as *Staphylococcus* isolates. Confirmed staphylococci isolates biochemically identified as shown in Table(9) according to Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994). Accordingly, the species were identified as *S. aureus* and *S. epidermidis* (Queck and Otto, 2008).

**Table 9: Morphological and biochemical reaction of *Staphylococcus spp* of studied fish product samples.**

Strain	<i>S. epidermidis</i>	<i>S. aureus</i>
Colony color	White	Yellow
Gram stain	+	+
Catalase	+	+
Coagulase	-	+
Oxidase	+	+
Mannitol	-	+
Lactose	+	+
Glucose	+	+
Gelatinase	-	+

**CONCLUSION**

Finally it could be concluded that *Staphylococcus aureus*, coliform group, *Clostridium* sp. and anaerobic spore-forming bacteria producing H<sub>2</sub>S were not detected in all canned fish samples. *E.coli* and anaerobic spore-forming bacteria producing H<sub>2</sub>S count were not detected in all smoked

samples. Frozen mackerel samples had total viable bacterial count less than the permissible limit. Salted fish samples were not compatible with EOS (2005<sub>c</sub> and <sub>d</sub>) from microbiological view, where it contained *Clostridium spp.* and these salted fish may be harmful in human nutrition.

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التقييم الكيماوي و الميكروبيولوجي لعينات من منتجات الأسماك  
رزق أحمد الدنجاوي<sup>1</sup> ، شادي محمد الشهاوي<sup>2</sup>، أحمد عزت منصور قاسم<sup>2</sup>،  
شريف محمد القاضي<sup>3</sup> و زينب صبري فرج<sup>1</sup>  
<sup>1</sup> قسم الصناعات الغذائية - كلية الزراعة - جامعة دمياط - مصر  
<sup>2</sup> قسم الصناعات الغذائية - كلية الزراعة - جامعة المنصورة - مصر  
<sup>3</sup> قسم الميكروبيولوجي - كلية الزراعة - جامعة دمياط - مصر

تم تجميع أربعة أنواع من منتجات الأسماك المصنعة و هي الأسماك المعلبة و المملحة و المدخنة و المجمدة من الأسواق المحلية لمدن محافظة دمياط. و تهدف الدراسة إلى تقرير ما إذا كانت هذه المنتجات متوافقة مع المواصفات القياسية المصرية أم لا. و بالتالي التأكد من أن هذه المنتجات الغذائية التي تستهلك بكثرة في محافظة دمياط أثناء عيد الربيع آمنة غذائياً. فقد تم دراسة العد الكلي للبكتريا الحية ، البكتريا الهوائية المتجترمة *Staphylococcus aureus* ، *Bacillus spp* ، مجموعة بكتريا القولون ، *Clostridium spp.* و البكتريا المتجترمة اللاهوائية المنتجة لكبريتيد الهيدروجين. و قد أوضحت النتائج أن جميع المنتجات السمكية تحت الدراسة كانت متوافقة مع المواصفات القياسية المصرية الخاصة بها من الناحية الكيمائية و الميكروبيولوجية فيما عدا المنتجات السمكية المملحة (السردين المملح - البوري المملح المسمى الفسيخ) لا تتوافق مع المواصفة القياسية من الناحية الميكروبيولوجية و قد تكون ضارة للمستهلكين.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة  
كلية الزراعة - جامعة دمياط

أ.د / عايده حافظ عفيفي  
أ.د / حسين عبد الله محمد الفضالي