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Some quality parameters of marketed frozen Mackerel and Sardine fish in Kafr El-Sheikh governorate

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Abstract

Fish has high consumer preference due to its inherent nutritive value, taste and easy digestibility. It is one of the most important sources of animal protein available and has widely been accepted as a good source of animal protein and other elements. The present study was planned to estimate the quality of frozen mackerel and sardine for that aim, a total of 70 samples from frozen fish (35 Mackerel and 35 Sardine) were collected from different retail markets in Kafr El-Sheikh governorate and subjected to bacteriological &chemical examination. The results of bacterial examination revealed that the mean of total bacterial count (TBC) was $8.3\times10^5\pm1.2\times10^5$cfu/g and $2.8\times10^5\pm5.2\times10^4$cfu/g, while the mean of total *coliform count* (TCC) were $1.4\times10^2\pm2.1\times10^1$cfu/g and $1\times10^2\pm1.1\times10^1$cfu/g, for Mackerel and Sardine respectively. Their identification revealed that *P. aeruginosa* were present by 25% and 28.6%, *K. pneumonae* were present by37.5% and 37.1% and *S. liquefaciens* were present by 22.5% and 22.9%, in both Mackerel and Sardine samples respectively. While specific fish pathogens such as *E. coli* was detected in Mackerel samples under the investigation by 17.1% and identified as *E. coli O111* (15.4%), *E. coli O158* (23%), *E. coli O78* (30.8%) and *E. coli O27* (30.8%), while it was detected in 11.4% in Sardine samples and identified as *E. coli O166* (33.3%) and *E. coli O125* (66.7%). Also, the *Staphylococci* were determined in Mackerel samples by 77% and in Sardine samples by 71% and the mean count of *Staphylococci* were $2.4\times10^4\pm1\times10^4$ cfu/g and $5.7\times10^3\pm1.1\times10^3$cfu/g, in Mackerel and Sardine samples respectively. Their identification revealed that *Staph aureus* was present by 7.4% and 0%, *Staph vitulanus* were present by 48.2% and 60% and *Staph warneri* were 44.4% and 40%, in Mackerel and Sardine samples respectively. While the results of chemical examination cleared that the mean value of pH were 6.03±0.07 and 5.67±0.06 for Mackerel and Sardine samples respectively, while the mean value of TVB-N were 30.06±1.63 mg/100 mg and 27.41±1.12 mg/100mg and the percentage of rejected samples were 57 and 49 in Mackerel and Sardine respectively. More over the mean value of TMA-N were 8.89±0.38 mg/100 mg and 9.81±0.39 mg/100 mg, and the percentage of rejected samples were 34 and 40 in Mackerel and Sardine respectively. Finally the mean value of TBA were 4.58±0.09 mg/kg and 4.55±0.15 mg/kg and the percentage of rejected samples were 34 and 37 and the percentage of rejected samples for all parameters were 42 and 43 in Mackerel and Sardine respectively, Also in this study the histamine content were lower than 5 mg/100 gm in all examined samples.

Keywords: *Frozen mackerel, frozen sardine, quality, freshness*

Introduction

Fish are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world **(Abisoye *et al*., 2011).** In tropical countries, any short fall in fish availability will affect the animal protein intake of people **(Salawu *et al*., 2004).** The fisheries sector plays an important socio-economic role because it provides a big part of the needed animal protein intake. It is also a source of income for families and the govern-

ment **(Portaria, 2009).**

Consuming fish provides an important source of protein, polyunsaturated fatty acids (PUFA), liposoluble vitamins and essential minerals, which are associated with health benefits and normal growth **(Verbeke *et al*., 2007).** According to FAO statistics, fish accounted for about 16% of the global population's intake of animal protein and 6% of all protein consumed **(FAO, 2010).**

Microbiological methods are used to estimate bacterial numbers, in order to determine fish freshness, hygiene and or evaluate the possible presence of bacteria or organisms of public health importance **(Huss, 1994).**

Several studies have been done on the effect of freezing on the quality of fish. However, less attention has been given to the changes of frozen-thawed fish (sensorial, microbial, chemical and physical) during storage in ice **(Magnusson and Martinsdottir, 1995).**

The freezing of fish is an effective way of long -term preservation and diminishing spoiling rate. For fatty fish controlling the lipid oxidation is vital and the best way of doing that is by freezing the fish before damages begins and keeping the temperature as low as possible from catching until final storage **(Huss, 1995).** Also freezing is one of the easiest, quickest, most versatile and most convenient methods of food preserving. Properly frozen food maintains more of their original colour, flavor, texture and nutrients than foods preserved by other methods **(Julie, 2013).** To meet the requirements for healthy human diet it is important not only to acquire meat with desirable fatty acids but also to preserve it the best, thus freezing is considered to be an excellent mean aid for maintaining meat quality for long periods and frozen storage has been regarded as useful technological aid **(Mateo-Oygue and Perez-Chabela, 2004)).**

Freshness is the one of the most important aspects of fish and fish products and it is a key element in the quality assessment of fish by consumer **(Luten and Martinsdottir, 1997** and **Martinsdottir, 2004).** The freshness of the raw material used for processing is essential for the over all quality of the final product so, the freshness of seafood can be evaluated by chemical, physical, microbiological or sensory methods. However, the sensory analysis has been the primary way to evaluate seafood freshness by the fisherman, producers, researchers and consumers. **(Oehlenschlager and Sorensen, 1997).**

Temperature and handling practices are the most important factors in determining the shelf life of the fish. If the fish is handled carefully and the temperature kept low and stable the shelf life is extended **(Doyle, 1995).**

Mackerel is a highly perishable commodity recording considerable losses in quality before consumption. As factors influence spoilage of mackerel as ambient temperature, age of raw material before processing, and storage conditions in addition to biological factors like seasonal variations in lipid content and sea temperature. Storage time and temperature are the major factors implicated in the loss of quality and shelf life of fatty fishes **(Huss, 1995).**

Usually, small and medium size fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. In general, these species are chilled or frozen whole soon after capture. Storage time depends on the fat content of the fish and the amount of food in the gut. Shelf life of fat fish species is generally shorter (around 2-8 days) than of low fatty fish species (7-15 days) **(Huss, 1995 and Martinsdottir and Magnusson, 2001).**

Certain species deteriorate faster than others mainly due to the presence of chemical constituents in their body tissue **(Jhaveri *et al*., 1982).** Handling of fresh mackerel has remained a problem because of its soft flesh, high lipid content and the skin of fatty pelagic fish is often very thin, this allows enzymes and bacteria to penetrate more quickly **(Hyldig *et al*., 2007).**

The chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning **(Huss, 1995).** The edible portion of mackerel is composed of 18.7% protein and 11.4% fat **(FAO, 1989).**

Histamine poisoning is a food-borne chemical intoxication resulting from the ingestion of food that contains unusually high levels of histamine. The scombroid fish are commonly involved in histamine poisoning because they possess large amounts of free histidine in their muscle tissues that serve as a substrate for bacterial histidine decarboxylase **(Okuzumi *et al*., 1982** and **Taylor 1986**).

The primary symptoms of histamine intoxication are cutaneous (rash, urticaria, oedema, localized inflammation), gastrointestinal (nausea, vomiting and diarrhea), hemodynamic (hypotension) and neurological (headache, oral burning and blistering sensation etc.) **(Taylor, 1986** and **Huss *et al*., 2003).** So, the present study aims to evaluate the microbiological and biochemical analysis of imported frozen Mackerel and Sardine fish from a view of public health safety and international trade.

Material and Methods

Collection of samples:

Seventy fish samples (35 mackerel and 35 sardine) were randomly collected from Kafr El-Sheikh markets. Each sample was packed in polyethylene bags and put in an insulated ice-box filled with crushed ice, then immediately transferred without delay to laboratory for bacteriological and chemical evaluations.

A- Sensory analysis of frozen fish owing to morphological characters: was assessed according to the method of **Paulus (1979).**

Quality parameter		Mackerel	Sardine
Appearance, texture	*Skin, back *Skin, abdomen *Texture, back	Strong blue and iridescent color Pearly/white colour Firm and elastic	Bright with metallicsheen and iridescent color
Eyes	*Pupil *Cornea *Shape	Black Bright Flat	Sparkling black clear convex
Gills	*Filaments *Odour *Colour	Close - coherent Seaweed, metallic, ocean Liver red	Close - coherent With absence of red spots blood-red
Abdomen	*Texture	Slightly soft	strong
Peritoneum		Adherent	Adherent and strong
Viscera	*status, bones	Whole and bright (bone embedded within flesh)	Whole and bright

B- Bacteriological examination:

Preparation of the Samples (ISO/IEC, 1999):

Each closed (sealed) sample package was thawed by holding it in refrigerator overnight at 5°C then, accurately 10g from each sample was aseptically cut and blended with 90ml sterile normal saline, then one ml of the homogenate was aseptically transferred into 9 ml normal saline in test tube. Similarly, further dilutions required for inoculation was prepared by this serial dilution process. Then all samples subjected for the following examination:

1-Total bacterial count according to **USDA (1998):** Colonies were enumerated on standard plate count agar after incubation at 37°C overnight.

2- Total *Coliform* count according to **USDA (1998)**: Colonies were enumerated on Violet Red Bile Agar (VRB agar) media and incubated at 37°C over night.

3- Biochemical Identification of *Coliform* Bacteria: It was carried out according to **(Kreig and Holt, 1984).**

4-Isolation and identification of *E. coli*: It was carried out according to **AOAC (1999)**. Using Eosin-Methylene Blue agar (EMB) at 37°C for 24 hrs.

5-Serodiagnosis of *E. coli* according to **Kok *et al.* (1996):** The isolates were serologically

identified by using rapid diagnostic *E. coli* **O** antisera sets. In Animal Health Research Institute (El-Doki).

6- Total *staphylococci* count according to **FDA (2001).** By using Baird parker agar media and incubated at37°C for 48 hrs.The obtained colonies were picked up and stored in semisolid agar for further identification, morphologically, microscopically and biochemically according to **MacFaddin (2000).**

C- Chemical evaluation:

1- Determination of Hydrogen ion concentration (pH): It was carried out according to (**Allen *et al.*, 1997**). The pH value was determined by using an electrical pH meter (Bye model 6020, USA).

2- Determination of Histamine: according to **ES 2006/1-2760.**

3- Determination of Total Volatile Nitrogen (TVB-N): It was carried out according to **ES 2006/1-2760**. For determination of total volatile nitrogen, the magnesium oxide method was used in which the samples contain ammonia, mono-methyl amine, diethyl amine and trimethyl-amine and another volatile amine and expressed as mg TVB-N per 100 gm muscle.

4- Determination of Tri-Methyl-Amine (TMA-N): it was carried out according to **ES 2006/1-2760.**

5- Determination of Thiobarbituric acid Number (TBA) mg/gm: It was carried out according to **ES 2006/1-2760**.

Results

Table (1). Percentage of accepted and rejected samples of examined frozen Mackerel and Sardine according to morphological characters (No. 35 for each)

Fish type	Accepted %	Rejected %
Mackerel	77.1	22.9
Sardine	82.9	17.1

Table (2). Statistical analytical results of Total bacterial, *coliform* and *staphylococci* count of examined frozen Mackerel samples

Bacterial count	Total bacterial count (CFU/g)			Rejected percent	
	Min.	Max.	Mean ±SE.	No.	%
Total bacterial count	1×10^4	3×10^6	$3.8\times 10^5\pm1.2\times10^5$	3	8.6
***Coliform* count**	4×10^1	4.5×10^2	$1.4\times10^2\pm2.1\times10^1$	18	51.4
***Staphylococci* count**	1×10^3	3×10^4	$2.4\times10^4\pm1\times10^4$	-	-

Table (3). Statistical analytical results of Total bacterial, *coliform* and *staphylococci* count of examined frozen Sardine samples

Bacterial count	Total bacterial count (CFU/g)			Rejected percent	
	Min.	Max.	Mean ±SE.	No.	%
Total bacterial count	5×10^4	1.2×10^6	$2.8\times 10^5\pm5.2\times10^4$	2	5.7
***Coliform* count**	2×10^1	2.2×10^2	$1\times10^2\pm1.1\times10^1$	7	20
***Staphylococci* count**	1×10^3	1.9×10^4	$5.7\times10^3\pm1.1\times10^3$	-	-

Table (4). Identification of *coliform* bacteria in examined frozen Mackerel and Sardine samples (No. 35 for each).

Type of bacteria	Mackerel		Sardine	
	No.	%	No.	%
Pseudomonas aeruginosa	10	25	10	28.6
Klebsiella pneumonae	15	37.5	13	37.1
E. coli	6	17.1	4	11.4
Serratia liquefaciens	9	22.5	8	22.9

Table (5). Serological identification of *E. coli* from frozen Mackerel(13 isolates) and Sardine fish (6 isolates)

Fish type	No. (%) of *E. coli* serotypes					
	O111	***O166***	***O125***	***O158***	***O78***	***O27***
Mackerel	2 (15.4%)	0	0	3 (23%)	4 (30.8%)	4 (30.8%)
Sardine	0	2 (33.3%)	4 (66.7%)	0	0	0

Table (6). Result of *Staphylococci* in examined frozen Mackerel and Sardine samples (No.35 for each).

Fish type	*Staphylococci* +ve samples	
	No.	%
Mackerel	27	77
Sardine	25	71

Table (7). Identification and percentage of isolated *Staphylococci* bacteria in examined frozen Mackerel and Sardine samples

Type	Mackerel	Sardine
Staphylococcus aureus	7.4%	0
Staphylococcus vitulinus	48.2%	60%
Staphylococcus warneri	44.4%	40%

Table (8). Mean values of some biochemical parameters of imported mackerel and sardine fish

Items	Fish species	
	Mackerel	Sardine
pH	6.03 ± 0.07^{a}	5.67 ± 0.06^{b}
Total volatile basic-nitrogen (TVB-N, mg/100g)	30.06 ± 1.63^{a}	27.41 ± 1.12^{a}
Tri-methylamine nitrogen (TMA-N, mg/100g)	8.89 ± 0.38^{a}	9.81 ± 0.39^{a}
Thiobarbituric acid (TBA, mg of malonaldehyde/kg)	4.58 ± 0.09^{a}	4.55 ± 0.15^{a}
Histamine	$<5^{a}$	$<5^{a}$

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P \leq 0.05$). Permissible limit of pH, TVB-N,TMA-N,TBA and histamine were 6.7, 30 mg/100 mg ,10 mg/100 gm, 4.5 mg/kg and up to 10 mg/100 gm flesh respectively according to **ES 889/1 (2005).**

Table (9). Correlation between different studied biochemical parameters of mackerel fish.

	TVB-N	TBA	TMA-N
pH	0.85	0.94	0.94
TVB-N		0.90	0.93
TBA			0.96
TMA-N			

Table (10). Correlation between different studied biochemical parameters of sardine fish

	TVB-N	TBA	TMA-N
pH	0.89	0.94	0.91
TVB-N		0.96	0.96
TBA			0.97
TMA-N			

Table (11). Rejected index of imported frozen mackerel and sardine fish.

Items	Fish species	
	Mackerel	Sardine
TVB-N		
Rejected%	57	49
Relative rejected%	118	85
TMA-N		
Rejected%	34	40
Relative rejected%	86	177
TBA		
Rejected%	34	37
Relative rejected%	92	108
Pooled items index		
Rejected%	42	43
Relative rejected%	98	102

Discussion

Sensory evaluation is the most important method today for freshness evaluation in the fish sector and the fish inspection services. **(Olafsdottir *et al*., 1997 and Martinsdóttir, 2004)**. Fish quality (freshness) is often assessed by sensory methods based on changes in appearance, odour, colour, flavour and texture. **(Lakshmanan, 2000).** So in table (1) the results of examined frozen Mackerel showed that about 77.1% from samples were accepted as had blue and iridescent colour of back skin, pearly/white colour of abdominal skin, firm texture of back, closed filaments, liver redcolour of gills, viscera being whole and bright and the bone embedded in flesh, peritoneum was adherent and finally the odour was fresh ocean. While 22.9% were rejected that had varied signs of spoilage changes as pale blue colour of back skin, golden colour of abdominal skin, Soft texture with slowly disappears of finger mark, brownish and sunken eye, splited and

brownish gills and finally the viscera was-moderately dissolved and the bone stared to loose from flesh with rancid and oilyodour. These signs of spoilage agree with **(Keay, 2001).** And these signs of spoilage occurred due to autolysis resulting from digestive enzymes, lipases, microbial spoilage from surface bacteria and oxidation. That confirms the view of **(Huss, 1995)** who stated that in fatty fish, the digestive enzymes are very active and begin to attack the walls of the digestive tract soon after capture which makes the tissues even more susceptible to rupture by rough handling. Also **(FAO, 2005)** mentioned that the digestive enzymes cause extensive autolysis that results in meat softening, rupture of the belly wall and drain out of the blood water, which contains both protein and oil.

While in Sardine samples 82.9% were accepted as had general appearance with bright, metallic sheen and iridescent colors, pupil of eyes were sparkling black, clear and convex cornea, gills had blood red colour, scales very adherent and transparent, abdominal coat strong and finally the peritoneum was adherent and strong. While 17.1% of samples were rejected and had signs of spoilage nearly similar to the spoilage signs of Mackerel. So, we noticed that the percentage of rejected samples of mackerel were higher than that of sardine and this may attributed to long period of abused temperature for Mackerel due to long period of marketing and increasing the turnout of consumers on it than sardine.

Results in table (2&3) showed that the TBC ranged from 1×10^4 to 3×10^6cfu/g, with mean value of $8.3\times10^5\pm1.2\times10^5$cfu/g, for frozen mackerel, while the results of frozen Sardine were 5×10^4 to 1.2×10^6 cfu/g, with mean value of $2.8\times10^5\pm5.2\times10^4$ cfu/g which meet the acceptable limit of International Commission of Microbiological Specification for Food **ICMSF** (**1998**) which is (5×10^5-10^7cfu/gm) and also meet the acceptable limit of Egyptian Standard **ES 889-1/2005** which is10^6cfu/g for both types and with rejected percent of 8.6 and 5.7, respectively. These results were lower than **Nickelson *et al.* (1980)** (7.9 x 10^6cfu/g), **Gorette *et al.* (1982)** (4.6 x 10^6cfu/g), **Bennour *et al*. (1991)** (1 x10^6cfu/g) and **Eze *et al*. (2011)** (1.1 x10^6cfu/g) and agree with that recorded by **Adebayo *et al.* (2012)** (6.3 x10^5cfu/g), while our results were higher than that recorded by **Slabyj *et al*. (1981)** (1.3x10^5cfu/g).

The results of total aerobic count of frozen fish attributed to inadequate and continuous chilling and freezing, in addition to unsatisfactory sanitation during handling ,distribution and marketing also, contamination of using materials used in these steps play an important role in increasing these existing organisms.

The microbial measurements can be used to evaluate the freshness of fish and this can be by determining the numbers of specific spoilage organisms (SSO) as well as classical total viable counts (TVC) measurements as the microbial counts within the flesh have higher correlation to sensory evaluation of freshness **(Olafsdottir *et al*., 1998).**

The total number of organisms varies greatly. A normal range on skin surface is 10^2-10^7cfu/g. The gills and the intestine both contain between 10^3 and 10^7cfu/g. while in tissue the count up to 10^5cfu/g. When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely and during storage, they invade the flesh by moving between the muscle fibers **(Huss, 1995).**

Also results in table (2 & 3) revealed that the total *coliform count* (TCC) ranged from 4×10^1 to 4.5×10^2cfu/g with mean value of $1.4\times10^2\pm2.1\times10^1$cfu/g, for frozen mackerel. That results were higher than the acceptable limit of Egyptian Stander **ES 889-1/2005** which is10^2cfu/g, with rejected percent 51.4%,while TCC in Sardine samples ranged from 2×10^1 to 2.2×10^2 cfu/g, with mean value of $1\times10^2\pm1.1\times10^1$ cfu/g. which meet the acceptable limit of Egyptian Standard **ES 889-1/2005** with rejected percent 20%. These results were lower than that recorded by **Slabyj *et al*.(1981)** (4.6 x10^5cfu/g), **Adebayo *et al.* (2012)** (2x 10^5cfu/g) and **Arannilewa *et al*., (2005)** (3 x 10^3cfu/g) who observed an increasing of total *coliform count* with a prolonged storage of fish, and agree with **Nickelson *et al.***

(1980) (1.1 x 10^2cfu/g), these results may be resulted from direct or indirect faecal contamination which come from either animal or human sources which indicate poor sanitation and miss handling of fish. The presence of coliform is indicator of sewage contamination which may also occur during different processing steps such as transport and handling. Moreover, the contamination may also be caused by the water used for washing or icing **(Boyd, 1990).** And may also attribute to temperature fluctuations, and time taken to transport fish **(Mhango *et al*., 2010).**

In table (2 & 3) the results showed that the S*taphylococci* were ranged from 1×10^3 to 3×10^4 with mean value $2.4\times10^4\pm1\times10^4$cfu/g and 1×10^3 to 1.9×10^4 with mean value $5.7\times10^3 \pm 1.1\times10^3$cfu/g for Mackerel and Sardine, respectively. These results were lower than that recorded by **El-Dengawy *et al. (*2017)** (1 x10^5cfu/g) and **Tayo *et al.* (2012)** (2.1x10^5) and higher than that recorded by **Sohad *et al.* (2008)** (2.3x10^3 and 1.5x10^3 in mackerel and sardine respectively)**.** So the high presence of these organisms may be attributed to fish handling with contaminated hands and unsatisfactory personal hygiene.

In table (4) these organisms were identified as the following *Pseudomonas aerugenosa* by 25%, *Kleseiella pneumonae* by 37.5%, *Serratialiquefaciens* by 22.5% and *E. coli* by 17.1% in Mackerel samples, while these organisms were identified in Sardine samples *as Pseudomonas aerugenosa* by 28.6%*, Kleseiella pneumonae* by 37.1%, *E. coli* by 11.4% and *Serratialiquefaciens* by 22.9%. In table (5) the strains of *E. coli* were serotyping as *E. coli O111, O158, O78* and *O27* were present by (15.4%, 23%, 30.8% and 30.8%) in Mackerel samples, While *E. coli O166* and *O125* were present by (33.3% and 66.7%) in Sardine samples. Higher finding for isolation of *E. coli* were recorded by **Adebayo *et al.* (2012)** and **Eze *et al*. (2011)** who isolated *E. coli* by 20% and 25%, while nearly similar finding showed by **Elhadi *et al.* (2016)** which was 18.6%, and lower finding recorded by **Sohad *et al.* (2008)** which was 14%. So their presence in fish indicate faecal contamination which come from external origin as contamination with faecal matter and unsatisfactory personal hygiene. This held the view reported by (**Ogbondeminu, 1993**) who said that the isolation of *E. coli* from fish is taken to indicate contamination coming from an external origin and assumes that the bacterium is not usually present in the fish itself. However, it has been found in the intestinal tract of fish, on the gills, in muscle and on the skin.

E. coli is reported as one of the commonest causes of food poisoning in main three countries throughout the world, including Europe (**Pennings *et al*., 1994)**. United States (**Beuchat, 1996**), South America (**Utsunomiya *et al*., 2001**) and the Far East (**Haque *et al*., 1994**). Their presence in fish intended for human consumption may constitute a potential danger, not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to those infecting humans (**Olayemi *et al*., 1991**).

E. coli O111, *O125,* strains from Enterohaemorrhagic *Escherichia coli* (EHEC) strains that are the cause of haemorrhagic colitis which may be complicated by subsequent haemolyticuraemic syndrome (HUS), thrombotic-thrombocytopenic purpura (TTP), and neurologic disorders. The organisms produce at least three biochemically and immunologically distinct cytotoxins which are designated as Shiga-like (SLT) or verotoxins **(Karch and Bockemühl, 1992).**

E. coli O166, *O158, O78 and O27* strains are of Enterotoxigenic *Escherichia coli* (ETEC) which are of the leading bacterial causes of diarrhea in the developing world as well as the most common cause of travelers' diarrhea. **(Bourgeois *et al*., 2016).** Infection with ETEC can cause profuse, watery diarrhea with no blood and abdominal cramping. Fever, nausea with or without vomiting, chills, loss of appetite, headache and muscle aches **(US Centers for Disease Control and Prevention, 2014).**

In table (6) and (7) the S*taphylococci* species were detected in 77% for Mackerel and 71% for Sardine samples. In table (8) these organ-

isms were identified as following *Staphylococcus aureus* by 7.4%, *Staphylococcus vitulinus* by 48.2% *and Staphylococcus warneri* 44.4% for Mackerel samples. While in Sardine samples the pervious *Staphylococcus spp.* were isolated by 0%, 60% and 40%,respectively.The results of *Staph aureus* isolation were lower than that recorded by **Eze *et al*., (2011)** (60% in mackerel)**.** And agree with to that detected by **El-Dengawy *et al. (*2012)** who cannot detect it in sardine). The presence of *Staph aureus* was attributed to the contamination of the fish samples by man. *Staph aureus* entered into the foods during handling, processing or vending. It also due to the fact that it forms the normal microflora present on the skin and in the nose and throat of most healthy people. So, contamination of foods with coagulase- positive *staphylococci* is largely as a result of human contact **(Nester *et al*., 2001).** *Staph aureus* is considered the third most important cause of disease in the world amongst the reported food-borne illnesses **(Tamarapu *et al*., 2001).**
Staphylococcus species are one of the most important food borne opportunistic bacteria which isolated from fish samples and some of *Staphylococcus species* are potential pathogens and the high population of these bacteria indicates the general quality of fish and the degree of the spoilage it might have undergone, **(Albuquerque *et al*., 2007).** The high prevalence of *Staphylococcus* in fish samples indicate the unhygienic handling of fish and this leading to high degradation of fish as presence of *Staphylococcus* associated with aquatic environments as well as contamination during post - harvest handling **(Purvis, 2002).**
So, the objective of microbiology analysis of fish is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of temperature abuse and hygiene during handling and processing.

Chemical examination

During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavour and texture of the fish meat. This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids **(Mahmoud *et al*., 2006).**
Our results in table (8) showed that the mean value of pH of frozen Mackerel were 6.03±0.07 and 5.67±0.06 in Mackerel and Sardine samples respectively which were within the accepted limits. As the accepted limit was 6.7 according **ES 889/1(2005).** Our results were lower than that recorded by **Sohad *et al.* (2008)** (6.2 and 6.1 in mackerel and sardine respectively) and **Mahmoud (1994)** (6.3). The decrease in pH value may be attributed to the breakdown of glycogen with the formation of lactic acid and the increase of pH may be due to the partial proteolysis and formation of ammonia by fish spoilage bacteria **(Pearson and Gillette, 1996).**
The pH value is not a suitable index on its own to determine quality of fish, it can be useful as a guideline for quality control of fish when used with other quality parameters **(Ruiz -Capillas and Moral, 2001).** Post mortem pH has been reported to vary from 6.0 to 7.1, depending on season, species and other factors **(Simeonidou *et al*., 1998).**
In Table (8) the mean values of TVB-N were 30.06 ± 1.63 mg/100 gm and 27.41 ± 1.12 mg/100 gm in Mackerel and Sardine samples respectively, which within the accepted limits (30 mg/100 gm) according **ES to 889-1/ 2005.** Our results higher than that recorded by **Mokrani *et al*. (2012)** (16.08 ± 0.43 mg N/100 g in sardine samples) and **Bennour *et al*., (1991)** (23 mg/100 gm in mackerel samples), while our results were lower than that recorded by **Marrakchi *et al*. (1990)** (47.18 mg/100 gm in sardine samples)**.**
Ammonia is one of the most spoilage end products of spoiled meat and meat products which is directly responsible for spoilage odors and flavors, it is considered as an indicator for amino acid degradation by bacteria and it can be measured as total volatile basic nitrogen. Accordingly, TVB-N can be considered as a reliable indicative measure for the quality of various food articles **(Gill, 1983)**, Also TVB-N can be considered as a reliable measure indi-

cating the quality of various food articles depending on the breakdown of their proteins **(Warries, 2000).**

The TVB-N value is index and generally used to determine the stage of freshness of fish (along with TMA). In Mackerel a level of 35-40 mg N/100 g of fish muscle is in general regarded as the limit of acceptability, beyond which the fish can be regarded as spoiled **(Lakshmanan, 2000).**

Results in table (8) cleared that the mean value of TMA-N were 8.89 ± 0.38 mg/100 mg and 9.81 ± 0.39 mg/100 mg in Mackerel and Sardine samples respectively Which agree the accepted limit (10 mg/100 mg) according to **ES 889/1(2005)**. Our results higher than that cleared by **Mokrani *et al.* (2012**) 0.58 ± 0.14 mg N/100 g in sardine samples) and **Bennour *et al.* (1991)** (5 mg/100 mg), while our results nearly similar that reported by **Marrakchi *et al.* (1990) (**9.9 mg/100 g) after 24 h and lower than reported by **Marrakchi *et al.* (1990) (**24.9 mg/100 g) after 48 h of storage on ice, respectively). So, the elevated results of TMA-N in the rejected samples may be owing to protein degradation result from repeated thawing and freezing process during handling and marketing.

TMA-N is considered a valuable tool in the evaluation of the quality of fish stored in ice mainly because of its rapid accumulation in the muscle of refrigerated fish, **(Kjosbakken *et al.*, 1983).** So, it is possible to use the TMA-N assay not only to assess the overall quality of fish but also to differentiate between fish of good and moderate freshness. Thus, it is possible to grade sardines according to their content of TMA. First grade: TMA-N <1, very good freshness. Second grade: TMA-N 1-3, good freshness. Third grade: TMA-N 3-5, intermediate freshness**. (Marrakchi *et al.*, 1990).**

TMA-N content found at different stages of storage was significantly different. Thus, its content can be used not only as an index of mackerel deterioration but also is a valuable tool to determine freshness classes **(Bennour *et al.*, 1991).**

The results in Table (8) showed that the mean value of TBA were 4.58±0.09 mg MD/kg and 4.55±0.15 mg MD/kg in Mackerel and sardine samples respectively. Which agree with the accepted limit (4.5 mg MD/kg) according to **ES 889-1/2005**. These results higher than that studied by **Erkan & Özden (2008)** (2.86±0.71 mg MD/kg in sardine samples). This elevated results of TBA may be attributed to high lipid oxidation which is a major cause of deterioration and spoilage for the pelagic fish species with high lipid content and that agree with **Hultin (1994)** who recorded that Fish lipids contain high amount of polyunsaturated fatty acids (PUFAs) and are therefore, are highly susceptible to oxidation**.**

Lipid peroxidation leads to low quality, rancidity and accumulation of potentially toxic substances in foods. So, spoilage in fish caused by auto-oxidation and evaluated by measuring Thiobarbituric acid (TBA) value which is used as an indicator of degree of lipid oxidation. **(Gorelik *et al.*, 2008).**

Results in Table (8) showed that the histamine content in examined Mackerel and Sardine samples were lower than 5 mg/100 gm, which agree with the accepted limit (10 mg/100 gm) according to **ES 889/1(2005)**. Our results similar to **Bennour *et al.* (1991)** (2 mg/100 gm)**, Ababouch *et al.* (1996)** (3.5 mg/100 gm), while our results lower than that recorded by **Erkan & Özden (2008)** (12.3 ± 071 mg /100 gm).

Fish have been implicated in most of the outbreaks of histamine poisoning and the majority has been from comorbid fish. Mackerel is most frequently involved, and this partially due to the greater consumption of those fish worldwide **(Taylor, 1986** and **Emborg, 2007).**

The consumption of Mackerel and other marine fish can result in histamine poisoning. This can happen as a result of time and temperature abuse and inappropriate handling. To prevent this intoxication, the fish must be rapidly cooled down to a temperature as close to 0°C as possible after catch, and a high standard of handling (Good Manufacturing Practices and Good Hygiene Practices) during processing. **(Codex, 2008).** It is not possible to eliminate

the histamine when it has developed in the fish. Any lot that has demonstrated elevated levels of histamine should be destroyed or diverted to a non-food use.

In table (9) there were high correlation between pH, TVB-N, TMA-N and TBA by 0.85, 0.94 and 0.94 and there were high correlation between TVB-N, TBA and TMA-N by0.90 and 0.93 also there was high correlation between TBA and TMA-N by 0.96 in mackerel fish.

Our results in table (10) revealed that there were high correlation between pH, TVB-N, TMA-N and TBA by 0.89, 0.91 and 0.94 and there were high correlation between TVB-N, TBA and TMA-N by 0.96 and 0.96 also there was high correlation between TBA and TMA-N by 0.97 in sardine fish. So this mean that estimation of any chemical parameters could be judge the freshness and quality of frozen mackerel and sardine.

Our results in table (11) showed that the percentage of rejected samples of TVB-N were 57 and 49 for Mackerel and Sardine respectively. More over the percentage of rejected samples of TMA-N were 34 and 40 for mackerel and sardine respectively. Finally the percentage of rejected samples of TBA were 34 and 37 for mackerel and sardine respectively, the percentage of rejected samples for all parameters were 42 and 43 for Mackerel and Sardine respectively. From the previous results we conclude that the mackerel fish had great risk on human health hazard than sardine fish.

Conclusion

From this study, it was concluded that the frozen fish must have great attention during handling and marketing as repeated thawing and freezing may lead to unacceptable changes in appearance, odour, colour, flavour and texture, increase in microbial load and presences of dangerous specific pathogens in addition to high degradation of protein and lipid which constitute a high risk on general health of fish consumers.

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