



RESEARCH ARTICLE

Quality Parameters of Some Marketed Frozen Fish in Dakahlia Governorate, Egypt

Mohamed A. Hussein, Hala M. Fouda*, Karima M. Eissa

Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

* Corresponding author: hala.foda317@gmail.com Article History: Received: 23/01/2022 Received in revised form: 16/02/2022 Accepted: 24/03/2022

Abstract

Fish deteriorative changes could occur directly after catching, storage, and retailing. One hundred samples of Scomber scombrus (S. scombrus), Clupea pilchardus (C. pilchardus), Pagrus pagrus (P. pagrus), Oreochromis niloticus (O. niloticus), and Mugil cephalus (M. cephalus) (20, each) were randomly collected from different fish markets at Dakahlia Governorate, Egypt, The respective mean values of pH, total volatile basic nitrogen (TVB-N mg/100g), and thiobarbituric acid (TBA mg/kg) were 6.52 ± 0.11 , 37.75 ± 4.79 and 3.91 ± 1.7 in S. scombrus, 6.45 ± 0.28 , 41.41 ± 5.62 and 3.25 ± 1.24 C. pilchardus, 6.58 ± 0.06 , 45.97 ± 6.61 and 3.25 ± 1.4 in *P. pagrus*, 6.75 ± 0.22 , 23.5 ± 3.35 and 1.20 ± 0.42 in *O. niloticus*, 6.68 ± 0.16 , 30.82 ± 4.32 and 1.75 ± 0.38 in *M. cephalus*. The histamine value in *S. scombrus* was significantly higher (p < 0.05) than other species, but all examined samples were within the permissible limit. The *P. pagrus* species contained significant higher (P < 0.05) aerobic plate count (APC), Pseudomonas spp, and Staphylococcus aureus (S. aureus) in comparison to other species. The O. niloticus contained the lowest microbial counts, chemical changes, and histamine. The examined fish was contaminated with S. aureus and Pseudomonas; in addition to some samples have high level of TVB-N and TBA than the recommended level. The level of histamine in the examined fish was below the level that induces public health hazard or toxicity.

Keywords: Staphylococcus aureus, frozen fish, TVB-N, TBA, Pseudomonas, Histamine

Introduction

Fish is a good source of protein, fat, and minerals that are necessary for a healthy body's stability, and it is regarded as a good food component for a huge portion of the processors. world's population. Food customers, and public health officials are all concerned about the quality of fish. From a food safety standpoint, it is critical to produce safe and acceptable fish and fish products by contamination. Fish controlling quality decreases as a result of a complex process including sensory, chemical. and microbiological deterioration Fish [1]. accounts for over 60% of the world's protein, with 60% of the developing world getting more than 30% of their animal protein from fish. Because of its great biological value and high protein retention in the body, fish protein

has excellent nutritive value. Low fat content and cholesterol levels in most fish products are important aspects of people's health, especially nations where cardiovascular disease in mortality is high and amino acids are scarce [2]. After a fish is caught, it begins to spoil quickly, and rigor mortis is to blame for the changes in the fish after it has died. The odor, flavor, and texture changes that occur during the spoilage process are caused by the breakdown of various components and the formation of new compounds. Deterioration occurs quickly due to various mechanisms triggered by microorganism metabolic activity, endogenous enzymatic activity (autolysis), and chemical oxidation of lipids [3]. The first process that causes fish to deteriorate is microbial growth, which is the rotting factor that has the greatest impact on the quality of fresh or minimally preserved fish. The fish muscles are initially sterile, however, they become infected after death due to the microbial population found on the fish skin [4]. Because of the high-water activity, low acidity (pH > 6), and high number of nonprotein nitrogenous substances found in fish, bacteria proliferate quickly, the causing undesired changes in appearance, texture, flavor, and odor, and lowering the product's quality [5]. Microorganisms produce volatile amines, biogenic amines, organic acids, sulphides, alcohols, aldehydes, and ketones, all of which have unpleasant and unacceptably strong off-flavors. Biogenic amines, such as histamine. cadaverine. tyramine, and putrescine, are the major chemicals created during microbiological spoilage. They are produced by bacterial decarboxylation of certain free amino acids during storage and are used to assess fish safety and quality [6]. The current study aimed to detect the bacteriological and chemical parameters in addition to histamine to assess the quality and safety of marketed frozen fish in the study area.

Material and methods

Sample collection

One hundred samples of Scomber scombrus, Clupea pilchardus, Pagrus pagrus, Oreochromis niloticus, and Mugil cephalus (20, each) were randomly collected from different fish markets at Dakahlia Governorate, Egypt, during February–August 2021. The fish samples were identified and packaged separately in sterile plastic ziplock bags (Mayank Plastics, Delhi, India), then maintained in an insulated box with ice packs (4 °C) and then delivered to the Control Laboratory, Food Faculty of veterinary Medicine, Zagazig university, Egypt within 2 hours. Using sterile scissor and scalpel, the scales and skin of each fish sample were removed aseptically. Between each fish scissor and sample, the scalpel were disinfected with % ethyl 70 alcohol. Thereafter, 25 g of each fish sample was placed in a sterile Whirl-Pak sample bag made of polyethylene (Thomas Scientific, Inc.) with 225 mL sterile buffered peptone water (Oxoid, CM509). Each bag was sealed, and then homogenized for 1 minute using a stomacher (Merck, Darmstadt, Germany) according to International Standard Organization (ISO) [7]. A sterile pipette was used to transfer the homogenate. One milliliter was put into a sterile test tube containing 9 mL of 0.1 percent peptone water (Conda, Spain), and a 10-fold serial dilution up to 10^7 was prepared.

Bacteriological parameters

According International Standard to Organization (ISO) [7], the aerobic plate counts (APCs) were detected using 0.1mL of sample homogenate from the selected dilutions onto duplicate sterile plates pre-poured with standard plate count ager (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and incubated at 35 °C for 48 h. The technique of Staphylococcus aureus count was applied according to ISO 6888-1[8]. Briefly, 0.1 ml from each of the prepared dilutions was spread onto duplicate plates of Baird Parker (BP) agar (Oxoid CM 275, Canada), supplemented with egg volk tellurite emulsion (50 ml/L, Oxoid SR54, Canada) and incubated at 37°C for 24-48 h. Pseudomonas counts were carried out according to ISO 13720 [9] on the pseudomonas agar base (CM 559; Oxoid, Canada) supplemented with cetrimide, fucidin, and cephaloridine supplements (SR 103; Oxoid, Canada). Pseudomonas colonies were counted after incubation of plates at 25 °C for 48 h.

Chemical parameters

The total volatile basic nitrogen (TVB-N) level of fish was detected according to Malle and Tao [10]. The TVB-N content was measured in milligram of nitrogen per 100 gram of fish. For each tested fish sample, four replicates were created. The thiobarbituric acid (TBA) assay was carried out according to Schmedes and Holmers [11]. The fish sample (10 g) was combined with 25 mL of 20% trichloroacetic acid (w/v) and homogenized for 30 seconds in a blender. The filtrate (2 mL) was added to 2 mL of 0.02M aqueous TBA in a test tube after filtering. The test tubes were incubated at room temperature in the dark for 20 h. The absorbance was measured using a UV-vis spectrophotometer (Thermo fisher Scientific, USA) at 532 nm. The RIDASCREEN histamine kit (R-Bipharm AG, Germany) was used to determine histamine according to the recommendations. manufacturer's The detection limit for frozen fish is 2.5 ppm. On the enzyme linked immunosorbent assay (ELISA) plate reader, optical density was estimated at 450 nm according to the manufacturer instructions (R-Bipharm AG, Germany). The quantity of histamine in the fish sample was inversely proportional to the amount of complex bound to the plate and the optical density.

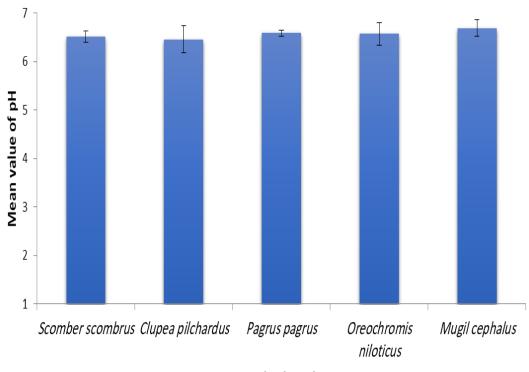
Statistical analysis

The results of bacterial counts, TVB-N, TBA, and histamine were analyzed using oneway ANOVA, followed by a post hoc Duncan's test to compare bacterial counts and chemical parameter values between different fish species (IBM SPSS Statistics, version 22). With a p value of 0.05, the test was significant. The APC, *S. aureus*, and Pseudomonas count data were reported as a log₁₀ standard deviation average (SD). While the data for TVBN, TBA, and histamine were reported as an average of value mean SD, the data for TVBN, TBA, and histamine were reported as an average of value mean SD.

Results and discussion

The natural pH in live fish is just above 7.0, typically about 7.3, but this value falls distinctly after death as the fish goes through rigor mortis and glycogen is converted into lactic acid. In most species, the postmortem pH is between 6.0 and 6.8 then rises again with spoilage [12]. The presented data in Figure 1 showed that pH mean values were $6.52 \pm 0.11, 6.45 \pm 0.28, 6.58 \pm 0.06, 6.75 \pm$ 0.22 and 6.68 \pm 0.16 in examined S. scombrus, C. pilchardus, P. pagrus, O. niloticus and M. cephalus, respectively. There were no significant differences (P < 0.05) between examined species attributed to the similarity in freezing condition. Freezing not only slows down the rate of bacterial growth but also the chemical changes responsible for quality deterioration [13]. The acceptability of examined fish according to the maximum permissible pH limit (6.5) of frozen fish established by Egyptian standard (ES) [14] was 70%, 80%, 45%, 70% and 65% of the examined *S. scombrus, C. pilchardus, P. pagrus, O. niloticus,* and *M. cephalus,* respectively (Table 1).

Total volatile basic nitrogen (TVB-N) is a key criterion for evaluating seafood quality and is one of the most common chemical indicators of marine fish deterioration. Inspectors must utilize TVB-N as a chemical check if the organoleptic test reveals any uncertainty about the freshness of fish according to a European Union directive on fish hygiene [15]. The level of TVB-N in descending manner was arranged as follow P. pagrus $45.97 \pm 6.61 > C.$ pilchards $41.41 \pm$ 5.62 > S. scombrus $37.75 \pm 4.79 > M.$ cephalus $30.82 \pm 4.32 > O$. niloticus $23.5 \pm$ 3.35mg/100g (Figure 2). Nearly similar values for frozen fish 27-52 mg/100g was obtained by Jinadasa [16]. The O. nilotius was significantly lower (P < 0.05) than other examined species, which may be due to low deteriorative changes as this type of fish stays for a short period in the distribution places, because it is a popular fish among the consumers. In the current study, the acceptability of frozen fish (Table 1) according to TVB-N content was 40%, 20%, 35%, 100% and 55% in S. scombrus, C. pilchardus, P. pagrus, O. niloticus and M. cephalus, respectively according to Egyptian standard (ES) [14], which established 30mg/100g as maximum permissible limit for TVB-N of frozen fish.



Examined species

Figure 1: Potency of hydrogen ions (pH) (Mean value ± SD) of examined frozen fish species.

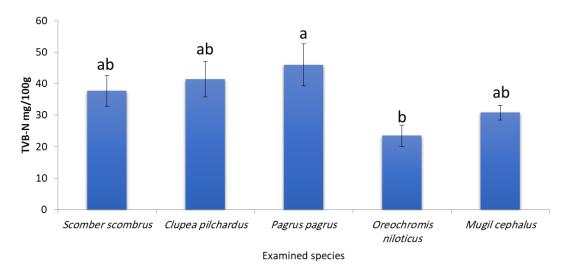


Figure 2: Total volatile basic nitrogen (TVB-N) mg/100g (Mean value \pm SD) of frozen fish species. Means of the same column carrying different superscript letters (a,b,c) are significantly different (P <0.05).

The TBA test is commonly used to determine lipid oxidation in food. Lipid oxidation is a major cause of food quality degradation and has long been a source of consternation for both manufacturers and food scientists. Because of the high vulnerability of long-chain poly unsaturated fatty acid (PUFA) to oxidation and the significant requirement of these PUFA in fish diets, concerns regarding lipid oxidation have also been addressed in the aquaculture business [17]. In current study, Figure (3) showed that TBA value was

significantly higher (P < 0.05) in S. scombrus $(3.91 \pm 1.7 \text{mg MDA/kg})$ and *P. pagrus* $(3.25 \pm$ 1.4mg MDA/kg) than O. niloticus (1.20± 0.42mg MDA/kg) and M. cephalus $(1.75\pm$ 0.38mg MDA/kg). The differences may be attributed to the increase of oxidative deterioration in oily fish than other fishes in addition to the long period of freezing in retail outlets. A lower TBA value (0.30-1.06) was recorded in fish collected from Egyptian fish market [18]. The acceptability was 65%, 75%, 85%, 100%, and 100% for examined S. scombrus, C. pilchardus, P. pagrus, O. niloticus, and M. cephalus, respectively (Table 1). The Egyptian standard [14] declared that 4.5 mg MDA/kg fish flesh is usually considered the limit, beyond that frozen fish unfit for human consumption.

The amount of histamine in a sample indicates whether it is likely to spoil or cause scombroid toxicity. Histamine food poisoning can be caused by ingesting human to 100 mg/100 g of fish meat [19]. The Food and Drug Administration FDA has set a maximum acceptable level of 50 mg/kg for histamine [20]. In addition, Egyptian standard [14] provide that the maximum permitted quantity of histamine for frozen fish, cannot exceed 100 mg/kg. The acceptability among examined samples was 100% according to the current Egyptian legislation (Table, 1). A maximum daily intake of histamine (50 mg) in meals has been published by the European Food Safety Agency for healthy adults [21]. The mean value of histamine was 33.22 ± 2.07 , $25.35 \pm$ $3.09, 11.69 \pm 0.93, 8.56 \pm 0.91$ and $10.02 \pm$ 0.76mg/kg in S. scombrus, C. pilchardus, P. pagrus, O. niloticus and M. cephalus, respectively. Higher histamine values of 206 -230, 175 - 230, 125 - 237 and 89 - 97 mg/kgwere respectively recorded in S. scombrus, C. pilchardus, P. pagrus, and O. niloticus by Refai *et al.*, [22]. Histamine value in S. scombrus was significantly higher (P < 0.05) than other examined species (Figure 4). These differences could have been caused by a higher concentration of the amino acid histidine, which is a precursor to histamine in the presence of bacterial carboxylation. This result was completely consistent with Kim et al., [23], who reported that mackerel and tuna fish had higher levels of histidine.

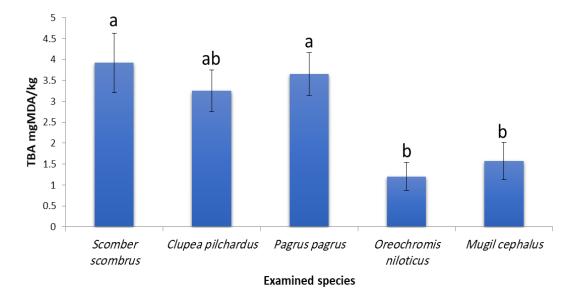


Figure 3: Thiobarbituric acid (TBA)mg Malondialdehyde (MDA) / kg (Mean value \pm SD) of frozen fish species. Means of the same column carrying different superscript letters (a,b,c) are significantly different (P <0.05).

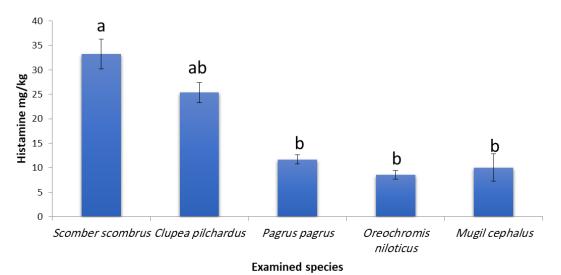


Figure 4: Histamine level mg/kg (Mean value \pm SD) of frozen fish species. Means of the same column carrying different superscript letters(a,b,c) are significantly different (P <0.05).

The APC count in S. scombrus, C. pilchardus, P. pagrus, O. niloticus and M. *cephalus* was 6.11 ± 0.56 , 6.28 ± 0.47 , $7.23 \pm$ $0.69, 5.18 \pm 0.43$ and $6.09 \pm 0.52 \log_{10} \text{CFU/g}$, respectively as shown in Table 2. The obtained counts were comparable to APC count in fish samples from Spain 5.27 log₁₀ CFU/g [24]. Also, 5.7 ± 2.1 , 6.0 ± 1.98 , $7.2 \pm$ 2.96, and 5.1 \pm 1.36 log₁₀ CFU/g in the examined T. nilotica, M. cephalus, P. pagrus, and S. scombrus, respectively in fish from Egypt [18]. Meanwhile, lower APC in Italy as 31% of examined raw fish presented an APC count in the range of 10^3 - 10^4 CFU/g, 34 % in the range of 10^4 - 10^5 CFU/g, and 24% higher

than 10^5 CFU/g [25]. In comparison to the other fish species studied, the mean value of APC in *P. pagrus* was significantly higher (*P* <0.05), which may be due to the species' poorer marketability in Dakahlia fish markets, more time spent in contact with contaminated ice and utensils, and temperature fluctuations in retail establishments. The acceptability was 45%, 100% and 55% for examined *S. scombrus, O. niloticus* and *M. cephalus*, respectively. Meanwhile, all examined *C. pilchardus* and *P. pagrus* were rejected according to the APC when compared with the Egyptian standard [14].

Egyptian standard (889-1/ 2005)		S. scombrus	C. pilchardus	P. pagrus	O. niloticus	M. cephalus
pH (6.5)	within	14 (70%)	16 (80%)	9 (45%)	14 (70%)	13 (65%)
	exceeded	6 (30%)	4 (20%)	11 (55%)	6 (30%)	7 (35%)
TVB-N (30mg/100g)	within	8 (40%)	4 (20%)	7 (35%)	20 (100%)	11 (55%)
	exceeded	12 (60%)	16 (80%)	13 (65%)	-	9 (45%)
TBA (4.5mg MDA/ kg)	within	13 (65%)	15 (75%)	17 (85%)	20 (100%)	20 (100%)
	exceeded	7 (35%)	5 (25%)	3 (15%)	-	-
Histamine (100mg/kg)	within	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
	exceeded	-	-	-	-	-

Table (1): Acceptability of the examined samples according their chemical parameters in relation to Egyptian standards of frozen fish (889-1/2005).

S. scombrus: Scomber scombrus, C. pilchardus: Clupea pilchardus, P. pagrus: Pagrus pagrus, O. niloticus: Oreochromis niloticus, , M. cephalus: Mugil cephalus TVB-N: total volatile basic nitrogen, TBA: thiobarbituric acid

Fish species	APC Min- Max	<i>S. aureus</i> Min- Max	Pseudomonas Min- Max	
	Mean ± SD	Mean ± SD	Mean ± SD	
Scomber scombrus	5.24 - 7.23	<2- 4.22	2.68 - 3.95	
	$6.11\pm0.56^{\text{b}}$	3.24 ± 0.43^{ab}	3.31 ± 0.39^{b}	
Clupea pilchardus	6.11 - 7.09	<2-3.84	2.45-3.69	
	6.28 ± 0.47^{ab}	3.11 ± 0.39^{ab}	2.98 ± 0.37^{bc}	
Pagrus pagrus	6.54 - 7.98	<2-4.56	3.42- 5.11	
	$7.23\pm0.69^{\rm a}$	$3.81\pm0.49^{\rm a}$	$4.29\pm0.51^{\rm a}$	
Oreochromis niloticus	4.28 - 5.61	<2-3.22	2.16 - 3.44	
	$5.18\pm0.43^{\rm c}$	$2.57\pm0.41^{\text{b}}$	$2.45\pm0.32^{\rm c}$	
Mugil cephalus	5.36 - 6.64	<2-4.08	2.54- 3.95	
	$6.09\pm0.52^{\text{b}}$	3.16 ± 0.44^{ab}	$3.34\pm0.38^{\text{b}}$	

Table (2): Statistical analytical results of bacterial counts log ₁₀ CFU/g of examined f	frozen
fish samples $(n = 20 \text{ for each})$.	

Means of the same column carrying different superscript letters (a,b,c) are significantly different (P <0.05). APC: aerobic plate count, *S. aureus: Staphylococcus aureus*, Min: Minimum, Max: Maximum, SD: standard deviation

Microorganisms of the genus *S. aureus* are found in the environment and are linked to human sources. As a result, the count of *S. aureus* is regarded as a crucial indicator of fish processing sanitation [26]. The *S. aureus* count was 3.24 ± 0.43 , 3.11 ± 0.39 , 3.81 ± 0.49 , 2.57 ± 0.41 , $3.16 \pm 0.44 \log_{10}$ CFU/g in examined *S. scombrus, C. pilchardus, P. pagrus, O. niloticus* and *M. cephalus*, respectively as shown in Table 2. Nearly similar counts were obtained in Spain as $3 \log_{10}$ CFU/g [24] and in Egypt from 2-4 \log_{10} CFU/g [18]. On contrary, *S. aureus* was not detected in fish samples collected from Italy [25]. The results in Table (3) declared that the acceptability was 60%,

70%, 35%, 85% and 40% for examined S. scombrus, C. pilchardus, P. pagrus, O. niloticus and M. cephalus, respectively according to the Egyptian standard [14] which established that 3 log₁₀CFU/g of fish flesh is usually considered the limit, beyond that frozen fish unfit for human consumption. The S. aureus count was significantly lower in examined O. niloticus, which may be attributed to the higher rate of selling than other species that decrease the chance of handling and contamination. Additionally, O. niloticus freshwater fish habitat and Staphylococcus species grows at optimum conditions (3.5% NaCl and 37C) [27].

Table (3): Acceptability of the examined samples according their bacteriological parameters in relation to Egyptian standards of frozen fish (889-1/2005).

Egyptian standard (889	9-1/ 2005)	S. scombrus	C. pilchardus	P. pagrus	O. niloticus	M. cephalus
APC (6 log ₁₀ CFU/g)	within	9 (45%)	-	-	20 (100%)	11 (55%)
	exceeded	11 (55%)	20 (100%)	20 (100%)	-	9 (45%)
S. aureus (3 log ₁₀	within	12 (60%)	14 (70%)	7 (35%)	17 (85%)	8 (40%)
CFU/g)	exceeded	8 (40%)	6 (30%)	13 (65%)	3 (15%)	12 (60%)

APC: aerobic plate count, S. aureus: Staphylococcus aureus

Pseudomonads are a good spoilage index in fishes and a marker for fish deterioration. Furthermore, they have to be the specific spoilage organism (SSO) in ice-stored freshwater fish. Additionally, the microbial population of fish stored aerobically under chilling condition consists almost exclusively of *Pseudomonas* spp..[28]. Data in Table 2 showed that the mean standard deviation of Pseudomonas spp. count was 3.31 ± 0.39 , 2.98 ± 0.37 , 4.29 ± 0.51 , 2.45 ± 0.32 , 3.34 ± 0.38 \log_{10} CFU/g in examined *S. scombrus, C.* pilchardus, P. pagrus, O. niloticus and M. cephalus, respectively. Nearly similar counts were obtained in fish by Angis and Oğuzhan [29], Badee et al., [30], Li et al., [31] and Hussein et al., [18]. Meanwhile, lower count obtained by Khalafalla et al., [12]. The P. significantly contained pagrus higher Pseudomonas count (P < 0.05), which may be attributed to lower marketability and long time in retail outlet that enhance growth and multiplication of Pseudomonas spp. No available regulation for Pseudomonad count in fish.

Conclusion

The current study declared that examined fish harbored *S. aureus* and Pseudomonas, which may induce public health hazard to consumers in addition to some samples have high level of total volatile basic nitrogen and thiobarbituric acid than the recommended level indicating the lower nutritional quality. The level of histamine in the examined fish is below the public health hazard or toxicity limits.

Conflict of Interest

The authors have no conflict of interest to declare.

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الملخص العربى

مقاييس الجودة لبعض الأسماك المجمدة المسوقة بمحافظة الدقهلية محمد عبدالله حسين، هالة محمد فوده و كريمة محمد عيسى قسم مر اقبة الأغذية كلية الطب البيطري جامعة الزقازيق مصر

تتدهور حالة الأسماك مباشرة بعد الصيد والتخزين وأثناء البيع بالتجزئة. فى هذة الدراسة تم تجميع مائة عينة من الماكريل، السردين، المرجان، البلطى والبورى 20) ، لكل منها) جمعت عشوائياً من أسواق الأسماك المختلفة بمحافظة الماكريل، السردين، المرجان، البلطى والبورى 20) ، لكل منها) جمعت عشوائياً من أسواق الأسماك المختلفة بمحافظة الدقهلية ، مصر بفحص اسماك الماكريل تبين أن متوسطات الأس الهيدروجيني وإجمالي النيتروجين المتطاير وحمض الثيوباربيتوريك 6.5± 1.0 و 37.5± 9.4 و 19.5± 1.7 على التوالى ؛فى السردين كانت المتوسطات 5.6 للثيوبار بيتوريك 15.5± 1.0 و 37.5± 1.27 و 19.5± 1.7 على التوالى ؛فى السردين كانت المتوسطات 5.6 للثيوبار بيتوريك 5.5± 1.21 و 37.5± 1.29 و 19.5± 1.7 على التوالى ؛فى السردين كانت المتوسطات 6.45 للثيوبار بيتوريك 1.25 للذي عاد 1.20 و 37.5± 1.29 و 37.