

Assessment of the microbial quality of imported frozen fish fillets in Sulaimani markets

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Abstract

In order to assess the microbial quality of frozen fish fillet sold in Sulaimani markets, 64 samples of frozen fish fillets belonging to 4 foreign trademarks were collected. The label information were conducted for all samples then the standard bacterial count, psychrophilic bacterial count, psychrotrophic bacterial count, enterobacteriaceae, *E.coli*, and *Vibrio spp.* were conducted. Although there were significant differences among the tested but all marks bacterial count were within the Iraqi standard regulations. All inspected marks were free from *Vibrio cholera*. In conclusion all four marks fish Samples were judge as safe food for human consumption.

التقييم النوعي المايكروبي لشرائح الاسماك المجمدة والمستوردة والمتوفرة في اسواق

السليمانية

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الخلاصة

لاجل اجراء التقييم الميكروبي لشرائح السمك المجمد في اسواق مدينة السليمانية، تم جمع 64 عينة سمك لاربعة علامات تجارية من مناشىء أجنبية. جمعت كافة المعلومات المثبتة على بطاقة الدلالة لتقييمها ثم خضعت كل العينات للفحوصات البكتيرية والتي تضمنت العد الكلي البكتيري، عد البكتريا الالفة للبرودة، عد البكتريا المتحملة للبرودة، عد البكتريا المعوية، بكتريا *E.coli* وبكتريا الكوليرا المعوية. على الرغم من وجود اختلافات معنوية بين العلامات التجارية المفحوصة، الا ان النتائج اظهرت بان كل العينات كانت ضمن الحدود الميكروبية القياسية، طبقا للمواصفة العراقية. كذلك سجلت العينات المفحوصة خلوها من بكتريا الكوليرا المعوية. نستنتج ان الاسماك من الاربع علامات امنة للاستهلاك البشري.

Introduction

Fishery products 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 (1). Fish contributes about 60% of the world's supply of protein and that 60% of the developing world derives more than 30% of their annual protein from fish that asserted by (2). In tropical countries, any shortfall in fish availability will affect the animal protein intake of people (3). The number of bacterial gastroenteritis associated to seafood products has been increased considerably during the last decades by the rapid globalization of the food market, the increase of personal and food transportation, and profound changes in the food consumption habits (4). Aquatic animals from some areas of the world can carry microorganisms such as *Vibrio spp.* that are harmful to human health and must be destroyed or removed before selling the products (5). *Vibrio Spp.*, are predominant spoilage species isolated from chilled fresh fish under aerobic conditions (6), and they are a halophilic bacterium capable of causing food and

waterborne gastroenteritis, wound infections and septicemia in humans (7). Spoilage of fish under aerobic conditions becomes apparent when specific spoilage bacteria (SSO) reaches the values of 10^8 - 10^9 /g flesh (8). Normally, this occurrence is after a lag phase, the time-span of which mainly depends on the temperature before the bacteria enter into exponential growth particularly in tropical fish species stored under low temperatures (0°C) (9). 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 (10). Estimation of bacterial numbers, in order to serve the purpose of food safety and shelf life determination (11). (12) found that the pathological bacterial contamination were very low which could be occurs due to unsanitary conditions and using improper preparation, bad handling or air contaminations at packaging. The aim of this investigation at evaluates and examines the bacterial pathogens associated with some foreign trademarks of frozen fish fillets commercially available or sold in Sulaimani market.

Material and Methods

Sampling and labeling: The ultimate inspection included a total number of 64 samples of frozen fish fillets pertaining to 4 foreign trademarks were collected from different parts of Sulaimani city markets. Microbiological tests were made in the laboratories of Animal Production Department, Faculty of Agriculture, University of Sulaimani, Kurdistan region, Iraq. The labeling information available on samples covers had been read, for the purpose of complaisance with Iraqi Standard regulation which deals with food labeling, IQS (230) (13), and with the IQS (1847) which deals with food expiry date (14).

Microbiological tests:

Sample preparation: 25g flesh meat, to 225 ml of Butterfield's phosphate diluent were added and homogenized, this is the first 10^{-1} dilution, series of dilutions were prepared in 9ml Butterfield's phosphate diluent until 10^{-7} . One ml was removed from each dilution and put in 3 petri-dishes, Plate Count Agar (Biolife, Italy) was cooled in a water bath to $45 \pm 1^\circ\text{C}$ and poured into the petri-dishes, mixed gently by swirling or tilting each plate. Incubated for periods and temperatures according to the bacteria detected.

Total plate count were enumerated in standard plate count agar after incubation at 37°C for 48 h as described by (15).

Psychrophilic count were enumerated in standard plate count agar after incubation at a $5 \pm 1^\circ\text{C}$ for 10 days as described by (16).

Psychrotrophic count were enumerated in standard plate count agar after incubation at a $15 \pm 1^\circ\text{C}$ for 4-5 days as described by (16).

- **Enterobacteriaceae count:** Fish tissue 10 g was aseptically excised, mixed with 90 ml of buffered peptone water (BPW) in sterile polythene bags. The samples were decimally diluted in series up to 10^{-4} . The dilutions were incubated at 35°C for 24 (17). A 1 ml from the liquate transferred to petri dishes and 15 ml of cooled violet red bile Agar (VRBA) (Oxoid) was added and immediately mixed with the sample. After the agar had set, a second layer 10 ml of VRBG agar was added, allowed to set and the plates were incubated at 35°C for 24 h (18) after which the number of pink colonies were recorded.
- **E. coli (19):** Eosin-methylene blue agar (EMB), were used, which was selective and differential for fecal Coliforms (*Escherichia coli*), after incubation at 37°C for 24 hours, the plates were examined for typical *Escherichia coli* colonies, which are metallic green sheen. A number (2-3) of isolated colonies were picked out and purified on EMB, then subcultured to Nutrient agar slants and incubated at 35°C for 18 -24 hours to be tested completely through the complete test.

- ***Vibrio* spp.:** Thiosulphate citrate bile salt sucrose (TCBS) agar for pathogenic *Vibrio* spp. The plates were incubated at 35±2°C for 24h. (20).
- ***Aeromonas* spp.:** For enumerate and identified of *Aeromonas* spp., Twenty five grams of flesh were blended in 225 ml of Tryptic soy broth enriched with 10 µg/ml ampicillin (TSBA) with a blender for 2 minutes, and then incubated at 28°C for 24 h. After incubation of enrichment cultures, serial dilutions were prepared in Butterfield's phosphate diluent (BPD). Aliquots of 0.1 ml from the dilutions 10⁻⁴ to 10⁻⁶ were transferred in duplicates onto the surface of starch ampicillin agar (SA) plates and spread on. The plates were incubated at 28°C for 18 to 24h (15).
- **Statistical analyses:** All data were subject to one-way analysis of variance (ANOVA) using SPSS 18.0 and XL Stat program for Windows. Differences Between the means were tested by Duncan's multiple range tests. The level of significance was chosen at P<0.05 and the results are presented as mean (21).

Results and Discussion

Table (1) shows the label information of different inspected frozen fish fillets samples collected from four trademarks. The results showed that all marks had the required label information. Labeling considered as a basic request in food marketing, because it's the consumer rights to know what's he is buying. The results showed that all trademarks were reported the required labeling information as recommended by (22 and 14). But using languages in labeling were, the manufacturer country mother language plus Arabic or English in some of marks, and that may not meet the local Iraqi standards (13). Although, this could be avoided by, obligating the manufacturer to label in Arabic, and in Kurdish if possible, in addition to English. Lack of awareness of some manufacturers to the importance of label information for consumers may lead to shortage in label information.

Table (1) The labeling form of the fishes fillet

Test	Information	Fillet (1)	Fillet (2)	Fillet (3)	Fillet (4)
Label	Trade Mark	Flander	Hasoon	white fish fillet <i>Pangasius</i> <i>Hypophthalmus</i>	Myanmar <i>Pangasius</i> <i>Hypophthalmus</i>
	Origin	Iran	Iran	Vietnam	-
	Pro. Date	+	+	+	+
	Exp. Date	+	+	+	+
	Net Weight	+	+	+	+
	Batch no.	+	+	+	+
	Using Language	Per., Ar., Eng.	Per., Ar., Eng.	Ar., Eng.	Ar., Eng.
	Storage Condition	+	+	+	+

Per.: Persian

Ar.: Arabic

En.: English

As been shown in Table (2), the mean value for standard plate count were 125.394, 49.675, 61.313 and 57.625 × 10² CFU/gm fish meat for marks Flander, Hasoon, white fish fillet and Myanmar respectively. Mark (Flander) was significantly different (P<0.05) from the other three marks. The same Table (2) showed the mean value for psychrophilic bacterial count which were 30.672, 28.125, 57.750, 56.313 × 10² CFU/gm fish for mark (Flander, Hasoon, white fish fillet and Myanmar) respectively. Mark (Flander and Hasoon) were not significantly different in between but they were significantly different (P<0.05) than both mark (white fish fillet and Myanmar) which were not different in between. Also table (2) showed the mean value for psychrotrophic bacterial count. Although, there were no significant differences among the four marks, but the higher count recorded in mark Hasoon, then mark white fish fillet followed by mark Myanmar, and lower count recorded in mark Flander (67.894, 66, 57.875 and

28.563×10² cfu/gm). Table (2) also showed the mean value of Enterobacteriaceae count, which recorded 70.031, 71.103, 26.563 and 25.25×10² cfu/gm for marks Flander, Hasoon, white fish fillet and Myanmar respectively. No significant differences recorded among the inspected marks. The aerobic bacteria count could reflect the quality of food sanitation during manufacturing, shipping and storage, and also provides an index of food freshness (23). The results of standard plate count of four marks were not exceed the standard range (5×10⁵-10⁷cfu/gm) which determined by (24). As whereas, the results were not exceeds the maximal limit of the Iraqi standards for fresh fish which is 10⁷ (25). While, the regulations of France and Japan determined the aerobic bacteria counts in frozen seafood as 5×10⁴ - 1 ×10⁵ CFU/g respectively. Our results were lower than the results obtained by (23) where they recorded the average of aerobic bacteria count for all test samples as 1.2×10⁵ CFU/g, for samples obtained from northern, central and southern Taiwan were (7.8×10⁴ ,1.4 ×10⁵ and 1.5×10⁵CFU/g respectively) and for samples collected from shopping malls, supermarkets and conventional markets were 1.1×10⁴, 1.4 ×10⁵ and 1.5 ×10⁵ CFU/g respectively). Food with lower aerobic bacteria is not necessarily safer to consume as the food pathogen may still exist in the food with lower aerobic bacteria count. Lowering the temperature could inhibit the pathogen as well as other microorganisms (23). The total psychrophilic counts of four inspected marks were lower than the results recorded by (26), which were (10⁶ cfu/gram fish meat). Psychrophils become decreased after fishing and extraction of fish from water, but the high psychrophilic count may indicate a pollutant water source. With respect to psychrotrophic count, all inspected marks recorded results lower than what be mentioned by (27) as 10⁹ cfu/gram meats. The importance for recording the psychrotrophic count is due to that, they may be related to level of bacterial contamination of fish which depended on the, environment source which include mesophiles that can tolerate and grow in low temperatures, and bacterial quality of water where fish was caught (28). And according to (27), the count of Psychrotrophic bacteria increased after prolonged storage time of the fish on ice, so can uses as a good indicator for storage time. Enterobacteriaceae levels permitted by Croatian microbiological standards for food and food products which are <10² CFU/g for fresh and frozen fish. The Enterobacteriaceae count is considered as another index of fish quality because it is related to storage condition in ice, washing and evisceration (29). A monitoring of these microorganisms has been suggested as a measure of fish quality. Also, risk management decisions should take into account the whole food chain from primary production to consumption (30).

Table (2) Bacterial count for four marks of frozen fish fillets(CFU/ g meat)

Traits	Marks of fish (10 ²)			
	Flander	Hasoon	white fish fillet <i>Pangasius Hypophthalmus</i>	Myanmar <i>Pangasius Hypophthalmus</i>
Standard plate count	125.394 a ±36.640	49.675 b ±24.001	61.313 b ±6.639	57.625 b ± 5.451
Psychrophilic. bacterial count	30.672 b ±9.069	28.125 b ±3.751	57.750 a ± 6.567	56.313 a ±7.760
Psychrotrophic. bacterial count	28.563 a ±7.223	67.894 a ±27.933	66.000 a ±5.122	57.875 a ±8.334
Enterobacteriaceae count	70.031a ±21.000	71.103 a ±31.047	26.563 a ±8.958	25.250 a ±6.113

Value are presented as mean ± standard errors.

Means having different letters in the same raw are significantly different.

The differences were significant at (P<0.05).

Table (3) showed the results of miscellaneous bacterial indicators for four marks of fish fillets. *E. coli* were isolated from two marks (Flander and Hasoon) but not from marks white fish fillet and Myanmar of fish fillets. Pathogenic microorganisms *Vibrio spp.* not isolated from the four under study fish fillets marks, *Aeromonas* were isolated from mark Flander and Hasoon, but not from marks white fish fillet and Myanmar (Table,3). Coliform (including *E.coli*) is a microorganism that can cause food infection inducing sickness in the digestion system, and is unavoidable without proper sterilization method (23). (31) have provided evidence for that, the ice used to refrigerating seafood may be contaminated with coliform bacteria which cause human infection, as they discovered the presence of high numbers of coliforms, heterotrophic indicator microorganisms and pathogenic strains in ice used for chilling fish and other seafood; therefore, some of the contamination detected in the current study could be due to the ice used for chilling purposes at processing. (32) indicated that all tissue samples except muscle tissues were contaminated with fecal coliform where *Escherichia coli* is the most common contaminant and is often encountered in high numbers. *Vibrio Spp.* are predominant spoilage species which has been isolated from chilled fresh fish under aerobic conditions (6). *Vibrio spp.* is a halophilic bacterium capable of causing food and waterborne gastroenteritis, wound infections and septicemia in humans (7). Fortunately no presence of pathogenic *Vibrio Spp.* were found in all inspected marks, which concenter as a health hazard (7), and this reflx the good processing procedures for the products. Instead colonies of *Aeromonas* isolates were found, this kind of microorganism found universally in all tested foods, including sea foods, raw milk, chicken, and meats such as lamb, veal, pork, and ground beef. Initial counts in these foods ranged from 10^2 to 10^5 cfu/g at 5°C (33). With this respect, although some inspected marks recorded presence of this kind of bacteria (Flander), but it concenter as un harmful for human. All four marks Samples were judge as safe food for human consumption from microbiological point of view.

Table (3) Miscellaneous bacterial evaluation of four marks of fish fillets

Traits	marks of fish	replicates																
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	
<i>E.coli</i>	Flander	ND	+	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Hasoon	ND	ND	ND	ND	ND	ND	+	+	+	+	+	+	+	+	+	+	+
	white fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Myanmar	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Vibrio spp.</i>	Flander	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Hasoon	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	white fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Myanmar	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Aeromonas count 10</i>	Flander	0	0.3	0.1	3.6	0.32	0.1	0	5.2	ND	ND	0	0	ND	ND	ND	ND	ND
	Hasoon	ND	ND	ND	ND	0.1	0.2	0.3	0.25	ND	ND	ND	ND	ND	ND	ND	ND	ND
	white fish fillet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Myanmar	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not detected

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