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Chemical and microbiological quality of imported chilled, frozen, and locally cultured fish in Saudi Arabian markets

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Abstract

Fish meats comprise an important part of diets; however, their rich nutrient composition and various sources mean these products can be spoiled quickly. The present study assessed the chemical and microbiological quality of imported chilled, frozen, and locally cultured fish in Saudi Arabian markets. Physiochemical, heavy metal, and microbial analysis were performed for 50 samples of fish from three sources. The results indicated that locally cultured fish were of better quality than imported fish. All of the locally cultured fish samples were under the limit of total volatile basic nitrogen TVB-N (30 mg/100 g) for fish, while 22.73% and 7.16% of frozen imported and chilled imported fish respectively were over TVB-N limit. Furthermore, chemical and microbial contamination in some fish samples exceeded the recommended permissible levels. We conclude that there was poor sanitation practice and unsuitable conditions during the production and handling processes of fish.

Keywords: fish quality; chilled fish; frozen fish; microbiological quality; fish safety.

Practical Application: Providing a potential information about harm caused by poor sanitation and unsuitable conditions during the production and handing processing of fish.

1 Introduction

Fisheries play a significant role in the food sector and international trades. The total fish production in 2016 reached an all-time high of 171 million tonnes, of which 88 percent was utilized for direct human consumption, thanks relatively stable capture fisheries production, reduced wastage and continued aquaculture growth. This production resulted in a record-high per capita consumption of 20.3 kg in 2016. The sector's contribution to economic growth and the fight against poverty y is growing. Strengthened demand and higher prices increased the value of global fish exports in 2017 to USD 152 billion, 54 percent originating from developing countries (Food and Agriculture Organization, 2018). The continuous growth of fish production, canal development for its distribution, and supplementation with fish products has dramatically increased over the past 50 years, with an annual growth rate of 3.2% during the period from 1961 to 2009, overtaking the global population growth of 1.7% per year (The State of World Fisheries and Aquaculture, 2012). Diets containing a wide range of fish and shellfish may improve heart health and growth of children. Fish meat and fish products are good sources of important nutrients, such as protein, minerals, vitamins, and omega-3 fatty acids, which provide the main nutrients for brain development (Jaclyn et al., 2010), as well as a large amount of amino acids, such as arginine, histidine, and proline (Dabrowski et al., 1969). All seafood species had high levels of EPA and DHA and much higher total n-3 PUFA than n-6 PUFA content (Durmuş, 2019).

It was reported that Rubian (tiger shrimp) *Penaeus semislcatus*, which is consumed as a regular part of the diet in the Arabian Gulf, could be beneficial due to its high content of phosphorus, zinc, calcium, copper, magnesium, and protein (Musaiger & D'Souza, 2008). The quality of fish and fish products is multifaceted and includes a wide range of issues, such as safety and freeness from health risks, nutritional value or quality, convenience, availability, and integrity, as well as the freshness of fish, sensory characteristics, physical features, type, and quantity of products. Furthermore, fishing, storage, handling, processing, and procedures, in addition to conditions such as time and temperature that affect the fish products' safety and quality are significant factors (Abbas et al., 2008). A lack of information about the enhanced fish management and post-harvest processes contributes to low-quality fish products and fisheries (Food and Agriculture Organization, 2020). In order to be confident about health and safety, it is essential to maintain high levels of fish quality throughout the whole food chain, from catching to consumption (Hyldig & Green-Petersen, 2005), Consumers' requests for excellent, healthy, and safe foods is growing globally (Sen, 2005).

Fish meat and seafood products are moderately unsteady in fresh and frozen preservation. The organoleptic characteristics of fish occur quickly due to multiple factors. Biochemical (i.e., enzymatic) changes and microbial effects yield quantifiable alterations in fish during fresh (iced) storage, while frozen preservation induces physical, chemical, and biochemical reactions that reduce fish quality (Woyewoda et al., 1986). Fish foods are known carriers of health risks, such as foodborne disease microbes including *Salmonella sp.*, *Vibrio sp.*, fungi, parasites,

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mycotoxins, natural toxins, metals, and other contaminants (Venugopal, 2002).

Fish quality can be assessed using sensory, chemical, physical, and microbial approaches, such as assessing volatile materials, lipid oxidation, breakdown of ATP products, and formation of biogenic amines (Ozyurt et al., 2009). While various analytical methods have been used to determine fish quality, sensory assessment is still recognized as the greatest operative method to evaluate the freshness of fish and decline in quality (Alasalvar et al., 2011). The quality index method is an informal, quick, and effective approach to evaluating the storing age and approximate fish shelf life, and is used to meet the growth of consumers' demand for quality and freshness information and the growing electronic trade. The application of regular techniques for estimating fish freshness and quality in each step of the fish production chain can assist the manufacturing to provide safe, good quality, and healthy fish meats, giving it a distinctive price (Bernardi et al., 2013).

Consumption of fish and shellfish products could lead to illnesses due to intoxication or infection and certain diseases associated with antibiotic-resistant pathogenic microorganisms (Adebayo-Tayo et al., 2012). It is estimated that there are >80 million cases per annum of seafood-borne diseases in the USA, with the cost of these infections reaching several billions of dollars per year (Adebayo-Tayo et al., 2012). Therefore, it is important to control the quality of fish and fish products to avoid microbial and chemical contamination. Since the seafood-borne diseases ratio is growing, it is essential to assure the quality and safety of fish. Therefore, the present study was aimed to assess the chemical and microbiological quality and safety of imported frozen and locally cultured fish in Saudi Arabian markets.

2 Materials and methods

2.1 Sample collection and preparation

A total of 50 samples of chilled imported fish samples (18 Hamor, 11 Shaor, 9 Harid, 8 Sigan, and 4 Biadh), and 50 samples of frozen imported fish (41 Bengasios Filleah, 5 Bolty, 4 Hamor), which had been imported during 3 weeks from India and Vietnam, and 50 samples of locally cultured fish samples were (25 Bolty, 14 Karros Asioy, and 11 Danese (Seasea bream)) Which is caught from the port of Yanbu, Saudi Arabia. They were kept refrigerated, and were analyzed within 24 hours of catching them. All samples were purchased from the fish market at Jeddah, Saudi Arabia during May 2019. The Arabic common names and scientific names of fish samples are listed in Table 1. Fish samples were iced (1:1 ratio fish: ice) immediately and delivered in a sterile container in hygienic conditions to the laboratory. The fish samples were then further prepared for physiochemical and microbial analyses by cutting the samples using sterile cutters and homogenizing prior to analysis.

2.2 Chemical analysis

Standard analytical techniques were implemented to measure moisture, protein, lipid, carbohydrate, and ash content of the fish samples. All chemicals were of analytical grade and purchased from Fischer (USA) and Merck (Darmstadt, Germany).

Determination proximate chemical composition

Moisture, Ash, crude lipid and crude protein content of fish samples were determined as described early (Horwitz & Latimer, 2006). All tests were performed in triplicate for all fish samples. The sugar content of the fish samples was calculated using the following Equation 1 (Mathew et al., 2014):

$$Carbohydrate\ (\%) = 100 - \begin{pmatrix} Lipids\ (\%) + Protein\ (\%) + \\ Moisture\ (\%) + Ash\ (\%) \end{pmatrix} \quad (1)$$

Determination of pH value

Samples were prepared by homogenizing 4 g of muscle in 20 mL of distilled water. The pH was measured using a digital pH meter (EUTECH Waterproof Multi Parameter, Singapore, Model - PCD-650) (Yeasmin et al., 2010). All measurements were performed in triplicate.

Free fatty acids determination

Free fatty acid (FFA) content was measured to indicate lipid oxidation. Samples of 0.8 g of fish were homogenized in 10 mL of neutralized ethanol and the FFA content was determined by

Table 1. Arabic and scientific names of fish samples.

Scientific name	Source	n	Local name
Pangasius hypophthalmus	**FI**	**41**	**Filleah (Sutchi catfish fillet)**
Oreochromis spilurus Oreochromis niloticus	**FI**	**5**	**Bolty bahry**
Epinephelus	**FI**	**4**	**Hamor**
Sparus aurata	**LC**	**11**	**Danese (sea bream)**
Oreochromis niloticus	**LC**	**25**	**Bolty niley (Nile tilapia)**
Lates calcarifer	**LC**	**14**	**Karros Asioy**
Siganus rivulatus, Siganus luridus	**CI**	**8**	**Sigan safy**
Carangoides ferdau	**CI**	**4**	**Biadh**
Epinephelus polyphekadion	**CI**	**18**	**Hamor sman**
Scaridae parrotfishes	**CI**	**9**	**Harid**
Lethrinidae sp.	**CI**	**11**	**Shaor**

CI, chilled imported; FI, frozen imported; LC, locally cultured.

titration with 2N KOH (Merck) and expressed as FFA%/100 g of lipid (Sadasivam & Manikam, 1996). FFA% was calculated using the following Equation 2:

$$FFA\% = \left(V \times N \times F \times 100 / sample\,weight\right) \times 1000 \quad (2)$$

where V represents volume of KOH (mL), N represents KOH normality, and F represents the equivalent weight of common FFA (e.g., the equivalent weight of oleic acid is 282/1 or 282).

Measurement of heavy metal content

Mineral elements were measured in samples according to recommended procedures (Horwitz, 2002; Nisbet et al., 2010). Lead, cadmium, copper, nickel, iron, mercury, and zinc were measured using a Buck Scientific 205 Atomic Absorption Spectrophotometer. Blank samples were treated in the same manner as the samples. All metals were determined against standards.

Determination of Total Volatile Basic Nitrogen (TVB-N)

The determination of TVBN was carried out as described earlier (Gassem, 2019), with few modifications, in brief 20 g of each samples were blended with 100 mL of distilled water; the mixture was transferred to distillation flask and completed to 250 mL with distilled water and 2 g of MgO was added to the mixture. TVB-N was liberated by boiling the mixture with magnesium oxide, which prevented volatile acids from distilling over into the boric acid and 0.04 mL of methyl red and bromocresol green indicator. The volatile nitrogen was received in 25 mL of 2.0% boric acid then titrated by 0.1N sulfuric acid. The TVB-N (mg N/100 g) was calculated by the following Equation 3:

$$TVB - N\left(mg\,N / 100g\right) = volume\,of\;H2SO4 \times Normality\,of\;H2SO4 \times 14 \quad (3)$$

2.3 Microbiological analysis

Total microbial counts

Total microbial counts were determined for all fish samples using standard methods (Food and Drug Administration, 2015), Briefly, 10 g of fish sample was homogenized for 4 min in 90 mL of sterile (0.1%) peptone water. Sequential dilutions were made, and 1 mL of all dilutions was plated in a sterile petri dish using a pipette and inoculated in Plate Count Agar (Merck) in triplicate. The plates were incubated for 3 days at 35 °C.

Total coliform count

The total coliform count was carried out on violet red bile agar (Oxoid) by pour plate method the samples were overlaid with the same agar after solidifying. Plates were incubated aerobically for 24-48h at 37 °C (Gassem, 2019).

Staphylococci spp. count

Staphylococci spp. were determined on Baird Parker Agar enriched by egg yolk tellurite at 37 °C for 24-48h, according the manual on Laboratory Testing of Fishery Products (Froese, 2016).

The presence of pathogenic bacteria

The presence of pathogenic bacteria such as *vibrio* spp., *Campylobacter* spp, and *Listeria monocytogenes* was checked using methods described in Food and Drug Administration (FDA) Bacteriological Analytical Manual (Food and Drug Administration, 1998).

2.4 Statistical analysis

Data were analyzed to determine the means and standard deviation (SD), and one-way analysis of variance test was used to measure differences, followed by Duncan multiple range test at level ($P \leq 0.05$) using Statistical Package for Social Science version 22.0 (SPSS, 2018, SPSS Inc., Chicago, Illinois, USA). The level of significance was indicated at the 95% confidence level. All results were expressed as mean ± SD of three trials.

3 Results and discussion

3.1 Chemical composition

The chemical composition of locally cultured, frozen imported, and chilled imported fish revealed that protein contents were 16.16% ± 3.7% to 23.02% ± 1.12%, 12.5% ± 1.05% to 19.05% ± 1.4%, and 12.5% ± 1.38% to 22.5% ± 1.04%, respectively (Table 2). Protein contents were increased and were significantly different between locally cultured fish and both the chilled and frozen imported fish.

The protein content of chilled imported fish was significantly increased compared with that of frozen imported fish ($P < 0.05$). The lipid content of locally cultured fish was threefold higher than that of chilled and frozen imported fish, and significant differences were shown between locally cultured fish and both types of imported fish, which did not differ in lipid content

Table 2. Chemical composition of locally cultured, frozen imported and chilled imported fish types [the minimum (Min) and Maximum (Max)] (%).

Content (%)	Locally cultured fish		Frozen imported fish		Chilled imported fish	
	Min	Max	Min	Max	Min	Max
Total protein	16.16 ± 3.7	23.02 ± 1.12[a]	12.5 ± 1.05	19.05 ± 1.4[c]	12.5 ± 1.38	22.5 ± 1.04[b]
Total lipids	1.83 ± 0.52	18.75 ± 1.5[a]	0.75 ± 0.13	4.8 ± 0.6[b]	1.21 ± 0.03	5.5 ± 0.58[b]
Carbohydrates	0.2 ± 0.04	0.66 ± 0.16	0.3 ± 0.05	0.48 ± 0.02	0.41 ± 0.03	0.7 ± 0.06
Ash	1.32 ± 0.3	1.9 ± 0.26[a]	0.27 ± 0.02	0.48 ± 0.006[c]	0.73 ± 0.04	1.21 ± 0.05[b]
Total solids	18.52 ± 2.29	43.56 ± 2.88[a]	13.82 ± 1.3	26.76 ± 1.3[b]	14.24 ± 0.9	28.7 ± 1.7[b]
Moisture	56.44 ± 3.61	81.48 ± 5.01[b]	73.24 ± 4.64	86.18 ± 4.03[a]	71.3 ± 3.34	85.76 ± 3.2[a]

The results represent mean ± SD (n = 50). Different letters in the same row indicate significantly different results ($P < 0.05$).

($P < 0.05$). Carbohydrate levels ranged between 0.2% and 0.7%, and showed no differences between fish from the different sources. Percentage ash levels were very high in locally cultured fish compared with imported fish, but there were higher ash levels in chilled fish than in frozen fish, and significant differences were recorded for local fish followed by chilled fish, and then frozen fish ($P<0.05$). Conversely, the moisture content of imported frozen fish samples were highest, followed by chilled imported fish samples, and local fish samples showed the lowest in moisture content. The moisture in both of types of imported fish samples did not differ significantly, but were significantly increased compared with that of local fish ($P < 0.05$).

The composition centesimal of the "*Vila Franca*" shrimp *in natura* under three freezing time intervals were found to be 79.91-80.19, 14.57-16.83, 1.48-1.60, 0.92-1.58 and 0.89- 2.46 1.48 (g/100 g) moisture, protein, ash, lipid and carbohydrates respectively, the storage under freezing did not affect ash and protein content of shrimp samples (Lira et al., 2019). The chemical content of the fish varied significantly depending on several factors, including the type of fish, sex, environment, age, size, season, nutrition quality, and reproduction cycle (Reza et al., 2009). The mean crude protein and lipid results of whole, gutted, and fillets of *Dicentrarchus labrax* were 18.57% ± 0.34%, 18.49% ± 0.001%, 19.21% ± 0.06%, and 7.45% ± 0.69%, 8.19% ± 0.35%, 6.62 ± 0.22%, respectively (Baygar & Alparslan, 2015), Aquatic food products deteriorate rapidly post-mortem as a consequence of biochemical and microbial breakdown mechanisms (Food and Agriculture Organization, 2014). Storage of fish products allows larger opportunities for chemical and enzymatic changes in marine foods (Crawford et al., 1979). Reddy et al. (1990), reported a moisture composition of 70.8% and 72.0% for fish fingers made from croaker and pink perch, respectively. Comparable to our results, the centesimal composition: moisture, protein, lipids, carbohydrates and ashes of white fresh hake was 77.59, 19.85, 1.47, 0.04 and 1.07 (g/100 g) respectively on a wet basis (Brito et al., 2019).

In addition, the moisture content of red tilapia on ice was 74.47% ± 1.54% to 78.33% ± 1.53%, and 76% ± 1.73% to 77.67% ± 0.58% for gutted and ungutted tilapia, respectively (Ruíz-Osorio et al., 2015). Ash is calculated as the sum of minerals present in seafood, and the ash content of fish often ranges from 0.4% to 2% in seafood (Balachandran, 2001), Nutritionally, protein is considered the most vital component of food. The quantity of protein in most seafood is 18% to 20%, and typically ranges from 15% to 24% (Balachandran, 2001). Changes in lipid content of *Rastrelliger kanagurtae* during iced and refrigerated storage ranged from 0.57-1.45 g/100 g (Chudasama et al., 2018). These differences may be explained by deviance in lipids depending on age, sex, and types feeding from fish to fish, even within the same types of fish.

A study reported that protein content was (18-22%), while the lipid content was < 3%, while moisture and ash were74.90-79.50% and 1.01-1.50%, respectively, in important finfish and shellfish in Arabian Gulf, Saudi Arabia (El-Faer et al., 1992). In the important fish in Makkah central fish market, Saudi Arabia, moisture content range was 58.27% in 79.41%, and ash % (dry weight) ranged between 1.20% and 5.78%, while crude oil was 1.17% to 38.00%, and crude protein was between 54.19% and 90.75% (dry weight) (El Shehawy et al., 2016).

pH values

The mean pH values for locally cultured, frozen imported, and chilled imported fish samples were 6.48 (range, 5.84-7.12), 7.42 (range, 6.94-7.90), and 7.05 (range, 6.23-7.78), respectively (Figure 1). There were statistically significant differences ($P < 0.05$) between locally cultured and both types of imported fish, which differed significantly at the same level.

Similar finding were also reported for the pH of wire-netting reef cod (*Epinephelus merra*), which increased significantly during storage in ice ((Jeyasekaran et al., 2005), suggesting that changes in pH during fish refrigeration varied according to species as

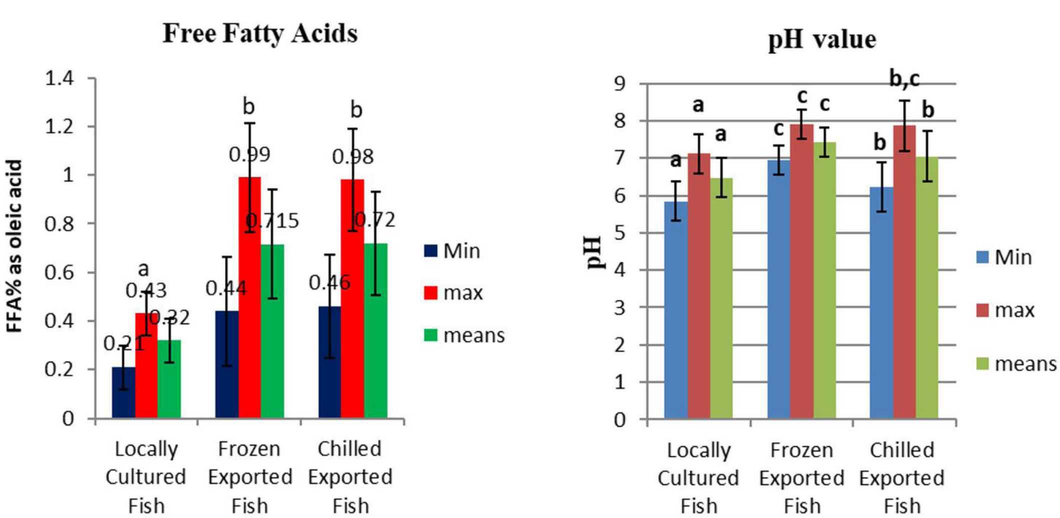

Figure 1. PH value and Free Fatty Acids of locally cultured, frozen imported and chilled imported fish. The pH values and Acidity (FFA% as oleic acid) of locally cultured, frozen imported, and chilled imported fish. The results represent mean ± SD (n = 50). Different letters in the same column indicate significant differences ($P < 0.05$).

well as additional factors. The increase in pH during storage could be ascribed to the production of basic compounds, such as trimethylamine, ammonia, and additional biogenic amines produced by bacteria during spoiling (Ruiz-Capillas & Moral, 2001).

Recently, the type and concentration of biopolymers as wall materials affected the droplet size, zeta potential, pH, moisture, surface oil content and encapsulation efficiency for nanoencapsulation of Freez dried nanoencapsulated fish oil (Vahidmoghadam et al., 2019). The mean pH of whole, gutted, and fillets of *Dicentrarchus labrax* were 6.46 ± 0.002, 6.45 ± 0.001, and 6.45 ± 0.002, respectively (Baygar & Alparslan, 2015). A previous study showed that pH increased during storing, in gutted tilapia, pH increased from 6.09 to 6.51 during 17 days of storage (Ruíz-Osorio et al., 2015). The pH values were 6.1 in dry *prawn masala*, 5.7 in dry *prawn sambal*, and 6.23 and *prawn pepper fry* (Jeyasanta et al., 2019). The observed increase in pH values in the present study could have been due to microbial contamination and enzymatic release of oxygen and hydrogen, leading to an increase in hydroxyl ion concentration, thus triggering a rise in pH (Turhan et al., 2001).

Free fatty acids

The acidity values (FFA% as oleic acid) of the locally cultured, frozen imported and chilled imported fish are shown in Figure 1. The lowest FFA% was found in locally cultured fish (0.21% to 0.43%), followed by frozen imported fish samples (0.44% to 0.99%), and chilled imported fish (0.45% to 0.98%). The mean FFA% was 0.32%, 0.715%, and 0.72% for locally cultured, frozen imported, and chilled imported fish, respectively. Locally cultured fish showed significantly decreased exposure compared with frozen and chilled imported fish ($P < 0.05$), which showed no significant differences between the two types.

Also, the lipid hydrolysis in the fish tissue resulted in the accumulation of FFA (Careche et al., 1999). FFA aggregation up to 500 mg/g of wet weight in meat during storage does not reveal the effects on the sensory quality the meat (Geromel & Montgomery, 1980). Thus, an increase in acid value is attributed to the liberation of FFA as a result of lipid oxidation. In agreement with the results of locally cultured fish in the study, the content of FFA as a percentage of oleic acid of fish fingers made from croaker and pink perch were 0.43% and 0.41%, respectively (Reddy et al. (1990). Another study reported that the FFA content of a fish sausage that incorporated dry powder potato starch increased to 3.45% oleic acid (Hegde et al., 1992). Oleic acid levels of approximately 0.5% to 1.5% start to become detectable to the palate (Pearson, 1976). A recent study showed that the FFA rate of ready-to-eat marine shrimp was highest (0.32%), followed by dry prawn masala (0.269%) and prawn pepper fry and dry prawn sambal (0.11%). These levels are within the satisfactory boundary of product lipolysis (Jeyasanta et al., 2019).

Total Volatile Basic Nitrogen (TVB-N)

The results of the Total volatile basic nitrogen (TVB-N) (mg/100 g) in locally cultured, frozen imported and chilled imported fish (Table 3), showed that TVB-N values were ranged between 8.43-19.01 with mean of 14.21 ± 1.01, 14.70-33.6 with mean of 18.69 ± 2.98 and 24.02-42.94 with mean of 31.20 ± 3.51 (mg/100 g) in locally cultured frozen imported and chilled imported fish respectively. All of the locally cultured fish samples were under the limit of TVB-N (30 mg/100 g) for fish, while 22.73% and 7.16% of frozen imported and chilled imported fish respectively were over TVB-N limit, and considered unacceptable. The locally cultured fish were lower with significant differences in compare with the imported fish samples at $P < 0.05$ level, and chilled imported fish were decreased in TVB-N from that of frozen imported fish $P < 0.05$ level.

Table 3. The Total volatile basic nitrogen (TVB-N) (mg/100 g) in locally cultured, frozen imported and chilled imported fish.

	Total volatile basic nitrogen (TVB-N) (mg/100 g)		
	Min	Max	Mean ± SD
Locally cultured fish	8.43	19.01	14.21 ± 1.01[a]
Frozen imported fish	24	42.94	31.20 ± 3.51[c]
Chilled imported fish	14.70	33.6	18.69 ± 2.98[b]

Different letters in the same row indicate significant differences ($P < 0.05$).

TVBN values do identify the later stages of spoilage and therefore can be used as a routine method to determine if chilled seafood is spoiled (Sykes et al., 2009). Similar with this study, the values of TVB-N of Imported Frozen Mackerel Fish were between 19.4-26.83, with mean of 23.01 mg/100 g (Makharita et al., 2015). And in contrast with the results of this study, determined the quality of the fish in a supply chain in Negombo, Sri Lanka and found that values of TVB-N were in the range 1-67 mgN/100 g and 25.10-104.30 mgN/100 g, and the unacceptable samples were 79%, 78% in the large and small fish respectively. TVB-N were 5.6, 2.4 and 7.12 (mg N/100 g) in Dry prawn masala, Dry prawn sambal and Prawn pepper fry respectively (Jeyasanta et al., 2019). It was reported that the mean total volatile basic nitrogen (TVB-N of Lake Malawi Tilapia fish from local and super markets were 15.40 ± 0.00 mg/100 g (Kapute et al., 2012). Recent studies was agreed with the present study, it found that TVN value in the most common consumed fresh fish in Saudi Arabia were between 12.71-30.33 mg/100 g, and 9.09% of samples were exceeded 30 mg/100 g. And the average of TVB-N content (mg/100 g) of samples stored in ice increased to 32.9 ± 1.45 mg/100 g and 41.3 ± 1.47 mg/100 g on the 5th and 7th day respectively and in the refrigerated samples it were 25.9 ± 0.61 mg/100 g even after storage for 7th days (Chudasama et al., 2018).

Heavy metals determination

The heavy metal concentrations in locally cultured, frozen imported and chilled imported fish are illustrated in Table 4. The highest concentration of heavy metals determined in locally cultured, frozen imported, and chilled imported fish samples from the fish market of Jeddah, Saudi Arabia were found for iron (25.9-39.31 µg/g) followed by copper (0.72-4.32 µg/g), lead (0.93-2.34 µg/g), mercury (0.62-0.94 µg/g), and cadmium (0.09-0.35 µg/g). The results showed increases in iron, with significant differences between locally cultured fish, chilled

Table 4. Concentration of heavy metals (μg/g) in locally cultured, frozen imported and chilled imported fish.

	Locally cultured fish			Frozen imported fish			Chilled imported fish		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Iron	25.9	39.31	32.605 ± 2.1[a]	18.47	30.86	24.66 ± 4.2[c]	22.16	37.05	29.60 ± 33[b]
Cadmium	0.024	0.21	0.117 ± 0.011[c]	0.09	0.35	0.22 ± 0.03[a]	0.03	0.33	0.18 ± 0.04[b]
Copper	0.69	3.36	2.025 ± 0.99[b]	0.72	4.32	2.52 ± 0.54[a]	0.35	2.45	1.4 ± 0.10[c]
Lead	0.55	0.86	0.705 ± 0.14[c]	0.93	2.34	1.635 ± 0.82[a]	0.84	2.12	1.48 ± 0.66[b]
Mercury	0.31	0.57	0.44 ± 0.07[c]	0.53	0.96	0.745 ± 0.052[b]	0.62	0.94	0.78 ± 0.02[a]

SD is the standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

imported fish, and frozen imported fish, and significantly higher concentrations of cadmium and lead in frozen imported fish, followed by chilled imported fish, and locally cultured fish ($P \leq 0.05$). Copper levels in frozen imported fish were highest, and were significant different from locally cultured fish and chilled imported fish. Mercury concentrations were significantly higher in chilled imported fish than frozen imported and locally cultured fish ($P \leq 0.05$).

The maximum permissible levels of heavy metals are 1.0 mg/kg of mercury in meat, meat products, and fish (GCC Standardization Organization, 2013), and 0.3 mg/kg of lead and 1.0 mg/kg of cadmium in fish according to the GCC Standardization Organization (2013). Mercury concentrations in all samples were below the permissible limits according to the GSO, and levels of lead in this study were above the upper acceptable limits of lead in fish proposed by the GSO and CAC, which is 0.3 μg/g (General Standard for Contaminants and Toxins in Food and Feed, 2015), but were below the limits recognized by the World Health Organization (WHO) and Food and Drug Administration (FDA), which are 1.5-1.7 μg/g and 2 μg/g, respectively. Cadmium levels in the present study were below the maximum acceptable limits of cadmium in fish proposed by the GSO and FDA, which are 1.0 μg/g and 3-4 μg/g, respectively, while levels of copper were lower than the WHO's upper acceptable limit in fish of 30 μg/g (Center for Food Safety and Applied Nutrition, 1998; Food and Drug Administration, 1998; Muzyed, 2011). In a previous study in Saudi Arabia, levels of vanadium, zinc, nickel, cadmium, mercury, lead, and arsenic in the most common fish species available in the Saudi markets were measured. The results revealed that levels of these minerals in fish were below the maximum permissible limits allowed by both Saudi and international legislatures for human consumption (Al Bader, 2008).

In agreement with our results, the maximum copper concentration was 0.907 μg/g in *Mugil cephalus*, and the upper concentration of lead was 0.552 μg/g in *Micropogonias furnieri* (Muzyed, 2011). Similar to the results found in the present study, iron concentrations ranged from 21.17 to 33.78 μg/g (Nisbet et al., 2010), and were 25.48 μg/g in *Clupea sprattus* (Tüzen, 2003). The limits of the detected metals were 15.4-320.6 mg/kg for aluminum, 45.5-86.1 mg/kg for zinc, 0.17-20.8 mg/kg for copper, and 31.9-743 mg/kg for iron (Ismaniza & Idaliza, 2012). Also, Mercury is a chemical that bioaccumulates; therefore, older fish and fish that live higher up the food chain have higher concentrations of mercury in their systems. Children, pregnant women, and women of childbearing age are advised to avoid some fish, such as shark, swordfish, king mackerel, and tilefish due to their high levels of mercury (Redman, 2007). A study conducted in Finland quantified the role of methyl mercury from fish products in the development of heart disease. The results showed a large range of hair mercury concentrations, including undetectable levels in 3.3% of the study participants. The levels of Mercury concentration were strongly correlated with fish consumption, and the authors reported that each 1-mg rise in hair mercury led to an 11% increase in risk of acute coronary incident and a 13% increased risk of death from coronary heart disease (Levenson & Axelrad, 2006).

Furthermore, Heavy metal levels were assessed in commercially available fish in markets in the Gaza Strip, and showed detectable concentrations of copper (0.251-0.907 μg/g), nickel (0.453-0.978 μg/g), manganese (0.376-0.834 μg/g), and zinc (3.705-20.535 μg/g). All values were below the limits proposed by the WHO for fish consumption (World Health Organization, 1996), while lead and cadmium concentrations in *M. furnieri* exceeded the EC-2005 limits of the fish (Muzyed, 2011). Five metals, including chromium, cadmium, copper, zinc, and lead, were detected in fish species from two Egyptian coasts areas, with zinc showing the highest levels (57 μg/g) in all fish, followed by chromium, cooper, lead, and cadmium. Chromium concentrations exceeded the upper acceptable limits in most fish, followed by lead and cadmium. On the other hand, copper and zinc levels were below the permissible amounts recommended by the FAO (Abdallah, 2008).

3.2 Microbiological analysis

The total plate count (TPC) results represent colony forming units per gram of sample (CFU/g). The acceptable percentages of bacteria in locally cultured, frozen imported and chilled imported fish based on the aerobic plate count (APC) are presented in Table 5, and the results are categorized according to the Saudi Arabia Standardization Organization (SASO) microbiological standards for foodstuffs. The numbers of samples with counts $>10^4$ were 50, 43, and 38 samples in locally cultured, chilled imported, and frozen imported fish, respectively, and 0, 3, and 4 samples, respectively, had counts $>10^5$. Three frozen and eight chilled imported samples had counts $>10^7$, whereas no locally cultured fish exceeded the limit of $>10^7$ counts. The SASO specifies 1.0×10^7 and 5.0×10^5 CFU/g as the upper (rejectable) and lower (marginal) levels of acceptability, respectively (Saudi Standards, Metrology and Quality Organization, 2014). All locally cultured fish were considered acceptable, while 6% and 16% of chilled and frozen imported fish samples, respectively, were unacceptable.

Table 5. Total plat count (TPC) (CFU/g), acceptable percentages and TPC of bacteria in locally cultured, frozen imported and chilled imported fish based on aerobic plate count (APC).

Fish type	n	Number of samples with count				Unacceptable samples (%)	TPC (CFU/g)			
		> 10^4	> 10^5	> 10^6	> 10^7		Minimum	Maximum	Mean	SD
Locally cultured fish	50	50	–	–	–	0	7.43×10^2	2.59×10^4	2.67×10^4c	0.74×10^4
Frozen imported fish	50	38	4	–	8	16 [a]	4.62×10^4	8.87×10^7	4.42×10^7a	3.61×10^7
Chilled imported fish	50	43	3	–	3	6 [b]	6.37×10^3	2.89×10^7	1.45×10^5b	2.69×10^5

Different letters in the same column indicate statistically significant differences ($P < 0.05$).

The TPC was significantly higher in frozen imported fish than in chilled imported fish ($P \leq 0.05$).

The microbiological qualities of individual samples vary widely depending on the animal species sources, as well as the method and conditions of preservation and storage. A previous study evaluated the microbial quality of seafood from a fish market in Dammam, Saudi Arabia. The TPC values of prawns exceed the marginal levels of acceptability and the total coliform counts exceeded the limits in all analyzed samples. It was reported that 66.6% of frozen fish were unacceptable in summer, and winter sampling frozen fish yielded >10^5 CFU/g (Popović et al., 2010). The results of TPC of bacteria in locally cultured, frozen imported and chilled imported fish are shown in Table 5. The results clearly show that the mean TPC values in the examined fish samples varied, with 2.67×10^4 CFU/g, 1.45×10^5 CFU/g, and 4.42×10^7 CFU/g for locally cultured, frozen imported, and chilled imported fish, respectively. A significant difference ($P < 0.05$) was shown between frozen imported fish and chilled imported fish and locally cultured fish, as well as between chilled imported fish and locally cultured fish.

In microbiological analysis, the utilized to approximate numbers of microbes to define the freshness of fish, sanitation, and/or estimate the likely existence of organisms that are important to public health (Huss, 1994). The International Commission on Microbiological Specifications for Foods (ICMSF) stated that the APC is an important factor for evaluation of microbial quality assessment in food products and is an indicator of the overall degree of microbial contamination of foods (International Commission on Microbiological Specifications for Foods, 1996). Estimating the numbers of microorganisms is used to determination food safety, shelf life, and quality (Dalgaard, 2000). With representative sample units of at least five, plate counts below 5×10^5 are considered of good quality, between 5×10^5 and 10^7 is marginally accepted quality, and plate counts $\geq 10^7$ are considered unacceptable quality (International Commission on Microbiological Specifications for Foods, 1986).

In the present study, the overall mean values of TPC of locally cultured fish and chilled imported fish were considered to be of good microbial quality according to the SASO and ICMSF specifications. Frozen imported fish samples that exceeded 10^7 counts were considered unacceptable or poor microbial quality. In contrast, the total viable aerobic count of frozen fish and fish processing materials from Bangladesh samples were within the acceptable limits, ranging from 2.8×10^5 to 4.9×10^5 CFU/g (Sanjee & Karim, 2016). The mean values for standard plate counts were 125.394, 49.675, 61.313, and 57.625×10^2 CFU/g fish meat for marks Flander, Hasoon, white fish fillet, and Myanmar, respectively (Murad et al., 2013). The maximum bacterial count was found in prawn ($6.15 \pm 1.22 \times 10^6$ CFU/g) followed by cuttlefish ($3.23 \pm 5.50 \times 10^5$ CFU/g), while the lowest count was obtained from fish collected from a fish market in Dammam, Saudi Arabia ($2.95 \pm 2.37 \times 10^5$ CFU/g) (Al Shabeeb et al., 2016). Raccach & Baker (1978), reported that the TPC of mechanically deboned pollock, cod, and whiting ranged from 4.7×10^5 CFU/g to 7.0×10^5 CFU/g. Furthermore, it was reported that the TPC in fish balls was 2.2×10^5 CFU/g (Yu & Lee, 1995). Arannilewa et al. (2006), examined the effects of freezing storage period on the sensory, chemical, and microbiological properties of tilapia fish (*Sarotherodun galiaenus*). Their results showed reductions in protein and fat by 27.9% and 25.92%, respectively. The total count of coliform bacteria was augmented from 3×10^3 to 7.5×10^6 during storage. Furthermore, a study by Altahir (2017) revealed bacterial loads of *Orechromis sp.*, *Bagrus sp.*, and *Clarias sp.* of $5.9 \times 10^5 \pm 0.18 \times 10^5$, $4.05 \times 10^5 \pm 0.31 \times 10^5$, and $4 \times 10^5 \pm 0.47 \times 10^5$ CFU/g in fresh fish. Accordingly, the microbial quality of fish meats varies according to several factors including the source of fish, fish types, the type and efficiency of implemented processing, types of water the fish are cultured in or caught from, the temperature of storage, and the moisture quantity in the fish.

Table 6 illustrates the results of microbiological Coliforms Count, Staphylococci sp, Vibrio spp., Listeria monocytogenes, Campylobacter and Yersinia spp., of experimental fish. The Total Coliforms Count 1.26 x ± 0.77x101 CFU/g, 1.45 ± 1.1x 104 CFU/g and 1.11 ± 0.95 x 102 CFU/g in the locally cultured fish, Frozen imported fish and Chilled imported fish respectively. In addition, *Staphylococci spp* were found in the locally cultured fish, while it were 2.16 and 2.01 MPN/g in Frozen imported fish and Chilled imported fish respectively. The locally cultured fish was free of each of *Vibrio spp., Listeria monocytogenes, Campylobacter and Yersinia spp.* While *Vibrio spp. and Listeria monocytogenes* were found in 7 and 5 samples of the frozen imported fish, and 3, 3 samples of the chilled imported fish respectively. The statistical analysis revealed high significant differences between locally cultured fish followed by chilled imported fish and then the frozen imported fish in the Total Coliforms Count at P≤0.05.

As fish can spoil more rapidly than many other foods, post-harvest handling, processing, preservation, packaging, storage and transportation require particular care to maintain its quality and nutritional attributes and avoid waste and losses. Preservation and processing can reduce the rate of spoilage and thus allow fish to be distributed and marketed worldwide in a wide range of product forms destined for food or non-food uses, from live organisms to more complex preparations (Food and Agriculture Organization, 2018).

Table 6. The results of microbiological Coliforms Count, Staphylococci sp, Vibrio spp., Listeria monocytogenes, Campylobacter and Yersinia spp., of experimental fish.

Microorganisms	Fish types		
	Locally cultured	Frozen imported	Chilled imported f
Coliforms Count CFU/g	$1.26 \pm 0.77x10^{1\ a}$	$1.45 \pm 1.1x\ 10^{4c}$	$1.11 \pm 0.95\ x\ 10^{2b}$
Staphylococci spp, MPN/g	ND	2.16	2.01
Vibrio spp.	ND	7 +ve	3 +ve
Listeria monocytogenes	ND	5+ve	3+ve
Campylobacter	ND	ND	ND
Yersinia spp.	ND	ND	ND

Different letters in the same column indicate statistically significant differences (P <0.05. ND= not detected; + ve= positive.

The acceptable limits of total coliforms (TC) for fresh and frozen fish are <100MPN/g (International Commission on Microbiological Specifications for Foods, 1986). In the present study, the locally cultured fish samples were accepted, while 5 and 3% of chilled and frozen imported fish were upper the limits and considered unaccepted regarding the Total Coliform count. In the frozen fish and fish processing materials from Bangladesh the total coliforms count ranged from5MPN/g to 28MPN/g and fecal coliforms count was from3MPN/g to 8.3MPN/g. Total coliform observed in raw samples of Chapila and processed frozen Chapila, were 29-43 MPN/g, and 6.1-9.2 MPN/g consecutively. And in raw Tengra and processed frozen, the mean value of total coliform 27.00 ± 5.57 MPN/g and 9.4 ± 3.75 MPN/g respectively (Quaiyum et al., 2013). Also, fecal coliform in raw Chapila were 3-6.2 MPN/g, while in case of frozen Chapila, <3MPN/g, And the fecal coliform observed in raw Tengra was 3- 6.1MPN/g, while in frozen Tengra fecal coliform it was <3 MPN/g in all samples (Quaiyum et al., 2013).

A study determined the microbiological quality of marketed fresh and frozen seafood in Croatia reported that the microbiological quality of single samples varied widely between animal species and also between seasons in total counts of aerobic mesophilic and psychrophilic bacteria.They found that *Staphylococcus aureus* were present at < 100 CFU/g in all samples. In addition, (0.41%) shellfish sample showed a level of *E. coli* CFU exceeding the given guideline of < 10^2 per g by four-fold.

Moreover, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella sp.*, were found in tissues of three fish species samples of Locally produced marine fishes in Al-Faw City, Basrah, Iraq (Al-Sheraa, 2018). Faecal coliform counts ranged between 0 and 90 and 0 and >1100 MPN/g in large and small fish respectively. *E.coli* were found in 5% and 70% of large and small fish respectively, and *Salmonella spp* were detected in 9 samples. While *L. monocytogenes* was found in one Sardinella albella and 8 Katsuwonus pelamis fish (Ariyawansa et al., 2016).

The microbial mean contents of Hout-Kasef samples were the total aerobic bacteria 3.77 $\log_{10}$ CFU/g, Halophile bacteria count 4.32 $\log_{10}$ CFU/g, *Staphylococcus* 3.23 $\log_{10}$ CFU/g, and Yeasts and molds count 1.33 $\log_{10}$ CFU/g, Total Coliform count was found less than 1.0 $\log_{10}$ CFU/g. No *Listeria monocytogenes, Campylobacter Yersinia spp.* and *Vibrio spp.,* were detected (Gassem, 2019). Several species of 8 bacterial families were isolated from seafoods obtained from the Eastern Province of Saudi Arabia, and the most predominant were *Vibrionacea, Aeromonadaceae, Shewanellaceae, Pasteurellaceae, Caulobacteriaceae, Pseudomonadaceae, Enterobacteriaceae and Burkholderiaceae* with various rates (Ibrahim et al., 2016).

The prevalence of foodborne pathogens in imported frozen fish marketed in Eastern Province of Saudi Arabia was assessed, it found that about 49.1% of samples were positive for foodborne pathogens, The foremost bacterial contaminations and foodborne pathogens were: *Enterococci* (14.4%), *Salmonella* (16.8%), *E. coli* (18.6%), and *Pseudomonas* (14%) (Elhadi et al., 2016). In addition, The mean Total Coliforms Count gave the following, 7.31 ± 3.98 x 10^3 CFU/g, 4.13 ± 5.19 x 10^3 CFU/g and 8.04 ± 1.01 x 10^3 CFU/g for Fishes, Prawns and Cuttlefishes respectively, And species of different bacterial isolates were identified such as, *Enterobacteriaceae*, *Staphylococci* spp, *Aeromonas* spp,*Shewanella putrifaciens*, *Aerococcus viridans*, *Vibrio* spp, *Ralstonia pickettii, Psuedomonas luteola*, *Pasteurella aerogenes*, and *Acinetobacter baumannii* (Al Shabeeb et al., 2016).

4 Conclusions

The present study showed that the chemical composition of locally cultured, frozen imported and chilled imported fish was within the acceptable ranges, whereas there was contamination of heavy metals in some fish samples that exceeded the standard levels. The microbial quality of some fish samples in the imported fish was poor and the total count of bacteria and coliform exceeded the upper recommended limits, in addition to the presence of some pathogenic bacteria in numbers of imported fish samples. This could be attributed to poor sanitation practice and unsuitable conditions during the production and handling processes. Therefore, immediate action should be taken to mitigate the contamination of toxic heavy metals, microorganisms, and other contaminants by frequent analysis and inspections in markets and at the entrance of foods to the food chain, and by strict implication of the rules and regulations with respect to food safety. At the same time, it is important to increase local people's awareness of the risks of contaminants and their effects on health.

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