Study of Bacterial contamination of Vacuum packaged Fish obtained from Ahvaz market

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Abstract

Keeping health and nutritional value of the packed foods has a great value in food industries. Among the diverse methods of food processing and packaging, vacuum packaging is one of the best methods for a variety of food products, such as fish. In order to evaluate the effectiveness of such method in preventing of bacterial growth during preservation of fish fillets, four vacuum packed fish of *Scomberomorus commerson*, *Saurida tumbil*, *Otolithes ruber*, and *Oreochromis niloticus* were investigated. Using microbial cultivation followed by species-specific culture media and staining showed negative growth of *Enterococcus sp.*, but positive growth of *Escherichia coli* and *Listeria sp.* Results showed that the highest microbial contamination of four studied fillets was as follow: *Otolithes ruber* > *Oreochromis niloticus* > *Scomberomorus commerson* > *Saurida tumbil*. Results also showed that as a variety of microbial species contamination, the most contaminated fillet belong to *Oreochromis niloticus*. Results of the present study showed that vacuum packaging was un-successful against the growth of the *Escherichia coli* and *Listeria sp.*, but fully successful against the *Enterococcus sp.* In conclusion, it seems that vacuum packaging needs to be paired with other hygienic methods for keeping bacterial growth out of the fish fillets.

Keywords: Bacterial contamination, Vacuum packaging, Fish, *E.coli*

1. Introduction

Keeping the health, preservation for long times, transport, and maintenance of the nutritional value of all food products were all possible due to suitable packaging which would be conservative for foods

against malicious and corrupting factors, such as microbial, physical and chemical factors. Vacuum packaging is one the most common and effective methods of food packaging which increases the product shelf life and to keep the quality of the packaged food (Mendes and Goncalvez, 2008). Aquaculture has the fastest growth among the various agricultural industries so that the global catch

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and aquaculture was predicted as 154 million tons (FAO, 2013). As well as raising awareness about the economic importance of fish farming, studies and research on bacterial diseases have also been increasingly growing. The microbial status of the caught fish is closely related to the environmental conditions and microbial quality of the water. Other factors include water temperature, salinity, a distance between fishing grounds and evacuation stations, infected areas of freezing, and treatments and post-harvest manipulations (Baker *et al.*, 2010). Fish aerobic corruption is mainly attributed to gram-negative bacteria, but with the advancement in fish-processing technologies, opportunities for the growth of gram-positive bacteria and their dominance are more likely to result in corruption (Gram, 2009). Ecologically and corruptible, gram-positive bacteria appear to be lower in number in comparison with other bacterial groups, but have this characteristic of mutual activity with other bacteria in corruption (Gram, 2009).

It has been revealed that maintenance and growth of the microorganisms in food are not only dependent on chemical properties of the food, but also to conservation conditions (Perez-Alonso *et al.*, 2004). Enzymatic and microbial activities are two major factors for corruption of the fish fillets. A wide range of pathogenic bacteria in food products have the ability of adherence to surfaces and remain active even after hygienic processes. It has also been showed that microbial pollution of the environment could affect the food products directly or indirectly (Lalitha *et al.*, 2005). Bacteria are the most abundant category of microorganisms that cause infections in humans through the consumption of infected fish. The most well-known food pathogenic bacteria are *Listeria monocytogenes*, *Aeromonas* spp., *Pseudomonas* spp., *Enterococcus faecalis*, and *Streptococcus* spp. (Bagge-Ravn *et al.*, 2003). In recent years, consumption of infected seafood to *Enterococcus*, *Streptococcus* and *Listeria* have

caused some major illnesses in humans (Arashisar *et al.*, 2004). Due to the importance of maintaining the health quality of packaged foods, we studied the microbial contamination of packed fish fillets obtained from Ahvaz market, Iran.

2. Materials and Methods

2.1. Collecting of packaged fish fillets

Vacuum packaged fish fillets of four fish species (*Otolithes ruber*, *Saurida tumbil*, *Oreochromis niloticus* and, *Scomberomorus commerson*) were collected from Ahvaz market (Iran) during summer 2017. All specimens were licensed from the health center and their expiration date was not over. The total number of 100 fillets was collected, as 25 specimens for each fish species. All samples were of the same size and weight and kept under the same temperature conditions.

2.2. Microbial examination

In order to determine the general presence of bacteria in examined fillets, and total bacterial count, swab samples taken from fillets were cultured on two culture media of Nutrient Agar (NA) and Plate Count Agar (PCA) (Tavakoli *et al.*, 2012). After 24h of incubation at 37°C, plates were examined for bacterial growth followed by gram staining (Tavakoli *et al.*, 2012). The specific eosin methylene blue (EMB) culture media was used for isolation of the *E. coli* (Goldberg, 2001). The specific Bile Esculin Agar (EBA) culture media of 6.5% salt was used for isolating the *Enterococcus* sp., followed by blood agar (BA) culture media and catalase test (Goldberg, 2001) (Table 1).

For the isolation of the *Listeria* sp., Blood agar culture media was used followed by *Listeria*-specific selective agar culture media, which the latter followed by blood agar culture media kept in 4°C for

24h and finally examined by catalase test (Erdogan *et al.*, 2002) (Table 2).

All cultures and growth tests included the negative control (same culture media without any samples for each step) for verification of the non-contamination

to ensure the correct operation of the laboratory tests (Erdogan *et al.*, 2002).

Statistical analysis was performed by one-way ANOVA test using SPSS 16.0.

Table 1: Biochemical tests used for identification of the *Enterococcus sp.*

Test	Growth in Esculin Bile Agar of 6.5% salt	Growth in blood agar	Catalase
Response	+	-	+

Table 2: Biochemical tests used for identification of the *Listeria sp.*

Test	Catalase	oxidase	Motility at 25°C	Growth in 25°C	BA at 4°C	Hemolysis
Response	+	+	+	+	+	+

3. Results

Results of the bacterial growth from different fish fillets showed that multiple culture media had positive growth in all fish fillet samples, except for the EMB for *O. ruber* and *S. tumbil* (Table 3). Results of this positive growth confirms the presence of bacteria in studied fillets.

Results of the bacterial count for each fish species are shown in Table 4. Fillets of *Otolithes ruber* showed the highest number of bacteria on PCA, illets

of *Saurida tumbil* on EBA, and fillets of *Oreochromis niloticus* on PCA and EBA, all with significant differences ($p < 0.05$). There was no significant difference for fillets of *Scomberomorus commerson* on all culture media ($p > 0.05$).

Results of the bacterial identification in fillets of four fish species is shown in Table 5. Results showed that *Listeria monocytogenes* and *Escherichia coli* were present on some fillets and the *Enterococcus sp.* on none of the samples.

Table 3: Bacterial growth in studied culture media from 4 fish species.

Culture media Fish species	PCA	NA	EMB	BA	EBA
<i>Otolithes ruber</i>	+	+	-	+	+
<i>Scomberomorus commerson</i>	+	+	+	+	+
<i>Saurida tumbil</i>	+	+	-	+	+
<i>Oreochromis niloticus</i>	+	+	+	+	+

PCA: Plate count agar; NA: Nutrient agar; EMB: Eosin methylene blue; BA: blood agar; EBA: Esculin Bile Agar.

Table 4: Bacterial count of different fish fillets in studied culture media (Log cfu/g)

Culture media Fish species	PCA	NA	EMB	BA	EBA
<i>Otolithes ruber</i>	$1.2 \times 10^5 \pm 0.0^b$	$1.2 \times 10^4 \pm 0.1^a$	-	$1.2 \times 10^4 \pm 0.0^a$	$1.2 \times 10^4 \pm 0.0^a$
<i>Scomberomorus commerson</i>	$1.1 \times 10^4 \pm 0.05^a$	$1.2 \times 10^4 \pm 0.1^a$	$1 \times 10^4 \pm 0.1^a$	$1.2 \times 10^4 \pm 0.0^a$	$1.2 \times 10^4 \pm 0.0^a$
<i>Saurida tumbil</i>	$0.5 \times 10^4 \pm 0.1^c$	$1.2 \times 10^1 \pm 0.0^a$	-	$1.2 \times 10^2 \pm 0.1^b$	$1.2 \times 10^4 \pm 0.1^d$
<i>Oreochromis niloticus</i>	$1.2 \times 10^4 \pm 0.0^c$	$1.2 \times 10^4 \pm 0.0^a$	$1 \times 10^3 \pm 0.1^b$	$1.2 \times 10^1 \pm 0.0^a$	$1.2 \times 10^4 \pm 0.0^c$

PCA: Plate count agar; NA: Nutrient agar; EMB: Eosin methylene blue; BA: blood agar; EBA: Esculin Bile Agar.

Table 5: Bacterial Identification in fish fillets.

Bacteria Fish	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i>	<i>Enterococcus sp.</i>
<i>Otolithes ruber</i>	+	-	-
<i>Scomberomorus commerson</i>	-	+	-
<i>Saurida tumbil</i>	+	-	-
<i>Oreochromis niloticus</i>	+	+	-

The total percent of each bacterium in all experimental specimens is shown in figure 1. As the results showed the most abundant species was *Listeria sp.*

Results showed that the presence of *Listeria sp.* in studied fillets was as follows: *Otolithes ruber* > *Saurida tumbil* > *Oreochromis niloticus*. No growth was observed for *Listeria sp.* in *Scomberomorus commerson* fillets. No significant differences were observed for the presence of *Listeria sp.* in three contaminated fillets (Figure 2).

Results showed that the presence of *E. coli* in studied fillets was as follows: *Scomberomorus commerson* > *Oreochromis niloticus*. No growth was observed for *E. coli* in fillets of *Otolithes ruber* and

Saurida tumbil. No significant differences were observed for the presence of *E. coli* in two contaminated fillets (Figure 3).

Results also showed that as the total microbial load of the observed fillets, *Oreochromis niloticus* was the most contaminated fillet. The mean total microbial load of *Oreochromis niloticus* was significantly higher than the other three observed fillets ($p < 0.05$) (Figure 4).

Results of the comparison between the abundance of observed bacteria in four experimental fish fillets showed that the most abundant species was *Listeria sp.* ($p < 0.05$), as previously described, no *Enterobacter* species was observed in experimental fillets (Figure 5).

Fig 1: Presence of each bacterium in experimental fish fillets.

Fig 2: Contamination of observed fish fillets to *Listeria* sp.

Fig 3: Contamination of observed fish fillets to *E. coli*.

Fig. 4: The total microbial load of observed fish fillets

Fig 5: Comparison between the total abundance of observed bacteria in four experimental fish fillets

4. Discussion

Preventing the growth of pathogenic bacteria and increasing the shelf-life of fish as a spoilage food source by using safe methods is under the attention to the safety and quality of fishery products. It has been revealed that *Listeria monocytogenes* which causes the Listeriosis is one the most important pathogenic bacteria in meat products and one of the main concerns of health issues (Lyhs *et al.*, 2001). New methods of packaging are one of the main ways

for preventing the contamination of food products to *Listeria*, *E. coli*, and *Enterococcus*.

It has been recognized that vacuum packaging is one of the most suitable packaging methods for postponing the corruption of marine products (Yassoralipour *et al.*, 2016). Results of the present study showed no contamination of fillets to *Enterococcus*, which shows that vacuum packaging was fully successful in order to inhibit growth of such bacteria. On the other hand, the other two

observed fish fillets were contaminated as 8.33% and 12.5% to *E. coli* and *Listeria* sp. respectively.

In a previous study, the outbreak of *Listeria* sp. in local markets of silver carp and common carp in Iran were revealed as 10% and 17.5%, respectively (Akhondzadeh *et al.*, 2003) which closely compares to the results of the present study. It has been reported that 8% of carps and 2% of clupeids in a case study in Iran (Tavakoli, 2012) were contaminated to *E.coli*. The differences between the total bacterial load of previous studies and the present study can be explained by the differences in fish species, maintenance conditions and processing differences.

The presence of bacteria is one of the major causes of corruption and deterioration of the quality of fish fillets during storage time. Therefore, generally, total bacterial load estimates are used as an accepted indicator in standards (Arashisar *et al.* 2004). The ICMSF has determined the limit of Log 10^7 cfu/g for the bacterial load in a raw fish (ICMSF, 1986).

According to the presented results, the microbial load of the observed fish fillets of the present study were all in permitted range. In general, the rate of microbial decay of fish and its products depends on the microbial flora, ambient temperature and fish species (Guillern-Regost *et al.*, 2006). Several factors, such as manipulation during filleting, contamination of the equipment and the health of persons involved in the work, determine the initial rate of bacterial load of the fillet, which justifies the different microbial burden of the fillets. According to the results, vacuum packaging was successful in order to preventing the presence and growth of the *Enterococcus* sp., but not successful in the case of contamination to *E. coli* and *Listeria* sp.

5. Conclusions

There are numerous studies about heavy metal, and

other elements in the Persian Gulf. Although two ROPME cruises held in this region, but there are some unknown aspects about elements like REEs. This research carried out in order to the investigation some of REEs elements, and its relation to sediment texture, carbonate, and Tom. The result reveals that all elements have less value than average shale and upper crust. La has minimum concentration among elements ($0.01 \mu\text{gg}^{-1}$) and Ce has the maximum level ($19 \mu\text{gg}^{-1}$). REEs ratio La/Sc (1.6) and Ce/La ratio (2.1) were below the (UCC) and average shale (AS) ratios. Among selected elements, Ce show enrichment and other elements have $\text{EF} < 1$. The correlation between carbonate and REEs were negatively strong which is due to the REE depletion in the marine carbonates. Some REES like La and Ce have an anthropogenic source. Except La, other elements have CVs ($>30\%$), suggesting that their distributions might be influenced by anthological source. Spatial distribution of selected REEs in GIS approved the previous finding as well. Further investigation in REEs concentration in river flow as well as dry deposition can provide more accurate clue about actual source of REEs in the Persian Gulf.

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