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Original Article

Determination of the Risky Microorganisms in Frozen Ready-to-Eat Seafood Sold in Istanbul Market

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ABSTRACT

Since frozen ready-to-eat seafood has the potential to cause food poisoning, this study focuses on microorganisms that have the potential to be a health hazard found in frozen ready-to-eat seafood, which is sold to highly populous communities. Therefore, the most popular frozen ready-to-eat seafood, fish balls and surimi crab legs, from Istanbul market were studied. Samples were gathered from seven different branches of four major chain market brands, twice in winter (October-March) and twice in summer (April-September). As a result of seasonal conditions summer samples had higher microbial loads compared to winter samples. The average mesophilic and psychrophilic aerobic bacteria counts of fish balls and surimi crab legs were below ≤4 log cfu/g. All samples were safe in terms of *Salmonella*, *Vibrio parahaemolyticus* and *Vibrio cholerae*; and acceptable in terms of *Staphylococcus aureus Clostridium perfringens*, and *Bacillus cereus*. The microbiological load of fish balls was higher ($p<0.05$) than the other samples. It was observed that more than 80% of them are risky in terms of coliform bacteria. It is concluded that attention should be paid especially to minced and spice added products. It is essential to pay more attention to the marketing of ready-to-eat seafood during the summer seasons for the prevention of public health.

Keywords: Pathogen, seafood, surimi crab leg, fish balls, ready to eat, frozen

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INTRODUCTION

Frozen and ready-to-eat foods are increasingly more preferred due to their fast and easy preparation, proximity to qualities of fresh produce, storability for long periods after purchase, provision of an individual, complete meal to the consumer, and widespread frozen product storage facilities (Giannakourou & Taoukis, 2005). The importance of seafood is well-known in terms of nutrition. Since many consumers do not prefer to clean and cook seafood at home, they are very important in the ready-to-eat food sector (Mol & Varlik, 2004). In addition to this, currently it is of utmost importance that the product is hygienic and safe because contamination that may occur during preparation of the product or long haul transportation and inappropriate conditions that may occur during storage or sales can cause ready-to-eat food to pose critical health risks to humans (Christison et al., 2008). In the course of transportation, storage, and sales of frozen ready-to-eat food, temperatures below -18 °C should be maintained and fluctuations in the temperature should not be allowed; otherwise, microorganisms posing health risks to humans may develop (Giannakourou & Taoukis, 2005). World Health Organization/Food and Agriculture Organization's committees of food safety experts have indicated that the most important health problem of the modern world is the diseases due to the consumption of food. Existence of risky microorganisms in ready-to-eat meals made of seafood is a great danger to public health since it may lead to mass food poisoning. However, there is very limited information on ready-to-eat seafood sold to a highly

populous community. That is why it has become necessary and essential to give useful information on bacterial load of ready-to-eat seafood sold in Istanbul where a large population inhabits.

This study aimed to determine the risky microorganisms that lead to food poisoning in widely consumed frozen seafood. Results of this study will create awareness in the seafood sector and consumers, determine the bacteriological risks of frozen ready-to-eat seafood, and reveal the potential harms to public health.

MATERIAL AND METHODS

Materials and sample collection

In this study, two of the most consumed frozen ready to eat seafood, fish-balls and surimi crab legs, were purchased from the Istanbul market. Fish-balls (n=112) are procured from seven branches of four supermarkets twice in summer (April to September) and twice in winter (October to March) periods. Sampling of surimi crab legs was conducted following the same method (n=112). Considering the possible impact of seasonal temperature changes on sales conditions, sampling was conducted both in summer and winter. Sampling locations are demonstrated in Figure 1.

Figure 1. Sampling locations in İstanbul, Turkey.

Microbiological analysis

The frozen samples were thawed at 5˚C for 12 h before analysis. Under aseptic conditions, 50g of the samples were taken and diluted in 450 ml Butterfield's phosphate buffered water for total mesophilic aerobic bacteria count, total psychrophilic bacteria count, total coliform bacteria count, *Escherichia coli* count and *Staphylococcus aureus* enumerations. For *Clostridium perfringens* and *Bacillus cereus* analysis, the sample (50 g) was diluted in 450 ml Maximum Recovery diluent and serial dilutions were prepared. After serial dilutions, samples were tested by the pour plate method (Standard Plate Count Agar, Oxoid, CM463) to determine total mesophilic aerobic and psychrophilic bacteria. For mesophilic bacteria count, incubation was at 35˚C for 24 h; for psychrophilic bacteria count, incubation time was 10 days at 7˚C (Baumgart, 1986). Total Coliform and *Escherichia coli* count were made according to Feng et al. (2020). Dilutions were plated on VRB-MUG Agar (Merck 1.04030) and typical pink colonies were counted to determine the coliform count after 18h incubation at 37˚C. Also, pink colonies were detected under UV lamp (366 nm, Merck 1.13203.0001) to define *Escherichia coli*. The colonies showing fluorescence under UV light were confirmed by indole, methyl red, Voges Proskauer and citrate tests (Feng et al., 2020). Baird Parker Agar (Himedia M043) was used for *Staphylococcus aureus* enumeration. Spread plates are incubated at 35˚C for 48 h. Typical *Staphylococcus aureus* colonies with a clear zone were confirmed using coagulase test (Tallent et al. 2016). For *Salmonella spp.* detection, 25 g of the sample was pre-enriched in Lactose Broth (Merck 1.07661) then, Rappaport–Vassiliadis Broth (Merck 1.07700) and Tetrathionate Broth (Merck 1.05285) were used for selective enrichment. Selective media used were Xylose Lysine Deoxycholate (Merck 1.05287) agar, Bismuth Sulfite Agar and Hektoen Enteric Agar (Merck 1.05418). Suspected cultures were screened by preliminary and secondary biochemical tests according to Andrews et al. (2011). The method described by Kaysner et al., (2004) was used to determine *Vibrio spp.* Each sample was examined for the presence of *Vibrio* species. After overnight incubation at 37˚C in alkaline peptone water (Oxoid CM1028), a loopful from the top culture was streaked onto Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar, Merck 1.10263). Following incubation at 37˚C 24 h, the suspected colonies were purified and tested for Gram staining, catalase, oxidase, aerobic and anaerobic utilization of glucose, motility, sensitivity to 10 and 150 µg O/129, H_2S production, acid production from D-cellobiose, lactose, arabinose, D-mannose, D-mannitol and sucrose, ONPG, Voges Proskauer, arginine dihydrolase, lysine and ornithine decarboxilase, salt tolerance (0, 3, 6, 8, 10% NaCl), gelatinase, urease, indole and growth at 42˚C. For the detection of *Bacillus cereus*, Mannitol-egg Yolk-Polymyxin Agar (MYP Agar, HI-MEDIA, Kat No: M636) was used. At the end of incubation (48 hours at 28 ˚C), colonies with pink-violet centers were counted as *Bacillus cereus* (TSC Agar, MERCK, Kat No:1.11972) (Rhodehamel & Harmon, 2001a; Halkman, 2005). To determine the number of *Clostridium perfringens,* Tryptose Sulfite Cycloserine (TSC Agar, MERCK, Kat No:1.11972) Agar was used. Black colonies were detected as *Clostridium perfringens* in petri dishes, which were incubated anaerobically for 24-48 hours in TSC Agar medium (Rhodehamel & Harmon, 2001b).

Statistical analysis

Statistical evaluation (means, standard deviations, t-test) of the data obtained from microbiological analysis (in log CFU g/ml) was made using SPSS 16.0. The study was duplicated. T- test was applied to evaluate the differences between the mean results of the groups. Differences were accepted as significant when $p<0.05$.

RESULTS AND DISCUSSION

Mesophilic aerobic bacteria count is determinative for freshness of seafood. According to International Commission on Microbiological Specifications for Foods (ICMSF), the maximum total mesophilic aerobic bacteria value for breaded and precooked seafood is 7 log cfu/g (ICMSF, 1986). On the other hand, Mol et al. (2007) reported that spoilage is observed when the psychrophilic aerobic bacteria load exceeds 6 log cfu/g in fish. It is reported that high levels of total psychrophilic bacteria count indicate that proper temperature was not maintained during storage (Alvarez-Astoga et al., 2002). The above-mentioned values were not

exceeded in any of the samples in our study (Table 1). This result suggests that products did not lose their freshness; storage and sale conditions were satisfactory.

Coliform bacteria are the frequently used indicators for determining sanitation conditions (Kala, 2006). High levels of total coliform load indicate lack of hygiene and post-processing contamination (Gonzalez et al., 2003). In our study, especially fish ball samples contained a higher amount of coliform bacteria (Table 1). Spice addition and mincing was considered the cause of high levels of coliform bacteria in the fish balls. As a matter of fact, total coliform load of fish-ball mince was detected to be 3.30 log cfu/g (Suvanish et al., 2000). Since it is known that frozen storage does not have a detrimental effect on coliform bacteria load and these bacteria can grow well under low temperatures as well (Suvanish et al., 2000; Umoafia & Okoro, 2018), high levels of coliform load in our samples is of great importance.

It was observed that fish-balls have statistically significantly higher ($p<0.05$) *E. coli* load than Surimi crab legs (Table 1). On the other hand, 12% of Surimi crab legs and 39% of fish-balls exceeded the limits (2 log cfu/g) suggested by Forsythe (2010) for ready-to-eat foods (Table 2, 3). Yalçın (2020) studied the microbiological quality of surimi sold in the markets and reported *E. coli* counts between $3.2 * 10^2$ - $9 * 10^2$ cfu / g. Adebayo-Tayo et al, (2012) sampled frozen fish samples from three different markets and reported 20% of them positive for *E.coli*. As freezing does not destroy *E. coli*, it is important to make sure food is safe before freezing (Oranusi et al., 2014)

The limit value for *Staphylococcus aureus* was given to be 4 log cfu/g (Anon, 2011; Forsythe, 2010; ICMSF, 1986) (Table 3). None of the frozen ready-to-eat seafood samples exceeded these limits in this study. High levels of *Staphylococcus aureus* in the food indicates poor hygienic conditions in the preparation of the product, especially due to personnel (Karaboz & Dinçer, 2002). Our findings demonstrated that conditions in the preparation of these products were generally appropriate.

Clostridium perfringens and *Bacillus cereus* are spore-forming pathogens and it is known that they can be easily isolated from spices as well (Iurlina et al., 2006). As a matter of fact, we ob-

Table 1. Minimum, Maximum and Average bacterial load results (log cfu/g) of the frozen fish balls and surimi crab leg in different seasons.

		Surimi crab legs			Fish-balls		
		Min	**Max**	**Average**	**Min**	**Max**	**Average**
Total mesophilic bacteria	Winter	2.26	4.26	2.94 ± 0.42^{x}	2.49	5.33	3.47 ± 0.56^{x}
	Summer	2.36	5.4	3.62 ± 0.65^{y}	2.42	5.59	4.53 ± 0.76^{y}
	All samples			**3.28 ± 0.64^{a}**			**4.00 ± 0.85^{b}**
Total Psychrophilic Bacteria	Winter	1.93	4.13	2.85 ± 0.37^{x}	2.54	4.48	3.39 ± 0.54^{x}
	Summer	2.45	4.98	3.62 ± 0.58^{y}	2.73	5.39	4.40 ± 0.64^{y}
	All samples			**3.23 ± 0.62^{a}**			**$3.90\pm0,78^{b}$**
Total Coliform	Winter	0	3.73	1.99 ± 1.23^{x}	0	4.27	2.40 ± 1.33^{x}
	Summer	0	3.45	2.58 ± 1.04^{y}	0	3.79	3.11 ± 0.80^{y}
	All samples			**2.28 ± 1.17^{a}**			**2.75 ± 1.15^{b}**
Escherichia coli	Winter	0	2.7	0.09 ± 0.50^{x}	0	3.32	0.43 ± 0.95^{x}
	Summer	0	2.93	0.54 ± 1.01^{y}	0	3.27	1.63 ± 1.23^{y}
	All samples			**0.31 ± 0.82^{a}**			**1.03 ± 1.25^{b}**
Staphylococcus aureus	Winter	0	3.46	1.77 ± 0.85^{x}	1.18	3.14	2.12 ± 0.54^{x}
	Summer	0	3.54	2.38 ± 0.52^{y}	1.88	3.3	2.68 ± 0.47^{y}
	All samples			**2.08 ± 0.77^{a}**			**2.40 ± 0.57^{b}**
Bacillus cereus	Winter	0	2.3	0.17 ± 0.56^{x}	0	3.44	1.16 ± 1.30^{x}
	Summer	0	3.27	1.46 ± 1.23^{y}	0	3.44	2.33 ± 1.17^{y}
	All samples			**0.81 ± 1.15^{a}**			**1.74 ± 1.36^{b}**
Clostridium perfringens	Winter	0	3.51	0.14 ± 0.30^{x}	0	2.48	0.41 ± 0.86^{x}
	Summer	0	3.27	0.81 ± 1.06^{y}	0	3.58	2.04 ± 1.32^{y}
	All samples			**0.48 ± 0.93^{a}**			**1.23 ± 1.38^{b}**

x, y: the difference between lines; a, b: the difference between columns ($p<0.05$).

Table 2. Exceeding the limit percentages of samples and number of samples exceeded limit/total number of samples.

	Surimi Crab leg		Fish Ball	
	E. coli	***S. aureus***	***E. coli***	***S. aureus***
Anon 2011		0%		0%
ICMSF 1986	4% (4/112)	0%	16% (18/112)	6% (7/112)
Forsythe 2010	12% (10/112)	0%	39% (44/112)	0%

Table 3. Microbiological Limits (log cfu/g).

	E. Coli	***S. aereus***	***B. cereus***	***C. perfringens***
Anon 2011	No limit	4	4	No limit
ICMSF 1986	2.69	4	No limit	No limit
Forsythe 2010	2	4	4	5

served that both bacteria species were present at significantly higher levels ($p<0.05$) in the fish-balls which contained spices (Table 1). *C. perfringens* can be found in raw ingredients like spices used in food processing. *C. perfringens* outbreaks usually occur from improper handling, such as insufficient cooling at the home, retail, or during food service (Juneja et al., 2009). However, there are no official criteria established by the European Commission (EC) for *Bacillus cereus* and *Clostridium perfringens*. But according to Anon (2011) the *Bacillus cereus* limit is 4 log cfu/g. Also Forsythe, (2010) reported 5 log cfu/g and 4 log cfu/g as inappropriate values for *Bacillus cereus* and *Clostridium perfringens*, respectively. In our study, these bacteria were higher in the summer period ($p<0.05$).

Food codex urges that 25 g food should not have any *Salmonella* spp. and in the case of their presence these products should not be offered for human consumption (ICMSF, 1988), because *Salmonella* spp. is the most widely spread bacteria that causes food poisoning in the world (Gonowiak, 1990). Similarly, *Vibrio parahaemolyticus* and *Vibrio cholerae* should not be present in the 25 g of seafood (ICMSF, 1988). In our study, *Salmonella spp., Vibrio parahaemolyticus* and *Vibrio cholerae* were not detected in the Surimi crab legs and fish-ball samples.

As a result of our study, we observed that fish-balls have statistically significantly higher microbial load than Surimi crab legs ($p<0.05$). The mincing operation in the preparation of fish-balls leads to extension of the surface and microorganisms spread all over this surface (Vural & Yesilmen, 2003). Moreover, it is known that spices are contamination sources (Little et al., 2003) and it is concluded that spices present in fish-balls are effective at producing this result. As a matter of fact, an increase in the bacterial load of fish was reported after mixing with spices.

It was observed that there are differences between samples of both Surimi crab legs and fish-balls taken in summer and winter in terms of all bacteria ($p<0.05$) and bacterial load of samples taken in summer were always higher (Table 1). It was also reported as a result of a study conducted on shrimps stored at different temperatures that increasing temperature led to increased microbial activity and quality of the food degraded rapidly (Umoh & Odoba, 1999). Likewise, the microbial load of foods is estimated to be higher in summer by Vural & Yesilmen (2003).

CONCLUSION

It was concluded in our study that samples of Surimi crab legs and fish-balls were marketed without losing their freshness and they were safe in terms of *Salmonella* spp., *Vibrio parahaemolyticus*, and *Vibrio cholerae*, and acceptable in terms of *Staphylococcus aureus Clostridium perfringens* and *Bacillus cereus* which are generally observed due to inappropriate processing conditions. In general, fish-balls have a higher microbiological load. It was also detected that bacterial load of samples taken in summer time is always higher ($p<0.05$). The fact that microbial load of fish-balls is higher than surimi crab legs indicates that this product can pose hazard on public health if the conditions of storage and sale are inappropriate. The minimum and maximum values recorded for each parameter demonstrated variability within samples. This variation can be defined by the fact that the samples were collected from various sellers where the selling conditions are very important. According to the findings of our study, careful action should be taken in terms of working conditions and raw material procurement in the production of especially minced food with spice addition; special attention should be paid to the sales of ready-to-eat frozen seafood in terms of public health especially in summer.

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