BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2016. Vol. **13**(3), 1811-1820

Study the Bacterial and Fungal Quality and Physicochemical Properties of Cold Smoked Salted Fishes of Caspian Sea, Iran

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http://dx.doi.org/10.13005/bbra/2334

(Received: 22 June 2016; accepted: 20 August 2016)

High consumption of cold salted smoked fish beside the low quality of processing of this products caused us to do the present study in order to investigate the bacterial and fungal quality and physicochemical characteristics of cold salted smoked fishes of Caspian Sea, Iran. Two hundred cold salted smoked fish samples were randomly purchased and microbial (total coliforms, *E. coli*, *Listeria monocytogenes*, vibrio spp. and *Staphylococcus aureus* and molds) and physicochemical analysis were done. Total of coliforms, *S. aureus*, *E. coli*, *L. monocytogenes* and Vibrio spp. and molds in cold salted smoked fishes were 50%, 45%, 2%, 18%, 23% and 93%, respectively. *Aspergillus fumigatus* were the most commonly detected fungi. Frequency of coagulase gene and kanagawa factor in *S. aureus* and *V. parahaemolyticus* isolates were considerable. There were no STEC strains in the *E. coli* isolates. Group 1 was the most commonly detected serotype in *L. monocytogenes* strains (55.55%). Mean ± standard error of moisture, TVN, pH and salt were 45.02±0.90, 79.45±4.75, 6.07±0.02 and 16.93±0.41, respectively. The growth of *S. aureus* and V. *parahaemolyticus* in samples with lower pH and moisture contents was significantly low. Accurate hygienic surveillance should perform to improve the chemical and microbial quality of cold salted smoked fish of Iranian markets.

Keywords: Clod smoked salted fish, Physicochemical, Microbial, Caspian Sea, Iran.

Fish is highly nutritious, easily digested and tasty foodstuff. It is much sought after by a broad cross-section of the world's population, particularly in developed countries. Today, extensive increasing in the consumption of fish and marine products has been occurred in developing countries like Iran[1,2]. Adeli et al. (2011)[1] reported the growing status of fish consumption per capita among Iranian people from 40 years ago up to now.

Proved reports represented that fish and marine foods are risks for variety of foodborne diseases[3-5]. During recent years, 188 outbreaks of seafood-associated diseases, causing 4,020 illnesses, 161 hospitalizations, and 11 deaths, were reported[6].

The microbial quality of fish and marine foods is closely related to environmental conditions and microbiological quality of the water. Water salt content, temperature, microbial load and post-harvest handling or processing conditions can effect on the microbiological quality of seafood products[7].

Amount of the fish caught from the Caspian Sea have been processed to smoked fish. These products are prepared using salting followed by cold smoking. Fish is smoked more for appearance, flavor and preservation. Today's lightly salted and smoked fish is not a preserved product; the amounts of salt and smoke used are

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not sufficient to prevent bacterial spoilage. The primary quality of salts and even sources of smoke are so low. Besides, most food poisoning bacteria can grow under the conditions normally found in processing and storage of smoked fish.

There were several reports showed that consumption of smoked fish can cause several implications in human beings[7-9].

In addition to microbial load, numerous frauds that take place at the processing and storage stages of fish smoking have also changed the physicochemical characters of fishes.

From a clinical, microbiological and physicochemical prospective, consumption of these products, which are popular among Iranians and traditionally consumed undercooked, can cause severe diseases in people. Therefore, the present study was carried out in order to investigate the bacterial and fungal quality and physicochemical characteristics of salted cold smoked fishes of Caspian Sea, Iran.

MATERIALS AND METHODS

Samples, preparation and primary microbiological tests

From October 2014 to July 2015, a total of 200 fish were purchased from supermarkets and fishing centers of various parts of Mazandaran province, Iran. Fishes were caught from the Caspian Sea. All fishes were salted for two weeks (8-13% salted fish). Then cold smoking was performed in temperature lower than 32 ^{o}C. All samples were immediately transported to the Food Hygiene laboratory of Islamic Azad University of Karaj, Karaj, Iran.

Ten grams of each sample (steaks cut from the meat, skin and gill region of fishes) were weighed into a sterile stomacher bag with 90 ml of sterile diluents containing 0.1% peptone, 0.8 % NaCl with pH adjusted to 7.2. The mixture was then macerated for 2 minutes in a stomacher (Lab Blender, Model 400). 1 ml of the homogenate was serially diluted in aseptic conditions and used for enumeration of microorganisms. Total coliforms, *E. coli*, *L. monocytogenes*, *V. parahaemolyticus* and *S. aureus* were enumerated according to the Nordic Committee on Food Analysis official methods[10, 11] using Violet Red Bile Agar (Merck, Germany), *Eosin Methylene Blue* agar (*EMB*, Merck, Germany), Listeria selective agar (LSA, Merck, Germany), Thiosulphate Citrate Bile Salt Sucrose agar (TCBS agar, Merck, Germany) and Baird Parker (BP, Merck, Germany) respectively. Plates for coliform were incubated for 24 h at 30°C. EMB agar, TCBS and BP plates were incubated for 24 to 48 h at 37°C. LSA media were incubated for 24 h at 35°C.

Specific isolation of bacteria

Vibrio parahaemolyticus

Totally, 10-g of each samples were cultured on *Tryptic Soy Broth* (TSB, Merck, Germany; 3% NaCl, pH 8.3) and incubated at 37°C for 18 to 24 h. A loopful of the enrichment culture was streaked onto Thiosulphate Citrate Bile salt Sucrose agar (TCBS agar, Scharlau Microbiology, Spain) used for the selective isolation of Vibrio strains. Typical colonies with green color were randomly selected. Several supplementary tests including gram staining, cell morphology, the citrate, oxidase, catalase, indol production, urease activity, Oxidation-Fermentation (O-F) test, motility (mannitol-motility agar, Pronadisa, Madrid, Spain), fermentation of selebiose, salicin, glucose, sucrose, arabinose, mannitol, ornithine and arginine and ortho-Nitrophenyl-^{2}-galactoside (*ONPG*) were also used for identification of *V. parahaemolyticus*. Results of the culture and biochemical tests were also confirmed using the Polymerase Chain Reaction (PCR) method described by Panicker et al. (2004)[12].

Wagatsuma agar (Merck, Germany) containing horse or human erythrocytes was inoculated with a 0.001-ml spot of a 0.5 McFarland standardized suspension of each *V. parahaemolyticus* strain isolated from cold salted fish. Each plate was also inoculated with Kanagawa-positive and negative control strains. The plates were incubated overnight at 35°C, and positive reactions were recorded as a zone of beta hemolysis surrounding the spot of growth on the human blood plate but not on the horse blood plate. Hemolysis on both media was considered a nonspecific reaction. In all tests, the positive-control strain produced a beta-hemolytic reaction on medium containing human blood but not on medium containing horse blood, and the negative-control strain was non hemolytic on both media.

Escherichia coli

Totally, 10-g of samples were

homogenized for 2 min in 90 ml of Peptone Water (PW, Merck, Germany). Then the samples were cultured on 5% sheep blood and MacConkey agar (Merck, Germany) and incubated for 18 to 24 h at 37 °C. Colonies with the typical color and appearance of *E. coli* were picked and streaked again on blood agar plates and re-streaked on *Eosin Methylene Blue* agar (*EMB*, Merck, Germany). The green metallic shiny colonies were considered as *E*. coli. The presumptive colonies were biochemically tested for growth on triple sugar iron agar (TSI) and lysine iron agar (LIA), oxidative/ fermentative degradation of glucose, citrate utilization, urease production, indol fermentation, tryptophan degradation, glucose degradation (methyl red test) and motility. Results of the culture and biochemical tests were also confirmed using the PCR method described by Sabat et al. (2000)[13].

E. coli strains were inoculated on Cefixime-tellurite Sorbitol Mac Conckey Agar (CT-SMAC) (Oxoid) and incubated for 24 h at 37 °C. Typical colourless colonies on CT-SMAC were consider as *E. coli* O157:H7.

Staphylococcus aureus

Twenty-five grams of each samples were weighed into sterile stomacher bags and diluted with 225 mL of sterile Butterfield's phosphate buffered dilution water (BPD, Merck, Germany). All samples were homogenized in a stomacher for about 1 min. Ten milliliters of BPD homogenized was inoculated into tryptic soy broth (TSB, Merck, Germany) with 10% NaCl and incubated at 37°C for 18 h. One loopful of the TSB was streaked on Baired-Parker agar (Merck, Germany) supplemented with egg yolk-tellurite emulsion and incubated at 37°C for 24 h. From each plate, typical colonies of *S. aureus* were isolated and cultured separately on brain-heart infusion agar (BHI, Merck, Germany). The identification was carried out using the following tests: Gram staining, production of coagulase, and fermentation of mannitol. Results of the culture and biochemical tests were also confirmed using the PCR method described by Alarcón et al. (2006)[14].

To confirm the presence of coagulase enzyme in the *S. aureus* strains isolated from cold salted fish samples, strains were incubated overnight in brain heart infusion broth (Merck, Germany) at 35 °C. The slide coagulase test was performed by adding 0.2 ml of the overnight brain heart infusion broth culture to 0.5 ml of rabbit plasma (Difco) in a tube. After 15 seconds of mixing, presence of coagulation represents the positive results.

Listeria monocytogenes

For *L. monocytogenes* isolation 1 g of each sample was aseptically taken, cultured in 9 mL of *Listeria* enrichment broth (UVM I) (Merck, Germany) and incubated at 37 °C for 24 h. One mL of primary enrichments were transferred to 9 mL of UVM II (Frazer broth) (Merck, Germany) and incubated at 37 °C for 24 h. Secondly enrichments were streaked onto Oxford agar (Merck, Germany) and Palcam agar (Merck, Germany) and incubated at 35 °C for 48 h. The plates were examined for *Listeria* colonies (black colonies with black sunken) and at least 3 suspected colonies were subcultured on Trypton Soy agar supplemented with 0.6% of yeast extract (TSAYE) (Merck, Germany) and incubated at 37 °C for 24 h. All the isolates were subjected to standard biochemical tests including Gram staining, catalase test, motility test at 25 °C and 37 °C, acid production from glucose, manitol, rhamnose, zylose, ±-methyl-D-mamoside, and nitrate reduction, hydrolysis of esculin, MR/VP test, ß-hemolytic activity, and CAMP test. *L. monocytogenes* isolates were identified using methods described in the Bacteriological Analytical Manual (BAM 2012)[15]. Results were also confirmed using the PCR method described by Elizaquível and Aznar, (2008)[16].

Serotypes were determined by a combination of multiplex PCR and antisera agglutination tests according to the method described by Burall et al. (2011)[17]. Briefly, overnight brain heart infusion agar (BHI-A) cultures were used to make lysates used for multiplex PCR analysis as well as antisera agglutination assays using Difco Listeria O antisera types 1 and 4 (BD Diagnostic Systems, Sparks, MD). The agglutination assay protocol was modified by suspending the bacteria in approximately 1 mL of FA buffer (BD Diagnostic Systems) and then followed based on the manufacturer's protocol.

Mycological examination

Twenty-five g of the sample was blended with 225 ml of 0.1% peptone water and 0.1 ml of the appropriate dilutions of samples were placed on Sabouraud Dextrose Agar (SDA, Merck, Germany) supplemented with chloramphenicol and

streptomycin (65 µg/ml) and were incubated at 28±1 °C for 3-5 days and the colonies were counted for total fungal count and the count was expressed as cfu/g. Growing colonies were examined using optic microscope.SO, Color, texture, growth rate, morphology and pigmentation of colonies were examined macroscopically. Tease mount using the lactophenol cotton blue was applied and microscopic features such as spore and hyphae morphology were studied (Ochei and Kolhatkar, 2000)[18].

Physicochemical analyses

Water activity, pH, TVN, sodium chloride and moisture

The water activity was determined using a water activity meter (AQX-2, Nagy mess system, Germany) calibrated at the ambient temperature (20 °C) with distilled water (aw=0.999) and saturated solution of NaCl (aw=0.756) and KCl (aw=0.853). The pH was analyzed on homogenates of 5.0 g sample in 45 ml of distilled water using a pH meter (Model 230, Mettler-Toledo GmbH, and Switzerland). For TVN, 10g of sample was blended and added to 300 ml tap water and 2 g magnesium oxide in a distilling flask of macro-Kjeldahl for distillation. The distillate collected in 2 % boric acid was titrated with 0.1 NH_2SO_4. The TVN as mg N/100g was obtained multiplying the titre (less blank) by 14. Sodium chloride content in fish samples was determined by volumetric method of Volhard[19] Official method 937.09. Moisture content was determined by oven drying of 5g of fish fillet at 105°C until a constant weight was obtained[19].

Statistical analysis

Statistical analysis was performed using SPSS/18.0 software for significant relationships. The incidences of each bacteria, fungi, chemical characters and the number of bacteria and fungi isolated from cold salted fish samples were statistically analyzed. Statistical significance was regarded at a P value < 0.05.

RESULTS

The results of our investigation showed that 186 out of 200 samples (93%) were contaminated with various types of molds. The minimum count of molds were 1.1×10^3 CFU/g and the maximum count were 6.5×10^4 CFU/g .Fungi belonging to 5 species were isolated from the

Table 1. Total prevalence of molds in the salted cold smoked fishes of Caspian Sea

Types of samples	No. samples collected	No. positive samples (%)	Distribution of various types of molds (%)				
			Aspergillus spp.		*C. albicans*	*P. expansum*	*F.venenatum*	*C. herbarum*
			A. fumigatus	*A. flavus*				
Cold salted smoked fish	200	186 (93)	69 (37.09)	31 (16.66)	17 (9.13)	22 (11.82)	19 (10.21)	28 (15.05)

Table 2. Total prevalence of bacteria in the salted cold smoked fishes of Caspian Sea

Types of samples	No. samples collected	Coliforms	Distribution of various bacteria (No. positive)								
			S. aureus (90)		*E. coli* (4)		*L. monocytogenes* (36)		Vibrio (46)		
			Coa+ (%)	*Coa-* (%)	STEC (%)	Non-STEC (%)	Group 1 (%)	Group 2 (%)	*V. parahaemolyticus* (6) K+ (%)	*V. parahaemolyticus* (6) K- (%)	Other species (%)
Salted cold smoked fish	200	100 (50)	30 (33.33)	60 (66.66)	-	4 (2)	5 (13.88)	4 (11.11)	2 (33.33)	4 (66.66)	40 (86.95)

samples of cold salted smoked fish (table 1). Of 186 cultures isolated, 100 belonged to the genus of *Aspergillus* (53.76%) with *A. fumigatus* being predominant (69 of 100 (69%)). The incidence of *A. flavus* among all *Aspergillus* isolates were 31% (31 of 100). The most commonly detected molds in the salted cold smoked fishes of Caspian Sea were *C. herbarum* (15.05%). Statistical analysis showed significant differences (P=0.039) between the prevalence of *A. fumigatus* and *A. flavus* and also between the prevalence of *C. herbarum* and *C. albicans* (P=0.046) and *A. fumigatus* and *C. albicans* (P=0.040).

Table 2 shows the total prevalence of bacterial pathogens in tested samples. Results showed that total prevalence of coliforms, *S. aureus*, *E. coli*, *L. monocytogenes* and *Vibrio spp.* in cold salted smoked fishes were 50% (100 out of 200 samples), 45% (90 out of 200 samples), 2% (4 out of 200 samples), 18% (36 out of 200 samples) and 23% (46 out of 200 samples), respectively. Of 90 *S. aureus* isolates, 30 strains harbored the coagulase gene (33.33%). There were no STEC strains in two *E. coli* isolates. The prevalence of group 1 and group 2 among the *L. monocytogenes* isolates were 55.55% (5 of 9) and 44.44% (4 of 9), respectively. Of 46 Vibrio isolates, 6 samples were positive for *V. parahaemolyticus* (13.04%). Two out of 6 *V. parahaemolyticius* isolates were kanagawa-positive (33.33%). Statistically significant differences were seen between the prevalence of coliforms and *E. coli* (P=0.019) and between *S. aureus* and *L. monocytogenes* (P=0.027). Significant differences were also seen between the incidence of *V. parahaemolyticus* and other species of Vibrio (P=0.034), coagulase positive and negative strains of *S. aureus* (P=0.042) and kanagawa-positive and negative strains of *V. parahaemolyticius* (P=0.037).

Table 3 represents the mean±standard error, minimum and maximum microbiological and physicochemical indicators in salted cold smoked fishes. Based on the results of the Pearson's correlation test, inverse relationship between pH and moisture values were found in salted cold smoked fishes ($P < 0.05$). Samples with the higher acidic levels had the higher moisture. According to the results of ANOVA method, the growth of *S. aureus* in samples with lower pH and moisture contents was significantly lower than the other samples. The growth of coliforms in samples with lower moisture contents was significantly lower than other samples. The growth of *L. monocytogenes* in samples with higher moisture contents was significantly higher than other samples. The growth of V. *parahaemolyticus* in samples with lower pH and moisture contents was significantly lower than the other samples.

Total physicochemical characters in salted cold smoked fish samples infected with various types of pathogenic agents are shown in tables 4, 5, 6 and 7.

Table 3. Average, standard error of average and minimum and maximum microbiological and physicochemical indicators in salted cold smoked fishes

Characters	Moisture (%)	Aw	TVN ((mg $100g^{-1}$)	pH	Salt (%)	Coliforms (CFU/gr)	*S. aureus* (CFU/gr)	*E. coli* (CFU/gr)	*L. monocytogenes* (CFU/gr)	*V. parahaemolyticus* (CFU/gr)
Average	45.02	42.02	79.45	6.07	16.93	3.36×10^{2}	2.17×10^{4}	2.56×10^{2}	2.32×10^{4}	2.89×10^{2}
Standard error of average	0.90	0.86	4.75	0.02	0.41	2.04×10^{2}	8.35×10^{3}	2.00×10^{2}	2.12×10^{3}	2.00×10^{2}
Minimum	9.82	11.25	22.40	5.42	8.78	-	-	-	-	-
Maximum	75.72	71.83	154.00	6.63	28.67	2.00×10^{4}	6.00×10^{5}	2.00×10^{3}	5.62×10^{5}	6.25×10^{3}

Table 4. Physicochemical characters in salted cold smoked fish samples infected with *S. aureus*

Characters	Moisture (%)	pH	Salt (%)
Mean±SE	41.93±1.46	6.13±0.03	17.11±0.83
Minimum	9.82	5.70	8.78
Maximum	75.72	6.63	28.67

Table 5. Physicochemical characters in salted cold smoked fish samples infected with coliforms

Characters	Moisture (%)	pH	Salt (%)
Mean±SE	40.02±1.54	6.05±0.04	10.36±0.65
Minimum	9.82	5.49	9.95
Maximum	75.72	6.60	21.29

Table 6. Physicochemical characters in salted cold smoked fish samples infected with *L. monocytogenes*

Characters	Moisture (%)	pH	Salt (%)
Mean±SE	45.12±1.54	6.57±0.04	9.39±0.83
Minimum	9.82	5.90	5.24
Maximum	75.72	6.82	14.33

Table 7. Physicochemical characters in salted cold smoked fish samples infected with *V. parahaemolyticus*

Characters	Moisture (%)	pH	Salt (%)
MeanSE±	40.02±1.54	6.05±0.04	11.46±0.92
Minimum	9.82	5.49	7.33
Maximum	75.72	6.60	15.79

DISCUSSION

Our results represents that cold salted smoked fishes are sources of *S. aureus*, *E. coli*, *L. monocytogenes* and Vibrio spp. Our studied samples have also been contaminated with various types of molds. Microbial contaminations increase after filleting, decrease after smoking and increase during subsequent packaging, storage and marketing. There are several hypothesis which can explain the high prevalence of studied bacteria and molds in the salted cold smoked fishes of our investigation.

First may be due to the low microbial quality of primary fishes. Busani et al. (2007)[20] examined the prevalence of *L. monocytogenes*

in 2086 fish samples of Italian markets. They showed that the total prevalence of *L. monocytogenes* was 0.3%. Their results have been confirmed our findings for the low prevalence of *L. monocytogenes* in salted cold smoked fishes (2%). Animal manure is commonly used for fertilizing Iranian fish farms. Safarpoor Dehkordi et al. (2013)[21] showed that the total prevalence of *L. monocytogenes* in the fecal samples of Iranian bovine, ovine, caprine, buffalo and camel species were 4.79%, 14.78%, 12.58%, 11.11% and 8.91%, respectively. High prevalence of *S. aureus* and *E. coli* in animal fecal samples have also been reported previously[22, 23]. Therefore, it is not surprising that such numbers of our samples were contaminated with *L. monocytogenes*, *S. aureus* and *E. coli*. In addition to this, high prevalence of *L. monocytogenes*, *E. coli* and *S. aureus* in smoked fish samples have also been reported previously[5, 7, 24-27]. An important point that should not be forgotten is the fact that the water sources used for washing of primary fish samples before smoke processing maybe contaminated with *L. monocytogenes*, *E. coli* and *S. aureus*. Several investigations showed the high prevalence of these pathogens in drinking water[28-30].

In the cases of Vibrio spp. and *V. parahaemolyticus*, numbers of reports showed that these bacteria are predominance in fish and marine foods[31-33]. Another cause of the high prevalence of Vibrio spp. in the fish samples of our investigation can relates to the microbial quality of water used from washing of fishes before smoke processing. Momtaz et al. (2013)[28] reported that 2.08% of the drinking water samples of Isfahan city (a developed city of Iran) were contaminated with Vibrio spp. Other researchers have also been reported the high prevalence of Vibrio spp, in water samples[34, 35]. In addition, Vibrio spp. can handle usual salt concentrations normally found in smoked fish. Tavakoli et al. (2012)[5] reported that the total prevalence of *S. aureus* and *V. parahaemolyticus* in smoked silver crap and smoked shad were 24% and 16%, 16% and 12%, respectively. There were no positive results for *E. coli* in their samples.

Second may be due to the fact that the process of salting and smoking of fishes have not been done properly. Fish to be smoked are just gutted and washed and then cut into smaller pieces, and left in the open to drip-dry so as to prevent hardening during smoking. Some of these stages can play important roles in contamination of fish samples but based on our results this claim can be correct especially for the final stages of processing. It is because of the chemical characters of fishes were not suitable for growth and maybe survival of bacteria and molds.

Cold salted smoked fishes of our study had high concentration levels of NaCl (>15%). The moisture contents of these fishes were 45.02% (An average). According to the slightly low acidic pH (6.07) and moderate Aw (42.02), cross contamination especially in the final stages of smoking process maybe the exact cause of high presence of bacteria and fungi in studied samples. The hypothesis of cross contamination especially in the final stages of processing is more practical in the cases of coliforms. It is because of these strains are sensitive to environmental condition and as we said the chemical characters of fishes were not suitable for growth of them. *S. aureus* is not the natural microflora of the fish and marine foods. Therefore, the main route of contamination of fish samples with this bacterium is cross contamination. *S. aureus* was the most commonly isolated bacteria in our study. This is maybe due to its high ability to growth on salty and low water activity-containing foods like cold salted smoked fish.

The initial quality of the wood used to produce smoke is probably the third hypothesis for the high prevalence of bacteria and fungi in studied samples. Wet, inferior and low quality woods are not capable to produce high quality smoke contains various phenolic components as an antimicrobial agents. The relationship between bacteria, the composition of the volatile fraction of smoke and the sensory quality of smoked fishes was studied by Rósa et al. (2008)[36].

Cold-smoked fish are not cooked, because the temperature generally does not exceed 43° C. Therefore, the most common causes of spoilage in smoked fish are mold growth, with Penicillium and Aspergillus species the initial offenders. The minimum count of molds of our study were 1.1×10^3 and the maximum count were 6.5×10^4. Our results showed that the total prevalence of *A. fumigatus*, *A. flavus* and *P. expansum* in the tested

samples were 37.09%, 16.66% and 11.28%, respectively. Similar results have been reported by Daramola et al. (2014)[37].

Another part of our study focused on the characterization of bacterial isolates. Presence of coagulase factor in 33.33% of *S. aureus* strains of cold salted smoked fishes represents higher pathogenicity and virulence in these isolates. Coagulase-positive *S. aureus* are used as a process hygiene criterion in some seafood products[38]. In the cases of *L. monocytogenes*, the most commonly detected serotypes were group 1 (55.55%) and group 2 (44.44%). In the study had been conducted by Momtaz and Yadollahi (2013)[4] 4b, 1/2a and 1/2b serotypes were detected in 66.66%, 5.55% and 27.77% of *L. monocytogenes* isolates, respectively which was similar to our results. Of the studies that have been conducted in the prevalence of *L. monocytogenes* in cold smoked fish[39, 40], all have shown a high prevalence of group 1 and group 2 serotypes. Out of 13 known serotypes of *L. monocytogenes*, three (1/2a, 1/2b, and 4b) are known to be responsible for >90% of human listeriosis cases[41]. Kanagawa-positive strains were detected in 33.33% of total *V. parahaemolyticus* isolates of cold salted smoked fishes of our investigation. Kanagawa-positive strains are primarily involved with human disease. Similar results were reported by Ebrahimzadeh Mousavi et al. (2011)[42], Santos et al. (2013)[43] and Harth et al. (2009)[44]. In fact, clinical isolates of *V. parahaemolyticus* are associated with the Kanagawa factor, in contrast to most environmental isolates, and consequently this is used most often as a virulence indicator.

The temperatures and times used in processing cold-smoked fish are very favorable for the proliferation of food spoilage and food-poisoning types of microorganisms. Therefore, particular attention to proper sanitation, brining, handling, process control and prompt refrigeration after smoking is essential. The results of the present investigation showed that the cold salted smoked fished were reservoir for coliforms, various fungi particularly Aspergillus species, coagulase positive *S. aureus*, *E. coli*, *L. monocytogenes* and kanagawa-positive *V. parahaemolyticus*. Since these products are consumed raw or undercooked in Iran, consumption of them maybe caused to severe clinical syndromes.

ACKNOWLEDGMENTS

The author of this study like to thank from Dr. F. Safarpoor Dehkordi and Prof. A. Akhondzadeh Basti for his clinical and epidemiological supports. This work has been supported by the research committees of the Islamic Azad University of Karaj (Grant number 13790fw).

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