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OCCURRENCE OF *LISTERIA* SPECIES IN PROCESSING EQUIPMENTS, UNITS AND FROZEN FISH OF FISH PROCESSING FACTORIES

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Abstract:

This study was performed to determine the presences of *Listeria* species in three fish processing factories in İzmir, Turkey. Gilt head seabream (*Sparus aurata*) has been processed in three factories and exported as frozen to other foreign countries. *Listeria* spp. expecially *Listeria monocytogenes* can be a point under consideration of fish processing factories while exporting. For this purpose; A total of 300 samples were examined for *Listeria spp* in three fish processing factories to determine the contamination levels of fish processing factories *with Listeria spp.* Samples were taken from units of fish processing factories such as (boxes, processing tables, floors) and equipments (processing coats, gloves) and also processed products (frozen fish). According to the results of this study, *Listeria monocytogenes* was isolated from 21 or 7% of the samples *Listeria ivanovi* was isolated from 15 or 5% of the samples and *Listeria welshimeri/innocua* was isolated from 2 or 0.6% of the samples collected from three factories. *Listeria welshimeri/innocua* was only isolated from the processing coats (2 or 11%) in factory A. However, *Listeria monocytogenes* was isolated from boxes (1 or 6%), processing tables (2 or 11%), floor (4 or 22%), processing coats (3 or 17%), gloves (6 or 33%) and frozen fish (5 or 50%) samples taken from the factory C. Except for frozen fish, *Listeria ivanovi* was isolated from boxes (7 or 39 %), processing tables (3 or 17%), floor (3 or 17%), processing coats (1 or 6 %) and gloves (1 or 6%) taken from the factory B. The incidence of *Listeria* species in the production line of fish processing factories points out that contamination can occur during fish processing stage. Therefore, *Listeria* spp. must be controlled during processing of fishery products. Proper cleaning and sanitation programme of fish processing factories must be applied. Samples must be taken and examined regularly from every units and equipments of fish processing factories to avoid the contamination and spread of *Listeria* spp in fish processing factories. Cleaning of fish processing equipments and fish processing units could be very important in order to avoid the occurrence of cross contamination of the fishery products. It must be taken hygienic precautions because of the contamination of *Listeria* spp. Besides HACCP plan must be applied to prevent recontamination of *Listeria* species to fishery products and it must be also applied to eradicate *Listeria* species from the fish processing factories.

Keywords: *Listeria spp,* Contamination, Fish processing factories, Fishery product

Introduction

Listeria monocytogenes has been regarded as a foodborne pathogen since the early 1980s and has been indicated as the causative agent in several foodborne outbreaks of listeriosis Dillon and Patel (1993). *L. monocytogenes* is a widespread microorganism in the environment which can be isolated from a variety of foods including fish. Fresh, frozen, undercooked, dried-salted, marinated, cold and hot smoked fish and fishery products are associated with the contamination *Listeria* spp. (Motes 1991, Farber 1991, Jemmi and Keusch 1992, Huss 1997, Beumer 1997, Poysky et al. 1997, Jorgensen and Huss 1998, Kilinc, 2001, Miettinen and Wirtanen, 2006, Porsby et al. 2008, Kovacevic et al. 2012).

Biofilm formation of *Listeria* spp. at various environmental conditions significantly impairs cell variation and certain strains are capable of dominating others in colonization of surfaces. Planktonic cells tend to proliferate faster than detached cells and even more than attached, especially at stringent conditions and low contamination levels. However, at high initial contamination levels and conditions close to optimal, such differences are less pronounced (Belessi et al. 2011). The importance of preventing pre-and postprocessing contamination of *L. monocytogenes* are also necessary. Because a significant increase of *L. monocytogenes* is measured during storage, there might be an increasing risk of infection for the consumer by storing such fish for a long time (Guyer and Jemmi 1991). Up to 75 % of retail packages of sliced smoked salmon have been shown to be contaminated by *Listeria monocytogenes* (Fletcher and Rogers 1991). Contrary to some literature data, it was concluded that *L monocytogenes* is able to grow significantly on refrigerated vacuum-packaged cold smoked salmon within the shelf-life of the product (Hudson and Mott 1993). *L. monocytogenes* contamination in smoked seafoods which are not cooked prior to consumption, may pose a health risk to the consumer (Dillon and Patel 1993). The growth of the psychrotrophic pathogens *L. monocytogenes* during refrigerated storage on aquacultured fish fillets could increase the food hazard risk, particularly where there is a possibility of cross-contamination with ready–to–eat food products (Fernandes et al. 1998). Most processors carry out appropriate food safety practices, but some improvements are needed in order to minimize the risk of *Listeria* contamination. It was found that the larger processors achieved better temperature control than the smaller processors. Approximately half of the visited premises needed to improve their refrigerated storage. The risk of ceiling condensation dripping onto product was a common problem, but the smaller premises were the most affected. Small food business operators require additional information on how cleaning and sanitation throughout the process can reduce contamination of the final product. Furthermore, guidance describing the best way of determining shelf life was requested by small processors (Rotariu et al., 2014). Behavior of planktonic, attached and detached *L. monocytogenes* cells in response to changes in the environmental conditions. This may be associated with cross-contamination scenarios occurring between surfaces and products in a food industry or even between products with different physicochemical parameters, and could contribute to the development of biotraceability models. Further knowledge on such physiological changes will markedly assist in risk assessment of *L. monocytogenes*, as well as in the development of efficient HACCP plans (Belessi et al. 2011). Processors having the highest *Listeria* prevalence were also those most concerned about what microbiological testing should be carried out and how to evaluate the quality of their products. Most processors rarely exceeded (i.e. once every several years) the statutory limit set by the European Union (>100 cfu/g or presence in 25 g). The small producers did not undertake product testing for *Listeria* because of high test costs and lack of technical expertise. Hence, it was concluded that sharing expertise between producers, especially to smaller processors would be beneficial in terms of consumer protection (Rotariu et al., 2014). In recent years, consumer attention has centered on the acquisition of very fresh food. Therefore, the food industry has focused not only on meeting the safety regulations in this field, but also in keeping customers by providing safe and healthy products (Calanche et al., 2013). Microbiological assessment along the fish production chain of Norwegian pelajic fisheries sector were studied by (Svanevik et al., 2015). This study has revealed that the quality of pelagic fish can be optimised by improving the hygiene conditions at some critical points at an early phase of the production chain.

Thus, the proposed assessment scheme may provide a useful tool for the industry to optimise quality and maintain consumer safety of pelagic fishery products (Svanevik et al., 2015).

Microbial fish safety is getting a close attention from regulatory agencies and consumers. Therefore, fish farm raising rainbow trout and affiliated slaughterhouse and smoking plants were evaluated for the occurrence of *Listeria monocytogenes* in Turkey (Kisla et al., 2007).

There are many studies in the literature made about occurrence of *Listeria* spp. in food processing plants (Korsak et al., 2012; Campdepadrós, et al., 2012; Almeida et al., 2013; Strydom et al., 2013; Martin et al., 2014; Ortiz et al., 2014; Ruckeri et al., 2014; Rodriguez-Lopez et al. 2015). There are a few studies made about the presence of L. *monocytogenes* in fish processing factories in the other countries (Duarte, et al., 1999; Miettinen and Wirtanen, G 2006; Skara et al., 2011). However, in Turkey there are very limited studies made about regarding the presence of *L. monocygones* in hot-smoked fish processing plant (Kisla et al., 2007).

The hygienic qualities of processed fishery products have been affected from the hygienic qualities of fish processing factories. For this purpose; the aim of this study was to examine the hygienic qualities of three fish processing factories accociated with *Listeria* spp.

Materials and Methods

Samples

A total of 300 samples were examined for *Listeria spp* in three fish processing factories. In each factory a total of 100 samples were examined. Each plant was visited two times while processing of gilt head seabream (*Sparus aurata*). A total of 100 samples were taken from each fish processing factory in two different processing time. Samples were collected from the same places in each factory. Samples were taken from boxes, processing tables, floors, processing coats and gloves by swapping (5x5 cm^2 of area). Each site was swabbed 3 times. Frozen fish samples were also taken. All swapped samples were put into preenrichment broth and transported to the Microbiology Laboratory of Ege University Fisheries Faculty, Fish Processing Technology Department under refrigeration in 30 minutes.

Microbiological analyses

Horizontal method (ISO 11290-1:1997) was used for determining *Listeria* spp. Brilliance™ *Listeria* Agar can be used following a variety of enrichment procedures i.e. ISO, NMKL, BAM, etc. The following is a suggested protocol using ONE Broth-*Listeria.* This method has been validated by AFNOR and been shown to give equivalent results to (ISO 11290-1:1997). One Broth *Listeria* Base (CM 1066, Oxoid, Basingstoke, Hants, England) were used for the enrichment step of the *Listeria* species method. One Broth *Listeria* Selective Supplement (SR0234E) were added as supplement. Brilliance™ Listeria Agar is a medium for isolation, enumeration and presumptive identification of *Listeria* species and *Listeria monocytogenes* from food samples. Brilliance ™ *Listeria* Agar Base (CM 1080 Oxoid, Basingstoke, Hants, England) were prepared. After the sterilization period, Brilliance™ *Listeria* Differential Supplement (SR0228E) and Brilliance™ *Listeria* Selective Supplement (SR0227E) reconstituted as directed mixed well and poured into sterile petridishes.

Each 25 g of sample was put in stomacher bag and added 225 mL of One Broth *Listeria* Base (CM 1066, Oxoid, Basingstoke, Hants, England). Samples were homogenised by using stomacher (IUL, Barcelona, Spain) for 30 sec. and incubated at 30ºC for 24 ±2h. Inoculum (10 µL) was spreaded on Brilliance *Listeria* Agar Base (CM 1080 Oxoid, Basingstoke, Hants, England). Plates were incubated at 37ºC for 24 ±2 hours. The plates were examined for blue colonies with and without opaque white halos. When testing frozen fish samples, incubated negative plates for a further 24 ±2 hours and examined again according to method of (ISO 11290-1:1997).

All cultures were tested and identified using the API *Listeria* identification kit (BioMerieux, Basingstoke, Hants, England) which comprises a gallery of 10 microtubes containing dehydrated substrates for enzymatic or sugar fermentation tests. The API *Listeria* identification test kit (BioMerieux, Basingstoke, Hants, England) includes an amino acids peptidase substrate (DIM reaction) which is hydrolysed by all *Listeria* species with the exception of *Listeria monocytogenes*. Kits were used in accordance with the manufacturers' instructions. (McLauchlin, 1997).

Statistical Anaysis

The Fisher's Exact Test was used to determine the statistical differences between the three fish processing factories. Statistically significant differences according to the existance of *Listeria* spp. in the three fish processing factories between units, equipments and frozen fish samples were indicated as ($p<0.05$ and $p<0.10$), no significant differences were indicated as ($p>0.10$).

Results and Discussion

A total of 300 samples were examined and 38 different isolates of *Listeria* species were identified in three fish processing factories. The species isolated from three fish processing factories were different. In factory A, *Listeria welshimeri/innocua* was isolated only from 2 samples taken from processing coat. However, the other samples taken from the factory A was not found positive for *Listeria* species (Table 1).

Listeria ivanovi was only detected in factory B. From the samples examined about *Listeria* species, *Listeria ivanovi* which detected from 15 of the 38 (39,5%) in factory B. Except for frozen fish, *Listeria ivanovi* was isolated from boxes (7 or 39%), processing tables (3 or 17%), floor (3 or 17%), processing coats (1 or 6%) and gloves (1 or 6%) taken from the factory B (Table 2).

The species most often isolated was *Listeria monocytogenes*, which accounted for 21 of the 38 (55.3%) isolates. *Listeria monocytogenes* was isolated all the samples taken from the factory C. *Listeria monocytogenes* was isolated from boxes (1 or 6%), processing tables (2 or 11%), floor (4 or 22%), processing coats (3 or 17%), gloves (6 or 33%) and frozen fish (5 or 50%) samples taken from the factory C (Table 3).

The existance of *Listeria* spp.in the fish processing factories between units, equipments and frozen fish were determined by using Fisher's Exact test. According to the results of this statistical test, there was significant difference between factory B and factory C at $\alpha=0.05$ level for boxes (p–value= 0.041) and frozen fish (p–value = 0.033). This statistical difference was obtained at $\alpha=0.10$ level for gloves (p–value= 0.088). According to the results of this statistical analysis, it was not be obtained any statistical significant difference between Factory B and C for processing tables and floors (p–value= 1.000) and for processing coats (p–value= 0.603).

Table 1. Incidence of *Listeria* species in fish processing equipments, units and frozen fish of factoryA

Samples taken from fish processing areas	The number of examined samples	The incidence number of *Listeria spp*	*Listeria* spp
Boxes	18	--	--
Processing tables	18	--	--
Floor	18	--	--
Processing coats	18	2 (11%)	*Listeria welshimeri/innocua*
Gloves	18	--	--
Frozen fish	10	--	--

Table 2. Incidence of *Listeria* species in fish processing equipments, units and frozen fish of factory B

Samples taken from fish processing areas	The number of examined samples	The incidence number of *Listeria spp*	*Listeria* spp
Boxes	18	7 (39 %)	*Listeria ivanovi*
Processing tables	18	3 (17%)	*Listeria ivanovi*
Floor	18	3 (17%)	*Listeria ivanovi*
Processing coats	18	1 (6 %)	*Listeria ivanovi*
Gloves	18	1 (6 %)	*Listeria ivanovi*
Frozen fish	10	--	--

Table 3. Incidence of *Listeria* species in fish processing equipments, units and frozen fish of factory C

Samples taken from fish processing areas	The number of examined samples	The incidence number of *Listeria spp*	*Listeria* spp
Boxes	18	1 (6%)	*Listeria monocytogenes*
Processing tables	18	2 (11%)	*Listeria monocytogenes*
Floor	18	4 (22%)	*Listeria monocytogenes*
Processing coats	18	3 (17%)	*Listeria monocytogenes*
Gloves	18	6 (33%)	*Listeria monocytogenes*
Frozen fish	10	5 (50%)	*Listeria monocytogenes*

Similarly, Dhanashree, Ottab, Karunasagar, Goebel and Karunasagar (2003). were found *L. innocua* in 30,8% and *L. monocytogenes* in 1,3% of fresh raw fish samples. Other species of *Listeria* were not isolated in this study. *L. monocytogenes* was isolated from 4,2% of raw clams and 2,9% of raw flat fish. It is interesting to note that among all food samples studied, highest incidence of *L. innocua* was observed in seafood. *L. monocytogenes* was also isolated only from seafood. This suggests that the risk of acquiring listeriosis is higher through seafood in India. Samples that were positive for *L. monocytogenes* were raw seafood which could be cooked before consumption. Nevertheless, presence of this organism in raw seafood poses a health risk in kitchen where raw and cooked seafood may be stored and handled. Encinas, Sanz, Garcıa-Lopez. and Otero, (1999) reported that counts of *Listeria* spp. were determined during the manufacture and drying of 21 lots of five chorizos (fermented spanish sausage) varieties produced by three different manufacturers. Manufacturing procedure and smoking significantly affected presumptive *listeria* counts. Thirteen strains recovered from F1 (factory 1) batches were identified as: *Listeria monocytogenes*, *Listeria innocua* and *Listeria welshimeri*. *Listeria* strains from F2 (factory 2) were assigned to *L. innocua* and *L. welshimeri*.

Miettinen and Wirtanen, (2006) focused on the ecology of *Listeria monocytogenes* in a fish farm by following the changes in its occurrence in different types of samples for a three-year period. Weather conditions were found to have a strong influence on the probability of finding *Listeria* spp. in a fish farm environment. The number of samples contaminated with *Listeria* spp. was typically bigger after rainy periods. Brook and river waters as well as other runoff waters seemed to be the main contamination source at the farm studied. The farmed fish originally found to carry *L. monocytogenes* become gradually *Listeria* free. In another study, *L. monocytogenes* is introduced into meat processing plants through raw meat. To overcome such contamination, suppliers of raw material should adhere to specific microbiological control measures. In addition, more attention should be focused on the appropriateness and compliance with procedures of cleaning and disinfection. (Thévenot et al. 2006). Other investigators from New Zealand assessed the contamination pattern of *L. monocytogenes* in Greenshell mussel processing plants. It clearly demonstrated that factories harbor different populations of *L. monocytogenes*, but also that some of these may occur in more than one plant. (Cruz and Fletcher 2011*). Listeria* spp. are also found in smoked fish and smoked plants. Rorvik et al. (1997) reported that forty smoked salmon processing plants were examined for the occurrence of *Listeria monocytogenes* and other *Listeria* spp. in the smoked salmon and the drains. *L. monocytogenes* was detected in smoked salmon from 13 (33%) and in the drains samples from 25 (63%) of the plants. Other *Listeria* spp. were found in smoked salmon samples from 16 (40%) and in the drains of 30 (75%) of the plants. Multivariate analyses of data on hygiene, management, production facilities of the plants and bacteriological results showed that job rotation was the strongest expressed risk factor for isolation of *L. monocytogenes* from the smoked salmon. Well-maintained facilities and use of vats for salting of the fillets, showed a preventive effect. *L. monocytogenes* in the drains was found to be a sensitive predictor for the presence of *L. monocytogenes* in the smoked salmon. In general, detection of other *Listeria* spp. in the smoked salmon or the drains could not be demonstrated to have any association with detection of *L. monocytogenes*. Incidence and sources of *Listeria monocytgones* in a traditional hot-smoked rainbow trout processing plant in Turkey were studied by (Kisla et al., 2007). In this study; samples including raw fish, swabbings of equipment or other surfaces, as

well as processing water, salt, fish feed and fish samples taken after various stages of processing were collected from thirty different locations in the plant. For the detection of *L. monocytogenes*, both conventional and *Listeria* Rapid Test (LRT) were used. *L. monocytogenes* was detected in thirty out of sixty samples (50%) by LRT, while it was detected in thirty-four out of sixty samples (57%) by conventional method. No *L. monocytogenes* was detected from raw fish, smoked fish (before handling) and processing water, but it was detected in all environmental samples including swabbings of equipment or other surfaces and smoked fish samples after filleting.

Gudbjornsdottir et al. (2004) detected *L. monocytogenes* in meat processing plants varied from 0% to 15,1%, in poultry plants from 20,6% to 24,1% and in seafood plants from 5,9% to 22,1%. In raw products the average incidence was 15,6% for meat, 22,2% for poultry and 39,0% for seafood products. The heating steps during the production of RTE (ready- to- eat) products eliminated *Listeria.* On average, 2,3% of RTE meat and 4,8% of RTE seafood products were recontaminated with *L. monocytogenes.* In the seafood sector almost all *Listeria* positive samples also included *L. monocytogenes* (91,1% of the positive samples), whereas in the meat and poultry sectors other *Listeria* species (mainly *L. innocua*) dominated. In most plants, the implemented cleaning procedures were insufficient to eliminate *Listeria*.

The prevalence of *Listeria monocytogenes* in ready-to-eat products of markets in Northern Spain was studied by Garrido et al. (2009), they were being analyzed 783 samples of deli meat products, smoked fish and pâté. RTE smoked fish was the most frequently contaminated food category (25% positive), with high occurrence in some brands (60% of lots positive). Significant differences in prevalence were found in in-store-packaged deli meat products (8,5%) with respect to manufacturer vacuum-packaged presentation (2,7%). These results reflect the need to improve hygiene and disinfection programs by addressing more accurate cleaning practices and continuous education of food workers. The occurrence of *Listeria* spp. and *Listeria monocytogenes* in retail RTE meat and fish products in Vancouver, British Columbia (B.C.) was investigated by Kovacevic et al., (2012). In this study conventional methods were used to recover *Listeria* spp. from deli meat (n =40) and fish (n= 40) samples collected from 17 stores. *Listeria* spp. were recovered only from fish samples (20%); 5% harboured *Listeria innocua*, 5% had *L. monocytogenes* and 10% contained *Listeria welshimeri.* Liu and Su (2006) indicated that food processing gloves were typically used to prevent cross-contamination during food preparation. However, gloves could be contaminated with microorganisms and become a source of contamination. This study investigated the survival of *Listeria monocytogenes* on gloves and determined the efficacy of electrolyzed oxidizing (EO) water for reducing *L. monocytogenes* contamination on seafood processing gloves.

Keeratipibul and Techaruwichit (2012) reported that the surfaces from which *Listeria* spp. were most frequently recovered were the liquid N_2 chiller exhaust pipe, the metal detector conveyor belt and the freezer drain. Therefore, the cleaning and sanitizing procedures were revised and strictly implemented to reduce and eliminate the real sources of *Listeria* contamination in the cooked frozen chicken meat process. The other investigators reported that *Listeria monocytogenes* was able to remain in specific places, particularly floor, in the factory, despite the sanitization treatments performed, although it was not detected on food contact surfaces. The identification of these *L. monocytogenes* survival points could be of value for improving their control as part of HACCP program. Both sanitizing protocols managed to reduce the LM load but not to eradicate this microorganism completely (Campdepadrós et al. 2012).

L. monocytogenes contamination of the hot-smoked rainbow trout in the plant seemed to have originated from the processing environment. There was a postprocess contamination in the plant during the period of study because all the samples after smoking were contaminated with *L. monocytogenes*. Morever, detection of *L. monocytogenes* from cleaned and sanitised equipments indicated that insufficient cleaning and sanitising procedures ignoring the possibility of biofilm were applied in the plant. It is therefore important to take hygienic precautions at different steps of the process to prevent colonization and spread of *L. monocytogenes* in processing plants. Application of a control system as HACCP will help to assure the microbiological safety and quality of the finished product (Kisla et al., 2007).

In the present study, *Listeria welshimeri/innocua* was isolated only from two samples taken from processing coats in factory A. In this factory the main contamination source was determined on processing coats. Gloves were contaminated with

Listeria spp in two factories. *Listeria ivanovi* was isolated from gloves (1 or 6%) taken from the factory B and *Listeria monocytogenes* was isolated from gloves (6 or 33%) taken from the factory C. Likewise, in the other study Liu and Su (2006) reported that gloves could be contaminated with microorganisms and become a source of contamination.

In our study, *Listeria ivanovi* was detected from all the samples taken from factory B except for frozen fish. *Listeria monocytogenes* was isolated from boxes (1 or 6%), processing tables (2 or 11%), floor (4 or 22%), processing coats (3 or 17%), gloves (6 or 33%) and frozen fish (5 or 50%) samples taken from the factory C. The presence of *L. ivanovi* in processing equipments in factory B and the presence of *L. monocytogenes* in all the samples taken from the factory C indicated that the need for frequently monitoring at the fish processing factories. Cleaning of fish processing equipments and fish processing units could be very important in order to avoid the occurrence of cross contamination of the fishery products.

Conclusion

In the present study, a total of 300 samples were examined from the three fish processing factories. 12.7% of samples were positive for *Listeria* species. *L. welshimeri/innocua* was found in 0.7% of the samples, *L. ivanovi* was detected in 5% of the samples, *L. monocytogenes* was isolated from 7 % of samples. In factory A, surface samples from workers' gloves, processing tables, boxes, floor, and frozen fish were negative for *Listeria.* On the other hand, all the samples taken from factory B (except for frozen fish samples) and in factory C were found to be positive for *Listeria* spp. The incidence of *Listeria* species in the production line of fish processing factories points out that contamination can occur during fish processing stage. Therefore, *Listeria* spp. must be controlled during processing of fishery products. Proper cleaning and sanitation programme of fish processing factories must be applied Samples must be taken and examined regularly from every units and equipments of fish processing factories to avoid the contamination and spread of Listeria spp in fish processing factories. Cleaning of fish processing equipments and fish processing units could be very important in order to avoid the occurrence of cross contamination of the fishery products. It must be taken hygienic precautions because of the contamination of *Listeria* spp. Besides HACCP plan must be applied to prevent recontamination of *Listeria* species to fishery products and also it must be applied to eradicate *Listeria* species from the fish processing factories.

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