

HYGIENIC STANDARDS AND PRACTICES IN NORWEGIAN SALMON PROCESSING PLANTS

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Abstract

The farmed salmon industry is important economically for several countries with Norway as the main producer constituting 53% of the world total. Bacterial contamination of salmon products may occur during processing, constituting potential life-threatening health hazards (e.g. listeriosis). The L. monocytogenes threat and thus strict legislation on ready-to-eat salmon products (i.e. smoked salmon) makes plant cleaning and hygiene important issues in the salmon industry. The present situation regards measured hygienic quality (i.e. cleanliness as means of total bacterial counts and the presence of L. monocytogenes), and hygiene standards and procedures in Norwegian salmon processing plants were investigated through visits and interviews at plants. The aim of the study was to identify potential sources of cross-contamination through the processing line and critical points for cleaning.

Four salmon processing plants were visited during the autumn of 2015. A total of 91 samples were collected. Sampling was performed during full operation from: gutting machines and drains, water tanks, conveyor belts, floors, and from round fish (skin and gills) using Sodi-box cloths, FloqSwabs and water samples. Total aerobic bacteria and *Listeria* spp. were enumerated by plate counting and the presence of *L. monocytogenes* confirmed.

From 91 samples, 6 were positive of *L. monocytogenes*. *L. monocytogenes* was found in one gutting machine at 2 out of the 4 plants, occasionally on floor, drains, and conveyor belts, once in a water tank, but not on ungutted fish. There was not found any correlation between the level of *Listeria* spp. and the total bacteria count ($R^2 = 0,026$, n = 30). Even though the levels were low, the findings of *L. monocytogenes* in processing equipment may potentially pose a threat to food safety. *L. monocytogenes* is a ubiquitous bacterium that is easily introduced from different sources. The main challenge is to hinder plant colonization through improved hygienic practice and hygienic design.

Key words: Salmon, Listeria, Hygiene, Hygienic design, Cleaning, Processing plants.

1. Introduction

Approximately 80% of the salmon farmed and slaughtered in Norway is exported unprocessed beyond slaughtering and gutting to other countries, where final processing and further distribution takes place. The consequence of this is that Norway loses a potential valorization of the salmon raw material, including by-products and side streams.

For the Norwegian salmon industry to fully exploit the salmon raw material, there is a need for modernization in the industry, in order to be competitive regards customs barriers and cheap labor. This implies fully automated lines including the whole process from: killing, bleeding, gutting, filleting and secondary processing, and by-product harvesting and processing. Through automation, one may limit the present use of buffer tanks for: cooling, rinsing and grading of the fish, and rather implement hygienic controllable lines focused on following single individuals through all processing steps. The use of fully automated processing will lead to reduced human labor, increased profitability, and



allow for full processing in Norway. The advantages will be better quality control in all steps, reduced transport costs and increased valorization. In such a process, hygiene is an important element, especially considering *Listeria monocytogenes* and other pathogenic bacteria that can establish in slaughterhouses and processing plants. An automated processing design handling fish individually may prevent bacterial cross contamination. It is important to secure good hygienic practices to achieve sustainability in the salmon processing industry.

The purpose of the present study was to identify sources of bacterial contamination along the present processing lines. The identification of critical steps and spots may allow for improved hygienic design connected to killing, slaughtering and processing in processing lines facilitating automation. The present situation regards measured hygienic quality (i.e. cleanliness as means of total bacterial counts and the presence of *L. monocytogenes*), and hygiene standards and procedures in Norwegian salmon slaughterhouses were investigated through sampling and interviews at four plants along the west coast of Norway.

1.1 The Salmon processing line

At present, the typical salmon slaughterhouse can be schematically outlined as in Figure 1.

Live farmed salmon is pumped either directly from the well boat transporting the salmon to the slaughterhouse, or from a sea net pen adjacent to the slaughterhouse, temporarily holding the salmon. Inside the slaughterhouse, the fish first enters a live chilling tank, with temperature close to 8 °C. The purpose of this tank is to lessen stress, to some extent sedate the fish, and to facilitate further processing by rectifying the fish. Typical residence time in this tank is 45 minutes.

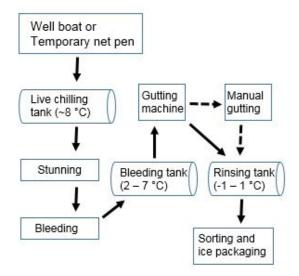


Figure 1. Typical salmon slaughterhouse operations

The fish is then stunned, normally in an electrical stunner [1]. The majority of Norwegian slaughterhouses do not have the live chilling tank, in these cases fish is pumped directly from the well boat/temporary net pen, and conveyed to the electrical stunner. Conveyor belts then transport the fish to the bleeding station, where the throat pulmonary artery is cut, in most cases manually. Bleeding out proceeds in seawater tanks with temperature of 2 - 7 °C and residence time 30 to 45 minutes. Next, fish is mechanically gutted, typically using a Baader[®] machine. A small fraction of the fish is bypassing the gutting machine and subject to manual gutting. This is due to deviant size (too small or big for the gutting machine). After gutting, the fish are conveyed to a rinsing tank with temperature of -1 - 3 °C. After approx. 25 minutes, depending on the final product format, fish are decapitated, filleted, or packed round. Fish, regardless of end-product, are finally packed on ice and stored before transport.

The Baader machine is according to plant operators a problematic source of recontamination, e.g. with L. monocytogenes, which is frequently isolated from the machine. Cleaning of the gutting machine is complicated since it is constructed of several small movable parts, lubrication points and vacuum suction, in addition to hard-to-reach areas for the cleaner. For thorough cleaning and disinfection, the gutting machine must be disassembled, which is not practically to do after each use, but rather as a part of e thorough cleandown of the processing plant, typically performed a couple of times per year. Other areas less accessible for daily cleaning, like under conveyor belts and other areas not directly accessible, may also be problematic. Conveyor belts and the transition zones between plastic and steel may form a good starting point for the formation of biofilms, especially when worn [2].

The water tanks in salmon slaughterhouses, especially the bleeding- and rinsing tanks, are easily contaminated with organic material, i.e. blood, and to a lesser extent skin mucus, scales, and gut content. L. monocytogenes is frequently observed in water high in organic material [3], and is able to survive at least 6 days in water with salmon blood at 2 - 7 °C [4]. The water tanks consist of tube systems and helixes that may function as a niche for *Listeria* spp., and due to the large size, helixes and nozzles, full control of Listeria decontamination may be difficult. Based on this, it was hypothesized that the tanks may act as reservoirs and even facilitate the persistence of L. monocytogenes. However, after analyzing the tanks in four slaughterhouses, we did not find conclusive evidence for this hypothesis with respect to Listeria. A more general conclusion is rather that fish and seawater entering the slaughterhouses have undetectable levels of *L. monocytogenes* and that contamination occurs mainly during processing after the gutting step.



1.2 Cleaning, legislation and internal routine controls

The salmon slaughterhouses are cleaned at nighttime after one or two shifts of production (depending on season and demand). This cleaning typically consists of an initial rough flushing with clean water to get rid of fish residuals and blood before it starts sticking which it will do if it starts drying. Then the area is foamed with acid or alkaline based soap and sprayed with disinfection chemicals in various forms. All cleaning is done by manual labor at present. Depending on the size of the plant, several workers walk around flushing the surfaces with a hose. Typically, the operators on the different machines do a crude flushing of the equipment and machines with cold water when their shift is finished. Then the cleaning shift comes in when the production is finished for the day. The cleaners spray on soap-foam, which covers the different machines and production surfaces. This foam should work for some time before water is sprayed on to rinse off the soap. Mostly hot water is used, but it should not be too hot because that will make it difficult to rinse of protein coatings. The last step is to apply disinfectants to inactivate microorganisms. The disinfectant is normally left to vaporize until the production starts again in the morning. The time estimated for the cleaning shift for flushing, foaming, rinsing and disinfection of the area defined as the 'slaughter line' (approx. 60 m²) in a specific slaughterhouse slaughtering > 100 tons of salmon per day is 3.5 hours. The slaughterhouses have differing routines for disassembly of equipment and full plant wash downs. This largely depends on the type of equipment and amount of use.

The Norwegian Food Safety Authority must approve: establishment, operation, moving and change of operation at slaughterhouses and processing plants. Application for approval must be followed by a description of internal control systems securing sufficient hygiene and prevention of spread of disease, and plan for journaling and documentation. The contagious hygiene demands are general, and simply stating that it must be secured that personnel, workwear, equipment, machines, used packaging etc. does not constitute a hygiene risk, there must be a barrier between by-products and wastewater, and all processing water and wastewater must be disinfected [5]. Norwegian food industry is further subject to the EU enforced Regulation (EC) 178/2002 [6], laying down the General Principles and requirements of food safety, and later Regulation (EC) 852/2004 [7], for Hygiene of foodstuffs, and other related Regulatives and Directives as reviewed by Kakurinov et al., [8]. The food safety that applies to the consumers is in the end secured through general food safety regulations. The recent EU-rules sets a limit of 100 cfu g⁻¹ at the end of the shelf life in products where L. monocytogenes is able to proliferate, like for example cold smoked salmon (CSS) [9].

There is no formal demands on the internal control systems except that it must be understood to secure sufficient hygiene and prevent spread of disease, and it is supervised, controlled and legislated by the Food Safety Authority. Systems approved can include a program for daily environmental and food product sampling for Listeria and coliform bacteria and less frequent (weekly - monthly) sampling for e.g.: total bacterial count (TBC), Salmonella, etc. in: products, specific equipment, ice and water. The samples are either analyzed in the slaughterhouses own laboratories on site, or they are sent to extern laboratories. It is very much in the slaughterhouses and their owners own interest to have a strict hygiene control because there will be serious consequences if there should be recalls or shut down, both economically and on public relations.

2. Materials and Methods

Four salmon processing plants (designated A, B, C, D) were visited during the autumn of 2015. Sampling was performed during full operation using Sodibox cloths (Sodibox, La Forét-Fouesnant, France), FloqSwabs (Copan, Italy), and water samples. Sampling was performed according to Table 1.

Approx. 2500 cm² were sampled with Sodibox cloths, and 25 cm² with FloqSwabs. Water sample volumes were 0.5 to 1 L. Only round ungutted salmon was sampled (skin samples behind the gills and above the centerline, and gills). Samples were stored at 4 °C and processed within 24 h. Sodibox cloths were placed in stomacher bags (Seward Medical, UK), suspended in 250 mL of buffered peptone water (Oxoid) and homogenized in a Starblender LB400 stomacher machine (VWR) for 3 minutes. For detection of L. monocytogenes, 45 mL of the homogenate was filtered onto a 0.45 μ m Mixed Cellulose Ester (MCE) filter with a diameter of 47 mm. The MCE filters were placed onto Listeria-selective Brilliance agar plates (Oxoid), and incubated for 24 h at 37 °C. Colonies suspected to be L. monocytogenes were transferred to new Brilliance plates and incubated as above. Presumptive L. monocytogenes on the secondary plates were again transferred to sheep blood plates (Oxoid) to observe for hemolysis, and confirmed to be L. monocytogenes by using the API Listeria kit (Bio-Merieux) according to the manufacturer's instructions.

Water samples was filtered and assessed as above, except that samples containing much blood and other organic material was prefiltered with a Steriflip vacuum-driven filtration system (Millipore, USA) with a 20 μ m pore size. FloqSwab samples from ungutted fish skin and gills were transferred to 15 mL Falcon tubes prefilled with 5 mL buffered peptone water (Oxoid) directly after sampling. FloqSwabs were left to resuspend by shaking (250 rpm) at room temperature for 30 min.

Table 1. Sampling scheme

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	Total			-

and then aliquots of the liquid were plated directly on Brilliance plates and assessed as above. Gill samples were only analyzed for the presence of *L. monocytogenes* and not quantification of bacteria.

For enumeration of total aerobic bacteria in Sodibox cloths and Floqswabs, aliquots of the homogenates were spread plated onto Plate Count Agar (PCA; Oxoid). Water samples were filtered onto MCE filters and placed on PCA plates. PCA plates were incubated for 48 h at 30 $^{\circ}$ C.

After sampling, the operators in charge at each plant was given a questionnaire with the following 12 questions as an e-mail attachment (translated from Norwegian):

- 1. What temperatures (°C) are in the water tanks inside the slaughterhouse?
- 2. How often is the water in the tanks changed?
- 3. How is seawater rinsed before use?
- 4. From what depth (m) is seawater taken?
- 5. How many persons work per shift in production (inside the slaughterhouse including packaging area)?
- 6. How many shifts per day?
- 7. How much (tons) salmon are slaughtered per day?
- 8. Is salmon entering the slaughterhouse via sea net pen or well boat?

	Table 2. Results of L	. monocvtoaenes	detection	per p	olant
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Plant #	Total samples	Positive for <i>L.</i> monocytogenes	% positive for L. monocytogenes
Α	24	0	0
В	27	2	7.4
С	14	3	21.4
D	26	1	3.8
Total	91	6	6.6

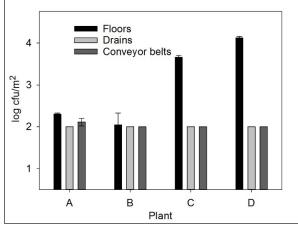


Figure 2. Presumptive *Listeria* spp. on surfaces and drains in salmon slaughterhouses. The dotted line denotes the detection limit of log 2 cfu/m2

- 9. How is the processing plant cleaned at the moment?
- 10. Do you have procedures for disassembly and washing of all machines and equipment (how often)?
- 11. What microbiological control do you apply (i.e. daily/weekly sampling, amount of samples of water, equipment, floor etc.)?
- 12. What is the most challenging area with regards to *Listeria* control?

The questionnaires were filled in within two months and delivered back by e-mail.

3. Results and Discussion

Results of *L. monocytogenes* detection are shown in Table 2 and 3 divided on premises and sample type, respectively.

The level of presumptive *Listeria* spp. is shown in Figure 2 (installations) and Figure 3 (water tanks). Total bacteria counts are shown in Figure 4 (Installations), and Figure 5 (water tanks). Note that the dimensions in the y-axis in Figures 2 and 3 are cfu per m² and L, respectively as opposed to cm² and mL in Figures 4 and 5.

The questionnaire-based surveillance is presented in Table 4.

 Table 3. Results of L. monocytogenes detection divided

 by sampled item

Sample type	Total samples	Positive for L. monocytogenes	% positive for <i>L. monocytogenes</i>
Installa- tions	42	5	11.9
Fish skin/gills	34	0	0
Water	15	1	6.7
Total	91	6	6.6

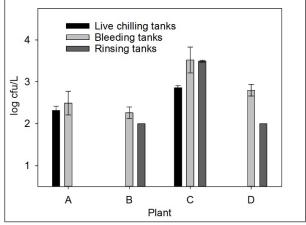
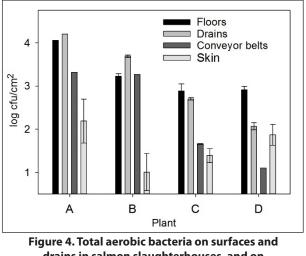


Figure 3. Presumptive *Listeria* spp. in water tanks in salmon slaughterhouses. The dotted line denotes the detection limit of log 2 cfu/L. Plant A did not have rinsing tank(s) and Plant B and D did not have live chilling tanks



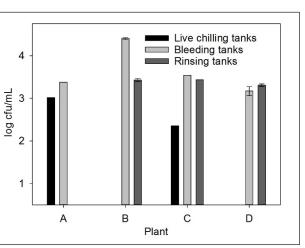




After linear regression of 30 samples positive of *Listeria* spp., there was no correlation between the amount of presumptive Listeria spp., and the total aerobic bacteria count ($R^2 = 0.026$). However, we were not able to distinguish L. monocytogenes from presumptive Listeria spp. as defined by characteristic growth on Listeria selective Brilliance plates (Oxoid), so that L. monocytogenes is only reported as positive or negative as verified by API-typing, and not quantified. Anyway, we were able to identify the closely related, but non-pathogenic L. welshimeri and L. innocua in one of the gutting machines in plant B, and in floor samples from plant C and D, respectively. The results of presumptive *Listeria* spp. quantification implies that *Listeria* spp. other than *L*. monocytogenes were comparatively frequent. It should also be noted that Bacillus spp. was found to grow with similar characteristics on the Brilliance plates. Although these could be readily disregarded by microscopy and the presence of spores, we cannot rule out that they have interfered with the analysis.

Of the 91 samples collected, only six were confirmed positive for *L. monocytogens*, and out of these, three were from gutting machines, and one each from the floor in a packaging area, conveyor belt after gutting machine, and a rinsing tank (Table 1 - 3). This means that *L. monocytogenes* was found only at the site of gutting, or after gutting in the processing line. This underpins that gutting machines, under conveyor belts, and drains are problematic areas for *Listeria* control as pointed out by the plant operators (Table 4) and that they are hard-to-reach spots for cleaning.

The present study did not sample the processed products, but it is shown that 5% of Norwegian retail CSS is positive of *L. monocytogenes* [10], and the mean prevalence in retail CSS worldwide is close to 10% [9]. In the EU in 2015, 3.9% of ready-to-eat (RTE) fish, 2.5% of RTE meat, and 1.1% of cheese were *L. monocytogenes*



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Figure 5. Total aerobic bacteria in water tanks in salmon slaughterhouses. Plant A did not have rinsing tank(s) and Plant B and D did not have live chilling tanks

positive [11]. It is well known that L. monocytogenes is a ubiguitous bacteria, and can very easily be transferred to various surfaces within a processing plant. Its saprophytic behavior allows it to decay moist plant material, and soil environments may be an important reservoir for this pathogen [12]. L. monocytogenes is very rarely isolated, however, from clean (unpolluted) seawater and from fish bred in pure water, meaning that the many positive samples from salmon products clearly indicates contamination during processing [13]. The present study is in accordance with this view, since no L. monocytogenes was found on skin or in gill of ungutted fish, and was only observed in a water tank after gutting and at the end of the slaughtering line (Table 1 and 3). Recontamination in the processing plant is often seen as the main problem [14, 15]. Some slaughterhouses may be colonized by L. monocytogenes, while others are free of the bacteria. Thus, raw material from particular producers may act as vectors for bacteria into smokehouse facilities, and it is therefore important to avoid L. monocytogenes contamination of slaughterhouses and slaughtered salmon.

Mechanical systems, e.g. gutting machines (Table 4) are difficult to clean and disinfect. Recontamination is therefore difficult to prevent. Autio *et al.*, [14] showed that by removing colonized equipment followed by thorough disinfection of remaining equipment and processing area by including hot steam, hot water, and hot air (80 °C) were effective measures for eliminating *L. monocytogenes* which was established on the processing line. Some bacteria, including *L. monocytogenes*, are capable of forming biofilms on material like for example stainless steel, which is widely used in processing equipment. Cells in this condition may be resistant against sanitary measures and thereby able to establish itself in processing lines [16]. Vogel *et al.*, [15] concluded that since salmon, although to a lim-

Table 4. Summary of surveillance based or	n questionnaire to plant operators
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Questions*		Plant				
		В	С	D		
Water tanks	Q1	0 - 2	0,5	Normally 0 – 2	Bleeding tank: 2 - 7, Rinsing tank: -1 - 2	
	Q2	Daily	Daily	Daily	Daily	
	Q3	UV treatment	No rinsing	Filter and UV treatment	UV treatment	
	Q4	30	ca 70	ca 35	ca 60	
	Q5	17-18	22 on 1 st shift, 15 on 2 nd shift	ca 40	40-45	
Production	Q6	2	2 (April 15 th - June 15 th). 1 (rest of year).	1	2	
Prod	Q7	210-215	ca 150 when two shifts, ca 90 when one shift	130-150	300	
	Q8	Well boat	Usually net pen	Usually net pen	Well boat	
Cleaning and microbiology	Q9	Daily flushing, foaming, flushing, disinfection. The plant is washed down 4 times a year.	Daily foaming, circulation wash and disinfection	Daily acid/alkaline chemicals and disinfection	Daily flushing, alkaline foam, flushing, disinfection. Switching regularly to acid foam.	
	Q10	Fixed program. Depending on type of equipment	No fixed program	Fixed program. Semiannually	Fixed program. Depending on type of equipment	
	Q11	Daily: Environmental sampling with regards Listeria (approx. 30 samples) and coliform bacteria. 3 times a week: ice sampling Weekly: Salmonella, sulfite reducing bacteria, Clostridia, and TBC. Water intakes (fresh and seawater), and from ice machine.	Daily skin and environmental sampling (sent to extern laboratory).	Daily: Product sampling, and equipment according to plan. Sampling of water 4 times per year.	Daily: Listeria in production environment and product. Twice a week: ATP sampling Weekly: Listeria and TBC in clean areas. Monthly: TBC and coliform bacteria in fresh/sea water and ice.	
	Q12	Areas less accessible for daily cleaning with risk of biofilm formation (gutting machine, under conveyor belts, transitions between plastic and steel, etc.)	Gutting machines	Gutting machines	Vacuum systems and gutting machines, floors and drains.	

Legend:

*: Q1: What temperatures (°C) are in the water tanks inside the slaughterhouse?; *Q2: How often is the water in the tanks changed?; *Q3: How is sea water rinsed before use?; *Q4: From what depth (m) is sea water taken? ; *Q5: How many persons work per shift in production (inside the slaughterhouse including packaging area)?; *Q6: How many shifts per day?; *Q7: How much (tons) salmon are slaughtered per day?; *Q8: Is salmon entering the slaughterhouse via sea net pen or well boat?; *Q9: How is the processing plant cleaned at the moment?; *Q10: Do you have procedures for disassembly and washing of all machines and equipment (how often)?; *Q11: What microbiological control do you apply (i.e. daily/weekly sampling, amount of samples of water, equipment, floor etc.)?; *Q12: What is the most challenging area with regards to Listeria control?



ited extent, is a carrier of *L. monocytogenes*, it will be impossible to prevent this pathogen from being introduced into processing plants. Focus should therefore be directed to sanitary measures and product conditions preventing growth. As reviewed by Rørvik [2], a significant risk factor is job rotation of the workers in the plant between different departments.

In order to eliminate *L. monocytogenes* from the processing environments, good production practices are needed, and the implication of Hazard Analysis and Critical Control Point (HACCP) programs [2, 9]. It is however pointed out, that the HACCP systems is the preferred strategy in most quality assurance programs, and it is recommended that microbiological criteria are only applied as guidelines in the verification of the HACCP system, and not for official control purposes [17].

Considering that seawater used in the tanks in the slaughterhouses was treated by UV, filtered and/or taken from depths \geq 60 m (Table 4), the total aerobic count may be regarded as relatively high in the live chilling tank (Plant A and C only; Figure 5), especially when compared to the level on fish skin (Figure 4). The levels in bleeding and rinsing tanks are naturally higher than in live chilling tanks (Figure 5). Temperatures in all tanks are kept low to minimize growth of bacteria (Table 4). A comparison between the four different plants are not feasible because they were all sampled during full production, at different times in the day, and had different capacities. Also the fact that the prehistory of the fish is not known, as time since delousing, transportation time, and other factors influencing their internal and external microbiota composition and level, complicates a comparison.

4. Conclusions

- The pathogen bacterium *L. monocytogenes* was detected at three out of four visited slaughterhouses.

- *L. monocytogenes* was present in low concentrations, i. e., under the quantification limit of 100 cfu per L or m^2 .

L. monocytogenes was not detected on fish skin or gills, and it is not suspected that water tanks acts as reservoir for this pathogen.

- *L. monocytogenes* was detected in the gutting machines, and on conveyor belts, floors and drains downstream of gutting, implicating the gutting machine and the gutting area as hot spots for cross contamination.

- Detection of *Listeria* in machines and equipment, as in the present study from salmon slaughterhouses, represents a risk of contamination of salmon products, and the pathogen may be transferred to the final product meant for human consumption. Salmon products can thus not be ruled out as a potential source of listeriosis.

- It is important to stress, however, that it has never been documented that people have been infected by *L. monocytogenes* through consumption of Norwegian salmon products. Nonetheless, *Listeria* control is also important regards, public relations and to avoid recalls. In terms of food safety, the presence of *L. monocytogenes* represents a food safety risk by the present hygiene practices.

- Prevention of *Listeria* colonization in salmon slaughterhouses and processing plants is necessary in order to secure the production of safe food, and to maintain a good reputation for the industry. Since *L. monocytogenes* is a ubiquitous bacterium, it will be introduced from different sources. The design of processing machines and equipment minimizing colonization and with sufficient cleanability is therefore of utmost importance.

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5. References

- Roth B., Moeller D., Veland J. O., Imsland E., and Slinde E. (2002). The effect of stunning methods on rigor mortis and texture properties of Atlantic salmon (Salmo salar). Journal of Food Science, 67, (4), pp. 1462-1466.
- [2] Rørvik L. M. (2000). Listeria monocytogenes in the smoked salmon industry. International Journal of Food Microbiology, 62, (3), pp. 183-190.
- [3] Benembarek P. K. (1994). *Presence, detection and growth of Listeria monocytogenes in seafoods a review*. International Journal of Food Microbiology, 23, (1), pp. 17-34.
- [4] Hsu J. L., Opitz H. M., Bayer R. C., Kling L. J., Halteman W. A., Martin R. E., and Slabyj B. M. (2005). *Listeria monocytogenes in an Atlantic salmon (Salmo salar) processing environment*. Journal of Food Protection, 68, (8), pp. 1635-1640.
- [5] Norwegian Ministry of Trade, Industry and Fisheries (2006). *Regulations on slaughterhouses and aquaculture production facilities*.
- [6] European Commission. (2002). Regulation 178/2002 on the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. O.J., L 31/1.



- [7] European Commission. (2004). *Regulation 852/2004 on Hygiene of foodstuffs*. O.J., L 139/1.
- [8] Kakurinov V., Jankovski V., Cilev G., and Gatzovska M. (2012). Legislation and other issues about hygienic engineering aspects in EU and Balkan countries. Journal of Hygienic Engineering and Design, 1, pp. 22-26.
- [9] Løvdal T. (2015). The microbiology of cold smoked salmon. Food Control, 54, pp. 360-373.
- [10] Hofshagen M., Nygård K., and Kruse H. (Eds.). (2004). Zoonoses reports for 2003. A report on diseases which can infect between animals and humans (in Norwegian).

<URL: http://www.vetinst.no/Arkiv/Zoonosesenteret/ Zoonoserapport03-no.pdf. Acessed 10 June 2017.

- [11] European Surveillance System (TESSy) and EFSA zoonoses database. (2016). European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2015-Listeria infections in the EU (2016). doi:10.2903/j.efsa.2016.4634.
- [12] Hansen C. H., Vogel B. F., and Gram L. (2006). Prevalence and survival of Listeria monocytogenes in Danish aquatic and fish-processing environments. Journal of Food Protection, 69, (9), pp. 2113-2122.
- [13] Huss H. H., Benembarek P. K., and Jeppesen, V. F. (1995). Control of biological hazards in cold smoked salmon production. Food Control, 6, (6), pp. 335-340.
- [14] Autio T., Hielm S., Miettinen M., Sjöberg A. M., Aarnisalo K., Björkroth J., Mattila-Sandholm T., and Korkeala H. (1999). Sources of Listeria monocytogenes contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Applied and Environmental Microbiology, 65, (1), pp. 150-155.
- [15] Vogel B. F., Huss H. H., Ojeniyi B., Ahrens P., and Gram, L. (2001). Elucidation of Listeria monocytogenes contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Applied and Environmental Microbiology, 67, (6), pp. 2586-2595.
- [16] Møretrø T., and Langsrud S. (2004). *Listeria monocytogenes: biofilm formation and persistence in food-processing environments*. Biofilms, 1, pp. 107-121.
- [17] Huss H. H. (1997). Control of indigenous pathogenic bacteria in seafood. Food Control, 8, (2), pp. 91-98.