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Modelling transfer of *Listeria monocytogenes* during slicing of 'gravad' salmon

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Abstract

Transfer of a rifampicin-resistant mutant of *Listeria monocytogenes* from an inoculated slicing blade to slices of 'gravad' salmon (*Salmo salar*), and from inoculated salmon fillet to the slicing machine and subsequently to slices of uninoculated fillet was studied. The effect of slicing temperature (0 °C, 10 °C and room temperature), inoculum level (approx. 3, 5 and 8 log CFU/blade), and attachment time of inoculum to blade (10 min and 2.5 h) were investigated and predictive models of the transfer were produced. In the tests of transfer from inoculated blade (5.9–9.0 log CFU/blade) initially 2.5–5.3 log CFU/g was present on the slices, slowly decreasing to an overall average decrease of 1.6±0.2 log CFU/g during slicing of 39 slices; the lowest reduction being 1.3 log CFU/g at 0 °C. In tests of transfer from contaminated salmon (7.6±0.1 log CFU/fillet) to uninoculated blade and further to uninoculated salmon, the reduction in number of *L. monocytogenes* in slices was 1.5 log CFU/g during slicing of 39 slices. For example 5.3±0.3 log CFU/g was transferred to second slice when the inoculum level was 8.4±0.4 log CFU/blade, but clearly ($p<0.05$) lower total number of *L. monocytogenes* were transferred to slices when the inoculum level was lower, the temperature was colder or the attachment time was longer. There was a progressive exponential reduction in the quantity of *L. monocytogenes* transferred and, based on statistical parameters, an exponential model ($y=a*e^{(-x/b)}$) fit the data from different test conditions and was suitable for predicting an expected number of *L. monocytogenes* on the salmon slices. Based on the predicted values, the logarithmic reduction in number of *L. monocytogenes* in slices was highest at room temperature with an inoculum level of 8.4±0.4 log CFU/blade (attachment time 10 min); the other test conditions differed significantly from this ($p<0.05$). Despite statistically significant differences, in all test conditions the number of bacteria were predicted to reduce quite rapidly (i.e. after slicing of the fourth fillet) to <1 log CFU/g, though this prediction was an extrapolation after 39 slices. The predictive models described herein can assist salmon processors and regulatory agencies in assessing cross-contamination from contaminated slicing machines to product and in designing risk management strategies.
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Keywords: *Listeria monocytogenes*; 'Gravad' salmon; Transfer; Predictive modelling

1. Introduction

Listeria monocytogenes is a problematic bacterium for the fish processing industry. It is ubiquitous and causes listeriosis, a serious illness, especially for people belonging to higher risk groups such as the very young, old, pregnant, or immunocompromized (Farber and Peterkin, 1991). 'Gravad' (i.e. cold-salted) and cold-smoked rainbow trout are popular food products in Finland. These products have also been associated with *L. monocytogenes* (Loncarevic et al., 1996; Ericsson et al., 1997; Autio et al., 1999; Miettinen et al., 1999, 2001) and can be considered high risk products because they are usually prepared ready-to-eat (RTE). In Finland, 14.4% (41/285) of 'gravad' fish (mainly rainbow trout and salmon) products at retail have been reported to contain *L. monocytogenes* during 2003–2004 (Aalto et al., 2006). Almost 25% (78/315) of reported listeriosis cases in Finland between 1995 and 2004 have been caused by certain sero- and genotype or closely related genotypes, which have also been found from vacuum-packed cold-smoked or 'gravad' fish products (Lyytikäinen et al., 2006).

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In the study of Miettinen and Wirtanen (2005) the prevalence of *Listeria* spp. and *L. monocytogenes* in fresh, unprocessed rainbow trout was 35.0% and 14.6%, respectively (Miettinen and Wirtanen, 2005), which suggests raw material to be a source of *L. monocytogenes* contamination in fish processing. Slicing machines are also a source of *Listeria* contamination (Autio et al., 1999; Miettinen et al., 2001; Tompkin, 2002), and are one of the most difficult types of equipment to clean in the food industry (Aarnisalo et al., 2006). Slicers can also contaminate products after the salting and/or smoking process (Autio et al., 1999).

In a study by Lundén, Autio and Korkeala (2002) transfer of *L. monocytogenes* contamination between food-processing plants was associated with a dicing machine. The mechanism and level of bacterial transfer from production surfaces to products and vice versus is important though yet an understudied factor for contamination routes of *L. monocytogenes* in food plants. Only a few studies have recently been done of the transfer of bacteria from production surfaces to meat or vice versus (Midelet and Carpentier, 2002; Flores et al., 2006; Lin et al., 2006; Vorst et al., 2006; Rodríguez and McLandsborough, 2007) or of slicing hygiene (Holley, 1997). Vorst et al. (2006) reported recently an extensive study about the transfer of *L. monocytogenes* during slicing of turkey breast, bologna and salami. Similar studies on transfer of *L. monocytogenes* from different processing surfaces (e.g. slicing blades) to fish, as well as the impact of slicing temperatures or the attachment time for inocula, have not been reported.

Predictive models can be used as tools for process development and optimization, such as in predicting the effect of cross-contamination on levels of bacteria in final product as described by Ivanek, Gröhn, Wiedmann and Wells (2004) for *L. monocytogenes* in a fish processing plant, or shelf-life of products as described for salmon fillets by Rasmussen, Ross, Olley and McMeekin (2002). Models for describing growth and inactivation of micro-organisms have been published (van Gerwen and Zwietering, 1998; Ross and McMeekin, 2003), including the Pathogen Modeling Program (PMP, http://www.arserrc.gov/mfs/pathogen.html), Combase Predictor (http://www.combase.cc/predictor.html), and Seafood Spoilage and Safety Predictor (http://www.difres.dk/micro/sssp/); data underlying many predictive models can be found in ComBase (http://www.ifr.ac.uk/combase/). These programs do not include models describing transfer of pathogens from food production surfaces to products due to lack of information about transfer rates and mechanisms. Recontamination, that being the introduction of pathogens onto the product after an inactivation step from the factory environment (den Aantrekker et al., 2003), has been identified as a significant cause of contamination of foods and thereby foodborne illnesses. Thus further investigations on routes and mechanisms for transfer of pathogens should be conducted before they can be used systematically as part of microbial risk assessments (den Aantrekker et al., 2003; Reij and den Aantrekker, 2004).

The objectives of the present study were to 1) investigate transfer of *L. monocytogenes* from a slicing blade to 'gravad' salmon slices, and from a contaminated salmon fillet to a slicing machine and uninoculated salmon slices; and 2) describe how the data on transfer can be used to produce a predictive model of the transfer. The effects of slicing temperature, inoculum level, and attachment time of the inoculum to the blade were also investigated. Based on these results, slicing practices were assessed and recommendations for processors were given for reducing the risk of *L. monocytogenes* contamination and product recalls.

2. Materials and methods

2.1. Bacteria

As the aerobic bacterial flora on salmon fillets disturbed detection of *L. monocytogenes*, a rifampicin resistant *L. monocytogenes* strain was produced to facilitate *L. monocytogenes* enumeration. Strain F2365, a serotype 4b isolate from the 1985 Mexican-style soft cheese outbreak (Linnan et al., 1988) was made resistant to 0.1% rifampicin (100 μg/ml) following the method of Kaspar and Tamplin (1993). The strain was transferred from frozen culture (−80 °C) to 10 ml brain heart infusion (BHI, Difco, Sparks, MD, USA) broth, incubated overnight at 37 °C, transferred to another BHI tube, and then incubated a second time. For each trial, a fresh overnight culture incubated at 37 °C was used.

2.2. 'Gravad' salmon fillets

Fresh Atlantic salmon (*Salmo salar*) fillets, approx. 1.8 kg / fillet, were bought from a local retail market and stored for no more than one week at 0 °C prior to experimentation. The 'gravad' fillets resembling commercial products were prepared. The fillets were salted by sprinkling manually 1% (w/w) sugar (America's Choice, Great Atlantic and Pacific Tea Company, NJ, USA) and 4% (w/w) sea salt (Cerulean Seas, HL Benndorf Corp, NJ, USA), both bought from retail as well, evenly on whole fillet. The fillets were set on HD aluminium foil (Acme Aluminum Foil, ID, USA) and covered with thin plastic film (America's Choice, Great Atlantic and Pacific Tea Company, NJ, USA) and a small plastic plate press (<1 kg). These were left at 0 °C for approximately 20 h before the trials.

The compositions of both fresh and 'gravad' salmon fillets were analyzed by a commercial laboratory from two (each approximately 250 g) pooled samples, each taken from 4–5 fillets, for fat with ether extraction method of AOAC International, no. 960.39; protein with Kjeldahl method, no. 928.08; salt with Volhard method, no. 941.18; sugar with sugar inversion method no. 931.07 and water content with drying method no. 950.46 (AOAC, 1998). Water activity was additionally analyzed from two fillets by the authors by using the Water Activity Meter Aqua Lab CX 2 (Decagon Devices, WA, USA).

2.3. Slicer and blade

The slicer used in the present study was a common delicatessen slicer (Globe 3975 Variable Speed Automatic Slicer,

Globe Food Equipment Co., Dayton, OH, USA) made of one-piece #304 stainless steel, seamless construction and with rounded corners. The blade was made of #304 stainless steel as well. The roughness (A'/A ratio, which is the scanned rough surface area/unit surface area) and sharpness of the blade were measured at the beginning and end of the tests by reflection confocal microscopy as described by Flores et al. (2006). In the present study, the reference unit surface area used was 250,000 $(\mu m)^2$ and at least three scans were performed on the sharp blade surface at different locations on the blade but were selected at random. The diameter of the blade was 291 mm. The slicer was equipped with a press (1.36 kg) to provide consistent pressure during slicing. The thickness of the slices was set at 3.5 mm and slicing was done manually.

2.4. Transfer of *L. monocytogenes* from inoculated slicing blade to uninoculated 'gravad' salmon slices

The blade was contaminated with *L. monocytogenes* by inoculating it with 5 drops (in 20 µl portions) of whole or diluted bacterial enrichment broth on both sides of blade, specifically on a 2.3-cm wide area on the outer edge of the blade. This resulted in adding approximately 8, 5 or 3 log CFU/blade. The inoculum was spread with a plastic spreader on the blade and the blade was dried in a laminar flow cabinet for 5–10 min. To test for the effect of attachment time, following inoculation, the blade was lightly covered with aluminum foil and held in the laminar flow cabinet for 2.5 h at room temperature (~20±0.5 °C).

The effect of slicing temperature was tested at room temperature, 10±0.5 °C, and 0±0.2 °C. The salmon fillets were cut in approximately 7 cm wide pieces, 4–5 pieces per fillet and sliced one after another. For tests at room temperature, the fillets were taken from storage (0 °C) and left at room temperature for 2–3 h before beginning the tests. Tests at 0 °C or 10 °C were done in a walk-in cold room. The slicer was maintained at the test temperature for 1 h before experimentation. The blade was pre-cooled to the test temperature before inoculation. For 10 °C tests, the fillets were transferred from 0 °C and maintained at 10 °C 1 h before experimentation.

For experiments, the first 11 slices and then every odd slice through the 39th slice were collected. Surface samples were also collected from the slicer holding plate using sterile cotton-tip swabs at the beginning, during slicing and at the end. In addition, surface samples (from approximately 10 cm^2 area) were taken from the blade safety guard and the blade (two samples) after slicing was completed (see Fig. 1). Because salmon has a high fat content (See "Composition of salmon fillets") and the tissue is soft, it was relatively easy to see the main product contact sites. Each trial was repeated three times on different days using replicate agar plates for each slice.

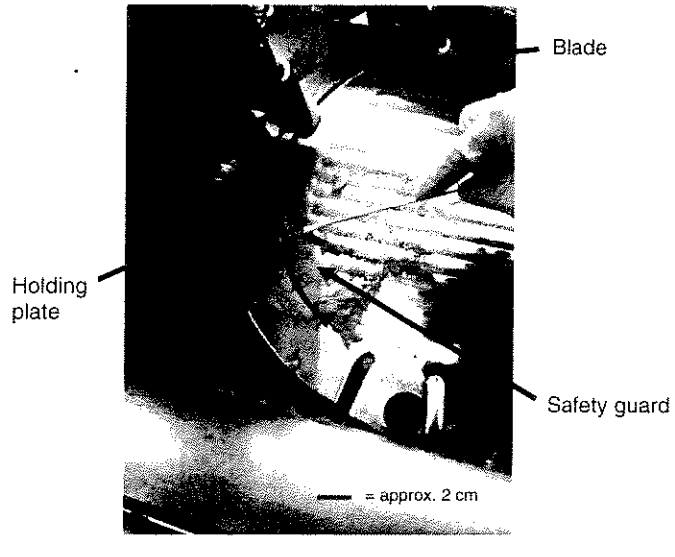

Fig. 1. Swab surface sampling sites of the slicing machine.

2.5. Transfer of *L. monocytogenes* from inoculated 'gravad' salmon fillet to slicing machine and to slices of uninoculated fillets

Fillets at room temperature were surface-inoculated to contain approximately 8 log CFU/fillet of *L. monocytogenes*. The first piece of salmon was inoculated with the *L. monocytogenes* suspension on both sides (20 µl twice to each side) and an additional amount (20 µl six times) on the top of the piece. Then, the following 3–4 pieces were inoculated with 20 µl twice to each side only. The inoculum was spread with a plastic spreader. The trials were done at room temperature.

After slicing the inoculated fillet, an uninoculated fillet was sliced into 39 slices and the slices analyzed. The sampling and analyses of the slices were done in the manner described above. Surface samples were taken from the salmon holding plate at the beginning of the experiment before slicing anything, after the first fillet was sliced, during the slicing, and at the end of slicing. The surface of the blade was tested after the first fillet was sliced. The blade and guard were swabbed after both fillets were sliced (Fig. 1). The trial was repeated three times on different days using two replicate plates for each slice.

2.6. Analyzing the samples

The slices (samples) were transferred directly to sterile filter (280 µm mesh) stomacher bags (Spiral Biotech, Norwood MA, USA) and weighted. Peptone water (0.1% w/w) was added to the sample at a 5-to-1 ratio (wt/wt) and the mixture was processed in a stomacher (Model Bag Mixer 400, Interscience Inc., Weymouth, MA, USA) at room temperature for 30 s. If the samples were not cultured directly onto Modified Oxford agars (MOX, Sigma Aldrich, St Louis, MO, USA) after stomaching, they were stored at 4 °C for a maximum of 2 h.

The swabs used for surface samples were placed individually into 10 ml of peptone water, mixed in a vortex mixer for 30 s, and then 10-fold serial dilutions (100 µl from each tube) were plated on two plates of MOX agar containing 0.1% rifampicin (Sigma Aldrich, St Louis, MO, USA). The plates were incubated at 37 °C for 48 h and the colonies on counted.

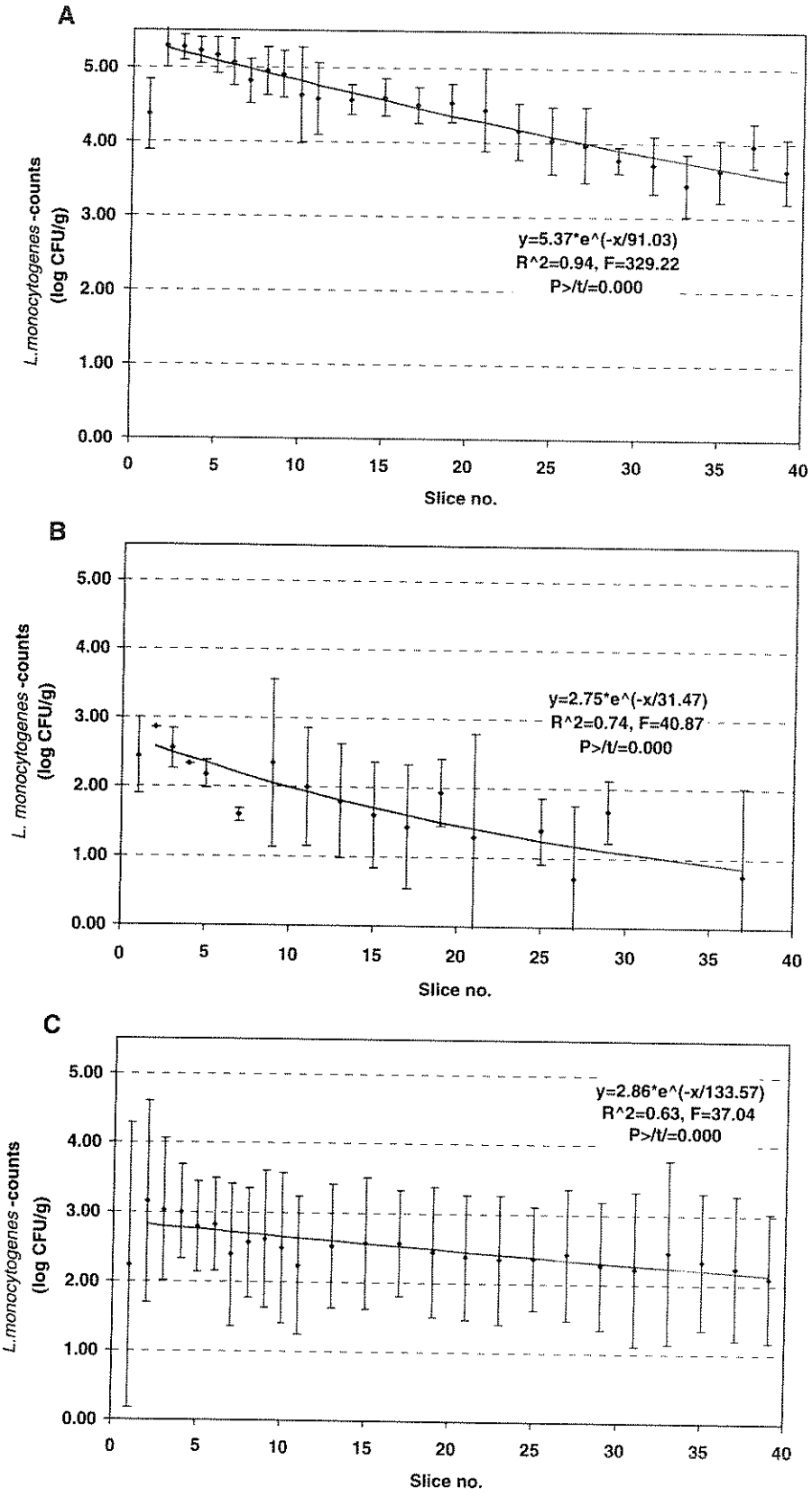

Fig. 2. Transfer of *L. monocytogenes* from inoculated slicing blade to uninoculated 'gravad' salmon fillet with different inoculation levels and standard deviations [SD] for three replicate tests; temperatures and attachment times. Average results of three replicate tests are presented as data points and predictions with lines. Statistics describing the prediction are presented: As a fit becomes more ideal, the r^2–values (coefficient of determination) approach 1.00 (0.00 represent a complete lack of fit), the *F*-statistic goes toward infinity and the standard error of parameters decreases toward zero (i.e. $p>/t/(<0.000)$). A) Inoculation level 8.41 [0.36] log CFU/blade, 20 °C, attachment time 10 min. B) Inoculation level 5.91 [0.59] log CFU/blade, 20 °C, attachment time 10 min. C) Inoculation level 9.00 [0.40] log CFU/blade, 0 °C, attachment time 10 min. D) Inoculation level 8.10 [1.20] log CFU/blade, 10 °C, attachment time 10 min. E) Inoculation level 8.07 [0.71] log CFU/blade, 20 °C, attachment time 2.5 h.

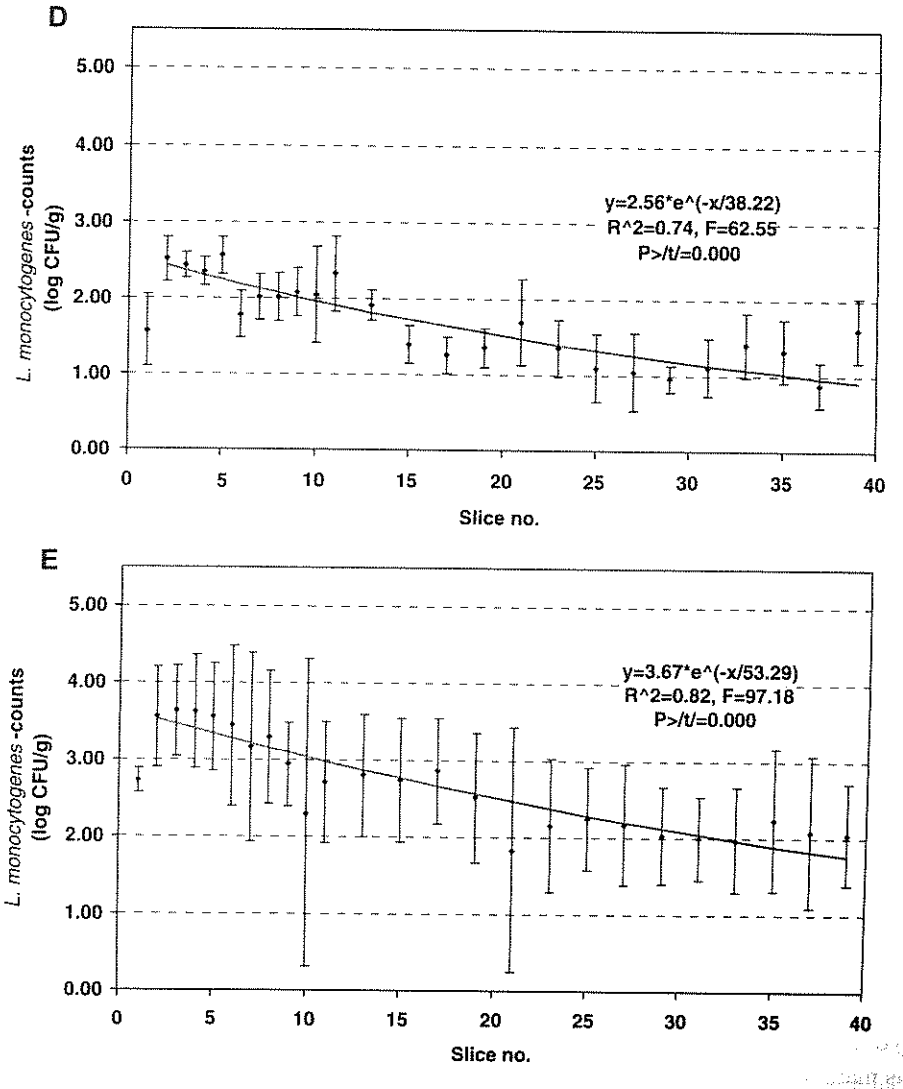

Fig. 2 (*continued*).

2.7. Cleaning and disinfecting the slicing blade and the machine

After each trial, the slicer and blade were sprayed with 70% ethanol and held for a minimum of 10 min at room temperature. The excess salmon waste on slicer surfaces was first wiped off with paper towels and the blade and other detachable parts (guard, table) were soaked in BacDown Detergent Disinfectent (BDD; Decon Labs, Inc Bryn Mawr PA, USA) that was diluted approximately 1:10 in warm tap water for a minimum of 15 min. Next, the slicer parts were cleaned with a soft brush, rinsed with tap water, further rinsed with deionized water and left to air-dry. After drying, the blade was wrapped in aluminium foil and sterilized by autoclaving (121 °C, 15 min). The other parts of the slicer were sprayed once more with 70% ethanol and air-dried.

2.8. Recovery rates

Surface recovery efficiency was determined for *L. monocytogenes* inoculated on blades at room temperature and 0 °C and measured on MOX containing 0.1% rifampicin and MOX without rifampicin. Both temperatures were tested to determine, if inoculation on cold stainless steel surface reduced the number of *L. monocytogenes* being able to be transferred to slices. Specifically, six samples were taken from both sides of the blade using a sterile cotton-tip swab and analyzed as previously described. The blade was inoculated as described earlier. In addition, after both top and bottom blade surfaces were swabbed, Kim Wipe laboratory tissue (Kimberly Clark Professional, GA, USA) was moistened with 10 ml of peptone water and the moistened tissue was dabbed around the entire surface of blade, placed back into the stomacher bag, stomached for 120 s, and 100 µl of fluid plated on both MOX and MOX agar containing rifampicin.

2.9. Statistical analyses and model development

Differences in transfer of *L. monocytogenes* between different test conditions were analyzed. The significances of difference between the results on transfer in different test conditions were analyzed with a general linear model univariate analysis using results for different slices as dependent variable and test number as a fixed factor and further by multiple comparisons Tukey-test. Differences in recovery rates of

L. monocytogenes on MOX and MOX agar with rifampicin, differences in recovery from the blade at 0 °C and room temperature as well as differences in blade surface roughness before and after all experiments were calculated by nonparametric Mann–Whitney test. Statistical analyses were conducted using SPSS for Windows v. 12.0.1. (Chicago, IL, USA). The level of significance was at $p<0.05$.

TableCurve 2D Version 5.01 (SYSTAT Software Inc., Richmond, CA, USA) was used to select an empirical model to best fit the distribution of experimental data, based on the simplicity, applications (predictions vs. time in slicing — convergence and no singularity in long-time prediction), fitted coefficients with standard error and *t*-test results, $p>|t|$, r^2-value and *F*-value.

3. Results and discussion

3.1. Composition of salmon fillets

The average compositional values for fresh salmon fillets were: moisture 67.2%, protein 20.0%, fat 12.4%, salt 0.1% and sugar 0.2%. The composition of 'gravad' salmon fillets were: moisture 60.0%, protein 19.7%, fat 16.1%, salt 3.4% and sugar 0.5%. The water activity (a_w) for the products was 0.98 and 0.93, respectively. Salt content of 'gravad' salmon was approximately the same as for commercial 'gravad' salmon fillets (Loncarevic et al., 1996). As expected, the addition of salt reduced both the moisture content and a_w of the product.

3.2. Roughness of the slicing blade

The roughness (A'/A) of the slicing blade was 2.3±0.1 on the sharp side (the blade was manufactured with the sharpened side facing down during slicing; the side facing up was smoother) and 1.7±0.1 on the flat side in the beginning of the tests, and 2.0±0.2 and 2.7±0.6, respectively, at the end of the tests. Pictures taken of the new blade and the blade at the end of the tests (pictures not shown) by reflection confocal microscopy showed a major change in the blade rim/edge structure which deteriorated after use. The A'/A on the sharp side of blade became smaller ($p<0.05$) and the flat side became larger ($p<0.05$) after use. The used blade A'/A (both sides) had a larger standard deviation (see above) than the new blade. Thus, blade wear could be demonstrated clearly over the course of the experiments (approximately 1.5 months, 3–4 trials per week), which emphasizes the importance of regular maintenance of the blade in food processing operations.

3.3. Transfer of L. monocytogenes from inoculated slicing blade to uninoculated 'gravad' salmon slices

In the present study, potential cross-contamination from a contaminated blade to uncontaminated 'gravad' salmon slices was simulated, and calculated over a total of 39 slices. This number was based on the typical number of slices obtained from one whole salmon fillet (approximately 1.8 kg/fillet). There was a progressive exponential reduction in the quantity of *L. monocytogenes* transferred (Fig. 2A–E). For example 5.3± 0.3 log CFU/g was transferred to second slice when the inoculum level was 8.4±0.4 log CFU/blade (Fig. 2A). Based on the results obtained from the samples, when compared to the inoculum level of the blade, clearly ($p<0.05$) lower total number of *L. monocytogenes* were transferred when the inoculum level was lower (Fig. 2B) (however approx. proportional number), the temperature was colder (Fig. 2C–D) or the attachment time was longer (Fig. 2E). There seemed not to be, however, clearly statistically significant differences in the logarithmic reduction of *L. monocytogenes* number in slices (2–39) among the different tests ($p>0.05$), though a marginally lower reduction was detected at 0 °C (Fig. 2C) compared to room temperature: In the tests of transfer from inoculated blade (5.9 log–9.0 CFU/blade) (See Fig. 2A–E) initially 2.5–5.3 log CFU/g was present on the slices, slowly decreasing to an overall average decrease of 1.6 log CFU/g during slicing of 39 slices (calculated from results of 5 tests with 3 replicates each). The lowest reduction was 1.3 log CFU/g at 0 °C. The transfer percentage (the amount of *L. monocytogenes* in 39 slices compared to inoculum) varied between 0.00011 and 0.17%, being the lowest at 0 °C (See Table 1). The reduction in quantity of *L. monocytogenes* transferred was lower than that reported for turkey breast, bologna and salami (2 log CFU/20 slices) reported by Vorst et al. (2006). The results showed, that the reduction in transfer for salami at inoculation levels of 3 and 5 log CFU/blade was less ($p<0.05$) than that observed for turkey breast and bologna. The authors concluded that a fat layer which developed on the blade during slicing most likely prolonged *Listeria* transfer to salami slices. During slicing of salmon in our study, a clearly visible layer of soft salmon material (salmon fillets consisting mainly of protein, fat and moisture, see "Composition of salmon fillets") could also be seen on slicer surfaces, though the fat content of 'gravad' salmon (16%) was lower than that of salami (36%) (Vorst et al., 2006). This indicated that other than fat, other product material also has an effect. In addition, solidification of fat may have had an effect on the slower transfer of *L. monocytogenes* at colder temperatures.

When calculated with the predicted values (See "Model development"), instead of using the results of 39 slices directly, a significantly ($p<0.05$) lower logarithmic reduction in the number of *L. monocytogenes* between slices (2–39 and 2–78) was found when slicing was done at 0 °C, indicating that colder temperature prolonged bacterial transfer. No significant ($p>0.05$) differences were found between tests done at room temperature with an inoculum level of 8.4±0.4 log CFU/blade (attachment time 10 min, Fig. 2A) and tests done at 10 °C (Fig. 2D) with lower inoculation level (Fig. 2B) or with a longer attachment time (Fig. 2E). Differences in logarithmic reductions were found at higher number of slices, when calculated with predicted values. According to predictions, after slicing 156 slices (i.e. approximately four fillets), results of the test made at room temperature with a high 8.4±0.4 log CFU/g inoculum level and a short (10 min) attachment time differed significantly ($p<0.05$) from the results of the other tests. Despite statistically significant differences, in all test conditions the number of bacteria was predicted to reduce quite rapidly (i.e. after slicing of the fourth fillet) to <1 log CFU/g, though this prediction was an extrapolation after 39 slices (see a combined Fig. 4 of all

Table 1
The percentage (%) of *L. monocytogenes* transferred from the blade to salmon slices and the number of *L. monocytogenes* (log CFU/10 cm^2) on the blade, guard and holding plate surface of the slicing machine during the transfer tests of *L. monocytogenes* from inoculated blade to uninoculated salmon

Sampling site	Inoculum level and test conditions					
	8.41[0.36] log CFU/blade[a] 20 °C, 10 m[b]	5.91[0.59] log CFU/blade[a] 20 °C, 10 min[b]	8.10[1.20] log CFU/blade[a] 10 °C, 10 min[b]	9.00[0.40] log CFU/blade[a] 0 °C, 10 min[b]	8.07[0.71] log CFU/blade[a] 20 °C, 2.5 h[b]	5.53[0.79] log CFU/blade[a] 20 °C[c]
Slices (no. of *L. monocytogenes* transferred to all 39 slices)						
Log CFU/slices	7.29 (0.076%)[d]	5.15 (0.17%)[d]	4.50 (0.00025%)[d]	5.04 (0.00011%)[d]	5.70 (0.0043%)[d]	5.07 (0.35%)[d]
Blade after 39 slices						
Log CFU/blade	5.81 [2.58] (0.25%)[d]	2.82 [2.58] (0.081%)[d]	3.47 [3.76] (0.0023%)[d]	4.19 [2.22] (0.0015%)[d]	4.51 [2.45] (0.027%)[d]	4.38 [3.24] (7.05%)[d]
Blade after 39 slices						
Log CFU/10cm^2	4.22 [0.99]	1.23 [0.99]	1.88 [2.17]	2.60 [0.63]	2.92 [0.86]	2.79 [1.65]
Guard after 39 slices	5.55 [0.13]	2.42 [0.67]	1.58 [2.24]	2.10 [1.92]	3.18 [0.76]	3.48 [1.06]
Holding plate						
After 6–9 slices	5.87 [0.02]	3.00 [0.21]	NA[e]	NA[e]	NA[e]	NA[e]
After 16–19 slices	5.35 [0.68]	2.69 [0.21]	1.71 [2.42]	2.58 [0.81]	4.30 [0.66]	4.10 [1.16]
After 26–29 slices	5.15 [0.01]	2.46 [0.65]	NA	NA	NA	NA
After 39 slices	4.75 [0.70]	2.62 [0.11]	1.87 [2.64]	2.78 [1.02]	3.28 [1.12]	3.11 [1.99]

[a] Inoculum on the blade, standard deviation of three replicate tests presented in brackets [SD].
[b] Attachment time.
[c] Blade inoculated by slicing an inoculated fillet (7.63 [0.07] log CFU).
[d] No. of bacteria/inoculum level on the blade (%); for calculating the transfer-% normal scale was used instead of LOG-scale.
[e] NA = not analyzed.

predictions). Validation data would provide more accurate estimates of the model performance.

According to Midelet and Carpentier (2002) who studied transfer of bacteria, including *L. monocytogenes*, from various materials to pieces of beef, in most cases the inoculation concentration had the strongest influence on the total number of CFU detached. Also, Midelet and Carpentier (2002) stated that the attachment strength of bacteria on different materials had a significant ($p<0.05$) effect on the rate that *L. monocytogenes* was transferred to beef. In our study as well, higher number ($p<0.05$) of *L. monocytogenes* was transferred to slices from the slicing blade when the inoculum was higher (Fig. 2A compared to Fig. 2B) or when the attachment time was shorter (Fig. 2A compared to Fig. 2E). When the blade inoculum was 2.9 log CFU/blade at room temperature and 3.2 log CFU at 10 °C, a total of only three colonies (at 10 °C) were found on the first 10 slices (not shown in Figs.) and no colonies were detected on the slicer surfaces, including the blade. Levels below the agar detection limits were likely present but were not tested by enrichment.

Simultaneously when studying the transfer of *L. monocytogenes* from the slicing blade to the salmon slices, changes in amount of *L. monocytogenes* on slicer surfaces were studied. The highest number of *L. monocytogenes* was detected on the surfaces of the

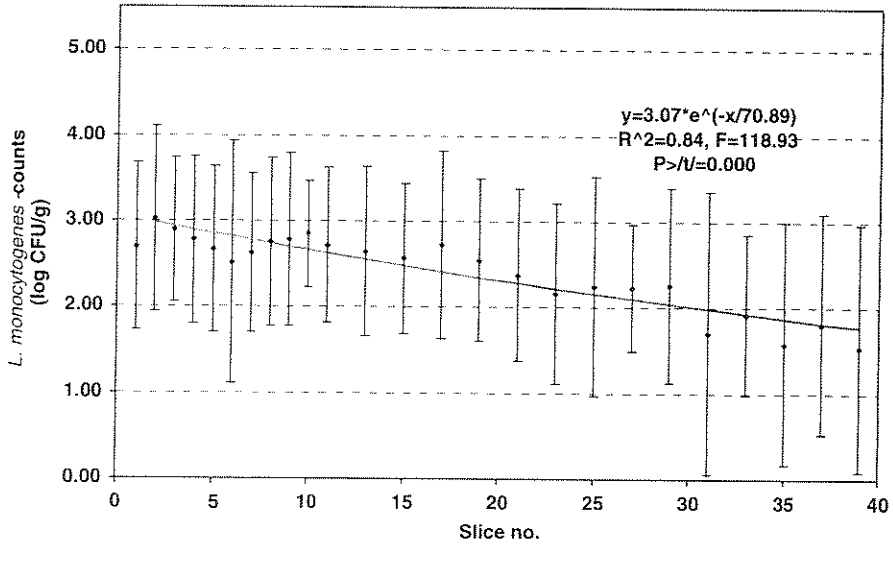

Fig. 3. Transfer of *L. monocytogenes* from inoculated (7.63 [0.07] log CFU/fillet) 'gravad' salmon fillet to uninoculated blade and further to uninoculated fillet (in figure) at 20 °C.

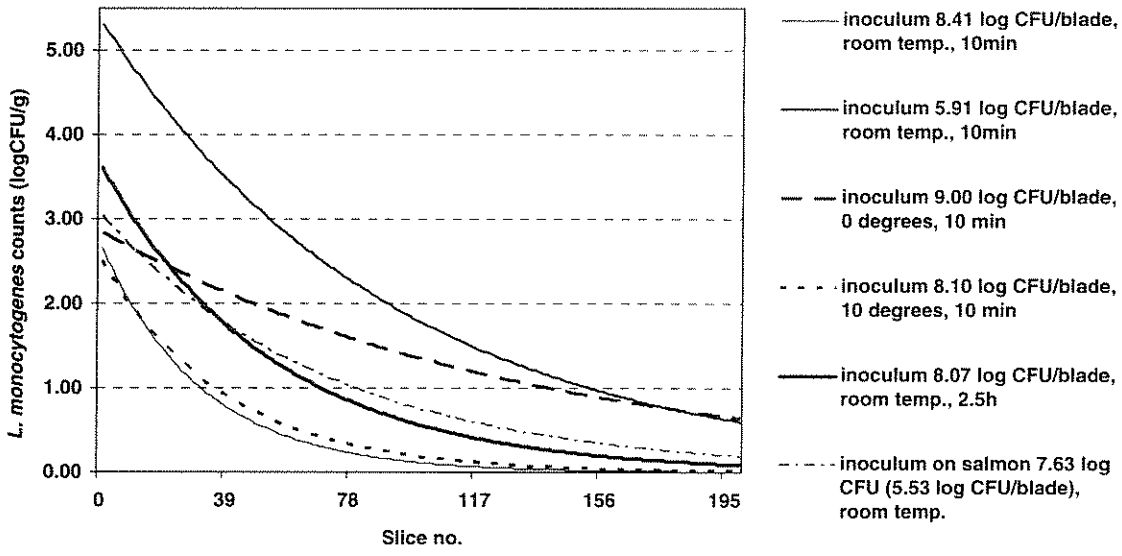

Fig. 4. Predicted transfer of *L. monocytogenes* from inoculated blade to uninoculated salmon fillets during slicing of 200 slices in different conditions. After slice 39 the predictions are extrapolations.

holding plate and the cover of the slicing machine when the blade inoculum was 8.4±0.4 log CFU. On the contrary, the greatest reductions in number of *L. monocytogenes* on the blade after 39 slices were at 0 °C and 10 °C (i.e 4.6–4.8 log units) and at these temperatures the lowest % of *L. monocytogenes* were present on the blade after slicing (Table 1). When the trial was performed at room temperature, the reduction was approximately 2.6–3.6 log units. This is partly explained by the fact that colder slicing temperatures stressed the bacteria and reduced their subsequent growth on selective agar. Based on the recovery test performed, the temperature of the blade affected recovery, in that approximately 1.1 log CFU/g less bacteria were recovered at 0 °C when compared to room temperature ($p<0.05$) (results not shown). The contamination level on the holding plate and cover depended on the level of *L. monocytogenes* on slices, especially in tests made at room temperature (Table 1 and Figs. 2A,B,E and 3), i.e. the higher the level was on slices, the higher the level was on equipment surfaces.

The recovery of *L. monocytogenes* from the blade, using swabs and Kim Wipes, on MOX-agar containing rifampicin was approx. 0.3 log CFU/g lower than recovery on MOX without rifampicin ($p>0.05$) at room temperature. Likewise, Vorst et al. (2006) reported a 12% difference in recovery of dessication-injured *L. monocytogenes* cells on MOX-agar compared to a non-selective agar (mTPA).

3.4. Transfer of *L. monocytogenes* from inoculated 'gravad' salmon fillet to the slicer surfaces and uninoculated fillet

When a salmon fillet was inoculated with *L. monocytogenes* (surface inoculum of fillet was 7.6±0.1 log CFU *L. monocytogenes* per fillet) and sliced, 3.0±1.1 log CFU/g was initially transferred to the subsequent sliced uninoculated fillet and the overall transfer was 1.5 log CFU/g after 39 slices (Fig. 3). After slicing the first contaminated fillet, the blade contained 3.9±0.8 log CFU/10 cm^2 (i.e. 5.5±2.4 log CFU/blade). The reduction was only marginally ($p>0.05$) lower compared to reduction when the blade was initially contaminated. When the predicted values (See "Model development") were used with higher (156) slice number (See Fig. 4), significantly smaller logarithmic reduction was observed compared to the test, where the blade was directly inoculated (8.4±0.4 log CFU/blade, 10 min) at room temperature (Figs. 2A and 4), but bigger ($p<0.05$) reduction was observed compared to the test when the blade was inoculated with 5.9±0.6 log CFU/blade (Figs. 2B and 4). The shorter attachment time of the inoculum to blade (in this test the time between slicing the inoculated and uninoculated fillet) compared to 10 min (time the inoculum was let to attach to blade in the other test) may explain this observation.

The reduction in number of *L. monocytogenes* on the blade during slicing a single fillet was 1.2 log CFU/10 cm^2 (compared to 1.5 log CFU/g reduction in the slices) (Table 1). On the slicer holding plate, the reduction in the number of *L. monocytogenes* was 1.0 log CFU/10 cm^2 after the 39th slice.

3.5. Model development

An exponential model (Eq. (1)) of transfer as a function of slice number provided a reasonable fit across all treatments (e.g. average $r^2>0.7$, except for the 0 °C study the r^2-value was 0.63). A difference in bacterial counts on salmon was observed between the first and second slices (Figs. 2 and 3). To normalize this effect on curve-fitting, data for the first slice were not included. We believe this effect resulted from the absence of contamination prior to the first slice, and which was, contrary to subsequent slices, cut only on one side.

$$y = a^{*}e^{(-x/b)}. \quad (1)$$

The values for constants a and b, as well as the statistical parameters for each test, are shown in Figs. 2 and 3. The model describes a microbial decay curve as a function of slice number. Deviations in the results of three replicate tests were likely due to variability in the size of individual salmon slices (on average 19.2±5.8 g) and to the lack of firmness (soft texture) in salmon

that resulted in material contaminating many surfaces on the slicer. Other possible factors which may have had an effect included blade sharpness, speed (rpm) of the blade and cutting force. We would anticipate greater variability in contamination for large scale slicers equipped with several blades, which further complicate cleaning procedures. To be able to investigate the transfer phenomenon and to compare transfer among different slicing conditions, the initial number of *L. monocytogenes* used in our study were high and represented a worst case scenario.

Based on the resulting models can be e.g. estimated, that if the initial contamination level of the blade would be 5.9 log CFU/blade (i.e. approximately 2.1×10^3 CFU/cm^2, 10 min attachment time), the amount of *L. monocytogenes* bacteria on slices would be approaching zero (<1 log CFU/slice, if the size of the slice is 20 g) after slicing of 127 slices (i.e. during slicing the fourth fillet), when the slicing is done at room temperature. If the inoculum level would be 3.0 log CFU/blade (i.e. approximately 2.6 log CFU/cm^2), *L. monocytogenes* bacteria would appear sporadically mainly in the slices of the first fillet, the probability of occurrence and highest number being highest on the second slice. The highest number of *L. monocytogenes* on slices was detected in the beginning of the slicing (except in the very first slice), and to minimize the occurrence and numbers of *L. monocytogenes* transferred to salmon slices, one approach would be to discard the first slices (e.g. first five) at the beginning of the operation. The blade, the blade guard and holding plate should be periodically dismantled, cleaned and sanitized to avoid attachment of and prolonged product contamination with *L. monocytogenes*. Additionally, the blade should be sharpened regularly.

In the present study we have presented how limited data from microbiological analysis can be used to assess transfer of *L. monocytogenes* among processing surfaces and product. The results of the predictions are most reliable when the data is obtained from tests made in conditions prevailing in the process where the model will be applied (e.g. temperature, attachment time of inoculum, type of slicer etc.). Assessing transfer of *L. monocytogenes* during slicing is especially important, since ready-to-eat products such as 'gravad' salmon, are consumed without further heat treatment. The resulting models can be used as part of plant-level risk assessments to guide risk management decisions. Further investigations should include the effect of strain variability, product composition and large scale slicers.

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