Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Control 17 (2006) 609–616
FOOD CONTROL
www.elsevier.com/locate/foodcont

Inactivation by ozone of *Listeria innocua* on salmon-trout during cold-smoke processing

M. Vaz-Velho [a,b,*], M. Silva [a], J. Pessoa [a], P. Gibbs [a,c]

[a] *Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200 Porto, Portugal*
[b] *Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Av. Atlântico, 4900 Viana do Castelo, Portugal*
[c] *Leatherhead Food International, Randalls Road, Leatherhead Surrey KT22 7RY, UK*

Received 28 July 2004; received in revised form 13 March 2005; accepted 14 March 2005

Abstract

This study was conducted to evaluate the efficacy of gaseous ozone exposure on *Listeria innocua* 2030c growth during cold-smoke processing of *Oncorhynchus mykiss* (salmon-trout). Three sets of experiments were performed: inoculation with *L. innocua* 2030c followed by a 20 min ozone exposure were applied to (a) fresh salmon-trout after filleting, (b) to fresh whole fish, and (c) to fresh whole fish after removal of the fish slime. In sets (a) and (b) fillets were subsequently cold-smoked but not in set (c). The ozone concentration inside the exposure chamber after 20 min reached 0.1×10^{-3} g/l.

Counts of *L. innocua* 2030c were performed after treatment for sets (a), (b) and (c), after smoking and weekly during 21 days in vacuum packs at 5 °C, for sets (a) and (b). Sampling for total Aerobic Plate Counts (APC) of non-inoculated samples was also performed in set (a). The percentage of salt in the water phase, peroxide values and the effect of treatment on sensory properties of cold-smoked salmon-trout fillets were also determined in the first and second set. In the first set, a decrease of less than $1 \log_{10}$ in *L. innocua* numbers occurred on ozone treated samples in all sampling occasions. APC was slightly lower on fresh fillets after treatment and on smoked fillets at 3 weeks of storage at 5 °C (less than $1 \log_{10}$ CFU/g). In the second set, a reduction greater than $1 \log_{10}$ *L. innocua*/g occurred on smoked fillets at the end of the storage period. In the third set, the slime removal resulted in a $1 \log_{10}$ *L. innocua*/g reduction on fresh treated samples.

Ozone treatment had no significant ($p > 0.05$) effect on *L. innocua* counts on samples compared to those on untreated fish. In both sets, no significant differences among ozone treated/untreated samples were noticeable by sensory evaluation ($p > 0.05$).

Keywords: *Listeria* spp.; Ozone; Cold-smoked fish

1. Introduction

Listeria monocytogenes is an ubiquitous psychrotrophic bacterium responsible for foodborne infections worldwide. Human listeriosis is predominantly a foodborne disease caused by *L. monocytogenes*, and although rare, has a high mortality. The disease most often affects unborn or newly delivered infants, pregnant women, and the immunocompromised (Farber & Peterkin, 1991). The pathogen has been consistently isolated from production lines of fresh to cold-smoked fish (e.g., Ben Embarek, 1994; Dillon, Patel, & Ratnam, 1992; Eklund et al., 1995; Farber, 1991; Jemmi & Keusch, 1994; Johansson, Rantala, Palmu, & Honkanen-Buzalski, 1999; Jørgensen, 2000; Jørgensen & Huss, 1998; Rørvik, Caugant, & Yndestad, 1995; Vaz-Velho, Duarte, &

* Corresponding author. Address: Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Av. Atlântico, 4900 Viana do Castelo, Portugal. Tel.: +351 (258) 819700; fax: +351 (258) 827636.
E-mail address: mvazvelho@estg.ipvc.pt (M. Vaz-Velho).

0956-7135/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodcont.2005.03.007

Gibbs, 2000; Vaz-Velho, Duarte, & Gibbs, 1998). A number of small outbreaks associated with smoked fish and shellfish have also been reported (Brett, Short, & McLauchlin, 1998; Ericsson et al., 1997; Hall, Pelerin, Soltanpoor, & Gilbert, 1995; Miettinen et al., 1999; Misrachi, Watson, & Coleman, 1991; Mitchell, 1991). The mild temperatures (<30 °C) and low salt concentrations [<5% (water phase salt)] used in the cold-smoked processing of fish are not sufficient to inactivate *L. monocytogenes*. Growth of the organism may occur in the product during storage and cold smoked fish does not undergo cooking before consumption (final report of Spoilage and safety of cold-smoked fish, 2000).

The use of ozone as a surface disinfectant of meats (Greer & Jones, 1989; Sheldon & Brown, 1986) and for the preservation of shrimps (Chen, Huang, Moody, & Jiang, 1992) has been reported. The extension of the shelf-life of perishable foods using ozone to reduce microbial activity has also been reported by Rice, Farquhar, and Bollyky (1982). As reported by Nelson (1982), the fresh quality of ozone-iced pacific salmon could be maintained for up to 6 days. The applications of ozone to fish preservation have been studied by several authors (e.g., Ravesi, Licciardello, & Racicot, 1987; Rice & Graham, 2001; Rice et al., 1982; Silva, Gibbs, & Kirby, 1998). Ozone concentrations as low as 0.01 ppm are toxic to bacteria and Gram-positive bacteria are more sensitive to ozone than Gram-negative bacteria (Mielcke & Ried, 2004).

In the United States ozone has received in 1997 GRAS (generally recognised as safe) classification, and in 2001 the FDA officially approved media containing ozone for use in the food industry, also for direct contact with food products, including fish, meat and poultry (Mielcke & Ried, 2004).

Until now, no information was available regarding the effect of ozone on *Listeria* spp. associated with cold-smoked fish processing. In this study, *L. innocua* 2030c, a tetracycline resistant strain, was used to replace the pathogen *Listeria monocytogenes* due to processing plant constraints and because all the *Listeria* strains isolated from Portuguese cold-smoked fish products were, to date, tetracycline sensitive (Vaz-Velho, Duarte, McLauchlin, & Gibbs, 2001). The behaviour of this strain, already studied, is similar to the major types of Portuguese *L. monocytogenes* strains (serotypes 4b and 1/2c) with respect to its growth/survival patterns under exposure to gaseous ozone. The best time/concentration combination of gaseous ozone for reducing *L. innocua* 2030c and *L. monocytogenes* 4b and 1/2c pure culture numbers was 20 min of exposure to ozone concentration of 0.1×10^{-3} g/l resulting in a 3–3.5 $\log_{10}$ *Listeria* spp./ml reduction (Vaz-Velho, Fonseca, Silva, & Gibbs, 2001).

This remarkable effect of ozone on pure cultures of *Listeria* spp. created great expectations for challenge studies with cold-smoked fish processing. Therefore, the purpose of this study was to ascertain if the application of gaseous ozone to raw fish has any effect on *Listeria* numbers during cold-smoked processing and further chilled storage for 3 weeks at 5 °C in vacuum packs. Such treatment could be a potential measure for reducing *L. monocytogenes* numbers on products intended to be maintained "traditional" but safe.

2. Materials and methods

Fresh, premium quality salmon-trout (*Oncorhynchus mykiss*) with an average weight of 1.5 kg, farmed and slaughtered in the north of Portugal were placed in polystyrene boxes covered with ice and transported to the laboratory and stored overnight at 5 °C. The next day the fish were eviscerated, beheaded and filleted. Three sets of experiments were performed.

For the first set of experiments (a), inoculation and treatment were applied to fish after filleting and fillets were then cold-smoked. In the second experiment (b), inoculation and treatment were applied to the whole fish before filleting to avoid the potential interference of muscle exposure and, after filleting, fish was subsequently smoked. Another experiment (c), inoculating and treating the whole fish with or without the presence of slime, was performed. Slime was removed from the fish surface by washing the fish with water for 15 min before inoculation and treatment, but the fish fillets were not smoked.

2.1. Ozone generation and measurements

Ozone was produced by a domestic ozone generator model PR1 (TRIOZON, Spain) using atmospheric air as the source of oxygen. The ozonated air produced at a constant flow rate by the apparatus (0.76 l/min) was passed via a silicone tube to a pump, and then to a plastic chamber of 26 dm^3 volume. The ozone in the air flow produced by the apparatus (0.32 mg/l) was measured experimentally by the iodometric method as described Silva et al. (1998). In addition, the ozone concentration in the air flow inside the box was calculated as described by Silva et al. (1998). The ozone concentration inside the chamber after 20 min reached 0.1×10^{-3} g/l.

2.2. Ozone treatments

An overnight 20 ml culture (30 °C, 18 h) of *L. innocua* 2030c a tetracycline resistant strain from Public Health Laboratory Services (PHLS, London/UK) private collection, yielding 10^{8-9} CFU/ml in Tryptone Soy Yeast Extract agar (Tryptone Soy Broth + 6 g/l yeast extract + 12 g/l agar (TSB-YE, Lab M, Bury, Lancashire/UK)) was centrifuged (Centromix P Selecta, Spain;

2400 g, 10 min) and the cells resuspended in 20 ml of sterile 1% (w/v) NaCl (Merck, Darmstadt/Germany). This suspension was further diluted in 1 l of sterilised water with 1% of NaCl (w/v). Fish fillets (set a) or whole fish (sets b and c) were introduced into this bath for 30 s. A cell concentration of about 10^6, 10^4 and 10^6 CFU/g was obtained in the raw fish of set (a), set (b) and set (c), respectively.

In the first set of experiments (a) the trials were as follows:

Trial 1. Two inoculated fillets subjected to ozone exposure for 20 min.
Trial 2. Two inoculated fillets not subjected to ozone exposure.
Trial 3. Two fillets not inoculated but exposed to ozone for 20 min.
Trial 4. Two remaining fillets were not inoculated nor treated with ozone.

Fillets were left with the belly skin and the lug bone and were further dry-salted and cold-smoked (Vaz-Velho, Duarte, & Gibbs, 2001). After smoking the fillets were sliced and portions of slices were vacuum packed and stored at 5 °C. This experiment was performed three times (three smoking batches).

For the second experiment (b), where inoculation and treatment were applied to the whole fish, the procedure was as follows: two fish were inoculated, one fish being subjected to a 20 min ozone exposure and the other fish not treated; two fish were not inoculated, one fish being subjected to ozone treatment and the other fish not. Fish were filleted before salting and smoking. Number of trials and procedures were as in set (a) but just one smoking batch was performed.

A third experiment (c) was performed by inoculating and treating the whole fish before and after removal of the slime to evaluate whether slime on the raw fish might interfere with ozone activity. The procedure was as follows: two fish inoculated before removal of the slime, one being subjected to a 20 min ozone exposure and the other fish not; two fish inoculated after removal of the slime, one being subjected to a 20 min ozone exposure and the other fish not. Fish were filleted but not further smoked.

2.3. Sampling and enumeration

25 grams of fish fillets (fresh and smoked material) were homogenised with 225 ml of sterile Maximum Recovery Diluent (MRD, Lab M, Bury, Lancashire/UK) in a Stomacher (Seward 400) for 2 min. One milliliter of this suspension was placed in tubes containing 9 ml of MRD. Serial decimal dilutions to 10^{-4} were made in MRD.

Sampling was done after inoculation and/or ozone treatment, after smoking, and weekly during the 3 weeks of storage in vacuum packs at 5 °C. Two samples per treatment were analysed on each occasion.

Enumeration of *L. innocua* on fish fillets was by spread plating onto PALCAM agar (Merck, Darmstadt, Germany) with 8 μg/ml tetracycline–HCl (Sigma, Steinheim/Germany) incubated at 30 °C for 48 h before counting the typical colonies (black colonies with a dark halo). Total Aerobic Plate Counts (APC) were also determined in non-inoculated samples using Long and Hammer's (1941) plating medium (modified by Van Spreekens, 1974) incubated at 25 °C for 5 days.

2.4. Cold-smoking processes

For the first set of experiments (a), three batches of smoked fish were produced. Differences between the processes were for practical reasons in obtaining a reasonable time of salting/smoking and saving time in the experiment and still producing an acceptable commercial smoked product.

For both sets of experiments (a, b) dry-salting was done for 6 h at 16 °C, with a sugar:salt mix of 1:6 by weight. The weight of salt + sugar corresponded to one third of the weight of the fillet. After salting, the fillets were washed/rubbed with running water to remove the surplus of salt, and were hung to drain overnight at 5 °C. Smoking was done for 8, 6 and 5 h, respectively for the first, second and third batches of the first set (a) and for 5 h for the second experiment (b). A vertical smoker (AGK, Type 135/12, Wallersdorf, Germany) was used but a more sensitive thermostat and timer (lae® electronic, MTR12, Oderzo, Italy) replaced those supplied with the smoker. Smoke was produced by smouldering beech wood chips (Räucher Gold®, type Kl 2/16, Rettenmaier & Söhne, Rosenberg, Germany). Time/temperature profiles of the processes were recorded on a portable microprocessor (Hanna instruments, HI 92804c Bedfordshire, UK). Although smoke temperature occasionally exceeded 30 °C, temperatures of the fillets were always below 28 °C. Relative humidity, recorded by an hygrometer (Rotronic AM3, Knoxville, USA) decreased from 80% to 70% during the first three hours, decreasing further to 52% at the end of the smoking process.

The smoked fillets were cooled overnight at 5 °C. The following day, lug and pin bones and belly flaps were removed and the fillets were then sliced by hand. Portions of slices (skin off), of about 100 g each, were vacuum packed (Multivac Sepp Haggenmüller KG, A300/41/42, Germany; 1 mbar/10 s) and stored at 5 °C for weekly analysis. The permeability of the packs to O_2, CO_2 and N_2 were respectively 4, 13 and 4 mol/m^2 d bar.

2.5. Chemical analysis

Sodium chloride and moisture contents and peroxide values of the finished products were evaluated following respectively, the Portuguese NP 2929 (Fish and fish products—determination of chlorides content, 1988), NP 2282 (Fish and fishing products—determination of the water content (Reference Method), 1991) and NP 3142 (Fish and fish products—determination of peroxide value, 1990) protocols for fish products. These analyses were performed after 3 weeks of storage in vacuum packs at 5 °C.

2.6. Sensory evaluation

After 3 weeks storage at 5 °C, sensory evaluation of non-inoculated ozone-treated and untreated samples was carried out with 10 semi-trained panelists. Judges were asked to classify the samples for overall acceptability regarding the flavour and aroma, choosing one of these five hypotheses: I like very much; I like; I don't like nor dislike; I dislike, I dislike very much, scored respectively with 5, 4, 3, 2 and 1 points. Samples scored with totals of $\geqslant 30$ points were considered acceptable. Judges were requested to justify their classification specifying why they liked or disliked. The protocol used was that adopted by the EC FAIR Project CT/95 1207 "Spoilage and safety of cold-smoked fish".

2.7. Statistical analysis

The effect of ozone treatment on sensory properties of the smoked fillets, within the different batches of sets (a) and (b) at the end of the third week of storage, was evaluated by analysis of variance. The ANOVA test was applied for quantitative analysis of the taste panel results. The effect of ozone treatments on *L. innocua* 2030c and APC numbers within the different batches and for all sampling times was evaluated by analysis of variance (ANOVA). It was accepted there was a significant difference between ozone treated/untreated samples if $P < 0.05$.

3. Results and discussion

As previously mentioned, differences in smoking times of the three batches from the first set (a) were for practical reasons only, yet still producing an acceptable commercial-like smoked product. However, as no significant differences in *L. innocua* numbers and APCs were found among the three batches ($P > 0.05$) of set (a), the average was considered.

The effect of prior ozone exposure on growth of *L. innocua* 2030c and APC during the cold-smoke processing and storage of set (a) is shown in Figs. 1 and 2.

In the first experiment, and in the first batch, the % of salt in the water phase (w/w) was too high (average = 7%) and decreased to 5.7% and 5.8% respectively in the second and third batches. In the second experiment the average % of salt in the water phase was 5.8% (w/w). According to Huss, Embarek, and Jeppesen (1995), a commercially acceptable level of salt should be below 5% in the water phase and for safety reasons this percentage must be at least 3%. Although the amount of salt in the water phase exceeded the recommended 5%, as sugar was added to the salt in the proportion 1:6 sugar:salt, the saltiness was not noticeable. All the products had levels of salt in the water phase $\geqslant 3.5\%$ (w/w) (Tables 1 and 2) but *L. innocua* 2030c is known to grow well at those levels of salt (Vaz-Velho, Durate & Gibbs, 2001).

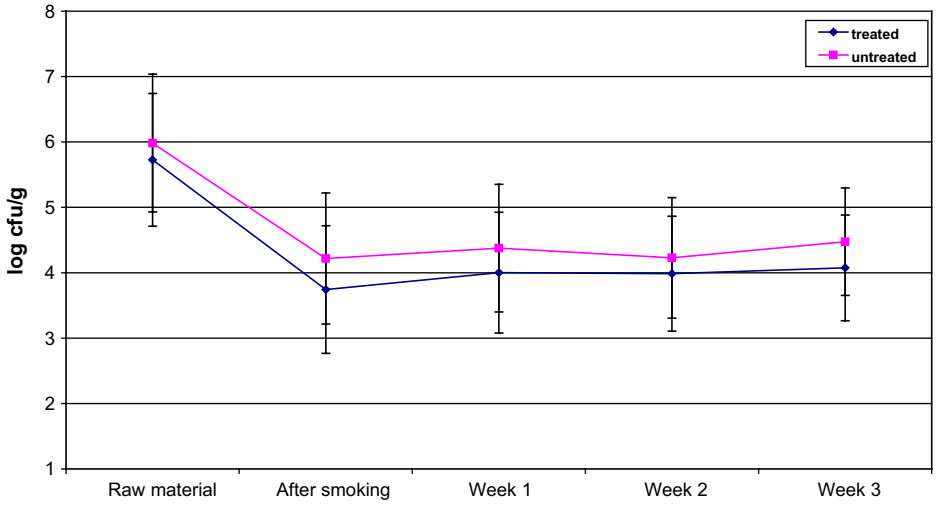

Fig. 1. The effect of gaseous ozone on *Listeria innocua* 2030c numbers during cold-smoke processing of salmon-trout (set a—ozone applied to fresh fish after filleting).

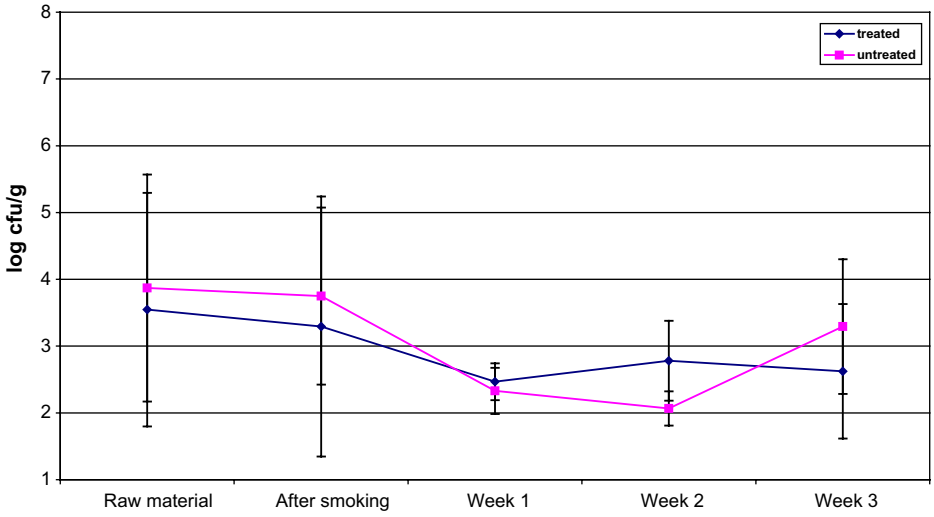

Fig. 2. Effect of gaseous ozone on APC during cold-smoking processing of salmon-trout (set b—ozone applied to fresh fish after filleting).

Table 1
First set (a)—sensory scores, peroxide values and % salt in the water phase of the three batches

Samples	Batch 1			Batch 2			Batch 3		
	Sensory scores	Peroxide value (meq/kg of fat)	%NaCl (w/w)	Sensory scores	Peroxide value (meq/kg of fat)	%NaCl (w/w)	Sensory scores	Peroxide value (meq/kg of fat)	%NaCl (w/w)
Inoc./treated	nd	2	7.1	nd	5	5.7	nd	Not detected	4.6
Inoc./treated	nd	3	8.3	nd	15	5.7	nd	3	5.4
Inoc./untreated	nd	Not detected	7.1	nd	7	5.5	nd	4	6.1
Inoc./untreated	nd	Not detected	6.1	nd	Not detected	6.2	nd	2	8.3
Treated	35	Not detected	4.7	43	Not detected	5.1	36	Not detected	5.7
Treated	nd	Not detected	8.7	nd	6	6.7	nd	Not detected	5.0
Untreated	39	Not detected	8.0	40	3	5.1	41	Not detected	4.4
Untreated	nd	Not detected	6.0	nd	Not detected	5.2	nd	3	6.7

nd: not determined.

Table 2
Second set (b)—sensory scores, peroxide values and % salt in the water phase

Sample	Panel score	Peroxide value meq/kg of fat	%NaCl (w/w)
Inoc./treated	nd	2.6	7.9
Inoc./treated	nd	10	8.8
Inoc./untreated	nd	3.6	6.7
Inoc./untreated	nd	6.1	3.8
Treated	nd	5.2	5.4
Treated	36	2.6	3.5
Untreated	38	2.3	4.3
Untreated	nd	10.7	6.4

nd: not determined.

Jørgensen (2000), found values of water phase salt in commercial Danish cold-smoked salmon ranging from 3% to 12.5%. Portuguese commercial cold-smoked swordfish always presented levels of salt (water phase salt) ≈8% (w/w) and *L. monocytogenes* has been consistently isolated from this kind of products (Vaz-Velho, Durate, McLauchlin et al., 2001).

In the first set of experiments (a) the best sensory scores were obtained by the untreated, treated and untreated samples respectively in the first, second and third batches (Table 1). In the second set (b) the untreated sample was scored best (Table 2). However, in both batches statistical analysis did not differentiate the products ($P > 0.05$).

No relationship between peroxide values and ozone treatment or moisture content was found in the samples tested, and the concentration of ozone used in this study did not significantly change the sensory characteristics of the smoked products.

As shown in Fig. 1, exposure to gaseous ozone at the levels used did not significantly reduce the numbers of *L. innocua* inoculated on salmon-trout fillet surfaces (a reduction of less than 1 $\log_{10}$ CFU/g in all sampling occasions). With respect to APC, although at the end of the storage period treated samples showed slightly

lower numbers (Fig. 2), no significant effect on APC was found—statistical analysis did not differentiate the treated/untreated products ($p > 0.05$).

The required ozone concentration and, of course, the disinfection rates depend in every case on the type of microorganism, on the degree of biological contamination, on the temperature, pH, turbidity and on the presence of ozone-oxidisable substances (Mielcke & Ried, 2004).

Restaino, Erampton, and Hemphill (1995), reported on the efficacy of ozone to reduce the numbers of *L. monocytogenes* and other pathogenic microorganisms. These authors showed that *L. monocytogenes* was more sensitive to ozonated water than the other Gram-positive and Gram-negative bacteria studied, either in the presence or absence of organic material.

Silva et al. (1998) reported gaseous ozone to be effective in reducing numbers of scad spoilage flora. These authors compared two ozone treatments on whole-eviscerated fresh fish: (i) the fish subjected just to a single ozone treatment before ice storage and (ii) subjected to a daily ozone treatment during iced storage. This last option, performed in the laboratory, was more effective in reducing the spoilage flora than the former. On the other hand, continuous ozonation of fish in boat holds (ozone rate maintained constant), was shown to be more effective than in the reported laboratory experiments, by increasing the lag phase and reducing the numbers of all tested microorganisms (Silva et al., 1998).

The interference of organic matter (exposure of fish muscle on fillets) was considered as the main potential reason for the results obtained in this first set of experiments. It has been reported that sensitivity of microorganisms to ozone is affected by several factors including the presence of organic matter (Glaze, 1986; Hoigné & Bader, 1975). A high and persistent level of organic substances will have a negative impact on ozone disinfection rate (Mielcke & Ried, 2004).

The applicability of ozone treatments in the cold-smoked fish industry, a major objective of this work, made it necessary to choose the best practicable time to apply the treatment to the fish. It was assumed for a smoking plant, the best moment would be after filleting and washing (less contamination by further handling) and just before salting.

In the literature reviewed, ozone treatments were applied to the whole fish rather than to fillets, hence the decision to treat whole salmon trout (set b). However, if this treatment shows greater potential for reducing *Listeria* numbers, the raw fish suppliers should apply the treatment before the fish arrive at the smoking plants, which may not be well-accepted by the fish farmers unless reliable raw material guarantees become mandatory.

As in set (a) ozone treatment did not significantly reduce *L. innocua* 2030c numbers at any stages of the processing chain and during storage, it was assumed that organic matter might have been the reason by interfering with ozone activity. The effect of ozone exposure on subsequent growth of *Listeria innocua* 2030c in the second experiment (b) is shown in Fig. 3. In this second experiment (b), fish was treated before filleting therefore the potential interference of the organic matter due to the exposure of the muscle was reduced; ≈1 log reduction in *L. innocua* numbers was found in the raw fish after treatment and at the end of the storage period this reduction was greater than 1 log (Fig. 3). No differences between treated/untreated samples were noticed after smoking until week 2 when treated fish showed higher numbers than untreated fish (≈1 log cycle) and, at week

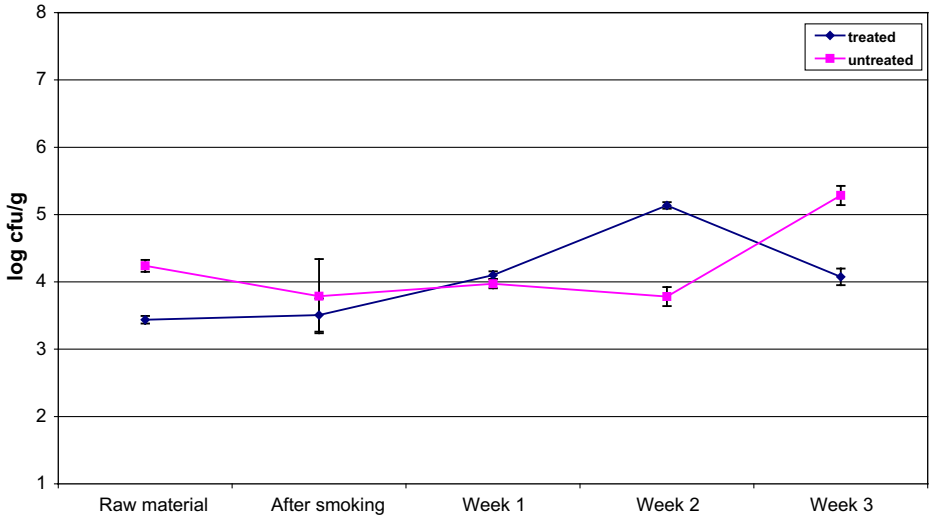

Fig. 3. The effect of gaseous ozone on *Listeria innocua* 2030c numbers during cold-smoke processing of salmon-trout (set b—ozone applied to fresh whole fish).

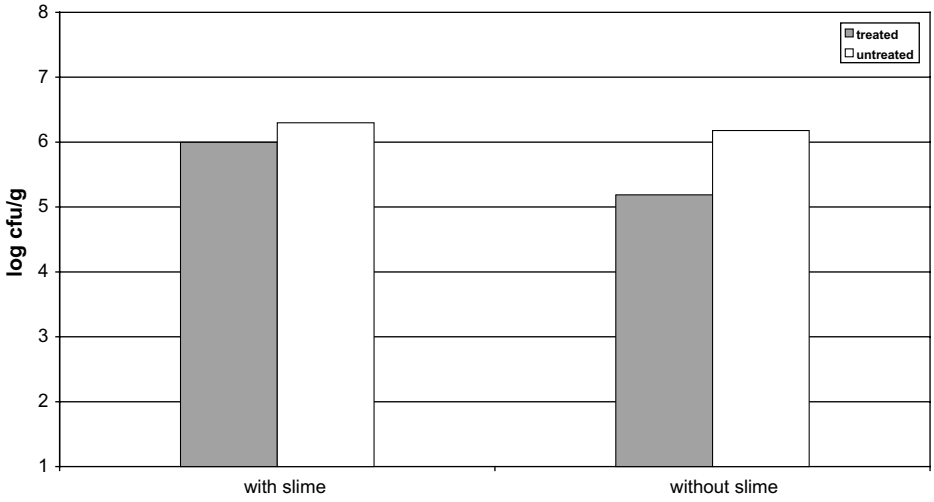

Fig. 4. The effect of gaseous ozone on *Listeria innocua* 2030c numbers in fresh whole salmon-trout with and without slime (set c).

3 the opposite happened. No clear explanation was found for these contradictory counts after 2 and 3 weeks of storage (standard deviations were very low). Statistical analysis did not differentiate the treated/untreated samples ($P > 0.05$).

Since it was possible that the slime layer present on salmon-trout skin but not in salt water fishes such as scad (Silva et al., 1998), might have reduced the efficacy of ozone treatment, a third experiment (c) with fresh whole salmon-trout was then performed. Inoculation with *L. innocua* and ozone treatments were applied to the fresh whole fish before and after removal of the slime layer, but the fillets were not then smoked. The results are presented in Fig. 4. When slime was removed before the inoculation and treatment ≈1 log reduction of *L. innocua* 2030c numbers on the raw fish was observed compared to the untreated samples. However, statistical analysis did not differentiate samples treated before removal of the slime from those treated after removal of the slime ($P > 0.05$).

Contrary to the results of this study, Rice and Graham (2001) reported ozone as a very effective microbicidal agent, reducing bacterial numbers in various types of food including fruits, vegetables, poultry and fish.

Previous studies using bacteria representative of normal fish spoilage flora (Silva et al., 1998) and *Listeria* spp. pure cultures (Vaz-Velho, Fonseca et al., 2001) exposed to various ozonation times, indicated that the survival rate of the tested bacteria was not linearly related to ozonation time, the higher death rate occurring during the first 15 and 20 min of exposure with a level of ozone inside the desiccators of about 0.25×10^{-3} g/l and 0.1×10^{-3} g/l respectively for the first and last studies. Of course, those experiments were performed on pure cultures of *Listeria* spp. or for reducing the total viable flora of smaller fish. For fish of greater dimensions such as salmon-trout, the length of exposure to ozone used in this study might have been too short. Additionally, care must be taken when using ozone applications to prevent the oxidation reactions that can occur making the product unacceptable. Since in the present study no relation between peroxide values and ozone treatment or moisture content was found in the samples analysed, higher concentrations of ozone or longer exposure, could be tested in future experiments.

The maintenance of a reliable and healthy food supply is the greatest challenge for food industry. Nowadays, it is possible to produce ozone in an economical and very reliable way—0.90 to 1.60 €/kg of ozone (Mielcke & Ried, 2004). The utilisation of ozone for direct contact with food products has been approved by FDA, in 2001, therefore it is likely that individual and special ozone application practices have been developed or will be processed quite soon.

References

Ben Embarek, P. (1994). Presence, detection and growth of *Listeria monocytogenes* in seafoods: A review. *International Journal of Food Microbiology, 23*, 17–34.

Brett, M. S. Y., Short, P., & McLauchlin, J. (1998). A small outbreak of listeriosis associated with smoked mussels. *International Journal of Food Microbiology, 43*, 223–229.

Chen, H. C., Huang, S. H., Moody, M. W., & Jiang, S. T. (1992). Bactericidal and mutagenic effects of ozone on shrimp (*Penaeus monodon*) meat. *Journal of Food Science, 57*, 923–927.

Dillon, R., Patel, T., & Ratnam, S. (1992). Prevalence of *Listeria* in smoked fish. *Journal of Food Protection, 55*, 866–870.

EC FAIR Project CT/95 1207 (2000). *Spoilage and safety of cold-smoked fish*. Final report.

Eklund, M. W., Poysky, F. T., Paranjpye, R. N., Lashbrook, L. C., Peterson, M. E., & Pelroy, G. A. (1995). Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. *Journal of Food Protection, 58*, 502–508.

Ericsson, I. L., Eklow, A., Danielsson-Tham, M. L., Loncarevic, S., Mentzing, O., Persson, I., et al. (1997). An outbreak of listeriosis suspected to have been caused by rainbow trout. *Journal of Clinical Microbiology, 35*, 2904–2907.

Farber, J. (1991). *Listeria monocytogenes* in fish products. *Journal of Food Protection, 54*, 922–924, 934.

Farber, J. M., & Peterkin, P. I. (1991). *Listeria monocytogenes*, a foodborne pathogen. *Microbiological Reviews, 55*, 476–511.

Fish and fish products—determination of chlorides content (1988). NP 2929—*Instituto Português da Qualidade*. Lisbon, Portugal.

Fish and fish products—determination of peroxide value (1990). NP 3141—*Instituto Português da Qualidade*. Lisbon, Portugal.

Fish and fishing products—determination of the water content (Reference Method) (1991). NP 2282—*Instituto Português da Qualidade*. Lisbon, Portugal.

Glaze, W. H. (1986). Reaction products of ozone: A review. *Environmental Health Perspectives, 69*, 151–157.

Greer, G. G., & Jones, S. D. M. (1989). Effects of ozone on beef carcasse shrinkage, muscle quality and bacterial spoilage. *Canadian Institute of Food Science and Technology Journal, 22*, 156–160.

Hall, S. M., Pelerin, M., Soltanpoor, N., & Gilbert, R. J. (1995). A case control study of listeriosis in England and Wales. In *Proceedings of the XII international symposium on the problems of listeriosis*, Perth, Australia, pp. 157.

Hoigné, J., & Bader, H. (1975). Ozonation of water: Role of hydroxyl radicals as oxidising intermediates. *Science, 190*, 782–784.

Huss, H. H., Embarek, P. K., & Jeppesen, V. F. (1995). Ben Control of biological hazards in cold smoked salmon production. *Food Control, 6*(6), 335–340.

Jemmi, T., & Keusch, A. (1994). Occurrence of *Listeria monocytogenes* in freshwater farms and fish-smoking plants. *Food Microbiology, 11*, 309–316.

Johansson, T., Rantala, L., Palmu, L., & Honkanen-Buzalski, T. (1999). Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *International Journal of Food Microbiology, 47*, 111–119.

Jørgensen, L. V. (2000). *Spoilage and safety of cold-smoked salmon*. Ph.D. Thesis, Royal Veterinary and Agricultural University of Copenhagen. Edited by Danish Institute for Fisheries Research, Technical University of Denmark.

Jørgensen, L. V., & Huss, H. H. (1998). Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. *International Journal of Food Microbiology, 42*, 127–131.

Long, H. F., & Hammer, B. W. (1941). Classification of the organisms important in dairy products. III *Pseudomonas putrefaciens*. *Research Bulletin Iowa Agriculture Exposition, 285*, 173–195.

Mielcke, J., & Ried, A. (2004). Current state of application of ozone and UV for food processing. In *Proceedings of the food protection international conference 2004*. Monte da Caparica, Portugal, 20–22 of May 2004.

Miettinen, M. K., Siitonen, A., Heiskanen, P., Haajanen, H., Bjorkroth, K. J., & Korkeal, H. J. (1999). Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold smoked rainbow trout. *Journal of Clinical Microbiology, 37*, 2358–2360.

Misrachi, A., Watson, A. J., & Coleman, D. (1991). *Listeria* in smoked mussels in Tasmania. *Communicable Disease Intelligence, 15*, 427.

Mitchell, D. L. (1991). A case cluster of listeriosis in Tasmania. *Communicable Disease Intelligence, 15*, 427.

Nelson, W. (1982). The use of ozonated ice to extend the shelf life of fresh Alaskan fish. Alaska Department of Commercial and Economic Development, Officially Commercial Fish Development Anchorage.

Ravesi, E. M., Licciardello, J. J., & Racicot, L. D. (1987). Ozone treatments of fresh Atlantic cod, *Gadus morhua*. *Marine Fisheries Review, 49*, 37–42.

Restaino, L., Erampton, E. W., & Hemphill, J. B. (1995). Efficacy of ozonated water against various food-related microorganisms. *Applied and Environmental Microbiology*, 3471–3475.

Rice, R. G., Farquhar, J. W., & Bollyky, L. J. (1982). Review of the applications of ozone for increasing storage times of perishable foods. *Ozone Science and Engineering, 4*, 147–163.

Rice, R. G., & Graham, D. M. (2001). US FDA regulatory approval of ozone as an antimicrobial agent—what is allowed and what needs to be understood. *Ozone News, 29*(5), 22–31.

Rørvik, L. M., Caugant, D. A., & Yndestad, M. (1995). Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. *International Journal of Food Microbiology, 25*, 19–27.

Sheldon, B. W., & Brown, A. L. (1986). Efficacy of ozone as a disinfectant for poultry carcasses and chill water. *Journal of Food Science, 51*, 305–312.

Silva, M. V., Gibbs, P. A., & Kirby, R. M. (1998). Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*). *Journal of Applied Microbiology, 84*, 802–810.

Van Spreekens, K. J. A. (1974). The suitability of a modification of Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Archive Lebensmittelhygiene, 25*, 213–219.

Vaz-Velho, M., Duarte, G., & Gibbs, P. (1998). Occurrence of *Listeria* spp. in salmon trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*). *Food Science and Technology International, 4*, 121–125.

Vaz-Velho, M., Duarte, G., & Gibbs, P. (2000). Evaluation of mini-VIDAS rapid test for detection of *Listeria monocytogenes* from production lines of fresh to cold-smoked fish. *Journal of Microbiology Methods, 40*, 147–151.

Vaz-Velho, M., Duarte, G., & Gibbs, P. (2001). Effect of different salting and smoking procedures on *Listeria innocua* 2030c and TVC during chilled storage of vacuum-packed cold-smoked salmon-trout. Oral presentation. In *Proceedings of ISOPOL XIV international symposium on problems of Listeriosis*, Mannheim, Germany 13–16 of May 2001.

Vaz-Velho, M., Duarte, G., McLauchlin, J., & Gibbs, P. (2001). Characterisation of *Listeria monocytogenes* isolated from production lines of fresh and cold-smoked fish. *Journal of Applied Microbiology, 91*, 556–562.

Vaz-Velho, M., Fonseca, F., Silva, M. V., & Gibbs, P. (2001). Is *Listeria innocua* 2030c tetracycline resistant, a suitable marker for replacing *Listeria monocytogenes* in challenge studies with Portuguese cold-smoked fish? *Food Control, 12*(6), 361–364.