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Short communication

A comparison between E-beam irradiation and high pressure treatment for cold-smoked salmon sanitation: microbiological aspects

M. Medina [a,*], M.C. Cabeza [b], D. Bravo [a], I. Cambero [b], R. Montiel [a], J.A. Ordóñez [b], M. Nuñez [a], L. Hoz [b]

[a] Departamento Tecnología de Alimentos, INIA, Carretera de La Coruña Km 7, 28040 Madrid, Spain
[b] Departamento Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

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ABSTRACT

The effectiveness of electron beam irradiation and high pressure treatment for the sanitation of cold-smoked salmon from two points of view, microbial safety and shelf-life extension, was compared. From the response of *L. monocytogenes* INIA H66a to irradiation, a *D* value of 0.51 kGy was calculated. For samples stored at 5 °C, 1.5 kGy would be sufficient to attain a Food Safety Objective (FSO) of 2 $\log_{10}$cfu/g *L. monocytogenes* for a 35-day shelf-life, whereas 3 kGy would be needed in the case of a temperature abuse (5 °C + 8 °C). Pressurization at 450 MPa for 5 min was considered to be an insufficient treatment, since the FSO of 2 $\log_{10}$cfu/g *L. monocytogenes* was only attained for a shelf-life of 21 days at 5 °C. However, treatment at 450 MPa for 10 min achieved this FSO for samples held during 35 days at 5 °C, or during 21 days under temperature abuse (5 °C + 8 °C) conditions. Irradiation at 2 kGy kept the microbial population of smoked salmon below 6 $\log_{10}$cfu/g after 35 days at 5 °C, with negligible or very light changes in its odor. Pressurization at 450 MPa for 5 min also kept the microbial population below 6 $\log_{10}$cfu/g after 35 days at 5 °C and did not alter odor, but affected negatively the visual aspect of smoked salmon.

1. Introduction

One of the most popular fish products worldwide is cold-smoked salmon, commonly prepared for consumption as an RTE product. Current technology results in a less salty, dry and smoked final product than that manufactured some decades ago. Hurdles preventing microbial growth in cold-smoked salmon have been minimized, thus favoring the development of spoilage and pathogenic microorganisms. The presence of *Listeria* in cold-smoked salmon can be mainly ascribed to contamination during processing rather than to contamination from raw fish (Rorvik, 2000; Vogel et al., 2001).

Strategies proposed to prevent *L. monocytogenes* growth in cold-smoked salmon include lactic acid bacteria (Nilsson et al., 1999), bacteriocins (Duffes et al., 1999), carbon dioxide in modified atmosphere packaging (Nilsson et al., 1997) or the combination of sodium diacetate with potassium lactate (Yoon et al., 2004; Vogel et al., 2006).

Non-thermal physical technologies, E-beam irradiation (Patterson et al., 1993; Zhu et al., 2005) and high pressure (HP) treatment (Amanatidou et al., 2000; Lakshmanan et al., 2003; Lakshmanan and Dalgaard, 2004) are effective tools to eliminate pathogens present in foods. Low E-beam doses of 1 kGy reduced *L. monocytogenes* counts in cold-smoked salmon and 2.0 kGy eliminated the pathogen (Su et al., 2004). However, HP treatment at 150–250 MPa did not inactivate *L. monocytogenes* in cold-smoked salmon (Lakshmanan and Dalgaard, 2004). Process variables such as the irradiation dose or the pressure level, together with the temperature and time of treatment, may affect the sensory properties of food.

Since *L. monocytogenes* is clearly a public health concern, risk management actions are required to reduce its prevalence and levels in smoked salmon. On this aim, a Food Safety Objective (FSO) can be used as a risk management tool for *L. monocytogenes* in smoked salmon.

In the present work, the effectiveness of E-beam irradiation and HP treatment to attain an FSO for *L. monocytogenes* in cold-smoked salmon, together with their effect on shelf-life extension, were compared.

2. Methods

2.1. Microorganism

Listeria monocytogenes INIA H66a from the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria,

* Corresponding author. Tel.: +34 9134 76774; fax: +34 913 572293.
E-mail address: mmedina@inia.es (M. Medina).

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Madrid, Spain) culture collection was used. Its radioresistance (D=0.46 kGy) was higher than the average, among five strains belonging to different serovars tested in cooked ham (Cabeza et al., 2007). Its baroresistance in cold-smoked salmon pressurized at 450 MPa for 10 min was comparable to that of another eight *L. monocytogenes* strains tested (Medina et al., unpublished data). The strain was maintained at −40 °C in trypticase soy broth (TSB, Difco, Sparks, MD, USA) with 10% glycerol.

Fresh cultures were prepared in TSB incubated at 32 °C for 24 h. The culture was then centrifuged at 4 °C and the pellet suspended in two different volumes of sterile saline solution, to yield bacterial loads of approximately 3×10^{10} and 3×10^{8}cfu/ml for E-beam irradiation and HP treatments, respectively.

2.2. Preparation of samples

Cold-smoked salmon fillets were sliced at a local industry, vacuum-packaged, and transported to the laboratory at 4 °C. Samples (20 g) were obtained by cutting pieces of uniform thickness from the slices. Samples were contaminated by immersion for 10 s in the cell suspensions described above, to achieve initial loads of approximately 8×10^{8} and 3×10^{6} cfu/g for E-beam irradiation and HP treatments, respectively. Afterwards, samples were vacuum-packaged and held at 4 °C during 24 h prior to treatments.

2.3. Product characteristics

Smoked salmon pH was determined in duplicate with a pH meter (model GPL 22, Crison Instruments, Barcelona, Spain). The a_w value was determined in triplicate with an Aqua Lab Water Activity Meter Series 3 (Decagon Devices Inc., Pullman, USA). Sodium chloride was determined in triplicate by a standard procedure (AOAC, 1995).

2.4. Treatments

For the irradiation treatment, triplicate samples were transported under refrigeration to the irradiation plant (Ionmed S.A., Tarancón, Spain), placed on stainless steel trays and irradiated in triplicate under an E-beam irradiation source operating at 10 MeV. Radiation doses employed ranged from 1 to 4 kGy. The dose absorbed by the salmon was checked by determining the absorbance of cellulose triacetate dosimeters simultaneously irradiated with the samples. For HP treatment, triplicate samples were pressurized at 450 MPa and 12 °C during 5, 10, 15, 20 and 25 min in a model ACIP 6000 apparatus (ACB, Nantes, France).

Non-inoculated samples were subjected in duplicate to irradiation and HP treatments for shelf-life determination. After irradiation and HP treatments, samples were stored at 5 °C.

2.5. Microbial analyses

Salmon samples (20 g) were homogenized with 10 ml of sterile saline solution in a Stomacher (Seward Laboratory, London, UK) and 10-fold diluted. Counts of *L. monocytogenes* were determined in duplicate on PALCAM Listeria Selective Agar (Merck KgaA, Darmstadt, Germany) plates incubated at 37 °C for 48 h.

Total mesophilic counts in non-inoculated samples were determined in duplicate on Plate Count Agar (Liofilchem s.r.l., Teramo, Italy) plates incubated at 30 °C for 72 h.

2.6. Shelf-life determination

The shelf-life of control and treated non-inoculated samples was estimated by periodically determining the total bacterial population and by sensory analysis of samples stored at 5 °C. From a microbial point of view, shelf-life was considered to end when the bacterial count exceeded 10^{7}cfu/g. The odor and visual aspect of salmon samples were assessed in duplicate by three trained panelists just after opening the bag, before microbial analysis.

3. Results and discussion

Before the treatments, the average pH of cold-smoked salmon used in the present work was 6.28, its a_w 0.965 and its salt content 3.85%. No significant changes were detected immediately after E-beam irradiation or high pressure treatment.

3.1. Food safety objective (FSO) and performance criteria (PC)

Risk assessment by USDA recommends a "zero tolerance" policy for *L. monocytogenes* in RTE meat products, i.e. a FSO of absence in 25 g. However, the ICMSF (2002) concluded that a FSO of 10^{2} cfu/g *L. monocytogenes* at the time of consumption would be valid for general purposes. The SCVPH (2005) of the European Union agreed with this FSO value.

Listeria present in the final smoked salmon come mainly from contamination during processing (Vogel et al., 2001). It may be assumed that contamination of cold-smoked salmon by *L. monocytogenes* along the manufacturing process is 10 cfu/g in the worst case. The average daily increases of our *L. monocytogenes* strain in control (untreated) salmon were 0.035 $\log_{10}$cfu/day at 5 °C and 0.094 $\log_{10}$cfu/day at 8 °C (unpublished data). Average daily increases ($\log_{10}$cfu/day) for other *L. monocytogenes* strains calculated from the results of various authors (FDA, 2003) would be 0.114 at 5 °C and 0.280 at 8 °C. On the basis of those data, more unfavorable than ours, *L. monocytogenes* counts in cold-smoked salmon would increase, in the absence of any treatment, from 1 $\log_{10}$cfu/g to 3.39, 4.19 and 4.99 $\log_{10}$cfu/g after 21, 28 and 35 days at 5 °C, respectively, and to 6.88, 8.84 and 10.80 $\log_{10}$cfu/g if stored at 8 °C. However, it is not to be expected that a temperature abuse at 8 °C occurs along the whole storage period but repeatedly at short periods of time, which could be estimated, in the worst case, to account for 50% of the total shelf-life. Under these simulated temperature abuse (5 °C + 8 °C) conditions, an average daily increase of 0.197 $\log_{10}$cfu/day might be estimated for *L. monocytogenes*. Presumptive counts after 21, 28 and 35 days under these conditions would be 5.14, 6.52 and 7.90 $\log_{10}$cfu/g.

The performance criterion (PC) is the effect on the frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to an FSO or adequate level of protection, as applicable (Gorris, 2005). Assuming the above mentioned contamination of 1 $\log_{10}$cfu/g immediately after processing, PC values of 1.39, 2.19 and 2.99 decimal reductions in *L. monocytogenes* numbers would suffice to attain an FSO of 2 $\log_{10}$cfu/g for shelf-lives of 21, 28 and 35 days at 5 °C, respectively (Table 1). Similarly, PC values of 3.14, 4.52 and 5.90 may be calculated for shelf-lives of 21, 28 and 35 days, respectively, under temperature abuse conditions (5 °C + 8 °C).

3.2. Process criteria for E-beam irradiation

The response of *L. monocytogenes* INIA H66a to irradiation (Fig. 1) fitted first-order inactivation kinetics, with the following regression equation : $y = 9.247 - 1.947x$ ($R^2 = 0.982$), where y represents the logarithm of the number of survivors and x the dose absorbed. From this equation, a D value of 0.51 kGy was calculated, slightly higher than the 0.46 kGy determined for the same strain in cooked ham (Cabeza et al., 2007). This difference may be considered as normal since it has been observed that the matrix influences bacterial radioresistance in foods (Augustin, 1996). Furthermore, it falls within the range reported for different *L. monocytogenes* strains in