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Effect of applying crust-freezing after skin-packaging on the natural microflora of Atlantic salmon (*Salmo salar*) during storage at low temperatures

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Abstract

The aim of the present study was to evaluate the effect of crust-freezing (CF) on fresh salmon fillets in skin-packaging during storage at −2.0°C. After CF, all treated samples and untreated controls were stored in a refrigerated cabinet for 20 d. Sampling was carried out at days 0, 2, 6, 8, 10, 14 and 20 in order to analyse total volatile basic nitrogen (TVB-N) and levels of mesophilic and psychrophilic viable counts (MVC and PVC). Enterobacteriaceae (ENT), lactic acid bacteria (LAB), H_2S-producing bacteria (SPB) and Pseudomonas spp. (PSE). No significant differences in TVB-N were found between samples except for those taken on day 20 where TVB-N levels of CF samples were lower than controls. Our results suggest that ENT might be the limiting microbial group to determine the end of shelf-life. Thus, if this group is used as an indicator of acceptability, the shelf-life of salmon can be extended from 8 to 20 d when skin-packed and then treated with CF.

Keywords

Crust-freezing • Enterobacteriaceae • salmon • shelf-life • skin-packaging • superchilling

Introduction

Atlantic salmon (*Salmo salar*) (Linnaeus, 1758) is one of the most economically important fish species due to its favourable organoleptic and nutritional properties. Within the European Union it is one of the most commonly consumed fish (1.97 kg/capita per yr) with a value of €5.4 billion in 2014 of which fresh salmon accounted for 69% of the total value (EUMOFA, 2015). Fresh fish is a highly perishable product and methods to improve preservation and extend shelf-life have become a priority for many research groups and the fish industry in order to reduce losses and feed increasing world populations. Spoilage can be defined as a series of microbial, enzymatic and chemical changes in fish, which cause sensory changes to a degree where it becomes unacceptable to the consumer. Microbial growth has been identified as the main cause of spoilage resulting in deterioration in sensory attributes (Leisner & Gram, 2014). Although a wide range of microorganisms are capable of growing on fresh fish, the methods of preservation are usually selected for particular groups of organisms during storage. These microbial groups are known as “specific spoilage organisms” (SSO) and are mainly responsible for off-odours and flavours making the fish organoleptically unacceptable (Gram & Huss, 1996).

To extend the shelf-life of fish, it is necessary to maintain the sensory characteristics of the product and ensure its safety. In this respect, one of the most important challenges is to maintain stable and sufficiently low temperatures throughout the whole processing and distribution chain (Kaale *et al*., 2011). Crust-freezing (CF), also known as “superchilling”, “deep-chilling”, “light-freezing” or “supercooling”, is a method for preserving food by allowing partial ice-crystallisation and subsequent low-temperature storage during distribution and retail sale (Kaale & Eikevik, 2014). Partial ice formation on the product surface can also absorb heat from the interior of the product, allowing more rapid chilling than with most traditional methods and also creating a “cold sink” or “reservoir” that can help to prevent temperature increases if the product is subjected to breaks in the cold chain.

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The CF process can be divided into two parts, an initial surface freezing (or CF itself) followed by storing the fish at temperatures just below the initial freezing point. Both stages are achieved at the product surface to a depth of 1–3 mm, depending on the degree of CF required, the thickness of the product and the CF rate applied (Kaale & Eikevik, 2014).

Vacuum skin packaging (VSP), also known as skin-pack, is a vacuum method consisting of a pre-made tray where the product is placed followed by the heating of the top film which makes it shrink tightly around the product, avoiding the formation of bubbles, wrinkles and the movement of the product within the tray. This method makes the product more visible to the consumer and reduces storage space needed for both packaging final product, and it has been increasingly used for meat and fish preservation (Li *et al*., 2012; Admin, 2016; Bellés *et al*., 2017). It has also been demonstrated that the use of VSP is particularly useful for extending the shelf-life of different fish species, maintaining the sensory, biochemical or microbiological quality of the product (Pérez-Alonso *et al*., 2004; Kumar & Ganguly, 2014).

The aim of the present study was to assess the effect of CF on the natural microflora of salmon after skin-packaging during storage at low temperatures. The growth of several microbial groups was used as indicators of shelf-life and compared to equivalent control samples.

Materials and methods

Raw material and sampling

Farmed Atlantic salmon (*S. salar*) from three different batches were acquired from a local fish processing company. The salmon were received gutted in polystyrene boxes in ice, approximately 48–72 h post-mortem, and were immediately cut into fillet portions of 150–200 g with skin using aseptic conditions. Only fillets with similar dimensions from the same location on the fish were selected for the study. Fillets were then individually packed in vacuum skin (Multivac R570 CD; MULTIVAC Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany) on pre-made trays (Cryovac® EGA 008, Sealed Air S.L., Abrera, Spain) of 200 µm thickness with an O_2 permeability rate (at 23°C) of 21 cm^3/m^2 per 24 h per 0% relative humidity (RH), and with a top film (Cryovac® VST 0250 skin top web; Sealed Air S.L.) of 100 µm thickness with an O_2 permeability rate (at 23°C) of 1.5 cm^3/m^2 per 24 h per 0% RH. The fillets were then stored at 5 ± 1°C for 4–5 h to ensure temperature homogeneity prior to treatment. The packed fillets in trays (experimental unit) were then randomly separated into two groups within each batch: control and crust-frozen. After CF, both groups were stored in a refrigerated cabinet (Zafrío S.L., Zaragoza, Spain) at −2°C for 20 d. Two trays per treatment (control–CF) and batch were taken to perform all analyses at different sampling points: 0, 2, 6, 8, 10, 14 and 20 d. Total volatile basic nitrogen (TVB-N) analysis was performed in triplicate and microbiological analysis in duplicate for each sampling point. The experimental design is summarised in Figure 1.

Freezing equipment and treatment conditions

Crust-freezing treatments were carried out in an air-freezer (ITA 100-SYEH/Tarre, S.A., Navarra, Spain) with a power of 1.0 kW, two evaporator fans, a capacity of 50 kg product/h at −25°C. The process parameters such as tunnel temperature (−20°C) and air velocity (3.3 m/s) were chosen after previous optimisation to set the most efficient equipment settings. Subsequently, the treatment time of skin-packed samples was selected after performing freezing curves (Figure 2). Two thermocouples were introduced inside packaged fillets with one on the surface (1–3 mm depth) and the other in the fillet core (geometric centre), to record temperature decrease over time. This allowed the time necessary to

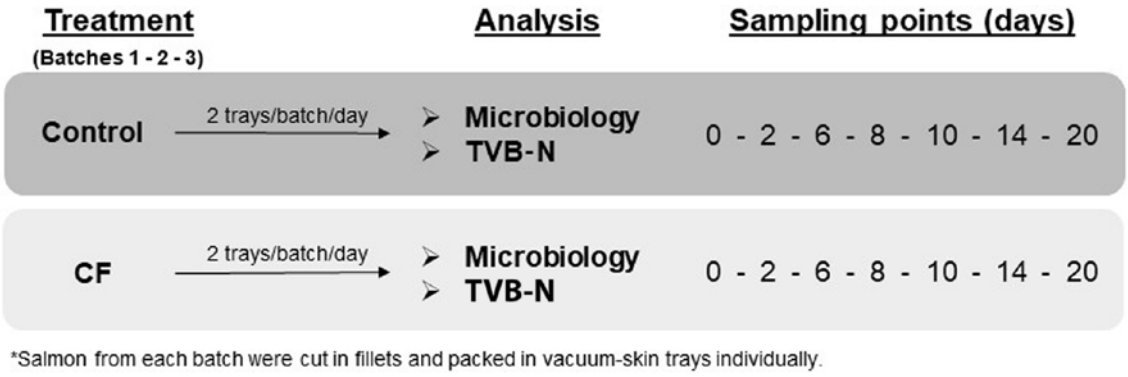

Figure 1. Summary of experimental design for evaluating the effect of crust-freezing (CF) on fresh salmon fillets packed under vacuum-skin conditions during storage at –2°C for 20 d.

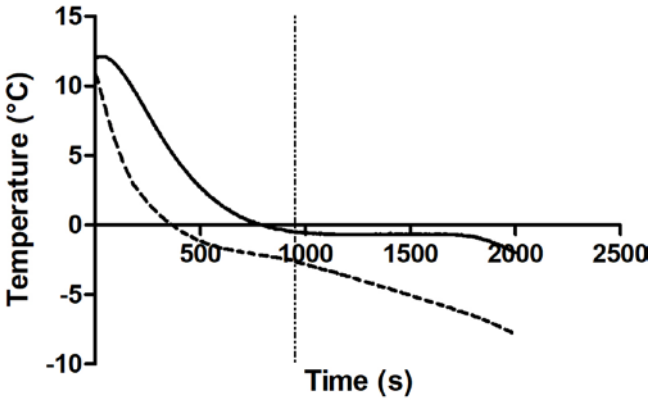

Figure 2. Freezing curves of skin-packed salmon fillets stored at –20°C and 3.3 m/s in a mechanical freezer. The solid black line represents the fillet core temperature and the dashed line corresponds to the surface temperature. The dotted vertical line represents the crust-freezing point (~900s). Data points are means of three replicates..

freeze the surface of the fillet while ensuring the centre remained unfrozen.

Microbiological analysis

Salmon samples (25 g) from each treatment and day were transferred aseptically to sterile stomacher bags (Seward Ltd., Worthing, West Sussex, UK) containing 225 mL of maximum recovery diluent (MRD; Oxoid Ltd, ThermoFisher Scientific Inc., Basingstoke, Hampshire, UK) and then homogenised in a masticator (Basic Masticator; IUL Instruments S.A., Barcelona, Spain) for 1 min. For microbial enumeration, 10-fold dilution series of fish homogenates were prepared and 0.1–1 mL aliquots were spread or poured on petri plates, depending on the culture media used. Total mesophilic and psychrophilic viable counts (MVC and PVC) were determined using molten plate count agar (PCA; Oxoid) containing 1% NaCl and were incubated for 48 h at 30°C and 7 d at 10°C, respectively. H_2S-producing bacteria (SPB) were enumerated by counting black colonies on Lyngby iron agar (Laboratorios Conda, S.A., Torrejon de Ardoz, Madrid, Spain) after 48 h at 25°C. Enterobacteriaceae (ENT) counts were carried out using violet red bile glucose agar (VRBGA; Oxoid) incubated for 48 h at 30°C (ISO, 2017). Lactic acid bacteria (LAB) were cultured under anaerobic conditions (AnaeroPack™, AnaeroGen™; Oxoid) in MRS (de Man, Rogosa and Sharpe) agar (Oxoid) for 3–4 d at 30°C, while *Pseudomonas* spp. (PSE) counts were performed on *Pseudomonas* CFC (Cetrimide, Fucidin, Cephalotin) selective agar (Oxoid) incubated at 25°C for 48 h.

TVB-N

TVB-N analysis was carried out following the method described in Chapter III of Commission Regulation (EC) No. 2074/2005 entitled “Determination of the concentration of TVB-N in fish and fishery products” (EU, 2005). The results were expressed as TVB-N (mg/100 g).

Statistical analysis

Initially, normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene’s test) were confirmed for the physico-chemical and microbiological data obtained. A preliminary test using a univariate and balanced general linear model was performed using a three-way analysis of variance (ANOVA; batch–treatment–storage time). As no significant differences were found between batches, two-way ANOVA (treatment–storage time, and their interaction) was carried out, using a post hoc Fisher’s (least significant difference [LSD]) test ($P < 0.05$). Pearson’s correlations to investigate the relationship between variables (microbial counts and TVB-N) were also evaluated. All statistical analyses were performed using XLSTAT-2018 software for excel (XLSTAT by Addinsoft, Addinsoft Inc., New York, NY, USA). Linear regression was performed using GraphPad PRISM® 5.0 software (GraphPad Software, Inc., San Diego, CA, USA).

Results

In the present study, some of the most relevant bacterial groups for fish spoilage were evaluated to observe the effect of CF treatments on the shelf-life of salmon. Preliminary statistical analysis found that there were no significant differences among the three different batches for any of the microbial groups or in the TVB-N. Therefore, the study focused on evaluating the effect of the CF treatment with respect to controls and over time. Table 1 shows the counts of SPB, PSE, PVC and MVC during the storage of CF-treated salmon and their corresponding controls after skin-packaging. Interestingly, levels of LAB were not represented as they were not detectable (<10 cfu/g) at any of the sampling points in either control or CF groups.

As shown in Table 1, SPB counts were detected from the second and sixth days of storage onwards for control and CF-treated samples, respectively. Furthermore, significant differences ($P < 0.05$) were found between treatments from day 10, where CF-treated samples contained lower counts than controls. After 20 d of storage, the SPB group reached 4.9 log cfu/g in control samples while in CF the counts were significantly lower at 4.2 log cfu/g ($P < 0.05$).

Table 2 shows the linear regression analysis for bacterial counts in control and CF-treated samples from day 8 to day 20. For SPB there were no significant differences ($P > 0.05$) between the slopes of the control and CF regression lines, which indicates that the treatment did not affect the microbial growth rate. However, differences in line elevations were

Table 1: Mean log cfu/g counts of H_2S-producing bacteria (SPB), *Pseudomonas* spp. (PSE), psychrophilic viable count (PVC) and mesophilic viable count (MVC) on skin-packed salmon treated with crust-freezing with their respective controls during storage at −2°C

Treatment	Bacterial group	Time (d)						
		0	2	6	8	10	14	20
Control	SPB	nd	0.9 ± 0.0^{a}	1.9 ± 0.0^{b}	2.7 ± 0.1^{c}	3.9 ± 0.0^{d1}	4.6 ± 0.1^{e1}	4.9 ± 0.0^{f1}
	PSE	nd	nd	3.2 ± 0.2^{a1}	4.1 ± 0.0^{b1}	4.5 ± 0.1^{c1}	4.8 ± 0.1^{d}	5.0 ± 0.1^{d1}
	PVC	2.1 ± 0.1^{a}	2.4 ± 0.1^{a}	3.5 ± 0.1^{b}	4.5 ± 0.1^{c1}	5.1 ± 0.2^{d1}	6.1 ± 0.1^{e1}	6.1 ± 0.1^{e1}
	MVC	2.3 ± 0.2^{a}	2.5 ± 0.1^{b}	3.4 ± 0.1^{c}	4.4 ± 0.2^{d1}	5.2 ± 0.2^{e1}	5.6 ± 0.3^{e1}	6.0 ± 0.3^{f1}
CF	SPB	nd	nd	1.6 ± 0.1^{a}	2.4 ± 0.1^{b}	3.1 ± 0.2^{c2}	4.0 ± 0.2^{d2}	4.2 ± 0.0^{d2}
	PSE	nd	nd	2.4 ± 0.1^{a2}	3.2 ± 0.1^{b2}	3.9 ± 0.2^{c2}	4.4 ± 0.2^{d}	4.5 ± 0.0^{d2}
	PVC	1.9 ± 0.0^{a}	2.1 ± 0.0^{a}	3.1 ± 0.1^{b}	3.8 ± 0.1^{c2}	4.5 ± 0.1^{d2}	5.1 ± 0.1^{e2}	5.6 ± 0.1^{f2}
	MVC	2.0 ± 0.0^{a}	2.4 ± 0.1^{b}	3.1 ± 0.1^{c}	3.7 ± 0.4^{d2}	4.6 ± 0.1^{e2}	4.8 ± 0.2^{ef2}	5.1 ± 0.3^{f2}

Values represent mean ± s.d.
^{a,b,c}Different superscript letters in the same row denote statistical differences between storage time points ($P < 0.05$).
^{1,2,3}Different superscript numbers in the same column denote statistical differences between treatments for each microbial group ($P < 0.05$).
CF = crust-freezing; nd = not detected, under the detection limit. SPB: <10 cfu/g, PSE: <100 cfu/g.

Table 2: Linear regression of H_2S-producing bacteria (SPB), *Pseudomonas* spp. (PSE), psychrophilic and mesophilic viable counts (PVC and MVC) and Enterobacteriaceae (ENT) counts in crust-frozen salmon stored at −2°C from day 8 to day 20 and controls

Microbial group	Treatment	Equation	R^2	RMSE
SPB	Control	log cfu/g = 0.200 *t* + 1.583	0.80	0.37
	CF	log cfu/g = 0.111 *t* + 1.800	0.68	0.33
PSE	Control	log cfu/g = 0.064 *t* + 3.756	0.76	0.17
	CF	log cfu/g = 0.098 *t* + 2.749	0.68	0.31
PVC	Control	log cfu/g = 0.138 *t* + 3.652	0.72	0.39
	CF	log cfu/g = 0.142 *t* + 2.894	0.88	0.25
MVC	Control	log cfu/g = 0.161 *t* + 3.301	0.84	0.26
	CF	log cfu/g = 0.124 *t* + 2.983	0.70	0.34
ENT	Control	log cfu/g = 0.102 *t* + 3.439	0.61	0.37
	CF	log cfu/g = 0.105 *t* + 1.956	0.93	0.13

CF = crust-freezing; R^2 = coefficient of determination; RMSE = root mean square error; *t* = time (d).

highly significant between groups ($P < 0.001$), indicating that CF treatments would extend the lag phase and extend the time to reach the maximum values for salmon.

PSE were not detected (<100 cfu/g) until after 6 d of storage, and counts were significantly different ($P < 0.05$) between control and CF samples for the remaining days in storage (Table 1). The initial low count of PSE within the first 6 d would be expected as the CF treatment and storage conditions likely caused an extension of the lag phase. Statistical analysis (Table 2) for PSE showed similar growth rates, but for CF samples the exponential phase began later than for controls. Counts of PSE by day 20 were found to be 5.0 and 4.5 log cfu/g on control and CF samples, respectively, which can be considered as acceptable from a quality perspective, since the levels of some SSO such as PSE at the end of the shelf-life can be around 8 log cfu/g (Boziaris & Parlapani, 2017).

Total PVC results are presented in Table 1 and showed low initial levels for control (2.1 log cfu/g) and CF (1.9 log cfu/g) samples. After 8 d of storage and beyond, the CF-treated salmon samples contained significantly lower PVC levels than corresponding controls ($P < 0.05$), and even after 20 d of storage levels in both groups remained relatively low. Their growth during storage was also significantly different ($P < 0.05$) between CF-treated and controls from day 6 onwards. This could again indicate a short extension on the lag phase for PVC growth during the first days of storage under these conditions.

Mesophilic viable counts during storage (Table 1) showed significant differences between treatments from day 8, and after 20 d, while CF samples had approximately a 1-log/cfu lower count than corresponding controls ($P < 0.05$). By using equations in Table 2, it is possible to extrapolate the time that the stored salmon fillets would exceed MVC recommended levels of 6–7 log cfu/g. For CF-treated samples, the estimated end of shelf-life would occur between 24 and 32 d, with controls having a predicted shelf-life of 17–23 d, corresponding to an additional 7–9 d of shelf-life when CF is applied.

Enterobacteriaceae counts are presented in Figure 3. Counts were lower in CF-treated samples than in their respective controls. From day 8 onwards, highly significant differences

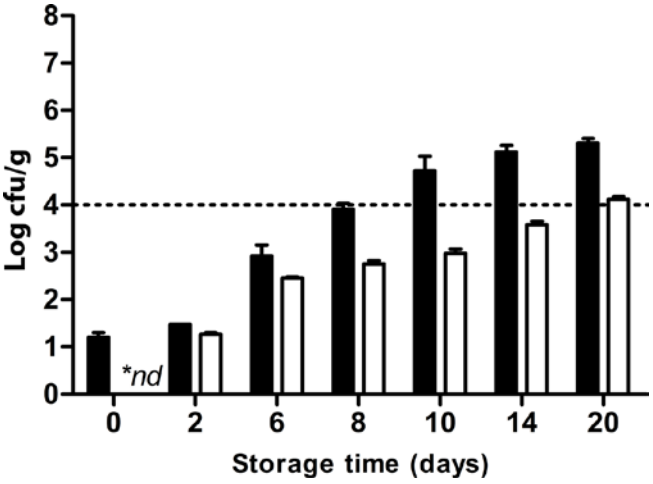

Figure 3. Mean log cfu/g of Enterobacteriaceae (ENT) in salmon after crust-freezing treatments (white) with their respective controls (black) over 20 d of storage in skin-packaging at −2°C. The horizontal dotted line represents the recommended limit of the shelf-life. *nd: not detected, under the detection limit (<10 cfu/g). Each bar represents mean ± s.e.

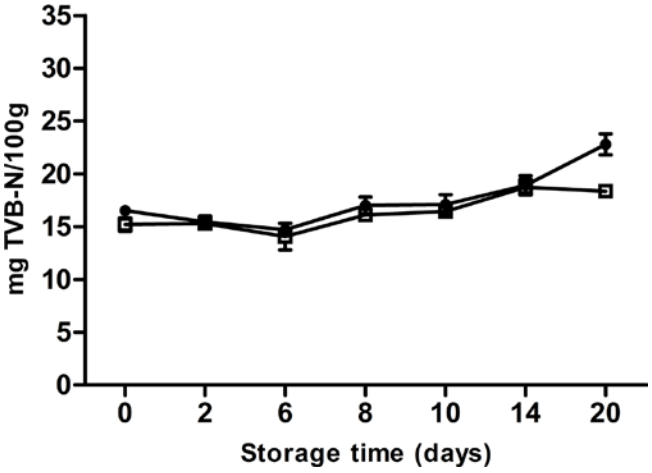

Figure 4. Total volatile basic nitrogen (TVB-N) mean values of salmon skin-packed after crust-freezing treatments (□) with their respective controls (●) over 20 d of storage at −2°C. Each point represents mean ± s.e.

(P < 0.001) were observed between control and CF-treated samples, and these differences were higher than for the other microbial groups investigated (1.2–1.8 log cfu/g). At day 0, ENT were not detected in any of the CF samples (<10 cfu/g), while initial counts in control samples were 1.2 ± 0.1 log cfu/g. This could indicate a simple variability between samples or an actual effect of CF on the initial load of ENT. Again, the growth rates (Table 2) of the estimated control and CF lines showed no significant differences between groups, but the difference between elevations was considered extremely significant (P < 0.001). After 20 d, the ENT counts for control samples were 5.3 log cfu/g while for CF-treated samples the levels were 1.2 log cfu/g lower (4.1 log cfu/g). This could indicate a delayed growth in CF-treated samples when compared to controls, reinforcing the assumption of a lag phase extension, or perhaps a reduction in initial numbers of viable organisms caused by CF.

The results for TVB-N are presented in Figure 4. At day 0 and up to day 20, the TVB-N values increased from 16.5 to 22.8 mg/100 g for control and from 15.2 to 18.3 mg/100 g in CF samples. Some differences were found between storage days (P < 0.05) within each group. However, no significant differences were observed between treatments (control and CF) except for samples analysed on day 20 where the CF values were lower than corresponding controls.

Two-way ANOVA also showed significant interactions (P < 0.05) between treatment applied and storage time for TVB-N. This was also detected for all microbial counts and differences were highly significant (P < 0.001). ENT group was the most sensitive group to this interaction and highly significant (P < 0.000) differences were observed between treatments from day 6 onwards, where control sample mean values exceeded 1 log cfu/g difference for each sampling point.

Finally, Pearson's coefficients demonstrated that all bacterial counts were correlated significantly with storage time (r^2 = 0.91 average). Similarly, TVB-N showed a good correlation with storage time (r^2 = 0.74) and with bacterial counts, for example, MVC (r^2 = 0.71), PVC (0.70) and SPB (r^2 = 0.68). A high and significant correlation was also observed among the different microbial groups (r^2 > 0.92), with the highest recorded among MVC, SPB and PVC (r^2 = 0.98).

Discussion

Many parameters can be used to define fish quality, but microbial counts are one of the most relevant and have been demonstrated to correlate with sensory acceptability and the end of shelf-life (Ólafsdóttir *et al*., 1997). Although no compulsory limits have been established for microbial counts of spoilage organisms on fresh fish to determine shelf-life, the literature contains a range of parameters that can be used. In general, limits have been based on guideline specifications (ICMSF, 1986), SSO levels (Gram & Huss, 1996), sensory analysis (Sveinsdottir *et al*., 2003) or on several institutional recommendations (Centre for Food Safety, 2014; FSAI, 2014). The predominant microflora of fish can depend on many factors, including the origin of the fish and the preservation methods employed. The most commonly identified SSO in fish and fish products are *Photobacterium phosphoreum*,

LAB, PSE and the genus *Shewanella*, which have been widely reported as the most relevant SPB (Vogel *et al*., 2005; Serio *et al*., 2014).

Lactic acid bacteria have been reported as the dominant flora on fish stored under anaerobic or vacuum package conditions (Gram & Dalgaard, 2002). However, the low levels of LAB observed in the current study may have resulted from the low initial counts combined with the low-storage temperature used, which is in agreement with Hansen *et al*. (2009) who reported low levels of LAB after 2-wk storage at 0.4 ± 0.15°C for salmon in air and modified atmosphere packaging (MAP).

Regarding SPB, Duun & Rustad (2008) considered that levels between 3 and 5.7 log cfu/g indicated "good" quality for salmon. Following this criterion, and due to the fact that those levels were never exceeded in this study, it can be concluded that the SPB group was not the main cause of spoilage of our product after 20 d.

Under aerobic conditions, PSE and *Shewanella* spp. are frequently reported to be the dominant spoilage organisms on fish irrespective of the origin (Gram & Huss, 1996). However, it has been reported that CO_2 packaging and the removal of oxygen, similar to conditions found in skin-vacuum packaging, inhibit the growth of these bacteria, particularly PSE. Nevertheless, they can still be found in salmon fillets due to the presence of low concentrations of oxygen after vacuum packaging which explains the presence and growth of these organisms in our samples albeit at low levels (Hansen *et al*., 2009; Macé *et al*., 2012).

Macé *et al*. (2012) reported that *P. phosphoreum* represented about half of the PVC group in salmon stored under MAP and vacuum-packaged conditions. This fact could explain the low growth rate of this group in the present study. It has been reported that *P. phosphoreum* is sensitive to sudden cooling or freezing, causing cold shock and slower initial growth or an extension of the lag phase (Olafsdottir *et al*., 2006; Hansen *et al*., 2009).

With regard to ENT, there is no maximum limit for fish intended for human consumption, but levels no higher than 10^3–10^4 cfu/g are recommended. Levels exceeding 10^4 cfu/g have been considered as "unsatisfactory" (FSAI, 2014). According to our results, these levels were exceeded after 20 d of storage for CF samples and just 8 d for control samples.

ENT and MVC are considered important hygiene indicator organisms in food and low levels of both provide some assurances with regard to food safety. However, as it has been widely reported, a high level of MVC does not necessarily mean a high spoilage potential and it would be necessary to investigate the bacterial flora to ascertain the prevalence and levels of important spoilage bacteria (Erikson *et al*., 2011). Consequently, in the present study, although several microbial groups were assessed, including the most relevant spoilage bacteria (SPB, PSE, LAB), the limiting factors considered were the MVC and ENT groups. As mentioned above, none of the most relevant SSO (SPB, PSE, LAB) assessed grew sufficiently during storage to be considered significant causes of spoilage under the conditioned used. Macé *et al*. (2012) reported that SSO have to be present at concentrations of at least 7 log cfu/g to produce sufficient quantities of metabolites associated with spoilage, and these levels were never reached in the current study.

Several authors have developed microbial growth models, alone or in combination with other parameters, in order to predict the shelf-life of salmon and found a strong relationship between total viable counts and sensory analysis (quality index method [QIM]) than with other SSO (Sveinsdottir *et al*., 2002; Churchill *et al*., 2016). Similar conclusions have also been reported previously for other fish species (Hernández *et al*., 2009; Özyurt *et al*., 2009). The predominant flora on fish can be diverse and can evolve in many ways contributing to spoilage (Gram *et al*., 2002). For instance, under vacuum or MA packaging conditions, the dominant microflora is often composed of LAB and Gram-negative fermentative bacteria, such as psychrotrophic ENT and *Shewanella* spp. (Gram & Huss, 1996). Lactic acid bacteria were not detected throughout the study; therefore, it could hardly be defined as the predominant flora. However, possibly due to the low temperatures of storage and anaerobic conditions used in our study, mesophilic and psychrotrophic ENT could have become one of the dominant groups due to the inhibition of other competing bacterial species. So, although initial levels of ENT were low in samples, their presence increased substantially and may have become the microbial group to define the end of shelf-life of salmon after CF treatments instead of MVC. The maximum recommended levels of MVC (6–7 log cfu/g) were not reached after 20 d of storage. To the best of our knowledge, this is the first time that ENT have been identified as the microbial group limiting the shelf-life of skin-packed salmon subjected to CF. According to our results, the combination of CF and skin-packaging would extend the salmon shelf-life by up to 12 d when ENT levels (>10^4 cfu/g) are used as a limit of acceptability.

Considering TVB-N, according to the current European legislation as defined in Regulation (EC) No. 2074/2005 (EU, 2005), the established limit for human consumption of Atlantic salmon is 35 mg/100 g, and our results demonstrated that levels in samples within all treatment groups were significantly lower than the maximum legal criterion.

In conclusion, our results suggest that ENT are the limiting group for determining product shelf-life when treatment and storage conditions used in this study are considered. CF treatment extended the shelf-life of skin-packed salmon by up to 12 d when ENT were used as an indicator of acceptability.

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References

Admin. 2016. "New Vacuum Skin Packaging Format from PA Helps Fish Producer Reduce Costs". Food & Drink Business Europe. Available online: https://www.fdbusiness.com/new-vacuum-skin-packaging-format-from-pa-helps-fish-producer-reduce-costs/.

Bellés, M., Alonso, V., Roncalés, P. and Beltrán, J.A. 2017. The combined effects of superchilling and packaging on the shelf-life of lamb. *Meat Science* **133**: 126–132.

Boziaris, I.S. and Parlapani, F.F. 2017. Specific spoilage organisms (SSOs) in fish. In: "The Microbiological Quality of Food – Foodborne Spoilers" (eds. A. Bevilaqua, M.R. Corbo and M. Sinigaglia), Woodhead Publishing, Elsevier, pages 61–91.

Centre for Food Safety. 2014. "Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items)". Food and Environmental Hygiene Department, Hong Kong. Available online: https://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf.

Churchill, O.J., Fernandez-Piquer, J., Powell, S.M. and Tamplin, M.L. 2016. Microbial and sensorial models for head-on and gutted (HOG) Atlantic salmon (*Salmo salar*) stored from 0 to 15 °C. *Food Microbiology* **57**: 144–150.

Duun, A.S. and Rustad, T. 2008. Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at −1.4 and −3.6. *Food Chemistry* **106**: 122–131.

Erikson, U., Misimi, E. and Gallart-Jornet, L. 2011. Superchilling of rested Atlantic salmon: different chilling strategies and effects on fish and fillet quality. *Food Chemistry* **127**: 1427–1437.

European Market Observatory for Fisheries and Aquaculture Products (EUMOFA). 2015. The EU fish market 2015 edition. Directorate-General for Maritime Affairs and Fisheries of the European Commission (Ed). European Union.

European Union (EU). 2005. Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004.

Food Safety Authority of Ireland (FSAI). 2014. Guidance note No.3 Guidelines for the interpretation for results of microbiological testing of ready-to-eat foods placed on the market (Revision I). Dublin-Ireland.

Gram, L. and Dalgaard, P. 2002. Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology* **13**: 262–266.

Gram, L. and Huss, H.H. 1996. Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology* **33**: 121–137.

Gram, L., Ravn, I., Rasch, M., Bruhn, J.B., Christensen, A.B. and Givskov, M. 2002. Food spoilage – interactions between food spoilage bacteria. *International Journal of Food Microbiology* **78**: 79–97.

Hansen, A.Å., Mørkøre, T., Rudi, K., Langsrud, Ø. and Eie, T. 2009. The combined effect of superchilling and modified atmosphere packaging using CO_2 emitter on quality during chilled storage of pre-rigor salmon fillets (*Salmo salar*). *Journal of the Science of Food and Agriculture* **89**: 1625–1633.

Hernández, M.D., López, M.B., Álvarez, A., Ferrandini, E., García-García, B. and Garrido, M.D. 2009. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. *Food Chemistry* **114**: 237–245.

International Commission on Microbiological Specifications for Foods (ICMSF). 1986. Sampling plans for fish and shellfish. In: "Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications" (ICMSF), University Toronto Press, Toronto, pages: 181–196.

International Organization for Standardization (ISO). 2017. *Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobateriaceae – Part 2: Colony-count technique* (ISO Standard No. 21528-2:2017).

Kaale, L.D. and Eikevik, T.M. 2014. The development of ice crystals in food products during the superchilling process and following storage, a review. *Trends in Food Science &Technology* **39**: 91–103.

Kaale, L.D., Eikevik, T.M., Rustad, T. and Kolsaker, K. 2011. Superchilling of food: a review. *Journal of Food Engineering* **107**: 141–146.

Kumar, P. and Ganguly, S. 2014. Role of vacuum packaging in increasing shelf-life in fish processing technology. *Asian Journal of Bio Science* **9**: 109–112.

Leisner, J.J. and Gram, L. 2014. Spoilage of fish. In: "Encyclopedia of Food Microbiology" (eds. C.A. Batt and M.-L. Tortorello), Academic Press, Elsevier Ltd., London, UK, pages 932–937.

Li, X., Lindahl, G., Zamaratskaia, G. and Lundström, K. 2012. Influence of vacuum skin packaging on color stability of beef longissimus lumborum compared with vacuum and high-oxygen modified atmosphere packaging. *Meat Science* **92**: 604–609.

Macé, S., Cornet, J., Chevalier, F., Cardinal, M., Pilet, M.F., Dousset, X. and Joffraud, J.J. 2012. Characterisation of the spoilage microbiota in raw salmon (*Salmo salar*) steaks stored under vacuum or

modified atmosphere packaging combining conventional methods and PCR-TTGE. *Food Microbiology* **30**: 164–172.

Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J. and Nilsen, H. 1997. Methods to evaluate fish freshness in research and industry. *Trends in Food Science & Technology* **8**: 258–265.

Olafsdottir, G., Lauzon, H.L., Martinsdóttir, E., Oehlenschläger, J. and Kristbergsson, K. 2006. Evaluation of shelf life of superchilled cod (*Gadus morhua*) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. *Journal of Food Science* **71**: S97–S109.

Özyurt, G., Kuley, E., Özkütük, S. and Özogul, F. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry* **114**: 505–510.

Pérez-Alonso, F., Aubourg, S.P., Rodríguez, Ó. and Barros-Velázquez, J. 2004. Shelf-life extension of Atlantic pomfret (*Brama brama*) fillets by packaging under vacuum-skin system. *European Food Research and Technology* **218**: 313–317.

Serio, A., Fusella, G.C., López, C.C., Sacchetti, G. and Paparella, A. 2014. A survey on bacteria isolated as hydrogen sulfide-producers from marine fish. *Food Control* **39**: 111–118.

Sveinsdottir, K., Martinsdottir, E., Hyldig, G., Jørgensen, B. and Kristbergsson, K. 2002. Application of quality index method (QIM) scheme in shelf-life study of farmed Atlantic salmon (*Salmo salar*). *Journal of Food Science* **67**: 1570–1579.

Sveinsdottir, K., Hyldig, G., Martinsdottir, E., Jørgensen, B. and Kristbergsson, K. 2003. Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (*Salmo salar*). *Food Quality and Preference* **14**: 237–245.

Vogel, B.F., Venkateswaran, K., Satomi, M. and Gram, L. 2005. Identification of *Shewanella baltica* as the most important H_2S-producing species during iced storage of Danish marine fish. *Applied and Environmental Microbiology* **71**: 6689–6697.