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Effects of High Pressure, Subzero Temperature, and pH on Survival of *Listeria monocytogenes* in Buffer and Smoked Salmon

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ABSTRACT

High pressure processing is a novel food preservation technology, applied for over 15 years in the food industry to inactivate spoilage and pathogenic microorganisms. Many studies have shown the differential resistance of bacterial cells to high pressure. *Listeria monocytogenes* is a bacterium able to grow at refrigerated temperature and to survive for a long time in minimally processed foods such as raw smoked fish. The freezing process does not cause significant decline of *L. monocytogenes.* The phase diagram of water under pressure permits a pressure treatment under subzero temperature, without the disadvantages of freezing for food quality. The aim of this study was to estimate if combined effects of pressure and subzero temperature could increase the destruction of *L. monocytogenes* in buffer and in smoked salmon. We investigated effects of high pressure processing (100, 150, and 200 MPa) combined with subzero temperatures (−10, −14, and −18°C) and pH (7.0 and 4.5). Results showed that the most effective high-pressure treatment to inactivate *L. monocytogenes* was 200 MPa, −18°C, and pH 4.5. The relevance of pressure holding time and the synergistic effect of pressure coupled with the subzero temperature to inactivate bacteria are highlighted. Modifications of physical properties (color and texture) were a lightening of color and an increase of toughness, which might be accepted by consumers, since safety is increased.

While world production of Atlantic salmon has increased, problems related to quality still persist. The temperature in the cold-smoking process of salmon never exceeds 28 to 32°C, which limits inactivation of enzymes in the tissue and does not inactivate microorganisms *(9). Listeria monocytogenes* is a major problem for the smoked salmon industry. There is no guarantee of the prevention of *L. monocytogenes* in cold-smoked salmon and, due to the lack of any bactericidal step in the current smoked process *(8),* an alternative process is required to eliminate this foodborne pathogen.

Research on high pressure processing of foods has received attention due to demand for high quality of food products. High pressure processing (HPP) can inactivate microorganisms at room temperature or in combination with heat or cold treatment while retaining the nutritional and the organoleptic properties of foods *(15).* An advantage of HPP over heat treatments is that the pressurization time is independent of the sample volume because the pressure acts instantly and uniformly on the food. In addition, keeping the sample under pressure for an extended period of time does not require any additional energy *(18).* HPP is a very promising technology for the preservation and development of many food products, and it uses relatively low amounts of energy. HPP treatments can induce distinct effects on the texture and structure of a given food and therefore, can be used for the development of new products or to increase the functionality, nonthermal coagulation of proteins and selective enzymatic inactivation. However, beyond some levels of pressure (>200 MPa) irreversible modifications of some proteins may cause color changes, leading to a cooked product appearance *(3, 6, 10).*

Many works since Hite's results *(11)* have studied the effect of high pressure on the microorganisms. Results show that pressure sensitivity of bacteria depends on the applied pressure level. The main effect of pressure is at the cell membrane *(23).* Furthermore, pressurization induces changes in morphology and biochemical reactions *(24).* Microorganisms differ in their sensitivity to pressure treatment, depending on their biological structure *(12, 13).* Though the destruction of microorganisms is often more efficient in buffer systems, many studies have shown the reduction of pathogenic bacteria by high-pressure treatments in food systems *(19).*

L. monocytogenes is widely recognized as a foodborne pathogen. A linear model was previously established for inactivation of *L. monocytogenes* with four main parameters

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of high pressure, pH, time, and temperature, and interactions between these variables *(23).* The effect of pressure on bacterial inactivation has been well documented *(2, 13, 25).* Inactivation of *L. monocytogenes* is dependent on pressure level *(28).* The interaction between holding time and pressure was previously demonstrated an equivalent inactivation with different combinations as 400 MPa at 20 min and 600 MPa at 10 min pressurized at 20°C *(23).* The treatment under 200 MPa at 20°C did not show significant *Listeria* reduction, even with a holding time of 20 min.

L. monocytogenes is a bacterium able to grow at refrigerated temperatures and to survive in adverse environments, including those conditions that occur in minimally processed food such as raw smoked salmon *(16, 20).* Even if freezing does not induce a marked inactivation of *L. monocytogenes (20),* application of HPP at subzero temperature might provide effective pathogen destruction and also improve quality. The phase diagram of water under pressure permits application of a pressure treatment at subzero temperatures, without the quality disadvantages of freezing *(14).* As functions of pressure and temperature, the phase state of freezing water changes and leads to the formation of different states of ice crystals (ice I, II, V, or VI). For example, between 100 and 200 MPa, water is still under liquid state at −10 and −20°C, respectively, and consequently the treated product is not frozen. The purpose of this study was to investigate the combined effects of treatments at 100 or 200 MPa with a long holding time (180 min), at pH 7.0 and 4.5, and subzero temperatures from −7 to −18°C. The pressure and temperature used did not induce ice nucleation, and were applied to *L. monocytogenes* in buffer and smoked salmon. Color and texture modification of the smoked salmon were also evaluated.

MATERIALS AND METHODS

Bacterial strain and cultural conditions. *L. monocytogenes* Scott A was obtained from Collection de l'Institut Pasteur (Paris, France) and stored at −30°C in cryobeads (AES, Combourg, France). The initial bacterial culture was obtained by inoculating a cryobead into brain heart infusion (Biokar, Beauvais, France) incubated at 37°C for 24 h. This culture was used to prepare a subculture by inoculating 1:1,000 (vol/vol) into fresh brain heart infusion for incubating 18 h at 37°C.

Preparation of the fish fillets. Fillets (1,100 g) of smoked salmon (*Salmo salar*) were collected from a local curing manufacturer (Wilmar, Nantes, France). Fillets were cut into sections of 250 g for color and texture analyses and 25 g for microbial enumeration.

Preparation of pressurized samples. Two different buffers were used, a phosphate buffer (pH 7.0) composed of 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 (Merck, Darmstadt, Germany) and citrate buffer (pH 4.5) composed of 0.2 M Na_2HPO_4 and 0.1 M citrate (Merck). The pressurized samples were composed of bacterial culture diluted 1/10 (vol/vol) with these two buffers to give approximately 10^8 CFU/ml. Bacterial suspensions were placed in a sterile polyethylene thermosealed bag (AES). One part of these samples was directly pressurized, and another part of samples was added to the cut fillets (wt/wt) before pressurization.

Enumeration of viable *L. monocytogenes.* The number of cells was determined before and after treatments, and the efficiency of treatment was calculated by the difference between these two counts in log CFU per milliliter. *L. monocytogenes* were enumerated by plating a 0.1-ml volume on plate count agar in duplicate (Biokar) in the case of buffer samples and on Oxford agar (Merck) for food samples, which were then incubated for 48 h at 37°C. In the case of small expected numbers of survival cells, 10 ml of cell buffer suspension was divided between two plates. Each plate received 5 ml of sample mixed with molten plate count agar. Thus, the threshold was 0.1 CFU/ml. The absence of *Listeria* on smoked salmon before the inoculation has been checked by the same method.

HPP. A 3.5-liter reactor unit (ACB Pressure Systems, Nantes, France) equipped with temperature and pressure regulator devices was used to achieve HPP. Aluminum cylinders were used to reduce the volume of the pressure vessel (i.e., reduce time of pressurization) and avoid heating of sample during pressurization. The temperature of the cooling jacket that surrounded the pressure vessel was controlled at a corresponding temperature during pressure treatment. One K-type thermocouple (0.3-mm diameter; Omega, Stamford, Conn.) positioned close to the sample provided temperature monitoring during treatment, and controlled the unfreezing of the sample during processing. Variation of temperature due to adiabatic heating effect during pressure increase was recorded by a data logger (model SA32, AOIP, Evry, France) every 5 s (Fig. 1). For all treatments, kinetic parameters were 3 MPa/s speed for pressurization and less than 1 s for depressurization. Samples stored at 4°C for the entire duration of the experiment were considered as standards for the bacterial reduction assessment.

Pressure treatments combined with subzero temperatures. Three pressure levels were used: 100, 150, and 200 MPa. The pressure was applied on samples for 180 min. The setting under pressure during this time (Fig. 1) allowed temperature variations to avoid pressure shift freezing. The chosen temperature of each pressure treatment was determined from the diagram of Kalichevsky *(14),* so that temperature was above the change of state for water. For treatments at 100, 150, and 200 MPa, the selected temperatures were, respectively, −7, −14, and −18°C. After 100 min under pressure, the temperature of the cooling jacket reached 20°C, to bring the product to 4°C so that depressurization would be obtained without ice nucleation. After pressure treatment, samples were stored at 4°C (maximum of 2 h) until analysis.

Treatment at subzero temperature without pressure. *L. monocytogenes* cultures in the two different buffers were frozen in an air-blast freezer (Servathin, Poissy, France). Three air temperatures, −7, −14, and −18°C, were used in the air-blast freezer with an air speed of 4 $m \cdot s^{-1}$ for 180 min. After freezing, the *L. monocytogenes* culture was thawed at ambient temperature (20°C) within 15 min, and then immediately analyzed to estimate separately the effect of freezing on its own.

Color measurements. Color of smoked salmon fillets was measured after pressure processing and without buffer using a Minolta CM-3500d colorimeter (Minolta, Carrières-sur-Seine, France). Measurements were achieved in the referential CIE 1976 (L^*, a^*, b^*) color space. L^* denotes lightness on a 0 to 100 scale from black to white; a^* corresponds to the indication of red when its value is positive and to green when it is negative; b^* corresponds to the indication of yellow when its value is positive and

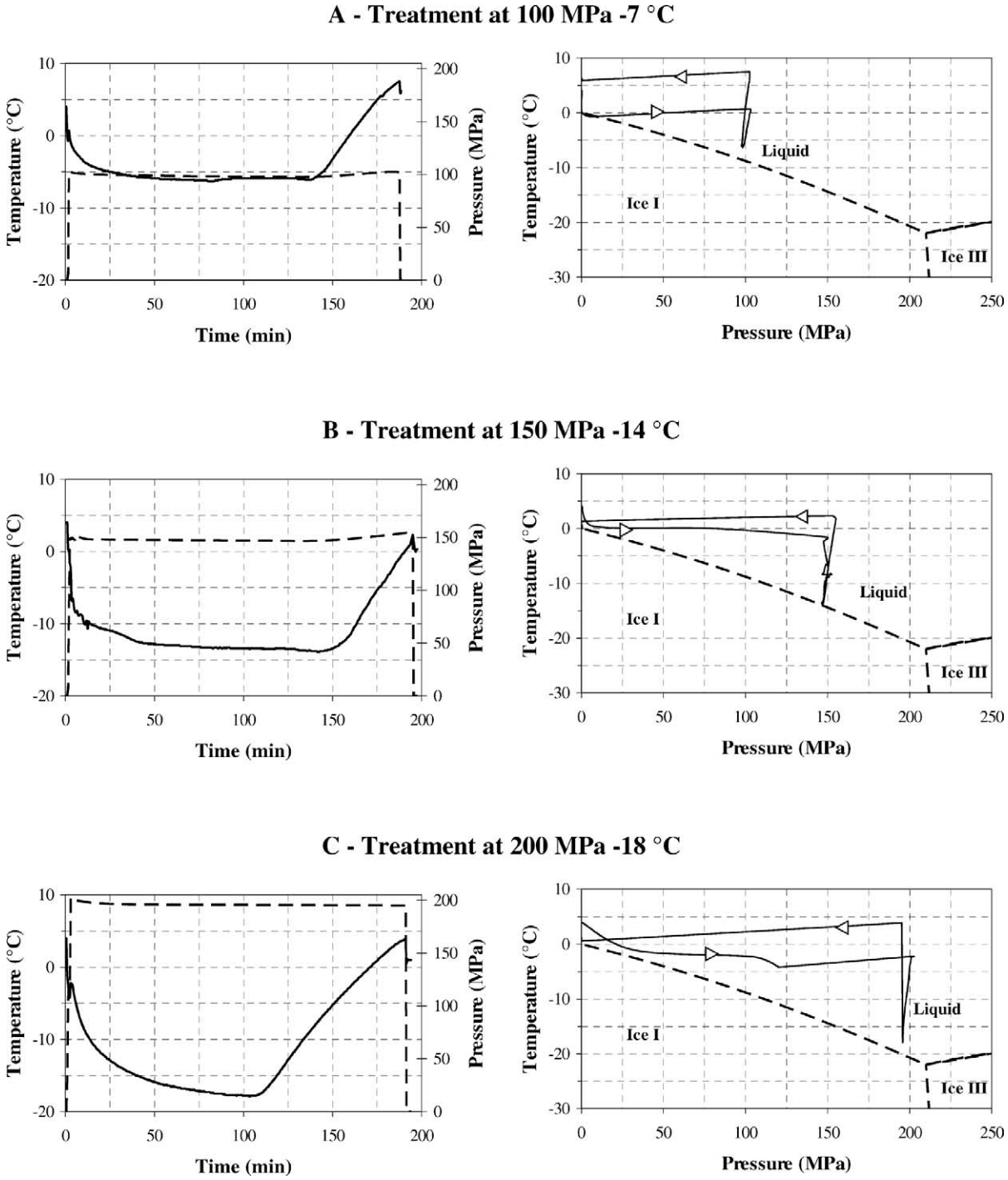

FIGURE 1. *On the left, change of pressure (dotted line) and temperature (dark line) according to time of HPP. On the right, temperature versus pressure (dark line), represented on Kalichevsky et al. (14) water phase diagram (dotted line). (A) Treatment at 100 MPa, −7°C for 180 min, with heating (7°C) before pressure release. (B) Treatment at 150 MPa, −14°C for 180 min, with heating (5°C) before pressure release. (C) Treatment at 200 MPa, −18°C for 180 min, with heating (5°C) before pressure release.*

to blue when it is negative; and ΔE corresponds to the total color difference and is calculated using L^*, a^*, and b^*:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

The control was untreated smoked salmon. Measurements were repeated 10 times on different positions of four fish fillets.

Texture profile analysis. Texture measurements, according to texture profile analysis defined by Bourne *(4),* were performed at room temperature with a texture testing instrument (LR5K, Lloyd Instruments, Fareham, Hants, UK) equipped with a sensor of 50 N. Ten cylindrical samples of 20-mm diameter for each treatment parameters were cut in the same way on the longitudinal orientation of the muscular fibers of salmon. Prior to analysis, samples were allowed to reach room temperature (20°C, 1 h). Each sample was compressed between stainless steel plates (diameter of 40 mm). The texture measurement was composed of two consecutive compressions of 25% in parallel way of muscle fiber orientation, at a constant speed of 1 mm/s, with delay of 30 s between the two compressions.

TABLE 1. *Inactivation of* L. monocytogenes *in buffer at various pressure, temperature, and pH conditions*

	Reduction $\log(N/N_0)$[a]		
	Storage (°C):		
pH	−7	−14	−18
7.0 (phosphate buffer)	−0.03 ± 0.07 A a[b]	0.33 ± 0.03 A b	0.19 ± 0.05 AB c
4.5 (citrate buffer)	−0.09 ± 0.07 AB a	0.05 ± 0.02 D b	−0.07 ± 0.08 A ab
	High pressure at 4°C (MPa):		
pH	100	150	200
7.0 (phosphate buffer)	0.23 ± 0.08 B a	0.77 ± 0.09 B b	0.59 ± 0.05 B c
4.5 (citrate buffer)	−0.76 ± 0.09 C a	−4.00 ± 0.10 E b	−5.55 ± 0.00 D c
	High pressure at subzero temp (MPa/°C):		
pH	100/−7	150/−14	200/−18
7.0 (phosphate buffer)	0.20 ± 0.13 AB a	−2.07 ± 0.19 C b	−4.18 ± 0.45 C c
4.5 (citrate buffer)	−2.57 ± 0.17 D a	−6.37 ± 0.43 F b	−8.91 ± 0.00 E c[c]

[a] The chosen temperatures were greater than the limit for freezing under pressure (see Fig. 2). All values are means ± standard deviations of three values. N_0 reference measured after storage of *L. monocytogenes* at 4°C in buffer pH 7.0 or 4.5.

[b] Same capital letters in the same column indicate no significant differences ($P < 0.05$). Same lowercase letters in a row indicate no significant differences ($P < 0.05$).

[c] Total reduction of *L. monocytogenes.*

The following parameters were determined from the resulting force–time curve: (i) hardness, corresponding to the maximum force required to compress the sample; (ii) cohesiveness, extent to which the sample could be deformed prior to rupture [$(A_3 + A_4)/(A_1 + A_2)$, where $A_1 + A_2$ is the total energy required for the first compression, and $A_3 + A_4$ is the total energy required for the second compression]; (iii) springiness, ability of sample to recover its original form after the deforming force is removed (L_2/L_1, were L_1 is the lengthening of the first compression, and L_2 is the lengthening of the second compression); (iv) gumminess was the force needed to disintegrate a semisolid sample to a steady state of swallowing (hardness × cohesiveness); and (v) chewiness, the work needed to chew a solid sample to a steady state of swallowing (springiness × gumminess).

Statistical analysis. All experiments were performed in triplicate. The data were recorded as the means of experiments calculated from 3 replicate measurements for microbial reduction and 10 replicated for texture and color parameters. Data was analyzed using a one-way analysis of variance according to the general linear model procedure with least-square means effects to determine significant differences between treatments. Duncan's multiple range test was applied to determine which means were significantly different according to Fisher's least significant difference test. Significant differences of inactivation were determined with 5% level of significance ($P < 0.05$) by the Student's t test. The analysis was calculated using STATGRAPHICS Plus, version 2.1, software (Statistical Graphics Corp., Princeton, N.J.).

RESULTS

Pressure and temperature changes during processing. Processing parameters during pressurization of *L. monocytogenes* at 100, 150, and 200 MPa at respectively −7, −14, and −18°C are presented in Figure 1. The graphs on the left in Figure 1 show typical pressure and temperature curves according to time of treatment. All temperatures decreased during the process to reach the cooling jacket temperature. Subzero temperatures were maintained for 120 to 140 min before the warming up of the system necessary to avoid the ice nucleation induced by the depressurization at subzero temperature. Pressure release of the system took place after 180 min, with the sample temperature between 4 and 8°C. This rapid depressurization induced a decrease of 1 to 2°C of the sample temperature. The graphs on the right in Figure 1 represent pressure versus temperature, with the dotted line corresponding to equilibrium freezing–melting curve of pure water. Experimental results demonstrated that buffer samples remained liquid throughout processing.

Reduction of *L. monocytogenes* in buffer systems. The results obtained are presented in Table 1 and Figure 2. With the aim to estimate the effect of temperature alone without pressure treatment, the survival of *L. monocytogenes* was previously tested at 4°C and subzero temperature at pH 4.5 and 7.0. No significant bacterial reduction was observed for these conditions without pressure (data not shown). Therefore, the unpressurized samples (stored at 4°C for the duration of pressure treatment) served as valid controls.

Pressure treatments at 4°C had no significant effect at pH 7.0, whereas the treatments in pH 4.5 resulted in a significant reduction of survival (Fig. 2). In this pH condition, increasing the pressure level led to an increase in the inactivation of *L. monocytogenes* up to 5.5 log CFU·ml^{-1} at 200 MPa (Table 1). In the case of combined treatments (high pressure–subzero temperature) at 100 MPa at −7°C, 150 MPa at −14°C, and 200 MPa at −18°C, the reductions of survivors were greater than those observed at 4°C at both pH, respectively. For example, maximal reductions reached 4.2 log CFU·ml^{-1} at pH 7.0 and 8.9 at pH 4.5. Neverthe-

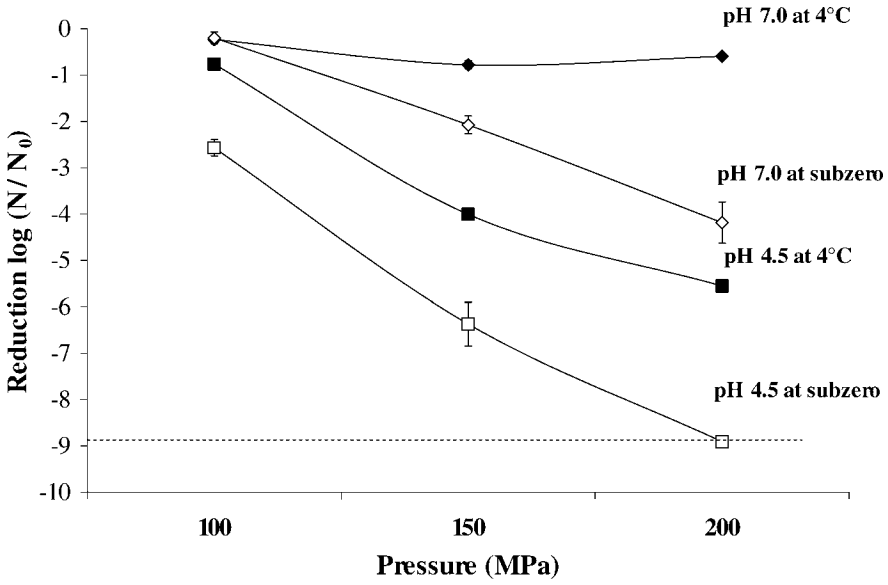

FIGURE 2. L. monocytogenes *inactivation obtained after HPP in buffer at pH 7.0 and 4.5, combined with HPP at 4°C and subzero temperature (−7°C at 100 MPa, −14°C at 150 MPa, and −18°C at 200 MPa) for 180 min. Samples were heated under pressure before releasing pressure to avoid the freezing of sample. The dotted line indicates the limit of* L. monocytogenes *detection.*

less, we noticed that all the treatments at 4°C and pH 4.5 remained more effective than those at −18°C and pH 7.0 (Fig. 2).

Inactivation of *L. monocytogenes* in smoked salmon. From treatments tested in buffers, the most effective treatments (150 MPa at −14°C and 200 MPa at −18°C) were selected for the experiments in smoked salmon. The inactivation observed in smoked salmon was very significant: reduction $\log(N/N_0)$ at pH 7.0 was 1.13 ± 0.24 and 4.22 ± 0.24 (respectively, at 150 MPa and 200 MPa), and reduction $\log(N/N_0)$ at pH 4.5 was 2.96 ± 0.30 and 4.89 ± 0.14 (respectively, at 150 MPa and 200 MPa). In fact, at pH 7.0, after a pressure treatment of 200 MPa at −18°C, there was no difference between inactivation in buffer (Table 1) and smoked salmon. However, at pH 4.5, inactivation was less in smoked salmon (4.89 log CFU·ml^{-1}) than in the buffer alone (8.91 log CFU·ml^{-1}). The results indicate that whatever the pH of treatment, the inactivation of *Listeria* was greater at 200 MPa than at 150 MPa. The pH of the smoked salmon before any treatment was 6.1. After pressure treatment, the pH of salmon was equal to 6.7 with buffer at pH 7.0, and to 4.71 with buffer at pH 4.5.

Modification of color and texture of marinated smoked salmon. Results of color measurements are presented in Table 2. The ΔE of control is more important for salmon with buffer pH 4.5 than for salmon with buffer pH 7.0. The acidification of the salmon provoked a bleaching (with an increase of the lightness L^*) of the fillet, due to the partial denaturation of sarcoplasmic and myofibrillar proteins. The total color difference is higher at pH 4.5. Whatever the pH, freezing induced few modifications of color; only a slight increase of L^* and b^* is observed with the lowest temperature.

HPP at 100 MPa and 200 MPa induced significant modifications of color, particularly an increase of the lightness. Whatever the initial pH of the salmon, the visual aspects of products were similar after HPP: effect of high-pressure treatment on color was higher than was effect of acidic pH.

Results of texture profile analysis are presented in Ta-

TABLE 2. *Color measurements of smoked salmon in buffer at different combinations of pressure, temperature, and pH*[a]

Treatment applied to salmon	Color features				
	Control	Freezing at −14°C	Freezing at −18°C	150 MPa at −14°C	200 MPa at −18°C
pH 7.0					
L^*	32.3 ± 1.7 A a[b]	34.8 ± 1.5 A b	38.3 ± 3.7 A c	50.5 ± 2.4 A d	52.3 ± 1.7 A d
a^*	13.2 ± 1.4 A a	12.3 ± 1.5 A a	13.4 ± 1.3 A a	17.5 ± 2.1 A b	16.2 ± 0.5 A b
b^*	11.4 ± 1.1 A a	10.8 ± 2.2 A a	14.4 ± 1.8 A b	17.9 ± 2.3 A c	17.6 ± 2.1 A c
ΔE	2.0 ± 1.3 A a	3.7 ± 1.5 A a	7.2 ± 3.6 A b	20.2 ± 1.1 A c	21.3 ± 1.3 A c
pH 4.5					
L^*	39.6 ± 7.2 B a	36.2 ± 4.2 A a	40.7 ± 5.1 A a	52.1 ± 1.6 A b	51.9 ± 1.0 A b
a^*	13.8 ± 1.3 A a	12.5 ± 1.1 A a	13.8 ± 1.5 A a	18.8 ± 1.3 A b	18.1 ± 0.8 B b
b^*	9.9 ± 3.3 A a	12.0 ± 1.9 A a	15.6 ± 3.4 A b	21.0 ± 1.6 B c	21.2 ± 0.9 B c
ΔE	7.3 ± 2.7 B a	6.0 ± 1.5 B a	7.7 ± 3.3 A a	17.5 ± 0.5 B b	17.2 ± 1.3 B b

[a] All values are means ± standard deviations of 10 values.

[b] Same capital letters in a column indicate no significant differences ($P < 0.05$) of each color feature (L^*, a^*, b^*, ΔE). Same lowercase letters in a row indicate no significant differences ($P < 0.05$).

TABLE 3. *Texture parameters obtained by texture profile analysis of smoked salmon in buffer at various pressure, temperature, and pH conditions*

Treatment applied to salmon	Texture parameters				
	Control	Freezing at −14°C	Freezing at −18°C	150 MPa at −14°C	200 MPa at −18°C
pH 7.0					
Hardness	4.34 ± 0.55 A a[b]	7.71 ± 0.86 A b	5.94 ± 1.36 A a	13.16 ± 1.95 A c	15.80 ± 2.07 A d
Springiness	0.94 ± 0.04 A a	0.78 ± 0.02 A bc	0.76 ± 0.03 A b	0.87 ± 0.04 A d	0.82 ± 0.05 A c
Gumminess	2.99 ± 0.34 A a	4.99 ± 0.69 A ab	4.05 ± 0.77 A b	8.85 ± 1.46 A c	10.03 ± 1.08 A d
Chewiness	2.81 ± 0.34 A a	3.91 ± 0.57 A b	3.07 ± 0.59 A ab	7.69 ± 1.39 A c	8.24 ± 1.14 A c
pH 4.5					
Hardness	9.06 ± 1.25 B a	8.43 ± 1.99 A a	5.18 ± 0.65 A b	14.03 ± 1.26 A c	16.14 ± 2.12 A d
Springiness	0.79 ± 0.03 A ab	0.76 ± 0.03 B b	0.69 ± 0.05 B c	0.82 ± 0.04 A a	0.84 ± 0.07 A a
Gumminess	5.98 ± 0.80 B a	5.26 ± 1.35 A a	3.49 ± 0.46 A b	8.92 ± 0.75 A c	10.39 ± 0.94 A d
Chewiness	4.76 ± 0.77 B a	4.01 ± 1.10 A a	2.37 ± 0.16 B b	7.34 ± 0.89 A c	8.76 ± 1.40 A d

[a] All values are means ± standard deviations of 10 values.

[b] Same capital letters in a column indicate no significant differences ($P < 0.05$) of each feature. Same lowercase letters in a row indicate no significant differences ($P < 0.05$).

ble 3. Acid buffer induced an increase of hardness, gumminess, and chewiness on control samples. Freezing at −14 and −18°C slightly affected texture: modifications were significant only for treatment at −14°C with pH 7.0, and at −18°C with pH 7.0 and 4.5.

Application of a HPP at subzero temperature was associated with an increase of all texture characteristics, in accordance with previous work *(27),* all the more important since pressure level was high. Modification of texture was independent of the pH.

DISCUSSION

The effects of pressure processing on the inactivation of microorganisms present in foods are well documented, but mainly for pressure treatments above 0°C *(26).* Novak and Juneja *(20)* mentioned that the freezing at −7, −14, and −18°C did not induce any inactivation of *L. monocytogenes.* A similar result was obtained in this study. In addition, the present treatments exposed food samples to pressure at subzero temperatures, without freezing. As predicted by the phase diagram of water under pressure *(7),* the samples were cooled under pressure, without ice crystals formation. The synergistic effect of pH and pressure observed by Ritz et al. *(22)* was confirmed in this study in both buffer media and food samples. Simpson and Gilmour *(26)* previously described the baroresistance of *L. monocytogenes* in foods, and they noticed the protective effect of foods in comparison with buffer media. Even if the efficiency of treatment is lower in salmon than in buffer media, the reductions obtained at pH 4.5 were highly significant: 2.96 and 4.89 log CFU·ml^{-1} at 150 and 200 MPa, respectively. These reductions were higher than those observed by Picart et al. *(21).* Two main reasons could explain these differences. In the present study, the samples were treated for 180 min versus 23 min. In the study carried out by Picart et al. *(21),* the pressure was released in 17 min to avoid ice nucleation. In our case, the temperature was progressively increased to reach 4°C, and then the pressure was released in 2 s (Fig. 1). These conditions of high-pressure treatment lead to an optimized reduction of *L. monocytogenes.* These results confirm our previous results that imply that holding time is important when considering the effect of pressure on microorganism reduction *(5).*

The most effective high-pressure treatment to inactivate *L. monocytogenes* (200 MPa at pH 4.5) led to a salmon fillet with higher *L**, *a**, and *b** values, and with higher hardness, gumminess, and chewiness.

The color changes are linked with the effect of high pressure on both myoglobin and myofibrillar proteins (of which partial denaturation implies a translucency modification), even if the exact mechanisms underlying color changes are not understood *(1, 17).* Color changes may not induce consumer rejection, even if visible with the naked eye. This should be assessed by sensory analysis. Furthermore, the increase of the toughness of the smoked salmon flesh might be considered as an advantage, as smoked salmon is often too soft.

We can conclude that high-pressure treatment at 200 MPa, pH 4.5, and subzero temperature (−18°C) induces a significant reduction of *L. monocytogenes.* We evidenced a synergetic effect between pressure, pH, and temperature. Further studies seem necessary to scale-up the process, since long pressurization times may not be practical. This report indicates that significant microbial reductions can be achieved, with a slight modification of the sensory quality of the salmon, which might not induce a consumer rejection.

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