Effects of a storage at -2°C/-3°C before retail displaying on the qualities of smoked salmon

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Abstract

Chilling is a technology consisting in storing smoked salmon at -2°C/-3°C during several weeks before retail displaying. This approach is an important point for controlling production and retail chain logistics.
In agro-food industry, cold storage is made either at positive temperature in order to slow down the growth of bacteria or at negative temperature (freezing) in order to stop the growth of bacteria. Storing food between 0°C and the freezing point is an alternative and the results on microbial and sensorial qualities have been investigated in a research program aiming at :

- highlighting determinant factors for the growth of *Listeria monocytogenes* (L.m): salt content, cold stiffening before slicing, kinetic characteristics of the strain, chilling duration
- assessing the consequences of chilling on *Listeria monocytogenes* growth
- assessing the impact of chilling on sensory changes.

Results permitted to assess the impact of this technology on the final product and will help to determine the commercial shelf life of the smoked salmon. They will also contribute to collect scientific data for a Quantitative Risk Assessment (QRA).

1. Introduction

Deep chilling is an innovating technology consisting in storing a food just above the initial freezing temperature (i.e; at -2°C/-3°C for smoked salmon) during several weeks before retail displaying. It is certainly of a great interest for the producers but, consequences on growth of *Listeria monocytogenes and* sensory changes have to be investigated.

2. Material and method

2.1. Experimental design for testing the effect of 4 parameters on the level of *Listeria monocytogenes*

An Hadamard matrix with 4 parameters (salt content, cold stiffening before slicing, kinetic characteristics of the strain, chilling duration) was performed to assess the impact of these factors on the level of *Listeria monocytogenes* reached during storage at positive temperature. Two levels were selected for NaCl, characteristics of the strains, cold stiffening and 3 levels were selected for chilling duration. The complete factorial design experiments was used and then, 24 assays were performed.
For salt contents, low level and high level were equal to 3.24 g/100g and 3.76g/100g. Measured were performed according to procedures described in a previous article (Leroi et al., 2000).

For strains, low level and high level were characterized by the maximal growth rates of 0.93 D^{-1} and 0.27 D^{-1}; these µmax were obtained by fitting the model of Baranyi and Roberts (1994) on results of growth of *L. monocytogenes* on ionized ground salmon at 8°C.

For cold stiffening, the 2 levels were presence or absence of cold stiffening and the 3 levels of chilling duration were 0, 14 and 28 days. For each assay, a challenge test was realized. Four slices of smoked

salmon was thawed at 20-22°C during a maximal time of 1 hour and a rectangle of 17 g of salmon was cut out from each slice.
In order to prepare the inoculum, the content of one cryotube was thawed, 1/10 diluted in tryptone soya broth (AES, Combourg, France) and cultured 7 days at 10°C. This first preculture was 1/10 diluted in tryptone soya broth and cultured 7 days at 10°C. This suspension was 1/100 000 diluted in tryptone salt (AES), then ½ diluted to obtain the inoculum suspension at a mean level of $8.1.10^3$ cfu per milliliter. A 0.1-ml volume of this inoculation suspension was spread onto each sample, which was then folded, so that the inoculum was sandwiched between the two layers. The folded disks were vacuum-packaged in oxygen-impermeable film. A specific refrigeration system using Peltier elements was used to chill the products. Two programs were applied in order to achieve a negative temperature before storage at chilling temperature: either a drop in temperature after processing in order to reach –2°C (for "without stiffening samples") either a drop in temperature until –12°C during processing to facilitate slicing followed by a rise to –2°C corresponding to industrial practice (temperature rise during slicing and packaging). Smoked salmons were either stored at positive temperature just after processing either kept at –2°C during 14 or 28 days. Then, the products were stored at 4°C during 8 days and at 8°C during 20 days. Storage at 4°C was intended to simulate a controlled cold chain during transportation and storage at 8°C was intended to simulate a domestic setting.
The absence of *L. monocytogenes* in the product before inoculation was checked (Anonyme, 1997).

2.2. Industrial sample for the following of the development of *L. monocytogenes* in naturally contaminated smoked salmon

For each batch, 42 samples of vacuum smoked salmon were taken just after packaging in different companies per week. Forty total batches were analysed as following in Table 1.

Table 1. Storage temperature of smoked salmon samples and analysis days.

Analyses of the batch of smoked salmon at week 0		
Nine smoked salmon were analysed at reception at laboratory (week 0)		
If at least 1 sample out of 9 is positive in *Listeria monocytogenes* enumeration and/or detection, the batch is stored at the various cycles time/ temperature and the samples were analysed according to planning below.		
Samples storage (cycles time/temperature):		
Storage at positive temperature	Storage at negative temperature then at positive temperature	Storage at negative temperature then at positive temperature
10 days at +4°C then 18 days at +8°C	14 days at –2°C (C14) then 10 days at +4°C then 18 days at +8°C	28 days at –2°C (C28) then 10 days at +4°C then 18 days at +8°C
Analyses days during the sample storage :		
Control Week 2 Control Week 3 Control Week 4	C14 Week 0 C14 Week 2 C14 Week 3 C14 Week 4	C28 Week 0 C28 Week 2 C28 Week 3 C28 Week 4

2.3. Enumeration and detection of *L. monocytogenes*

Enumerationof *L. monocytogenes* was performed at the beginning of the positive storage and after 14, 21 and 28 days of storage. To quantify low concentrations of *L. monocytogenes*, a membrane filtration method was performed. This method (Gnanou Besse et al., 2004), permits to lower the enumeration limit to 0.2 $cfu.g^{-1}$. To quantify high numbers of *L. monocytogenes*, 17 g of salmon were homogenized with 153 ml of tryptone salt solution using a stomacher. Ten-fold serial dilutions were made in tryptone salt diluent. One ml of the first decimal dilution was spread on 3 plates of agar for *Listeria* according to Ottavioni and Agostini (ALOA),(AES Laboratories), and 0.1 ml of each subsequent dilution was spread on one ALOA agar plate. Plates were incubated at 37°C for 24 h and 48 h. Presumptive isolates were confirmed (Anonyme, 1998).

Detection of *L. monocytogenes* was performed by method "ALOA One Day". Twenty-five g of the sample was diluted at 1/10eme in Fraser ½. After 24h of incubation to 30°C, 0.1 ml of homogenate were spread over ALOA agar which was then incubated 24h at 37°C.

2.4. Experimental design for testing the effect of chilling on sensory evaluation

To test the effect of a period of chilling at –2°C on sensory characteristics of smoked salmon, three products have been processed, each one by a different industrial. Similar protocol has been followed for these products but results of only one sample will be presented here as example. This product was salted by injection technique and smoked during 2h15 at 22°C. Smoked salmon was presented in packages of four slices. The day of processing and after packaging, sample was divided into three sub set : the first one (Control) was the reference sample stored 10 days at +4°C then 18 days at 8°C, the second one (C14) and the third one (C28) was respectively kept 14 days and 28 days at –2°C, before being stored in the same refrigeration conditions as the reference sample. Each sample (Control , C14, C28) was evaluated by the trained panel after 2, 3 and 4 weeks of storage.

A descriptive test with conventional profiling (Stone and Sidel, 1974) was carried out on smoked salmon processed according to an industrial procedure. Samples were scored by twenty panellists belonging to the IFREMER staff with a large experience on evaluation of smoked salmon (Leroi et al., 2001; Cardinal et al., 2004; Brillet et al., 2005). Sessions were performed in individual partitioned booths (NF V-09-105, 1995) equipped with a computerised system (Fizz system, Biosystèmes, Dijon, France). Panellists rated the sensory attributes on a continuous unstructured line scale from low intensity (0) to high intensity (10).
The descriptors related to the odour, appearance, texture and flavour of smoked salmon slices were evaluated in the following order: odour: global intensity, smoke note, raw salmon, herring, vegetable / herb, amine, sour, rancid rubber; appearance: orange colour, pink colour, homogeneity of colour; fatty aspect, translucent appearance, tearing of the slices (brittleness); texture: firmness, crunchy texture, melting texture, fatty texture, pasty texture; flavour: global intensity, smoke note, raw salmon, herring, vegetable / herb, salty taste, acid taste, amine, sour, rancid and rubber.

Once scored the sensory criteria, panellists were asked to classify samples into three classes defined by a spoilage level on the basis of odour and flavour evaluation : class 1, no spoilage, class 2 : weak to medium spoilage, class 3 :strong spoilage. A quality coefficient (QC) was calculated as follows (Leroi et al., 2001): QC = [(1 X %class 1) + (2 X % class 2) + (3 X %class 3)]/100. A sample was rejected when QC was higher than 2 that means that at least 50% of the panellists have scored in class 3.
The day of sensory analysis, the packages of smoked salmon were opened and kept open for 15 minutes at ambient temperature, after that each slice was individually repacked in aluminium foil. Smoked salmon sample was presented in each session with the two other industrial samples. Products were assigned 3-digit numbers, randomised and served simultaneously.

2.5. Statistical analysis

Multifactor analysis of variance with Statgraphics Plus 3.1 software (Sigma Plus, Paris, France) was carried out on data concerning the level of *L. monocytogenes* during storage at positive temperature in artificially inoculated samples (Hadamard matrix) and on sensory data.
For data concerning the level of *L. monocytogenes,* after artificial inoculation (Hadamard matrix), multiple comparisons were also performed.
For sensory data, the significant statistical level was set at $P < 0.05$. The factors included in the treatment were “chilling” with levels 0, 14 and 28 days, “time of storage” with levels 2, 3 and 4 weeks of refrigerated storage and also the factor “panellist”. To avoid eventual sanitary problem with *listeria* after the longer storage at –2°C (28 days), these products were not consumed by the panel at week 4, so for texture and flavour parameters, analysis takes into account only week 2 and 3.

3. Results

3.1. Effect of 4 parameters on the level of *Listeria monocytogenes*

An analysis of variance (Table 2) was performed to test the effect of each parameter on the increase of *L. monocytogenes* during storage at positive temperature (4°C during 8 days then 8°C during 20 days) and the results were completed by multiple comparisons (results not shown). The most influential parameter is the contaminating strain and the multiple comparisons point out a positive effect of strain TQA 131 on the increase of *L. monocytogenes* during storage at positive temperature. Chilling has a positive effect on the increase of *L. monocytogenes* on and after the 14th day of storage at positive temperature. Rising salt content from 3.24 g/100g to 3.76g/100g has a negative effect on the result on and after the 21st day of positive storage. There is no influence of cold stiffening.

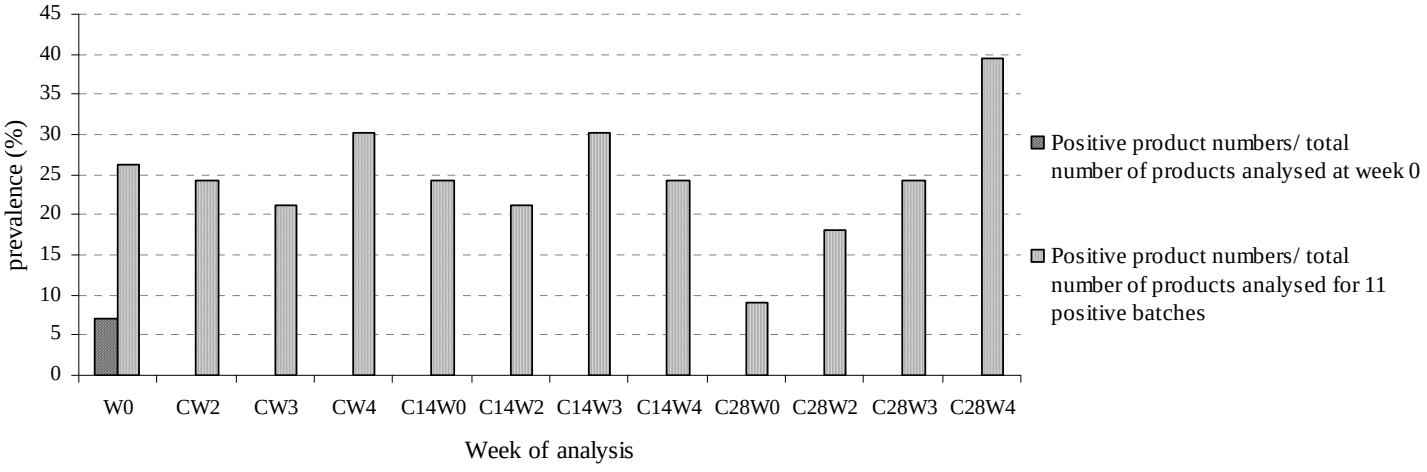
Table 2. Influence of 4 parameters on the increase of *Listeria monocytogenes* during storage at positive temperature (4°C during 8 days then 8°C during 20 days).

	Significance (P value) of parameters			
	W0	**W2**	**W3**	**W4**
Parameters				
Strain	0.9392	0.0001***	0.0000***	0.0002***
Chilling duration	0.3498	0.0484*	0.0637°	0.0131*
Salt content (g/100g)	0.2823	0.2292	0.0433*	0.0223*
Cold stiffening	0.4954	0.7991	0.1674	0.4734

$^{***}P<0.01$, very significant; $^{*}P<0.05$ and $^{\circ}P<0.1$, significant

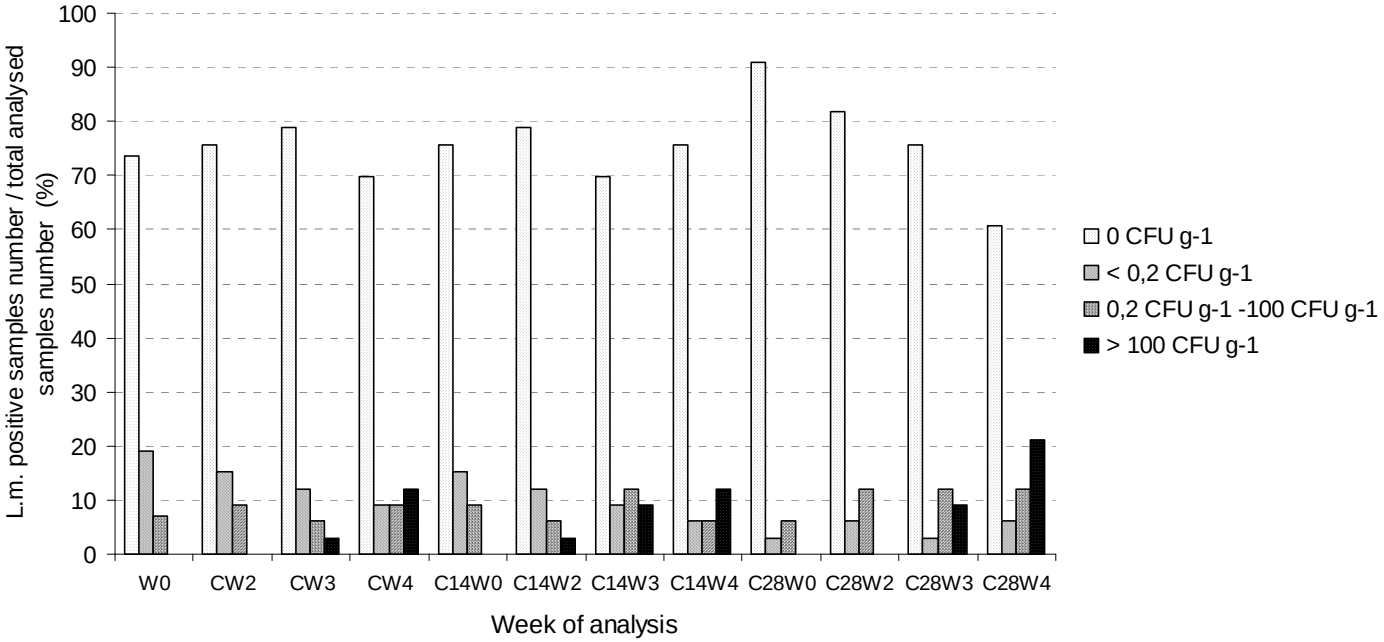
3.2. Effects of a storage at -2°C on the development of *Listeria monocytogenes* in naturally contaminated smoked salmon

Forty batches of smoked salmon samples were analysed and 11 batches (27.5% of the analysed batches) appeared positive in the enumeration and/or detection of *L. monocytogenes* at their reception at the laboratory (analysis week 0 (W0) (Figure 1). At analysis week 0, *L. monocytogenes* was detected and/or enumerated in 26 of the 360 analysed products (7.22%).



The prevalence in *L. monocytogenes* for the Control was between 21 and 30% during the totality of storage in positive temperature (CW2, CW3 and CW4). We observed that the prevalence of *L. monocytogenes* was the same in the seafood products stored during 14 days at -2°C then in positive temperature (C14W2, C14W3 and C14W4) comparing with the products only stored in positive temperature (CW2, CW3 and CW4). On the other hand, at analysis week0 after the conservation at -2°C during 28 days (C28W0), only 9.1% were contaminated by *L. monocytogenes*. Then the prevalence of *L. monocytogenes* increased during the storage at positive temperature and was approximately 39% at the analysis week 4 (C28W4).

At analysis week 0 (W0), the concentrations of *L. monocytogenes* were still below 100 CFU g^{-1} which is the maximum limit given by the International Commission on Microbiological Specification of Foods (Figure 2). This 100 CFU g^{-1} limit was exceeded in around 12% of products analysed after 28 days of storage at positive temperature (CW4). At analysis week 4 of positive temperature storage after the storage at –2°C during 14 days (C14W4), 12% of positive products were above 100 CFU g^{-1} limit. At analysis week 0 after the storage at –2°C during 28 days (C28W0), 9% analysed products were contaminated with less of 100 CFU g^{-1}. At analysis week 4 (C28W4),21% of positive samples were above 100 CFU g^{-1} limit with the concentrations which can be close to 100,000 CFU g^{-1}.



sample storage at positive temperature, C14: sample storage at –2°C during 14 days then at positive temperature, C28: sample storage at –2°C during 28 days then at positive temperature, W: week of analysis.

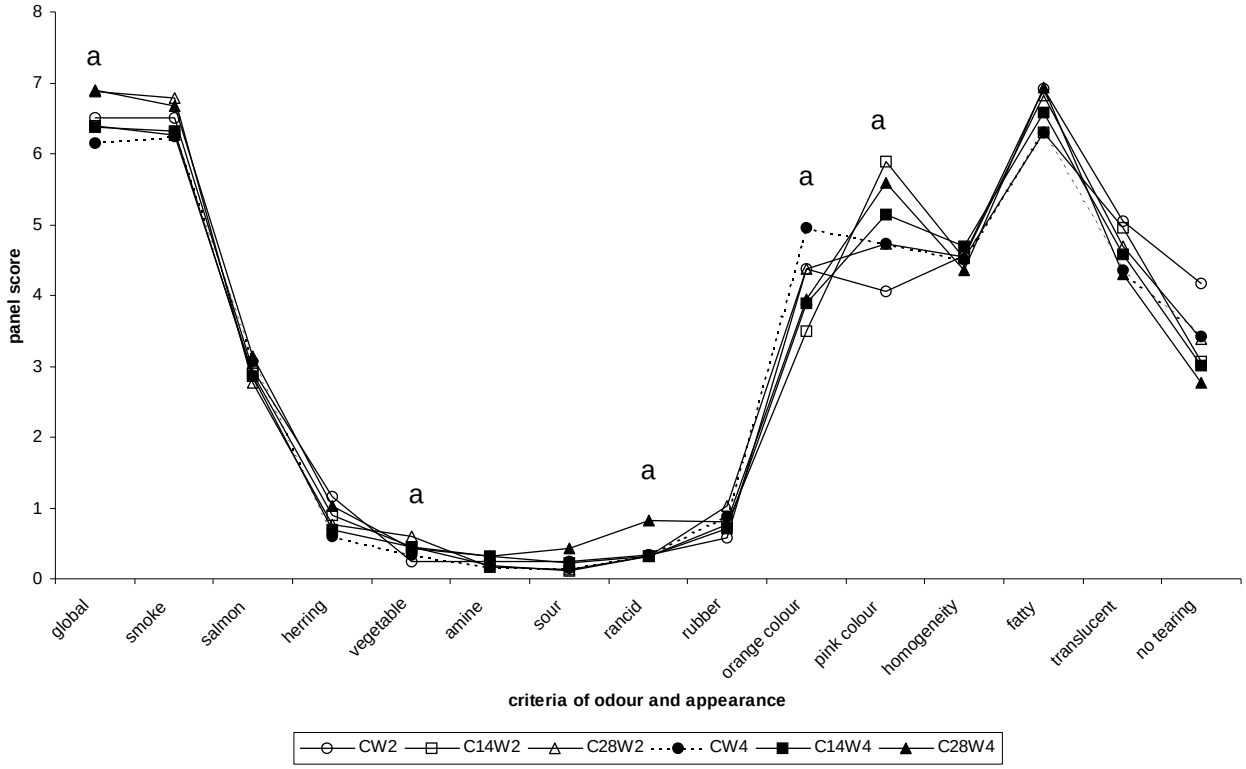
3.3. Effect of chilling on sensory characteristics

The analysis of variance allows the main sensory criteria affected by the “chilling” treatment to be identified. The main criteria affected are: global, vegetable and rancid odours as well as the pink colour. For the criteria of odours, the difference is significant between the control sample and the sample kept 28 days at –2°C and analyzed after 4 weeks. Regarding vegetable and rancid odour, even results are significant, scores are low all along the storage and the difference is generally due to only few panelists more sensitive to these criteria.

The pink colour seems more intense in products stored 14 or 28 days at –2°C and at the opposite, control sample are a little more orange colour.

Figure 3 illustrates the evolution of these criteria of odours and appearance in the three treatments of chilling (0, 14 days and 28 days) through the sensory profile after 2 and 4 weeks. The evolution of the quality coefficient QC with values which do not exceed 1.3, confirms that not one of the samples has developed sign of spoilage. The analysis of variance performed on data from weeks 2 and 3, shows only a small significant chilling effect on crunchy texture and on flavour: the flavour is slightly less salmon and the taste is slightly more acid.

The evolution of the quality coefficient QC with values which do not exceed 1.3, confirms that not one of the samples has developed sign of spoilage.



a : chilling effect was significant (P<0.05) between control and C28 sample

Figure 3. Effect of chilling on sensory descriptors of odours and appearance in smoked salmon after 2 and 4 weeks of refrigerated storage

4. Conclusion

The storage of smoked salmon samples at –2°C during 28 days has a positive effect on the development of *L. monocytogenes* (inoculated or naturally contaminated) and small effects on the sensory parameters comparing with the samples stored at positive temperature (Control).

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