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The effects of superchilled storage at −2 °C on the microbiological and organoleptic properties of cold-smoked salmon before retail display

Effets d'une conservation à −2 °C (super-réfrigération) avant la mise en vente sur les propriétés microbiologiques et organoleptiques du saumon fumé à froid

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Abstract:

The aim of this study was to investigate the impact of superchilling (−2 °C) on the evolution of *Listeria monocytogenes* and organoleptic characteristics of cold-smoked salmon samples. An Hadamard matrix experimental design was carried out on artificially inoculated samples stored at +4 °C for 10 d and at +8 °C for 18 d to know the influence of four factors: salt content, strain, cold stiffening and superchilling time, on the level of *L.monocytogenes* in cold-smoked salmon. The growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon and the organoleptic properties were investigated under superchilling conditions.
Superchilling (−2 °C for 28 d) had a limited impact on some of the organoleptic properties but the level of *L. monocytogenes* at the end of the shelf-life (4 °C for 10 d and 8 °C for 18 d) could exceed the microbiological criterion set by the European legislation.

Keywords: Salmon; Smoking; Review; Refrigerated storage; Quality; Microbiology

Mots clés: Saumon; Fumaison; Synthèse; Entreposage frigorifique; Qualité; Microbiologie

Nomenclature

CFU
: colony forming unit

C
: control

QC
: quality coefficient

SC
: superchilling

SD
: standard deviation

W
: week

μ_{max}
: maximum growth rate

1. Introduction

Cold-smoked salmon is consumed year-round by consumers, especially in France where it is highly regarded. Nevertheless, a peak of consumption is observed in December during the holiday period (Bergis, 2002). Cold-smoked salmon can be stored either above 0 °C or at freezing temperature at the industrial level before being dispatched to shops and supermarkets. Freezing and frozen storage permit an extended shelf-life, but freezing modifies the structure of the organoleptic properties of the product as compared to superchilling for mullet and yellowtail fish (Lee and Toledo, 1984). Superchilling may be considered to be an innovative technology in that it consists of storing food just above the initial freezing temperature (e.g. at -2 °C/-3 °C for cold-smoked salmon for which the initial freezing temperature is around –4 °C / -5 °C depending on salt content) for several weeks before retail display. Nevertheless, there is a lack of information regarding this technique in the available literature. The regulatory authorities are also sometimes embarrassed by this technique, in which the temperature domain may be considered to be quasi-freezing. This technique may be of interest for industry; however, undesired effects may occur such as the growth of *Listeria monocytogenes* which is one of the major pathogenic strain contaminating smoked salmon and also sensory changes. There is thus a need to investigated this technique. The objective of this paper is to investigate the impact of superchilling on the quality attributes of cold-smoked salmon and the possible benefit in terms of microbial quality.

2. Material and methods

2.1. Experimental design for testing the effect of four parameters on the level of *Listeria monocytogenes* on artificially contaminated samples

A Hadamard matrix with four parameters (water-phase salt, cold stiffening before slicing, kinetic characteristics of the strain, and superchilling duration) was performed to assess the impact of these factors on the level of *L. monocytogenes* reached during

storage at 4 °C for 10 days and 8 °C for 18 days. Cold stiffening consisted of subjecting samples to a partial freeze down to -12°C. This technique is common in industry to facilitate the slicing of cold-smoked salmon. Two levels were selected for NaCl, characteristics of the strain, cold stiffening and 3 levels were selected for superchilling duration. A complete factorial design (24 assays) was used. The first considered factor was the water-phase salt (WPS), which is the salt content (in g of salt per 100 g on a wet basis) divided by the water content. Low level and high level of WPS were equal respectively to 5.02 g per 100 ml and 6.27 g per 100 ml. Measurements were performed according to the procedures described by Leroi et al. (2000). Considering WPS, the 2 batches may be considered in the middle of the range of commercial the smoked salmon (Cornu et al., 2006).

For the second factor, 2 strains of *L. monocytogenes* of serovar 1/2a were used. These 2 strains were selected from the results of a previous study. In this previous study, 19 strains of *Listeria monocytogenes* isolated from cold-smoked salmon were compared for their maximal growth rate in ionized ground salmon at 8 °C. The μ_{max} were obtained by fitting the model of Baranyi and Roberts (1994) to the results of growth of *L. monocytogenes*. We selected for the present experiment the strain TQA 172 with the lowest μ_{max} (0.27 d^{-1}) and the strain TQA 131 with the highest μ_{max} (0.93 d^{-1}) .

For cold stiffening, the 2 levels of classification were presence or absence, and the three superchilling durations were 0, 14 and 28 days.

For the 2 batches used (one with the low WPS and one with the high WPS), the results obtained (from 2 samples per batch) for physicochemical characteristics were respectively 6.09 (σ = 0.03) and 6.12 (σ = 0.25) for pH, 5.02 (σ = 0.50) and 6.27 (σ = 0.11) for water-phase salt (in g per 100 ml) , 1.07 (σ = 0.03) and 1.02 (σ = 0.20) for phenolic compounds (in mg per 100 g). All the samples were frozen to – 18 °C and for each assay, a challenge test was performed. Four slices of cold-smoked salmon were thawed at 20-22°C over a maximal time of 1 h and a rectangle of salmon weighing 17 g was cut from each slice.

In order to prepare the inoculum, the content of one cryotube was thawed, diluted 1:10 in tryptone soya broth (AES, Combourg, France) and cultured for seven days at 10°C. This first preculture was diluted 1:10 in tryptone soya broth and cultured for seven days at 10°C. This suspension was diluted 1/105 in tryptone salt (AES), then diluted 1:2 to obtain the inoculum suspension at a mean level of 8.1 x 103 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 2 layers. The folded rectangles were then vacuum-packaged in oxygen-impermeable film. A specific refrigeration system using Peltier elements was used to superchill the products. Two programs were applied in order to achieve a negative temperature before storage took place: either a drop in temperature after processing to reach -2 °C (for "without stiffening samples") or a drop in temperature to -12 °C to facilitate slicing followed by a rise to -2 °C, which corresponds to the current industrial practice (temperature rise during slicing and packaging). Cold-smoked salmon was either stored directly at 4 °C for 10 days and at 8 °C for 18 days just after processing or kept at -2 °C for 14 or 28 days before being stored at 4 °C for 10 days and at 8 °C for 18 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 (Anonymous, 1997).

2.2. Industrial samples for following the development of *Listeria monocytogenes* in naturally contaminated cold-smoked salmon

Five processing plants owned by different companies were selected to obtain a large variety of samples. From each plant, 40 batches of vacuum-packed cold-smoked salmon (a batch = 42 cold-smoked salmon samples composed of 4 vacuum packed slices, processed the same day in a smokehouse) were brought back to the laboratory in chilled conditions (4 °C) immediately after processing. Nine samples per batch were analyzed (Week 0), 9 samples were stored at 4 °C, 12 samples were stored at –2 °C for 14 days and 12 samples were stored at –2 °C for 28 days and were analyzed as shown in Figure 1.

2.3. Detection and enumeration of *Listeria monocytogenes*

Enumeration of *L. monocytogenes* was performed at week 0 (W0) (the day of reception of products at laboratory) and after 14, 21 and 28 days of storage. To quantify low concentrations of *L. monocytogenes*, a membrane filtration method was performed. This method permits the enumeration of *L. monocytogenes* to a lower limit of 0.2 CFU g^{-1} (Gnanou Besse et al., 2004). Three different suspensions (5, 15 and 30 ml) of cold-smoked salmon homogenate were treated with 0.83 % Tween 80 at 10 % and 0.83 % trypsin 1:250 stock solutions prepared in phosphate buffer pH 7.5, for 20-25 min at 37 °C in a water-bath shaker. The suspensions were then filtered in single-use filtration units with an effective 12.25-cm² filtration area (MicroFunnel™ Filter Funnels, VWR, Fontenay sous Bois, France) with a 0.45 µm pore-size membrane (4.7 cm diameter) composed of mixed cellulose esters (Millipore, Saint-Quentin in Yvelines, France). The filters were laid on agar *Listeria* according to Ottaviani and Agosti (ALOA) (AES, Combourg, France) and incubated for 48 h at 37 °C. For enumeration, only filters containing less than 100 colonies were counted and the volume of suspension analysed was recorded. With this method, *L. moncytogenes* appears as blue colony without a halo. Ten typical blue colonies had to be spotted inoculated on an ALOA plate and incubated for 24 h at 37 °C to read the halo formation. To quantify high numbers of *L. monocytogenes*, 17 g of salmon were homogenized with 153 ml of tryptone salt solution using a Stomacher blender. Ten-fold serial dilutions were made in tryptone salt diluent. One ml of the first decimal dilution was spread on three plates of agar designed for *Listeria* growth 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 then incubated at 37 °C for 24 h and 48 h. Presumptive isolates were confirmed (Anonymous, 1998). Detection of *L. monocytogenes* was performed by the "ALOA One Day" method (International Reference Method NF EN ISO 11290-1, and validated analytical protocol AFNOR no. 10/3-09/00). Twenty-five grams of the cold-smoked salmon sample was diluted at 1:10 in Fraser 1:2 broth. After 24 h of incubation at 30°C, 0.1 ml of homogenate was spread over ALOA agar which was then incubated for 24 h at 37 °C.

2.4. Enumeration of aerobic psychrotrophic flora

Total aerobic psychrotrophic flora (TAPF) was plated on Long and Hammer (LH) agar after incubation for 5 days at 15 °C (van Spreekens, 1974).

2.5. Experimental design for testing the effect of superchilling on sensory qualities

To test the effect of a period of superchilling at -2 °C on the sensory characteristics of cold-smoked salmon, three products were processed, each one coming from a different company. The same protocol was followed for these products, but the results from only one sample will be presented here as an example. This product was salted by the injection technique (using a specific brine instead of dry salt salting) to a level of 5.6 g of salt for 100 ml of water phase (σ = 0.7) and smoked for 2 h 15 min at 22 °C to reach 1.20 (σ = 0.15) mg of phenol for 100 g of product. Cold-smoked salmon was presented in packages of four slices. The day of processing and after packaging, sample was divided into three sub sets : the first one (Control = C) was the control sample stored 10 days at 4 °C then 18 days at 8 °C, the second one (superchilling 14 days at -2 °C = SC14) and the third one (superchilling 28 days at -2 °C = SC28) was respectively kept 14 days and 28 days at -2 °C, before being stored in the same refrigeration conditions as the control sample. Each sample (Control, SC14, SC28) was evaluated by the trained panel after 2, 3 and 4 weeks of storage. The initial count of total psychrotrophic flora in the control sample (C) was 2.4 log CFU g^{-1}(σ = 2.1).

A descriptive test with conventional profiling was carried out on cold-smoked salmon processed according to an industrial procedure (Stone et al., 1974). The samples were scored by twenty panellists belonging to the IFREMER staff with substantial experience in evaluating cold-smoked salmon (Brillet et al., 2005; Cardinal et al., 2004; Leroi et al., 2001). The sessions took place in individually partitioned booths (Anonymous, 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).

Descriptors related to the odour, appearance, texture and flavour of the cold-smoked salmon slices were evaluated in the following order – for odour: global intensity, smoke note, raw salmon, herring, vegetable / herb, amine, sour, and rancid rubber; for appearance: orange colour, pink colour, homogeneity of colour, fatty aspect, translucent appearance, and no tearing of the slices (brittleness); for texture: firmness, crunchy texture, melting texture, fatty texture, and pasty texture; and for flavour: global intensity, smoke note, raw salmon, herring, vegetable / herb, salty taste, acid taste, amine, sour, rancid, and rubber.

Once the sensory criteria were scored, the 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; and 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, meaning that at least 50 % of the panellists scored the sample as belonging to class 3.

On the day of sensory analysis, the packages of cold-smoked salmon were opened and kept open for 15 minutes at ambient temperature, after which each slice was individually repacked in aluminium foil. A cold-smoked salmon sample was presented in each session with the two other industrially processed samples. Products were assigned 3-digit numbers, randomized, and served simultaneously.

2.6. Statistical analysis

Statistical t-test was performed to compare water phase salt (WPS), with α = 0.10. 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* found

during storage at 4 °C for 10 days and 8 °C for 18 days in artificially inoculated samples (Hadamard matrix) and on the sensory data.
For the data concerning the level of *L. monocytogenes,* after artificial inoculation (Hadamard matrix), multiple comparisons were also performed.
For the sensory data, the statistical significance level was set at $P < 0.05$. The factors included in the treatment were "superchilling" with levels of 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 sanitary risks 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, the analysis only takes weeks 2 and 3 into account.

3. Results

3.1. Effect of four parameters on the level of *Listeria monocytogenes* on artificially contaminated samples

The increases of *L. monocytogenes* on artificially contaminated samples of cold-smoked salmon during the storage at 4 °C for 10 days and at 8 °C for 18 days were calculated for the different combinations of factors (Tables 1 and 2). An analysis of variance (Table 3) was performed to test the effect of each parameter on the increase of *L. monocytogenes* during storage at 4 °C for 10 days and at 8 °C for 18 days, and the results were completed by multiple comparisons (results not shown). The most influential parameter is the inoculated strain and the multiple comparisons pointed out that the increase of *L. monocytogenes* was higher when the cold-smoked salmon was inoculated with the more rapid strain: the mean increase for a shelf-life of 21 days was 3.30 log CFU g^{-1} with strain TQA 131 whose μ_{max} = 0.93 d^{-1} when grown in ionised ground smoked salmon and 1.43 log CFU g^{-1} with the strain TQA 172 whose μ_{max} = 0.27 d^{-1}. This effect was observed on and after the 14th day of storage at 4 °C for 10 days and 8 °C for 18 days. The duration of superchilling affected the increase of *L. monocytogenes* too: for example, the mean increase at the end of a shelf-life of 28 days was 2.45 log CFU g^{-1} when there was no superchilling and 3.95 log CFU g^{-1} when the duration of the superchilling was 28 days. An increase of the water-phase salt from 5.02 g per 100 ml to 6.27 g per 100 ml resulted in a lower increase on and after the 21st day of storage above 0°C: the mean increase for a shelf-life of 21 days was 2.73 log CFU g^{-1} for a WPS of 5.02 g per 100 ml and 1.99 log CFU g^{-1} for a WPS of 6.27 g per 100 ml. Cold stiffening did not have any detectable effect.
Neither the physicochemical characteristics (except WPS) nor the total aerobic psychrotrophic flora between the 2 batches of cold-smoked salmon may explain the differences relating to the results. The physicochemical characteristics (pH, phenolic compounds) and the level of total aerobic psychrotrophic flora of the 2 batches were close. The mean number of total aerobic psychrotrophic flora (obtained from 12 samples) was 2.30 log CFU g^{-1} (σ = 0.29) for one batch and 2.24 log CFU g^{-1} (σ = 0.46) for the other (results not shown).

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

Forty batches (40 batches x 9 products analyzed at W0 = 360 products analyzed) of cold-smoked salmon samples were analyzed. Twelve of these 40 batches (27.5 % of the analyzed batches) and 26 products of 360 analyzed were positive for *L. monocytogenes* upon their reception at the laboratory at analysis week 0 (W0) (Fig. 2).

The prevalence of *L. monocytogenes* was between 21 and 30 % during the totality of storage at 4 °C for 10 days and 8 °C for 18 days (CW2, CW3 and CW4). We observed that the prevalence of *L. monocytogenes* was the same in the cold-smoked salmon stored for 14 days at -2 °C and then shifted at 4 °C for 10 days and 8 °C for 18 days (SC14W2, SC14W3 and SC14W4) compared to the products only stored above 0 °C (CW2, CW3 and CW4). On the other hand, at W0 after storage at -2 °C for 28 days (SC28W0), only 9.1% of samples were contaminated with *L. monocytogenes*. The prevalence of *L. monocytogenes* then increased during the storage at 4 °C for 10 days and 8 °C for 18 days and was approximately 39 % at the end of the storage (SC28W4).

At week 0 (CW0), the enumeration of *L. monocytogenes* was still below 100 CFU g^{-1} (Fig. 3), 100 CFU g^{-1} being the maximum limit given by the European Regulation for these products (Anonymous, 2005). This 100 CFU g^{-1} limit was exceeded in about 12 % of products analyzed at the end of the storage at 4 °C for 10 days and 8 °C for 18 days (CW4). At week 0 after storage at -2 °C for 14 days (SC14W0) and 28 days (SC28W0), the products were contaminated with less than 100 CFU g^{-1}. At week 4 of above 0 °C storage, following storage at -2 °C for 14 days (SC14W4), 12% of the positive products were above the 100 CFU g^{-1} limit. At analysis week 4 (SC28W4), 21 % of positive samples were above the 100 CFU g^{-1} limit.

3.3. Effect of superchilling on sensory characteristics

The analysis of variance allows the main sensory criteria affected by the “superchilling” treatment to be identified. The main criteria affected were global, the vegetable and rancid odours, and the pink colour. For the odour criteria, the difference between samples kept for 28 days at -2 °C and analyzed after 4 weeks and the control sample is significant. Regarding vegetable and rancid odour, even though the results were significant, the scores were low all along the storage and the difference is likely due to only a few panellists who were more sensitive to these criteria.
The pink colour seemed more intense in products stored 14 or 28 days at -2 °C, whereas the control samples were a little bit more orange.
Figure 4 illustrates the evolution of these odour criteria and the appearance of salmon in the three superchilling treatments (0, 14 days and 28 days) based on the sensory profile measured at the end of storage. The analysis of variance performed on data from weeks 2 and 3 showed only a small significant superchilling effect on crunchy texture and on flavour; the flavour is slightly less “salmonish”, and the taste is slightly more acidic.
The evolution of the quality coefficient (QC), with values that do not exceed 1.3 confirms that none of the samples developed any sign of spoilage.

4. Discussion

At the end of the storage of the products, the prevalence of *Listeria monocytogenes* as well as on the contamination levels were different in the products stored above 0 °C and those stored in superchillig for 28 days at –2 °C and above 0 °C. Other studies have observed different levels of initial contamination and prevalence in function of the industrial plant (Beaufort et al., 2007; Dauphin et al., 2001; Markkula et al., 2005; Midelet-Bourdin et al., 2007). In fact, the results observed for products stored for 14 days at -2 °C and at 4 °C for 10 days and 8 °C for 18 days were similar with those observed for the products only stored at 4 °C for 10 days and 8 °C for 18 days. In contrast, the presence of *L. monocytogenes* was important in the products stored for

28 days at -2 °C and at 4 °C for 10 days and 8 °C for 18 days. The drop in the prevalence of *L. monocytogenes* after a long time at -2 °C could involve stress of the bacteria and shows the limits of the methods used for detection (Asperger et al., 1999; Cornu et al., 2002; Curiale and Lewis, 1994; Gnanou Besse et al., 2005; Midelet-Bourdin et al., 2007; Petran and Swanson, 1993). However, it should also be stressed out that *L. monocytogenes* was present in the products during the storage at 4 °C for 10 days and 8 °C for 18 days; this result could be due to the adaptation of the bacteria to low temperatures. For example, after 42 days of storing *Parapercis colias* at -1.5 °C, Bell et al. (1995) showed an increase of *L. monocytogenes*. We observed slight sensory modifications (in appearance, texture and odour) for products that were stored for 28 days at -2 °C and then stored for three or four weeks at 4 °C for 10 days and 8 °C for 18 days, as has also been highlighted in another study (Dunn and Rustad, 2008) on fresh salmon stored at -1.4 °C.

Conclusion

The storage of cold-smoked salmon at -2 °C (superchilling process) for 14 days did not have any serious consequences on the quality of cold-smoked salmon compared to controls (absence of superchilling). However, the prevalence of *L. monocytogenes* was more important at the end of the storage of the products stored at -2 °C for 28 days (superchilling) and then above 0 °C at follow in comparison with the products stored above 0 °C or at –2 °C for 14 days and above then 0 °C at follow. Sensory parameters were modified at the end of the storage of the products at -2 °C for 28 days and above 0 °C at follow compared with the products stored above 0 °C or at –2 °C for 14 days and above 0 °C at follow. It may therefore be useful to initiate further studies related to superchilling, applying periods of storage at -2 °C ranging from 14 to 28 days.

These results should help cold-smoked salmon producers toward advances in superchilling technology. They should also be useful in providing scientific data for a Quantitative Risk Assessment (QRA).

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Figures

12 products conserved
28 days at –2°C (SC28)
then 10 days at +4 °C
then 18 days at +8°C

Analyzed of 3 products at:
- SC28W0
- SC28W2
- SC28W3
- SC28W4

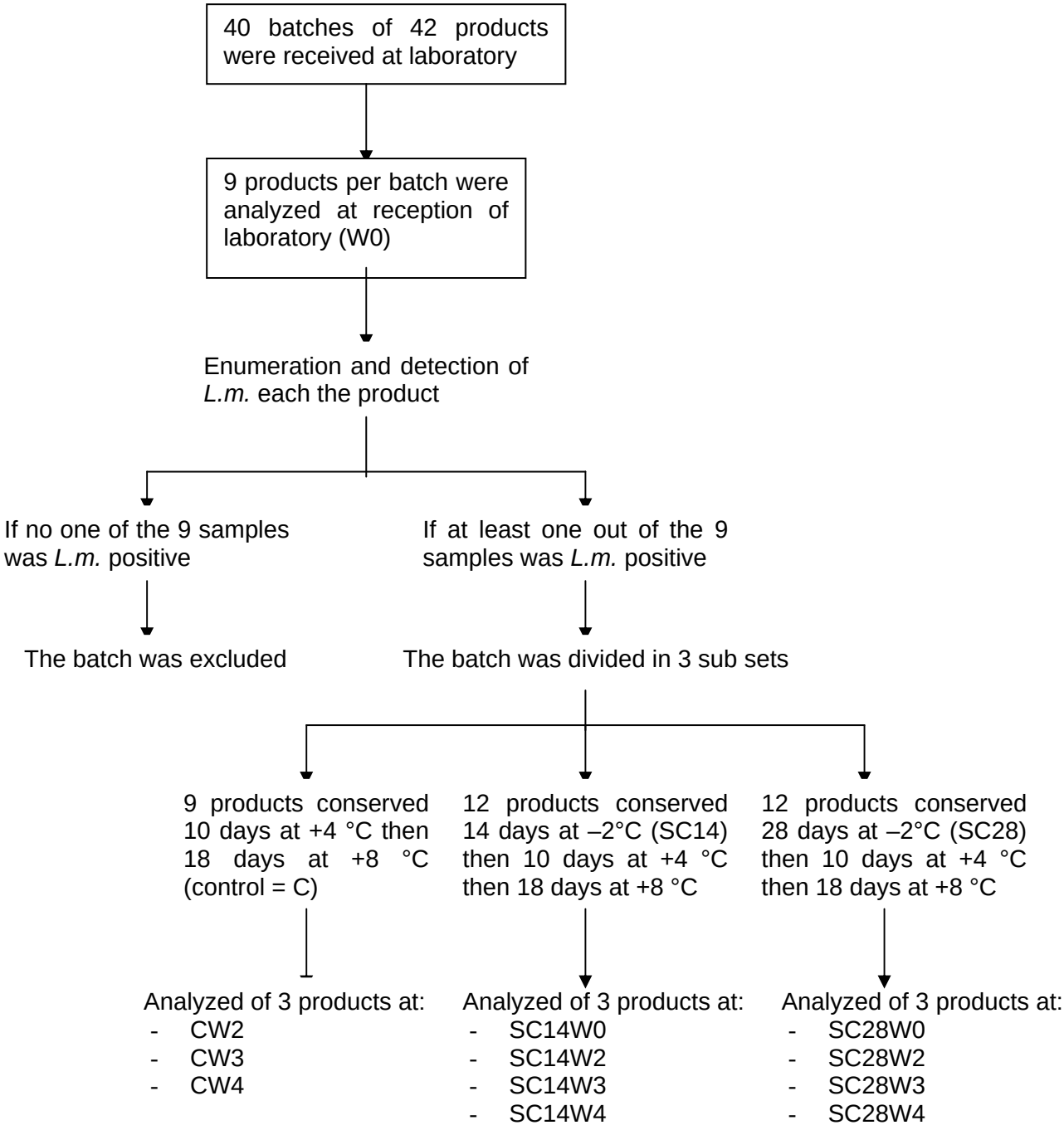


Fig. 1. Storage temperatures of cold-smoked salmon samples and days of their analysis C: sample storage at 4 °C/10 d + 8 °C/18 d (control), SC14: sample storage at −2 °C/14 d + 4 °C/10 d + 8 °C/18 d, SC28: sample storage at −2 °C/28 d + 4 °C/10 d + 8 °C/18 d, W: week of analysis.

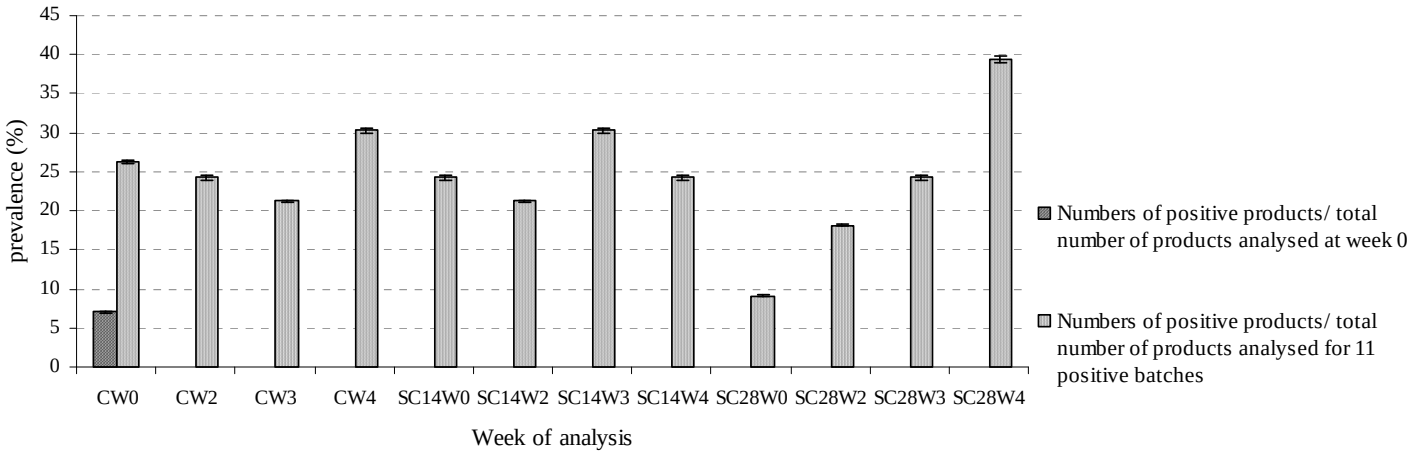


Fig. 2. Number of samples positive for *L. monocytogenes* as a function of the week of analysis (at W0, *n* = 360 for total number of samples analyzed and *n* = 99 for the total number of samples analyzed for 11 positives batches and *n* = 33 for other weeks of analysis). C: sample storage at 4 °C/10 d + 8 °C/18 d (control), SC14: sample storage at −2 °C/14 d + 4 °C/10 d + 8 °C/18 d, SC28: sample storage at −2 °C/28 d + 4 °C/10 d + 8 °C/18 d, W: week of analysis.

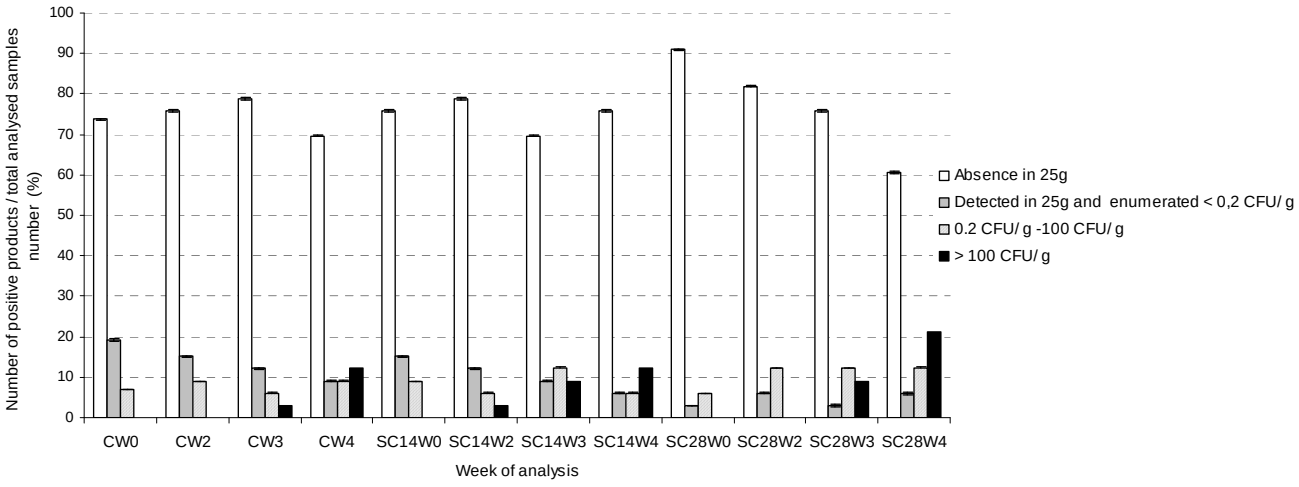
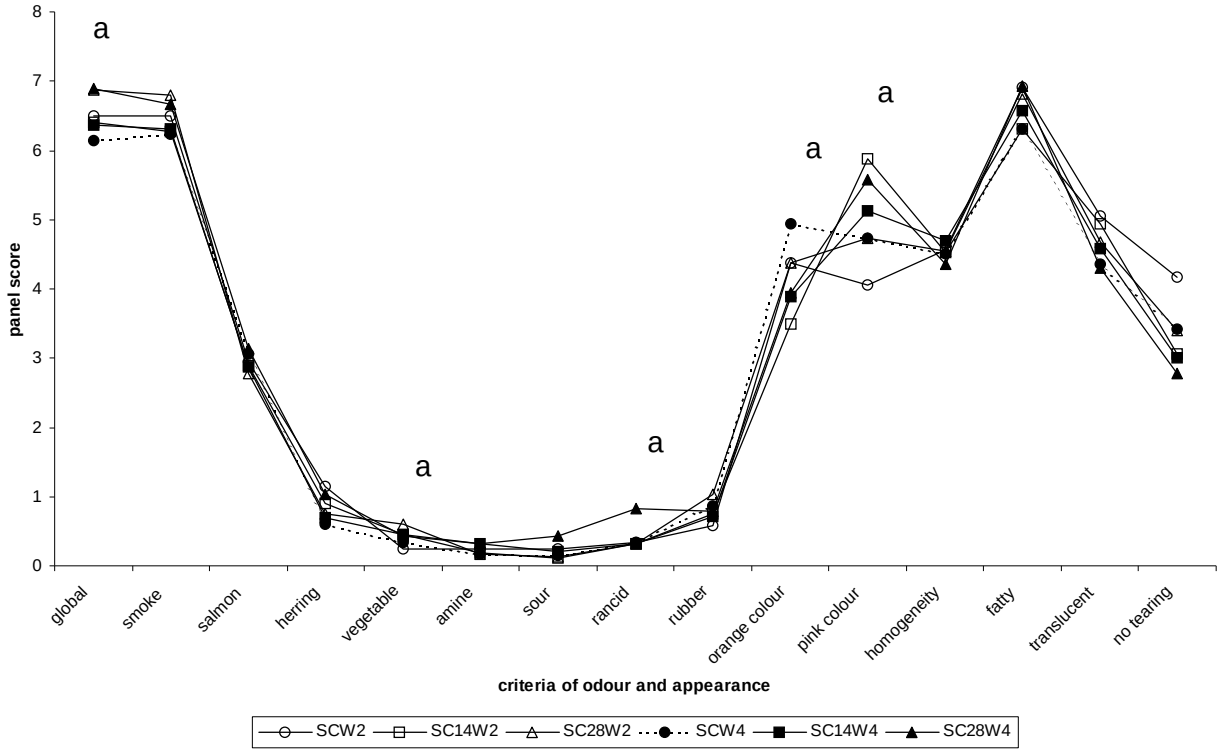


Fig. 3. *L. monocytogenes* contamination in naturally contaminated cold-smoked salmon samples (at W0, *n* = 99 for total number of samples analyzed in 11 positive batches and *n* = 33 for other times of analysis). C: sample storage at 4 °C/10 d + 8 °C/18 d (control), SC14: sample storage at −2 °C/14 d + 4 °C/10 d + 8 °C/18 d, SC28: sample storage at −2 °C/28 d + 4 °C/10 d + 8 °C/18 d, W: week of analysis.

Fig. 3



a : chilling effect was significant (P<0.05) between control and SC28 sample

Fig. 4. Effect of superchilling on the organoleptic descriptors of odors and appearance in cold-smoked salmon after two and four weeks of refrigerated storage. a: chilling effect was significant (P<0.05) between control and SC28 sample.

Tables

Table 1. Increase (log $CFUg^{-1}$) of *L. monocytogenes* TQA 131 on artificially contaminated samples of cold-smoked salmon during the storage at 4 °C for 10 days and at 8 °C for 18 days (W: week of analysis)

Cold stiffening	Water-phase salt	Superchilling duration (days)	Storage 4 °C for 10 days and at 8 °C for 18 days			
			W0	**W2**	**W3**	**W4**
CS-	**WPS-**	**0**	0	1.73	4.1	4.69
CS-	**WPS-**	**14**	-0.4	1.91	2.09	3.84
CS-	**WPS-**	**28**	0.47	2.85	4.57	5.11
CS-	**WPS+**	**0**	0	0.59	1.7	2.31
CS-	**WPS+**	**14**	-0.33	2.28	2.56	4.5
CS-	**WPS+**	**28**	-0.3	1.35	2.49	4.1
CS+	**WPS-**	**0**	0	1.54	2.96	4.73
CS+	**WPS-**	**14**	-0.11	1.05	4	5.13
CS+	**WPS-**	**28**	-0.1	2.98	5.6	6.51
CS+	**WPS+**	**0**	0.12	1.04	1.83	2.61
CS+	**WPS+**	**14**	-0.07	2.42	4.04	n.t.
CS+	**WPS+**	**28**	0	1.21	n.t.	2.6

CS: cold stiffening; CS-: without cold stiffening; CS+: with cold stiffening

WPS: water-salt phase; WPS-: 5.02 g/100 ml; WPS+: 6.27 g/100 ml

n.t.: not tested

Table 2. Increase (log $CFUg^{-1}$) of *L. monocytogenes* TQA 172 in artificially contaminated samples of smoked salmon during the storage at 4 °C for 10 days and at 8 °C for 18 days (W: week of analysis).

Cold stiffening	**Water-phase salt**	**Superchilling duration (days)**	**Storage 4 °C for 10 days and at 8 °C for 18 days**			
			W0	**W2**	**W3**	**W4**
CS-	**WPS-**	**0**	0.22	0.63	1.08	1.43
CS-	**WPS-**	**14**	-0.04	0.62	1.67	3.39
CS-	**WPS-**	**28**	-0.39	0.64	1.67	2.79
CS-	**WPS+**	**0**	0	0.31	0.96	1.34
CS-	**WPS+**	**14**	0.08	1.19	1.58	2.69
CS-	**WPS+**	**28**	-0.48	0.62	0.94	2.03
CS+	**WPS-**	**0**	-0.1	0.01	0.73	0.92
CS+	**WPS-**	**14**	0.39	n.t.	n.t.	n.t.
CS+	**WPS-**	**28**	-0.06	1.16	2.15	3.34
CS+	**WPS+**	**0**	0	0.36	0.93	1.6
CS+	**WPS+**	**14**	-0.12	0.6	1.9	3.9
CS+	**WPS+**	**28**	-0.31	0.71	1.34	2.02

CS: cold stiffening; CS-: without cold stiffening; CS+: with cold stiffening

WPS: water-salt phase; WPS-: 5.02 g/100 ml; WPS+: 6.27 g/100 ml

Table 3. Influence of four parameters on the increase of *Listeria monocytogenes* during storage at above 0 °C (4 °C for 10 days, then 8 °C for 18 days).

4.1.1. Significance (P value) of parameters			
W0	**W2**	**W3**	**W4**

Parameters				
Strain	0.9392	0.0001***	0.0000***	0.0002***
Superchilling duration	0.3498	0.0484*	0.0637°	0.0131*
Water-phase Salt	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 °$P < 0.1$, significant