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Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method

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Abstract

The aim of this work is to evaluated the microbiological quality and the sensory characteristics of salmon slices processed by the sous vide method under different conditions of time–temperature when stored at 2 and 10 °C.

Three different combinations of time–temperature were studied (5 min/65 °C, 10 min/90 °C and 15 min/90 °C). The product stored at 2 or 10 °C was evaluated periodically to study its sensory quality, microbiological condition and proximate composition. Batches stored at 2 °C had lower growth rates of mesophiles and psychrotrophs. *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Listeria monocytogenes* were not found in any of the samples. Neither aerobic nor anaerobic sporeforming bacteria were detected in the salmon processed at 90 °C for 15 min and stored at 2 °C after 45 days. The heat treatment of 90 °C for 15 min was the most effective for extending the shelf life of fish (>45 days). However, under these conditions the sensory characteristics were not optimal.

It can be conclude that the treatment at 90 °C for 15 min was the most effective one to ensure the safety and to extend the shelf life of sous vide salmon. However, under these conditions the sensory characteristics were not optimal immediately after processing.

This study emphasizes the importance of heat treatment and storage temperature in order to ensure the safety particularly in a fat fish, since fat can acts as a protecting agent in the heat resistance of microorganisms.

Keywords: Fish; Sous vide; Salmon; Microbiology; Safety

1. Introduction

The increase in consumer demands for minimally processed refrigerated convenience foods with characteristics closer to that of the fresh products has led to a growth in the use of sous vide processing technology to extend the shelf life and to keep the quality of fresh food (Schellekens & Martens, 1992).

Sous vide or vacuum-cooked foods are defined as "raw materials or raw materials with intermediate food, that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pouches" (Schellekens & Martens, 1992). Sous vide products are typically heated at relatively mild temperatures (65–95 °C) for a long period of time. After heating the products are quickly cooled and kept in chilled storage (1–4 °C). The shelf life of sous vide products varies within 6–42 days. There is a tendency to design heat treatments primarily in function of optimal quality retention rather than optimal bacterial destruction, particularly in protein-rich products (Goussault, 1993; Houben, 1999).

The three main factors which determine the microbiological safety of sous vide products are (i) the intensity of heat treatment, (ii) the rapidity of cooling and the temperature reached and (iii) the control of chilled storage (temperature and time) (SVAC, 1991).

The UK Advisory Committee on the Microbiological Safety of Food recommends for cook-chil products with an extended shelf life of more than 10 days and up to 42 days (⩽3 °C), a heat treatment of 90 °C for 10 min or equivalent lethality and strict chill conditions in order to control the *Clostridium botulinum* risk. In order to eliminate non-sporeforming pathogens such as *Listeria*

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monocytogenes a heat treatment of 70 °C for 2 min or equivalent heating process is required (ACMSF, 1992). An adequate heat treatment must achieve at least six log cycle reduction in the psychrotrophic strains of *Clostridium botulinum* and *Listeria monocytogenes*. The treatments necessary to achieve a significant reduction in *Cl. botulinum* spores cause unacceptable thermal damage in some products. Therefore, less severe heat treatments have been proposed, but additional hurdles should be incorporated (Genigeorgis, 1993).

Spain, together with Norway and Portugal are the main consumers of fish in Europe (71 g per person per day) (Varela, Moreiras, Carbajal, & Campos, 1991), a figure which is much higher than the one relevant for the rest of the European countries. Over the last few years, an increase in consumption has been observed. This change in foods habits has been caused by the knowledge of the beneficial effects on the health of fish consumption as it has a high protein quality and content similar to the one in meat and a high content in hydrosoluble and liposoluble vitamins, minerals and polyunsaturated fatty acids of the n-3 family (PUFA n-3) (Sánchez-Muniz, Viejo, & Medina, 1991; Simopoulus, 1998). However, fish and fish products are vulnerable to various biochemical, physical and microbial forms of deterioration on going through the production chain. Moreover, a substantial part of the quality of fresh or processed fish is often lost at consumer or retail level. In order to increase the average amount of fish eaten at home, good quality seafood properly prepared and conveniently packaged should be available (Schellekens, 1996).

Fish processed by the sous vide method keeps their intrinsic qualities (keeping seafood's natural and fresh-like appearance and extending their shelf life). However, applying high thermal treatments to fish gives an unacceptable decrease in its sensory quality. Therefore, a heat treatment in the order of 60–80 °C for 20–40 min is preferable. Thus, in sous vide fish cooked at low temperatures, the control of surviving microorganisms is an important safety issue (NACMCF, 1990).

Over the last 10 years, several authors have studied the microbiological quality of sous vide products (Betts, 1991; Gaze, Brown, Gaskell, & Bansk, 1989; Ghazala, Aucoin, & Alkanani, 1996; Ghazala, Cosworthy, & Alkanani, 1995; Light, Hudson, Williams, Barret, & Schafheitle, 1998; Meng & Genigeorgis, 1994; Miyazawa et al., 1994; Rybka et al., 1999). However, there is very little information available on fish processed by the sous vide method, particularly on its microbiological quality (Bem Embarek & Huss, 1993; Bergslien, 1996; García-Palacios, 1999; Gitleson, Salmarch, Cocotas, & McProud, 1992; Rosnes, Kleiberg, Bergslein, & Vidvei, 1999).

The most important pathogens in sous vide salmon are *Clostridium botulinum* type E and *Listeria monocytogenes* (Meng & Genigeorgis, 1994). Other pathogenic bacteria (*Bacillus cereus* and *Clostridium perfringens*) have been regarded as contaminants from ingredients, handling of raw materials or hygienic production conditions (Rosnes et al., 1999).

The aim of this work was to evaluate the shelf-life, the microbiological quality and the sensory characteristics of salmon processed by the sous vide method under different conditions of time–temperature when stored at 2 and 10 °C.

2. Material and methods

Slices from salmon (*Salmon salar*) were cut into portions of 100, 15 g of olive oil and 0.2 g of salt were added. Each portion was packaged in a polyethylene-polyamid pouch with O_2 permeability of 25–30 $cm^3\,m^{-2}$ per 24 h and water steam permeability of 5 $g\,m^{-2}$ per 24 h at 25 °C. The pouches were heat sealed using a vacuum sealing machine (TECNOTRIP, Barcelona, Spain). Heat processing was carried out in a steam oven (Surdry A-142, Bilbao, Spain). The heating profiles of vacuum packed foods were obtained by locating a thermocouple (TESTO, thermometer, Testo GmbH & Co., Lanzkirch, Germany) in the geometric centre of the sample.

Three different combinations time/temperature treatments were tested: 90 °C for 15 min (Batches Aa and Ab), 90 °C for 5 min (Batches Ba and Bb) and 65 °C for 10 min (Batches Ca and Cb).

After heat processing, products were immediately chilled using a blast chiller (Matachana, Barcelona, Spain), temperatures were measured by an Irinox register. Temperatures of 32 °C were reached in 21.5 min and 0–2 °C in 45 min. All the products were prepared by a local producer of "sous vide" meals (De Celis SA, Navatejera, León, Spain).

After chilling, Batches Aa, Ba and Ca were stored at 2 °C, whereas Batches Ab, Bb and Cb were stored at 10 °C for up to 45 days. Samples were taken from raw fish, immediately after cooling and after 0, 3, 14, 21 and 45 of storage.

Two experiments were carried out. The following determinations were made in each experiment: pH, water activity, proximate composition (protein, fat content, ash), microbiological analyses and sensory analysis.

2.1. Physicochemical analyses

For determination of pH, 10 g of fish were blended with 10 ml of distilled water. The pH of the homogenized sample was measured with a Crison Basic 20 pHmeter (Crison Instruments, Barcelona, Spain) (AOAC, 1995). Water activity was determined using an

Aqualab TCX-2 water activity instrument (DECAGON, Inc. USA) (AOAC, 1998). Determinations were performed in duplicate.

2.2. Proximate composition

The moisture was determined by drying four homogeneous samples (3 g) at 100 °C to a constant weight (method 240.03, AOAC, 1980). Total protein content was calculated in six homogeneous freeze-dried samples from Kjeldahl nitrogen using a 6.25 conversion factor (AOAC, 1984). Total fat content of four freeze dried samples (0.5 g of each sample) was extracted with petroleum ether (BP 40–60 °C) in an extracting unit (Soxtec System 1040 Tecator, Sweden) and gravimetrically determined. Ash was determined by heating at 500 °C to constant weight, using a muffle fumace model Vulcan 3-550 (NEY., California, USA) (method 310.12, AOAC, 1975).

2.2.1. Microbiological analyses

Twenty-five grams of salmon were weighed aseptically and homogenized in a Stomacher (IUL, Barcelona, Spain) for 2 min with 225 ml of sterile peptone water (0.1% peptone). Further decimal dilutions were made with the same diluent. The total number of mesophilic microorganisms was determined on Plate Count Agar (PCA, Oxoid) following the pour plate method, incubated at 30 °C for 72 h (ICMSF, 1978). Psychrotrophs were determined on Plate Count Agar with an incubation temperature of 7 °C for 10 days, following the pour plate method (ICMSF, 1978). Anaerobes were determined in PCA incubated under anaerobic conditions at 30 °C for 72 h (ICMSF, 1978). Aerobic spores were determined using PCA following the pour plate method and incubated at 30 °C for 72 h after a heat treatment at 80 °C for 10 min to destroy vegetative cells (ICMSF, 1978). Anaerobic spores were determined following the same procedure used for aerobic spores but with incubation under anaerobic condition at 30 °C for 72 h (ICMSF, 1978). Lactic acid bacteria were determined in MRS (Oxoid) and incubated at 30 °C for 72 h (de Man, Ragosa, & Sharpe, 1960). *Enterobacteriaceae* were determined on plates of Violet Red Bile Glucose agar (Difco, Detroit, MI). Plates were overlaid prior to incubation at 37 ± 1 °C for 18–24 h (ICMSF, 1978). *Staphylococcus aureus* was enumerated by plating on Baird-Parker agar (Oxoid) following the surface plate method. The incubation temperature used was 37 ± 1 °C (18–24 h). Suspected colonies were subjected to DNAse test (Difco, Detroit, MI) (ICMSF, 1978). *Bacillus cereus* was enumerated in Bacillus selective agar (Oxoid) incubated at 30 °C for 48 h (ICMSF, 1978). *Clostridium perfringens* was enumerated in SPS agar (Oxoid) incubated at 37 °C for 48 h under anaerobiosis (ICMSF, 1978). The presence of *Listeria* spp. was investigated by the following procedure: a 25 g sample was homogenized with 225 ml of Listeria Enrichment Broth (LEB, Merck) in a Stomacher. The enrichment broth was incubated at 30 °C for 48 h. LEB cultures were streaked onto Palcam agar and the plates were then incubated at 37 °C for 48 h and analyzed for the presence of *Listeria* colonies (Mossel, Corry, Struijk, & Baird, 1995; Varnam & Evans, 1996).

All analyses were performed in duplicate.

Table 1
Quality scale used in sensory analysis

Characteristic	Score						
	1	2	3	4	5	6	7
Appearance	Old						Fresh
Odour	Off odour						Fresh
Taste	Cloying						Fresh
Rancidity	Strong rancidity						No rancidity
Texture	Very soft						Firm

2.2.2. Sensory analysis

The sensory analysis was carried out using a panel of 10 judges selected and trained according to ISO-standard (ISO 8586-1, 1993). The quality of each sample was classified using characteristics describing: appearance, odour, taste, rancidity and texture. Each characteristic was scored on a point scale from 1 to 7, according to Table 1. Taste was only analysed on Day 0. If scores were lower than four, fish was unacceptable. Salmon was analyzed after heating for 2 min at full power in a microwave Teka model MW 206 of 800 watts and a frecuency of 2450 MHz (TEKA Industrial SA, Santander, Spain). A control sample, prepared by traditional cooking, was provided at each evaluation so the panelist could evaluate the storage samples against the control.

2.2.3. Statistical analysis

Analysis of variance was performed using the SPS program for Windows; version 100.1.3. The Student *t* test for comparison of means was performed using the same program. Significance was established at $p < 0.05$ level.

3. Results

3.1. Proximate composition

Raw salmon is a fat fish (13.71 ± 0.14 g/100 g edible portion) with a high pH close to neutral (6.24 ± 0.01) a moisture content of 66.66 ± 0.70 g/100 g edible portion and a protein content of 18.13 ± 0.37 g/100 g edible portion.

The average moisture decreased after heat treatment (61.44 ± 0.46 g/100 g edible portion). Significant differences ($p < 0.05$) were found between the raw salmon slices and the sous vide salmon. However, there were no significant differences ($p < 0.05$) between salmon slices

processed at 90 °C and storage at different temperatures and neither between salmon processed at different combinations of time/temperature.

The sous vide treatment decreased the moisture content and increased the other components. This increase was particularly evident in fat content after treatment and after 3 days of storage at 2 °C (fat content of 19.66 ± 0.42 g/100 g edible portion). However, a decrease in fat content was observed on Day 21 (15.33 ± 0.15 g/100 g edible portion) that remained on Day 45 (15.88 ± 0.21 g/100 g edible portion). In contrast, protein content decreased after 3 days of storage (15.49 ± 0.50 g/100 g edible portion), and an increase was observed on Day 21 (20.43 ± 0.22 g/100 g edible portion).

The pH did not change significantly throughout the storage period, although a slight increase of 0.24 units was observed after sous vide treatment. The water activity did not change significantly throughout the storage period (0.989 ± 0.002).

3.2. Microbiological quality

S. aureus, *Bacillus cereus*, *Clostridium perfringens* and *Listeria monocytogenes* were not detected in any sample.

Microbiological results are shown in Fig. 1. The raw salmon had initial mesophiles and anaerobes counts of 4.77 ± 0.42 and 4.18 ± 0.24 log cfu/g respectively (Figs. 1 and 2). The mean mesophile and anaerobes counts of the salmon slices processed at 90 °C for 15 min (Batches Aa and Ab) were lower than 2 log cfu/g after 14 days of storage at 2 or 10 °C. After 21 days of storage a slight growth higher at 10 °C was observed, but populations reached were always lower than 4 log cfu/g. The mesophiles and anaerobes populations in salmon slices processed at 90 °C for 5 min were above 3 log cfu/g after 14 days of storage at 10 °C (Batch Bb), whereas in salmon processed at 65 °C for 10 min populations were above 3 log cfu/g in all the samples stored at 10 °C (Batch Cb). However, when the storage temperature was 2 °C, mesophiles and anaerobes populations above 3 log cfu/g were reached after 21 days of storage in Batch Ca. The heat treatment had a significant effect ($p < 0.05$) on mesophile and anaerobes counts. The final mesophiles and anaerobes counts were significantly lower ($p < 0.05$) in samples stored at 2 °C than in those stored at 10 °C for all heat treatments studied. Indeed, the cells surviving heat treatments of 90 °C for 15 min were hardly able to grow at 2 °C after 14 days. Anaerobes counts were between 0.5 and 1 log unit lower than mesophile counts.

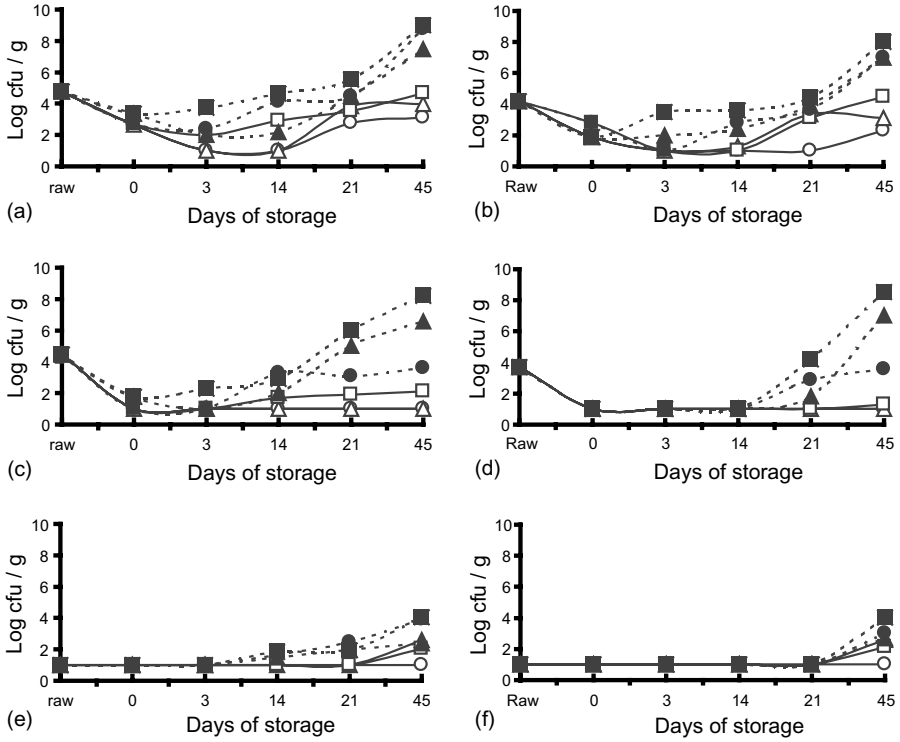

Fig. 1. Effect of processing conditions and storage temperature on microbial population of salmon processed by sous vide method. (○) salmon processed at 90 °C for 15 min and stored at 2 °C, (Δ) salmon processed at 90 °C for 15 min and stored at 10 °C, (□) salmon processed at 90 °C for 5 min and stored at 2 °C, (●) salmon processed at 90 °C for 5 min and stored at 10 °C, (▲) salmon processed at 65 °C for 10 min and stored at 2 °C, (■) salmon processed at 65 °C for 10 min and stored at 10 °C: (a) mesophile, (b) anaerobes, (c) psychrotrophs, (d) lactic acid bacteria, (e) aerobic spores and (f) anaerobic spores. The data are the mean values of two experiments sampled in duplicate.

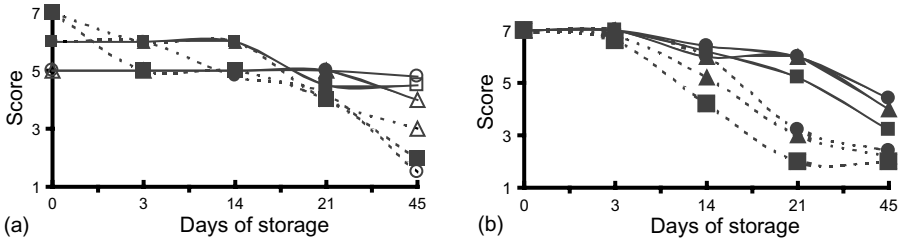

Fig. 2. Effect of processing conditions and storage temperature on sensorial characteristics of salmon processed by sous vide method. (○) salmon processed at 90 °C for 15 min and stored at 2 °C, (△) salmon processed at 90 °C for 15 min and stored at 10 °C, (□) salmon processed at 90 °C for 5 min and stored at 2 °C, (●) salmon processed at 90 °C for 5 min and stored at 10 °C, (▲) salmon processed at 65 °C for 10 min and stored at 2 °C, (■) salmon processed at 65 °C for 10 min and stored at 10 °C. (a) appearance and (b) odour. The data are the mean values of two experiments sampled in duplicate.

Mesophiles counts above 6 log cfu/g were only reached after 45 days of storage in Batches Bb, Ca and Cb.

Psychrotroph growth was lower when the heat treatment was more severe (Fig. 1c). Psychrotrophs counts were below 1 log cfu/g in salmon processed at 90 °C for 15 min after 45 days of storage at 2 or 10 °C. However, the psychrotroph counts increased by 4.75–6.5 log units between Day 0 and 45 in salmon slices processed at 65 °C depending on temperature of storage, whereas in salmon processed at 90 °C for 5 min the increase was of 1–2.6 log units during the same period. Significant differences ($p < 0.05$) were found between salmon processed at different temperatures after 14 days of storage. Also storage temperature had a significant effect ($p < 0.05$) on psychrotrophs counts after 14 days of storage.

Although *Enterobacteriaceae* were detected in raw salmon (2.66 ± 0.91 log cfu/g), no growth was observed after sous vide treatment.

Initial lactic acid bacteria contamination of raw salmon ranged from 3.52 to 4.2 log cfu/g. After heat treatment their levels were under the detection limit (1 log cfu/g) (Fig. 1d). No growth of LAB was observed in Batches Aa and Ab after 45 days of storage. However, these bacteria were able to recover their viability and attain a level of about 3.55 ± 0.3 log cfu/g in Batch Bb after 45 days of storage at 10 °C. In Batches Ca and Cb, LAB populations were above 6 log cfu/g on Day 45.

The initial level of aerobic and anaerobic sporeforming bacteria was below detection limit (Fig. 1e and f). Sporeforming anaerobic bacteria were only detectable after 45 days of storage in salmon processed at 65 °C for 10 min (Batches Ca and Cb) and those processed at 90 °C for 5 min (Batches Ba and Bc). No aerobic sporeforming bacteria were detected in salmon processed at 90 °C for 15 min and storage at 2 °C after 45 days. On the other hand, under inappropriate storage conditions at 10 °C the aerobic sporeforming bacteria were able to multiply and reach a level between 2.5 and 4 log cfu/g depending on the heat treatment. Both processing conditions and storage temperature influenced the sporeforming bacteria counts.

3.3. Sensory quality

The results obtained from the sensory analysis are shown in Fig. 2. On Day 0, the highest score, particularly in appearance was reached by the salmon processed at 65 °C. Significant differences ($p < 0.05$) were found between batches processed at 65 °C and those processed at 90 °C for 15 min on Day 0. Fish processed at 90 °C had a more pale color and the precipitation of protein was more pronounced. The protein coagulation was the main reason for the reduced score of the products processed at 90 °C. However, although on Day 0 the salmon slices processed at 90 °C for 15 min had a score lower than those processed at 65 °C, this score did not change substancially after storage at 2 °C, and retained a reasonable acceptability until the end of the storage period.

As regards the odour, on Day 0 salmon processed by sous vide system retained their sensory quality. The lowest scores in odour were reached by the samples of salmon processed at 65 °C and stored at 10 °C after 21 days. Taste was only analyzed on Day 0. Batches processed at 65 °C obtained the higher score on taste. Rancidity was observed after 45 days of storage. After 21 days of storage salmon processed at 65 °C was considered unacceptable by the panellists, whereas salmon processed at 90 °C for 10 min and stored at 2 or 10 °C was considered unacceptable after 45 and 21 days respectively.

Based on the numbers of days needed by mesophiles to reach populations above 6 log cfu/g and an appearance/odour/texture score below 4, sous vide salmon has a shelf-life of about 21 days when processed at 65 °C. When, salmon was processed at 90 °C for 5 min and stored at 10 °C shelf-life was also 21 days. This shelf-life could be extended to 45 days if the product was processed at 90 °C for 5 min and stored at 2 °C or processed at 90 °C for 15 min.

4. Discussion

The proximate composition of the raw salmon was similar to that reported by others authors (USDA,

1987), except in regards its fat content, which was higher in our samples (13.71% ± 0.14). These other authors have reported the following average composition for raw farmed *Salmon salar*: water content: 68.9, protein 19.9, fat 10,9 and ash 1.1 g/100 g, whereas figures for wild salmon are 68.5, 19.8, 6.3 and 2.5 respectively (USDA, 1987).

It must be considered that composition of fish can vary greatly according to the season and that it is related to spawing cycles, specially in fat and water content, since these components rise and fall inversely. This affects both texture and flavor, and probably microbial growth. On the other hand, the farming of fish generally increases their lipid content.

Mesophiles counts were significantly lower ($p < 0.05$) in salmon stored at 2 °C compared to those stored at 10 °C for all processing treatments, this fact emphasizes the importance of storage temperature to ensure the quality and safety of minimally processed food products.

Besides the little information on salmon sous vide, some microbiological discrepancies have been found in the literature. Meshophiles counts of this study were higher than those reported by Rosnes et al. (1999). These authors studied the microbiological quality of sous vide salmon processed at 70 °C for 15 min and storage at 4 and 10 °C. These researchers observed that mesophile counts were below 1 log cfu/g after 42 days of storage at 4 °C and above 6 and 8 log cfu/g after 17 and 42 days of storage at 8 °C respectively. The higher counts found for us in salmon processed at 65 °C/10 min after 45 days of storage at 2 °C (7.49 ± 0.52 cfu/g) could be explained by the lower temperature and time applied. However, even when heat treatments of 90 °C/5 min and 90 °C/15 min were applied, we found higher mesophiles counts after 45 days of storage at 2 °C (4.66 ± 0.22 and 3.11 ± 0.34 log cfu/g respectively). Since, the heat treatment was more severe, these higher counts could be due to the higher counts in raw salmon (about 1 log unit higher) and to some protective factor such as the high fat content, although the mentioned authors did not show the fat content. Nevertheless, on Day 45 the counts were higher in our study than those reported by Rosnes et al. (1999), they found higher mesophiles counts in salmon stored at 4 °C on Day 7 (approximately 3 log cfu/g) than in any of the samples of the present study after 14 days of storage at 2 °C.

With regard to microbial levels of raw fish, it must be considered that they vary according to water conditions, temperature and handling.

Schellekens (1996) stated that the nature of food (fat content, pH, water activity and essential amino acids) is an important determining factor of the lethality of a heat treatment and also of the possibility of pathogen growth. This author pointed outs that it would be important to study the influence that each factor has on the growth and inactivation of the microorganisms with the aim of establishing additional hurdles when the first barrier (refrigeration at adequate temperature) in sous vide foods is not fulfilled.

Studies on heat resistance carried out on fish with different fat contents and in broth, conclude that a greater fat content in fish (e.g. 20% as opposed to 0.5%) acts as a protecting agent in the heat resistance of pathogenic microorganisms (*Listeria monocytogenes* and *Escherichia coli*) VTEC (Bem Embarek & Huss, 1993). However, other studies carried out on ground beef with the same percentage of fat (20%) showed that no significant protective effect of fat could be demonstrated (Buncic, Voijnovic, Paunovic, & Radisic, 1992; Mackey, Pritchet, Norris, & Mead, 1990). This could be due to the fat added to the ground meat and does not have the same protecting effect than fatty meat-tissue or to the different type of food studied (fish and meat).

However, other authors have reported higher mesophiles counts. Bergslien (1996) observed mesophiles population in sous vide salmon processed at 65 °C for 10 min after 7 days of storage at 2 °C above 5 log cfu/g.

Our results agree with those reported by Simpson, Smith, Simpson, Ramaswamy, and Dodds (1994) who studied the evolution of aerobic, anaerobic and lactic acid bacteria on other sous vide products. Simpson et al. (1994) studied the shelf life of sous vide spaghetti and meat sauce subjected to a heat processing at 65 °C (71 and 105 min) and 75 °C (37 and 40 min). They also observed a gradual increase in total aerobic, anaerobic and lactic acid bacteria counts throughout storage. They found that products stored at 5 °C had a shelf-life of >35 days irrespectively of the processing treatment. However, for products stored at 15 °C, packages were visibly swollen after 14 or 24 days, depending on the severity of the heat processing treatment. This fact could be explained since minimally processed foods may contain a large proportion of thermally injured cells which are able to undergo repair throughout storage, particularly at temperature abuse conditions and reach levels of public health concern.

Counts of anaerobic bacteria were similar to total aerobic counts. This may be explained by a large proportion of facultatively anaerobic bacteria. These results agree with those reported by Carlin, Guinebretiere, Choma, Schmitt, and Nguyen (1999) who also found similar counts of anaerobic and aerobic bacteria in sous vide vegetables, being most of the anaerobes isolated capable of growing in air. On the other hand, after vacuuming of "sous vide" products, there is usually 1–5% oxygen left in the package at the beginning of the process, this allows facultative anaerobic bacteria to grow. This mechanism explains why clostridia, as obligate anaerobic microorganisms, can only be found after an extend storage period.

Psychrotrophs counts were lower than mesophiles counts and were only detectable after 14 days of storage

except in salmon processed at 90 °C for 15 min (Batches Aa and Ab). These low psychrotrophs counts have been also observed by other authors. Rosnes et al. (1999) did not found psychrotrophic bacteria in salmon sous vide after 42 days of storage except on Day 17 with a maximum figure of 2.8 log cfu/g. Psychrotrophs grow, albeit slowly at good refrigeration temperatures (<5 °C) and had an optimum of about 25 °C. The psychrotrophic nature of many of the indigenous bacteria found in cold water fish could result in rapid growth even at low temperature. However, microorganisms may be injured by milder treatments, being this injury characterized by increased nutritional requirements of microorganisms to grow (Montville, 1997). Moreover, under unfavorable values of other environmental factors, which include oxygen concentration, the minimal growth temperature increases considerably. In addition, injured cells are still viable, though their recovery by normal enumeration procedures is negatively affected in three ways. Firstly, lag times increase considerably, even when optimal recovery media are used. Secondly, sometimes generation times increase as well in comparison to fully viable cells under the same intrinsic and extrinsic conditions (Mossel et al., 1995). Finally, the incubation temperature may affect the recovery of debilitated populations, and note that heat injured non-sporeforming bacteria grow over a much narrower range than fully vital ones (Thomas, Reinbold, & Nelson, 1963). Thus, the great difference between mesophile and psychrotrophs populations could represent injured cells that could be not recovered at low temperature. It should be noticed that determinations of psychrotrophs are usually carried out at 7 °C and then require at least a week for completion, whereas incubation for a few hours at 20–25 °C before reducing their temperature to 5 °C gives visible colonies of psychrotrophs in a shorter time (Mossel et al., 1995). In extended shelf life foods, there may be sufficient time for this organisms to adjust to the chill environment and grow. On the other hand, since the temperature requirements of various microorganisms differ, refrigeration may considerably change the qualitative composition of the microbiota (Farkas, 1997). Although, the main concern of sous vide products stored at good refrigeration temperatures (<5 °C) are psychrotrophic bacteria, most of the studies carried out on microbiological quality of sous vide foods evaluate mesophiles counts rather than psychrotrophs (Carlin et al., 1999; Guerzoni, Gianotti, & Lopez, 1999; Simpson et al., 1994). Our results show that mesophile counts give a more accurate estimation of survivors than psychrotroph counts in sous vide products.

Our results agree with those reported by Guerzoni et al. (1999) who studied the evolution of lactic acid bacteria on sous vide meat products. These authors also observed that LAB were undetectable immediately after sous vide treatment in meat product, but could recover during storage. However, these authors reported that the growth of the lactic acid bacteria occurred only sporadically in a few samples. In contrast, Rosnes et al. (1999) did not detected viable lactic bacteria, neither aerobic and anaerbobic sporeforming bacteria during the storage time of 42 days.

Since *Listeria* is able to grow in fish, even at low temperatures (ICMSF, 1996), its possible presence in the samples was investigated. *Listeria* spp. was not found in any sample. The absence of pathogens could be expected, since the raw fish was not contaminated and batches were elaborated with strict hygienic conditions. Although, the raw fish studied was free of pathogens, several authors have detected *L. monocytogenes* in raw fish (Colburn, Kasyner, Abeyta, & Wekell, 1990; Fuchs & Surendra, 1989). On the other hand, this pathogen is known to be more heat resistant than normal vegetative cells and, hence, may survive some of the milder heating treatments, specially in products with a high fat content that could act as a protecting agent in the heat resistance of *Listeria monocytogenes* (Bem Embarek & Huss, 1993; Gorris, 1995; Mackey & Bratchell, 1989).

Further research on *Clostridium botulinum* type E is needed since this microorganism is capable of growing at low temperatures and can survive lower heat treatments. The lowest temperature limit established for growth and toxin production by strains of psychrotrophic *Cl. botulinum* is 3.3 °C (Post et al., 1985). Because of the difficulties to maintain the cold chain and the common temperature abuses throughout distribution, retail markets and consumer, additional hurdles must be included (Genigeorgis, 1993; Tolstoy, 1991). Our results show the thermal abuse at 10 °C reduces the shelf life of sous vide salmon and allows for the growth of sporeforming bacteria thus being a potential risk. The continuity in the cold chain apparently results in a reduction of the surviving microorganisms and the reduced ability to recovery from heat injury.

Salmon processed at 90 °C for 15 min had an acceptable microbiological quality after 45 days of storage at 2 °C, thus highlighting the importance of heat treatment and storage temperature. Indeed, the cells surviving that heat treatment were unable to reproduce at 2 °C. Salmon which received a heat treatment of 65 °C was not any more effective at 2 °C than at 10 °C.

The decrease in score of the sensory characteristic in the fish processed at 65 °C is comparable to the increase in bacterial numbers during the same period. Although on Day 0 the salmon slices processed at 90 °C for 15 min had a score lower than those processed at 65 °C, this score did not change substancially after storage, and retained a reasonable acceptability until the end of the storage period, since microbial growth was limited.

Salmon is vulnerable to heat treatment, since there is a protein precipitation during heat treatment at about 70 °C (Bergslien, 1996). A white protein layer on the fish

is unacceptable for the consumer, and a high heat treatment can also cause a hard and a dry texture of the fish products.

Off-odour in salmon processed at 65 °C could be related to bacterial and chemical changes during processing. Since salmon has a high fat content it is very vulnerable to oxidation and rancid taste development. However, using vacuum packaging most of the oxygen in the surrounding atmosphere is removed and rancidity delayed. As oxidation is delayed, sous vide technology is more adequate in maintaining the quality of fat fish than other technologies such as freezing.

It can be concluded that treatment of 90 °C for 15 min was the most effective for extending the shelf life of fish. However, under these conditions the sensory characteristics are not optimal, particularly with regards to appearance. The storage temperature has great importance in order to ensure the quality and safety of sous vide products, together with the heat treatment. Since, the sensory characteristics of salmon are vulnerable to high temperatures and safety of products processed at low temperatures cannot be ensure, additional hurdles must be applied in order to ensure the safety of sous vide salmon processed at low temperatures.

In order to established adequate processing combinations of time/temperature to ensure the microbial quality and the shelf life of sous vide processed fish, initial contamination and fat content should be considered.

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