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Effect of high pressure processing in reducing *Listeria* spp. and on the textural and microstructural properties of cold smoked salmon (CSS)

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Ágrip á íslensku:

Meginmarkmið verkefnisins var að rannsaka áhrif háþrýstings (400-900 MPa) á dauða bakteríunnar *Listeria monocytogenes* og gæðaþætti (myndbyggingu, áferð og lit) í kaldreyktum laxi eftir meðhöndlun í 10, 20, 30 og 60 sekúndur. Áhrif á heildarfjölda loftháðra baktería, mjólkursýrugerla og *Bacillus* gróa voru einnig rannsökuð. Tvær tilraunir voru framkvæmdar, önnur í Júlí 2005 og hin í Nóvember 2006.

Rannsóknin sýndi að meðhöndlun með háþrýsting í stuttan tíma væri árangursrík til að bæta gæði og öryggi kaldreyktra afurða. Vegna breytinga í útliti og áferð afurðanna er þörf á frekari rannsóknum. Þessi nýja aðferð lofar góðu til að mæta kröfum um lengra geymsluþol á reyktum laxi.

Rannsóknin hefur mikið gildi fyrir iðnaðinn, vegna þeirrar nýjungar að nota háþrýsting í stuttan tíma (sekúndur) til að eyða bakteríunni Listeríu í reyktum laxi og auka þannig geymsluþol þessarar verðmætu afurðar.

Lykilorð á íslensku: *háþrýstingur,Listeria,myndbygging,áferð,litur*

Summary in English:

The main object of this research was to study the effects of high pressure processing (400-900 MPa) on the survival of *Listeria monocytogenes* and the characteristics (microstructure, texture and colour) of cold smoked salmon when it was processed for 10, 20, 30 and 60 seconds. The changes in counts of total aerobic bacteria, lactic acid bacteria and *Bacillus* spores were also studied. Two experiments were carried out, one in July 2005 and the second in November 2006.

It is concluded here that the combination of high pressure and short time treatment is very effective to improve the quality and safety of cold smoked products. However, because of the changes in the visual appearance and texture, further studies are necessary. This new development is promising to meet requirements for prolonged shelf life of ready-to-eat cold smoked salmon with high microbiological quality and safety.

This study is of high industrial relevance because it combines the innovative approach of using high pressure processing for short time (seconds) to reduce the number of *Listeria* in cold smoked salmon and thereby extend the shelf life of this valuable product.

English keywords: *High pressure, Listeria, microstructure, texture, colour*

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1. INTRODUCTION

Global aquaculture production has increased in recent years and is now growing more rapidly than all other animal food production sectors. According to FAO statistics, the sector has increased worldwide at an average rate of 9,2 % per year since 1970, compared with only 1,4 % for capture fisheries and 2,8 % for terrestrial farmed meat production systems. Aquaculture production reached 45.5 million tonnes in 2004 compared to total world fish production of 140,5 million tonnes (FAO, 2005).

Among the various species cultured worldwide is Atlantic salmon (*Salmo salar*). The production of this species has trebled during the last ten years and reached 1.24 million tonnes in 2004, accounting for 2,74 % of the total aquaculture production. The biggest producers are Norway (566.000 tonnes), Chile (349.000 tonnes), Scotland (160.000 tonnes) and Canada (97.000 tonnes) (FAO, 2005). A great proportion of the farmed Atlantic salmon reaches the worldwide market as a cold smoked product, but smoking is one of the oldest processing methods that have been used to extend the shelf life of food. While the world production increases, problems related to bacterial contamination in smoked salmon still persist (Ben-Embarek *et al*., 1997; Hansen *et al*., 1998; Gram, 2001, Gombas *et al*., 2003; Gudmundsdottir *et al*., 2005). Due to the fact that the temperature during the smoking process never exceeds 28°C it does not have any significant effect on *Listeria monocytogenes*. *L. monocytogenes* has been recognized as a major food pathogen and has created problems for a long time for both producers of smoked salmon and consumers (Ericsson *et al*., 1997; Loncarevic *et al*., 1997; Farber *et al*., 2000). Many processing parameters have been tested, such as a combination of NaCl and low temperature but it could not prevent the growth of *L. monocytogenes* (Gimenez & Dalgaard, 2004). There is no control point during cold-smoking process that can guarantee the elimination of *L. monocytogenes* in the final product. However, the occurrence of *L. monocytogenes* in the finished cold-smoked fish products can be minimized by:

1) obtaining the primary product from known sources (for example, those with a history of non-contaminated fish);

2) following strict adherence to GMPs to prevent recontamination during processing; and

3) inhibiting growth of any survivors by marketing the product frozen, or by using salt

and other preservatives (e.g. diacetate and lactate) or by the use of bioprotective cultures that can inhibit growth at refrigerated temperatures (Huss *et al*., 1995; Huss *et al*., 2000; Tomé *et al*., 2006; Fonnesbech *et al*., 2006). If the organism cannot be eliminated and growth-inhibiting steps are not introduced, the hazard can be controlled by limiting shelf life (at 4°C) to ensure that no more than 100 cells/g are present at the time of consumption. Time limits may need to be established by each processor because the time limit should reflect the initial level of the organism in freshly produced products. Based on these facts it is necessary to develop a new processing method for smoked salmon, which ensures the safety of the product. Over the last decade, investigators have explored the possibilities of applying some novel technologies in the fight against this pathogen. High pressure processing is one of these promising technologies. It is a non-thermal preservation technique that depends on pressure, time, temperature and product characteristics and it allows microorganisms to be inactivated with fewer changes in texture, colour and flavour compared to conventional technologies (Knorr, 1993; Cheftel, 1995; Torres & Velazquez, 2005). Most of the studies related to the application of high pressure processing of seafood have been conducted on its effect on proteins (Angsupanich *et al*., 1999), muscle colour (Amanatidou *et al*., 2000; Ohshima *et al*., 1993), lipids (Chevalier *et al*., 2001; Ohshima *et al*., 1992) and on bacteria (Amanatidou *et al*., 2000; Smelt, 1998). The effects of high pressure processing on *Listeria monocytogenes* have been studied by several authors. Some have studied the effect of treatment time (Patterson *et al*., 1995; Simpson & Gilmour, 1997) or pressure (Shigehisa *et al*., 1991), while others (Ritz *et al*., 2000) have studied the minimum conditions of the three parameters (pressure, time, temperature) to maximize the reduction of cell viability. It has been stated that traditional high pressure processing is not efficient to prevent growth of *Listeria* and to keep the colour and texture in its initial condition. Laksman & Dalgaard (2004) showed that pressure at 250 MPa did not inactivate *L. monocytogenes* but lag phases of 17 and 10 days were observed at 5 and 10°C, respectively. Pressure at 200 MPa had a marked effect on both the colour and the texture of chilled cold-smoked salmon. Other studies have shown that high-pressure treatment of salmon spread extended shelf life from 60 to180 days at 3 or 8°C without any significant chemical, microbiological, or sensory changes and completely inactivated pathogens present in the inoculated sample (Carpi *et al*., 1995).

The resistance of microorganisms to pressure varies considerably depending on pressure, time and temperature. By increasing the pressure and time of treatment the number of *L. monocytogenes* in hard cheese, meat products and fruit juice decreased proportionally (Fonberg-Broczek *et al*., 2005). *L. monocytogenes* has shown to be very sensitive to pressure changes and because of the cost of high pressure processing it would be desirable to increase treatment pressure and keep the treatment time short (Chen *et al*., 2006). Most of the studies related to application of high pressure processing of seafood apply 200–700 MPa pressure for 3, 5, 10, 15 or 20 minutes (Torres &Velazquez, 2005). However, new development in high pressure technology enables the high pressure to be reached in 10 seconds. The objective of this research was to study the effect of high pressure processing (400–900 MPa) on the survival of *Listeria innocua* and the characteristics (microstructure, texture and colour) of cold smoked salmon when it was processed for 10, 20, 30 and 60 seconds. The changes in counts of total aerobic bacteria, lactic acid bacteria and *Bacillus* spores were also investigated. Two experiments were carried out, one in July 2005 and the second one in November 2006.

2. MATERIALS & METHODS

2.1. Preparation of bacterial culture

In order to decide which strain should be selected for this study, a pre-study was performed on six strains of *Listeria monocytogenes* and two strains of *Listeria innocua*. All strains were obtained from an IFL-collection. The strains were cultivated overnight in Tryptic Soy Broth supplemented with 0.6g Yeast Extract/100g (TSB-YE, Difco) at 35°C and subcultured twice. All these strains have been compared by a genetic typing technique using pulsed-field gel electrophoresis (Gudmundsdottir *et al*., 2005).

The following strains were tested for the efficacy of high pressure treatment on reducing the bacterial number:

Listeria innoca (EU2173/E-34)

Listeria innocua (EU2172/E-33)

Listeria monocytogenes (E1)

Listeria monocytogenes (E5)

Listeria monocytogenes (H-01-170-2)

Listeria monocytogenes (L-327)

Listeria monocytogenes (L-435)

Listeria monocytogenes (L-462)

2.2. Raw material and sample preparation.

Two experiments were carried out, one in July 2005 and another in November 2006. The salmon used as raw material was obtained from the same producer in both cases. Atlantic salmon (*Salmo salar*) was farmed and slaughtered at Rifos hf. on the north coast of Iceland. A sample of 50 fishes of 3-4 kg each was randomly selected from a large population on the slaughtering day. Two days later the salmon was filleted and cold-smoked at Reykofninn smokehouse, which is located in the Reykjavik area. Each fillet was packed in vacuum and delivered immediately to the Icelandic Fisheries Laboratory (IFL).

2.2.1. Experiment 1.

The day after the fillets was delivered to IFL, the fillets were cut into 30-50 g pieces (4 cm x 6 cm) and vacuum packed by Magic VacTM Champion. Before the packaging, one half of the samples were contaminated (spiked) with 1 mL of 2×10^5 CFU/mL *L. innocua* to obtain a final concentration of 10^3-10^4 CFU/g. Dilution was made in a Butterfield´s buffer. The other half were not contaminated, but high-pressure processed and used for textural and microstructural analysis.

The day after (day 5 from slaughter) the samples were transported to the Berlin University of Technology, Department of Food Biotechnology and Food Process Engineering. On day 6 and 7 the smoked salmon samples were processed with high-pressure. In this experiment 400 500, 600, 700, 800 and 900 MPa pressures were applied for 10, 20, 30, and 60 seconds.

On day 8 the samples were transported back to Iceland where they were examined for microbiology at IFL and texture and microstructure at the IceTec. The temperature during this transportation was documented with a logger (data not shown). Water content, water activity, lipid (fat) content, pH, NaCl content and TBA-value were determined to characterize the material used for this study. Samples for these analyses were taken from the raw material and at different steps throughout the process.

2.2.2. Experiment 2.

Similar methods were used to contaminate the samples as in experiment 1. The day after the fillets was delivered to the IFL, the fillets were cut into 30*50 g pieces (4 cm x 6 cm) and part of it were vacuum packed (VAC) by Magic VacTM Champion and same number were packed in modified atmosphere (MAP) (50% CO_2 and 50% N_2). Gas composition in the headspace of two packs was measured at packaging and at sampling. Septums were put on the MA-packs and the gas measured with PBI Dansensor (CheckMate 9900). During the transport of samples between Iceland and Berlin this ratio decreased and a higher ratio of oxygen was detected (data not shown). Before packaging, one half of the samples were contaminated (spiked) with inoculate so the final concentration of *L. innocua* was <1, 2,5, 25 and 250 CFU/g in the samples. Dilution was made in a Butterfield´s buffer. The other half were not contaminated, but high-pressure processed and used for textural, microstructural and sensory analysis. The temperature during this transportation was documented with a StowAway Tidbit temperature logger from Onset. Examples of the measurement of the ambient temperature during transport of cold smoked salmon from Reykjavik-Berlin-Reykjavik are shown in Figure 1. In this experiment there were 500 MPa and 700 MPa pressures applied for 30 and 60 seconds.

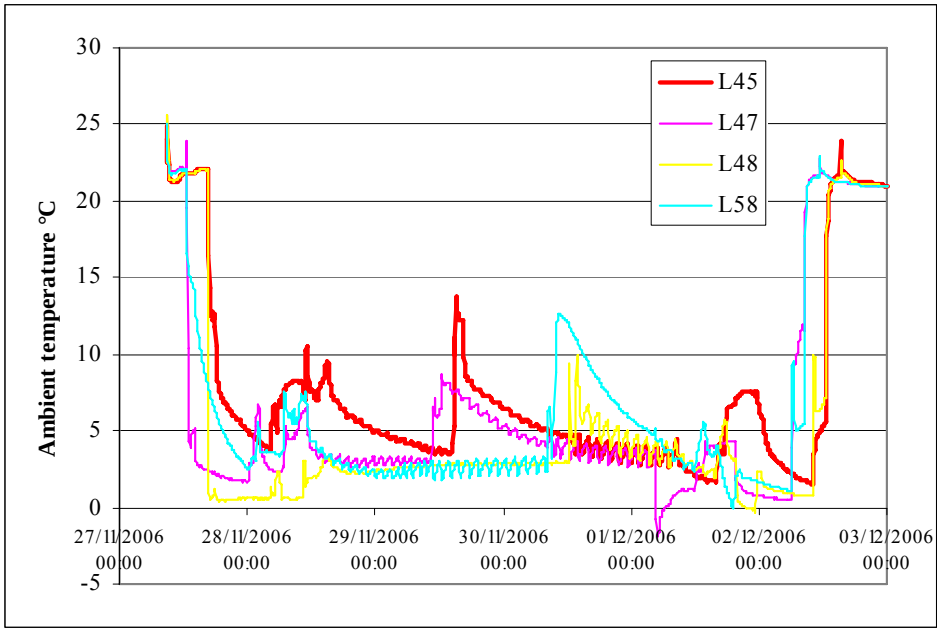


Figure 1. Examples of results from temperature measurements during transport of cold smoked salmon from Reykjavik - Berlin - Reykjavik.

2.3. Water -, fat- and salt content, lipid oxidation, water activity and pH analysis

Water content was measured according to ISO 6496 (1999). The sample was heated in an oven at 103°C +/-2°C for four hours. Water loss corresponds to the weight loss.

Fat content was measured according to AOCS (1997) with modifications according to Application note Tecator no AN 301. The sample was extracted with petroleum ether, boiling range 40-60°C. The extraction apparatus was 2050 Soxtec Avanti Automatic System.

Salt content was measured according to AOAC (2000). Soluble chloride was extracted from the sample with water containing nitric acid. The chloride content of the solution was titrated with silver nitrate and the end point was determined potentiometrically.

Lipid oxidation: Thiobarbituric (TBARS) test was used to measure the lipid oxidation or TBA values. TBARS reactive substances were determined by a modified version (Sørensen & Jørgensen, 1996) of the extraction method described by Vyncke (1970, 1975) with a few modifications. The sample size was reduced to 15 g and homogenized with 30 mL of 7,5% trichloroacetic acid solution containing 0,1% of both propyl gallate and EDTA. The absorbance of samples and standards were measured at 530 nm. TBARS,

expressed as µmol malondialdehyde per kilogram of sample (µmol MDA/kg), was calculated using malondialdehyd-bis-(diethyl acetate) as standard.

Water activity was measured by using Aw - Wert - Messer (Durotherm) capsule at 22°C and kept in an incubator for at least four hours before a reading was taken. Calibration and temperature corrections were made according to the manufactures instructions.

pH analysis. Approximately five grams of minced tissue was mixed with the same amount of water (weight), and pH measurement was made using the PHM 80 Portable Radiometer Analytical Copenhagen, with an immersed electrode according to instructions of the manufacturer's manual.

All analysis were done in duplicate or triplicate (lipid oxidation).

2.4. Microbial analysis

Aerobic plate count (APC) was determined by spread plating on modified Long and Hammer (LH) agar according to van Spreekens (1974) with 1 g NaCl/100 g (15°C for 5-7 days). Lactic acid bacteria (LAB) were determined by spread plating on Nitrite Actidione Polymyxin (NAP) agar for 22°C for 5 days (Modified from: Davidson & Cronin (1973). To confirm the existence of LAB, catalase test was performed. MPN technique was used in the enumeration of Listeria and the media used and steps were according to USDA (2002). UVM was used as the pre-enrichment step followed by inoculation to Fraser broth with a subcultured to a plate of MOX from all black tubes. In this case the 3-tube 3 dilution (3x3) format was used. The detection limit for this method is MPN 0,3 CFU/g or 1 CFU/100 ml (solid or liquid sample). One millilitre of the homogenates was transferred into the first of three test tubes, each containing 10 ml of UVM in double concentration. The next three consecutive dilutions were made in single or recommended concentration of UVM. This made it possible to achieve sampling sizes of 1, 0,1 and 0,01 g of salmon per 10 ml.

For Bacillus spore count 10 mL of the 1/10 mixture were heated at 75°C for 30 min. The pour plate technique was done on Plate Count Agar (Difco). Plates were incubated at 35°C for two days. All analyses were done in duplicate.

2.5. High pressure processing (HPP)

Efficacy of high pressure treatment in reducing Listeria spp. (pre-study).

The pre-study of high-pressure processing was carried out at IceTec using an Autoclave Engineering (Erie, PA, USA) high pressure system. Maximum design pressure for the system was 500 MPa at room temperature. The size of the sample holder was 2,4 L. Pressure transmitting medium was 5 % oil (Kutwell 42 from Exxon) in water solution. Cell suspensions (1 mL) inoculated to a gauze were placed in a plastic bag and vacuum packed by Magic VacTM Champion. The bacterial strains were high-pressure treated at 350 MPa for 5 and 20 min at 22 °C. A total of five minutes were needed to reach 350 MPa whereas pressure decompression took 30 s.

High pressure processing of smoked salmon.

High pressure processing of smoked salmon was carried out at The Berlin University of Technology, Department of Food Biotechnology and Food Process Engineering, Berlin, Germany, in a designed and constructed lab-scale high pressure system (High Pressure Research Center, Unipress Equipment Division, Sokolowska 29/37, Warsaw, Poland). Maximum design pressure for the system was 1000 MPa at an operating temperature range of –25 to 100°C. The volume of the sample holder was 0,75 L. A mixture of water and glycol (propylene glycol (1.2 propanediol; 50:50) was used as a pressure transmitting medium. The most HPP-resistance strains (L. innocua (E-34)) from the pre-study were chosen to continue the main study. In the first experiment spiked and unspiked vacuum packed salmon samples were high-pressure processed at 400, 500, 600, 700, 800 and 900 MPa pressure for 10, 20, 30 and 60 s. Temperature during holding time was 42°C in all trials. Unpressurized samples were used as a control. Ten seconds were needed to reach 400, 500 and 600 MPa and 20-25 s to reach 700, 800 and 900 MPa. In the second experiment the vacuum- and MA-packed samples were high-pressure processed at 500 and 700 MPa pressure for 30 and 60 s. For few samples the temperature during the holding time was lowered to 5°C. These samples were just used for sensory analysis to see if the texture and the appearance would change by decreasing the temperature.

Unpressurized samples were used as a control as in experiment 1. Following the high-pressure processing the samples were transported back to Iceland where they were examined for microbiology at IFL and texture and microstructure at IceTec.

2.6. Storage trials with spiked samples of cold smoked salmon

The storage trial was just carried out in experiment 1 and it consisted of samples after two HPP treatments (500 and 900 MPa), as well as untreated samples used as a control. The samples were tested for microbial changes (APC, LAB and *Bacillus* spores), the survival of *Listeria innocua* and for lipid oxidation.

2.7. Microstucture

Samples for microstructure analysis were collected from the HPP treated salmon pieces using a cork knife, 11 mm in diameter. They were embedded in plastic tubes 15 mm in diameter, 30 mm length (Kartell, Novigilo, Italy) containing O.C.T. compound (embedding medium) (Tissue Tek, Sakura, Torrance, CA) and frozen in liquid nitrogen. Freezing (below –80°C) occurred in approximately 40 s. The frozen specimens were stored at –80°C until cryosectioning and staining. The specimens were sectioned frozen at –27°C in a Leica CM1800 cryostat (Leica, Heidelberg, Germany) for transverse cuts, 10 μm thick. Sections were mounted on glass slides and stained using Orange G (0,5g CI 16230 (Polysciences, Warrington, PA), 99,0 mL distilled water, 1,0 mL acetic acid). The sections were washed with distilled water and stained for five minutes in methyl blue solution (0,07 g CI 42780 (Sigma, St. Louis, MO), 99,0 mL water 1,0mL acetic acid). The stained samples were washed for five minutes with distilled water, dried at room temperature and mounted with MOUNTEX (Histolab, Göteburg, Sweden). The samples were examined in an optical microscope, Leica DM RA2 at 100 x magnification and images captured using a Leica DC300F digital camera mounted on the microscope. The pictures of the microstructure of samples from this experiment where analysed in Leica QWin software, where the amount of non cellular material was measured. In other words, the spaces between cells where analysed.

2.8. Texture measurements

Warner-Bratzler shearing blade with a thickness of 3.21 mm, length of 125 mm and width of 70 mm was assembled to the TA.XT2® Texture Analyser (Surrey, England). It shears or cuts through the sample with a test speed of 2.5 mm/s. The computer software was set to plot a force versus time plot and the results were expressed as the maximum peak force (shear force in N) required to shear through the sample. Other parameter calculated was the area under the curve which describes the total amount of work required to cut through the sample. This test method incorporates compression of fibres beneath the blade, tension in the adjoining fibres and shearing of the fibres (Veland & Torrissen, 1999; Bouton *et al*., 1975). Textural measurements were performed on pieces of pooled samples from the fillet in experiment 1 and under the dorsal fin of the salmon fillet in experiment 2. Each sample was measured three times.

2.9. Colour measurements

The intensity of the flesh colour was measured by using the MiniScan XE plus from HunterLab using the D65 light source. The instrument records the L* (lightness – intensity of white colour), a* (redness – intensity of red colour) and b* (yellowness) values. Each sample was measured three times.

2.10. Sensory analysis

Samples of smoked salmon were evaluated by four sensory experts with regard to appearance and texture. Untreated sample was used as a control. Samples of cold-smoked salmon were HPP treated at 500 Mpa at two different times (30 and 60 s) and two different temperatures (5°C and 22°C). The panellists evaluated the samples using the "**difference from control"** (Degree of difference) method originally described by Aust *et al*., (1985). This method is used when the objective is the combination of determining if a difference exists between samples and a reference or control sample and estimating the size of such a difference.

2.11. Statistical analysis

Analysis of variance (ANOVA) was carried out on microbial- and textural data together with the data from colour measurements in the statistical program NCSS 2000 (NCSS, Utah, USA) to compare the effects of different treatments. The program calculates multiple comparisons using Turkey-Kramer Multiple-Comparison Test. Significance was established at $p \leq 0.05$

3. RESULTS FROM EXPERIENT 1

3.1 Characteristics of cold smoked salmon

The cold smoked salmon contained 37,25 ± 0,4% dry matter and 3,25 ± 0,1% (w/w) NaCl corresponding to 4,92 ± 0,2% (WPS) water phase salt. The fat content was 9,6 ± 0,4% and pH was 6,12 ± 0,1. The TBARS content was 3,9 ± 0,09 μmol/kg. Robles-Martinez and Cervantes (1982) proposed a TBARS content of 18 μmol/kg as a general indicator of rancidity in frozen fish. The authors are not aware of limits or guidelines for rancidity in cold smoked salmon.

Figure 2 shows distribution of aerobic plate count (APC) on LH and total count of lactic acid bacteria (LAB) on NAP in cold smoked salmon at different processing steps. The bacterial count increased from <1 log CFU/g to 2,6 ± 0,2 log CFU/g and to 2,7 ± 0,1 log CFU/g, respectively. The increase of APC occurred after the salting and washing step while count of LAB increased steadily during the whole process and in the final product lactic acid bacteria were the dominant flora of the total count. Neither *Listeria* nor *Bacillus* spores were detected in any of these samples.

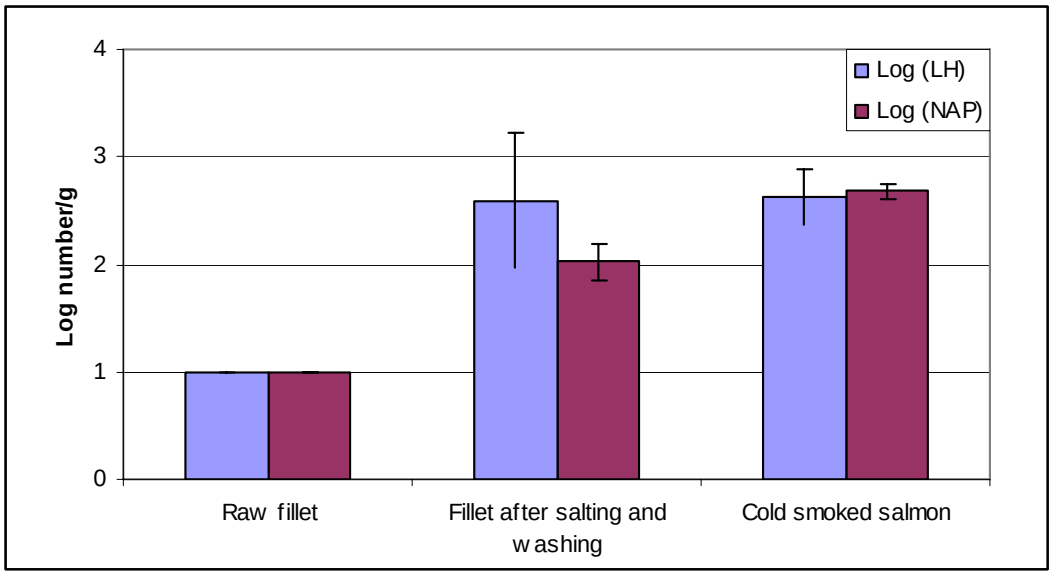


Figure 2. Distribution of bacteria during processing of cold smoked salmon

3.2 Challenge test with *Listeria innocua*

The number of *L. innocua* in overnight culture was $2{,}03\times10^{9}$ CFU/mL. Cold smoked salmon was spiked with 10^{4} dilution of overnight culture and the final number of *L. innocua* in the spiked samples was $4{,}5\times10^{3}$ CFU/g. The inactivation of *L. innocua* by HPP is shown in Table 1. Increasing the pressure from 400 MPa to 900 MPa had a significant effect ($p<0{,}05$) on the reduction of the bacteria with the 900 MPa most efficient. At 400-500 MPa the reduction of bacteria was less then 1-1.5 log cycle. By increasing the pressurization time at 600 and 700 MPa the reduction increased. These results indicate that the pressure needs to be up to 700-900 MPa to be efficient enough to reduce the number of *Listeria innocua* in cold smoked vacuum packed salmon with regard to the safety of the product. The initial number of the bacterium in the spiked product tested was rather high, around 4500 CFU/g, which is maybe not the real situation on the market.

Table 1. Survival of *L. innocua* in HPP treated cold smoked salmon . Most Probable Number (MPN).

		CFU/g (MPN)			
	High pressure (MPa)	**10 sec**	**20 sec**	**30 sec**	**60 sec**
spiked	**400 MPa**	**>110**	**>110**	**>110**	**>110**
spiked	**400 MPa**	**>110**	**>110**	**>110**	**>110**
spiked	**500 MPa**	**>110**	**>110**	**>110**	**110**
spiked	**500 MPa**	**>110**	**>110**	**>110**	**110**
spiked	**600 MPa**	**>110**	**24**	**1,5**	**0,36**
spiked	**600 MPa**	**>110**	**9,3**	**2,3**	**<0,3**
spiked	**700 MPa**	**0,73**	**<0,3**	**0,36**	**<0,3**
spiked	**700 MPa**	**0,91**	**<0,3**	**<0,3**	**<0,3**
spiked	**800 MPa**	**0,3**	**<0,3**	**<0,3**	**<0,3**
spiked	**800 MPa**	**<0,3**	**<0,3**	**<0,3**	**<0,3**
spiked	**900 MPa**	**<0,3**	**<0,3**	**<0,3**	**<0,3**
spiked	**900 MPa**	**<0,3**	**<0,3**	**<0,3**	**<0,3**
unspiked	**500 MPa**	**<0,3**			**<0,3**
unspiked	**500 MPa**	**<0,3**			**<0,3**
unspiked	**900 MPa**	**<0,3**			**<0,3**
unspiked	**900 MPa**	**<0,3**			**<0,3**

3.3 Storage trials with spiked samples of cold smoked salmon

3.3.1 Microbial analysis

The pressurised samples were stored at 5,5°C and tested for psychrotrophs (aerobic plate count at 15°C), LAB count, *Listeria* and *Bacillus* spores on days 5, 12, 26 and 41. The initial counts in the samples are described in chapter 3.1. The changes in the level of APC and LAB count are shown in Tables 2 and 3. The growth of *L. innocua* is shown in Figures 3 and 4. Four different pressure times were tested. When the pressure applied was 500 MPa the number of *L. innocua* was only reduced significantly ($p<0,05$) after 60 s as shown in Figure 3. One log reduction was observed and the number decreased during storage indicating some damaged cells which could not recover. No reduction was observed after 10 s and it was unchanged until after more than 26 days. By increasing the treatment time to 20 and 30 s it seems like the bacteria were damaged in some way because the total number of *Listeria* decreased during the first 12 days of storage but after

that the number increased unlike what happened after 60 s treatment time. No *Listeria* spp. was detected in unspiked unpressurised control samples, not even after 41 days storage at 5,5°C (data not shown), indicating a good quality and safety of the raw material used in this experiment. Figure 4 shows the reduction of *L. innocua* at 900 MPa for 10, 20, 30 and 60 s. No *Listeria* was detected immediately after HPP treatment at 900 MPa but after storage at 5,5°C for 26-41 days low level (0,3-20 CFU/g) was detected. That indicates that some of the cells survived and could recover after some storage time. The initial concentration of APC on LH was 420 CFU/g and counts of lactic acid bacteria on NAP was 480 CFU/g which indicates that by using LH at 15°C some lactic acid bacteria are missed as shown in Figure 2 in chapter 3.1.

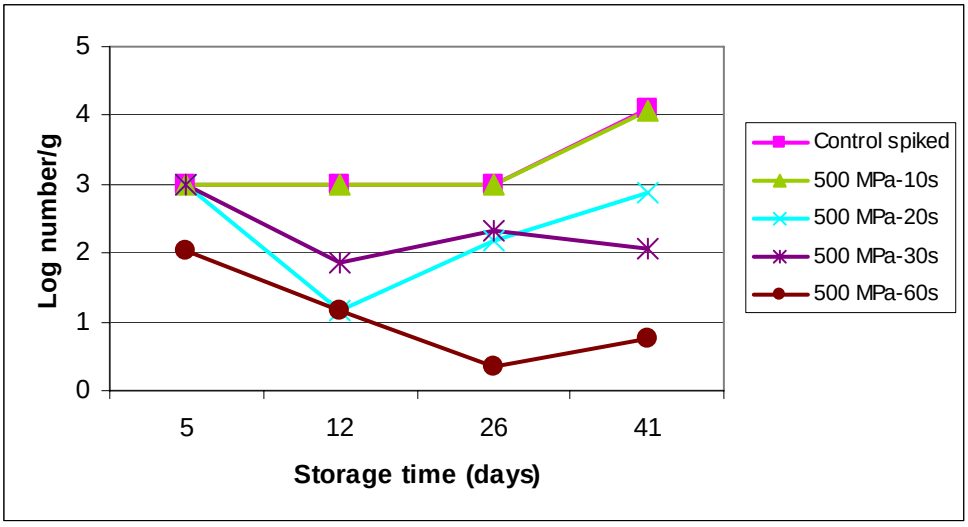


Figure 3. Growth of *Listeria innocua* (MPN) during storage of cold-smoked salmon at 5,5°C. Cold smoked salmon had been treated with 500 MPa for 10, 20, 30 and 60 s. Samples not treated with HPP used as control. Means of two samples.

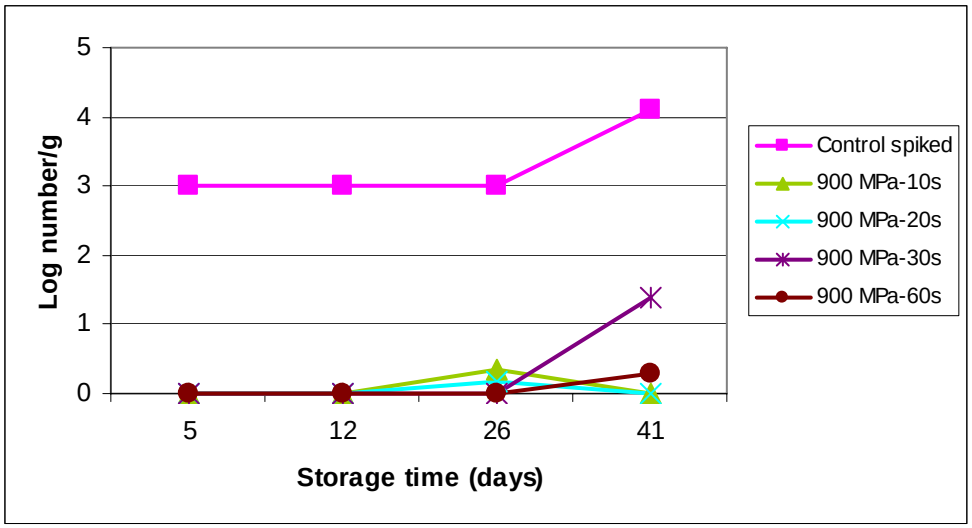


Figure 4. Growth of *Listeria innocua* (MPN) during storage of cold-smoked salmon at 5,5°C. Cold smoked salmon had been treated with 900 MPa for 10, 20, 30 and 60 s. Samples not treated with HPP used as control. Means of two samples.

Aerobic plate counts on LH and counts of lactic acid bacteria on NAP (log no./g) during storage period are shown in Tables 2 (500 MPa) and 3 (900 MPa). At the beginning of the storage trial the APC was 0,5-1 log lower in HPP treated samples at 500 MPa samples compared to untreated samples. During the storage the number increased steadily and after 41 days storage time about 2 Log increase was observed. Lowest number was observed in samples treated with 500 MPa for 60 s indicating more effect related to longer treatment time. The number of lactic acid bacteria had reduced significantly compared to untreated samples but during storage 4-5 log increase was observed indicating that the lactic acid bacteria seems to be very quick to recover after HPP treatment. When the samples were treated at 900 MPa pressure a significant reduction of APC of log 2-3 was observed (on day five) and some increase was observed after treatment time for 10 and 20 s but not after 30 and 60 s indicating a good effect when the treatment time was extended. Similar effects were observed when results from LAB count were evaluated. The LAB count was however considerably higher than APC or log 5,87, compared to log 4,71 in untreated samples. The HPP treatment had significant effect, both at 500 and 900 MPa and it was clear that the recovery of LAB bacteria after 900 MPa treatments was difficult. A reduction of 4-5 logs was observed after treatment time for 20-60 s at 900 MPa. During storage for 26 days these samples showed 4-6 log lower number/g compared with number in untreated samples, indicating that lactic acid

bacteria are sensitive to HPP treatment. The increase of LAB was considerably faster after HPP treatment, indicating that they can recover more quickly perhaps because of the lack of competition from other background flora. The recovery of LAB after HPP treatment at 900 MPa was difficult as can be seen from the result in Table 3. Bacillus spores were not detected in any samples.

Table 2. Microbial evaluation in vacuum packed cold smoked (spiked) after HPP treatment at 500 MPa during storage at 5,5°C.

	Aerobic total count					Lactic acid bacteria				
Treatment time (sec)	0	10	20	30	60	0	10	20	30	60
Storage time (days)										
5	3,68±1,21	2,86±0,64	2,52±0,04	3,49±0,96	2,80±0,12	5,55±0,77	1,45±0,64	2,45±0,04	2,69±0,56	2,18±0,04
12	3,47±0,38	2,27±0,52	2,48±0,14	2,86±0,28	3,24±0,80	5,40±3,03	3,02±2,01	2,24±0,14	3,58±1,8	3,77±2,81
26	4,71±0,56	3,65±0,04	3,22±0,71	5,28±1,44	4,27±0,18	8,14±0,09	6,10±0,72	3,42±1,43	6,09±1,54	5,38±0,18
41	4,80±0,96	4,60±1,69	5,20±0,12	5,22±0,57	3,56±0,79	8,78±0,24	7,58±0,12	6,44±1,22	7,79±0,91	6,30±0,85

Table 3. Microbial evaluation in vacuum packed cold smoked (spiked) after HPP treatment at 900 MPa during storage at 5,5°C.

	Aerobic total count					Lactic acid bacteria				
Treatment time (sec)	0	10	20	30	60	0	10	20	30	60
Storage time (days)										
5	3,68±1,21	1,45±0,21	1,00±0	2,11±1,57	1,79±1,12	5,55±0,77	na	1,30±0	1,57±0,81	1±0
12	3,47±0,38	1,45±0,21	1,30±0,43	1 ±0	1 ±0	5,40±3,03	4,52±1,01	1,15±0,21	1,39±0,55	1±0
26	4,71±0,56	2,29±0,30	2,99±0,27	1,69±0,12	1,45±0,21	8,14±0,09	3,11±0,86	4,00±0,48	2,00±0	2,37±0,52
41	4,80±0,96	na*	na	na	1,00±0	8,78±0,24	na	na	na	1,00±0

* na - not available

3.3.2 Lipid oxidation

Figure 5 shows the results from measurements on lipid oxidation. The results indicate that HPP accelerates slightly lipid oxidation if treated at 900 MPa for 30 s or longer. This difference was not significant ($p>0.05$). When treated at 900 MPa for 60 s, the TBA value decreased during storage but the difference was not significant ($p>0.05$). This value was probably due to the individual difference between samples. All these values are considered low and safe for consumption if compared to limits for frozen fish which is 18 µmol/kg according to Robles-Martinez & Cervantes (1982).

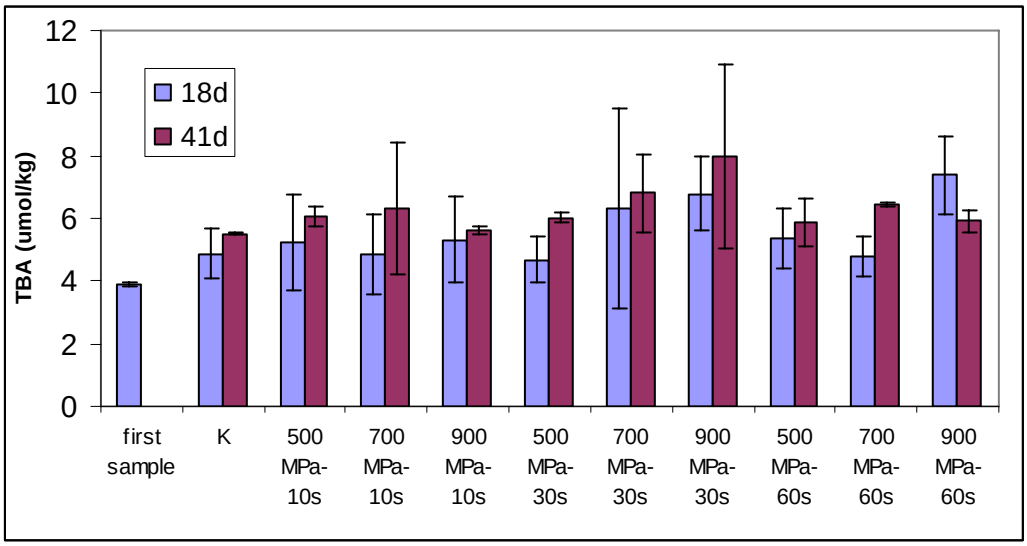


Figure 5. Changes in TBA-value as an indicator for rancidness during storage at 5,5°C for 18 and 41 days after HPP treatment at 500, 700 and 900 MPa for 10, 30 and 60 s.

3.4 Microstructure

The effect of high-pressure processing on the microstructure of cold smoked salmon is shown in Figure 6. With increased pressure and longer processing time the space between the cells increased. Effects were only minor at 400 and 500 MPa, but at 600 MPa and higher and for processing time of 30 and 60 s the effects were clear.

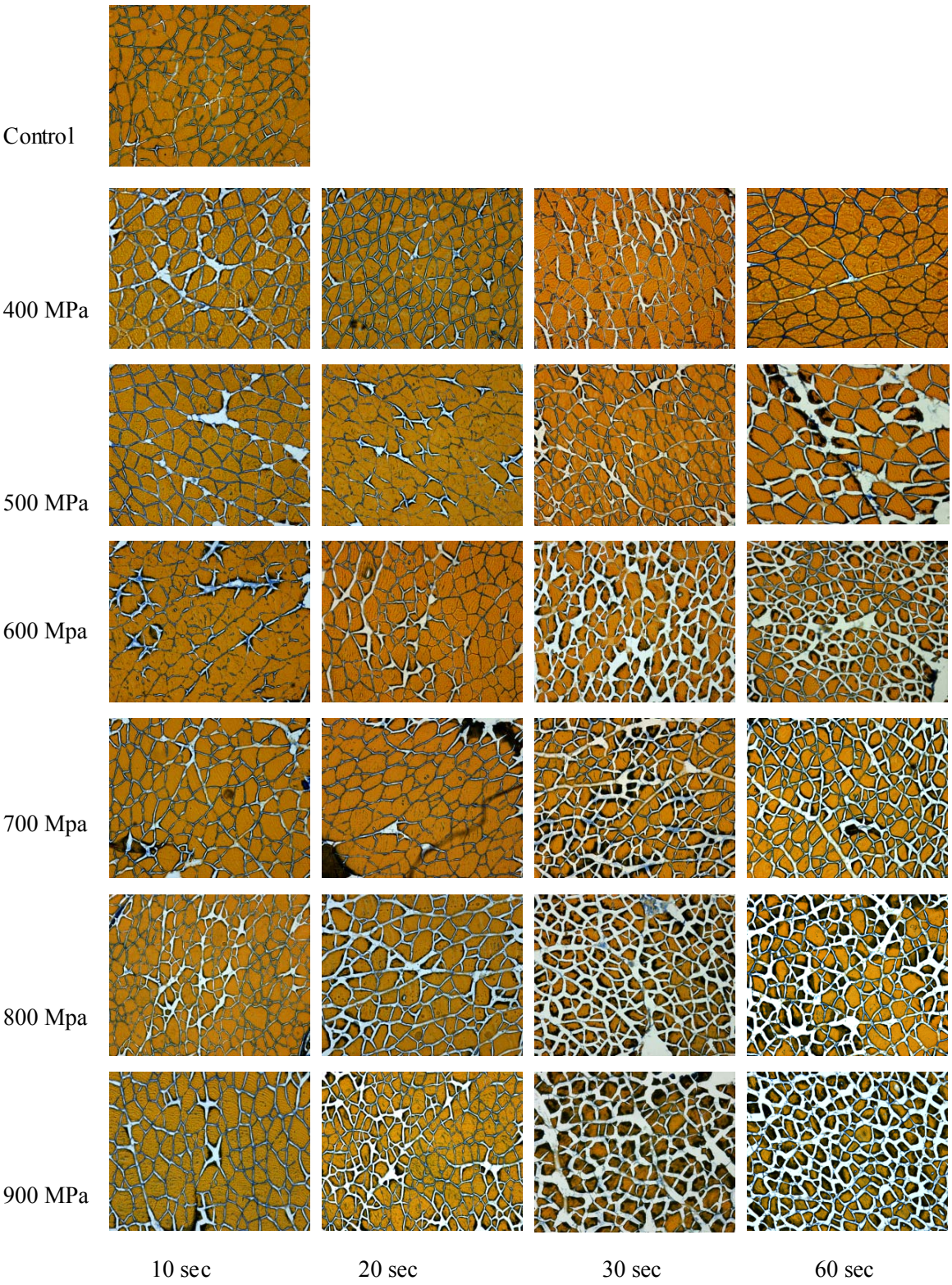


Figure 6. Effect of high pressure on the microstucture of cold smoked salmon

3.5 Textural properties

The effect of high-pressure processing (20 s) on the texture of the cold smoked salmon, measured as the maximum peak force required to shear through the sample, is shown in Figure. 7. Less force was needed to shear through the samples processed at 400 MPa than the control sample. The reason is that the control samples were pooled collected from various part of the fillet, but not from the same location on the fillet. Generally there was a gradual increase in toughness as the pressure was increased. Sample processed at 900 MPa in 10, 20 and 60 s was significantly tougher ($p<0.05$) than sample processed at 400 MPa. Each sample was measured three times. Similar effects were also observed for 10, 30 and 60 s processing time, for different HPP treatments.

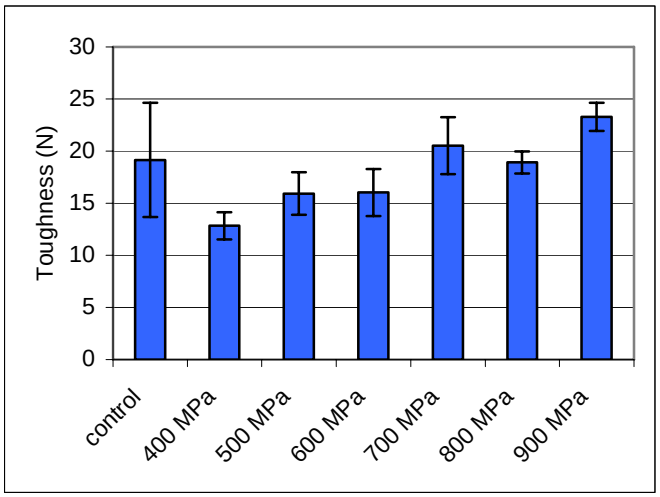


Figure 7. Effect of high pressure process for 20 s on smoked salmon.

3.6 Colour measurements

Figure 8 demonstrates the effect of high-pressure processing on the appearance of the smoked salmon when it is processed for 20 s. A clear gradient was in visible effects from control to 900 MPa. Similar gradient was also visible from 10 s to 60 s. Minor effects were already visible at 400 MPa. The effects were more visible for the interior of the salmon than the surface. The effect of high pressure treatment for 20 s on the lightness (L) is shown in Figure 9 and the effect on redness (a) is shown in Figure 10. The results in Figure 9 demonstrate an increase in lightness in all samples. Generally the increase in lightness was significant ($p<0.05$) at 600, 700, 800 and 900 MPa, compared to other

pressure treatments. The highest values at 700 and 800 MPa at 20 s were more likely due to variability between samples. Figure 10 demonstrates that the high-pressure process does not have any effects on the redness (a value) of the smoked salmon.

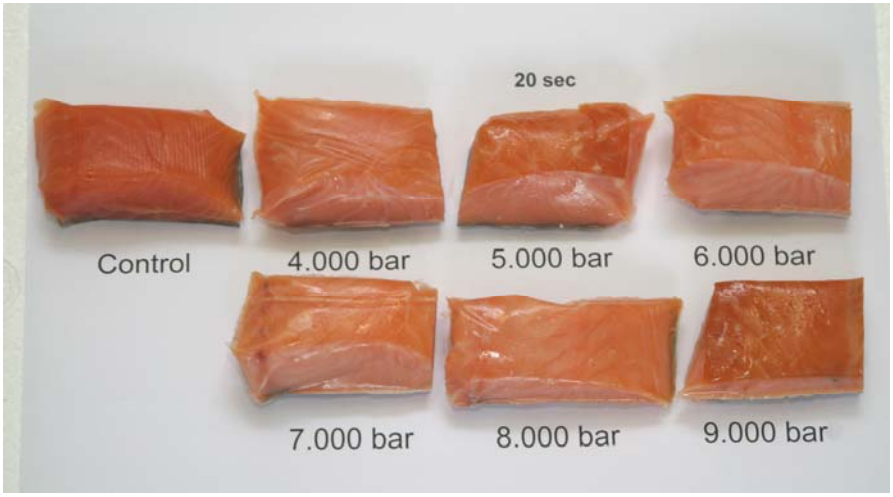


Figure 8. Photographs of high-pressure processed smoked salmon for 20 s.

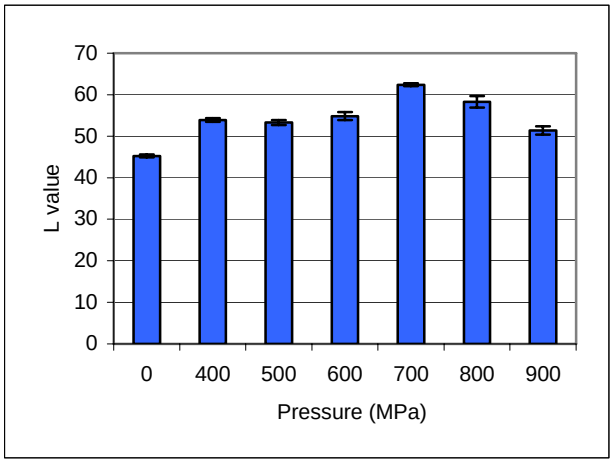


Figure 9. The lightness (value) of the high-pressure processed smoked salmon (20 s) measured by MiniScan XE plus from HunterLab.

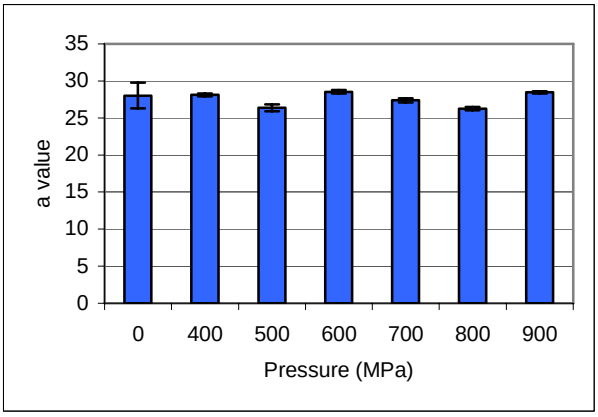


Figure 10. The redness (a-value) of the high-pressure processed (20 s) smoked salmon measured by MiniScan XE plus from HunterLab.

4. RESULTS FROM EXPERIMENT 2

4.1 Microbial analysis

In the second experiment the initial APC on LH in cold smoked salmon was Log 3.74 ± 0.77 CFU/g and count of lactic acid bacteria on NAP Log 2,51 ± 0 CFU/g. Neither *Listeria* nor *Bacillus* spores were detected in these samples. The level of *Listeria innocua* contamination was lowered down to >1 - 250 CFU/g of cold smoked salmon from 4500 CFU/g like it was in experiment 1. The pressure needed to decrease the number of *Listeria* from as low as 25 CFU/g down below the detection limit of the method used was still 700 MPa, which is the same as was needed in experiment 1 to ensure the safety of the product (Table 4). The samples packed in MAP showed a lower count but the difference was not significant ($p>0,05$).

Table 4. Survival of *L. innocua* in HPP treated cold smoked salmon at 25°C. Most Probable Number (MPN).

Treatment (MPa)	Time (s)	Package	Innoculation level (CFU/g)			
			<1	**2,5**	**25**	**250**
Control		MAP	<0,3	<0,3	3,3	78
500	30	MAP	<0,3	<0,3	0,36	67
500	60	MAP	<0,3	<0,3	0,30	46
700	30	MAP	<0,3	<0,3	<0,3	<0,3
700	60	MAP	<0,3	<0,3	<0,3	<0,3
Control		VAC	<0,3	<0,3	1,21	78
500	30	VAC	<0,3	<0,3	0,55	78
500	60	VAC	<0,3	<0,3	0,93	20
700	30	VAC	<0,3	<0,3	<0,3	0,36
700	60	VAC	<0,3	<0,3	<0,3	<0,3

4.2 Microstructure

The effect of HPP on the microstructure of cold smoked salmon packed in modified atmosphere and vacuum is shown in Figures 11. and 12. The results are given in percentage of non cellular material as a ratio of the total area of the picture. In other words, the results indicate the amount of spaces between the cells after each treatment. The space between the cells depicts the stage of gaping in the fillet. A larger space between the cells causes more gaping in the fillet.

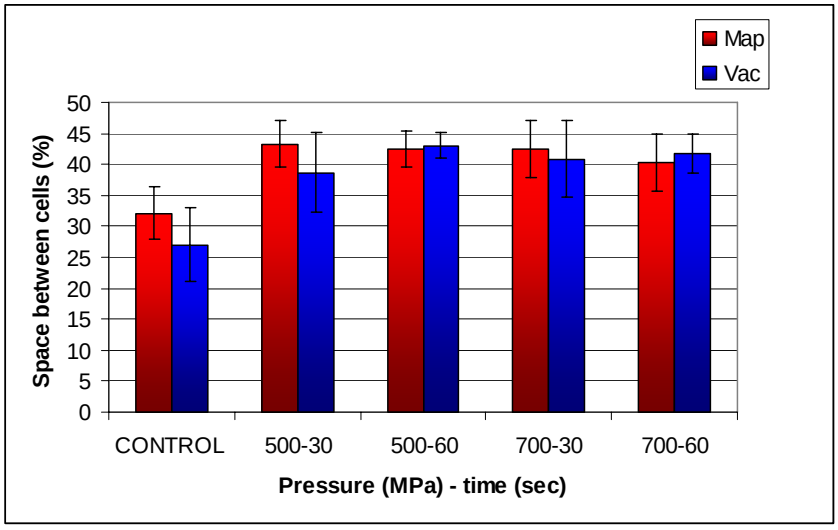
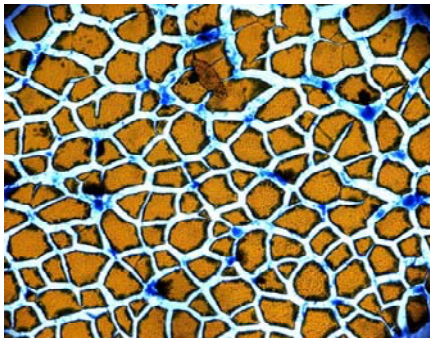
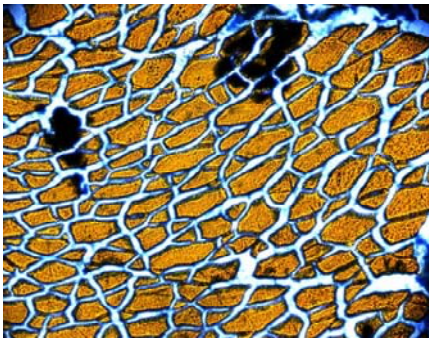


Figure 11 Average spaces between cells for HPP, both for MAP and VAC packaging.

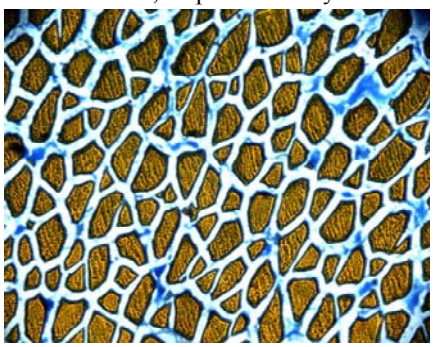
The results showed that the space between the cells in HPP samples packed in modified atmosphere were significant ($p<0.05$) compared to the control sample. There was no significant difference between HPP samples.



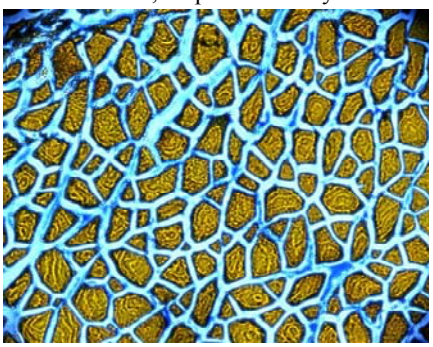
Control MAP
Results (this pic.): 33,44%
Results (group average): 32,05%
Picture nr. 173, 25 pictures analysed.



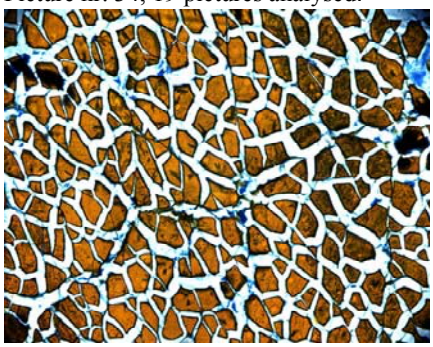
Control VAC
Results (this pic.): 29,68%
Results (group average): 26,87%
Picture nr. 192, 14 pictures analysed.



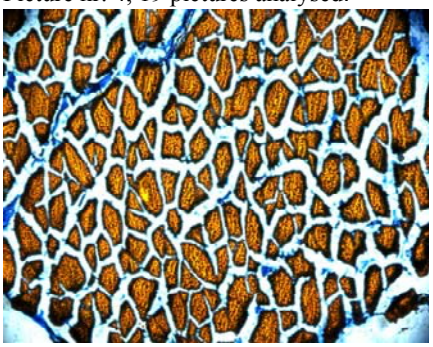
500-30-MAP
Results (this pic.): 42,72%
Results (group average): 43,25%
Picture nr. 34, 19 pictures analysed.



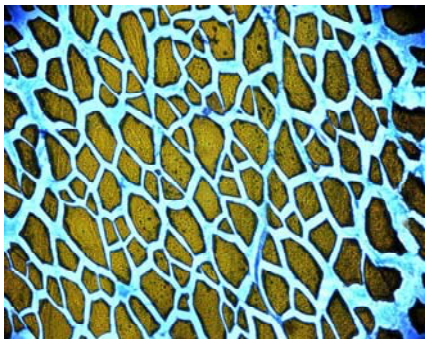
500-30-VAC
Results (this pic.): 38,51%
Results (group average): 38,66%
Picture nr. 4, 19 pictures analysed.



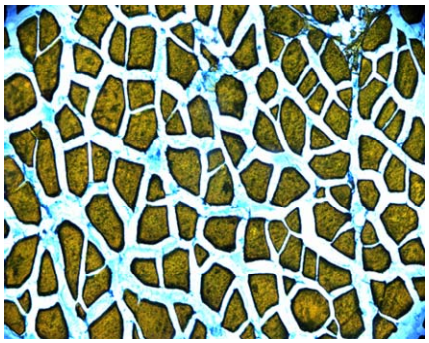
500-60-MAP
Results (this pic.): 41,83%
Results (group average): 42,49%
Picture nr. 76, 17 pictures analysed.



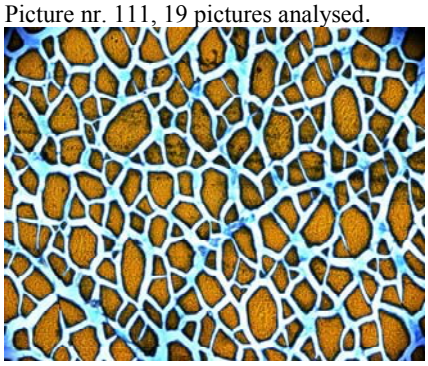
500-60-VAC
Results (this pic.): 43,45%
Results (group average): 43,08%
Picture nr. 45, 13 pictures analysed.



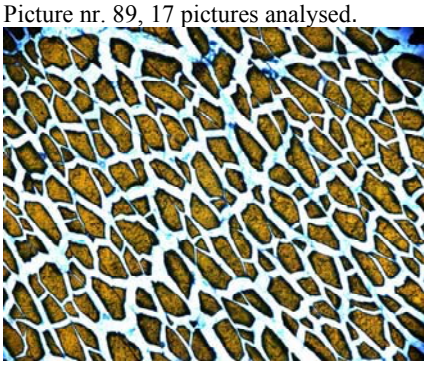
700-30-MAP
Results (this pic.): 42,13%
Results (group average): 42,47%
Picture nr. 111, 19 pictures analysed.



700-30-VAC
Results (this pic.): 41,25%
Results (group average): 40,89%
Picture nr. 89, 17 pictures analysed.



700-60-MAP
Results (this pic.): 40,02%
Results (group average): 40,26%
Picture nr. 151, 22 pictures analysed.



700-60-VAC
Results (this pic.): 41,2%
Results (group average): 41,67%
Picture nr. 4 of 20 pictures analysed.

Figure 12. Effect of high pressure treatment on the microstructure of cold smoked salmon. Each picture describes the results from each treatment and the control group.

The results were the same for HPP samples packed in vacuum as for the MAP samples, according to the significant difference. However there was no significant difference between the MAP and VAC samples for each HPP treatment.

Texture measurements

From experiment 2 the effect of HPP on textural properties of cold smoked salmon packed in vacuum and modified atmosphere (MAP) is shown in Figure 11 and 12. There was a gradual increase in toughness as the pressure was intensified in samples packed in MAP (Figure 11). Significant difference ($p<0.05$) was observed between high-pressure processing samples at 500 MPa for 30 and 60 s and 700 MPa for 60 s. There was no significant difference ($p>0.05$) between the control group and the high-pressure processing samples at 500 MPa. The same trend was observed between the vacuum packed samples after different high-pressure processing. T-test showed that the difference in toughness between samples packed in vacuum and MAP treated with the same pressure was no significant ($p>0.05$). Unlike in experiment 1, samples were taken from the same location on the fillets for all HPP treatments.

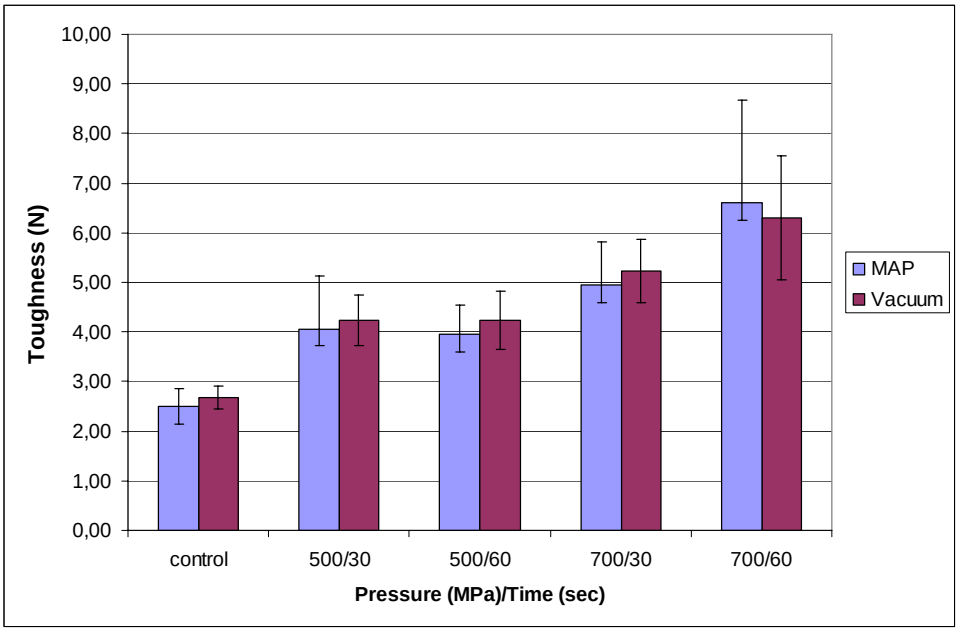


Figure 11. Effect of high pressure process on textural properties in smoked salmon.

The effect of HPP on the total amount of work needed to cut through the samples packed in vacuum and MAP is shown in Figure 12. There was a gradual increase in the work required as the pressure and the processing time increased. Significant difference ($p<0.05$) was observed in samples packed in MAP at different high-pressure processing.

The work required was significant higher in samples, processed at 700 MPa pressure, compared to other samples. No significant difference was observed between control sample and samples processed at 500 MPa pressure. Same trend was observed in vacuum packed samples, but there was also significant difference between control sample, except that there was also significant difference between control sample and samples high-pressure processed at 500 MPa pressure.

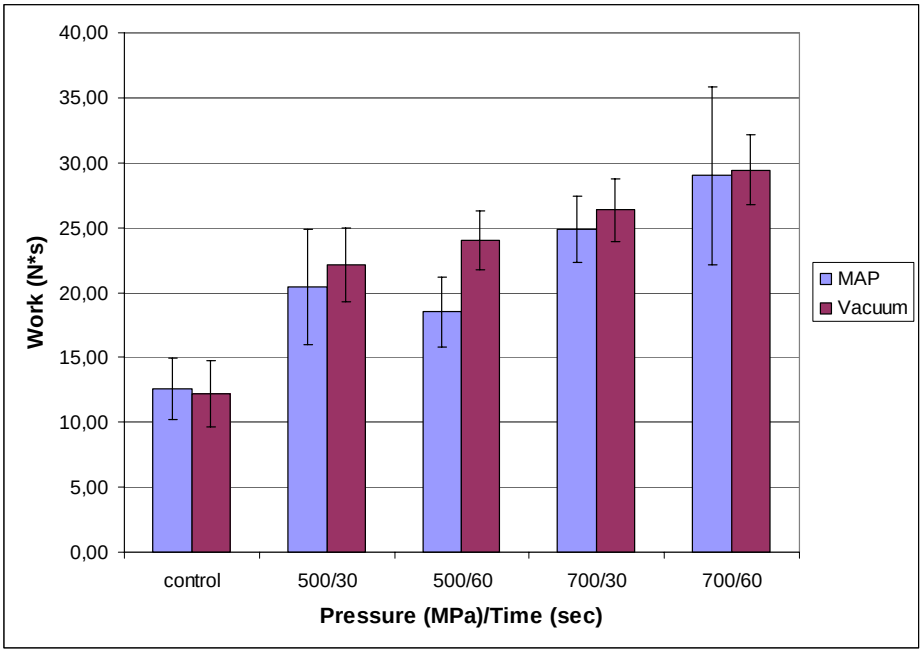


Figure 12. Effect of high pressure process on textural properties in smoked salmon.

4.4. Colour measurements

The effect of HPP on the lightness (L value) is shown in Figure 13, and the effect on redness (a value) is shown in Figure 14. The results in Figure 13 demonstrates significant ($p<0.01$) increase in lightness between all high-pressure processed samples of smoked salmon packed in MAP. The same trend was observed in vacuum packed samples. The effect of different time for each high-pressure processed sample on lightness was not significant ($p>0.05$). There was no significant difference in lightness between smoked salmon packed in vacuum and MAP processed at the same high-pressure, still samples of smoked salmon showed more lightness in MAP. Figure 14 demonstrates that the high-

pressure process did not have any effects on the redness of the smoked salmon, neither in vacuum packed nor MAP samples.

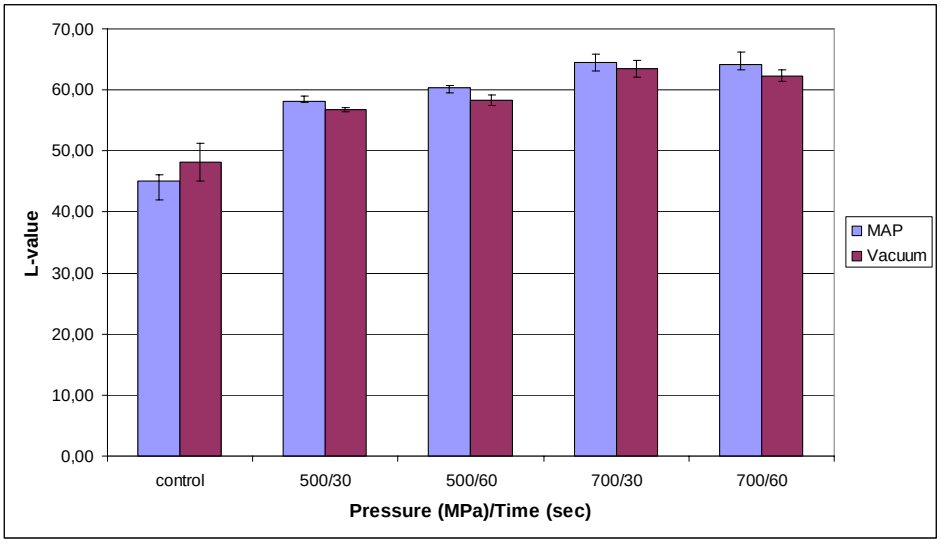


Figure 13. The lightness (L value) of the high-pressure processed smoked salmon measured by MiniScan XE plus from HunterLab.

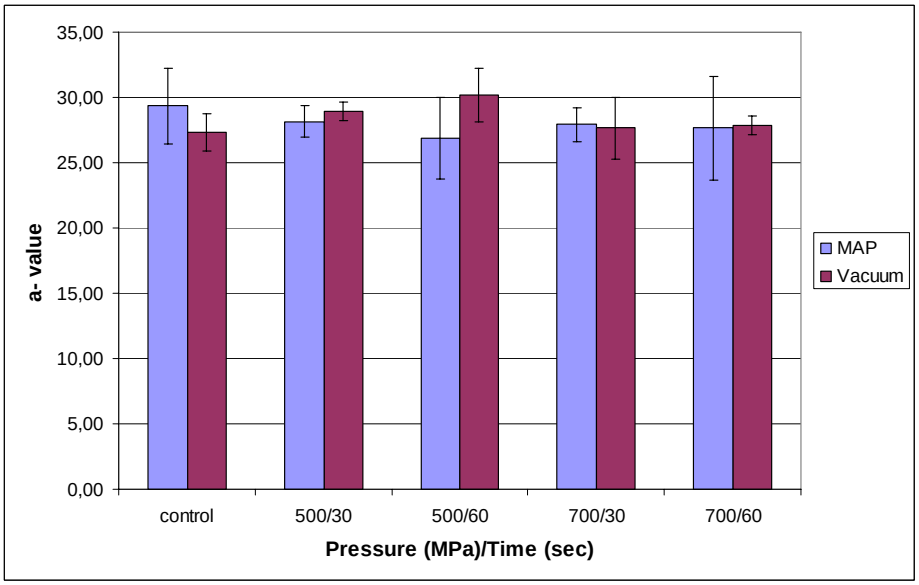


Figure 14. The redness (a value) of the high-pressure processed smoked salmon measured by MiniScan XE plus from HunterLab.

4.5 Sensory analysis

Deviations from control sample were clearly noticeable or strong in all samples. The surface of the treated samples was much drier and harder (shell-like) and the colour was lighter and more yellow compared to the control sample. After cutting the samples the internal colour was more pearl-shiny with a bluish hue and lighter than the control sample. Samples VAC 500/30 s-60 s at 5°C and MAP 500/30 s at 22°C were a bit tougher than other samples evaluated with a fork. The colour of the treated samples was similar, light brown on the outer surface and the cutting area was bluish. Samples VAC 500/60 at 22°C and MAP 500/60 at 22°C were lighter and the cutting area was pearl-shiny. Sample VAC 500/30 at 22°C was quite different compared to others as the internal colour was more similar to the surface colour. In addition, this sample had a cooked appearance. From these results it can not be seen that lower temperature decreases the effect on the appearance.

5. DISCUSSION

High pressure processing (HPP) of foods was first reported by Hite (1899) when he used this technology to increase the shelf life of milk. Since then several studies on different food items have been published. The emphases have been both on the texture as well as microbiological aspect (Amanatidou *et al*., 2000; Chevalier *et al*., 2001; Angsupanich *et al*., 1999; Ohshima *et al*., 1993; Ohshima *et al*., 1992). The effects of HPP on microorganism in food are of great interest and it has been shown that the effect of the HPP treatment on *Listeria innocua* is depending on processing parameters time and pressure (Ritz *et al*, 2000). The results from this study indicate that the pressure needs to be as high as 700 – 900 MPa to reduce the number of *Listeria innocua* to the level where the safety of the product can be ensured. HPP above 600 MPa delays the lag phase and because of that the storage time can be prolonged. However, the initial number of the *Listeria innocua* in the final product tested was rather high or 4500 CFU/g which might not reflect the situation in the industry**.** Jørgensen and Huss (1998) reported that initial

numbers of *L. monocytogenes* in cold-smoked salmon were <10 cells / g (53 out of 64 positive) and only two samples (of 32 positive) contained between 10^3 and 10^4 CFU/g after 3-7 weeks of storage. Another study carried out by Cortesi *et al*., (1997) showed no growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon stored up to five weeks at 2 or 10 °C (36 or 50 °F). Similar results were observed in this study where *Listeria* spp. was not detected in a naturally contaminated salmon after storage for 41 days at 5,5°C. Due to these facts it is necessary to investigate the effect of pressure and time where the initial concentration of bacteria is lower. Analysis of the data from this study indicates that *Listeria* spp. are much more sensitive to high pressure treatment than other background flora tested. But it should be noticed that HPP treatment at 500 MPa did not have any effect on the number of *Listeria* spp. except when treatment time was increased to 60 s. Because of the cost of high pressure processing it would be desirable to increase treatment pressure and keep the treatment time as short as possible (Chen *et al*., 2006). These results showed that when the applied pressure was 700-900 MPa a shorter time can be used, e.g. 10 s. The effect on the count of aerobic bacteria, LAB and *Listeria* as well as *Bacillus* spore were also evaluated. HPP treatment at 500 MPa did only decrease the number of TPC on LH agar of 1 log compared to 2 log decrease when treated at 900 MPa. More effect was observed when evaluating the decrease in number of LAB where the treatment had significant effect at both pressure levels. In almost all cases the recovery of LAB after treatment at 900 MPa was difficult, but there was one exception, a sample exposed to 900 MPa for 30 sec where all bacterial group started to multiply after 26 days storage. The results show that LAB became predominant in the spoiled cold smoked salmon flora for all the samples. Lactic acid bacteria often dominate the microbial flora in smoked fish products during refrigerated storage (Magnusson & Traustadottir, 1982). As a result smoked fish products have a prolonged shelf life, since the Gram-negative spoilage flora is somewhat inhibited (Jeppesen & Huss, 1993). This is in accordance with other studies showing that the LAB appear to be well adapted in vacuum packages and more resistant than Gram negative bacteria (i.e. *Pseudomonas* spp.) to the high salt content found in smoked salmon products (Hansen *et al*., 1995). The difference observed on the LH and NAP is relatively high in this study compared to the literature (Leroi *et al*., 2001). The number of LAB was up to 4 Log higher than TPC on LH. It is expected that the LAB will grow on the modified Long and Hammer's medium

(LH). It can however be speculated that processing parameters for smoked products such as dry salting and brine injection influence the microbial flora and the spoilage pattern (Hansen *et al*, 1995). It can also be assumed that some species of the lactic acid bacteria growing in these salmon samples did not grow well on LH, perhaps due to the lack of glucose in this medium, but the low incubation temperature (15°C) can also have affected the results. Another possibility why these results are observed might be that more than one species of lactic acid bacteria can grow in cold smoked vacuum packed salmon and the processing parameters can influence that. This difference was also detected in the untreated samples but it was not so big in the beginning, after five days it was 2 log which is more in accordance with previously reported results. The increase of LAB was considerably faster after HPP treatment, compared to TPC, indicating that they can recover more quickly, possibly because of the lack of competition from other background flora. Unfortunately, the species of LAB were not identified down to particular species. The purpose of evaluating the TPC on LH and LAB on NAP was only to gain some indication of the background flora and the results strongly suggest that the LAB are dominating in all cases and therefore no direct microscopic technique was used to evaluate the results on LH. These findings need to be re-evaluated with further studies where the emphasis will be on the composition of the background flora which was not the case in this study. Then it is good to have in mind that *P. phosphoreum* and *Vibrio* spp. are differentially counted on Long and Hammer's medium on the basis of colony size, colonies of *L. sakei* or *C. piscicola* being smaller (Joffraud *et al*., 2006) as well as carrying out the catalase test.

The results indicate that high pressure treatment can extend the microbial shelf-life when exposed to 900 MPa to 26 days compared to suggested limited shelf life for a period where growth of *Listeria* spp. is unlikely to take place or to reach levels >100 CFU/g (Huss *et al*., 2000). The product presented good microbiological quality and there was no indication of lipid oxidation. In the second experiment, the initial number of total count on LH was one LOG higher than in experiment 1, but the LAB count was similar. Neither *Listeria* nor *Bacillus* spores were detected in these samples, just as in experiment 1. In this case the initial number of *Listeria innocua* was much lower compared to the initial number in experiment 1, but despite that the pressure up to 700 MPa was needed to reduce the number to a satisfactory level where the safety of the product could be

ensured. The samples packed in MAP showed a lower count but the difference was not significant ($p>0,05$). It was expected that the MAP environment would allow lower pressure and a shorter treatment time to reduce the level of *L. innocua* to an acceptable level compared with vacuum packed product.

Because of high levels of polyunsaturated lipids, the salmon is susceptible to deterioration by oxidation that can affect the product quality of the salmon and affect the sensory quality, but values have not been found to be so high that the fish should be regarded as oxidized (Espe *et al*., 2002). The result from this study is in accordance with that.

The effect of HPP treatment on the microstructure of cold smoked salmon in experiment 1 was minimal at 400 MPa, but increased with both time and pressure and is most significant at 900 MPa and 60 s. When figure 6 is compared to results in Table 1, where the greatest reduction of *Listeria innocua* occur, it is clear that the effect on microstructure coincides with the reduction of the bacteria. In experiment 1, the effect from HPP on the sample that had the 500 MPa treatment was not as obvious. There were more drastic effects between the control sample and the samples which were treated with 500 MPa in experiment 2. It is known that HPP affects microstructure of meat and fish at pressure between 200 and 400 MPa, depending on the type of product (Ledward, 1998). Gudmundsson and Hafsteinsson (2001) have also reported the effect of HPP at 300 MPa on gaping in salmon fillets. Their results indicated more gaping as the space between the cells increased.

The effect of high pressure treatment on the textural properties is not clear in experiment 1. From Figure 7 shows that toughness decreases when treated at 400 and 500 MPa when treated for 10, 20, 30 and 60 s, compared to the control sample. The control sample had a large standard deviation and the lowest and highest value of toughness was 13 N and 25 N, respectively. When pressure was increased to 700 MPa and up to 900 MPa, the toughness was similar as in the control sample. The probable reason for this is that the measurement of the textural properties was performed on pooled samples, e.g. not on the same location of the fillets. Jonsson *et al*, (2000) and Sigurgisladottir *et al*., (1999) reported the difference in toughness on different location in fresh Atlantic salmon fillets. The study of Jonsson *et al*., showed variation in the value of maximum force, to cut through the samples along the fillet of fresh salmon from 15 N near the head and 42 N

near the tail. Different results from experiments 1 and 2 were detected. All samples were taken from on the same spot of the muscle under the dorsal fin. The results showed a clear gradual increase in toughness as the pressure and time of processing increased. The values for toughness in HPP smoked salmon were much lower in experiment 2, compared to experiment 1. The difference in toughness values could be caused by the sampling method, as mentioned before. Studies have shown increased tissue firmness in fresh bluefish at high pressure (200MPa for 10 minutes) (Simpson, 1998; Ashie & Simpson, 1996). Master *et al*., (2000) reported a cooked appearance in trout, cod, carp, plaice and pollock at high pressure levels of 150-200 MPa for five minutes. In general, the value of the peak force reflects the toughness of the fish sample. Increasing pressure generally causes an increase in the peak force for both experiences (Zhu *et al.,* 2004).

The colour of smoked salmon is an important factor (Sigurgisladottir *et al*., 1997) and it needs to be maintained until the product reaches the consumer. Negative effects on the colour, caused by high pressure processing of smoked salmon, could therefore be a hindering factor regarding the application of this new technology. The results of this study showed no effects on the redness, but Amanatidou *et al*. (2000) had earlier reported a significant reduction of redness. However, he studied fresh salmon and in his study the processing times were 10, 30, and 60 minutes compared to 10, 20, 30 and 60 seconds in our study. High pressure has immediate effect on the lightness. Even a treatment time of 10 sec and pressure of 400 MPa has some slight effect on the lightness of cold smoked salmon. Lightness then increased as a function of treatment time and pressure and coincides with the visual changes (Figure 8) where the salmon becomes lighter in colour as a function of both time and pressure. However, due to the short processing time the highest value for lightness never exceeded 62. A threshold value of acceptability for smoked salmon has not been reported, but for fresh salmon a threshold of value 70 is considered as unacceptable (Amanatidou *et al*., 2000). Studies on cod- and mackerel muscle showed that the colour of the muscle became lighter with increasing pressure (Ohshima *et al.,* 1993).

6. CONCLUSIONS

The conclusion is that the combination of high pressure and short treatment time is very effective to improve the quality and safety of cold smoked products. However, because of changes in the visual appearance and texture, further studies are necessary and hurdle effects of some parameters will be evaluated. The authors are especially thinking of further studies on the effect of MAP in combination with HPP. Decreasing the working temperature during HPP treatment will most likely reduce the effect on microstructure and texture. This new development is promising to meet the requirement for prolonged shelf life of ready-to-eat cold smoked salmon with high microbiological quality and safety. This study is of high industrial relevance because it combines the innovative approach of using high pressure processing for a short time (seconds) to reduce the number of *Listeria* in cold smoked salmon and thereby extend the shelf life of this valuable product. The knowledge from this study provides information for the industry in the development of new technology such as HPP where high pressure is reached in a very short time and the treatment time is short (10 s).

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