Comparative evaluation on the quality and shelf life of Atlantic salmon (*Salmo salar* L.) filets using microwave and conventional pasteurization in combination with novel packaging methods

Jørgen Lerfall[1,*], Anita Nordeng Jakobsen[1], Dagbjørn Skipnes[2], Lene Waldenstrøm[1], Sunniva Hoel[1], Bjørn Tore Rotabakk[2]

[1)] Norwegian University of Science and Technology (NTNU), Department of Biotechnology and Food Science, NO-7491 Trondheim, Norway

[2)] Nofima AS, Department of Processing Technology, P.O. Box 327, NO-4002 Stavanger, Norway

Corresponding author: Jørgen Lerfall, Norwegian University of Science and Technology (NTNU), Department of Biotechnology and Food Science, NO-7491 Trondheim, Norway, e-mail: Jorgen.lerfall@ntnu.no, phone: +47 73 55 97 49

Abstract

A comparative evaluation on the effect of CO_2 on quality and shelf life of Atlantic salmon loins pasteurized with microwave and conventional technology was conducted. The experimental design allowed CO_2 to enter the salmon muscle before (Soluble gas stabilization (SGS) + vacuum) or after pasteurization (CO_2-emitter + vacuum), whereas the control samples (vacuum only) were not presented for CO_2. This setup resulted in six different groups; three heated with microwaves and three with conventional pasteurization.

The core temperature of microwave samples was 58.8±2.2 °C whereas the surface temperature was equal to the oven temperature (62 °C) during conventional pasteurization and close to the core temperature during microwave pasteurization (57.6±1.4 °C). Microwave heated samples showed higher microbial growth, decreased shelf life, and darker (lower *L**-value), more reddish (higher *a**-value) and yellowish (higher *b**-value) color compared to conventional heated salmon. Lowest liquid loss (LL) was observed in salmon packaged with the CO_2-emitter, whereas a SGS step prior to pasteurization did not affect the LL negatively as compared to samples packaged in vacuum only. Treatment with CO_2, independent of the pre-step using SGS or an emitter, resulted in increased shelf life. Protein denaturation, microbial growth, product color, product shelf life and sensory properties of the salmon loin were significantly affected by the applied pasteurization method (microwave- or conventional pasteurization). However, the heat load was probably too high to detect differences resulting from the pre-treatment using SGS or packaging with CO_2-emitter.

Keywords: Atlantic salmon; soluble gas stabilization (SGS); CO_2-emitter; microwave pasteurization, Sous vide

Practical application:

Recent developments with increased time pressure from both work and past time activities has led to a tremendous increase in the demand for convenient, tasty ready-to-use food options. Furthermore, contemporary trends for consumption of fresh or lightly processed seafood, stresses the need to develop processing methods that allow a fulfillment of these demands, while still offering a reasonable shelf life. CO_2 in combination with either microwave- or conventional pasteurization are innovative processing technologies that can meet consumer's demand of such products.

1. Introduction

Lightly processed seafood is a growing segment ranging from raw products in vacuum- or modified atmosphere packages to lightly salted, or lightly pasteurized products. Several technologies including gentle salting (Gallart-Jornet et al., 2007; Åsli & Mørkøre, 2012), modified atmosphere packaging (MAP) (Sivertsvik, Jeksrud, & Rosnes, 2002), soluble gas stabilization (SGS) (Rotabakk, Birkeland, Lekang, & Sivertsvik, 2008; Sivertsvik, 2000), sous vide cooking (Baldwin, 2012), microwave pasteurization (Rosnes & Skipnes, 2018) and surface pasteurization (Bremer, Monk, Osborne, Hills, & Butler, 2002) have been used alone or in different combinations to improve the quality, safety and shelf life of seafood products.

Packaging has become an important hurdle against microbiological growth due to the use of milder processing technologies and reduced use of additives in the industry (Noseda, Vermeulen, Ragaert, & Devlieghere, 2014). Vacuum packaging, which can be considered as a specific case of MAP, can easily be combined with heat processing (Baldwin, 2012). On the other side, traditional MAP is often applied to fresh fish where the use of carbon dioxide (CO_2) inhibit bacterial growth (Sivertsvik et al., 2002). Traditional MAP is however difficult to combine with pasteurization due to the insulating and exponential nature of the present gasses. To utilize the positive effect of CO_2 in combination with pasteurization, alternative technologies combined with vacuum must be used. Interesting technologies are; SGS that allows CO_2 to enter the flesh before pasteurization, and the use of a CO_2 emitter that allows the CO_2 to enter the product after pasteurization (CO_2 will be released when the cook loss activate the emitter). Documentation of the synergic effect of CO_2 and heat is however limited to a study on milk, where dispersion of CO_2 in the milk before pasteurization was found to increase the thermic inhibition of *Bacillus cereus* and *Pseudomonas fluorescens* (Loss & Hotchkiss, 2002). Preliminary results (not published) has shown that SGS combined with sous vide cooking may increase the shelf life of ready-to-eat salmon products.

The industry is continuously searching easy and economical processing solutions. The best solution for pasteurization today is by conventional pasteurization with an autoclave (Dagbjørn Skipnes,

2014). Autoclaves provide a counter pressure and a temperature distribution that is much better at low temperatures (<90 °C) compared to alternative methods such as steam cabinets, water baths or traditional microwave ovens. The counter pressure is also important for the heat transfer due to minimization of the head space between the product and the packaging material that may occur during pasteurization (Skipnes, Øines, Rosnes, & Skåra, 2002). This is especially important when a SGS step is used prior to the pasteurization process.

Microwave pasteurization is an interesting technology that offers fast heating rates, decreased processing time, and often enhanced product properties (Thostenson & Chou, 1999). In microwave pasteurization the shape and the sample size are important (Ryynänen & Ohlsson, 1996). Materials containing polar molecules are rapidly heated, when exposed to microwave radiation, due to molecular friction generated by dipolar rotation in presence of an alternating electric field (Thostenson & Chou, 1999; Venkatesh & Raghavan, 2004). Microwave ovens have however several challenges due to uneven heating and a limited penetration depth (Ryynänen, 2002). To avoid such problems a lab scale microwave oven with possibilities for a pre-set counter pressure is now developed (Rosnes & Skipnes, 2017).

The aim of the present study was to investigate the effect of different CO_2 treatments in combination with microwave cooking or conventional pasteurization (autoclave) on the product quality and shelf life of a gently heated ready-to-eat Atlantic salmon (*Salmo Salar* L.) product. As controls, vacuum packaged salmon heated with microwaves or conventional pasteurization (autoclave) without added CO_2 was used.

2. Material and methods

2.1. Fish material and experimental design

Fresh vacuum packaged Atlantic salmon (*Salmo salar* L.) back loins were purchased from Bremnes Seashore AS (Bremnes Seashore AS, Norway). The raw material arrived the laboratory facilities at Nofima AS, Stavanger, Norway the day after slaughtering.

A full factorial design was set up to study the effect of CO_2 in combination with either microwave or conventional (autoclave) pasteurization on the product quality, including the shelf life. The experimental design allowed CO_2 to enter the salmon muscle before (SGS + vacuum) or after pasteurization (CO_2-emitter + vacuum), whereas the control samples (vacuum only) were not presented for CO_2. The CO_2 emitter (WOD8-XTC150, McAirlaids, UK) had an emmission capacity of 150 mL CO_2. This setup resulted in six different groups, whereof three were heated with microwaves (vacuum only, CO_2-emitter+vacuum, and SGS + vacuum, hereby annotated as **MV, ME** and **MS**, respectively) and three with conventional pasteurization (vacuum only, CO_2-emitter+vacuum, and SGS + vacuum, hereby annotated as **AV, AE** and **AS**, respectively). Raw material characteristics are presented in Table 1.

The experimental trial started two days after slaughtering when groups **MS** and **AS** underwent a SGS treatment (described subsequently). The subsequent processing were thereafter conducted 3 days post mortem. The average sample weight and fillet thickness were 61.9 ± 2.9 g and 18.5 ± 3.3 mm, respectively. All samples were, independent of the treatment used (SGS, CO_2-emitter or vacuum only), packaged in 20-µm polyamid (PA)/70-µm polyethylene (PE) bag (120 × 80mm, Star-pack produktie B.V., Waalwijk, The Netherlands) with a Webomatic Supermax-C (Webomatic, Germany) vacuum machine. The atmosphere was evacuated to an end pressure of 10mbar before the bags were sealed and thereafter heated with microwaves or conventional pasteurization, respectively. The oxygen transmission rate (OTR) for the bag was 30 $cm^3 \times m^{-2} \times d^{-1} \times atm^{-1}$.

The physio-chemical quality and the shelf life were examined during 24 days refrigerated storage (3.7 ± 0.3 °C) whereas the sensory quality was measured with Check-all-that-apply (CATA) tests (Ares & Jaeger, 2013) and acceptance, three and twelve days post processing. As a control of heat denaturation of muscle proteins, differential scanning calorimetry (DSC) was performed on a selection of samples (n=5) heated with microwaves and conventional pasteurization, respectively.

2.2. Soluble gas stabilization treatment (SGS)

The SGS treatment was carried out in batches of 25-26 samples per tray, placed inside a heat-sealed 20-µm polyamid (PA)/70-µm polyethylene (PE) bag (700 × 500 mm, Star-pack produktie B.V., Waalwijk, The Netherlands) where the atmosphere was evacuated (5000 Pa vacuum, CVP Fresh Vac Model A-600, Downers Grove, Il, USA) twice and flushed with 100% food-grade CO_2. Gas composition under the SGS treatment was 94.3 ± 0.6% CO_2, and the total pressure was equal to atmospheric pressure. The SGS treatment was carried out during 18h refrigerated storage at 2 °C. The SGS-bags were large enough to ensure excess availability of CO_2 (filling degree approximately 5% product per package volume).

2.3. Pasteurization

A novel microwave heated batch autoclave (Gigatherm AG, Switzerland) as shown by Rosnes and Skipnes (2018) was operated at 2450 MHz, 1 kW with 1.3 bar to induce volumetric heating of the samples. Continuous power was used for the first 50 s and then four intervals with 30 s for temperature equilibration and 5 s heating. The heating was followed by 2 min cooling by water spray (10 °C) and additional cooling in ice water for 10 min before the samples were stored in a refrigerator at 4 °C until analysis

Conventional pasteurization of samples by raining water was done in a batch autoclave (MicroShaka 900, Steriflow, France) operated in static mode and within 3 min heated to 62 °C and 1.3 bar pressure and held at these conditions for 12 min. The heating was followed by cooling to 30 °C within 5 min and to 20 °C within another 5 min before final cooling in ice water and stored at 4 °C in a refrigerator until analysis.

The sample temperatures were measured in a preliminary experiment by eight thermocouples (E-val Flex, Ellab AS, Denmark) and eight fiber optic probes (Optocon AG, Germany) to determine the heat load for the conventional autoclave and the microwave heated autoclave, respectively. During the following experiments, the power consumption of the microwave process was logged and combined

149 with the weight of the six samples in each run to determine the temperature increase. For this
150 purpose a specific heat capacity of salmon of 3 600 J × (kg × K)$^{-1}$ based on an empiric formula by Choi
151 and Okos (1983) was used for calculations.

152 The thermal load of the process was determined based on available data of *Lactobacillus sakei*, one
153 of the spoilage bacteria found in mild processed salmon, (Stohr, Joffraud, Cardinal, & Leroi, 2001). A
154 decimal reduction time (D-value) of 52.9 s at 57 °C (Franz & von Holy, 1996) and associated
155 temperature dependency (z-value) of 8.5 °C per log change in decimal reduction time were applied as
156 a reference in this study.

157 *2.4. Differential Scanning Calorimetry (DSC)*

158 Differential scanning calorimetry (DSC) was performed on five samples from **MV** and **AV** (in total ten
159 samples) at a heating rate of 5 °C per minute over a range from 20 °C to 110 °C using a Perkin Elmer
160 DSC 8500 instrument (Perkin Elmer, USA) as described by Skipnes, Van der Plancken, Van Loey, and
161 Hendrickx (2008). Analyses were performed on approximately 60 mg homogeneous muscle tissue
162 (free of fat and connective tissue) with an empty pan as a reference. The Pyris Software (Version
163 13.2.1.0007, Perking Elmer, USA) was used for data analysis. The total residual enthalpy (ΔH) was
164 defined as the area under the denaturation peak using a straight base line whereas the ΔH of myosin,
165 sarcoplasmic proteins (Peak II and Peak III) and actin was integrated based on the specific curve of
166 each thermogram.

167 *2.5. Liquid loss*

168 The liquid loss (LL) throughout processing and storage was calculated as the difference in fillet mass
169 between raw and processed samples measured at day 3, 6, 10, 13, 17, 19 and 24 (n=5), respectively
170 (Equation 1).

171 Equation 1:

172 LL = $\frac{m_0 - m_x}{m_0} \times 100\,\%$, where

173 m_0: initial sample mass at t_0 (raw sample)

174 m_x: sample mass at t_x (x=3, 6, 10, 13, 17, 19 and 24 days post processing)

175

176 *2.6. Texture*

177 Instrumental textural analyses were performed 3, 6, 10, 13, and 24 days post processing (n=5) using a
178 Texture Analyser TA-XT2 (SMS Ltd., England) equipped with a 25 kg load cell and a flat-ended cylinder
179 probe (20 mm diameter, type P/1SP). The force-time graph was recorded and analyzed by the
180 Texture Exponent light software for windows (version 4.13, SMS). The resistance force (N) was
181 recorded with a constant speed of 2 mm sec^{-1}, and the force required to press the cylinder down to
182 60% of fillet thickness (F60%) was applied to describe fillet firmness.

183 *2.7. Color*

184 Multispectral imaging was carried out on a Videometer Lab (Videometer AS, Denmark) system
185 measuring the light reflected from the sample surface at day 3, 6, 10, 13 and 24 post processing
186 (n=5). This system is based on a high-intensity integrating sphere illumination featuring light emitting
187 diodes (LED) together with a high-resolution monochrome grayscale camera (Dissing, Nielsen,
188 Ersbøll, & Frosch, 2011). The data acquisition was done by imaging the fillet surface at 18 different
189 wavelengths ranging from 405 to 970 nm. Before use, the system was calibrated radiometrically
190 using both a diffuse white and a dark target followed by a light setup optimized to fit the object of
191 interest. The data collected from the image at each wavelength represented an average of all pixels
192 recorded in the area of interest of each sample. The software Videometer Lab 2 - Multispectral
193 (second edition, version 2.6) was used to calculate *L*a*b**-values from RGB values obtained from the
194 sample image.

195 *2.8. Microbiological analyses*

A 10-g sample of fish muscle was aseptically transferred to a sterile stomacher bag and diluted 1:10 with sterile peptone water (1.0 g x L^{-1} bacteriological peptone (Oxoid, Norway) and 8.5 g × L^{-1} NaCl) and homogenized vigorously for 60 s in a Stomacher 400 Lab Blender (Seward Medical Ltd., UK). Appropriate serial dilutions were made in sterile peptone water and spread at their respective agar plates. Aerobic plate count (APC) and H_2S-producing bacteria were quantified as total and black colonies, respectively, on Lyngby's iron agar (IA) (Oxoid) supplemented with 0.04% L-cysteine (Sigma-Aldrich, Norway), and incubated at 22 °C for 72 h. Psychrotrophic aerobic plate count (PC) was quantified on Long and Hammer agar (LH) with 1% NaCl to support growth of *Photobacterium phosphoreum* (NCFA, No. 184, 2006), and incubated at 15 °C for six days. Lactic acid bacteria (LAB) were quantified on de Man, Rogosa and Sharp agar (MRS) (Oxoid) supplemented with 10 mg × L^{-1} amphotericin B (Sigma-Aldrich) and incubated in anaerobe atmosphere at 25 °C for five days. Anaerobic sulphite-reducing bacterial spores (SRS) were quantified on Shahidi-Ferguson Perfringens (SFP) agar base (Difco, Becton, Dickinson & Co, USA) according to NCFA (No. 56, 2015). In brief, the serial dilutions were heated at 80 °C for 10 minutes prior to plate pouring. The plates were incubated anaerobically at 15 °C for five days. Enterobacteriaceae were quantified on violet-red-bile-glucose agar (VRBGA) (Oxoid) that was incubated at 37 °C for 24 h. *Pseudomonas* spp. were quantified on Pseudomonas agar base (Oxoid) supplemented with Pseudomonas CFC selective supplement (Oxoid) and incubated at 25 °C for 48 h. *Brochothrix thermosphacta* was quantified using STAA agar base (Oxoid) supplemented with STA Selective Supplement (Oxoid), and incubated at 22 °C for 48 h.

Analysis of APC, H_2S-producing bacteria, PC and LAB were performed at day 0 (n=5) and at each sampling day (3, 6, 10, 13, 17, 19 and 24 days post processing, (n=3) and the remaining analyses were done at day 0 (n=5) and day 24 (n=3).

The log-transformed bacterial counts (APC, PC and LAB) after heat-treatment were fitted to the primary model of Baranyi and Roberts (1994) for estimation of the maximum specific growth rates (μ_{max}) and maximum population densities (Y_{max}).

221 *2.5.1. Final bacterial community by PCR-denaturing gradient gel electrophoresis (DGGE)*

222 Total genomic DNA was extracted from pooled salmon samples from the last sampling point (24
223 days). Pooling was done by mixing 1 ml from three parallels of homogenized samples. DNA was
224 extracted from 1 ml of the pooled samples using the DNeasy Blood and Tissue Kit (Qiagen, Norway),
225 as described by the manufacturer in the protocol for Gram-positive bacteria. A nested PCR strategy
226 was applied to avoid a possible co-amplification of 18S rRNA from the fish (Bakke, De Schryver, Boon,
227 & Vadstein, 2011). For the external PCR, the primers 7f (5'-agagtttgatymtggctcag-3') and 1510r (5'-
228 acggytaccttgttacgactt-3') were used to amplify almost the entire bacterial 16S rRNA (Lane, 1991). A
229 fragment of the variable region (v3) of the 16S rRNA gene was then amplified using primers 338f (5'-
230 actcctacgggaggcagcag-3') with a 40 bp GC clamp attached (5'-
231 cgcccgccgcgcgcggcgggcggggcgggggcacgggggg-3') and 518r (5'-attaccgcggctgctgg-3') (Muyzer, Dewaal,
232 & Uitterlinden, 1993). PCR products were analyzed on the DCode Mutation Detection System (BioRad
233 Laboratories AB, Norway) system with a 40-60 % denaturing gradient. The gel was run at 60 V for 18
234 h. As a marker for the DGGE (picture analysis), pooled 16S rDNA products from six different pure
235 cultures of bacteria (*Bifidobacterium bifidum* (CCUG 45217), *Lactobacillus pentosus* (DSMZ 20314),
236 *Aeromonas hydrophila* (CCUG 14551), *Leukonostoc mesenteroides* (CCUG 21965), *Shewanella*
237 *putrefaciens* (CCUG 13452), and *Pseudomonas aeruginosa* (CCUG 2080)) were used. For sequencing
238 of excised bands, the DNA were re-amplified using the linker PCR primer 338f-GC-M13R (5'-
239 caggaaacagctatgaccgcccgccgcgcgcggcgggcggggcgggggacgggggactcctacgggaggcagcag-3') (O'Sullivan,
240 Webster, Fry, Parkes, & Weightman, 2008). The PCR-DGGE procedure was performed twice with
241 conforming results, and the results presented here is from the second run. DNA sequencing was
242 performed by Eurofins Genomics (Ebersberg, Germany). Basic local alignment search tool (BLAST)
243 was used to assign taxonomy to the sequences. The sequences were submitted to the European
244 Nucleotide Archive (ENA, available at https://www.ebi.ac.uk/ena) with accession numbers
245 ERS2518265-ERS2518271.

246

2.9. Sensory description (Check-all that-apply) and acceptance

A consumer study was carried out three and twelve days post processing with 75 and 69 participants, respectively. All participants (mainly students, age 20-35 with a sex distribution of approximately 1:1) were recruited in the canteen of NTNU, Norway. At the recruitment stage, no information other than that the products were heated Atlantic salmon was provided. The participants were asked to evaluate the samples using a 9-point hedonic scale followed by a CATA questionnaire. The overall acceptability were measured by the same scale (1 = not acceptable and 9 = high acceptability). Ten grams salmon of each of the six experimental groups (**MV**, **ME**, **MS**, **AV**, **AE** and **AS**) were served to the consumers at room temperature in plastic cups labeled with a three-digit random number. Samples were presented monadically according to a balanced design. Still water was available for rinsing between samples. The participants were asked to complete the CATA questionnaire comprising 11 quality-related terms. Participants were asked to check all the terms that they considered appropriate to describe the quality of each salmon sample. The terms were selected based on expected changes in the salmon product related to the processing and the storage conditions. The terms considered were the following: cooked, uncooked, juicy, dry, tender, firm, fresh odor, unfresh odor, metallic, carbon dioxide (tingling) and rancid.

2.10. Statistics

The data were analysed by a general linear model (GLM) with the pasteurization technology, packaging technology and storage time as fixed factors (. A multivariate GLM with *L**, *a** and *b** as multiple Y were used to analyze fillet appearance. To compare different groups, one-way ANOVA and Duncan`s comparison test was used. Statistical analysis of microbiological plate counts were done on log-transformed data, and results presented are average ± standard error (SE). Sensory data analyses was performed by one-way ANOVA and a pairwise Cochran and McNemar test. All statistical analyses were performed using the IBM SPSS software (release 23, IBM corporation, USA), unless otherwise

stated. The alpha level was set to 5% ($P < 0.05$). All results are given as average ± standard deviation (SD), unless otherwise stated.

3. Results

3.1. Heat processing

The microwave pasteurization resulted in a temperature increase of 39.8 ± 1.3 °C from 18.9 °C to an end temperature of 58.8 ± 2.2 °C. The ambient heat loss was 12.6% of the average 67.8 KJ delivered to each batch of salmon with an average weight of 413.7 g. Average temperature curves are shown in Fig. 1 together with curves for heat load expressed as lethality of *L. sakei*. As expected, it was possible to reach processing temperature much faster by microwave pasteurization compared to conventional pasteurization.

Both microwave cooking and conventional pasteurization resulted in an approximately 8 log inactivation of *L. sakei* (of 20.3 min and 7.8 min for conventional and microwave pasteurization respectively) in the core of the samples (Fig. 1). At the surface of the samples, the temperature was equal to the ambient temperature (62 °C) during conventional pasteurization, while the surface temperature was close to the core temperature during microwave pasteurization (57.7 ± 1.4 °C).

3.2. Heat denaturation of proteins

The thermogram of the raw Atlantic salmon showed two well-defined endothermic transitions with two minor transitions in between (Table 2). The first peak was observed at a temperature of 46.1 ± 0.7 °C corresponding to denaturation of myosin, whereas peak II and peak III correspond to denaturation of the sarcoplasmic proteins (denaturation temperature of 58.7 ± 1.5 and 68.8 ± 0.8 °C, respectively). The fourth peak did moreover correspond to the denaturation of actin with a transition temperature of 79.0 ± 0.3 °C.

The total denaturation enthalpy ($J \times g^{-1}$) was reduced by the heat processes applied, resulting in significantly lower denaturation energy of heat-treated samples compared to the raw material (GLM,

295 $P < 0.001$). Total denaturation enthalpy ($J \times g^{-1}$) differed both in the core and in the sample surface
296 between microwave and conventional heated samples ($P = 0.001$), which was not expected based on
297 the temperature profile logged during processing (Fig. 1). The main contributor to the observed
298 difference, was peak II (58.7 ± 1.5°C), where microwave heated samples showed significantly higher
299 transition enthalpy compared to those heated with conventional pasteurization (0.10 ± 0.05 and 0.01
300 ± 0.01 $J \times g^{-1}$, respectively). The transition enthalpy of peak II of microwave heated samples did
301 moreover not differ from the raw material ($P > 0.26$).

302 *3.3. Liquid loss*

303 The LL was significantly affected by the experimental design (GLM, $P < 0.001$, Fig. 2) where significant
304 effects of storage time (GLM, $P < 0.001$) and the applied packaging technology (SGS, emitter or
305 vacuum only) was found (GLM, $P < 0.001$). The pasteurization method applied however did not affect
306 the LL during processing or storage (GLM, $P > 0.38$).

307 *3.4. Textural properties*

308 The fillet firmness was significantly affected by the experimental design (GLM, $P = 0.037$, data not
309 shown), with pasteurization method as the only significant factor (GLM, $P < 0.001$). There were no
310 effects of applied packaging technology or storage time (GLM, $P > 0.37$ and > 0.45, respectively).
311 Softest flesh was observed in microwave heated salmon (on average 7.1 ± 3.8 N) whereas
312 conventional heated salmon was the firmest (on average 10.5 ± 4.9 N).

313 *3.5. Colorimetric properties*

314 The flesh appearance (CIE, 1994) was affected by the experimental design (Multivariate GLM, Pillais`
315 Trays, $P < 0.001$, Table 3) where the multivariate discriminants were found to be pasteurization
316 method ($F = 161.55$, $P < 0.001$) and packaging technology ($F = 2.55$, $P = 0.021$). The storage time did
317 not affect the visual appearance of the samples ($F = 1.35$, $P > 0.19$).

318 Testing each parameter individually, microwave heated salmon (average of **MV**, **ME** and **MS**) were
319 found to be darker (lower *L**-value), more reddish (higher *a**-value) and more yellowish (higher *b**-
320 value) as compared to the conventional heated salmon (average of **AV**, **AE** and **AS**). The weak effect
321 of packaging technology observed in the multivariate approach, was not found on *L**, *a** or *b**-values
322 individually ($P > 0.13$, >0.070 and >0.30, respectively). The Duncan comparison test did however
323 range heated salmon packaged in vacuum only (**MV** and **AV**) to be more reddish (higher *a**-value,
324 28.4 ± 6.9) compared to those treated with SGS (**MS** and **AS,** 26.8 ± 7.4) whereas or samples
325 packaged with an emitter (**ME** and **AE**) were placed in between (26.8 ± 7.4).

326 The reflective properties in the visible- (405-700nm) and the near infrared spectra (700 to 970nm) of
327 the fillet surface were affected by the experimental design (Multivariate GLM, Pillais` Trays, $P <$
328 0.001, Table 3) where the multivariate discriminants were found to be applied pasteurization
329 technology ($F = 45.41$, $P < 0.001$), and packaging technology ($F = 2.92$,$P < 0.001$). The storage time
330 did however not affect the reflective properties of the fillet surface ($P > 0.20$-0.69).

331 The fillet surface of microwave heated salmon reflected significantly less light at all measured
332 wavelengths as compared to those heated with conventional pasteurization ($P < 0.004$). SGS treated
333 salmon (**MS** and **AS**) was moreover found to reflect significantly more light at 405, 505 and 525nm
334 compared to vacuum samples (**MV** and **AV**) or samples packaged with an emitter (**ME** and **AE**). In
335 addition, strong tendencies of higher reflection of SGS treated salmon were found at 435, 450, 470,
336 570 and 590nm ($P = 0.051$- 0.097).

337 *3.6. Microbiological analysis*

338 The mean initial concentration of APC, PC and LAB in raw salmon was 1.8 ± 0.30 log CFU × g^{-1}, 2.1 ±
339 0.52 log CFU × g^{-1} and 3.2 ± 0.04 log CFU x g^{-1} (n = 5), respectively. The pasteurization method
340 significantly affected the microbiological growth during storage (GLM, $P < 0.001$, for APC, PC and
341 LAB). APC, PC and LAB were only sporadically detected in samples after conventional pasteurization

342 throughout the storage period (data not shown). The effect of packaging technology is therefore
343 presented only for microwave-heated samples (Fig. 3).

344 After microwave pasteurization, growth of APC was significantly higher in **MV** samples than in the
345 CO_2-induced samples (**ME** and **MS**, Fig 3A; GLM, P = 0.040) during storage. The APC counts of **ME**
346 samples were significantly lower than for the other samples during the first ten days of storage (GLM,
347 P=0.009). From day ten, the **MV** group showed significantly higher counts throughout the storage
348 period (GLM, P =0.006). The primary model of Baranyi and Roberts (1994) described well the growth
349 of APC in vacuum samples after heat-treatment (R^2 =0.99). There was no difference in the growth
350 rates of APC in the **MV** and **ME** samples, whereas the growth rate in the **MS** samples was almost
351 halved (Table 4).
352 H_2S-producing bacteria, defined as black colonies on IA, were not detected. The only exception was
353 in **MV** samples between day 17 and 24. Quantitative determination was however difficult as the
354 black colonies only appeared in overgrown plates not suitable for counting.

355 After pasteurization, slow growth of PC occurred for all groups the first 10 days of storage (Fig 3B).
356 The concentration of PC was significantly higher in **MV** samples than in CO_2-induced samples (**ME** and
357 **MS**) between day 10 and 24 (Fig. 3, GLM, P = 0.021). **MV** samples reached its maximum population
358 density of 6.8 ± 0.5 CFU x g^{-1} at day 19, and the growth rate of PC in these samples was two times
359 higher than the samples subjected to CO_2 (**ME** and **MS**). No significant differences in PC counts or
360 growth rates among **ME**- and **MS** samples were detected and the **MS** samples reached the maximum
361 population density (Y_{max}) at day 24.

362 Numerically lower concentrations of LAB were observed in CO_2-induced samples between day 10 and
363 24 than in vacuum-samples, but the observed difference was not significant (GLM, P = 0.082). LAB
364 displayed the highest specific growth rate in **MV** samples, and lower growth rates of LAB were
365 observed in **ME**- and **MS** samples. All groups had LAB counts around 6 –7 log CFU × g^{-1} at the end of
366 storage (Table 4).

Sulphite- reducing bacterial spores, Enterobacteriaceae, *Pseudomonas* spp. and *Brochothrix thermosphacta* were not detected at day 0. At day 24, the **ME**- and **MS** samples were negative for the above mentioned organisms. However, for the **MV** group, at day 24, sulphite reducing bacterial spores, *Enterobacteriaceae* and *Pseudomonas* spp. were quantified at levels of 4.15 ± 0.16 log CFU × g^{-1}, 5.64 ± 0.52 log CFU × g^{-1} and 6.59± 0.56 log CFU × g^{-1}, respectively.

3.6.1. Identification of bacterial community species by PCR-DGGE

The microbial communities from the salmon subjected to different heat treatment and packing (**MV**, **ME**, **MS**, **AV**, **AE**, and **AS**) were analyzed by PCR-DGGE at the end of storage (24 days). A total of 15 bands were excised from the DGGE gel, and 11 were successfully sequenced. The DGGE profiles of samples **MV**, **ME**, **MS**, and **AV** were almost identical, with the exception of one double-band in sample **MS** that was of non-bacterial origin. The band richness was considerably higher in the autoclaved samples **AE** and **AS** but the majority of bands in these two samples were very weak, poorly separated, and thus not able to be sequenced. The dominating sequences, detected in all samples regardless of heat treatment or packaging were identified as *Carnobacterium* spp. or *Carnobacterium maltaromaticum*. *Yersinia enterocolitica* was detected in all samples, however with very weak bands in samples **AE** and **AS**. One unique band class, identified as *Aeromonas* spp. was detected in the **AE** and **AS** samples only.

3.7. Sensory perception

The sensory perception was affected by the experimental design (Multivariate GLM, Pillais` Trays, $F = 710.6$, $P < 0.001$) where the multivariate discriminants were found to be the pasteurization method ($F = 18.1$, $P < 0.001$), packaging technology used ($F = 2.55$, $P<0.001$) and storage ($F = 2.9$, $P = 0.001$). Observed perception of each characteristic, of the different groups, are presented in Table 5. Among samples from conventional heated groups (**AV**, **AE** and **AS**), packaging technology was found to affect the fillet juiciness, dryness, tenderness and firmness. This was not observed for microwave-heated samples (**MV**, **ME** and **MS**, Table 5). It is also noteworthy that the observed taste of carbon dioxide

392 (tingling) is almost ignorable, both for samples pre-treated with SGS (**MS** and **AS**) or packaged with a
393 CO_2 emitter (**ME** and **AE**).

394 The overall acceptability was not affected by the experimental design (GLM, P > 0.19) but a weak
395 effect of packaging technology was observed (P = 0.013). The judges did score samples packaged
396 with a CO_2 emitter (**ME** and **AE**) higher than those pre-treated with SGS (**MS** and **AS**) (on average 5.3
397 ± 2.3 and 4.8 ± 2.2, respectively). Samples packaged in vacuum only (**MV** and **AV**) were placed in
398 between (on average 4.9 ± 2.1). Considering storage, the result indicate that the differences in
399 acceptability as affected by packaging technology, increased from day 3 to day 12 (Table 5).

400 **4. Discussion**

401 The salmon back loins used in the present study were homogeneous regarding commercial quality
402 and divergences in quality of the raw material was equal to marked standards for commercial high
403 end salmon products. The initial contamination level of the raw material were low (ranging from 1.8
404 to 3.2 log CFU g^{-1} for APC, PC and LAB) and comparable to those found in similar studies (Mace et al.,
405 2012; Schirmer et al., 2009). The raw material used in the present study is therefore assumed to have
406 similar characteristics as expected in a traditional industrialized process.

407 The main reason for physiochemical changes in salmon flesh during thermal processing is protein
408 denaturation that is not affected by pasteurization technology applied, but by the heat load present.
409 The pasteurization technology do however affect the energy penetration. In conventional thermal
410 processing, energy is transferred to the material through convection, conduction, and radiation of
411 heat from the surfaces of the material. In contrast, microwave energy is delivered directly to
412 materials through molecular interaction with the electromagnetic field. In heat transfer, energy is
413 transferred due to thermal gradients, but microwave pasteurization is based on the transfer of
414 electromagnetic energy to thermal energy and is regarded as an energy conversion, rather than a
415 heat transfer (Thostenson & Chou, 1999). In the present study, different behavior of conventional
416 and microwave pasteurization resulted in an uneven protein denaturation as affected by the

417 pasteurization protocol, which further affects several quality parameters including texture, color,
418 microbial growth and sensory perception. The irreversible protein denaturation that occurs during
419 thermal processing, follows first order kinetics whereas color is known to follow a zero-order
420 mechanism (Ovissipour, Rasco, Tang, & Sablani, 2017). In the present study, the highest total
421 transition energy (lowest degree of protein denaturation, $J \times g^{-1}$) was observed in microwave-heated
422 salmon, which gave indicia of uneven water holding- and textural properties of the salmon muscle as
423 affected by the pasteurization technology used. This was however not observed on the LL, but by a
424 slight effect on the muscle texture. Observed difference in transition energy ($J \times g^{-1}$) was mainly
425 related to denaturation of sarcoplasmic proteins (Peak II) that is known to have minor effects on the
426 LL and textural properties as compared to denaturation of myosin and actin (Deng et al., 2002). The
427 LL was in our study, however affected by the CO_2 technology used. SGS processing is earlier found to
428 increase the LL of cod (*Gadus morhua*) due to an acidification of the muscle tissue (Sivertsvik, 2007)
429 whereas Rotabakk et al. (2008) observed lower LL of SGS treated farmed Atlantic Halibut
430 (*Hippoglossus hippoglossus*) compared to those packaged in MAP only. In the present study, equal LL
431 was observed between vacuum packaged samples and samples pre-treated with SGS. The exception
432 was on day six, where samples pre-treated with SGS show the highest LL. Salmon heated with a CO_2-
433 emitter as absorbent, showed lowest LL, which can be explained by a slight release of vacuum in
434 those samples caused by formation of CO_2. Based on our results, a pre-treatment with SGS did not
435 affect the LL negatively compared to samples packaged in vacuum only.

436 The heat load is known to affect the visual perception of heated salmon due to denaturation of
437 proteins (Kong, Tang, Rasco, & Crapo, 2007; Martens, Stabursvik, & Martems, 1982), and thereby a
438 change in light scattering properties of the fillet surface. In the present study, a higher heat load on
439 the fillet surface of conventional heated samples as compared to those heated by microwaves was a
440 result of the experimental setup. Differences in heat load between the respective technologies was
441 caused by the nature of the heat transition where the surface temperature of conventional heated
442 samples ended equal to the ambient temperature (62 °C). This was further found to affect the visual

perception (both colorimetric and reflection) of the products. It was moreover observed that the introduction of CO_2, independent of CO_2 technology used (SGS or emitter), affects the fillet redness (*a**). The introduction of CO_2 did however not affect fillet yellowness (*b**) or lightness (*L**). The reflection properties of the salmon muscle show high reflection above 570 nm as well as low reflection properties between 405 and 570 nm. This is in match with a high absorbance of light in the violet, blue and green area, while the yellow, red and dark area is highly reflected, giving the salmon muscle its characteristic pink color (Dissing et al., 2011). Higher reflection in the violet, blue, green and yellow area of heated salmon (pre-treated with SGS), supported that the use of CO_2 affects the visual perception to a more reddish hue compared to those heated in vacuum (**AV** and **MV**).

The evolution of microbiota during storage are highly dependent on the processing condition used, where LAB, *Pseudomonas* spp., *P. phosphoreum* and psychotropic Enterobacteriaceae, but also *Aeromonas* spp. and *Brochotrix thermosphacta* has been reported to dominate the microbiota of lightly processed salmon products (Hoel, Jakobsen, & Vadstein, 2017; Leroi, 2010; Løvdal, 2015). The effect of different packaging technology in combination with microwave cooking or conventional pasteurization as hurdles against microbiological growth to increase shelf life of ready-to-eat seafood is however poorly studied.

The heat load of both microwave cooking and conventional pasteurization in this study were designed to give an approximately 8 log reduction of *L. sakei* in the core of the samples. Uneven heat distribution may occur in microwave heated samples that results in uneven inactivation of microorganisms (Chandrasekaran, Ramanathan & Basak, 2013). However, the average heat loads for the total volume of the samples are quite different for microwave cooking and the conventional pasteurization method (Fig 1.). Together with higher heat load present at the surface of conventional heated samples, this can explain higher inactivation of microorganisms in those samples. PCR-DGGE-demonstrated one unique band class, identified as *Aeromonas* spp. in **AE** and **AS** samples. *Aeromonas* are H_2S- producing organisms than can be detected as black colonies on iron agar (NCFA,

468 No. 184, 2006). Absence of black colonies on iron agar sampled from **AE**- and **AS**-samples indicated
469 that the detected *Aeromonas* spp. was not viable.

470 In our study, the combination of microwave pasteurization and CO_2-induction enhanced the
471 microbiological shelf life of Atlantic salmon filets compared to microwave pasteurization in
472 combination with vacuum packaging. Vacuum-packed filets reached its maximum population density
473 for APC, LAB and PC at day 19 compared to day 24 for the CO_2-induced samples. The calculated
474 specific growth rates of PC and LAB were lower in samples subjected to CO_2 (ME and MS) than
475 vacuum. The same effect was not observed for APC. ME samples displayed an initial inhibition of
476 APC, but a fast regrowth from day 6 resulted in a high specific growth rate comparable to the level in
477 vacuum samples. Vacuum packaging also resulted in growth of several potential spoilage organisms
478 that were not detected in CO_2- induced samples; *i.e.* sulphite- reducing bacterial spores,
479 *Enterobacteriaceae* and *Pseudomonas* spp. and H_2S-producing bacteria. Initially, a longer lag-phase
480 for APC and LAB were observed in emitter- samples than for the others, but no real difference in
481 microbiological shelf life were observed between **MS**- and **ME**- samples stored for 24 days. Hansen et
482 al. (2009) demonstrated lower bacterial growth during storage in MA packaged raw prerigor-filleted
483 Atlantic salmon compared to vacuum packaged fillets.

484 The sequence analysis of excised DNA bands from the DGGE gel demonstrated that *Carnobacterium*
485 *s*pp. /*Carnobacterium maltaromaticum* and *Yersinia enterocolitica* were present in all samples,
486 independent of packaging methods*. Carnobacterium* species (*i.e. C. piscicola* and C. *divergens)*, in
487 addition to *Brochotrix thermosphacta*, were also identified as the dominant spoilage organisms in
488 raw Atlantic salmon stored at 1 °C and MAP (60% CO_2 and 40 % N_2) (Rudi, Maugesten, Hannevik, &
489 Nissen, 2004). Mace et al. (2012) demonstrated that MAP raw salmon microbiota were dominated by
490 LAB, *Pseudomonas* and *Photobacterium phosphoreum*. A. Å. Hansen, Mørkøre, Rudi, Olsen, and Eie
491 (2007) found *Carnobacterium* strains as the dominant microbiota of cod packed in MAP (60% CO_2
492 and 40% O_2) and with CO_2-emitter. *Carnobacterium* spp. develop off-flavours due to their ability to

493 metabolize amino acids to alcohols, aldehydes and H_2S and NH_3 (Leroi, 2010). Both *Aeromonas* spp.
494 and *Yersinia enterocolitica* are regarded as psychotrophic potential pathogens (Gupta, Gulati, Bhagat,
495 Dhar, & Virdi, 2015; Martino, Fasolato, Montemurro, Novelli, & Cardazzo, 2014) in MAP and ready-
496 to-eat products.

497 The sensorial perception is a key attribute and eating quality is an important determinant of the
498 overall impression of a food (Rasekh, Kramer, & Finch, 1970). In the present study, microwave
499 heated samples were perceived as less cooked and more tender compared to those heated by the
500 conventional method. Tenderness of thermal processed fish is known to achieve consumer's
501 acceptance (Kong, Tang, Lin, & Rasco, 2008) and might be an important success factor for microwave
502 pre-cooked salmon in the commercial marked. Small effects of storage was however observed
503 regarding sensory perception despite of a small increase of respondents checking the parameter
504 "unfresh odor" between day three and day twelve post processing. This correspond however to a
505 natural reduction of quality during storage of such products.

506 **5. Conclusion**

507 It is concluded that protein denaturation, microbial growth, product color, product shelf life and
508 sensory properties of the salmon loin were significantly affected by the applied pasteurization
509 method (microwave- or conventional pasteurization). It is moreover concluded that the LL was
510 significantly reduced by the use of a CO_2-emitter, whereas a SGS step prior to pasteurization did not
511 affect the LL compared to samples packaged in vacuum only. The color was most affected by
512 pasteurization technology applied where microwave cooked samples was found to be darker, more
513 reddish and more yellowish compared to those heated by the conventional method. Samples stored
514 with CO_2 independent of the use of SGS or emitter gave increased product shelf life. It was moreover
515 concluded that the heat load probably was too tough to differ between samples pre-treated with SGS
516 and those packaged with the CO_2-emitter.

517

Acknowledgment

This work was supported by the Regional Research Found Mid-Norway (project 248954). The authors wish to thank staff at Nofima AS, Stavanger and students and staff at Norwegian University of Science and Technology (NTNU, Trondheim) for excellent technical support.

Author Contributions section

Jørgen Lerfall: Project leader, corresponding author, writer, design, processing, colorimetric-, textural, DSC, and drip loss analyses

Anita N Jakobsen: Design, microbiological analysis and writing

Dagbjørn Skipnes: Design, processing and writing

Lene Waldenstrøm: Sensory analysis and writing

Sunniva Hoel: Identification of bacterial community species by PCR-DGGE and writing

Bjørn Tore Rotabakk: Design, processing and writing

References

Ares, G., & Jaeger, S. R. (2013). Check-all-that-apply questions: Influence of attribute order on sensory product characterization. *Food Quality and Preference, 28*(1), 141-153. doi:http://dx.doi.org/10.1016/j.foodqual.2012.08.016

Bakke, I., De Schryver, P., Boon, N., & Vadstein, O. (2011). PCR-based community structure studies of bacteria associated with eukaryotic organisms: a simple PCR strategy to avoid co-amplification of eukaryotic DNA. *J Microbiol Methods, 84*(2), 349-351. doi:10.1016/j.mimet.2010.12.015

Baldwin, D. E. (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science, 1*(1), 15-30. doi:http://dx.doi.org/10.1016/j.ijgfs.2011.11.002

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial-growth in food. *Int J Food Microbiol, 23*(3-4), 277-294.

Bremer, P. J., Monk, I., Osborne, C. M., Hills, S., & Butler, R. (2002). Development of a Steam Treatment to Eliminate Listeria monocytogenes From King Salmon (Oncorhynchus tshawytscha). *Journal of Food Science, 67*(6), 2282-2287. doi:10.1111/j.1365-2621.2002.tb09541.x

Chandrasekaran, S., Ramanathan, S. & Basak, T. (2013). Microwave food processing—A review. *Food Research International*, 52, 243-261

Choi, Y., & Okos, M. R. (1983). *Thermal properties of liquid foods – review.* Paper presented at the Winter meeting of the American Society of Agricultural Engineers, Chicago.

CIE. (1994). Survey of reference materials for testing the performance of spectrophotometers and colorimeters. In *Publication CIE nr. 114.1*. Vienna, Austria: Central bureau of the CIE.

Deng, Y., Rosenvold, K., Karlsson, A. H., Horn, P., Hedegaard, J., Steffensen, C. L., & Andersen, H. J. (2002). Relationship Between Thermal Denaturation of Porcine Muscle Proteins and Water-holding Capacity. *Journal of Food Science, 67*(5), 1642-1647. doi:10.1111/j.1365-2621.2002.tb08698.x

Dissing, B. S., Nielsen, M. E., Ersbøll, B. K., & Frosch, S. (2011). Multispectral Imaging for Determination of Astaxanthin Concentration in Salmonids. *PLoS ONE, 6*(5), e19032. doi:10.1371/journal.pone.0019032

Franz, C. M. A. P., & von Holy, A. (1996). Thermotolerance of meat spoilage lactic acid bacteria and their inactivation in vacuum-packaged vienna sausages. *International Journal of Food Microbiology, 29*(1), 59-73. doi:http://dx.doi.org/10.1016/0168-1605(95)00022-4

Gallart-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escriche, I., & Fito, P. (2007). Influence of brine concentration on Atlantic salmon fillet salting. *Journal of Food Engineering, 80*(1), 267-275. doi:http://dx.doi.org/10.1016/j.jfoodeng.2006.05.018

Gupta, V., Gulati, P., Bhagat, N., Dhar, M. S., & Virdi, J. S. (2015). Detection of Yersinia enterocolitica in food: an overview. *European Journal of Clinical Microbiology & Infectious Diseases, 34*(4), 641-650. doi:10.1007/s10096-014-2276-7

Hansen, A. A., Morkore, T., Rudi, K., Rodbotten, M., Bjerke, F., & Eie, T. (2009). Quality changes of prerigor filleted Atlantic salmon (Salmo salar L.) packaged in modified atmosphere using CO2 emitter, traditional MAP, and vacuum. *J Food Sci, 74*(6), M242-249. doi:10.1111/j.1750-3841.2009.01233.x

Hansen, A. Å., Mørkøre, T., Rudi, K., Olsen, E., & Eie, T. (2007). Quality Changes during Refrigerated Storage of MA-Packaged Pre-rigor Fillets of Farmed Atlantic Cod (Gadus morhua L.) Using Traditional MAP, CO2 Emitter, and Vacuum. *Journal of Food Science, 72*(9), M423-M430. doi:10.1111/j.1750-3841.2007.00561.x

Hoel, S., Jakobsen, A. N., & Vadstein, O. (2017). Effects of storage temperature on bacterial growth rates and community structure in fresh retail sushi. *J Appl Microbiol, 123*(3), 698-709. doi:10.1111/jam.13527

581 Kong, F., Tang, J., Lin, M., & Rasco, B. (2008). Thermal effects on chicken and salmon muscles:
582 Tenderness, cook loss, area shrinkage, collagen solubility and microstructure. *LWT - Food*
583 *Science and Technology, 41*(7), 1210-1222. doi:https://doi.org/10.1016/j.lwt.2007.07.020
584 Kong, F., Tang, J., Rasco, B., & Crapo, C. (2007). Kinetics of salmon quality changes during thermal
585 processing. *Journal of Food Engineering, 83*(4), 510-520.
586 doi:https://doi.org/10.1016/j.jfoodeng.2007.04.002
587 Lane, D. J. (1991). *16S/23S rRNA sequencing*. Chichester; United Kingdom: John Wiley and Sons Ltd.
588 Leroi, F. (2010). Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology,*
589 *27*(6), 698-709. doi:http://dx.doi.org/10.1016/j.fm.2010.05.016
590 Loss, C. R., & Hotchkiss, J. H. (2002). Effect of dissolved carbon dioxide on thermal inactivation of
591 microorganisms in milk. *Journal of Food Protection, 65*(12), 1924-1929.
592 Løvdal, T. (2015). The microbiology of cold smoked salmon. *Food Control, 54*(0), 360-373.
593 doi:http://dx.doi.org/10.1016/j.foodcont.2015.02.025
594 Mace, S., Cornet, J., Chevalier, F., Cardinal, M., Pilet, M. F., Dousset, X., & Joffraud, J. J. (2012).
595 Characterisation of the spoilage microbiota in raw salmon (Salmo salar) steaks stored under
596 vacuum or modified atmosphere packaging combining conventional methods and PCR-TTGE.
597 *Food Microbiol, 30*(1), 164-172. doi:10.1016/j.fm.2011.10.013
598 Martens, H., Stabursvik, E., & Martems, M. (1982). Texture and colour changes in meat during
599 cooking related to thermal denaturation of muscle proteins. *Journal of Texture Studies, 13*(3),
600 291-309. doi:doi:10.1111/j.1745-4603.1982.tb00885.x
601 Martino, M. E., Fasolato, L., Montemurro, F., Novelli, E., & Cardazzo, B. (2014). Aeromonas spp.:
602 ubiquitous or specialized bugs? *Environ Microbiol, 16*(4), 1005-1018. doi:10.1111/1462-
603 2920.12215
604 Muyzer, G., Dewaal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial-populations by
605 denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified
606 genes-coding for 16s Ribosomal-Rna. *Appl Environ Microbiol, **59***(3), 695-700.
607 NCFA. (2006). *Aerobic count and specific spoilage organisms in fish and fish products* (184). Retrieved
608 from
609 NCFA. (2015). *Sulphite-reducing Clostridia. Detemination in foods.* (56). Retrieved from
610 Noseda, B., Vermeulen, A., Ragaert, P., & Devlieghere, F. (2014). Packaging of Fish and Fishery
611 Products. In *Seafood Processing* (pp. 237-261): John Wiley & Sons, Ltd.
612 O'Sullivan, L. A., Webster, G., Fry, J. C., Parkes, R. J., & Weightman, A. J. (2008). Modified linker-PCR
613 primers facilitate complete sequencing of DGGE DNA fragments. *J Microbiol Meth, **75***(3),
614 579-581.
615 Ovissipour, M., Rasco, B., Tang, J., & Sablani, S. (2017). Kinetics of Protein Degradation and Physical
616 Changes in Thermally Processed Atlantic Salmon (Salmo salar). *Food and Bioprocess*
617 *Technology, 10*(10), 1865-1882. doi:10.1007/s11947-017-1958-4
618 Rasekh, J., Kramer, A., & Finch, R. (1970). Objective evaluation of canned tuna sensory quality.
619 *Journal of Food Science, 35*(4), 417-423. doi:10.1111/j.1365-2621.1970.tb00947.x
620 Rosnes, J. T., & Skipnes, D. (2017). Minimal processing technologies applied in fish processing. In D.
621 N. Borda, A.I.; Raspor, P.; Sun, D. (Ed.), *Trends in Fish Processing Technologies*. Boca Raton:
622 CRC Press.
623 Rosnes, J. T., & Skipnes, D. (2018). Minimal heat processing applied in fish processing. In D. N. Borda,
624 A.I.; Raspor, P. (Ed.), *Trends in fish processing technologies* (pp. 28-69). Boca Raton, Florida,
625 USA: CRC Press.
626 Rotabakk, B. T., Birkeland, S., Lekang, O. I., & Sivertsvik, M. (2008). Enhancement of Modified
627 Atmosphere Packaged Farmed Atlantic Halibut (Hippoglossus Hippoglossus) Fillet Quality by
628 Soluble Gas Stabilization. *Food Science and Technology International, 14*(2), 179-186.
629 doi:10.1177/1082013208092051
630 Rudi, K., Maugesten, T., Hannevik, S. E., & Nissen, H. (2004). Explorative Multivariate Analyses of 16S
631 rRNA Gene Data from Microbial Communities in Modified-Atmosphere-Packed Salmon and

632 Coalfish. *Applied and Environmental Microbiology, 70*(8), 5010-5018.
633 doi:10.1128/aem.70.8.5010-5018.2004
634 Ryynänen. (2002). *Microwave heating uniformity of multicomponent prepared foods.* (PhD),
635 University of Helsinki, PhD EKT series 1260.
636 Ryynänen, S., & Ohlsson, T. (1996). Microwave Heating Uniformity of Ready Meals as Affected by
637 Placement, Composition, and Geometry. *Journal of Food Science, 61*(3), 620-624.
638 doi:10.1111/j.1365-2621.1996.tb13171.x
639 Schirmer, B. C., Heiberg, R., Eie, T., Møretrø, T., Maugesten, T., Carlehøg, M., & Langsrud, S. (2009). A
640 novel packaging method with a dissolving CO2 headspace combined with organic acids
641 prolongs the shelf life of fresh salmon. *International Journal of Food Microbiology, 133*(1–2),
642 154-160. doi:http://dx.doi.org/10.1016/j.ijfoodmicro.2009.05.015
643 Sivertsvik, M. (2000, 10-14 October). *Use of soluble gas stabilization top extend shelf life of fish.*
644 Paper presented at the WEFTA, Leptocarya, Pieria, Greece.
645 Sivertsvik, M. (2007). The optimized modified atmosphere for packaging of pre-rigor filleted farmed
646 cod (Gadus morhua) is 63ml/100ml oxygen and 37ml/100ml carbon dioxide. *LWT - Food*
647 *Science and Technology, 40*(3), 430-438. doi:http://dx.doi.org/10.1016/j.lwt.2005.12.010
648 Sivertsvik, M., Jeksrud, W. K., & Rosnes, J. T. (2002). A review of modified atmosphere packaging of
649 fish and fishery products – significance of microbial growth, activities and safety.
650 *International Journal of Food Science & Technology, 37*(2), 107-127. doi:10.1046/j.1365-
651 2621.2002.00548.x
652 Skipnes, D. (2014). Heat Processing of Fish. In *Seafood Processing* (pp. 61-81): John Wiley & Sons, Ltd.
653 Skipnes, D., Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2008). Kinetics of heat
654 denaturation of proteins from farmed Atlantic cod (Gadus morhua). *Journal of Food*
655 *Engineering, 85*(1), 51-58. doi:http://dx.doi.org/10.1016/j.jfoodeng.2007.06.030
656 Skipnes, D., Øines, S., Rosnes, J. T., & Skåra, T. (2002). Heat Transfer in Vacuum Packed Mussels
657 (Mytilus edulis) During Thermal Processing. *Journal of Aquatic Food Product Technology,*
658 *11*(3-4), 5-19. doi:10.1300/J030v11n03_02
659 Stohr, V., Joffraud, J. J., Cardinal, M., & Leroi, F. (2001). Spoilage potential and sensory profile
660 associated with bacteria isolated from cold-smoked salmon. *Food Research International,*
661 *34*(9), 797-806. doi:https://doi.org/10.1016/S0963-9969(01)00101-6
662 Thostenson, E. T., & Chou, T. W. (1999). Microwave processing: fundamentals and applications.
663 *Composites Part A: Applied Science and Manufacturing, 30*(9), 1055-1071.
664 doi:http://dx.doi.org/10.1016/S1359-835X(99)00020-2
665 Venkatesh, M. S., & Raghavan, G. S. V. (2004). An Overview of Microwave Processing and Dielectric
666 Properties of Agri-food Materials. *Biosystems Engineering, 88*(1), 1-18.
667 doi:http://dx.doi.org/10.1016/j.biosystemseng.2004.01.007
668 Åsli, M., & Mørkøre, T. (2012). Brines added sodium bicarbonate improve liquid retention and
669 sensory attributes of lightly salted Atlantic cod. *LWT - Food Science and Technology, 46*(1),
670 196-202. doi:http://dx.doi.org/10.1016/j.lwt.2011.10.007

671

672

671 Table 1. Properties of the raw material used in the present study (n=5)

Analyses	*Parameter*	*Value*
DSC[1]	*Total enthalpy, J × g^{-1}*	*3.1±0.1*
	Myosin, J × g^{-1}	*0.9±0.1*
	Peak II, J × g^{-1}	*0.1±0.0*
	Peak III, J × g^{-1}	*0.1±0.0*
	Actin, J × g^{-1}	*0.2±0.0*
Texture	*$F_{60\%}$, N*	*21.1±6.2*
Color	*L**	*59.8±2.1*
	*a**	*39.4±1.2*
	*b**	*44.2±2.3*

672 [1] *Differential Scanning Calorimetry, DSC*
673
674

675 **Table 2.** Total transition enthalpy (J × g^{-1}) and the specific enthalpy (J × g^{-1}) of myosin, sarcoplasmic proteins
676 (peak II and III) and actin in the surface (n=5) and center (n=5) of salmon samples heated with conventional and
677 microwave pasteurization, respectively

Peak	*Denaturation temperature* [1]	**Conventional**		**Microwave**		*GLM*[2]
		Surface	Center	Surface	Center	
Total enthalpy		*0.95±0.18*^{b}	*1.08±0.22*^{b}	*2.04±0.52*^{a}	*1.79±0.49*^{a}	*P=0.001*
Myosin	*46.1±0.7*	*not detected*	*not detected*	*0.13±0.30*	*0.02±0.04*	*P>0.44*
Peak II	*58.7±1.5*	*0.01±0.00*^{b}	*0.01±0.01*^{b}	*0.09±0.02*^{a}	*0.11±0.08*^{a}	*P=0.001*
Peak III	*68.8±0.8*	*0.03±0.02*	*0.04±0.02*	*0.02±0.01*	*0.02±0.01*	*P>0.26*
Actin	*79.0±0.3*	*0.40±0.10*	*0.26±0.07*	*0.27±0.10*	*0.33±0.08*	*P>0.10*

678 [1] General Linear Model (GLM) analyses of variance, where *P* are the significance level for the effects of the model. Different superscripts
679 (^{abc}) within each row indicate significant variation ($P < 0.05$) between groups by a one-way ANOVA and Duncan's comparison test.
680 [2] The denaturation temperature (°C) is presented as the temperature at maximum endothermic heat flow of the respective peak.

681

682

683 **Table 3**. Colorimetric and reflective properties (independent of storage time) of Atlantic salmon heated with
684 microwaves or conventional pasteurization (autoclave) packaged in vacuum only, vacuum and CO_2 emitter, and
685 vacuum pretreated with SGS technology (n=25)

	Microwave (M)			Conventional (A)			GLM [1]			
Parameter	**V**acuum	**E**mitter	**S**GS	**V**acuum	**E**mitter	**S**GS	P_M	P_H	P_T	P_S [2]
Color										
*L**	68±3^b	69±3^b	69±3^b	77±1^a	76±2^a	77±2^a	<0.001	<0.001	>0.13	>0.68
*a**	35±4^a	34±3^a	33±4^a	22±3^b	22±3^b	20±4^b	<0.001	<0.001	>0.070	>0.54
*b**	38±4^a	37±3^a	37±5^a	25±3^b	24±1^b	23±4^b	<0.001	<0.001	>0.30	>0.66
Reflection										
405nm	23±4^c	24±4^c	24±5^c	35±3^b	35±3^b	37±4^a	<0.001	<0.001	=0.038	>0.20
435nm	18±4	18±4	19±5	31±3	31±1	33±1	<0.001	<0.001	>0.070	>0.41
450nm	16±4^c	17±4^c	18±5^c	31±3^b	32±5^{ab}	34±5^a	<0.001	<0.001	>0.053	>0.37
470nm	16±4^c	17±4^c	17±5^c	31±3^b	31±5^{ab}	34±5^a	<0.001	<0.001	>0.051	>0.36
505nm	15±4^c	16±4^c	17±4^c	32±2^b	31±5^{ab}	32±5^a	<0.001	<0.001	>0.047	>0.32
525nm	19±4^c	21±4^c	21±5^c	35±5^b	35±5^{ab}	38±5^a	<0.001	<0.001	>0.035	>0.31
570nm	44±5^b	46±5^b	46±5^b	57±2^a	56±2^a	58±3^a	<0.001	<0.001	>0.081	>0.24
590nm	56±4^b	58±5^b	58±5^b	57±2^a	66±1^a	67±2^a	<0.001	<0.001	>0.097	>0.46
630nm	72±3^b	73±3^b	73±2^b	75±1^a	75±1^a	75±0^a	<0.001	<0.001	>0.72	>0.51
645nm	72±3^b	73±2^b	73±2^b	74±0^a	74±0^a	74±0^a	<0.001	<0.001	>0.82	>0.50
660nm	74±1^b	74±2^b	74±1^b	75±0^a	75±0^a	75±0^a	<0.001	<0.001	>0.92	>0.57
700nm	75±1	76±2	76±1	76±0	76±0	76±0	<0.001	=0.004	>0.93	>0.69
780nm	76±2^b	77±2^b	77±1^b	78±0^a	78±0^a	78±0^a	=0.034	<0.001	>0.71	>0.58
850nm	77±2^b	77±3^b	77±1^b	79±0^a	79±0^a	79±0^a	=0.001	<0.001	>0.57	>0.50
870nm	76±3^b	78±3^b	77±3^b	81±0^a	81±0^a	81±0^a	<0.001	<0.001	>0.37	>0.46
890nm	72±3^c	74±4^b	73±3^{bc}	79±1^a	79±1^a	80±1^a	<0.001	<0.001	>0.20	>0.42
940nm	63±3^b	65±4^b	65±3^b	72±1^a	72±1^a	74±1^a	<0.001	<0.001	>0.061	>0.46
970nm	52±4^b	54±5^b	54±6^b	64±2^a	63±2^a	66±2^a	<0.001	<0.001	>0.032	>0.34

686 [1] General Linear Model (GLM) analyses of variance, where P_M, P_H, P_T, and P_S are the significance levels for the effects of the model, heat
687 technology applied, CO_2-technology used and storage time, respectively. Different superscripts (^{abc}) within each row indicate significant
688 variation ($P < 0.05$) between groups by a one-way ANOVA and Duncan's comparison test.
689 [2] P_S: data not shown

690

691

Table 4. Growth kinetic parameters (maximum specific growth rate (μ_{max}, d^{-1} ± SE) and maximum population density (Y_{max}, log CFU/g ± SE) for samples subjected to different pasteurization treatment and packaging technology (MV, MS, ME) for total aerobic plate count (APC), psycrotrophic plate count (PC), and lactic acid bacteria (LAB) The parameters were estimated from the primary model of Baranyi and Roberts (1994) using log-transformed bacterial counts after heat-treatment.

Group	μ_{max} (d^{-1})	Y_{max} (log CFU/g)	R^2	SE (fit)
APC				
MV	0.34 ± 0.019	7.20 ± 0.17	0.99	0.18
MS	0.19 ± 0.038	6.12 ± 1.1	0.88	0.48
ME	0.32 ± 0.16	NA	0.73	1.1
PC				
MV	0.67 ± 0.43	6.80 ± 0.53	0.85	0.90
MS	0.30 ± 0.16	6.07 ± 0.88	0.71	0.84
ME	0.37 ± 0.16	NA	0.67	1.3
LAB				
MV	0.50 ± 0.082	6.30 ± 0.34	0.95	0.55
MS	0.31 ± 0.047	6.13 ± 0.66	0.93	0.54
ME	0.35 ± 0.11	NA	0.90	0.81

R^2, coefficient of determination, SE (fit), standard error of fit to the model, NA: Not analyzed due to no asymptote.

692

693

694 **Table 5. Overall acceptability (scale 1-9),** and relative numbers (%) of respondents checking each characteristic
695 three and twelve days post processing (n=75 and 69, respectively)

Parameter	Day	Microwave (M)			Conventional (A)			P-value[a]
		Vacuum	Emitter	SGS	Vacuum	Emitter	SGS	
Overall acceptability	***3***	5.0	5.1	5.1.	5.1	5.3	4.6	>0.51
	12	5.0^{ab}	5.4^{a}	4.9^{ab}	4.7^{ab}	5.3^{a}	4.4^{b}	=0.045
Cooked	***3***	25.3^{d}	45.3^{bc}	32.0^{cd}	62.7^{a}	53.3^{ab}	58.7^{ab}	<0.001
	12	40.6^{c}	37.7^{c}	29.0^{c}	66.7^{ab}	59.4^{b}	75.4^{a}	<0.001
Uncooked	***3***	42.7^{a}	25.3^{b}	49.3^{a}	9.3^{c}	10.7^{c}	6.7^{c}	<0.001
	12	31.9^{a}	27.5^{a}	40.6^{a}	2.9^{bc}	11.6^{b}	1.4^{c}	<0.001
Juicy	***3***	46.7^{ab}	46.7^{ab}	54.7^{ab}	38.7^{b}	57.3^{a}	20.0^{c}	<0.001
	12	56.5^{a}	55.1^{ab}	50.7^{ab}	37.7^{bc}	52.2^{ab}	26.1^{c}	=0.001
Dry	***3***	13.3^{c}	25.3^{bc}	17.3^{bc}	30.7^{b}	20.0^{bc}	49.3^{a}	<0.001
	12	24.6^{c}	18.8^{c}	23.2^{c}	43.5^{b}	20.3^{c}	60.9^{a}	<0.001
Tender	***3***	60.0^{a}	54.7^{a}	56.0^{a}	34.7^{b}	54.7^{a}	20.0^{b}	<0.001
	12	60.9^{a}	68.1^{a}	62.3^{a}	33.3^{bc}	43.5^{b}	20.3^{c}	<0.001
Firm	***3***	16.0^{bc}	17.3^{bc}	6.7^{c}	22.7^{b}	13.3^{bc}	40.0^{a}	<0.001
	12	17.4^{bc}	5.8^{c}	14.5^{bc}	24.6^{ab}	15.9^{bc}	39.1^{a}	<0.001
Fresh odor	***3***	30.7	25.3	24.0	29.3	33.3	22.7	>0.51
	12	31.9	36.2	31.9	24.6	29.0	31.9	>0.77
Unfresh odor	***3***	4.0^{b}	10.7^{ab}	9.3^{ab}	16.0^{a}	17.3^{a}	18.7^{a}	=0.027
	12	17.4	17.4	14.5	18.8	13.0	21.7	>0.77
Metallic	***3***	9.3	20.0	12.0	17.3	21.3	12.0	>0.18
	12	11.6	23.2	15.9	124.6	21.7	21.7	>0.32
Carbon dioxide (tingling)	***3***	1.3^{ab}	8.0^{a}	2.7^{ab}	0^{b}	6.7^{ab}	1.3^{ab}	=0.015
	12	5.8	7.2	5.8	0	10.1	5.8	>0.20
Rancid	***3***	5.3	10.7	5.3	13.3	8.0	17.3	>0.079
	12	10.1	7.2	13.0	18.8	15.9	15.9	>0.31

696 [a] Different superscripts (^{abcd}) within each row indicate significant variation ($P < 0.05$) between groups by a pairwise Cochran and McNemar
697 test (CATA).
698

699

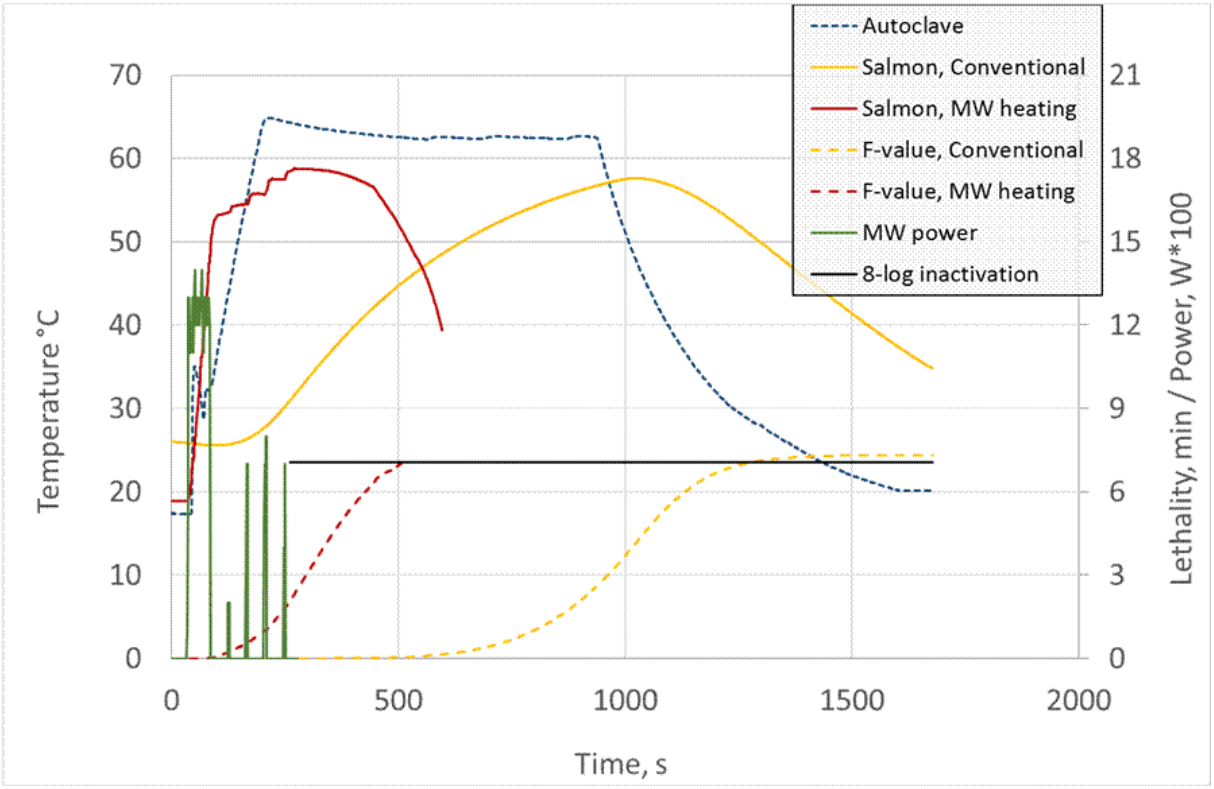
Figure captions:

Figure 1. Average core temperature of salmon samples during conventional pasteurization (yellow, continuous line, n=6) and microwave pasteurization (red, continuous line, n=26). The process water temperature (blue, dotted line) is shown for conventional pasteurization and microwave power (green continuous line) in hundreds of W. Lethality resulting from the heat load based on kinetic inactivation parameters for *L. sakei* is shown for conventionally heated samples (yellow, dashed line) and microwave heated samples (red, dashed line).

Figure 2. Main effects of packaging technology (SGS, emitter or vacuum only, n=28) on the liquid loss (% ± SD of initial weight) during 24 days of storage (4 °C) of microwave and conventional heated Atlantic salmon. Small letters (a and b) behind the legends indicate significant differences by GLM: $P_{model} < 0.001$, $P_{storage} < 0.001$, $P_{technolgy} < 0.001$, $P_{heating} > 0.38$).

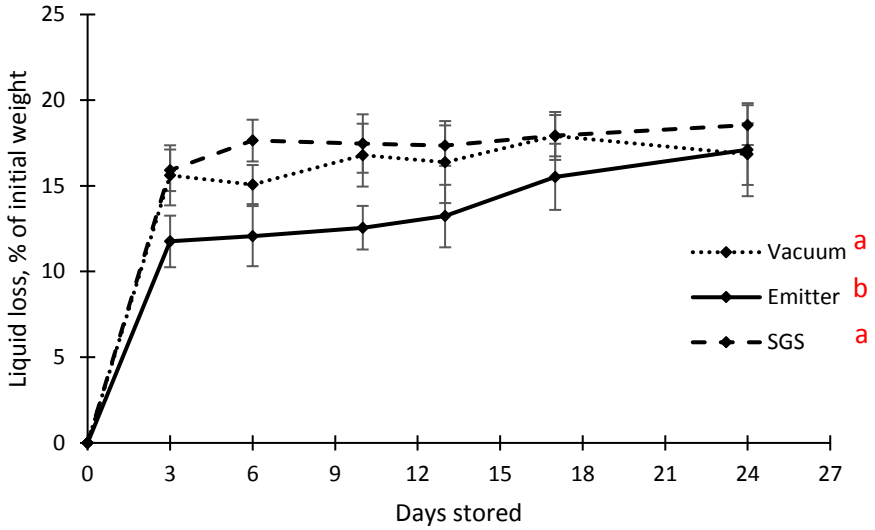
Figure 3. Growth of **A)** aerobic plate counts (APC) ($GLM_{overall}$: P = 0.040; GLM $_{day\ 10\text{-}24\ of\ storage}$: P = 0.009), **B)** Psychotropic counts (PC) ($GLM_{overall}$: P = 0.41; GLM $_{day\ 10\text{-}24\ of\ storage}$: P = 0.021) and **C**) Lactic acid bacteria (LAB) ($GLM_{pverall}$: P = 0.28; GLM $_{day\ 10\text{-}24\ of\ storage}$: P = 0.082) of microwave heated samples (vacuum only, **MV**; CO_2-emitter + vacuum, **ME** and SGS + vacuum, **MS**). Each sampling point represent the average value (n=3, except n=5 at day 0) whereas vertical bars indicate ± SE. Samples heated by the conventional method (**AV**, **AE** and **AS**) showed only sporicidal bacterial growth (data not shown).

721



722

723 **Figure1.**



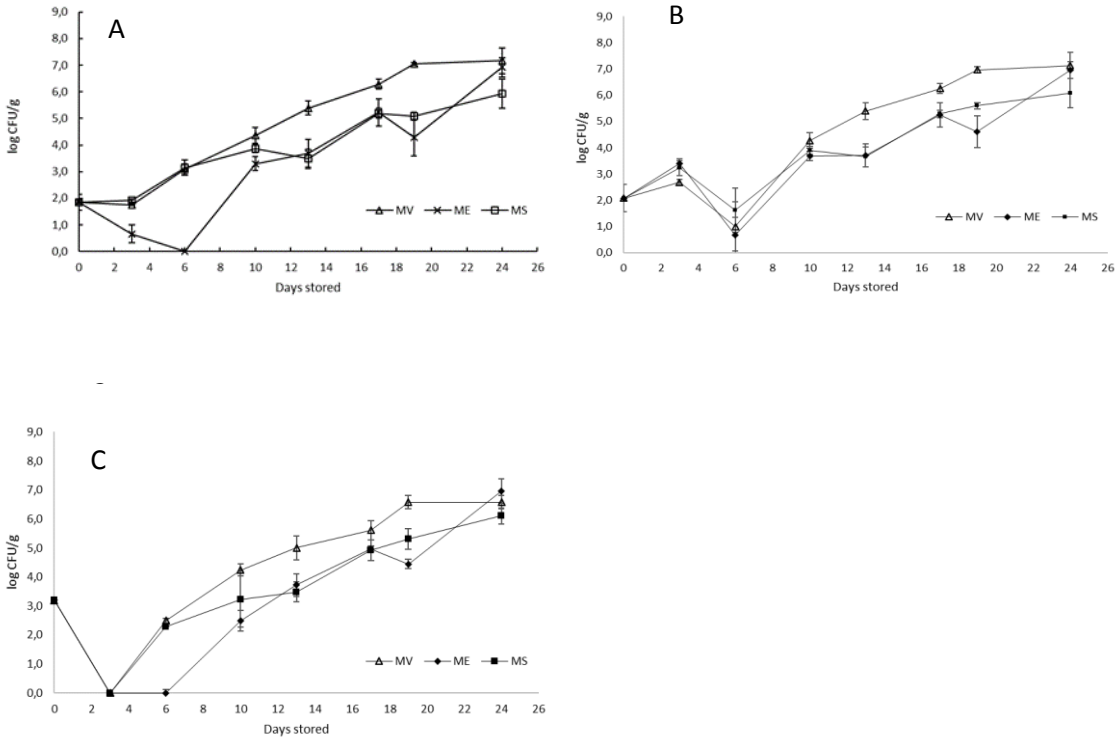
724

725 **Figure 2**.

726

727

728



729

730

731

732

733 **Figure 3.**

734

735

736