The effects of pre-processing sanitation and modified atmosphere packaging on microbial growth in bulk packs of Atlantic salmon (*Salmo salar*) fillets

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Abstract. This research was to extend the shelf-life of fresh Atlantic salmon fillets when packaged in bulk food service modified atmosphere packaging trays with low gas to product volume ratios. When head-on-gutted Atlantic salmon were washed in Neutral electrolyzed water sanitizer prior to filleting, the microbial load on the skin of HOGs treated at 20 ppm and 100 ppm chlorine equivalents decreased by 3.5 log CFU/cm^2 down to 2.0 and 1.5 log CFU/cm^2, respectively. Further trials washed the HOGs with 100 ppm of NEW before filleting. They were then packed in a different product gas to product (G/P) volume ratios (0.4:1, 1:1 and 2:1) and stored at 0 °C or 4 °C up to 20 days. The combinations of sanitation pre-processing and high G/P ratio were most effective for controlling the microbial count to 4.5 log CFU/g when stored at 0 °C compared to a microbial count of 7.2 log CFU/g for the unwashed fillets after 20 days under 4 °C storage. Other variable combinations were between these levels. A combination of improved pre-processing sanitation and a low temperature can therefore raise the hurdles for microbial growth to extend the shelf-life of bulk packed fresh salmon fillets packed at high volumetric densities for storage and shipping efficiency.

1. Introduction

The used of modified atmosphere packaging (MAP) to prolong the shelf-life of seafood products is widely used due to its effectiveness in inhibiting microbial growth and oxidative reactions. The efficacy of MAP for extending shelf-life is influenced by the microbial level of the raw material, the temperatures during processing and storage, and the resulting concentration of carbon dioxide (CO_2); nitrogen (N_2)) and oxygen (O_2) in the headspace of the pack [1]. Most research on the use of MAP for salmon products has focused on a combination of CO_2 and N_2 [2-4] or just using CO_2 [5]. The gas to product (G/P) volumetric ratio is one of the main factors for MAP to be effective. A G/P ratio of 2:1 or 3:1 was recommended for use by Sivertsvik, Rosnes [6]. However, bulk trays have a high packing density compared to retail MAP packs and consequently can only achieve a low G/P ratio. A high G/P ratio

allows adequate CO_2 to be used for the MAP packaging in order to efficiently inhibit the microbial load. For the commercial shipment of bulk packages (e.g. 1.5 – 2.5 kg) of fresh salmon fillets, the G/P ratio is constrained to have a low packaging density, which is corresponding with a high G/P ratio. A high G/P ratio is not feasible for the industry. It substantially raises the costs of transportation that is based on volumetric density. Moreover, a high level of CO_2 gas at low G/P ratios causes package deformation owing to adsorption of CO_2 into the product forming a partial vacuum within the sealed pack. Understanding the best G/P ratio to prolong the shelf-life of bulk salmon fillets in industrial shipping practice is required.

MAP will be most effective in combination with additional hurdles prior to packaging. MAP, along with low storage temperatures, noticeably prolongs the shelf-life of the products. However, even in conjunction with good cold chain practice, MAP can only inhibit microorganism growth [7-9] and not eradicate the microbes. A low microbial load level in the product before the packaging step needs to be assured using good hygiene during production and further growth must be minimized. Effective sanitation is required for good hygiene during the processing of fish products where natural microflora contamination comes from the fish skin, slime and gut contents. Neutral electrolyzed oxidizing water (NEW), can be an effective option to minimise microbial cross-contamination prior to packaging. NEW provides a stronger bactericidal effect compared to acidic electrolyzed water (AEW), but with less impact on sensory properties [10, 11]. However, the efficacy of electrolyzed oxidizing (EO) water declines with increasing amounts of organic substances and with storage time depending on conditions [12-14]. Using the ice form of NEW with head-on gutted (HOG) salmon could be an alternative as the ice will give a continuous release of sanitizer that would extend the bactericidal effect of a treatment solution to result in a low initial microbial number of the raw material prior processing and packaging. Previous research showed that a pre-processing stage using 100 ppm NEW/neutral electrolyzed oxidizing ice (NEI) was able to reduce microbial load on HOG salmon over three days of treatment by 1.5 log from the initial count This method was therefore used to improve the pre-treatment stage of HOGs to feed the best quality product to the MAP process.

The aim of this study was to prolong the shelf-life of fresh Atlantic salmon fillets when packaged in bulk. The approach was to determine the effects of combining the improved pre-treatment sanitation with MAP methods by determining the change in microbial load as a function of treatment and storage. A study was set up treating the HOGs with a NEW sanitation step after holding in ice-water for rigor reversal and varying the G/P ratio.

2. Materials and Methods

2.1. NEW and NEI preparation and chlorine oxidation properties analysis

NEW (pH 7.4, oxidation-reduction potential (ORP) 900 ± 12 mV free chlorine concentration (FCC) 500 ± 10 ppm) was generated from using an Envirolyte ELA 400 automated electrolytic generator (Envirolyte ®, Tallinn, Estonia). NEW was then transferred into 20 L closed polyethylene containers and stored at room temperature (17 – 20° C) prior to use. Concentrations lower than 500 ppm were made by diluting the NEW concentrate water with tap water (pH 7.1, ORP 200 ± 18 mV, FCC 2 ± 0.5 ppm) to the required final concentration of the desired FCC. The ORP was measured with a portable redox meter (IC-MV-500, Milwaukee, Szeged, Hungary). pH was measured using a pH meter (FE20, Mettler-Toledo, Columbus, OH, USA), and the FCC of NEW was measured with a modified titration method using thiosulfate modified from the procedure of Walding [15]. NEI was made by freezing NEW into ice blocks. Two litres of NEW was transferred into stainless steel containers (2.7 L, 49 x 137 x 152 mm). The containers were then frozen in a benchtop blast freezer (EF.101. Irinox, Tarzo, Italy) and stored in a freezer at -18° C until used. The ice was then shaved to ice flakes less than 2 mm thick immediately prior to use with a motorized ice shaver (HB32OA, Hatsuyuki, Mie, Japan).

2.2. Impact of sanitation concentration on microbial load of HOG Atlantic salmon

Fresh HOG Atlantic salmon, approximately 51 – 53 cm in length and weighing between 2.7 – 2.8 kg, were obtained directly from the commercial processor within 4 days of harvest. The salmon HOGs were transferred to the laboratory by shipment on ice in polystyrene foam boxes and kept in a cool room at 4° C to match to existing industry practice. A total of 12 HOGs that had been stored in flake ice for seven days were randomly selected for two treatment groups (n = 3 per group), as follows. A: Washed with 20 ppm of NEW and B: Washed with 100 ppm of NEW. HOGs were washed by dipping them in 20 L of NEW at 6 – 7° C for five seconds with manual agitation. Two identical separate experiments were carried out two weeks apart. HOGs were placed on a sanitized chopping board for five minutes to drain the remaining NEW solution from the skin after washing. Enumeration by total viable counts (TVC) of aerobic bacteria, lactic acid bacteria (LAB) and hydrogen sulphide producing bacteria (HSPB) was carried out on three separate samples from different HOGs. The enumeration of bacteria was also carried out on three independent samples before and after HOGs were washed with NEW.

2.3. Impact of sanitation and MAP combination on microbial load of HOG Atlantic salmon

HOGs of Atlantic salmon (between 51 – 53 cm in length and 2.7 – 2.8 kg) were commercially harvested from two separate locations in Tasmania, Australia, according to production sources at the time. In accordance with industry practice, the HOGs were kept in ice-water in insulated bins in a cool room at 4° C for three days pre-processing to achieve reversal of rigor mortis prior to use.

The HOGs then were transferred on ice to the laboratory. The HOGs were removed from the insulated boxes and sanitized by dipping them for five seconds in 20 L of 100 ppm NEW. The HOGs were briefly drained and manually filleting by starting from the tail and cutting along the body toward the head using a filleting knife. The HOGs for the control samples were filleted without any additional washing treatment. The fillets were then manually cut into portions of 100±5 g or 125±5. A total of 12 treatments in a combination of Washing treatments (unwashed and washed), gas to product (G/P) v/w ratio (0.4:1, 1:1 and 2:1) and storage temperatures (0° C and 4° C) were carried out.

2.4. MAP and gas setting

A single tray MAP machine (Multivac T100, Wolfertschwenden, Germany) was used for packaging the fillets. During the MAP packaging the gaseous air in the tray (high impact polystyrene (HIPS), L: 22mm; W:168 mm; D: 40 mm) BP97-40 black 1 L, Alto, Albany New Zealand) was evacuated to 5 – 7 mBar and flushed (800 – 980 mBar) with food-grade mixture of CO_2 and N_2 (70:30%) before heat sealing with a top barrier film (22 μm from Alto, Albany, New Zealand). Two independent samples from different trays were used to test for TVC on Days 0, 10, 15, 17 and 20. Another commercial sample control tray was made using chilled Atlantic salmon fillets obtained from a local supermarket that had undergone the commercial process of production, filleting, bulk MAP packaging and retail distribution. It was packaged with the same methods as mentioned above with 0.4:1 G/P ratio and stored at 4° C for 15 days. For this control, organoleptic and colour assessment, microbial analysis, and pH analysis were conducted on Day 0 and Day 15.

2.5. Organoleptic assessment

The organoleptic assessment was assessed by a trained panelist and carried out to evaluate appearance, off-odors production and color using specifications were based on industrial practice. At each assessment time, one tray per treatment was opened. Odor was assessed *in situ* in the tray by smelling the fillets as soon as the tray was opened. Slime was observed by swabbing the fillets using a sterile swab stick. Slime was recorded if slime was observed bridging between the swab and the fillets. The color of fillets was measured by comparison with the Salmon Fan Roche Color (DSM N.V, Heerlen, Netherlands) at each time point.

2.6. pH and headspace gas analysis

The pH of homogenized fillets (20 g of fillet in 0.1% peptone; 0.85% NaCl v/v) was evaluated at each time point with a pH meter (FE20, Mettler-Toledo, Columbus, OH, USA) at room temperature. A Checkmate II Gas Analyzer (PBI-Dansensor, Ringsted, Denmark) was used to analyze CO_2 and oxygen levels in the headspace of each pack in triplicate. Gas was sampled from the headspace by piercing the top of the pack with a syringe through a foam rubber septum (Pryde Measurement, Ashburton, Australia) on top of the cover film to avoid the introduction of false atmosphere into the gas analyzer. Average values from 2 samples were used for statistical analyses of the data.

2.7. Microbiological counts

A TPC was carried out at each sampling point on three separate samples from different fillet portions or skin area. To enumerate the microbial in the skin of salmon, a sterile square sampling template (4 x 5 cm (20 cm^2)), VWR™, Leicestershire, UK) was used to define the sampling area in each fillet. A single sterile absorbent cotton swab (plastic 15 cm, Westlab Pty. Ltd, VIC Australia) was rubbed across the designated square skin template area and rubbing continued until the whole template surface had been wiped. To enumerate a total microbial in salmon fillets, samples of approximately 20 g were stomached for one minute in diluent (0.1% peptone, 0.85% NaCl). Triplicate serial dilutions were spread onto Aerobic Petrifilm (3M™ Petrifilm™ Aerobic count plate) and LAB Petrifilm (3M™ Petrifilm™, LAB count plate). Petrifilms were incubated aerobically at 36±1° C for 48±3 hours prior to enumeration [16, 17]. The detection limit for the TPC and LAB petrifilm was 2.1 log CFU/g.

To enumerate HSPB triplicate serial dilutions were spread-plated onto iron agar (IA) (per litre: 20g bacteriological Peptone, 3 g Lab Lemco powder, 3 g yeast extract, 0.3 g ferric citrate, 0.3 g sodium thiosulphate, 5g NaCl, 15g agar and 0.6 g L- cysteine) [4]. The IA plates were incubated aerobically at 25±1° C for 48±3 hours prior to enumeration and only black-pigmented colonies on IA were counted [18]. The detection limit for the IA counts was 1 log CFU/g. On Day 0, TVC was determined before and after fillets were MAP packaged. At each point during storage (Days 1, 10, 15, 17 and 20) a microbial plate count was carried out to determine the total number of bacteria, HSPB and LAB.

2.8. Statistical analysis

The data were analysed by Multivariate ANOVA (MANOVA) with Tukey's Multiple Comparison Test. A significant difference was established at $P<0.05$ using SPSS, V25, (SPSS Inc., Chicago, Ill., USA).

3. Results and Discussion

3.1. Impact of sanitation concentration on microbial load of HOG Atlantic salmon

When the HOGs were washed after pre-processing storage there was a significant difference ($p<0.05$) in microbial load remaining between the control and HOGs washed with 20 or 100 ppm NEW. The microbial counts of HOGs washed with 20 ppm and 100 ppm NEW decreased from initial counts of 3.5 log CFU/cm^2 to 2 log CFU/cm^2 and from 3.5 log CFU/cm^2 to 1.4 log CFU/cm^2. No LAB or HSPB were detected in the skin swabs.

The first stages of fillet processing play a significant role in keeping a low microbial count in the final products after storage. While fish flesh is mostly free from bacteria before death, contamination occurs during the processing of the HOGs into fillets. In industrial practice, automated processing of the salmon HOGs takes less than 5 minutes to complete filleting, deboning, portioning and packaging. However, in many cases, HOGs are not sanitised to lower the microbial burden before going through the filleting machine. The study showed that having five seconds of washing the HOGs in sanitiser of at least 100 ppm FCC can significantly lower the microbial level on the skin. This would help to avoid cross-contamination of fillets during processing. Washing the HOGs with NEW 100 ppm chlorine equivalent successfully lowered the microbial number up to 2.1 log CFU/cm^2 (Figure 1). This finding had a similar result to the study that been reported by Khazandi *et al* [10]. They used a HOCl-containing water-based sanitation product (EO water, ECAS4 Adelaide, SA, Australia) at pH 7, 300 ppm of FCC

to wash the whole salmon with 15% or 50% v/v with water. They found both 15 % and 50 % v/v EO water lowered the microbial population at day 0 by 1 log CFU/g. Therefore, adding a sanitation washing step prior to filleting can help ensure a low number of counts in the fillets delay the on-set of log phase growth during storage of the packaged fillets [1, 8].

3.2. Impact of sanitation and MAP combination on microbial load of HOG Atlantic salmon

The current study showed no adverse effects with the fillets colour being above standard (27 Roche salmon colour scale) and no apparent slime or off-odour detectable by the end of storage time (20 days) regardless of the treatment. The colour of fillets on Day 20 was paler compared to Day 0 and Day 10 based on Roche Salmon Fan colour comparison. It was also observed that the packages became deflated under a slight vacuum with tray distortion occurring from Day 17 to Day 20 due to CO_2 absorption. Analysis of the pH and colour of the salmon fillets during storage showed that stored fillets packed in MAP with CO_2 70% and N_2 30% in both controlled temperatures (4 and 0 °C) gave a consistent pH value between 6.1 to 6.5. The colour of the fillets were between 30.7 – 31.0 and 28.0 – 29.7 Roche scale at day 0 and 20, respectively. For the commercial samples from the store, pH level were 6.2 and 6.3 for day 0 and day 15 fillets, respectively. The colour was decreased from 29.3 Roche scale on day 0 to 26.3 Roche scale on day 2.

The level of G/P ratio gave marked differences in gaseous CO_2 concentration in the packages over time (Figure 1). The concentration of CO_2 declined from 66% to 45, 32 and 20% for 2:1, 1:1, and 0.4:1 G/P ratio, respectively, over the 10 days. There was no significant difference ($p > 0.05$) between samples of unwashed and sanitised on the level of CO_2 in the packaging. The trend over time for the level of CO_2 in packages was highly similar. However, packages stored at 0 °C had 2 – 8% less gaseous CO_2 compared to that of packages stored at 4° C on the same storage day.

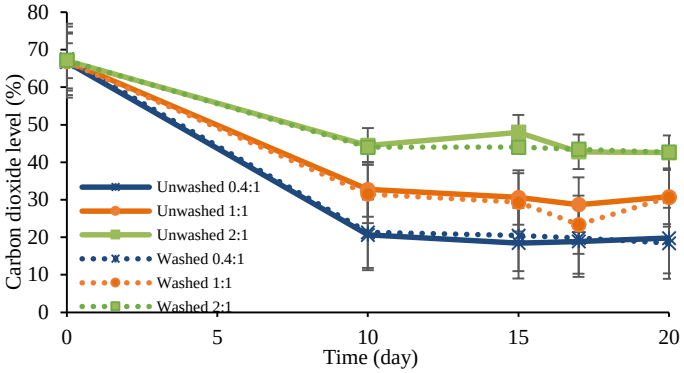

Figure 1. CO_2 concentration (%) of headspace packages of salmon fillets unwashed and sanitised (Samples were made at three levels of G/P ratio stored at 0°C for 20 days. The results represent mean (n = 2) and the error bars indicate 95% confidence intervals).

The different combinations of sanitising treatments, temperatures and G/P ratios resulted in significant differences ($p < 0.05$) in the final population of the bacteria on the fillets (Figures 2 – 3). There was no significant difference between G/P ratio and storage temperature at Day 0. The significant difference only occurred between unwashed and sanitation washed samples (Figures 2 – 3). There was a significant difference ($p<0.05$) of TVC among temperatures and treatments (unwashed and washed) at Day 10 (Figures 2 – 3). The TVC in the 0.4:1 G/P ratio was significantly different ($p<0.05$) compared to the G/P ratio of 1:1 and 2:1 (Figure 2). The TVC was not significantly different ($p>0.05$) between G/P ratio 1:1 and 2:1. The TVC of the sanitation washed sample stored at 0° C was 1 log CFU less than unwashed samples on Day 10 (Figure 2).

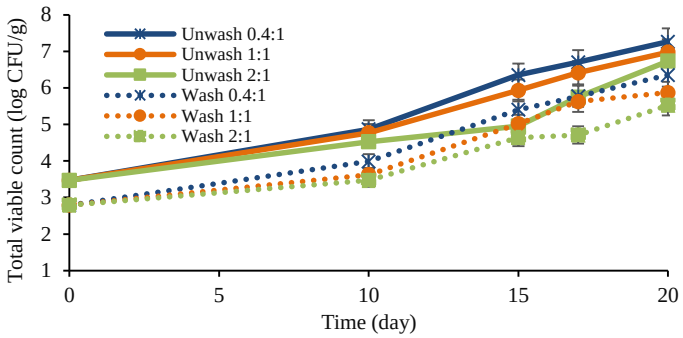

Figure 2. Total viable count (TVC) of aerobic bacteria of 4° C salmon fillets unwashed and sanitised (Samples were made at three levels of G/P ratio stored at 4° C for 20 days. The results represent mean (n = 4) and the error bars indicate 95% confidence intervals).

The TVCs of the sanitation washed samples were 1.5 and 1.7 log CFU/g less than the unwashed sample at Day 15 and 17, respectively (Figure 2). The TVC of the unwashed sample with a low G/P ratio (0.4:1) stored in 0° C was lower than 6 log CFU/g (Figure 3) on Day 20. On Day 20 TVC of the unwashed sample with a low G/P ratio (0.4:1) stored in 4° C was higher than 6 log CFU/g (Figure 3) or exceeded the maximum TVC for shelf-life determination. LAB was first detected in the plates on Day 17 and increased at Day 20, but LAB was not always found in all treatments particularly being absent in sanitation washed sample stored at 0° C. HSPB were also detected only after Day 17. No HSPB was found in fillets that were washed and packed in high G/P ratio (2:1) and stored at 0° C.

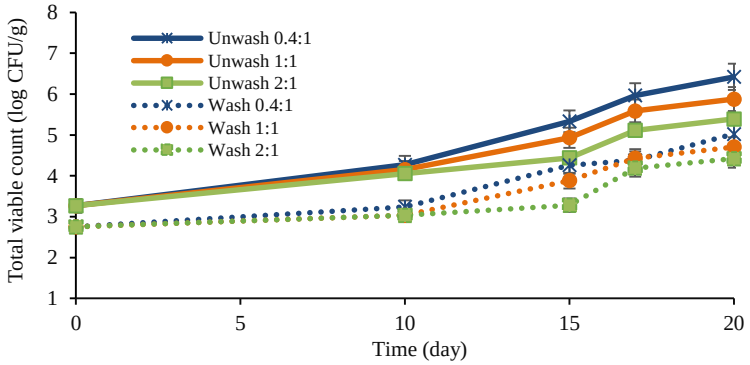

Figure 3. Total viable count (TVC) of aerobic bacteria of 0° C salmon fillets unwashed and sanitised (Samples were made at three levels of G/P ratio stored at 0° C for 20 days. The results represent mean (n = 4) and the error bars indicate 95% confidence intervals).

The sensory attributes such as colour, appearance and odour of fresh fillets from HOGs subjected to a 5 seconds wash with 100 ppm NEW before filleting were not appreciably better compared to the fillets from unwashed HOGs. The HOGs that were used had been handled from harvesting through the pre-treatment process and transportation on ice or ice/water. This industrial processing procedure had successfully maintained the freshness of the HOGs, and no improvement of the sensory attributes due to using sanitation washing of the HOGs was apparent over the storage time. However, the TVC of the unwashed sample at the lower G/P ratio stored at 0° C was higher indicating depression of the microbial growth due to sanitation washing. As the microbial load did not exceed 10^6 CFU/g it may not have been high enough to cause a sensory issue (Figures 2 – 3). The high G/P ratio was able to suppress microbial growth but this ratio cannot be achieved with industrial bulk packaged (1.5 – 2.5 kg) fillets. Therefore, washing in sanitiser may be desirable to make the shelf-life more robust after the fillets are taken out of

the MAP for sale at the fish bar in the supermarket. Further improvements might be achieved by having a longer washing sanitation time and/or higher sanitation concentration.

The counts of aerobic bacteria, HSPB and LAB of the salmon fillets sample obtained from the local supermarket were higher compared to salmon fillets packaged fresh in MAP with HOGs obtained directly from the Industry. The LAB and HSPB count at Day 0 was 4 log CFU/g and 1.6 CFU/g, respectively. The level of LAB and HSPB increased at Day 15, 5.7 CFU/g and 4.9 CFU/g, respectively. The presence of LAB and HSPB was only detected on Day 17 and 20 in this study. The mean generation time of HSPB, LAB, *Pseudomonas spp.*, *Brochothrix thermosphacta* and *Photobacterium* spp of salmon (*Salmo salar*) stored aerobically at 4 °C for 10 days was between 17.2 – 26 hours [19]. This indicates that the treatment was successful in reducing the growth of LAB and HSPB in salmon fillets.

4. Conclusions

A combination of improved pre-processing sanitation, a high G/P ratio and a low temperature can raise the hurdle for microbial growth and prolong the shelf-life of fresh Atlantic salmon fillets by at least two days and potentially up to six days above the current industry shelf-life of 14 days. A 100 ppm FCC is an adequate concentration of NEW/NEI for improving the pre-processing sanitation.

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