Combining Modified Atmosphere Packaging and Nisin to Preserve Atlantic Salmon

Dong Han[1], Inyee Han[2] & Paul Dawson[2]

[1]Auburn University, Auburn, AL, USA

[2]Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC, USA

Correspondence: Paul Dawson, Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC 29634, USA. Tel: 864-656-1138. E-mail: pdawson@clemson.edu

Abstract

Preservation effects of modified atmosphere package combined with nisin on fresh Atlantic salmon were evaluated. Farm-raised Atlantic salmon were purchased from the local market and packaged using either 19 % CO_2: 70 % N_2: 11 % O_2, 38 % CO_2: 51 % N_2: 11 % O_2, and under atmospheric air (with and without nisin at 400 IU/g) resulting in a total of 6 treatments. The microbiological (aerobic plate count, psychrotrophic bacteria, and lactic acid bacteria) and the total volatile basic nitrogen analyses were evaluated on Day 0, 2, 4, 7 and 10. Package headspace and sensory evaluation were also conducted on Day 0, 2 and 4. The presence of CO_2 effectively inhibited the growth of all three types of bacteria while nisin significantly inhibited the growth of aerobic microorganisms with less impact on lactic acid bacteria. The TVB-N test indicated that CO_2 delayed the spoilage of Atlantic salmon while nisin had a lesser but measurable impact on Atlantic salmon shelf-life. The experiments support the potential for combining modified atmosphere package and nisin as an effective method to limit the spoilage of Atlantic salmon compare to traditional preservation methods.

Keywords: Atlantic salmon, modified atmosphere packaging, nisin, shelf life

1. Introduction

Modified atmosphere packaging (MAP) has become a popular preservation technology (McMillin, 2008). The basis of MAP is a sealed food package with an altered headspace gas mixture. Both microbiological and chemical reactions continue during shipping and storage often altering the package gas headspace. Several researchers (Fagan et al., 2004; Wang et al., 2008; Economou et al., 2009; Tsironi et al., 2010) have studied the effect of the MAP on fresh seafood product shelf life. Other researchers (Jayasingh, et al., 2002; Sivertsvik et al., 2003; Fagan et al., 2004; Wang et al., 2008; Economou et al., 2009) have demonstrated that higher concentrations of CO_2 extend the microbiological shelf-life of fresh seafood. However, high concentrations of CO_2 can also lead to quality loss.

Salmon spoilage is multifaceted and one of the main factors causing spoilage is the growth of microorganisms (Rasmussen et al., 2002). In MAP preserved salmon, O_2 levels and temperature are lower than ambient conditions and these different environments have an impact on the growth of microorganisms. Beside microbiological growth, other chemical indexes can reflect the spoilage level or freshness of Atlantic salmon. For example, total volatile basic nitrogen (TVB-N) (Dhaouadi et al., 2007) and trimethylamine (TMA) have been used for this purpose (Gökoğlu et al., 2004; Erkan and Özden, 2008). TVB-N is an indicator of total nitrogen and during the storage of salmon, the reduction of trimethylamine oxide (TMAO) results in the production of trimethylamine (TMA) (Pena-Pereira et al., 2010). The measurement of TVB-N and TMA has been employed as spoilage indicators for decades and the ratio of TVB-N to TMA (TVB-N/TMA) has been an index of seafood freshness (Mitsubayashi et al., 2004; Howgate, 2010).

Nisin is a well-known bacteriocin that inhibits the growth of gram-positive bacteria and is generally recognized as safe (GRAS) as a food preservative (de Arauz et al., 2009). Researchers have confirmed that nisin (Jofré et al., 2008; Lu et al., 2010; Shirazinejad et al., 2010) and MAP (Raju et al., 2003; Economou et al., 2009; Tsironi et al., 2010; López‐Mendoza et al., 2007,) can extend the shelf life of seafood when used individually. However, little

has been published on combining MAP with nisin to extend the shelf life of fresh Atlantic salmon. Therefore the objective of this research was to determine the effect of nisin combined with MAP on Atlantic salmon shelf life.

2. Material and Methods

2.1 Salmon

Fresh, sliced farm-raised Atlantic salmon fillets were transported on ice (2:1 ice:salmon ratio)to the laboratory. Salmon was purchased as skinless and boneless fillets from a local grocery store and held under super-chilled conditions (-4±2 ℃) until experiments (up to 3 days). Frozen fillets were thawed at 4±2 ℃ for 12 hours. These fillets were cut into portions of approximately 150 g and then further cut a 25 g portion and a 10 g portion of the 150 g fillet. Thirty nine portions of 150 g Atlantic salmon samples were prepared and 3 were tested immediately. Processing and packaging procedures were conducted under strict hygienic conditions.

2.2 Nisin Activity

Commercial nisin (Nisaplin®, 10^6 IU/g) was purchased from the manufacturer (Danisco, New Century, KS, USA). Nisaplin® activity was determined in triplicate using a zone of inhibition assay. Nisaplin® sample was diluted 1:500 in sterile water, then a series of two-fold dilutions were tested against *Lactobacillus plantarum* ATCC 14917 by spotting 10 µL of each dilution on the surface of the MRS agar medium seeded uniformly with the suspension of *L. plantarum*. After 48 h incubation at 37 ℃ under 5% CO_2, the plates were examined for inhibition zones. The activity of nisin in Arbitrary Units per mL (AU/mL) was expressed as the reciprocal of the highest dilution showing a clear inhibition zone for each triplicate sample. The activity of nisin was expressed in AU/mg based on the weight of the nisin compounds used in serial dilution. Stock nisin (10^6 IU/mL solution was then prepared by dissolving Nisaplin® **i**n sterile water.

2.3 Modified Atmosphere Packaging

Final gas mixture ratios in packages were 19% CO_2 : 70% N_2 : 11% O_2 and 38% CO_2 : 51% N_2 : 11% O_2. Previous researchers have tested 60:40 CO_2:N_2 ratios and studies from our group with other meat types found lower CO_2 with N_2 and O_2 mixtures were successful in extending shelf life (Naas et al., 2013; Kalleda et al., 2013).Three salmon portions (10, 25 and 115 g) were placed in foam trays (C976 Sealed Air Cryovac, Duncan, SC) (8¾ * 6¾ * 15/8 ″). The nisin solution was spread on the surface of fillets using a sterile spreader to obtain a final nisin concentration of 400 IU/g. The six MAP -nisin combination treatments were; air, 19:70:11% CO_2:N_2:O_2 or 38:51:11% CO_2:N_2:O_2 each either without or with 400 IU/g nisin. A Ross Jr™ preformed tray MAP machine (Model No. S-3180, Robert Reiser & Co. Inc., Canton, MA 02021) was used for packaging all salmon. Gases used in the package were pure mixtures of CO_2, O_2 and N_2 (National Specialty Gases, Durham, NC 27713). A vacuum pressure of 150 mbar, gas pressure of 765 mbar, seal time of 2.1 sec, knife temperature of 143 ℃ and seal temperature of 141 ℃ were pre-set on the MAP machine. The trays were sealed using lid stock film (Lid 1050) (18.5″wide) to achieve a gas:product ratio of 5:1. All samples were refrigerated (2±4 ℃ C) until the analyses. At the same time, 7 empty packages were also sealed with the 3 gas mixtures and stored in the same environment as salmon samples to monitor the gas composition of empty packages.

2.4 Analyses - Headspace Gas Analysis

There were 4 major analyses (headspace gas analyses, microbiologic enumeration, TVB-N titration test, sensory testing) employed to evaluate freshness of salmon samples. All analyses were conducted on samples from each of the 6 treatments as described in Table 1 on days 0, 2, 4, 7 and 10, except for package headspace gas mixtures which were taken on days 0, 2 and 4. The gas mixture in the headspace of an airtight food package was monitored to determine how gas composition changed relative to the spoilage of the salmon. A gas chromatograph (series 200, Gow-Mac Inst.Co., Bethlehem, PA) fitted with CTR-1 gas analysis column (catalog no.8700, Alltech, Sanjose, CA) and TCD (thermal conductivity detector) was used to determine the package headspace gases (O_2, CO_2, N_2). An integrator (Hewlett Packard, Wilmington, DE) was used to plot chromatograms and calculate gas percentages from peak areas. A 0.05 mL package headspace gas sample was analyzed at each sampling interval by injecting a needle (syringe type) through a gas-tight septum placed onto the package film surface. The chromatograph was calibrated using standardized gas mixtures verified by manufacturer (Air Products and Chemicals Inc., Allentown, PA).

2.5 Microbiological Enumeration

Twenty-five g portion of fish samples were aseptically removed from trays, placed in sterile stomacher bags (model 400, 6041/STR, Seward Limited, London, UK) and homogenized for 2 min at 230 rpm in a laboratory blender (Model 400, Seward™, FL, USA 33330) containing pre-added 225 mL pre-chilled sterile peptone-physiological saline solution (0.1% peptone + 0.85% NaCl) (Difco™, Bactopeptone, Becton, Dickison

& Company, MD, USA 21152). Then decimal serial dilutions were prepared from this homogenate in the same chilled sterile diluent. Culture medium for aerobic microorganisms was Plate Count Agar (PCA) (Difco^{TM}, Bactopeptone, Becton, Dickison & Company, MD, USA 21152). The plates were incubated for 48 h at 37 ℃ and the population of psychrotrophic bacteria was determined by a spread plate counting method with PCA with 1% NaCl and incubated at 4 ℃ for 7 days. For estimation of potential lactic acid bacteria (LAB), diluted samples were plated on deMan, Rogosa, and Sharpe (MRS) agar and incubated at 37 ℃ for 72h in a CO_2 incubator with a continuous CO_2 flow. (Difco^{TM}, Bactopeptone, Becton, Dickinson & Company, MD, USA 21152) Prior to data analyses of microbiological data, bacterial populations were converted to logarithmic values (CFU/g).

2.6 Total Volatile Basic Nitrogen

Total volatile basic nitrogen (TVB-N) tests were prepared by homogenizing 10 g of fish from trays with 100 ml water in a laboratory blender (Model 400, Seward^{TM}, FL, USA 33330) for 1 min at 230 rpm. Then the salmon-water mixture was centrifuged at 3000 rpm for 5 min with the centrifuge at 10,000 x g (J-26 XPI, Beckman Coulter, Inc., CA, USA 92821). 10 ml of the supernatant was placed into a distillation tube, followed by 10mL of 1% (w/v) magnesium oxide suspension. Vacuum-distillation was conducted using a vertical distillation unit (Model RV 10 digital, IKA ® Works, Inc., NC, USA 28405) and the distillate was placed into 20mL of 2% (v/v) aqueous boric acid solution with the 7-8 drops of indicator solution. After five minutes, the distillation was ended and titrated. The titration was conducted using 0.005 mol/L sulphuric acid solution. The indicator solution was a mixture of 0.2% methyl red ethanol solution and 0.1% methylene blue solution added immediately before titration. The titration endpoint was a color change from green to blue/purple.

2.7 Sensory Test

All the assessors were trained using pre-spoiled salmon series. 5 samples were prepared for each of 7 days of training and held at 20 ℃ for different time periods to accelerate spoilage. 3 terms (general appearance, color, and odor) were used on a 5 point scale for the 5 samples so that assessors agreed on the different levels of salmon spoilage.

At least 7 assessors were involved in each day's sensory test. On each day of testing, packages were opened and each of the six samples was tested immediately by the trained panel using the 5-point scale. The minimal scoring difference was set at 0.5. After the grading, panelists were asked to decide if the samples were acceptable or not for consumption.

2.8 Five-Level Sensory Scale (Modified From Matis, 2016)

General Appearance:

level. 1: Firm texture with natural and fresh fish fillet appearance.

level. 2: Slight drip loss and minor reduction on firmness and appearance

level. 3: Soft texture and obvious reduction in appearance

level. 4: Extreme soft texture and critical appearance reduction

level. 5: Totally spoiled salmon texture and appearance

Color:

level. 1: Flesh-colored salmon tissue with almost no effect of spoilage.

level. 2: Minor change of flesh-colored salmon tissue.

level. 3: Obvious change from flesh-colored to deeper red colors.

level. 4: Extreme change of flesh-color deep red color.

level. 5: Totally spoiled salmon dark red color

Odor:

level. 1: Flesh seafood-like smell

level. 2: Slight fish odor

level. 3: Obvious change from flesh odor to fishy odor.

level. 4: Strong fishy odor.

level. 5: Completely spoiled fishy odor

2.9 Statistical Analysis

All analyses (headspace gas analyses, microbiological enumeration, TVB-N tests and sensory tests) were replicated three times in separate trials with different lots of salmon. Analysis of variance was conducted for each parameter to determine if there was a significant effect ($p \leq 0.05$) due to the treatments. When the treatment was determined to be significant for a parameter based on an analysis of variance, the Least Significant Difference (LSD) test was used to separate the means ($p \leq 0.05$) (SAS, Version 9.0, 2004).

3. Results

3.1 Nisin Activity Detection

An activity level of 1.6×10^6 AU/ml was confirmed for the commercial nisin sample. Nisin was first detected from its production by *Lactococcus lactis* subsp. *lactis* in 1928, and has become a popular bacteriocin with widespread commercial use (Ross et al., 2002). Nisin effectively inhibits the growth of Gram-positive bacteria, such as *Micrococcus*, *Lactococcus*, *Staphylococcus*, *Lactobacillus* and *Listeria* (Arauz et al., 2009). The gram-positive bacteria strain used to determine nisin activity was *Lactobacillus plantarum* ATCC 14917. The results verified that growth of *Lactobacillus plantarum* ATCC 14917 can be inhibited by nisin. Also, the commercial nisin sample was found to retain high activity throughout use in the study. Nisin doesn't generally inhibit the growth of gram-negative bacteria, fungi and virus (Arauz et al., 2009). This group of microorganisms can cause spoilage of food products which limits nisin preservation effects. Nisin has been reported to restrict some pathogenic bacterial growth. When *L. monocytogenes* was inoculated into long-life cottage cheese the number of viable *L. monocytogenes* cells was reduced by one log with nisin (Ferreira and Lund, 1996). *Clostridium sp.* can be susceptible to nisin and spore outgrowth of *Clostridium sp.* is more likely to be restricted than vegetative cell growth by nisin (Delves-Broughton, Blackburn, Evans & Hugenholtz, 1996).

3.2 Headspace Gas Analyses

During the storage of empty MAP sealed packages, there was no change in CO_2 and O_2 percentage concentrations for both 19% CO_2/70% N_2/11% O_2 and 38% CO_2/51% N_2/11% O_2 packages (P>0.05).This result indicates there was an airtight seal and stable gas environment throughout the Atlantic salmon preservation study. Most MAP is formed from one or more of these four materials: polyvinylchloride (PVC), polyethylene terephthalate (PET), polyethylene (PE) and polypropylene (PP), moreover, PE is usually the major component in a MAP film since it provides the hermetic seal. Polyethylene is also considered for the characteristics of anti-fogging ability, peel ability and the ability to seal under less than optimal conditions (Phillips, 1996).

The technique of sealing modified atmospheres in the polymeric film without further exchange of gasses between inside and outside of the package often generates lower O_2 and higher CO_2 concentrations compared to atmospheric conditions to extend food shelf life. These conditions can influence the biological activity (respiration, enzymatic reactions, and microbial growth) within the package which can, in turn, inhibit spoilage (Zhao et al., 1995; Mangaraj et al., 2009).

Initial O_2 concentration ranged from about 11% in MAP packages to 21% for air packages (Table 1). On Day 2, salmon samples packaged in the air with and without nisin had similar O_2 concentrations (p>0.05), however, by Day 4, there was a significant difference ($p \leq 0.05$) of the oxygen level between air/nisin and air/no nisin. Also, the 38% CO_2 MAP treatment inhibited O_2 consumption compared to the 19% CO_2 MAP ($p \leq 0.05$) at day 2. Thus as CO_2 concentration increased, the O_2 consumption rate of microorganisms present on salmon decreased at early sampling times. This implies that the microorganism's respiration rate was slowed with the increase in CO_2. Similar to the contrast between air/nisin and air/no nisin treatments, the 19% CO_2 package with and without nisin on day 4 also limited the metabolism rate of the microorganisms ($p \leq 0.05$). As the primary gas consumed by spoilage microorganisms during growth, reduced oxygen has been recognized as an important factor in MAP. Taylor, Davidson, & Zhong, (2007) reported commercial nisin samples with similar composition as the one used in the current study [2.5% pure nisin, 74.4% sodium chloride, 23.8% denatured milk solids and 1.7% moisture (w/w)] had similar activity (10^6 IU/g). Nisin limited the growth of spoilage microorganisms and reduced total oxygen consumption in both the present and study by Taylor, Davidson & Zhong (2007).

Table 1. The concentration of O_2 of farmed Atlantic salmon packaged in different air packages and nisin stored at 2-4 °C

Treatment (package condition)	Day 0	Day 2	Day 4
	Percentage of Oxygen package headspace		
Air + Nisin(400 IU/g)	20.60 ±0.26 a	18.08 ±0.57 a	8.92 ±0.42 b
Air without nisin (control)	20.60 ±0.26 a	17.76 ±0.15 a	5.89 ±0.44 b
38% CO_2/51% N_2/11% O_2 + Nisin(400 IU/g)	10.75 ±0.10 a	10.45 ±0.31 a	6.95 ±0.33 b
19% CO_2/70% N_2/11% O_2 + Nisin(400 IU/g)	11.01 ±0.18 a	8.14 ±0.28 b	6.5 ±0.46 b
38% CO_2/51% N_2/11% O2 without nisin	10.75 ±0.10 a	9.62 ±0.23 a	6.26 ±0.24 b
19% CO_2/70% N_2/11% O_2 without nisin	11.01 ±0.18 a	8.18 ±0.39 b	4.16 ±0.69 cb

a-c means within rows with different letters are significantly different (p≤0.05). n=3

3.3 Microbiologic Enumeration

In fresh food product preservation, especially fresh seafood, and meat ingredients, microorganism growth is the primary cause of spoilage (Hozbor et al., 2006). Enumeration of particular microorganisms' groups is important to understand factors affecting spoilage.

3.3.1 Aerobic Plate Count Cultured at 37 °C

The average starting population of aerobic microorganisms on salmon was 3.25 ± 0.06 log CFU/g. The total aerobic bacteria for the 2 MAP treatments differed from air-packed salmon (p≤0.05) on day 2 (Figure 1).
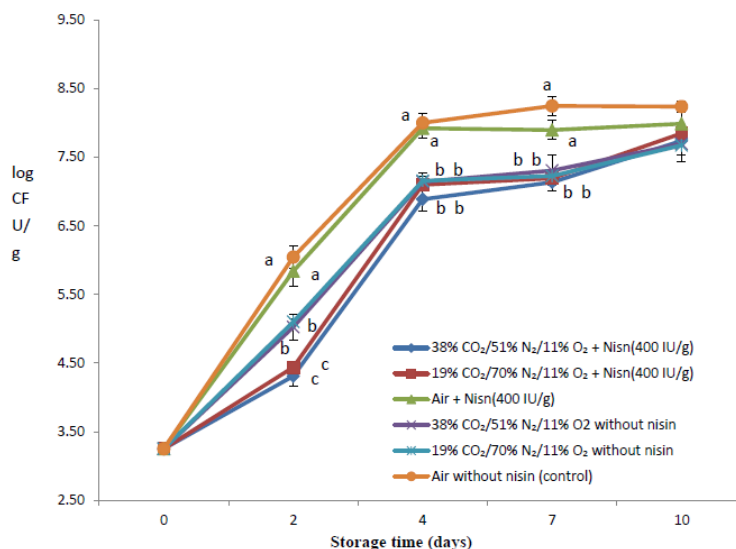

Figure 1. Total aerobic bacterial populations of farmed Atlantic salmon stored at 2-4 °C packaged in various modified atmosphere packaging with and without nisin (n=4) a-c means with a different letter are significantly different (p≤0.05)

Furthermore, the 2 MAP treatments with nisin had a lower population of aerobes on salmon on day 2 compared to the MAP treatments without nisin. No difference in aerobic bacteria population was observed between salmon packaged in CO_2 concentrations of 19% and 38% (p>0.05). On days 2, 4 and 7 the population of bacteria on salmon packaged in the air was greater than MAP-packaged salmon (p≤0.05). By day 10, MAP salmon treatments had reached ~ 8 log CFU/g aerobic, however, air-packed salmon reached this level by Day 4. Ibrahim Sallam (2004) used 7 log CFU/g aerobic plate count population as the spoilage indicator in fish products and following this standard, MAP-packaged salmon had approximately a 1-2 day longer shelf life compared to salmon packaged in air.

3.3.2 Psychrotrophic Bacteria Count

Similar to aerobic microorganisms, no difference in psychrotrophic bacteria population was observed between

salmon packaged in CO_2 concentrations of 19% and 38% (p>0.05) (Figure 2). Air-packaged salmon had higher psychrotrophic bacteria populations (about 1 log CFU/g) than the other four treatments at day 2 (p≤0.05). Thus, CO_2 inhibited the growth of psychrotrophic bacteria while nisin had no observable inhibition of psychrotrophic bacteria (p>0.05). As storage continued, psychrotrophic bacteria population differences due to the various treatments diminished (p>0.05).
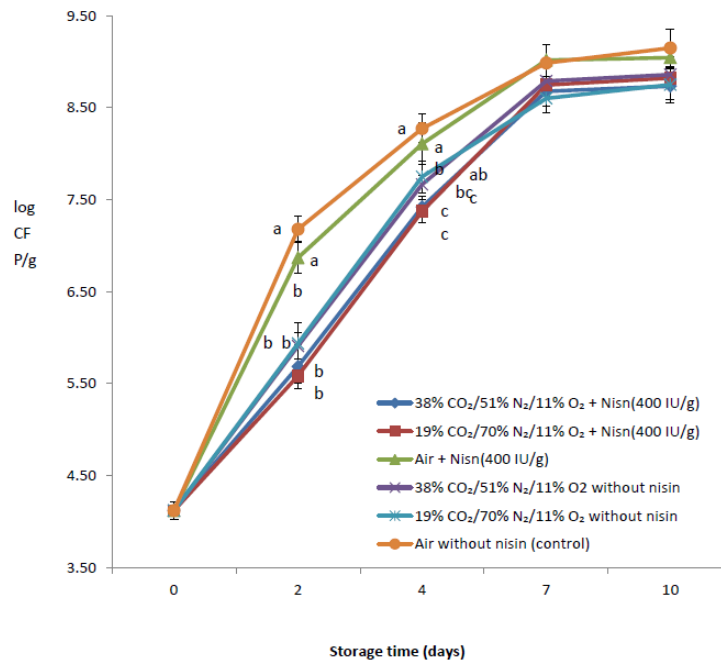

Figure 2. Psychrotrophic bacterial populations of farmed Atlantic salmon stored at 2-4 ℃ packaged in various modified atmosphere packaging with and without nisin (n=6). a-c means within rows with a different letter are significantly different (p≤0.05)

Due to the storage of fresh fish at refrigerated temperatures, psychrotrophic bacteria dominate the spoilage of refrigerated fish (Sivertsvik et al., 2002). Gram-negative psychrotrophic bacteria often dominate in the spoilage of refrigerated fresh food products and thus may reduce the efficiency for nisin to extend shelf life since nisin alone has a little inhibitory effect on Gram-negative bacteria. Nisin can target vegetative cells acting at the cytoplasmic membrane causing pores resulting in cell degradation (Bauer and Dicks, 2005). However, nisin is usually ineffective against gram-negative bacteria due to the presence of the lipopolysaccharide layer (LPS) (Millette et al., 2004).

3.3.3 Lactic Acid Bacterial Count

Initial lactic acid bacteria (LAB) populations were 1.25 ± 0.17 log CFU/g and compared to MAP packaged salmon, air packaged samples without nisin had higher populations at day 2 and 4 than any CO_2 packaged salmon (p≤0.05)(Figure 3). The 38% CO_2 preserved salmon inhibited LAB population compared to 19% CO_2 regardless of nisin application at day 2. As storage continued, LAB population differences due to the various treatments dissipated. Interestingly, there may be a synergistic effect of LAB and nisin on Gram-negative bacteria since LAB has been shown to disrupt the outer Gram-negative membrane (Alakomi et al, 2000), possibly making Gram negative bacteria more susceptible to nisin.
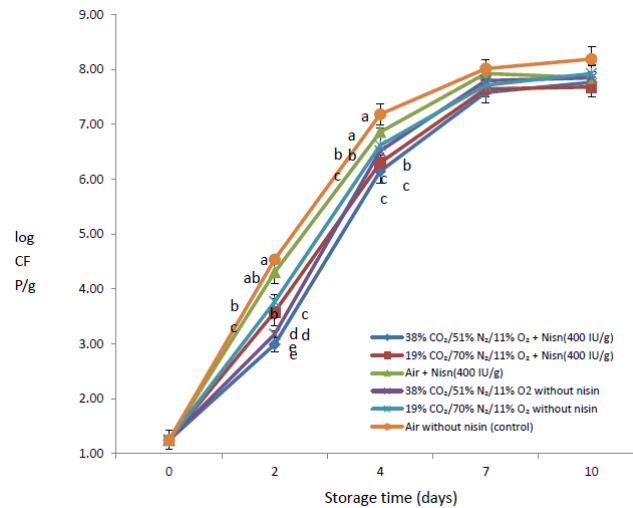
Figure 3. Lactic acid bacterial populations of farmed Atlantic salmon stored at 2-4 ℃ packaged in various modified atmosphere packaging with and without nisin (n=6). a-e means within rows with a different letter are significantly different (p≤0.05)

3.3.4 TVB-N Titration Test

The initial concentration of total volatile basic nitrogen (TVB-N) titration was 2.89 ± 0.01 mg/100 g. On all 4 sampling days, TVB-N concentration was higher in air-packed salmon compared to MAP packed salmon (Figure 4) ($p \leq 0.05$). The only difference in TVB-N between air/nisin and air/non-nisin packed salmon was on day 2 ($p \leq 0.05$). No significant TVB-N difference was detected between the 4 CO_2 MAP treatments ($p > 0.05$). The application of CO_2 delayed Atlantic salmon spoilage as determined by TVB-N. A significant difference was detected between the CO_2 MAP and the air packed samples on all four sampling days ($p < 0.05$). No significant difference in the production of TVB-N was found between the 38% and 19% CO_2 ($p > 0.05$). Nisin had no impact on the TVB-N concentration except for air packed samples on day 2 ($p \leq 0.05$). Application of nisin in the aerobic atmosphere has been extensively researched but nisin under anaerobic conditions has not received as much attention. Nisin was not as effective as CO_2 headspace in inhibiting the general spoilage of packaged Atlantic salmon. The increase in TVB-N shows a similar trend as the bacterial growth of the psychrotrophic bacteria. Previous research has verified a strong connection between the generation of TVB-N and the spoilage of refrigerated fresh products (Arashisar, Hisar, Kaya & Yanik, 2004, Ojagh, Rezaei, Razavi, & Hosseini, 2010)
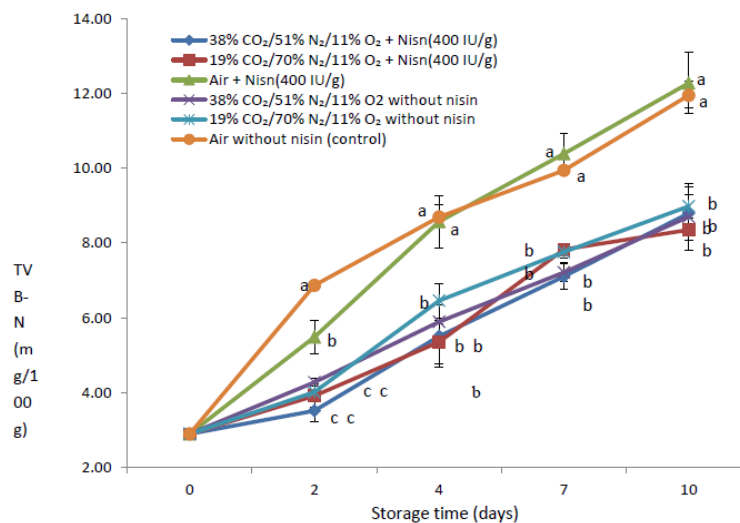

Figure 4. TVB- N of farmed Atlantic salmon stored at 2-4 ℃ packaged in various modified atmosphere packaging with and without nisin (n=6). a-c means within rows with a different letter are significantly different (p≤0.05)

Lactic acid bacteria are recognized as one of the major spoilage groups in MAP foods and controlling LAB can impact shelf-life. The higher package CO_2 concentration resulted in a slower growth of LAB ($p \leq 0.05$). Although LAB is recognized as a spoilage bacteria that can grow at low oxygen levels, the present of CO_2 successfully slowed the growth of LAB in the current experiment. Sivertsvik et al. (2002) concluded that CO_2 decreases the growth of microorganism especially aerobic bacteria and that this inhibition could not be explained only by the limited O_2 nor the pH changes caused by the CO_2. Other research has revealed that the presence of oxygen can inhibit the growth of LAB (Ibrahim Sallam, 2007). Sivertsvik et al. (2002) theorized that the effect of CO_2 on microorganisms could be described by changes in cell membrane function which effect nutrient absorption, the deactivation of enzymes, degradation of membranes and changes in the proteins. The results show that nisin was not as effective as CO_2 in limiting the growth of LAB.

3.5 Sensory Test

While sensory analysis can be affected by microbiological status, microbial safety is a priority during shipping and storage of fish.. No significant difference ($p > 0.05$) were detected for the first three evaluation terms (appearance, color, and odor) due to the 6 treatments. On the acceptability, a significant difference was observed that air packaged sample without nisin application displayed the lowest acceptable rate on day 4 ($p \leq 0.05$). Thus all the CO_2 or nisin -treated Atlantic salmon samples were more acceptable than control salmon samples. Fish tissue usually contains 60-80% (w/w) water (Ghaly et al., 2010) and the drip loss can cause the major decrease of fresh fish's appearance. The release of water from the fish tissue resulting from microbial growth can reduce the general appearance and overall quality of Atlantic salmon. Alfnes et al. (2006) mentioned the color of salmon is an essential factor of freshness. Ottestad et al. (2011) reported that the color of Atlantic salmon is dependent on the relationship between oxygen and myoglobin. No sensory color difference ($p > 0.05$) was observed in the current study. Inside and surface volatile and non-volatile amines (Bulushi et al., 2009) are the major spoilage amine produced by the blooming of all kinds of microorganisms. Although differences between TVB-N test results were observed, there was no significant difference ($p > 0.05$) in the sensory odor evaluation. However, a difference in salmon acceptability ($p \leq 0.05$) was observed between the control (no nisin packaged in air) and the other 5 storage treatments of Atlantic salmon.

4. Conclusion

Combining MAP with nisin can impact the spoilage rate of Atlantic salmon, however, little synergistic effect between MAP and nisin were observed in this study. In all three microbiological test methods (aerobic plate count, psychrotrophic bacteria, and LAB), the present of CO_2 effectively inhibited the growth of these microorganisms. However, the inhibition effectiveness difference between the medium concentration of CO_2 (38%) and low concentration of CO_2 (19%) was only found in LAB. Furthermore, nisin only significantly inhibited the growth of aerobic microorganisms. CO_2 can efficiently limit the spoilage of Atlantic salmon as measured by the TVB-N test but no difference was observed between 38% and 19% CO_2. The sensory evaluation found no differences in appearance, color, and odor. But more assessors tended to reject the non-nisin and non-CO_2 packed samples compared to the other treatments. Modified atmosphere packaging alone may be a more cost-effective than adding nisin to shelf life extension of fresh salmon.

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