12-2014

COMBINING MODIFIED ATMOSPHERE PACKAGING AND NISIN TO EXTEND THE SHELF LIFE OF ATLANTIC SALMON (SALMO SALAR)

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COMBINING MODIFIED ATMOSPHERE PACKAGING AND NISIN TO EXTEND THE SHELF LIFE OF ATLANTIC SALMON *(SALMO SALAR)*

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Food, Nutrition and Culinary Sciences

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by
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December 2014

Accepted by:
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ABSTRACT

Atlantic salmon is often used in laboratory experiments to represent marine fishes. Many researchers try to optimize the levels and dosage of preservatives to maintain the freshness and extend the shelf-life of Atlantic salmon. Little information has been provided about the effectiveness of combined method between MAP and nisin, especially on the preservation of Atlantic salmon. Strong evidences suggest that MAP associated with nisin may have a potential to improve Atlantic salmon storage quality.

Preservation effectiveness of combining modified atmosphere package (MAP) and nisin on fresh Atlantic salmon was determined using various methods. Six groups of farmed Atlantic salmon were purchased from local market and treated with using MAP (19 % CO_2 / 70 % N_2 /11 % O_2 , 38 % CO_2 / 51 % N_2 /11 % O_2 and air) and nisin (400 IU/g or not applied). Microbiological enumeration (aerobic plate count, psychrotrophic bacteria and lactic acid bacteria) and TVB-N test were conducted on Day 0, 2, 4, 7 and 10 of refrigerated storage. Package headspace gas composition and sensory evaluation were carried out on Day 0, 2 and 4. The presence of CO_2 effectively inhibited the growth of bacteria while nisin only inhibited the growth of aerobic microorganisms. TVB-N test indicated that CO_2 can efficiently delay the spoilage of Atlantic salmon while nisin has less of an impact on Atlantic salmon shelf-life. The experiments support the combination of modified atmosphere package and nisin as an effective method to limit the spoilage of Atlantic salmon compared to traditional preservation methods.

ACKNOWLEDGMENTS

I would like to present my gratitude to my program advisor Dr. Paul Dawson for the tremendous support and direction during my graduate study in Clemson especially in the research project of “Combining Modified Atmosphere Packaging and Nisin to Extend the Shelf Life of Atlantic Salmon *(Salmo Salar)*”. I would like to thank Dr. Inyee Han for her profound help in the research project. I would also like to thank my committee members, Dr. Xiuping Jiang and Dr. T. R. Jeremy Tzeng for their essential advice and fundamental help.

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CHAPTER ONE: LITERATURE REVIEW

Market of Atlantic salmon

Atlantic salmon *(Salmo salar)* is a migratory fish found widely in the northern Atlantic Ocean and adjacent freshwater. During the few past decades, it has become an important marine fish species in food markets (Fig.1). Atlantic salmon provides high nutritional value with low calories, and a variety of minerals and vitamins. It is also rich in omega-3 polyunsaturated fatty acids.

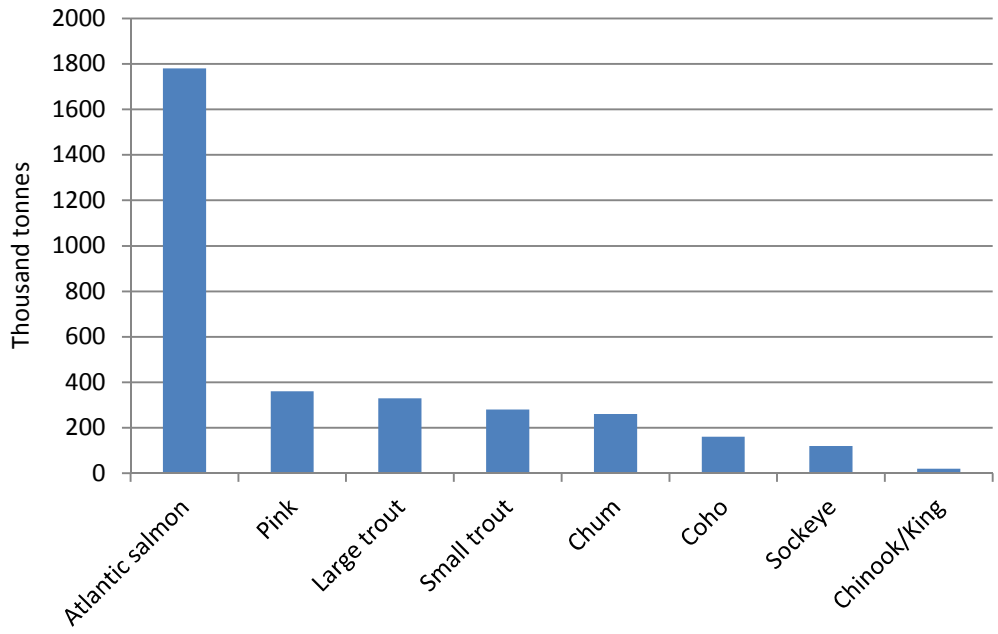

Fig.1. 2012 Worldwide Salmonids Harvest (species)

In 2012, the total harvest of Atlantic salmon was 1.78 million tones. As the largest species of edible salmonids, Atlantic salmon can be prepared in many ways such as smoking, grilling and sushi. Atlantic salmon processing industry requires high quality salmon, especially for sushi consumption. Inland farming of Atlantic salmon occurs in Norway, Chile, UK, North America and New Zealand/Tasmania (Fig.2). Recent data shows North America has become the second largest Atlantic salmon market in the world having only 37 present of entire demand fulfilled by the harvest of its own.

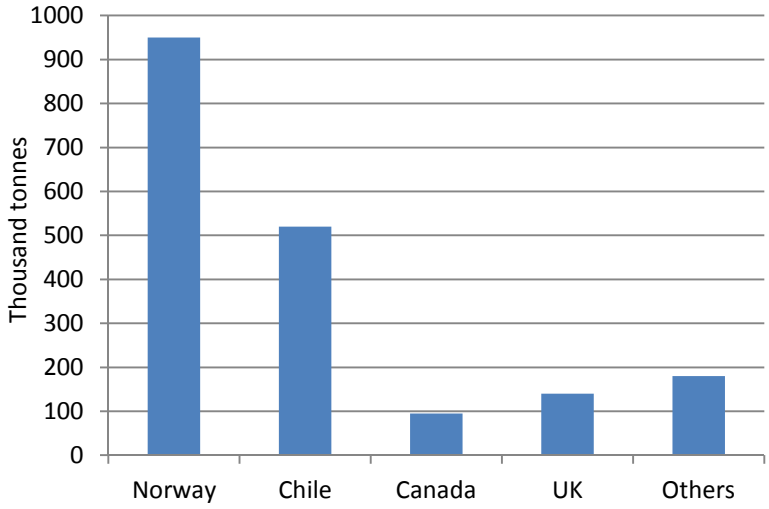

Fig.2. 2011 Worldwide Salmonids Harvest (Place of Production)

Salmon is harvested and frozen in Norway and South America then transported to North America by cargo ship. Generally, freezing of Atlantic salmon is necessary according to both industrial procedures and federal legislation. However, processing and retail

establishments have a high demand for fresh Atlantic salmon and the shelf-life of the fresh salmon is an important factor influencing the salmon industry.

Modified atmosphere packaging (MAP) has become a widely adopted preservation method during the last few decades because of increased demand for fresh products. MAP is replacement of air in the package with a different gas mixture. Low O_2 with high CO_2 limits microbiological growth and chemical reactions to extend food shelf-life. Nisin is known as an efficient, non-toxic, safe, and natural food preservative. Determining the preservation effect of MAP with nisin on fresh salmon is the main goal for this research. Improving quality retention of Atlantic salmon between harvest and consumption may have a significant impact on the Atlantic salmon market. Studies on salmon preservation may also benefit related food industrial fields.

Atlantic salmon preservation

As mentioned earlier, freezing is an important method of preservation of Atlantic Salmon. Modern refrigeration is often employed in the Atlantic salmon industry and research has revealed that freezing can maintain Atlantic salmon quality at a high level. Indergård et al., (2013) tested biochemical, structural, sensory and microbiological factors of Atlantic salmon after long-term frozen storage. -25 °C frozen storage maintained the salmon at an acceptable level after 12 months. -60 °C frozen storage

reduced drip loss but had no other significant quality improvements than the –25 °C frozen. Many steps during salmon processing are not performed in a low temperature environment such as slicing and packaging. Duun et al., (2008) tested the parameters of superchilled Atlantic salmon with vacuum package at -1.4 and -3.6°C. They reported that the shelf-life of vacuum packed salmon fillets was doubled with the utilization of superchilled storage at -1.4 °C and -3.6 °C. Some processes require thawed Atlantic salmon for preparation to retail markets. Repeated freezing and thawing will inevitably damage salmon fillet tissue. Einen et al., (2002) compared how freezing and thawing of fresh or frozen Atlantic salmon fillets affected drip loss, gaping, texture, color and rigor contraction. They concluded that freezing decreased color quality and firmness and unfrozen fillets had less fillet gaping, higher color score, lower drip loss and firmer texture. Moreover, as an indispensable step in Atlantic salmon industry, thawing is often implemented more slowly than freezing and usually causes damage to salmon tissue. Although many new freezing and thawing techniques have been utilized in food industry, particular characteristics of Atlantic salmon result in many difficulties in implementing these freezing and thawing techniques. For example, high-pressure thawing (HPT) has been applied in many food marketing aspects. However, Zhu et al., (2004) reported that HPT of Atlantic salmon resulted in significant drip loss and structural cracking compared to other thawing methods. Even though HPT significantly accelerated the thawing process, it is not a favorable method for Atlantic salmon thawing because of the quality loss.

There are several challenges and opportunities in the preservation of Atlantic salmon. Damage during repeated freezing and thawing has led to studies to protect stored salmon. Ice-chilling, vacuum and other fundamental preservation methods are often employed to preserve Atlantic salmon in an attempt to satisfy the rising demand for salmon. Many researchers are studying more efficient ways to preserve Atlantic salmon. Gallart-Jornet et al., (2007) showed that superchilling was more effective compared to freezing and ice-packing in preserving raw Atlantic salmon. Schirmer et al., (2009) evaluated the effectiveness of a packaging method with a CO_2 headspace combined with organic acids and found that CO_2 and acetic acid inhibited bacterial growth at 4 °C and reduced the population of *P. phosphoreum*. The CO_2 created a product that appeared similar to vacuum packaged fish so this group believed further study in this area was warranted.

Fernández et al., (2009) measured the shelf-life extension of Atlantic salmon using natural additives, superchilling and MAP and reported that combining MAP and superchilling dramatically extended the shelf-life of Atlantic salmon. Even though the natural additives (rosemary extract and Sea-i®) did not improve salmon shelf-life, the use of hurdle technology to preserve salmon was proposed by the authors. Hansen et al., (2009) analyzed effectiveness of MAP using a CO_2 emitter, traditional MAP and vacuum

package and found out *Photobacterium phosphoreum* dominated spoilage in all three packages. They concluded that MAP maintained higher salmon quality during storage and, MAP with a CO_2 emitter and a reduced gas-to-product volume (g/p) ratio improved the storage quality of Atlantic salmon. Sallam (2007) found that the addition of sodium acetate, sodium lactate or sodium citrate preserved salmon 10, 12, and 15 days, respectively compared to 8 days without added salt when salmon was held 1 °C.

Modified atmosphere packaging

Modified atmosphere packaging has become a popular preservation technology during the past few decades. Not only can MAP maintain food quality longer, MAP can add convenience to the consumer. The basis of MAP is a sealed food package with an altered headspace gas mixture. Once the gas is sealed by plastic film, minimal gas exchange occurs through the package film stabilizing the inside gas atmosphere. Both microbiological and chemical reactions will occur during the preservation period and the gas mixture composition can slow these reactions. MAP has a well-documented history on maintaining freshness of fish products. Wang et al., (2008) employed the MAP gas mixture (CO_2/N_2/O_2: 50%/45%/5%) on 3 cod samples and stored separately at chilled (1.5 °C) and superchilled (−0.9 °C) temperatures. Physical, chemical, and microbial analysis was conducted throughout 21 days of storage. The authors concluded that superchilling combined with MAP not only increased the shelf-life of cod sample, but

also maintained a higher flavor quality compared to control samples. It is worth noting that even though superchilling was the focus for this project, the effect of MAP on the freshness of cod products wasn't specifically studied, especially the hypothesis that MAP may contribute more to the quality improvement of the samples than the temperature variance. Fagan et al., (2004) evaluated the quality maintenance of three different preservation methods (MAP^1: 60% N_2/40% CO_2, MAP^2: 100% CO_2 and traditional method as control). These researchers reported that MAP had lower total microorganism population than samples in air and MAP did not influence odor or acceptability scores compare to the traditional method. The authors believed that technologies of MAP have benefits in product retailing.

The major gases used in the MAP for food preservation are oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2). For salmon fillets in MAP at chilled temperatures (2 °C to 4 °C), shelf-lives of 14 to 21 days have been observed. For fish products MAP, generally, the higher CO_2 ratio, the more effective a MAP is. This is mainly because CO_2 dissolves into the aqueous phase of the fish inhibits bacterial growth. However, high levels of CO_2 in the fish tissue may cause excessive lightening and exudate loss. Fagan et al., (2004) tested the drip loss and texture between two different MAP gas mixtures on Atlantic salmon (60% N_2/40% CO_2, 100% CO_2) and found out that higher levels of CO_2 resulted in a significant loss of moisture and texture quality (Fig.3).

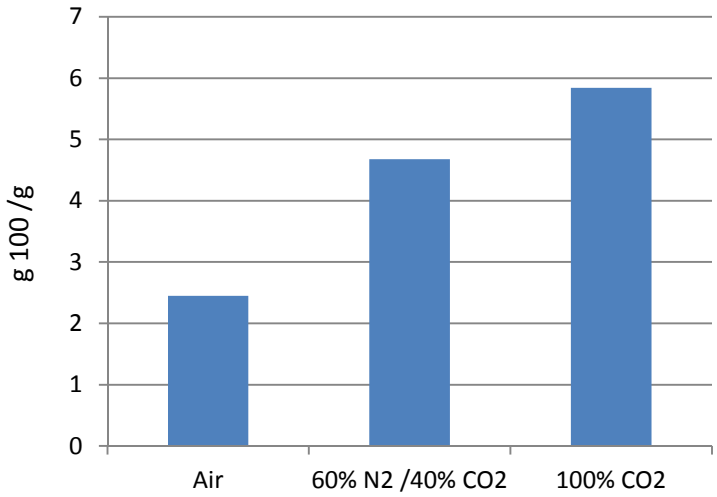

Fig.3. Drip loss of Atlantic salmon after 7 days' preservation by three methods (Fagan et al., 2004)

According to most studies, the desirable range of CO_2 in MAP is between 50% and 80%. Also, a minimum level of O_2 should be included in MAP of fish products to inhibit potential growth and toxin production of *Clostridium botulinum* type E which can survive and bloom under anaerobic conditions (Economou et al., 2009).

Microbiological issues of MAP preserved Atlantic salmon

Spoilage of fresh Atlantic salmon is inevitable no matter what processing and preservation methods employed. While spoilage is multifaceted, the main factor causing fresh salmon spoilage is growth of microorganisms. In MAP preserved salmon, O_2 level

and temperature are usually lower than typical preservation methods. Different environments promote different types of spoilage microorganisms. Studies on the microorganisms in Atlantic salmon MAP preservation have provided critical information to both researchers and the seafood industry. Macé et al., (2012) evaluated the spoilage potential of eight bacterial species for MAP Atlantic salmon through the indexes of characterization, enumeration, chemical, volatile compounds and sensory tests. Of the eight bacterial species were isolated from MAP Atlantic salmon, three bacterial species were characterized by the authors as rapid and strong spoilers: *C. maltaromaticum*, *H. alvei* and *P. phosphoreum*. They examined the bacteria both in alone and in co-culture environments then concluded that *P. phosphoreum* dominated the spoilage of MAP Atlantic salmon. They also mentioned that TMA and acetic acid from these bacterial species associate with amines and sour odors which are typical signs of spoiled MAP fish.

Table 1.

Fish products and Specific Spoilage Organisms (SSO)

Fish Product	Specific spoilage organism
Iced marine fish	*Shewanella putrefaciens*
Iced freshwater fish	*Seudomonas* spp.
MAP preserved fresh marine fish	*Photobacterium phosphoreum*
MAP *preserved thawed* marine fish	*Carnobacterium piscicola*

(adapted from Gram and Dalgaard, 2002; Emborg et al., 2002)

Macé al., (2012) applied enumeration and identification tests to bacteria from MAP preserved Atlantic salmon. In these bacterial experiments, total psychrotrophic viable counts, total lactic acid bacteria (LAB), *Brochothrix* spp. and *Enterobacteriaceae* spp. were the targeted bacterial groups. All organism types having at least 7 Log CFU/g total population was used as a marker of spoilage. Based on the 7 Log CFU/g criteria, spoilage occurred in 7 days with total psychrotrophic viable count and LAB as the dominant flora. The researchers concluded that MAP promoted the growth of LAB and a group of Gram-negative fermentative bacteria. Important information from this included that holding the number of spoilage bacteria below 7 Log CFU/g is essential to extend the shelf-life of Atlantic salmon and that limiting certain bacterial groups is a key principle to extend shelf life. Emborg et al., (2002) evaluated the microbial spoilage and formation of

biogenic amines of MAP preserved Atlantic salmon. They found that the most dominant spoilage organism, *Photobacterium phosphoreum,* limited the storage life of MAP preserved fresh Atlantic salmon to under 21 days and that freezing reduced the bloom of *P. phosphoreum* giving an additional 1-2 weeks of shelf life. Moreover, *Carnobacterium piscicola* was the dominant spoilage microflora of thawed MAP preserved Atlantic salmon. As for the formation of biogenic amines, 20 mg/kg histamine was detected in salmon with *P. phosphoreum* as the dominant spoilage organism and 40 mg kg^{-1} tyramine in salmon with *C. piscicola* as the dominant spoilage organism. They concluded that even though *P. phosphoreum* dominated the spoilage microflora of fresh MAP salmon, it produced relatively smaller amounts of biogenic amines compared to other major spoilage organisms. Also, freezing salmon below - 20 °C reduced the spoilage potential of *P. phosphoreum*, however, freezing did not contribute to reducing formation of biogenic amines. Novel microbiological techniques in salmon spoilage monitoring have yielded additional information. Powell and Tamplin (2012) combined culture-based and DNA-based methods to detect the microbial communities of carbon dioxide and nitrogen packaged Atlantic salmon. They reported that salmon microflora was dominated by *Shewanella* spp., *Carnobacterium* spp. and a variety of other microorganisms. They also concluded that combining culture-based and DNA-based techniques can expand the knowledge concerning microbial communities. Wu and Sun (2013) investigated the effectiveness of series-hyperspectral imaging (TS-HSI) for rapid and non-invasive determination of surface total viable count of salmon tissue during

storage. They extracted different spoilage stages of salmon and the reference TVC values of the same samples were measured using standard plate count and TS-HSI. Comparison of the data between the two methods led the authors to conclude that TS-HSI has a potential for determination of bacterial spoilage in fresh salmon.

Chemical aspects of MAP preserved Atlantic salmon

Beside microbiological parameters, many chemical indexes can also reveal the spoilage level or freshness of Atlantic salmon. For example, total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) are often employed for this purpose. The ratio between TVB-N and TMA (TVB-N/TMA) is an indicator of the freshness of sea-fish during storage. TVB-N measures the entire nitrogen present in the sample. Trimethylamine (TMA) is often determined using a picrate method. During the storage of salmon, the reduction of trimetlylamine oxide (TMAO) results in the production of TMA. This reaction occurs throughout the storage period resulting in TMA accumulation. Naturally, the amount of TVB-N and TMA has a critical relation to the spoilage and freshness level. Some dimethylamine may be included in the result. Gas-liquid chromatography and flow injection are part of these detection systems (Howgate, 2010). This and other research has suggested that TVB-N and TMA can be adopted as a feasible parameter to determine the freshness of sea-fish. Mitsubayashi et al., (2004) invented a TMA biosensor by immobilizing flavin-containing monooxygenase type-3 (FMO-3) for the

evaluation of sea-fish freshness. FMO-3 is one of the drug metabolizing enzymes found in human liver. They concluded that applications of FMO immobilized sensor for detecting TMA vapor has strong potential for indicating freshness of sea-fish.

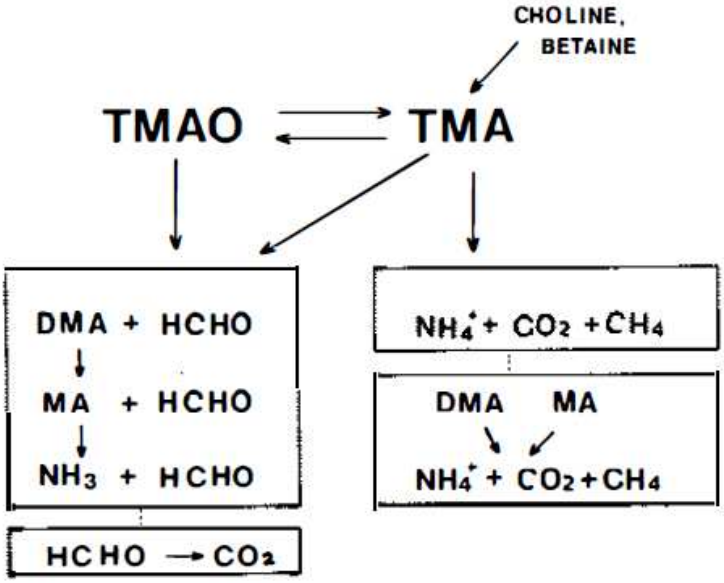

Fig.4. Bioreaction between Trimethylamine oxide (TMAO) and Trimethylamine (TMA) in fish tissues (Barrett and Kwan, 1985)

Pena-Pereira et al., (2010) employed a headspace-single-drop microextraction in combination with microvolume UV–vis spectrophotometry to enhance the determination of trimethylamine-nitrogen (TMA-N) in fish samples. They believed that this method was suitable for the determination of TMA-N in fish from short shelf life markets. The measurement of TVB-N and TMA has been employed as the spoilage and freshness evaluation method for decades. However, no publicly accepted procedures

have been standardized which may raise the concern about the accuracy and reliability of these TVB-N and TMA based detectors for commercial use (Howgate 2010).

Nisin: a preservative associated with fish products

Nisin is a polypeptide produced by *Lactococcus lactis*. It is well known as a bacteriocin that inhibits the growth of gram-positive bacteria and considered as a safe (GRAS) food preservative. Nisin has been revealed to efficiently effect bacteria peptidoglycan synthesis and obtained membrane permeability properties (Scott et al., 1981) and some studies (Rayman et al., 1983, Mazzotta et al., 1997) confirmed nisin can effectively impede the growth of *Clostridium botulinum*, which is an anaerobic spore-form bacteria species that can cause the production of a toxin in canned and pickled foods. Beside foodborne microbiological utilization, nisin also employed to maintain freshness and extend shelf-life of many food ingredients. Several studies have combined nisin with MAP. Economou et al., (2009) evaluated the effectiveness of nisin and EDTA to preserve MAP stored fresh chicken meat. They concluded that 500 IU/g nisin with 50 mm EDTA and 1500 IU/g nisin with 50 mm EDTA extended shelf 9-14 days. Also, the authors believed that further research on the combination of nisin-EDTA and MAP should be conducted. Jofré et al., (2008) compared the preservation effects of nisin, enterocins A and B and sakacin K on cooked and dry-cured ham spiked with *Listeria monocytogenes, Salmonella enterica* and *Staphylococcus aureus*. Only nisin inhibited the growth of

multiple bacteria in cooked ham exposed to temperature abuse. Lu et al., (2010) tested the shelf-life extension capacity of nisin and cinnamon with a calcium-alginate coating on northern snakehead fish fillets at refrigeration temperature. They measured microbiological populations, TVB-N and thiobarbituric acid (TBA) value and color of nisin-cinnamon integrated alginate-calcium coated northern snakehead fish fillets. They concluded that this nisin could efficiently maintain quality of northern snakehead fish fillets during 15 days of storage. The ability of nisin combined with lactic acid to reduce the population of naturally existing microorganisms on shrimp held at 4 OC and tested at 7 and 14 days was determined through measuring total aerobic plate count, psychrotrophic counts, population of *Pseudomonas* spp., H_2S producing bacteria and lactic acid bacteria (Shirazinejad et al., 2010). The treated groups were 2.91 and 2.63 log CFU/g lower for total aerobic bacteria than control groups after 7 days and 14 days' storage. Among the results, nisin combined with 2.0% lactic acid had the highest reduction. The author advocated nisin and lactic acid in the purpose of reducing microorganism and extending the shelf life of shrimp.

Tsironi et al., (2010) used kinetic modeling of MAP (50% CO_2 and 50% air) with nisin on the shelf life of gilthead seabream (*Sparus aurata*) fillets. They reported that "strict aerobic Gram-negative organisms, primarily *Pseudomonas* spp., were strongly inhibited by CO_2 and nisin resulting in a significant shelf life extension at all storage temperatures". They concluded that osmotic pretreatment with MAP extended the

shelf life and nisin can give additional shelf life extension when stored aerobically or under MAP. Moreover, they believe this novel method could potentially improve the commercial value of fresh chilled fish products. YunPeng (2009) applied nisin to fish products and evaluated multiple parameters to determine the most suitable dosage in practice. The author also observed a better effect on fish's taste, color and acceptability by adding 0.15 g/kg and 0.2 g/kg of nisin in fish.

Conclusion

Atlantic salmon, the major species of edible salmonids, is often used in laboratory experiments to represent marine fishes. Many researchers evaluated various preservation techniques to optimize the levels and dosage of preservatives to maintain the freshness and extend the shelf-life of Atlantic salmon. Among these studies, MAP and nisin have been reported repeated as efficient approaches to fulfill this demand. Nevertheless, little information has been provided about the effectiveness of combined method between MAP and nisin, especially on the preservation of Atlantic salmon. Strong evidences suggest that MAP associated with the GRAS preservative nisin may have a potential to improve Atlantic salmon storage quality.

REFERENCES

Barrett E L, Kwan H S. Bacterial reduction of trimethylamine oxide. Annual Reviews in Microbiology, 1985, 39(1): 131-149.

Delves-Broughton J, Blackburn P, Evans R J, et al. Applications of the bacteriocin, nisin. Antonie van Leeuwenhoek, 1996, 69(2): 193-202.

Duun A S, Rustad T. Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at− 1.4 and− 3.6° C. Food Chemistry, 2008, 106(1): 122-131.

Dziewulska K, Rzemieniecki A, Czerniawski R, et al. Post-thawed motility and fertility from Atlantic salmon (*Salmo salar*) sperm frozen with four cryodiluents in straws or pellets. Theriogenology, 2011, 76(2): 300-311.

Economou T, Pournis N, Ntzimani A, et al. Nisin–EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. Food chemistry, 2009, 114(4): 1470-1476.

Einen O, Guerin T, Fjæra S O, et al. Freezing of pre-rigor fillets of Atlantic salmon. Aquaculture, 2002, 212(1): 129-140.

Emborg J, Laursen B G, Rathjen T, et al. Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon *(Salmo salar*) at 2 °C. Journal of Applied Microbiology, 2002, 92(4): 790-799.

Fagan J D, Gormley T R, Uı Mhuircheartaigh M M. Effect of modified atmosphere packaging with freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. Innovative Food Science & Emerging Technologies, 2004, 5(2): 205-214.

Fernández K, Aspe E, Roeckel M. Shelf-life extension on fillets of Atlantic Salmon *(Salmo salar)* using natural additives, superchilling and modified atmosphere packaging. Food Control, 2009, 20(11): 1036-1042.

Gallart-Jornet L, Rustad T, Barat J M, et al. Effect of superchilled storage on the freshness and salting behaviour of Atlantic salmon *(Salmo salar)* fillets. Food chemistry, 2007, 103(4): 1268-1281.

Gram L, Dalgaard P. Fish spoilage bacteria–problems and solutions. Current Opinion in Biotechnology, 2002, 13(3): 262-266.

Hansen A Å, Mørkøre T, Rudi K, et al. Quality changes of prerigor filleted Atlantic salmon (*Salmo salar L.*) packaged in modified atmosphere using CO2 emitter, traditional MAP, and vacuum. Journal of food science, 2009, 74(6): M242-M249.

Howgate P. A critical review of total volatile bases and trimethylamine as indices of freshness of fish. Part 1. Determination. Electronic Journal of Environmental, Agricultural & Food Chemistry, 2010, 9(1).

Indergård E, Tolstorebrov I, Larsen H, et al. The Influence of Long-term Storage, Temperature and Type of Packaging Materials on the Quality Characteristics of Frozen Farmed Atlantic Salmon *(Salmo Salar)* International Journal of Refrigeration, 2013.

Jofré A, Aymerich T, Monfort J M, et al. Application of enterocins A and B, sakacin K and nisin to extend the safe shelf-life of pressurized ready-to-eat meat products. European Food Research and Technology, 2008, 228(1): 159-162.

Lu F, Ding Y, Ye X, et al. Cinnamon and nisin in alginate–calcium coating maintain quality of fresh northern snakehead fish fillets. LWT-Food Science and Technology, 2010, 43(9): 1331-1335.

Macé S, Cornet J, Chevalier F, et al. Characterisation of the spoilage microbiota in raw salmon *(Salmo salar)* steaks stored under vacuum or modified atmosphere packaging combining conventional methods and PCR–TTGE. Food microbiology, 2012, 30(1): 164-172.

Macé S, Joffraud J J, Cardinal M, et al. Evaluation of the spoilage potential of bacteria isolated from spoiled raw salmon (*Salmo salar*) fillets stored under modified atmosphere packaging. International journal of food microbiology, 2012.

Mazzotta A S, Crandall A D, Montville T J. Nisin resistance in Clostridium botulinum spores and vegetative cells. Applied and environmental microbiology, 1997, 63(7): 2654-2659.

Mitsubayashi K, Kubotera Y, Yano K, et al. Trimethylamine biosensor with flavin-containing monooxygenase type 3 (FMO3) for fish-freshness analysis. Sensors and Actuators B: Chemical, 2004, 103(1): 463-467.

Pena-Pereira F, Lavilla I, Bendicho C. Colorimetric assay for determination of trimethylamine-nitrogen (TMA-N) in fish by combining headspace-single-drop microextraction and microvolume UV–vis spectrophotometry. Food chemistry, 2010, 119(1): 402-407.

Powell S M, Tamplin M L. Microbial communities on Australian modified atmosphere packaged Atlantic salmon. Food microbiology, 2012, 30(1): 226-232.

Sallam K I. Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. Food Chemistry, 2007, 101(2): 592-600.

Schirmer B C, Heiberg R, Eie T, et al. A novel packaging method with a dissolving CO_2 headspace combined with organic acids prolongs the shelf life of fresh salmon. International journal of food microbiology, 2009, 133(1): 154-160.

Shirazinejad A R, Noryati I, Rosma A, et al. Inhibitory effect of lactic acid and nisin on bacterial spoilage of chilled shrimp. World Acad. Sci. Eng. Technol, 2010, 65: 163-167.

Sivertsvik M, Rosnes J T, Bergslien H. Modified atmosphere packaging. Minimal processing technologies in the food industry, 2002: 61-86.

Stevens K A, Sheldon B W, Klapes N A, et al. Nisin treatment for inactivation of Salmonella species and other gram-negative bacteria. Applied and Environmental Microbiology, 1991, 57(12): 3613-3615.

Tsironi T N, Taoukis P S. Modeling microbial spoilage and quality of gilthead seabream fillets: combined effect of osmotic pretreatment, modified atmosphere packaging, and nisin on shelf life. Journal of food science, 2010, 75(4): 243-251.

Wang T, Sveinsdóttir K, Magnússon H, et al. Combined application of modified atmosphere packaging and superchilled storage to extend the shelf life of fresh cod (Gadus morhua) loins. Journal of food science, 2008, 73(1): S11-S19.

Wu D, Sun D W. Potential of time series-hyperspectral imaging (TS-HSI) for non-invasive determination of microbial spoilage of salmon flesh. Talanta, 2013, 111: 39-46.

Yun P J. Application of nisin in fish research. China Condiment, 2009 (11): 67-69.

Zhu S, Ramaswamy H S, Simpson B K. Effect of high-pressure versus conventional thawing on color, drip loss and texture of Atlantic salmon frozen by different methods. LWT-Food Science and Technology, 2004, 37(3): 291-299.

CHAPTER TWO: COMBININGMODIFIED ATMOSPHERE PACKAGING AND NISIN TO PRESERVE ATLANIC SALMON

ABSTRACT

Preservation effects of modified atmosphere package (MAP) combined with nisin on fresh Atlantic salmon was determined. Six groups of farmed Atlantic salmon were purchased from local market and treated with using MAP (19 % CO_2 / 70 % N_2 /11 % O_2 , 38 % CO_2 / 51 % N_2 /11 % O_2 and air) with and without nisin (400 IU/g). Microbiological enumeration (aerobic plate count, psychrotrophic bacteria and lactic acid bacteria) and the TVB-N test were determined 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 and had less impact on lactic acid bacteria. 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 the combination between modified atmosphere package and nisin as an effective method to limit the spoilage of Atlantic salmon compare to traditional preservation methods.

Key words:

Atlantic salmon; Modified atmosphere package; Nisin; shelf-life

1. Introduction

Modified Atmosphere Packaging (MAP) has become a popular preservation technology. The basis of MAP is a sealed food package with an altered headspace gas mixture. Both microbiological and chemical reactions continue during the preservation period and the gas headspace composition may change due to these reactions. MAP has been shown to maintain freshness and delay spoilage of fresh fish products. 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. Also, some researchers (Jayasingh et al., 2002, Sivertsvik et al., 2003, Fagan et al., 2004, Wang et al., 2008, Economou et al., 2009,) have demonstrated that the higher concentration of carbon dioxide in MAP, the longer shelf-life of a fresh product. However, high concentrations of CO_2 can lead to quality loss.

Salmon spoilage is multifaceted and one of the main factors causing spoilage is the bloom of microorganisms. In MAP preserved salmon, O_2 level and temperature are lower than ambient conditions and these different environments have certain impact on the growth of microorganisms. Beside microbiological spoilage detection, many chemical indexes can also reveal the spoilage level or freshness of Atlantic salmon. Total volatile basic nitrogen (TVB-N) 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 Trimetlylamine 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 the spoilage and freshness evaluation method for decades and the ratio of TVB-N to TMA (TVB-N/TMA) has been an index of sea-fish freshness during storage (Howgate, 2010, Mitsubayashi et al., 2004).

Nisin is a polypeptide produced by *Lactococcus lactis*. It is well known as a bacteriocin that inhibits the growth of gram-positive bacteria and is generally recognized as safe (GRAS) as a food preservative. Researchers (Jofré et al., 2008, Lu et al., 2010, Shirazinejad et al., 2010) have confirmed that nisin is able to effectively extend the shelf-life of many fresh food products. Other research (Economou et al., 2009, Tsironi et al., 2010, López-Mendoza, 2007) have studied the seafood shelf life extension effects of MAP. However, little has been published on the preservation effectiveness of MAP combined with nisin on 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. Materials and Packaging

2.1. Salmon Samples

Sliced farmed Atlantic salmon fillets were transported on ice to the laboratory. Salmon was purchased as skinless and boneless fillets from a local grocery store and frozen until experiments.

2.2. Preparation of salmon

Frozen fillets were thawed at 4°C 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 from the 150 g fillet. 39 portions of 150 g Atlantic salmon samples were prepared and 3 were tested immediately. Processing and packaging procedures were under strict hygienic conditions.

2.3. Nisin Activity

Commercial nisin (Nisaplin®, 10^6 IU/g) was obtained from the manufacturer (Danisco, give city, state, country headquarters). Nisaplin® activity was detected in triplicate using a zone of inhibition assay. Nisaplin sample was diluted 1:500 with 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 °C under 5% CO_2, the plates were examined for inhibition zones. 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. 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^{-1}) solution was then prepared by dissolving Nisaplin® in sterile water.

2.4. 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. Three portions (25 g, 10 g and approximately 115 g) were placed in foam trays (C976 Sealed Air Cryovac, Duncan, SC) (8¾ * 6¾ * 15/8). And the nisin solution was spread on the surface of fillets with nisin {how was is spread on the surface?} to reach a final nisin concentration of 400 IU/g. MAP -nisin combination treatments are shown in Table 2. 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). Vacuum pressure of 150 mbar, gas pressure of 765 mbar, seal time of 2.1 sec, knife temperature of 143°C and seal temperature of 141°C were preset on the MAP machine. The trays were sealed using lid stock film (Lid 1050) (18.5wide) to achieve a gas/product ratio of ~5/1. All samples were refrigerated (2-4°C) until analyses. At the same time, 7 empty packages were also sealed with the 3 gas mixtures in Table 2 and stored in same environment as salmon samples to monitor the gas composition of empty packages.

3. Analysis Methods

There were 4 major analyses (headspace gas analyses, microbiologic enumeration, TVB-N titration test, sensory testing) employed to evaluate freshness of salmon samples. All tests (except package headspace) of samples from each of the 6 treatments according to

Table 2 were conducted before the packaging to determine the initial state of each group. Package headspace, microbiological, TMA /TVB-N titration and sensory tests were conducted on day 2, 4, 7 and 10.

Table 2.

Gas composition and Nisin treatment of 6 salmon groups

Gas Composition	CO_2/ N_2/ O_2 (%): 19/ 70/ 11	CO_2/ N_2/ O_2 (%): 38/ 51/ 11	air
nisin (400 IU/g)	Treatment. 1	Treatment. 2	Treatment. 3
Control	Treatment. 4	Treatment. 5	Treatment. 6

3.1. Headspace Gas Analyses

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 punching a needle (syringe type) through a gas tight septum placed onto the package film surface.

3.2. Microbiologic Enumeration

In fresh food product preservation, especially fresh seafood and meat ingredients, microorganisms' blooming is the primary cause of spoilage. Enumeration of particular microorganisms' groups is important to understand factors affecting spoilage. In the current study, 25 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 laboratorial blender (Model 400, Seward^{TM}, FL, USA 33330) containing pre-added 225 ml pre-chilled sterile peptone-physiological saline solution (0.1% peptone + 0.85% NaCl) (Difco^{TM}, 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°C. The population of psychrotrophic bacteria was determined by a spread plate counting method with PCA with 1% NaCl and incubated at 4 °C 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°C for 72 h in a CO_2 incubator with a continuous CO_2 flow. (Difco^{TM}, Bactopeptone, Becton, Dickison & Company, MD, USA 21152) Prior to data analyses of

microbiological data, bacterial populations were converted to logarithmic values (CFU/g).

3.3. TVB-N titration test

Total volatile base nitrogen (TVB-N) tests were prepared by homogenizing 10 g of fish from trays with 100 ml water in a laboratorial 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 (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.

3.4. Sensory test

All the assessors were trained using pre-spoiled salmon series. 5 samples were prepared for each day of training and held at 20°C for a 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.

3.5. 5-level sensory scale

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 on 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

3.6. Statistical analysis

All the experiments (Headspace gas analyses, microbiological enumeration, TVB-N testes and sensory testes) were replicated twice. Analysis of variance was conducted for each parameter to determine if there was a significant effect ($p<0.05$) due to treatments. When the treatment was determined to be significant on a parameter, Statistics Analysis System (SAS, Version 9.0, 2004) was employed to perform a Least Significant Difference (LSD) test to determine if there were significantly difference ($p<0.05$).

4. Result and Discussion

4.1. Nisin Activity Detection

1.6×10^6 AU/ml was confirmed as activity level of the commercial nisin sample. Nisin was first detected produced by *Lactococcus lactis* subsp. *lactis* by Rogers and other in 1928, after then, it 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). In this project, 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 spp*. can be susceptible to nisin and spore outgrowth of *Clostridium spp.* is more likely to be restricted than vegetative cell growth by nisin (Delves-Broughton et al., 1996).

4.2. Headspace Gas Analyses

During the storage of empty MAP sealed packages, there was no change in CO_2 and O_2 percentage concentration 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 for the Atlantic salmon preservation study. Most MAP are 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, peelability and the ability to seal under less than optimal conditions (Phillips, 1996).

The technique of sealing modified atmospheres in polymeric film without further exchange between inside and outside atmosphere often generates a low O_2 and high CO_2 concentration, especially with a designed gas mixture applied. These conditions can influence the metabolism biological components in the package which helps inhibit spoilage and pathogenic contaminants (Mangaraj et al., 2009).

Initial O_2 percentage concentration of 38% CO_2 / 51% N_2/ 11% O_2 was 10.75% ± 0.10%, initial O_2 percentage concentration of 19% CO_2 / 70% N_2/ 11% O_2 was 11.01% ± 0.08% and initial O_2 percentage concentration of Air was 20.60% ± 0.04%. On Day 2, salmon samples packaged in air with and without nisin show similar O_2 concentration ($p>0.05$),

however, on Day 4, there is a clear difference ($p<0.05$) of the oxygen level between air/nisin and air/no nisin. The 38% CO_2 MAP treatment inhibited O_2 consumption compared to the 19% CO_2 applied MAP ($p<0.05$) at day 2. Thus as CO_2 concentration increased, the O_2 consumption rate of microorganisms present on salmon decreased at early storage time. This implies that the microorganism's respiration rate was slowed with the increase in CO_2. With the comparison between air/nisin and air/no nisin treatments and between 19% CO_2 package with and without nisin on day 4 (Figure 7), the conclusion can be draw that nisin also limited the metabolism rate of the microorganisms ($p<0.05$) at relatively low CO_2 concentration. As the primary gas consumed by spoilage microorganisms during growth, has been recognized as an important gas component in MAP. Taylor et al., (2007) reported a commercial nisin sample with 10^6 IU/g containing 2.5% pure nisin, 74.4% sodium chloride, 23.8% denatured milk solids and 1.7% moisture (w/w) which showed similar composition compared to the commercial nisin sample used in the current study. Nisin limited the growth of spoilage microorganisms and reduced total oxygen consumption.

Table 3. Concentration of O_2 of farmed Atlantic salmon packaged in different air packages and nisin stored at 2-4 °C (n=2)

Treatment	Day 0	Day 2	Day 4
Air + Nisin(400 IU/g)	20.60% ± 0.26% a	18.08% ± 0.57% a	8.92% ± 0.42% a
Air without nisin (control)	20.60% ± 0.26% a	17.76% ± 0.15% a	5.89% ± 0.44% b

a-b means within rows with a different letter are significantly different ($p<0.05$)

Table 4. Concentration of O_2 of farmed Atlantic salmon packaged in different modified atmosphere packages and nisin stored at 2-4 °C (n=2)

Treatment	Day 0	Day 2	Day 4
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% a
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% a
38% CO_2/51% N_2/11% O2 without nisin	10.75% ± 0.10% a	9.62% ± 0.23% a	6.26% ± 0.24% a
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% b

a-b means within rows with a different letter are significantly different ($p<0.05$)

4.3. Microbiologic Enumeration

4.3.1. Aerobic Plate Count Cultured at 37 °C

3.25 ± 0.06 log CFU/g of aerobic bacteria were detected on Day 0 as a baseline population. The total aerobic bacteria for the 3 MAP treatments differed ($p<0.05$) on day 2 (Figure 5). Although the means for the MAP and air treatments with and without nisin

did not differ ($p>0.05$), the means of the MAP treatments and air were different ($p<0.05$). However, no significant aerobic microorganism difference was observed between salmon packaged in CO_2 concentrations of 19% and 38% ($p>0.05$). Furthermore, nisin-treated salmon reduced aerobic populations on day 2 ($p<0.05$). On days 2 and day 4, the population of bacteria on salmon packaged in air was greater than MAP-packaged salmon ($p<0.05$). At the end of storage, there was no difference in aerobic bacterial counts between all treatments ($p>0.05$). The presence of CO_2 (at least 19 %) limited the growth of aerobic microorganism compared to salmon packaged in air through Day 7 ($p<0.05$). 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 a 1 day longer shelf life compared to salmon packaged in air.

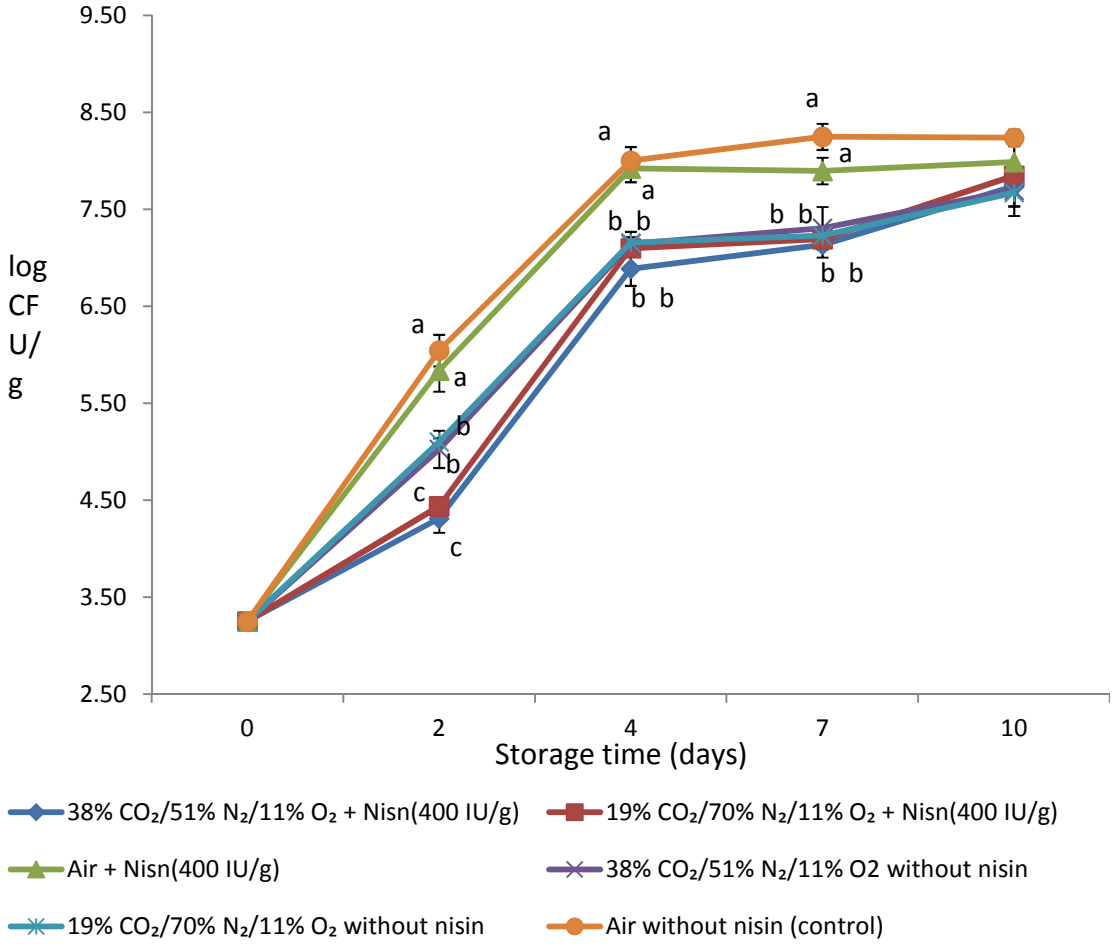

Fig.5. Total aerobic bacterial populations of farmed Atlantic salmon stored at 2-4 °C packaged in various modified atmosphere packaging with and without nisin (n=4) a-c means with a different letter are significantly different ($p<0.05$)

4.3.2. 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 6). 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 ($p<0.05$). Nisin had no observable inhibition of psychrotrophic bacteria ($p>0.05$). As storage continued, psychrotrophic bacteria population differences due to the various treatments diminished ($p>0.05$).

As the major spoilage bacteria at refrigerated temperatures, many specific spoilage organisms of psychrotrophic bacteria dominate the spoilage causes 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 does not inhibit the growth of Gram-negative bacteria. Nisin can target vegetative cells acting at the cytoplasmatic membrane and causing pores produced 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).

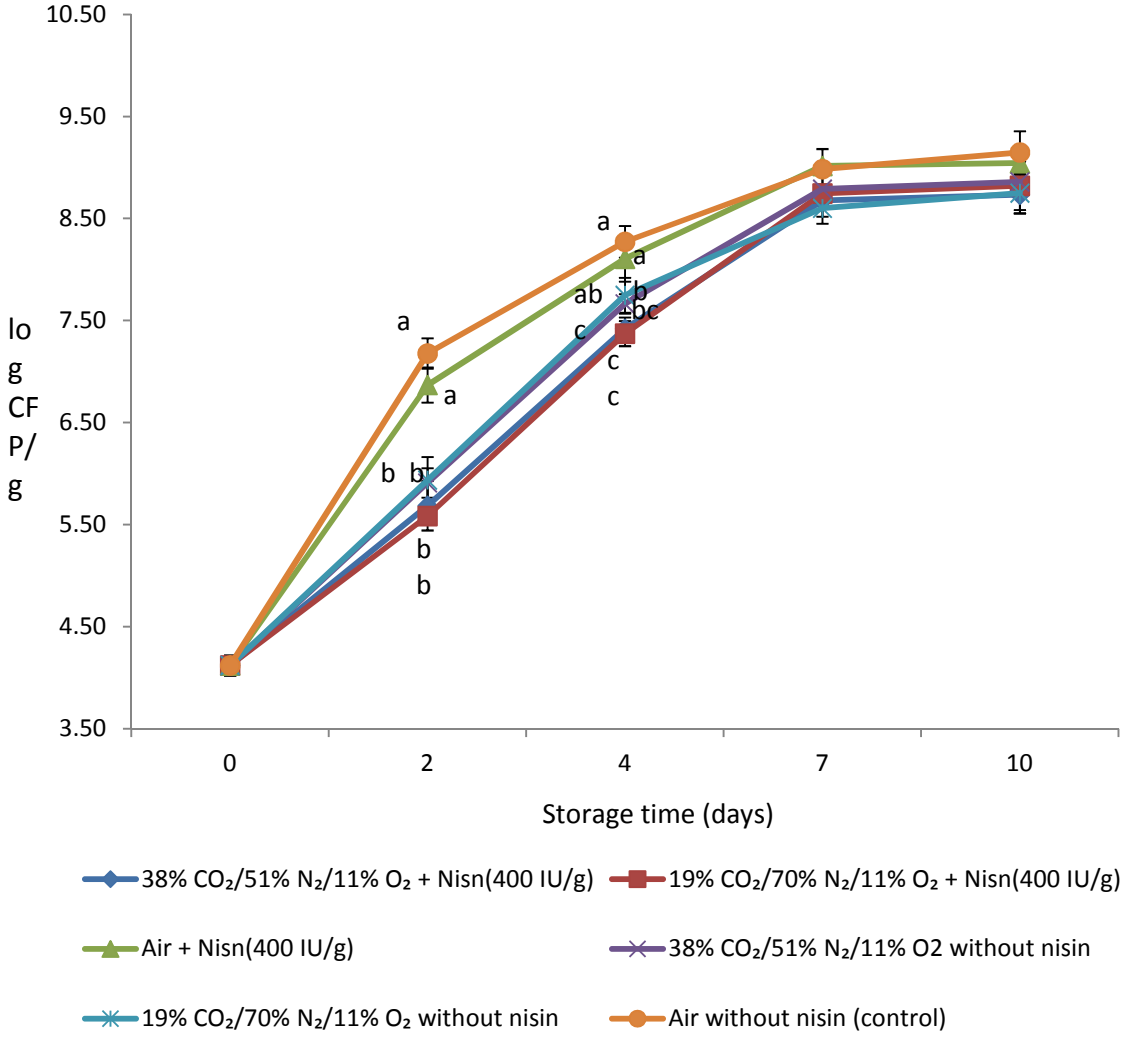

Fig.6. Psychrotrophic bacterial populations of farmed Atlantic salmon stored at 2-4 °C packaged in various modified atmosphere packaging with and without nisin (n=4). a-c means within rows with a different letter are significantly different ($p<0.05$)

4.3.3. Lactic acid bacterial count

Initial lactic acid bacterial (LAB) populations were 1.25 ± 0.17 log CFU/g and compared to MAP packaged salmon, air packaged samples without nisin treatment had higher populations at day 2 and 4 than any CO_2 packaged salmon ($p<0.05$)(Figure 7). 19% CO_2 preserved salmon displayed a clear advantage for inhibiting LAB population compared to 38% CO_2 regardless nisin application at day 2. As storage continued, LAB population differences due to the various treatments were not observed ($p>0.05$).

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 ($p<0.05$). Although LAB is recognized as a spoilage bacteria that can grow at lower 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. Another research has revealed that the presence of oxygen can inhibit growth of LAB and no more than 5 log cfu/g of LAB was observed in aerobic conditions (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 effects 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.

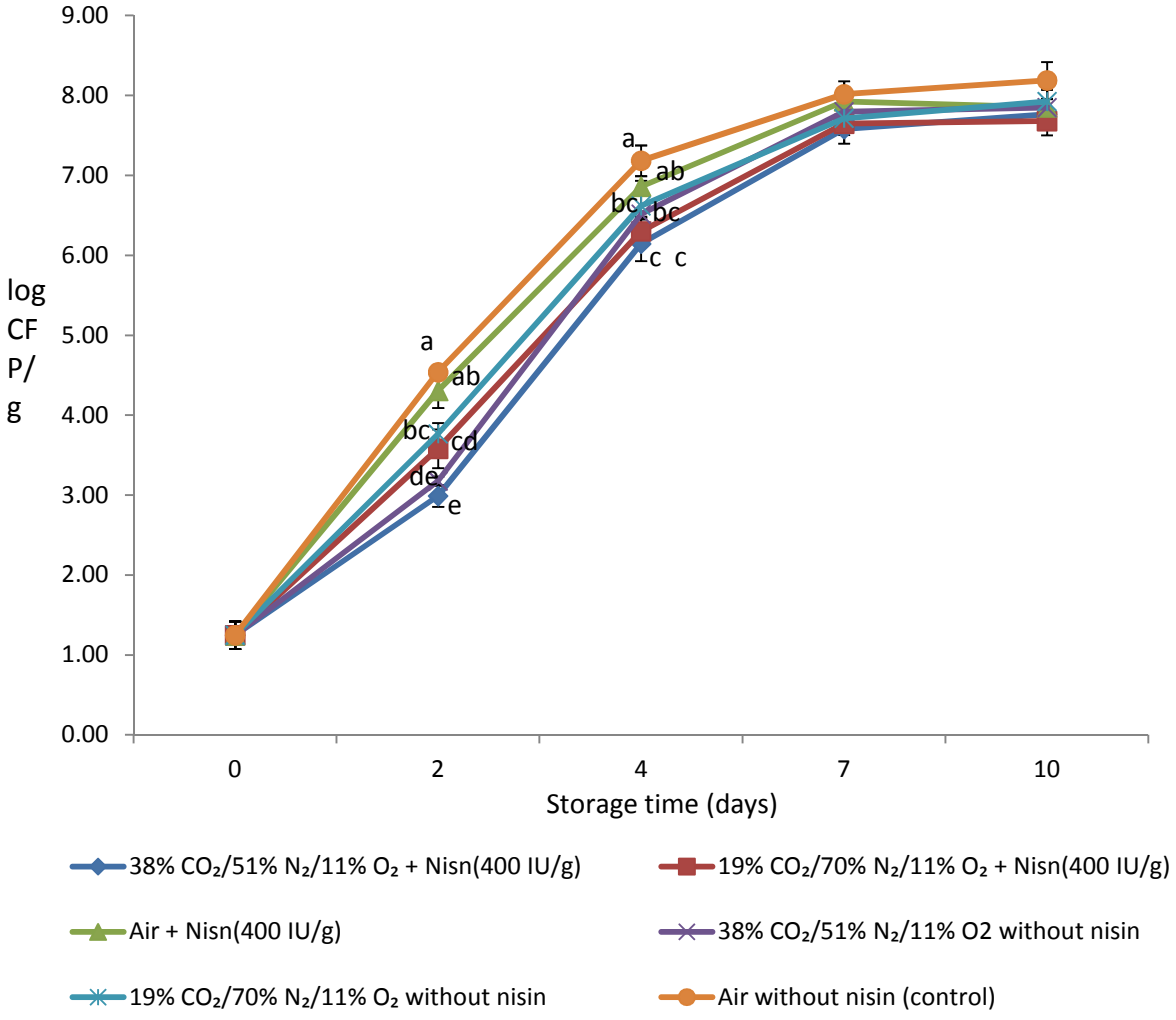

Fig.7. Lactic acid bacterial populations of farmed Atlantic salmon stored at 2-4 °C packaged in various modified atmosphere packaging with and without nisin (n=4). a-e means within rows with a different letter are significantly different ($p<0.05$).

4.4. 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 8) ($p<0.05$). And the only detected difference between air/nisin and air/non-nisin was on day 2 ($p<0.05$). 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 non-CO_2 MAP samples on all four sampling days ($p<0.05$). No significant difference on 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 air packed samples on days 4, 7 and 10 ($p>0.05$). Application of nisin in aerobic atmosphere has been extensively researched but nisin under anaerobic conditions has not been as extensively studied. 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 bacterial growth with the psychrotrophic bacteria population's growth which has the highest population bacteria group detected in the experiment. Previous research has verified a strong connection between the generation of TVB-N and the spoilage of refrigerated fresh products (Arashisar et al., 2004, Ojagh et al., 2010)

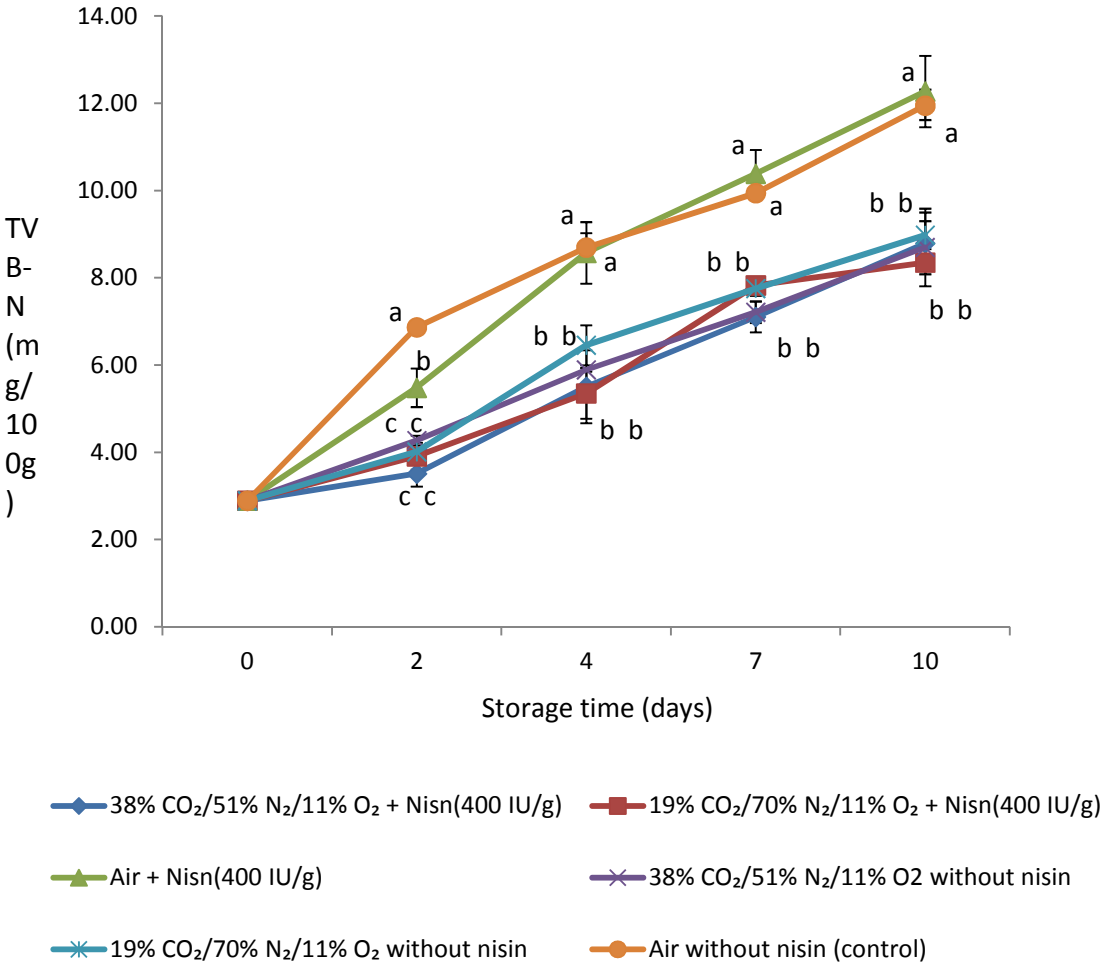

Fig.8. TVB- N of farmed Atlantic salmon stored at 2-4 °C packaged in various modified atmosphere packaging with and without nisin (n=2). a-c means within rows with a different letter are significantly different ($p<0.05$).

4.5. Sensory test

Sensory evaluation of different MAP packaged with nisin treated or non-nisin treated Atlantic salmon samples are listed in Appendix A. All six samples were evaluated by trained assessors at day 2 and day 4 during storage and at least seven assessors were involved in each day's evaluation. 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 ($p<0.05$). 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 freedom of water to move from the fish tissue resulting from microbial growth can reduce the general appearance quality of Atlantic salmon. However, no observable difference was detected in this study. 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 ($p<0.05$) was observed

between the control (no nisin packaged in air) and the other 5 storage treatments of Atlantic.

5. 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 medium concentration of CO_2 (38%) and low concentration of CO_2 (19%) was only found for LAB. And 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. Sensory evaluation found no difference 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. The experiments along with previous studies support the potential for combining MAP and nisin as an effective method to limit the spoilage of Atlantic salmon however, MAP alone may be a more cost effective approach to shelf life extension of fresh salmon.

REFERENCES

Alfnes F, Guttormsen A G, Steine G, et al. Consumers' willingness to pay for the color of salmon: a choice experiment with real economic incentives. American Journal of Agricultural Economics, 2006, 88(4): 1050-1061.

Arashisar Ş, Hisar O, Kaya M, et al. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. International journal of food microbiology, 2004, 97(2): 209-214.

Bauer R, Dicks L M T. Mode of action of lipid II-targeting lantibiotics. International journal of food microbiology, 2005, 101(2): 201-216.

Bulushi I A, Poole S, Deeth H C, et al. Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation—a review. Critical reviews in food science and nutrition, 2009, 49(4): 369-377.

Davies A R. Advances in modified-atmosphere packaging//New methods of food preservation. Springer US, 1995: 304-320.

de Arauz L J, Jozala A F, Mazzola P G, et al. Nisin biotechnological production and application: a review. Trends in Food Science & Technology, 2009, 20(3): 146-154.

Delves-Broughton J, Blackburn P, Evans R J, et al. Applications of the bacteriocin, nisin. Antonie van Leeuwenhoek, 1996, 69(2): 193-202.

Devlieghere F, Debevere J, Van Impe J. Effect of dissolved carbon dioxide and temperature on the growth of Lactobacillus sake in modified atmospheres. International journal of food microbiology, 1998, 41(3): 231-238.

Dhaouadi A, Monser L, Sadok S, et al. Validation of a flow-injection-gas diffusion method for total volatile basic nitrogen determination in seafood products. Food chemistry, 2007, 103(3): 1049-1053.

Erkan N, Özden Ö. Quality assessment of whole and gutted sardines (Sardina pilchardus) stored in ice. International journal of food science & technology, 2008, 43(9): 1549-1559.

Ferreira M, Lund B M. The effect of nisin on Listeria monocytogenes in culture medium and long-life cottage cheese. Letters in applied microbiology, 1996, 22(6): 433-438.

Ghaly A E, Dave D, Budge S, et al. Fish spoilage mechanisms and preservation techniques: review. American Journal of Applied Sciences, 2010, 7(7): 859.

Gökoğlu N, Cengız E, Yerlıkaya P. Determination of the shelf life of marinated sardine (*Sardina pilchardus*) stored at 4° C. Food Control, 2004, 15(1): 1-4.

Hozbor M C, Saiz A I, Yeannes M I, et al. Microbiological changes and its correlation with quality indices during aerobic iced storage of sea salmon (*Pseudopercis semifasciata*). LWT-Food Science and Technology, 2006, 39(2): 99-104.

Ibrahim Sallam K. Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food control, 2007, 18(5): 566-575.

Jayasingh P, Cornforth D P, Brennand C P, et al. Sensory Evaluation of Ground Beef Stored in High-oxygen Modified Atmosphere Packaging. Journal of Food Science, 2002, 67(9): 3493-3496.

López-Mendoza M C, Ruiz P, Mata C M. Combined effects of nisin, lactic acid and modified atmosphere packaging on the survival of Listeria monocytogenes in raw ground pork. International journal of food science & technology, 2007, 42(5): 562-566.

Mangaraj S, Goswami T K, Mahajan P V. Applications of plastic films for modified atmosphere packaging of fruits and vegetables: a review. Food Engineering Reviews, 2009, 1(2): 133-158.

Manju S, Jose L, Srinivasa Gopal T K, et al. Effects of sodium acetate dip treatment and vacuum-packaging on chemical, microbiological, textural and sensory changes of Pearlspot (*Etroplus suratensis*) during chill storage. Food Chemistry, 2007, 102(1): 27-35.

Mazzotta A S, Crandall A D, Montville T J. Nisin resistance in *Clostridium botulinum* spores and vegetative cells. Applied and environmental microbiology, 1997, 63(7): 2654-2659.

McMillin K W, Huang N Y, Ho C P, et al. Quality and shelf-life of meat in case-ready modified atmosphere packaging//Quality Attributes of Muscle Foods. Springer US, 1999: 73-93.

McMillin K W. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Science, 2008, 80(1): 43-65.

Millette M, Smoragiewicz W, Lacroix M. Antimicrobial potential of immobilized Lactococcus lactis subsp. lactis ATCC 11454 against selected bacteria. Journal of Food Protection®, 2004, 67(6): 1184-1189.

Ojagh S M, Rezaei M, Razavi S H, et al. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry, 2010, 120(1): 193-198.

Ottestad S, Sørheim O, Heia K, et al. Effects of storage atmosphere and heme state on the color and visible reflectance spectra of salmon (*Salmo salar*) fillets. Journal of agricultural and food chemistry, 2011, 59(14): 7825-7831.

Paul Ross R, Morgan S, Hill C. Preservation and fermentation: past, present and future. International Journal of Food Microbiology, 2002, 79(1): 3-16.

Phillips C A. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. International Journal of Food Science & Technology, 1996, 31(6): 463-479.

Raju C V, Shamasundar B A, Udupa K S. The use of nisin as a preservative in fish sausage stored at ambient (28±2 °C) and refrigerated (6±2 °C) temperatures. International journal of food science & technology, 2003, 38(2): 171-185.

Rasmussen S K J, Ross T, Olley J, et al. A process risk model for the shelf life of Atlantic salmon fillets. International journal of food microbiology, 2002, 73(1): 47-60.

Rayman K, Malik N, Hurst A. Failure of nisin to inhibit outgrowth of Clostridium botulinum in a model cured meat system. Applied and environmental microbiology, 1983, 46(6): 1450-1452.

Ringø E, Gatesoupe F J. Lactic acid bacteria in fish: a review. Aquaculture, 1998, 160(3): 177-203.

Scott V N, TAYLOR S L. Temperature, pH, and spore load effects on the ability of nisin to prevent the outgrowth of Clostridium botulinum spores. Journal of food science, 1981, 46(1): 121-126.

Sivertsvik M, Jeksrud W K, Rosnes J T. A review of modified atmosphere packaging of fish and fishery products–significance of microbial growth, activities and safety. International journal of food science & technology, 2002, 37(2): 107-127.

Sivertsvik M, Rosnes J T, Kleiberg G H. Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (Salmo salar) fillets. Journal of Food Science, 2003, 68(4): 1467-1472.

Taylor T M, Davidson P M, Zhong Q. Extraction of nisin from a 2.5% commercial nisin product using methanol and ethanol solutions. Journal of Food Protection®, 2007, 70(5): 1272-1276.

ZHAO Y, WELLS J H, McMILLIN K W. Dynamic changes of headspace gases in CO_2 and N_2 packaged fresh beef. Journal of Food Science, 1995, 60(3): 571-575.

APPENDIX A

Table.5.

Sensory test result under the term of "Appearance"

Treatment	Day 2	Day 4
38% CO_2/51% N_2/11% O_2 + Nisn(400 IU/g)	3.87 ± 0.92	3.21 ± 0.86
19% CO_2/70% N_2/11% O_2 + Nisn(400 IU/g)	4.41 ± 0.67	3.54 ± 0.85
Air + Nisn(400 IU/g)	3.83 ± 0.99	3.18 ± 0.96
38% CO_2/51% N_2/11% O2 without nisin	4.20 ± 0.75	3.43 ± 0.80
19% CO_2/70% N_2/11% O_2 without nisin	4.03 ± 1.12	3.36 ± 0.74
Air without nisin (control)	3.80 ± 1.24	3.68 ± 1.13

Values are presented as mean ± standard deviation.

No significant different was observed between the values within rows ($p>0.05$)

Detected initial grade at Day 0 is 4.78 ± 0.36 (n=9)

Table 6.

Sensory test result under the term of "Color"

Treatment	Day 2	Day 4
38% CO_2/51% N_2/11% O_2 + Nisn(400 IU/g)	4.23 ± 0.89	3.68 ± 0.70
19% CO_2/70% N_2/11% O_2 + Nisn(400 IU/g)	4.60 ± 0.58	3.61 ± 0.74
Air + Nisn(400 IU/g)	4.17 ± 0.91	3.61 ± 0.63
38% CO_2/51% N_2/11% O2 without nisin	4.37 ± 0.56	3.42 ± 0.89
19% CO_2/70% N_2/11% O_2 without nisin	4.07 ± 0.96	3.25 ± 0.62
Air without nisin (control)	4.07 ± 0.83	3.54 ± 0.77

Values are presented as mean ± standard deviation.

No significant different was observed between the values within rows ($p>0.05$)

Detected initial grade at Day 0 is 4.88 ± 0.28 (n=9)

Table 7.

Sensory test result under the term of "Odor"

Treatment	Day 2	Day 4
38% CO_2/51% N_2/11% O_2 + Nisn(400 IU/g)	3.80 ± 0.95	3.50 ± 0.71
19% CO_2/70% N_2/11% O_2 + Nisn(400 IU/g)	3.93 ± 0.93	3.39 ± 1.07
Air + Nisn(400 IU/g)	3.77 ± 0.77	2.71 ± 0.88
38% CO_2/51% N_2/11% O2 without nisin	3.97 ± 0.76	3.36 ± 0.74
19% CO_2/70% N_2/11% O_2 without nisin	3.77 ± 0.77	3.11 ± 1.10
Air without nisin (control)	3.73 ± 0.93	3.14 ± 1.39

Values are presented as mean ± standard deviation.

No significant different was observed between the values within rows ($p>0.05$)

Detected initial grade at Day 0 is 4.88 ± 0.32 (n=9)

Table 8.

Sensory test result under the term of "Accept or not"

Treatment	Day 2	Day 4
38% CO_2/51% N_2/11% O_2 + Nisn(400 IU/g)	0.64 ± 0.49	0.36 ± 0.49 a
19% CO_2/70% N_2/11% O_2 + Nisn(400 IU/g)	0.79 ± 0.42	0.36 ± 0.49 a
Air + Nisn(400 IU/g)	0.57 ± 0.50	0.36 ± 0.49 a
38% CO_2/51% N_2/11% O2 without nisin	0.64 ± 0.49	0.36 ± 0.49 a
19% CO_2/70% N_2/11% O_2 without nisin	0.79 ± 0.42	0.29 ± 0.46 ab
Air without nisin (control)	0.53 ± 0.50	0.13 ± 0.36 b

Values are presented as mean ± standard deviation.

a-b means within rows, a value with a different letter has a significant difference ($p<0.05$)

Detected initial grade at Day 0 is 1.00 ± 0.00 (n=9)