HIGH PRESSURE PROCESSING AND NISIN AS POSSIBLE NONTHERMAL TREATMENTS FOR CONTROL OF *LISTERIA* AND IMPACT ON QUALITY ATTRIBUTES IN COLD SMOKED PACIFIC SOCKEYE SALMON

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Lisa Dawn Desrocher

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MASTER OF SCIENCE

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Clifford Hall
Chair

Teresa Bergholz

Robert Maddock

Julie Garden-Robinson

Approved:

September 9, 2018	John McEvoy
Date	Department Chair

ABSTRACT

Cold smoked salmon products are considered high risk for *Listeria monocytogenes* contamination by the U.S. Food and Drug Administration due to lack of validated kill step. Currently, there are no commercialized post-packaging control measures to mitigate *Listeria spp*. in cold smoked salmon processing. Nisin applied during cold smoked salmon processing has been reported to reduce *L. monocytogenes* with no change in final product organoleptic properties. High pressure processing of cold smoked salmon post-packaging has also been reported to mitigate *L. monocytogenes*. However, the pressure and time required have adverse effects on cold smoked salmon such as lightening of flesh color. The effectiveness of nisin and high-pressure processing on the survival of *Listeria innocua* in cold smoked salmon was recently reported. The combination of Nisin and HPP was found to be more effective for controlling *L. innocua* than either treatment alone while maintaining desirable consumer attributes.

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I want to acknowledge my family for their support throughout this process. I also want to thank North Dakota State University for providing the opportunity to expand my knowledge in Food Safety.

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GENERAL INTRODUCTION

Cold smoked salmon (CSS) is a ready to eat (RTE) seafood product produced and consumed around the world. As a RTE food product, CSS can be consumed directly at point of purchase without the benefit of further preparation. Due to the lack of a validated kill step during processing, CSS is categorized as a high-risk food item for *Listeria monocytogenes* contamination by the U.S. Food and Drug Administration (FDA) (*17*). As compared to other RTE meat products that are thermally processed to greater than 74°C, the cold-smoking process only reaches temperatures between 10° and 32°C. At these processing temperatures *Listeria* spp. survives, thus characterizing CSS as a raw RTE meat product (*52, 8*). *Listeria monocytogenes* is a psychotropic, Gram -positive, facultative anaerobe, as well as being tolerant to high salt and low pH environments. These adaptations allow the microorganism to thrive in a variety of environments such as processing facilities.

Listeria monocytogenes is a bacterial pathogen that causes the foodborne illness listeriosis. Listeriosis has a low incidence rate affecting about 3 in 1 million individuals, but a high fatality rate reaching approximately 20% of all reported cases (*2, 11*). Immunocompromised individuals are the most susceptible to listeriosis. Of this group, populations older than 65 years comprise 50% of reported listeriosis cases (*54*).

Without the benefit of a post-packaging treatment to eliminate *L. monocytogenes* in CSS, in-process preventive controls are required. Upon implementation, these controls must not negatively impact the quality and sensory attributes of CSS. One method of interest that has been investigated is the application of bacteriocins. Nisin is an FDA-approved bacteriocin has been commercially applied to vegetable, dairy, and meat products (*58*). The reported efficacy of nisin in reducing *L. monocytogenes* in CSS while maintaining sensory attributes makes the

application as non-thermal preventive control for CSS highly plausible. High pressure processing (HPP) is another non-thermal treatment for the elimination of *Listeria* spp. in CSS that has been heavily investigated (*28, 35, 36, 47, 50*). As a commercialized process, HPP has been implemented to reduce microbial load and extend shelf life without detriment to product quality as seen in thermal treatments (*56*). The efficacy of HPP for pathogen reduction, depends upon the pressure and hold times applied. The longer the holding time and the higher the pressure, the greater the lethality of the treatment. Although higher pressures and longer holding times leads to greater reduction in pathogens, it also causes quality changes in the product (*56*).

The interest in developing a non-thermal treatment to eliminate *Listeria* spp. in CSS was the focus of a joint research project conducted by Nicole Lebow, graduate student at Washington State University, Department of Food Science, Pullman, WA and published in the *Journal of Food Science* (*39*). Lisa Dawn Desrocher a Food Safety graduate student at North Dakota State University played a supporting role in the joint research project. Desrocher conceptualized the overall scope of the research, procured commercial CSS, assisted in preliminary experiments and designed and conducted the peelability study in the third experiment. Preliminary experiments were executed to determine the efficacy of the combined treatments of nisin and HPP while maintaining CSS sensory attributes. Based on these results, the research project was designed to meet three objectives. The first objective was to determine the combined effects of nisin and HPP on *Listeria innocua* growth over a 36-day shelf life study at the refrigerated temperature of 4°C and at the abuse temperature of 7°C. The second objective was to determine the effects of nisin and HPP on flesh color and spoilage organisms in CSS. The third objective was to determine the impact of nisin and HPP on sensory attributes of CSS, including peelability of pre-sliced CSS.

LITERATURE REVIEW

Importance of salmon to food industry. Consumer demand for convenient, ready-to-eat (RTE) food products with high nutritional value and free from artificial ingredients and preservatives continues to increase (*14*). The nutritional benefit of fish, such as salmon, drives consumption with 60% in the form of smoked products (*55*). There are two main methods for smoking salmon: hot smoking and cold smoking. Both hot smoked salmon and cold smoked salmon are distributed and sold as RTE food products. Where hot smoking reaches internal temperatures of 60-70°C during the smoking process, cold smoking reaches internal temperature of 20-30°C. The temperature achieved during the cold smoking process is ineffective in eliminating pathogens dictating processors follow strict guidelines as set by the Food and Drug Administration (FDA) to minimize growth of said pathogens (*17*).

Cold smoked salmon production. Cold smoked salmon is a minimally processed food as presented in Figure 1 (*16*). Harvested whole salmon are washed and processed to remove the head and the internal organs upon receipt at production facility. To aid in the reduction of spoilage and pathogenic bacteria, the body cavities are also washed. The in-process salmon are then either flash frozen or held under refrigeration for immediate further processing. Prior to brining, the fillets are removed from the frame and skinned. The next stage is to cure the salmon fillets with either a liquid or dry brine. Brines can contain salt and possibly other additives, including sugar, spices, and flavorings. Following brining, the fillets are rinsed and may undergo a drying step under refrigeration prior to cold smoking. If the surface of the fillet is not dry enough it may prevent the absorption of smoke in the smokehouse. The smoke used in the production of CSS can be from combustion of wood, liquid smoke or a combination of both. During cold smoking the temperatures cannot exceed 32.2 °C for > 20 hours or 10 °C for > 24 hours, which is insufficient to kill pathogens that may be present.

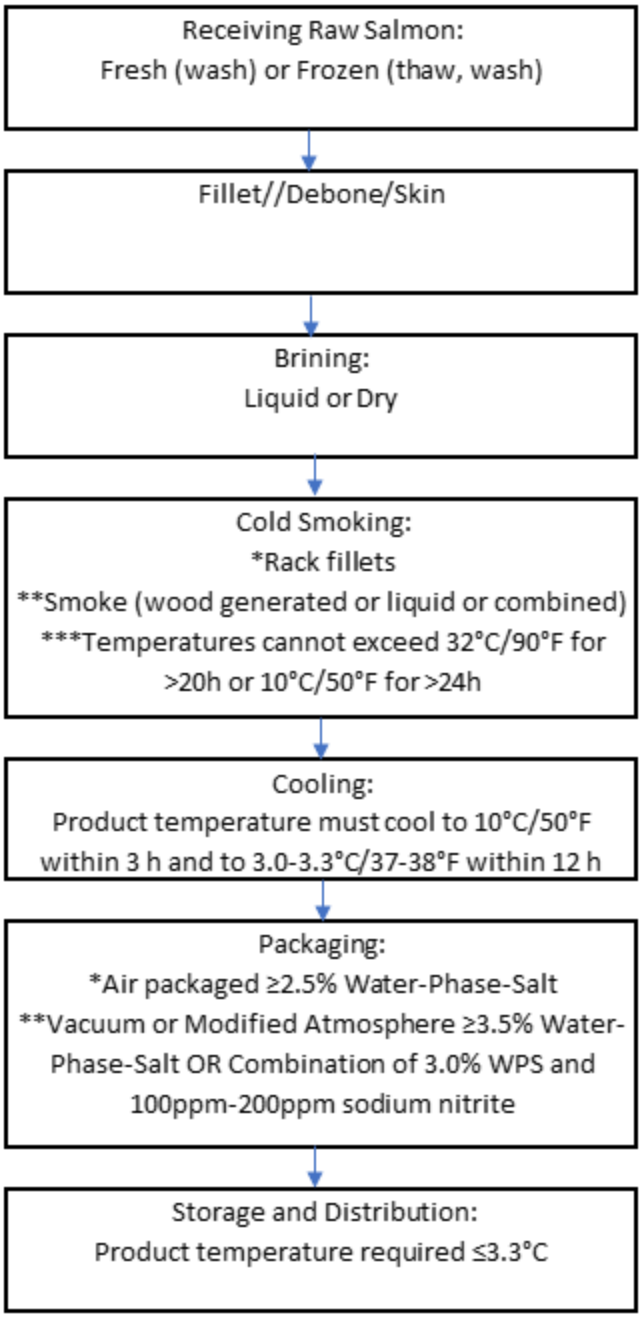


Figure 1: ***Cold-smoked salmon production flow diagram with required processing temperatures and water-phase salt concentrations. Adapted from the FDA (16)***

As such, CSS products are considered a minimally processed or raw RTE food. The salmon is then required to cool to 10 °C within 3 hours after the cold-smoking step, and 3.3 °C

within 12 hours to prevent microbial growth. In the U.S., most CSS fillets are pre-sliced, portioned, packaged under vacuum or modified atmosphere and frozen prior to commercial distribution.

Cold smoked salmon processing parameters for food safety. Control of spoilage and pathogenic growth in CSS is influenced by water-phase salt (WPS) concentration, type of packaging, water activity, and storage conditions of the finished product. The FDA (*16*) requires CSS products to achieve a WPS of 3.5% when packaged anaerobically (vacuum or modified atmosphere) or 2.5% when packaged aerobically for pathogen control such as *Clostridium botulinum*. The water activity of CSS between 0.94 and 0.98 prevents growth of foodborne pathogens such as *Acinetobacter* spp. (*37*). Freezing of the salmon either prior or post processing is required for the destruction of parasites, of which *Diphyllobothrium latum* and *Anisakis simplex* are the most common in salmon (*23*). Antimicrobial effects are also achieved by phenolic compounds deposited on the surface of CSS during smoking processing step (*48*). Strict control over storage temperature below 4°C throughout shelf life provides an additional food safety hurdle. The foodborne pathogens *Listeria monocytogenes* and *C. botulinum* have been detected in CSS products and are a major concern for consumers due to their high fatality rates (*16*).

Sensory attributes of cold smoked salmon. Cold smoked salmon is consumed globally whereby consumers have long established expectations of the products sensory characteristics. Key attributes include appearance, aroma, taste and texture. Consumers associate the color of seafood with freshness and quality (*57*). The CSS flesh is to appear as it would in the raw state exhibiting translucency and not the opacity as in cooked or thermally treated salmon. The muscle pigments astaxanthin and canthaxanthin provide for the desired red hue in salmon fillets, with

sockeye salmon having the highest concentration of these pigments (*6, 49*). As the majority of CSS is sold pre-sliced in the U.S., the slices should peel apart easily and maintain their integrity upon handling.

Spoilage of cold smoked salmon. The refrigerated shelf life of packaged CSS is typically self-limiting due to the growth of spoilage organisms. In the U.S., the major processors distribute CSS frozen to retailers where it is sold in the refrigerated state. Lactic acid bacteria (LAB) are the predominant cause of spoilage in CSS through the production of volatile compounds such as acetic acid and propyl acetate leading to off-odor and flavor (*32*). The FDA (*17*) reported that aerobic packaging of CSS reduced the risk of *C. botulinum* growth and increased the rate of spoilage. The competitive growth of LAB in CSS is thought of as a secondary control measure against potential pathogens such as *C. botulinum* (*17*).

***Listeria spp.* and listeriosis.** The genus *Listeria* is comprised of 17 species of which two are pathogenic (*7, 8, 45*). *Listeria monocytogenes* is pathogenic to humans and animals while *Listeria ivanovii* is predominantly pathogenic to animals. *L. monocytogenes*, a Gram-positive, non-spore forming, facultative anaerobic bacterium, has been isolated in environments such as soil, water, mammals, and fish. In humans, *L. monocytogenes* infections are caused by 3 serotypes (1/2a, 1/2b, and 4b) resulting in listeriosis, often transmitted via consumption of contaminated food (*2*).

The Foodborne Diseases Active Surveillance Network monitors foodborne illness in the U.S.A, and found listeriosis cases were rare between 2006-2013, affecting 3 in 1 million individuals, but with a high fatality rate of 19.5% in 2013 (*11*). Deaths from listeriosis are the third most prevalent cause of foodborne fatalities after nontyphoidal *Salmonella* and *Toxoplasma gondii* in the U.S.A. (*8, 53*). Immunocompromised individuals, the elderly, expectant mothers,

and newborns suffer from more severe symptoms when exposed to *L. monocytogenes*, requiring a minimum exposure dose of 5 log CFU/g, while the minimum exposure dose for healthy individuals was 7 log CFU/g (*13*). Over 50% of all listeriosis cases occur in individuals over the age of 65 years, causing bacteremia, meningitis, and death (*54*). Expectant mothers infected by *L. monocytogenes* typically experience flu-like symptoms, while the fetus suffers from more severe outcomes such as stillbirth or premature birth, with 29% of cases resulting in loss of the fetus or death of the newborn (*12*).

***Listeria* contamination in cold smoked salmon.** *Listeria* contamination is a major concern in ready-to-eat (RTE) foods as they are marketed to be consumed directly upon purchase. The ability of *L. monocytogenes* to grow in a broad range of pH (4.0-9.6), salt content (0 to 10%), temperatures -1.5°C (29.3°F) to 45°C (113°F), and in the presence or absence of oxygen makes the bacterium difficult to control in food production (*15, 20*). The widespread nature of *Listeria* spp. in the environment allows for the introduction into processing facilities from a variety of sources such as contaminated raw materials, air, water and processing personnel. Additionally, the CSS process involves several pieces of equipment which are difficult to clean, such as skinners, smokehouses and slicers, and can establish a niche for *Listeria spp.* (*44*). As the equipment is operated product can become contaminated, potentially spreading through the remaining processing stages. *Listeria spp*. has been reported to be more prevalent in fish processing plants than meat or dairy (*34*). Rotariu et al. (*52*) reported the frequency of *L. monocytogenes* contamination in smoked salmon as directly correlated to the processors sanitation practices. Gianfranceschi et al. (*26*) found 50% of food samples contaminated with *L. monocytogenes* were RTE foods, including seasoned meat, dairy, seafood, and vegetable products.

Products referred to as minimally processed or raw RTE (raw-RTE) are not intended to be cooked prior to consumption. These products are at higher risk of *Listeria* contamination due to the lack of validated kill step during processing. A validated kill step is generally considered to be a thermal process sufficient to provide a 6-log reduction of the target pathogen. Seafood products that fall under the purview of raw-RTE include sashimi, cold smoked fish, and caviar. With a contamination frequency > 5%, the FDA Center for Food Safety and Applied Nutrition (*9*) has declared cold smoked fish as high-risk for *Listeria* spp. contamination. Furthermore, the FDA continues to uphold zero-tolerance for *L. monocytogenes* in RTE foods, with detection in either of two 25-gram food samples to be considered adulterated (*9*). There were 13 recalls for *L. monocytogenes* in CSS products in 2016-2017 and two listeriosis outbreaks from CSS consumption in Canada and Denmark (*43*).

Control strategies for *Listeria* in cold smoked salmon. The lack of a validated kill step during CSS processing and the prevalence of *L. monocytogenes* in CSS is concerning to the salmon industry and consumers. Effective implementation of control methods, while maintaining desirable sensory attributes remains a top priority for processors. The antimicrobial effects of salt, temperature and smoke compounds on *L. monocytogenes* have been reported (*31, 40*). A smoking temperature of 55°C had the greatest effect on *L. monocytogenes* inactivation, while the addition of salt during brining and phenolic compounds during smoking resulted in a 3.5 log CFU/g reduction. When cold smoking salmon, Cornu et al. (*10*) also found salt and phenolic compounds reduced growth of *L. monocytogenes*. Kang et al. (*33*) found that freeze-thawing salmon prior to inoculation of *L. monocytogenes* led to marked growth of the pathogen when stored at 7°C. The effect of different curing method was also reported. Dry brined and

freeze-thawed CSS exhibited a lag phase of up to 11 days as compared to wet brined and freeze-thawed which *L. monocytogenes* grew within 24 hours.

Although the CSS processing steps have potential to reduce *L. monocytogenes*, they do not eliminate the pathogen (*29, 48*). Additional control steps such as the application of bacteriocins and post-packaging treatments are currently under investigation.

Bacteriocins. As consumers continue to demand foods free from artificial ingredients and chemical preservatives, antimicrobials such as bacteriocins are of significant interest to CSS processors. Bacteriocins are antimicrobial peptides or proteins produced by bacteria to fight against competing microorganisms in the surrounding environment (*58*). Nisin, an FDA-approved bacteriocin produced by LAB, has been commercially applied to vegetable, dairy, and meat products during processing prior to finished product packaging (*40, 58*).

Nisin presents a bactericidal effect on Gram-positive bacteria by forming pores in the bacterial cell wall and preventing synthesis of peptidoglycans (*19*). Nisin has been reported to inhibit growth of *Listeria spp., C. botulinum, Salmonella typhimurium* and spoilage LAB (*22, 24, 25*). Kang et al. (*33*) reported 20 ppm Nisin application in CSS resulted in a significant decrease in initial *L. monocytogenes* concentration of 10^6 CFU/g by approximately 2 log CFU/g. The impact of nisin on sensory attributes of food products has been evaluated. Nisin had no effect on appearance, taste or aroma in meat and seafood products, including Atlantic salmon (*30, 44*).

High pressure processing. Although processors continue to improve control strategies throughout each stage of CSS production, the opportunity for post-processing contamination remains a concern. Post-packaging treatments to eliminate *L. monocytogenes* but maintain desired sensory attributes of CSS is a high priority for processors. High-pressure processing (HPP) has been commercially applied post-packaging to heat sensitive products such as

smoothies, vegetable dips, meats, and seafood to reduce the microbial load and extend the shelf life (*21, 56*). The conditions of HPP vary depending on the food product. Higher pressures and longer hold times lead to a greater reduction in pathogens but also greater observed quality changes in the product including appearance (*56*).

High-pressure processing is a batch process, where packaged food is placed into a cylindrical chamber and suspended in a pressure-transmitting fluid that evenly distributes the applied pressure ranging from 50 to 1,000 MPa throughout the product. While HPP is considered a non-thermal process, the internal temperature of the system increases about 3 °C per 100 MPa (*3*). This rise in temperature has limited the use of HPP as heat decreases the quality and sensory attributes of CSS, leading to the exploration of HPP at sub-zero temperatures (*50*). Larger temperature increases have been observed in foods with high concentrations of lipids. High-pressure processing inactivates spoilage bacteria and pathogens by disrupting the cell membrane structure. Fluidity of the cell membrane decreases and undergoes a lipid bilayer phase change, leading to denaturation of membrane-bound proteins (*51*). Pressures over 200 MPa affect critical cellular structures and processes of bacteria, including motility, cell division, nutrient uptake, protein structure, and overall viability (*1*).

High pressure processing and cold smoked salmon. High-pressure processing reduces microbial growth but has been demonstrated to impact the lightness (L^*) and texture of CSS. Colorimeters assign numerical values for Lightness (L^*), green-red (a^*) and blue-yellow (b^*). Changes in these values correspond to changes that can be perceived visually. Lightness (L^*) is described as the luminosity, brightness or lightness of an object where a L^* reading of 0 is black and a L^* reading of 100 is white. Lakshmanan et al. (*36*) treated CSS at 100 MPa, 200 MPa, and 300 MPa for 10 min, 20 min, or 30 min at processing temperatures ranging from 20°C to 30°C.

The lightness (*L**) of the salmon flesh increased as the pressure and holding time increased. Picart et al. (*47*) treated smoked salmon mince inoculated with *L. innocua* to 207 MPa at -21°C or 4°C for 60 min. They observed a 1.1 log CFU/g reduction in *L. innocua* when pressurized at -21°C compared to a 0.7 log CFU/g reduction when processed at 4°C. Ritz et al (*50*) observed a 4.22 Log reduction of *L. monocytogenes* in CSS after applying 200 MPa at -18 °C for 180 min. Cold-smoked salmon was lighter (L*), redder (a*), and yellower (b*), while hardness, springiness, gumminess, and chewiness were increased as compared to untreated CSS. Gudbjornsdottir et al. (*28*) applied higher pressures to CSS, ranging from 400 MPa to 900 MPa, with shorter holding times (10 s to 60 s). The temperatures within the HPP chamber reached 42°C. High-pressure processing at 900 MPa for 10 s caused a 4.5 Log reduction of *L. innocua*, with an increase in *L**. In all studies, the *L** of salmon flesh was observed to increase as the holding time increased. The implementation of HPP at low product temperatures using high pressure and short holding times to mitigate *Listeria* contamination and impact on CSS color has not been studied.

MATERIALS AND METHODS[1]

Preliminary experiments and three independent experiments were conducted at Washington State University, Department of Food Science, Pullman, WA. The following is a brief narrative of the materials and methods with full details published in the Journal of Food Science by Lebow et al (*39*) in the paper titled: 'Influence of High-Pressure Processing at Low Temperature and Nisin on Listeria innocua Survival and Sensory Preference of Dry-Cured Cold-Smoked Salmon' and Lebow's Master's Thesis titled: 'The Effects of High-Pressure Processing and Nisin on Listeria Growth, Quality and Sensory Characteristics of Cold-Smoked Pacific Sockeye Salmon.'*(38).*

Experimental design. Commercially processed CSS from Trident Seafoods Corporation, Seattle, WA was used for the preliminary and experiments 1 through 3 as presented in Table 1. Three separate commercial production lots were secured for each independent experiment and transported to Washington State University, Department of Food Science, Pullman, WA. Each production lot of CSS was cut into uniform 25-gram portions from either side of the fat line. For samples receiving a nisin treatment, a topical application of a nisin solution was applied to top and bottom surface of 25g CSS portion to achieve a 10 µg/g dose of nisin.

Preliminary experiment. For the preliminary experiment, CSS with and without nisin treatment received a 6 Log CFU/ g inoculation of a *L. innocua* cocktail by topical application onto the surface of the CSS 25-gram portions. The *L. innocua* cocktail was comprised of three placing the frozen vacuum packaged samples in a pouch filled with ice slurry prior to insertion in

[1] The material in this chapter was co-authored by Noelle Kaitlin Lebow, Dr. Denise M. Smith and Lisa Dawn Desrocher. Lisa Dawn Desrocher had primary responsibility for conceptualizing research scope, procuring experimental samples, determining sample requirements, designing and conducting the peelability study. Lisa Dawn Desrocher also served as proofreader.

Table 1: ***Experimental treatments for cold smoked salmon[a].***

Experiment	Objective	Nisin (µg/g)	HPP Pressure (MPa)	HPP Holding Time (s)
Preliminary	Effect *L.innocua* inactivation	No	450	30, 60, or 120
		No	600	30, 60, or 120
		Yes	450	30, 60, or 120
		Yes	600	30, 60, or 120
		Yes	(none)	(none)
1	Effect on *L.innocua* growth	Yes	450	120
		Yes	600	120
		Yes	(none)	(none)
2	Effect on flesh color and spoilage organisms	Yes	450	120
		Yes	600	120
		Yes	(none)	(none)
3	Effect on sensory attributes and peelability	Yes	450	120
		Yes	600	120
		Yes	(none)	(none)

[a] *All experiments included Control CSS. Adapted from Lebow et al (39)*

the pressure chamber. Samples with and without nisin, inoculated with a 6 Log CFU/g dose of *L. innocua* were exposed to pressure at either 450 or 600 MPa for a holding time of 30, 60 and 120s. Enumeration of *L. innocua* post-processing was performed as reported by Lebow et al (*39*). Results of the preliminary experiment were used to select HPP treatment conditions for further study.

Experiment 1. For the first experiment, inoculated CSS sampled were treated with nisin alone (CSS+N), with nisin and HPP at 450MPa for 120s (CSS+N450) and with nisin and HPP at 600MPa for 120s (CSS+N600). The control CSS was inoculated with 6 Log CFU/g *L. innocua* cocktail, but did not receive nisin or HPP treatments (Table 1) (*39*). After low temperature HPP, Control CSS, CSS+N, CSS+N450 and CSS+N600 samples were divided into two sets and stored

at either 4°C or 7°C for 36 days. Enumeration of *L. innocua* was performed every six days to determine the impact of the treatments as per Lebow et al (*39*).

Experiment 2. The impact of nisin in combination with low temperature HPP at 450 and 600MPa at 120s, on CSS flesh color and naturally inherent spoilage organisms was investigated in the second experiment. Samples in the second experiment did not receive the *L. innocua* cocktail inoculation. The CSS flesh color was evaluated and reported in *L** (lightness), *a** (redness) and the *b** (yellowness) values. The impact of treatments on spoilage bacteria was evaluated through the enumeration of lactic acid bacteria (LAB) and psychrotrophic bacteria. Samples were evaluated for flesh color and spoilage bacteria over a 36-day shelf life study conducted at storage temperatures of 4°C and 7°C according to methods per Lebow et al (*39*).

Experiment 3. In the third experiment, the impact of nisin in combination with low temperature HPP at 450 and 600MPa with a holding time of 120s, on sensory attributes and peelability of CSS slices were evaluated. Samples in the third experiment did not receive the *L. innocua* inoculation. Post-processed, samples were stored frozen until evaluated.

RESULTS AND DISCUSSION

Effect of nisin and low temperature HPP on *L. innocua* inactivation. The treatment conditions resulting in the greatest reduction of *L. innocua* on Day 0 were CSS+N450 with a reduction of 2.82 log CFU/g ($P<0.0001$) and CSS+N600 with a reduction of 3.43 log CFU/g ($P<0.0001$) (*38*). The Food Safety and Inspection Service (*20*) requires post-lethality treatments to reduce the *Listeria* concentration by 2 Log for "increased control" of the bacteria. The typical concentration of *L. monocytogenes* detected on CSS in production facilities is relatively low (< 100 CFU/g), suggesting the nisin and HPP treatments may be sufficient to eliminate naturally occurring *Listeria* spp. on commercial CSS products (*4, 52*). Treatment conditions of nisin and HPP at 450 MPa or 600 MPa for 120 s were selected for further study.

Effect of nisin and low temperature HPP on *L. innocua* growth. On Day 0, *L. innocua* was reduced in CSS+N, CSS+N450 and CSS+N600 by 1.59, 2.63, and 3.99 log CFU/g, respectively (Table 2) and as reported by Lebow (*38*). During the 4°C shelf life study, *L. innocua* growth was observed in Control CSS that had been inoculated with 6 log CFU/g *L. innocua* cocktail, after 18 days of storage at 4 °C ($P = 0.0059$). Similar results reported in previous research showing substantial *L. monocytogenes* growth on CSS after 21 days at 4 °C (*41,42*). Growth of *L. innocua* was delayed in CSS+N when stored at 4 °C, with growth detected on day 36 at 6.35 log CFU/g ($P = 0.0055$). Levels of *L. innocua* did not change when CSS treated with CSS+N450 and CSS+N600 were stored for 36 days at 4 °C. The low population of *L. innocua* during the 36-day storage at 4 °C suggests CSS+N450 or CSS+N600 would further reduce the risk for *L. monocytogenes* growth during the refrigerated storage of 4°C up to 30 days, as is recommended by commercial manufacturers. Storage at 7 °C led to accelerated growth of *L. innocua* in CSS+N after 24 days as presented in Table 2 and reported by Lebow et al (*38*).

Table 2: ***Growth of Listeria innocua on cold smoked salmon stored at 4°C and 7°C.***

4 °C	Log CFU/g						
	Day 0[a]	Day 6	Day 12	Day 18	Day 24	Day 30	Day 36
Control	5.82 ± 0.11^{Ax}	5.86 ± 0.12^{Axy}	6.30 ± 0.17^{Axyz}	6.77 ± 0.78^{Axyz}	7.92 ± 0.26^{Az}	8.11 ± 0.23^{Az}	7.60 ± 1.23^{Ayz}
CSS+N	4.23 ± 0.15^{By}	3.89 ± 0.61^{By}	3.87 ± 0.40^{By}	5.46 ± 1.06^{Ayz}	5.01 ± 1.57^{Byz}	5.40 ± 2.33^{Byz}	6.35 ± 1.39^{Az}
CSS+N450	3.19 ± 0.33^{Bz}	2.16 ± 0.38^{Cz}	2.60 ± 0.45^{BCz}	2.61 ± 0.31^{Bz}	1.74 ± 0.67^{Cz}	2.05 ± 0.29^{Cz}	2.14 ± 0.69^{Bz}
CSS+N600	1.83 ± 0.75^{Cz}	1.13 ± 1.00^{Cz}	1.38 ± 1.06^{Cz}	1.86 ± 0.25^{Bz}	1.48 ± 0.49^{Cz}	1.80 ± 0.73^{Cz}	0.81 ± 1.02^{Bz}
7 °C	**Log CFU/g**						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	5.82 ± 0.11^{Ay}	6.95 ± 0.89^{Ayz}	8.53 ± 0.03^{Az}	8.48 ± 0.13^{Az}	8.44 ± 0.18^{Az}	8.28 ± 0.21^{Az}	8.25 ± 0.12^{Az}
CSS+N	4.23 ± 0.15^{Bx}	5.53 ± 0.90^{Axy}	6.91 ± 1.44^{Byz}	8.42 ± 0.21^{Az}	8.30 ± 0.10^{Az}	8.29 ± 0.15^{Az}	8.07 ± 0.59^{Az}
CSS+N450	3.19 ± 0.33^{Bx}	2.68 ± 0.32^{Bx}	3.29 ± 0.95^{Cx}	6.39 ± 0.19^{By}	6.98 ± 2.26^{AByz}	8.50 ± 0.21^{Az}	8.39 ± 0.34^{Az}
CSS+N600	1.83 ± 0.75^{Cx}	1.10 ± 1.07^{Bx}	2.73 ± 0.78^{Cx}	4.78 ± 1.26^{By}	6.52 ± 1.04^{Byz}	7.06 ± 1.42^{Az}	7.24 ± 1.28^{Az}

[a]Means of lots from 4 °C and 7 °C were combined for day 0 ($n = 6$).
Values are expressed as the mean of lots ($n = 3$) ± standard deviation. Capital letters (ABC) indicate significant differences across treatments within a particular day, and lower case letters (xyz) indicate significant differences across days within a treatment ($P < 0.05$). Adapted and reprinted from Lebow (*38*).

Growth of *L. innocua* in CSS+N450 and CSS+N600 stored at 7 °C was detected on day 18 ($P < 0.0001$ in both treatments) (*38*). At day 30, both CSS+N450 and CSS+N600 reached levels higher than original dose of 6 log CFU/g. All treatments contained similar concentrations of *L. innocua* on day 36 when stored at 7 °C ($P = 0.2473$). Results are consistent with those observed by Lakshmanan and Dalgaard (*35*), where *L. monocytogenes* increased by 4 log CFU/g in CSS treated with HPP of 250 MPa for 20 min and stored at 10 °C. In contrast, Kang et al. (*33*) observed 1.5-Log CFU/g reduction of *L. monocytogenes* when CSS containing 10 µg nisin/g was stored for 40 days at 7 °C. The effectiveness of nisin on CSS can vary with salt concentration, pH, or lipid content (*5*, *24*).

Effect of nisin and low temperature HPP on spoilage organisms. Initial concentrations of naturally occurring psychrotrophic, spoilage bacteria on CSS were low across all treatments ($P = 0.1008$), averaging < 1 log CFU/g (Table 3) (*38*). The low initial counts indicate the CSS

Table 3: ***Growth of psychrotrophic bacteria on cold smoked salmon stored at 4°C and 7°C.***

4°C	Log CFU/g						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	0.80 ± 0.55^{Ax}	1.73 ± 0.83^{Axy}	2.93 ± 0.47^{Ay}	3.08 ± 0.68^{Ay}	4.72 ± 1.02^{Az}	4.73 ± 0.30^{Az}	5.90 ± 1.18^{Az}
CSS+N	0.49 ± 0.39^{Ax}	1.86 ± 0.63^{Ax}	3.82 ± 0.78^{Ay}	3.97 ± 0.15^{Ay}	3.51 ± 1.51^{Ay}	5.05 ± 0.51^{Ayz}	6.12 ± 0.45^{Az}
CSS+N450	0.00 ± 0.00^{Az}	0.06 ± 0.10^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	1.24 ± 2.15^{Bz}	0.00 ± 0.00^{Bz}	0.32 ± 0.28^{Bz}
CSS+N600	0.00 ± 0.00^{Az}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.58 ± 1.00^{Bz}
7°C	**Log CFU/g**						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	0.80 ± 0.55^{Aw}	2.86 ± 0.68^{Ax}	4.34 ± 0.78^{Axy}	4.14 ± 1.76^{Axy}	5.79 ± 0.91^{Ayz}	6.00 ± 1.35^{Ayz}	7.87 ± 0.07^{Az}
CSS+N	0.49 ± 0.39^{Aw}	3.62 ± 0.21^{Ax}	4.39 ± 0.57^{Axy}	4.30 ± 1.53^{Axy}	6.88 ± 0.14^{Az}	6.16 ± 1.44^{Ayz}	7.05 ± 0.38^{Az}
CSS+N450	0.00 ± 0.00^{Ay}	0.00 ± 0.00^{By}	0.39 ± 0.68^{By}	0.06 ± 0.10^{By}	1.34 ± 2.33^{By}	1.53 ± 0.61^{By}	4.20 ± 0.29^{Bz}
CSS+N600	0.00 ± 0.00^{Az}	0.00 ± 0.00^{Bz}	0.36 ± 0.62^{Bz}	0.12 ± 0.10^{Bz}	0.16 ± 0.28^{Bz}	1.48 ± 2.56^{Bz}	0.72 ± 0.94^{Cz}

[a]Means of lots from 4 °C and 7 °C were combined for day 0 ($n = 6$).
Values are expressed as the mean of lots ($n = 3$) ± standard deviation. Capital letters (ABC) indicate significant differences across treatments within a particular day, and lower case letters (wxyz) indicate significant differences across days within a treatment ($P < 0.05$). Adapted and reprinted from Lebow *(38)*.

was processed following Good Manufacturing Practices (GMPs) and Standard Sanitation Operating Procedures (SSOPs). After 36 days, psychrotrophs in the Control CSS and CSS+N stored at 4 °C and at 7°C reached 10^6-10^7 CFU/g. Nisin is produced by *Lactococcus lactis*, belonging to the LAB genus, which may explain why LAB was unaffected by nisin (*58*). After 36 days, psychrotrophs in CSS+N450 and CSS+N600 at 4 °C were 0.32 and 0.58 log CFU/g, respectively. Growth of psychrotrophs did not occur at 7 °C until day 36 in CSS+N450, reaching 4.20 log CFU/g ($P < 0.0001$). Psychrotrophs were inhibited in CSS+N600 at 7°C, resulting in a concentration of 0.72 log CFU/g on day 36. Between day 6 and day 36 at 7°C, psychrotrophic counts were lower in CSS+N450 and CSS+N600 compared to the Control CSS and CSS+N (all $P < 0.0010$).

Lactic acid bacteria did not grow in CSS+N450 and CSS+N600 stored at 4 °C or 7 °C for 30 days as listed Table 4 (*38*). Growth was observed in CSS+N450 on day 36 at 7 °C with a

Table 4: ***Growth of lactic acid bacteria on cold smoked salmon stored at 4°C and 7°C.***

4°C	Log CFU/g						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	0.44 ± 0.45^{Aw}	1.63 ± 0.74^{Awx}	2.76 ± 0.26^{Axy}	3.75 ± 1.26^{Axyz}	4.30 ± 0.87^{Ayz}	3.92 ± 1.66^{Axyz}	5.81 ± 1.23^{Az}
CSS+N	0.00 ± 0.00^{Ax}	1.32 ± 1.26^{ABxy}	3.19 ± 1.40^{Ayz}	4.87 ± 0.87^{Az}	3.74 ± 1.91^{Ayz}	4.26 ± 0.63^{Az}	4.86 ± 1.06^{Az}
CSS+N450	0.00 ± 0.00^{Az}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.16 ± 0.28^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}
CSS+N600	0.00 ± 0.00^{Az}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Cz}	0.06 ± 0.10^{Bz}	0.00 ± 0.00^{Bz}
7°C	**Log CFU/g**						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	0.44 ± 0.45^{Ax}	0.28 ± 0.34^{Axy}	3.94 ± 0.57^{Ayz}	3.75 ± 2.05^{Ayz}	5.15 ± 0.59^{Az}	5.60 ± 1.07^{Az}	6.64 ± 0.94^{Az}
CSS+N	0.00 ± 0.00^{Ax}	0.00 ± 0.00^{Axy}	3.49 ± 1.30^{Ayz}	4.01 ± 3.47^{Az}	5.20 ± 1.21^{Az}	4.50 ± 3.90^{Az}	6.07 ± 0.55^{Az}
CSS+N450	0.00 ± 0.00^{Ay}	0.00 ± 0.00^{Ay}	0.00 ± 0.00^{By}	0.00 ± 0.00^{By}	0.00 ± 0.00^{By}	0.00 ± 0.00^{By}	2.46 ± 2.16^{Bz}
CSS+N600	0.00 ± 0.00^{Az}	0.00 ± 0.00^{Az}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Bz}	0.00 ± 0.00^{Cz}

[a]Means of lots from 4 °C and 7 °C were combined for day 0 ($n = 6$).
Values are expressed as the mean of lots ($n = 3$) ± standard deviation. Capital letters (ABC) indicate significant differences across treatments within a particular day, and lower case letters (wxyz) indicate significant differences across days within a treatment ($P < 0.05$). Adapted and reprinted from Lebow *(38)*.

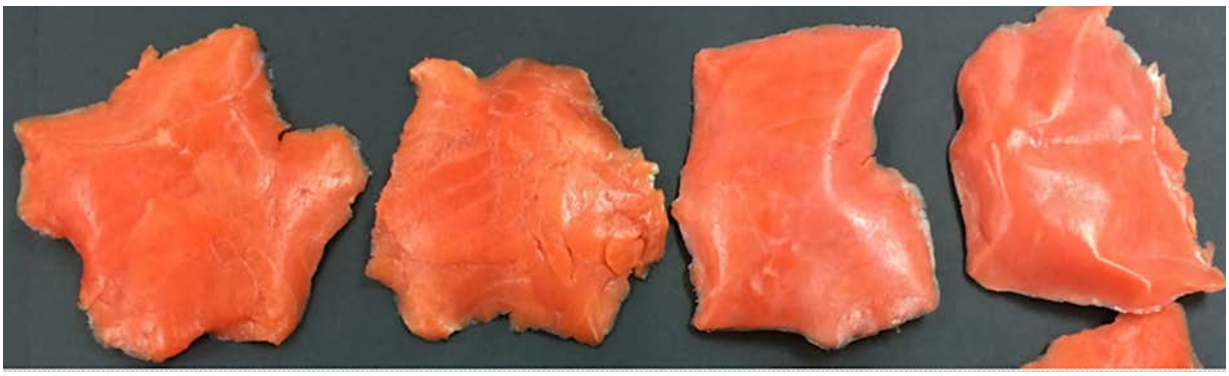
reading of 2.46 log CFU/g ($P < 0.0001$). However, LAB was not detected in CSS+N600 on the 36-day at 7 °C, suggesting CSS+N600 was the most effective treatment for controlling LAB growth in CSS.

The spoilage organisms in CSS have been identified as LAB, *Photobacterium phosphoreum*, and select *Enterobacteriaceae* spp. (*27*, *35*). Lakshmanan and Dalgaard (*35*), determined LAB to be the primary psychrotrophic organisms on CSS, identifying 74 of 75 spoilage isolates as LAB. Perceived acidic and sour aromas produced by LAB volatiles, such as acetic acid and propyl acetate, cause spoilage in CSS (*32*).

Nisin is produced by *Lactococcus lactis*, belonging to the LAB genus, which may explain why LAB was unaffected by nisin (*58*). After 36 days, psychrotrophs in CSS+N450 and CSS+N600 at 4 °C were 0.32 and 0.58 log CFU/g, respectively. Growth of psychrotrophs did not occur at 7 °C until day 36 in CSS+N450, reaching 4.20 log CFU/g ($P < 0.0001$). Psychrotrophs were inhibited in CSS+N600 at 7°C, resulting in a concentration of 0.72 log CFU/g on day 36.

Effect of nisin and low temperature HPP on flesh color. The appearance of CSS was impacted by HPP treatment (Figure 2 and Table 5) (*38*). Application of nisin had no effect on *L**, *a**, or *b** values of CSS flesh, as similarly reported by Han and others (*30*), who also found that nisin (0.4 mg/g) had no effect on the color of salmon. Redness (*a**) and yellowness (*b**) were not affected in CSS+N450 and CSS+N600; however, the treatments increased the lightness (*L**) of CSS on day 0 by 3.21 and 3.61 units ($P = 0.0471$, $P = 0.0197$, respectively), when compared to the control.

Previous research on the effects of ambient temperature HPP on CSS have shown increased lightness during processing (*28, 36, 57*). Change in lightness, appearance and color of HPP treated CSS is reported to be from partial denaturation of the myoglobin and myofibrillar proteins which modifies the translucency of the muscle tissue (*50*). Compared to control CSS and CSS+N, lightness was accelerated by HPP during 7 °C storage.



Control CSS CSS+N CSS+N450 CSS+N600

Figure 2: ***Appearance of CSS at Day 0 in experiment 2. Adapted from Lebow (38).***

Effect of nisin and low temperature HPP on sensory attributes and peelability. Consumer panelists ($n = 120$) compared the Control CSS product pairwise with 3 treatments consisting of CSS+N, CSS+N450, and CSS+N600 (Table 6) (*39*). Texture of CSS+N450 and

Table 5: ***Lightness (L*) of cold smoked salmon stored at 4°C and 7°C.***

4°C	*L**						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	39.79 ± 2.84^{Ay}	39.93 ± 1.49^{Ay}	40.56 ± 2.37^{Ay}	41.69 ± 1.76^{Ay}	43.57 ± 2.25^{Ayz}	46.99 ± 1.08^{Az}	47.87 ± 2.87^{Az}
CSS+N	41.54 ± 1.75^{ABy}	39.91 ± 4.69^{Ay}	43.52 ± 2.55^{Ayz}	42.16 ± 3.04^{Ay}	41.97 ± 3.71^{Ay}	47.91 ± 3.09^{Az}	48.49 ± 2.75^{Az}
CSS+N450	43.00 ± 2.04^{By}	48.94 ± 1.86^{Bz}	49.97 ± 2.68^{Bz}	47.39 ± 1.23^{Byz}	48.10 ± 2.38^{Bz}	50.77 ± 1.38^{ABz}	51.44 ± 2.39^{ABz}
CSS+N600	43.40 ± 3.80^{Bx}	47.77 ± 1.74^{Bxy}	48.85 ± 1.95^{Byz}	50.74 ± 0.87^{Byz}	49.53 ± 2.87^{Byz}	52.56 ± 0.68^{Byz}	53.53 ± 2.79^{Bz}
7°C	*L**						
	Day 0[a]	**Day 6**	**Day 12**	**Day 18**	**Day 24**	**Day 30**	**Day 36**
Control	39.79 ± 2.84^{Ax}	41.71 ± 3.12^{Axy}	44.57 ± 2.36^{Ay}	40.95 ± 2.36^{Axy}	45.21 ± 3.90^{Ay}	50.96 ± 2.22^{Az}	51.99 ± 0.94^{ABz}
CSS+N	41.54 ± 1.75^{ABx}	41.90 ± 3.19^{Axy}	43.09 ± 3.44^{Axy}	41.12 ± 1.66^{Ax}	45.92 ± 2.22^{Ayz}	49.87 ± 3.33^{Az}	50.52 ± 0.99^{Az}
CSS+N450	43.00 ± 2.04^{Bw}	47.84 ± 2.20^{Bxy}	46.74 ± 0.19^{ABwx}	50.01 ± 3.42^{Bxy}	50.42 ± 1.84^{Bxy}	52.34 ± 1.11^{Ayz}	55.85 ± 0.98^{Bz}
CSS+N600	43.40 ± 3.80^{By}	49.50 ± 3.32^{Bz}	48.83 ± 2.48^{Bz}	48.72 ± 2.62^{Bz}	50.38 ± 3.71^{Bz}	53.23 ± 0.48^{Az}	51.06 ± 1.56^{Az}

[a]Means of lots from 4 °C and 7 °C were combined for day 0 ($n = 6$).
Values are expressed as the mean of lots ($n = 3$) ± standard deviation. Capital letters (ABC) indicate significant differences across treatments within a particular day, and lower case letters (wxyz) indicate significant differences across days within a treatment ($P < 0.05$). Adapted and reprinted from Lebow *(38)*.

CSS+N600 was preferred to Control CSS at 62% and 61%, respectively ($P = 0.0093$, $P = 0.0012$, respectively). Sixty-two percent of panelists preferred the flavor of both CSS+N450 and CSS+N600 in comparison to Control CSS ($P = 0.0040$, $P = 0.0013$, respectively). For overall preference, CSS+N450 and CSS+N600 were preferred over control CSS by 61% and 62% of panelists, respectively ($P = 0.0049$, $P = 0.0019$, respectively).

In summary, CSS treated with nisin and HPP were preferred over the Control CSS for texture, flavor, and overall preference. Similarly, Yuste et al. (*59*) treated sausage with 500 MPa for 5 min, and found consumers preferred sausage treated with HPP for flavor and overall acceptance.

The impact of nisin and HPP treatments on the peelability of CSS slices were evaluated. A team of twelve individuals evaluated the peelability of the CSS samples by rating them on a scale of 1-9, where 9 was defined as most acceptable with complete separation of slices.

Table 6: ***Three-paired preference sensory evaluation (n=120).***

	Preference (%)		
	Control	**CSS+N**	**No Preference**
Appearance	34	38	28
Aroma	35	48	18
Texture	31	37	33
Flavor	38	38	24
Overall Preference	42	40	18
	Control	**CSS+N450**	**No Preference**
Appearance	66[a]	31	3
Aroma	57[a]	30	12
Texture	36	62[a]	2
Flavor	34	62[a]	4
Overall Preference	34	61[a]	5
	Control	**CSS+N600**	**No Preference**
Appearance	64[a]	27	9
Aroma	46	33	21
Texture	31	61[a]	8
Flavor	31	62[a]	6
Overall Preference	32	62[a]	6

[a] *Condition was preferred based on sensory attribute within pair of control and treatment (P<0.05) Adapted from Lebow et al (39).*

Treatments evaluated were Control CSS, CSS+N, CSS+N450 and CSS+N600. No significant difference between the peelability of Control CSS and CSS+N was reported (*P* = 0.1981) (*39*). Conversely, CSS+N450 and CSS+N600 were rated as least acceptable (*P* = 0.9995). High-pressure processing appeared to have induced adherence of the slices to each other and reducing peelability. A potential method to improve peelability would be to interleave and shingle the CSS slices with plastic sheets to prevent the slices from adhering together during HPP.

OVERALL SUMMARY AND FUTURE RESEARCH

Cold-smoked salmon is a high-risk RTE product that is frequently contaminated with *L. monocytogenes* during processing. The combined application of nisin (10 µg/g) and high pressure of 600 MPa for 120 s within an ice slurry was the most successful treatment in reducing *L. innocua*, at 3.99 log CFU/g. Nisin (10 µg/g) combined with high pressure of 600 MPa for 120 s maintained the lowest concentration of psychrotrophs and LAB throughout the 36-day storage at 4 °C and 7 °C. Low-temperature HPP increased lightness (L^*) of CSS, although, ambient-temperature HPP resulted in greater L^* increases in previous studies. Marked changes in lightness may have contributed to consumer panelists preferring the appearance of control CSS over HPP treated CSS. However, HPP treated CSS was preferred overall. In addition to the negative impact to appearance, HPP also decreased the peelability of sliced CSS. Future research is required to mitigate the changes in these two key product attributes if low temperature HPP is to be commercialized for CSS production.

The combined treatments of nisin and HPP reduced but did not eliminate *L. innocua* in CSS. However, as the inoculation concentration was higher than average found in contaminated CSS (<100 CFU/g), the combined treatments may be effective in eliminating contaminated CSS in a commercial processing environment. Future research could be conducted on combined treatments at varied inoculate concentrations, pressures/holding times and using *L. monocytogenes* versus *L. innocua*.

Even though the sensory panel preferred the HPP treated samples overall, the significant changes in lightness and peelability remain a deterring factor for implementation by processors. Future research could include adding natural colorants to the surface of the salmon prior to HPP to mitigate color change. Vacuum packaging severely compresses the pre-sliced CSS potentially

facilitating the adhesion of the slices post-HPP. Investigating interleaving the slices and/or replacing vacuum packaging with skin-packaging is also recommended to address peelability of CSS post-HPP.

Finally, partnering with current HPP manufacturers to determine the viability of commercializing low temperature HPP is recommended. A temperature controlled inner fluid chamber to prevent product temperature increase that occurs under higher pressures and holding times is not available in the marketplace today.

FOOD SAFETY PLAN RECOMMENDATIONS

The application of the FDA approved bacteriocin nisin during the slicing of CSS is recommended as an additional hurdle in the control of *L. monocytogenes*. The slicer is the last piece of equipment used on CSS prior to hand packaging and has been identified as a niche for the pathogen. Nisin provides for antilisterial benefit without negatively impacting CSS sensory characteristics. Currently, there is no commercialized post-packaging treatment to effectively eliminate *L. monocytogenes* from CSS while maintaining sensory characteristics. Until additional research is conducted to validate HPP, and effective implementation is achieved, CSS will remain a high-risk food product for *L. monocytogenes (18)*. The CSS industry collectively agrees that the primary cause of contamination is non-compliance to good manufacturing practices (GMPs) and standard sanitation operating procedures (SSOPs) during processing (*43*). Until a post-packaging validated kill step for *L. monocytogenes* in CSS is developed, it is imperative that CSS processors develop, implement and verify a specific pathogen control program for *L. monocytogenes.*

Seafood Hazard Analysis and Critical Control Point (Seafood HACCP) is the FDA mandated prevent control system for seafood products (*17*). The new FDA Hazard Analysis and Risk Based Preventive Controls for Human Food regulations require that facilities have a written environmental monitoring program for RTE foods that are likely to be contaminated with environmental pathogens such as *L. monocytogenes*. Seafood products are exempt due to Seafood HACCP, but processors are still expected to take additional steps to mitigate adulterated products. Several key elements of an effective pathogen control program are: 1) Raw material controls; 2) GMPs and SSOPs; and 3) Environmental monitoring.

Raw material controls. The raw salmon that enters the processing is a potential source for contamination. Raw material suppliers should be a partner with the value-added processor to implement treatments and handling procedures to minimize contamination. Some of these treatments can include chlorinated thaw tanks, treating with calcium hydroxide solution or washing with acidified sodium chlorite solution. Each of these treatments would take place prior to filleting, skinning and subsequent brining/curing.

Good Manufacturing Practices and Sanitation Standard Operating Procedures. For CSS processing facilities, the identification and elimination of reservoirs for pathogen growth is critical for an effective Good Manufacturing Practice (GMP) and Sanitation Standard Operating Procedure (SSOP) program. Both programs should target all food contact surfaces, personnel hygiene and product process flow to prevent cross-contamination. Properly designed equipment that can be effectively cleaned will facilitate pathogen control. Verification of implementation of GMPs and SSOPs for food contact surfaces can be accomplished with routine microbiological testing. Plant personnel are among the most significant vectors for contamination in a processing facility. Sustained training and monitoring of hygiene practices is key in the control program. A one-way product process flow of raw material to finished goods is important in reducing the risk of cross-contamination. This also includes travel paths of personnel, equipment and even air flow.

Environmental monitoring. The objective of environmental monitoring is to verify the effectiveness of the pathogen control program. Risk-based analysis should be used as the framework to assess areas of risk for pathogenic bacteria in the processing facility i.e. environment. The monitoring program is then designed to identify these areas, establish a sampling plan and frequency of testing. In addition, to verifying and identifying the presence and

location of *Listeria* in the processing facility, the monitoring program should also ensure the corrective actions, if implemented, are effective. This means that if found, corrective actions are taken, such as re-cleaning the site or equipment, and re-testing. Analysis of collected data at a specific frequency will aid in identifying trends. This allows for continuous improvement of the pathogen control program.

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