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Potassium Lactate Combined with Sodium Diacetate Can Inhibit Growth of *Listeria monocytogenes* in Vacuum-Packed Cold-Smoked Salmon and Has No Adverse Sensory Effects

BIRTE FONNESBECH VOGEL,[1]* YOKE YIN NG,[1] GRETHE HYLDIG,[1] MONA MOHR,[2] AND LONE GRAM[1]

[1]*Department of Seafood Research, Danish Institute for Fisheries Research, Søltofts Plads, DTU Buliding 221, DK-2800 Kgs. Lyngby, Denmark; and* [2]*Højmarklaboratoriet A/S, Adelvej 11, Højmark, 6940 Lem Street, Denmark*

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ABSTRACT

Growth of *Listeria monocytogenes* in ready-to-eat fish products such as cold-smoked salmon is an important food safety issue. The objective of this study was to evaluate the antilisterial activity of potassium lactate (PL) in combination with sodium acetate (SA) or sodium diacetate (SDA) in cold-smoked salmon and to determine whether these compounds could be incorporated easily into the formulations and technology currently used by processors. A commercial brine injector was used to inject salmon filets with either saturated saline brine or saturated saline brine supplemented with combinations of PL and SA (PURASAL Opti.Form PA 4) or PL and SDA (PURASAL Opti.Form PD 4). In the brine-injected cold-smoked salmon, 2.1% (water phase) PL and 0.12% (water phase) SDA delayed the growth of *L. monocytogenes* for up to 42 days of vacuum-packaged storage at 10°C. Storage at 25°C for 6 h resulted in only a 1-log CFU/g increase in *L. monocytogenes.* Treatments with lower concentrations of PL and SDA or similar concentrations of PL and SA resulted in an extended lag phase and slower growth of *L. monocytogenes.* It was not possible to incorporate more than 2% (water phase) PL while ensuring a minimum of 3% (water phase) NaCl in the finished product because PL decreased the solubility of NaCl. Sensory analyses revealed that the preservatives did not negatively affect flavor or odor. The combination of PL and SDA is therefore a viable technology for preventing *L. monocytogenes* growth on cold-smoked salmon.

Listeria monocytogenes is a foodborne pathogen of major concern because of the severity of listeriosis. Ready-to-eat foods with extended refrigerated shelf lives are possible sources of contamination because this organism may grow to high numbers in these products. Smoked seafood, including cold-smoked salmon, has been categorized as having a high risk of listeriosis per serving *(43).* Processing of cold-smoked salmon includes no recognized critical control point for *L. monocytogenes,* and this product probably cannot be produced completely free of this pathogen *(17).*

The prevalence of *L. monocytogenes* in Danish and U.S. cold-smoked salmon currently is approximately 4% *(16, 48),* but prevalences vary among smokehouses from less than 0.5% to as high as 10%. Contamination of cold-smoked salmon with *L. monocytogenes* occurs primarily during processing, and some subtypes of *L. monocytogenes* can persist for months or years in factories producing cold-smoked fish *(4, 10, 19, 34, 39, 46).* Such persistent types appear to be unique to each facility *(47)*; however, groups of genetically similar *L. monocytogenes* strains frequently dominate and persist in fish slaughter and smoking facilities *(48).*

Current U.S. regulatory policy considers a ready-to-eat seafood product adulterated if *L. monocytogenes* is detected in a 25-g sample. A new European Community regulation on microbiological criteria for foodstuffs became effective on 1 January 2006 *(12)* and differentiates between ready-to-eat foods that can support growth of *L. monocytogenes* and those in which the organism cannot grow (stabilized products). Products are considered stabilized when preservatives that inhibit growth are added or when shelf life is limited to 5 days of storage at 5°C. This regulation is risk based because it acknowledges that listeriosis is caused primarily by ingestion of high numbers of the organism *(43).* *L. monocytogenes* can grow in cold-smoked salmon *(17, 23, 43),* and such products would have to meet the criteria of no *L. monocytogenes* in a 25-g sample or a 5-day shelf life. Alternatively, manufacturers may be able to demonstrate that a *L. monocytogenes* limit of 100 CFU/g is not exceeded during the product shelf life. Some cold-smoked salmon products contain a combination of lactate, phenols, salt, and lactic acid bacteria that control growth of *L. monocytogenes,* but some manufacturers may need to consider addition of food preservatives to stabilize their product.

The addition of live (nonpathogenic and nonspoiling) lactic acid bacteria or pure bacteriocins can inhibit growth of *L. monocytogenes* in cold-smoked fish *(1, 29–31).* Sodium nitrite *(32)* and sodium lactate *(33)* have been tested in cold-smoked fish but cannot ensure complete growth inhibition.

According to Tompkin *(41),* the most widely used compounds for control of *L. monocytogenes* in foods are sodium lactate (SL) and sodium diacetate (SDA), used individually or in combination. SL and potassium lactate (PL) ex-

* Author for correspondence. Tel: +45 45 25 25 64; Fax: +45 45 88 47 74; E-mail: bfv@difres.dk.

TABLE 1. *Chemical characterization of injected cold-smoked salmon brine containing NaCl and a combination of potassium lactate (PL) plus sodium acetate (SA) or sodium diacetate (SDA)*

Treatment	Description	Dry matter (g)	NaCl (%, water phase)	pH	PL (%)		SA or SDA (%)	
					Water phase	Wt/wt	Water phase	Wt/wt
1	Standard NaCl %	38	4.9 ± 0.2	6.21	0.82 ± 0.08	0.51	0.04 ± 0.04	0.02
2	PA 4 2% (target 2% lactate)	39	3.7 ± 0.1	6.20	1.99 ± 0.12	1.24	0.12 ± 0.02	0.07
3	PD 4 2% (target 2% lactate)	37	3.4 ± 0.1	6.05	2.06 ± 0.07	1.32	0.12 ± 0.03	0.08
4	PD 4 1.5% (target 1.5% lactate)	40	4.3 ± 0.1	6.21	1.90 ± 0.18	1.16	0.09 ± 0.02	0.05
5[a]	Reduced NaCl %	36	3.8 ± 0.1	6.17	0.80 ± 0.05	0.51	0.00 ± 0.01	0.00
6	PA 4 2.5% (target 2.5% lactate)	37	2.7 ± 0.1	6.16	2.27 ± 0.23	1.46	ND[b]	ND

[a] Control for treatments 2 and 3.
[b] Not determined.

tend shelf life and increase the safety of meat and poultry products *(3)*. SDA also is used in foods as a flavoring and antimicrobial agent *(25)*. The antilisterial activity of SL or PL in combination with SDA in a dipping solution *(5, 13, 15, 24, 35)* or as part of the formulation *(5, 27, 28, 37, 38)* has been investigated in several meat products. When used in the formulation either alone or together, 2 to 3% SL and 0.125 to 0.25% SDA can control the growth of *L. monocytogenes* in cooked vacuum-packed meats *(5, 27, 28, 37, 38)*. The combination of PL and SDA absorbed into slices of cold-smoked salmon inhibited the growth of *L. monocytogenes* Scott A *(49)*.

The purpose of the present study was to evaluate the antilisterial activity of PL, sodium acetate (SA), and SDA against *L. monocytogenes* in cold-smoked salmon and to determine whether these compounds could be easily incorporated into the formulations and technologies currently used by processors. Few published studies have included evaluations of the sensory effects of effective treatments on the products tested. In the present study, we found that treatments that effectively inhibited growth of *L. monocytogenes* did not have adverse sensory effects.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *L. monocytogenes* strains La22 *(47)* and V518a *(46)* isolated from cold-smoked salmon were grown in brain heart infusion (BHI) at 25°C for 24 h and reinoculated into BHI containing 3% NaCl and incubated at 15°C for 24 h. The cultures were diluted to appropriate *L. monocytogenes* concentrations in sterile peptone saline, typically 10^4 CFU/ml. These dilutions were used to inoculate all model systems. *L. monocytogenes* counts were made by spread plating cultures on Palcam agar (PALCAM agar base, CM0877, Oxoid Ltd., Basingstoke, UK) with PALCAM selective supplement (SR150, Oxoid).

Cold-smoked salmon juice supplemented with SA, SDA, and/or PL. Salmon juice was prepared according to the method of Nilsson et al. *(29)*. Cold-smoked salmon was boiled in tap water for 10 min at a ratio of 2:1 (wt/vol). The suspension was filtered, and the salt concentration was adjusted to 4% (wt/vol). The juice was buffered with K_2HPO_4 (Merck, Darmstadt, Germany), and the pH was adjusted to 6.2 with 2 N HCl. The juice was heat treated by boiling for 30 min, divided into 20-ml aliquots in 50-ml sterile flasks, and then supplemented with SA (01-148, Macco Organques Inc., Valleyfield, Quebec, Canada), SDA (SDR,0100, Jungbunzlauer, Ladenburg, Germany), PL (Purasal P/HiPure 60, PURAC Biochem, Gorinchem, The Netherlands), PL in combination with SA at a ratio of 14:1 (56% PL and 4% SA; PURASAL Opti.Form PA 4, PURAC Biochem), or PL in combination with SDA at a ratio of 14:1 (56% PL and 4% SDA; PURASAL Opti.Form PD 4, PURAC Biochem). Flasks were inoculated with *L. monocytogenes* at 10^3 CFU/ml and stored at 10°C. *L. monocytogenes* counts were obtained after 0, 2, 5, 8, 12, 16, 21, and 28 days by surface plating on Palcam agar (Oxoid CM0877 and SR150) and incubation for 2 days at 37°C. All experiments were carried out in duplicate. During the experiments, temperatures were recorded with data loggers (Tinytag, Gemini Data Loggers Ltd., Chichester, UK).

Cold-smoked salmon homogenate supplemented with combinations of PL plus SA or SDA. Cold-smoked salmon homogenate was prepared by homogenizing (Mini Quick 6720, OBH Nordica, Taastrup, Denmark) 150 g of cold-smoked salmon for 1 min (four times for 15 s each) with 15 ml of a sterile 4% NaCl (wt/vol) solution and combinations of PL (56%) plus SA (4%) or PL (56%) plus SDA (4%). The cold-smoked salmon homogenate was inoculated by mixing 5 ml of a 10^5 CFU/ml suspension of *L. monocytogenes* into 150 g of cold-smoked salmon homogenate for 1 min (four times for 15 s each). Five-gram portions of the fish homogenate were distributed in plastic bags, vacuum sealed (Multivac model A300/16, Sepp. Haggenmuller GmbH & Co., Wolfertschwenden, Germany), and stored at 10°C. All model systems were stored vacuum packed at 10°C, and bacteria were enumerated after 0, 2, 6, 13, 16, 21, and 28 days. All experiments were carried out in duplicate. During the experiments, temperatures were recorded with data loggers.

Cold-smoked salmon brine injected with combinations of PL and SA or SDA. One batch of iced ocean-raised gutted salmon (*Salmo salar*) from Norway was head cut and filleted by commercial machines in one flow. Using a commercial brine injector, fillets were injected with either saturated saline brine or saturated saline brine supplemented with combinations of PL and SA (PA 4) or PL and SDA (PD 4). The brine used for dissolving PL-SA or PL-SDA had a lower NaCl concentration than the pure NaCl brine, and fillets were therefore also salted with brine at this lower concentration (Table 1). After cold smoking, the fillets were quick frozen and stored at −30°C.

Commercially prepared salmon was used for two types of challenge experiments in which the product was inoculated with *L. monocytogenes* cultures either by (i) homogenizing the fish with the bacterial culture or (ii) adding the culture between slices of salmon.

To homogenize the fish with the bacterial culture, 150 g of brine-injected cold-smoked salmon was inoculated with *L. monocytogenes* by mixing for 1 min (four times for 15 s each) with a 15-ml suspension of *L. monocytogenes* supplemented with 15 ml of a sterile 4% NaCl (wt/vol) solution and combinations of PL and SA (PA 4) or PL and SDA (PD 4) to the same concentration as targeted for the different treatments. Five-gram portions of the fish homogenate were placed in plastic bags, vacuum sealed, and stored at 10°C. Control samples of each treatment were similarly packaged without inoculation. All experiments were carried out in duplicate.

L. monocytogenes culture was added between two salmon slices to simulate contamination of commercially packaged cold-smoked salmon. Two cold-smoked salmon slices were weighed aseptically (5 g each). One of the slices was surface inoculated with 0.1 ml of *L. monocytogenes* suspension at multiple points with a pipette to yield a concentration of 10^2 CFU per package, and the other slice was placed on top of the inoculated slice. Packages were vacuum sealed and stored at 10°C. Three inoculated packages per treatment were assayed at each sampling time. During the experiments, temperatures were recorded with data loggers.

Chemical analyses of brine-injected cold-smoked salmon. For each of the six treatments, 100 g from one fillet was cut from the head, middle, and tail areas, respectively, and used for chemical analyses. One gram of fish was mixed with 9 ml of saline, and the pH was measured with a pH meter equipped with a combined glass electrode (PHM95 pH/ion meter, Radiometer, Copenhagen, Denmark). Salt concentrations were determined potentiometrically using AOAC method 976.18 (salt [chlorine as sodium chloride] in seafood, the potentiometric method) in combination with method no. 937.07 (fish and marine products, treatment and preparation of sample procedure) and method no. 971.27 (sodium chloride in canned vegetables, potentiometric method) *(2)*. Concentrations of acetate, diacetate, and lactate were determined using a previously described high-performance liquid chromatography method *(9)*. External standards were used for identification and quantification of the compounds.

Sensory analyses of brine-injected cold-smoked salmon. Cold-smoked salmon (Table 1) was analyzed by sensory profiling using a trained panel of nine assessors in sessions that included three samples with two replicates. The assessors were all selected, tested, and specifically trained in descriptive analysis (ISO 11035 *(21)*) of smoked salmon. A sensory vocabulary was developed using samples of smoked salmon from the same smokehouse from which the experimental samples were obtained. In three sessions, the assessors were trained to evaluate the sensory attributes on an unstructured 15-cm linear scale with anchor points. The sensory attributes and the definition are listed in Table 2. The evaluations were performed in separate booths under normal daylight and at ambient temperature according to ISO 8589 *(22)*. The assessors used water and flat bread to cleanse the palate between samples. Data were collected with a computer system (FIZZ Network version 2.0, Biosystems, Couternon, France).

Cold-smoked salmon was thawed at 2°C for 24 h and stored 0 or 21 days at 5°C. After cutting the tail portion from the smoked filet, the bones and skin were removed before the sample was mechanically minced (OLIO DENSO 41, 51-104, Maskinfabrikken Strømmen, Randers, Denmark) and mixed (model N-50G, series 14048901, Hobart Corp., Troy, Ohio). Portions (25 g) were divided into porcelain jars, coded with three random digit numbers, and then served in random order to the assessors.

The sensory profile data were corrected for the level effect (i.e., assessors using different parts of the linear scale) as described by Thybo and Martens *(40)*. The corrected value is the original value minus the mean of all replicates and products for the given assessor (i.e., the residuals). Sensory properties were linked to assessors and trials by partial least squares regression (PLSR) using The Unscrambler (version 7.6 SR-1, Camo ASA, Oslo, Norway) *(26)*. Both discriminant-PLSR and ANOVA-PLSR models were calculated, making it possible to determine significant variables in both sensory data (*x*) and process variables (*y*).

TABLE 2. *Sensory attributes and definitions used when assessing the sensory profile of minced smoked salmon*

Sensory attributes	Definition
Odor	
Smokiness	Intensity of smoky odor
Sourish	Acidic, acetic acid, citric acid
Sweet	Sucrose-like
Fat	Fresh fish oil, unripe hazelnut
Sour	Sour dishcloth
Flavor	
Smokiness	Intensity of smoky flavor
Oily	Fresh fish oil, unripe hazelnut
Salty	Typical taste of salt
Sweet	Sucrose-like
Sourish	Sourish-like in fruit
Bitter	Quinine- or caffeine-like
Metallic	Warm metal, blood
Texture	
Oiliness	Degree to which oil is perceived after chewing

Microbiological analyses of cold-smoked salmon homogenates or slices. At appropriate time intervals, two or three packages of each batch of minced (5 g) or sliced (10 g) cold-smoked salmon were taken for microbiological analyses. Each sample was diluted 10-fold in physiological peptone saline (PPS) and homogenized in a stomacher (model BA 7021, Struers Kebo Lab A7S, Roskildevej, Denmark) for 1 min. Additional serial dilutions were made in PPS. *L. monocytogenes* counts were obtained after surface plating on Palcam agar (Oxoid CM0877 and SR150) and incubation for 2 days of at 37°C. Aerobic colony counts were obtained after plating on Long and Hammer (L&H) agar *(44)* and incubation for 7 days at 15°C. To determine bacterial populations in the 10-fold dilutions of salmon, 0.5 ml was surface plated on two plates of Palcam or L&H agar. Numbers of lactic acid bacteria were determined after pour plating in nitrite polymyxin agar (NAP-agar 265430, Difco, Becton Dickinson, Sparks, Md.) supplemented with natrium-nitrite (231559-9, Merck), cycloheximide (C 7698, BDH, Poole, UK), and polymyxin B sulfate (p-1004, Sigma-Aldrich, St. Louis, Mo.), according to the method of Davidson and Cronin *(11)*, and incubation for 3 days at 25°C.

RESULTS

Effect of SA, SDA, and/or PL on growth of *L. monocytogenes* in cold-smoked salmon juice. Salmon juice without PL, SA, or SDA supported rapid growth of *L. monocytogenes* to populations exceeding 8 log CFU/ml after 8 days of storage at 10°C (Fig. 1). Incorporation of organic acid salts into cold-smoked salmon juice, either individually or in combination, resulted in different levels of inhibition

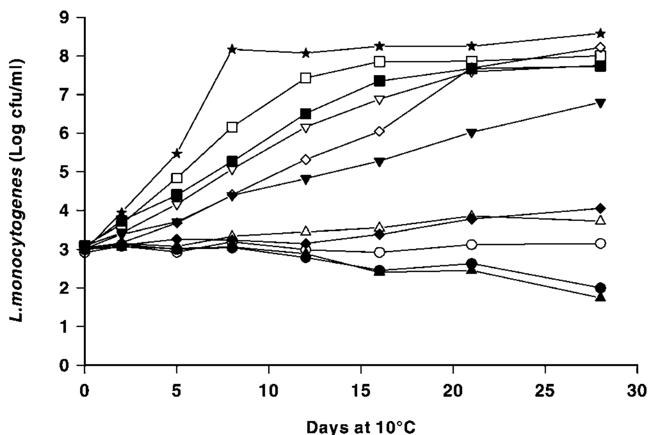

FIGURE 1. *Growth of* Listeria monocytogenes *(strain La22) at 10°C in cold-smoked salmon juice (★) supplemented with 0.14% sodium acetate (SA) (□), 0.21% SA (■), 0.14% sodium diacetate (SDA) (▽), 0.21% SDA (▼), 2% potassium lactate (PL) (◇), 3% PL (◆), and combinations of 2% PL plus 0.14% SA (△), 3% PL plus 0.21% SA (▲), 2% PL plus 0.14% SDA (○), and 3% PL plus 0.21% SDA (●). Points are averages of two determinations, and the percentage is given in the water phase.*

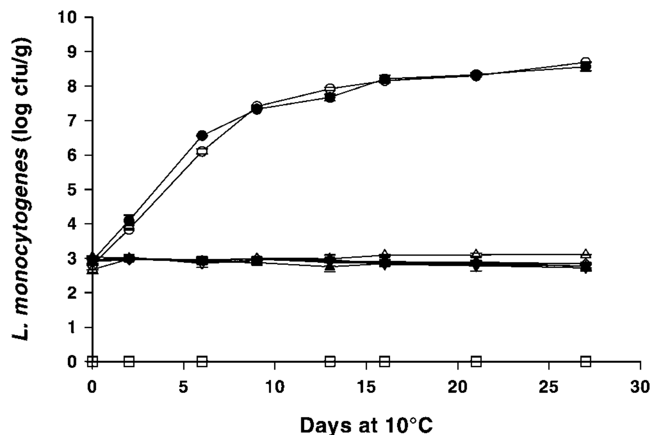

FIGURE 2. *Growth of* Listeria monocytogenes *(strains La 22 or V518a) in minced cold-smoked salmon supplemented with different combinations of potassium lactate (PL) and sodium acetate (SA) or PL and sodium diacetate (SDA): uninoculated control (□); control inoculated with strain La22 (○), supplemented with 2% PL and 0.14% SDA and inoculated with strain La22 (△), supplemented with 2.5% PL and 0.18% SDA and inoculated with strain La22 (▽), supplemented with 3% PL and 0.21% SDA and inoculated with strain La22 (▼), and supplemented with 2% PL and 0.14% SA and inoculated with strain La22 (◇); control inoculated with strain V518a (●) and supplemented with 2% PL and 0.14% SDA and inoculated with strain V518a (▲). Samples were stored vacuum packed at 10°C. Points are averages of two determinations, and error bars are standard deviations. PL, SA, and SDA percentages are given in the water phase.*

of *L. monocytogenes* (Fig. 1). The most effective treatments were 2.0% PL plus 0.14% SDA, 3.0% PL plus 0.21% SDA, and 3.0% PL plus 0.21% SA, which completely inhibited growth of *L. monocytogenes* during 28 days of storage at 10°C, followed by 2.0% PL plus 0.14% SA or PA 4 and 3% PL. Inhibition using either 0.14 or 0.21% SA or SDA alone or 2% PL was not as pronounced, with *L. monocytogenes* growth exceeding 8 log CFU/ml after 12 to 21 days of storage at 10°C. The pH was approximately 6.0 in all salmon juice samples containing added SDA and was 6.1 in all other samples.

Effect of SA, SDA, and/or PL on growth of *L. monocytogenes* in cold-smoked salmon homogenate. Figure 2 illustrates that 2, 2.5, and 3% (water phase [wp]) PD 4 and 2% (wp) PA 4 prevented growth of *L. monocytogenes* for up to 27 days of storage at 10°C, whereas control samples without PD 4 or PA 4 supported *L. monocytogenes* growth, with populations exceeding 7 log CFU/g after approximately 8 days of storage at 10°C. No differences in growth or inhibition were seen between *L. monocytogenes* strains La22 and V518a, which both originated from fish smokehouses but belong to different serogroups and lineages *(45)*. All samples supported growth of lactic acid bacteria, with growth only slightly affected by supplementation with PD 4 (results not shown).

Chemical analyses of brine-injected cold-smoked salmon. The pH ranged between 6.1 for treatment 3 (targeted 2.1% PL and 0.12% SDA) and 6.2 for treatment 1 (standard NaCl percentage) (Table 1). The natural content of lactate was approximately 0.8% (wt/wt) in treatments 1 and 5 in which only NaCl was used in the brine solution. In treatments 2 (target 2.0% PL and 0.14% SA) and 6 (target 2.5% PL and 0.18% SA), 2.0 ± 0.1% PL + 0.12 ± 0.02% SA and 2.3 ± 0.2% PL and no SA were detected, respectively. In treatments 3 (target 2.0% PL and 0.14% SDA) and 4 (target 1.5% PL + 0.11% SDA), 2.1 ± 0.1% PL + 0.12 ± 0.03% SDA and 1.9 ± 0.2% PL + 0.09 ± 0.02% SDA were detected, respectively. The concentration of NaCl in the water phase for all treatments ranged between 2.7 and 4.9% (Table 1). PL plus SDA or PL plus SA decreased the solubility of NaCl, and the concentration of NaCl in the mixed brine was lower than that in the normal brine. The concentration of NaCl was only 2.7% when the brine contained 2.3% PL. The highest concentration of preservative in combination with the minimum 3% NaCl was found in treatments 2 and 3, where approximately 2% PL and 0.12% SA or SDA were found in combination with 3.7 and 3.4% NaCl, respectively.

Sensory analyses of brine-injected cold-smoked salmon. Sensory properties of the product were not significantly influenced by the addition of PL plus SDA or PL plus SA, and only minor differences in the sensory profile were found among the six treatments (Fig. 3). Treatment 3 had a less intense oily flavor that might be caused by differences in lipid content and not by the preservatives in the brine. The lipid content of salmon ranged from 11.6 to 18.4% (wt/wt) (results not shown). After 21 days of storage, these differences were even smaller (Fig. 3b), and no off-flavors were detected in the smoked samples. Consumers most likely would not notice such small differences.

Challenge testing of brine-injected cold-smoked salmon. Counts of indigenous bacteria at the beginning of storage were less than 10 CFU/g for all uninoculated samples (results not shown), and no *L. monocytogenes* was de-

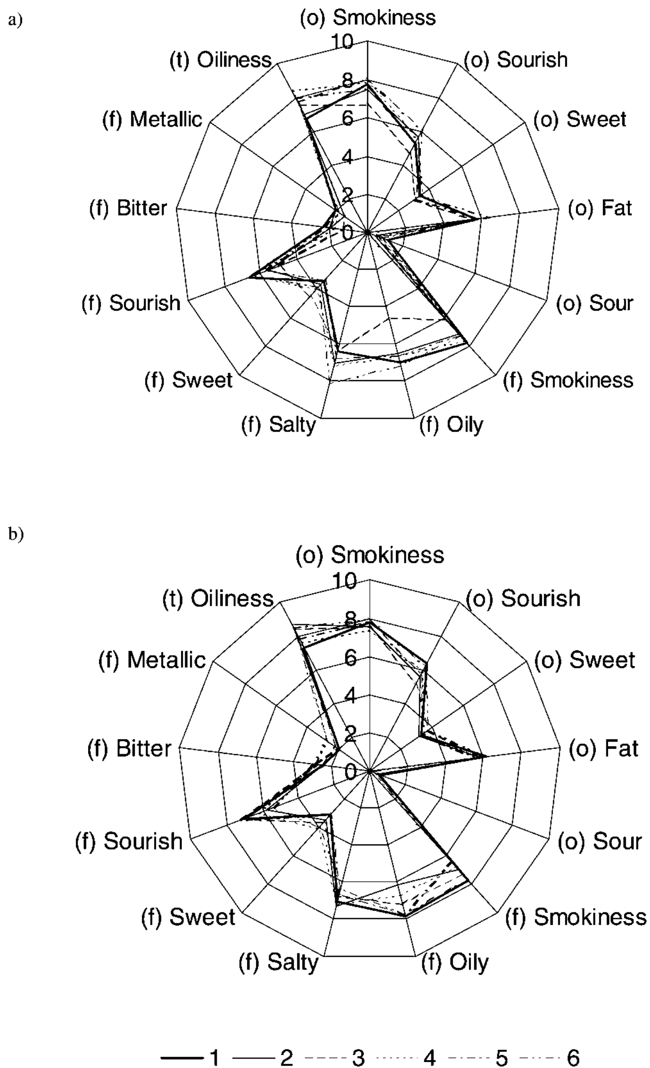

FIGURE 3. *The sensory attributes for odor (o), flavor (f), and texture of the six treatments of cold-smoked salmon stored for 0 days (a) and 21 days (b) at 5°C. Treatment 1, standard NaCl percentage; treatment 2, PA 4 2% (target 2% lactate); treatment 3, PD 4 2% (target 2% lactate); treatment 4, PD 4 1.5% (target 1.5% lactate); treatment 5, reduced NaCl percentage; treatment 6, PA 4 2.5% (target 2.5% lactate).*

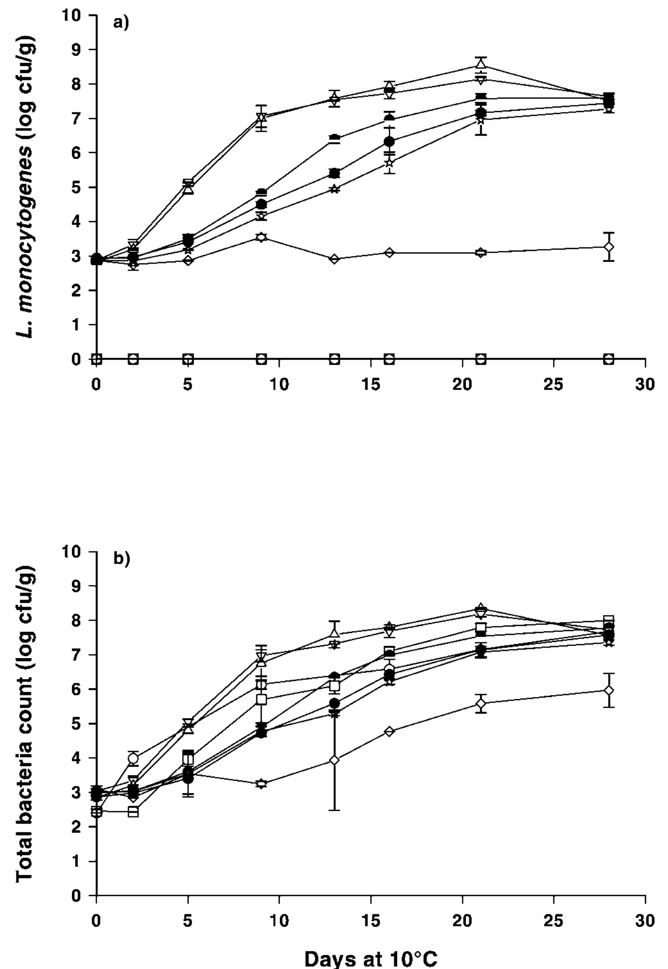

FIGURE 4. *Growth of* Listeria monocytogenes *(strain La22) (a) and total bacteria count (b) in minced cold-smoked salmon. The salmon were brine injected with NaCl and a combination of potassium lactate (PL) and sodium acetate (SA) or sodium diacetate (SDA). Uninoculated control of standard NaCl percentage (□); inoculated control of standard NaCl percentage (△); uninoculated control of reduced NaCl percentage (○); inoculated control of reduced NaCl percentage (▽), supplemented with 1.9% PL and 0.09% SDA (◓), supplemented with 2.1% PL and 0.12% SDA (◇), supplemented with 2.0% PL and 0.12% SA (★), and supplemented with 2.3% PL and SDA, but SDA content was not determined (●). Samples were vacuum packed and stored at 10°C. Points are averages of two determinations, and error bars are standard deviations. PL, SA, and SDA percentages are given in the water phase.*

tected. The initial *L. monocytogenes* population on inoculated cold-smoked salmon was approximately 10^3 CFU/g for all samples. The combination of 2.1% PL plus 0.12% SDA prevented growth of *L. monocytogenes* in minced fish at 10°C. In contrast, combining SA at the same concentration (2.0% PL + 0.12% SA) or a slightly higher concentration (2.3% PL + undetermined SA) resulted in slow growth (Fig. 4a). Lower concentrations of PL (1.9%) and SDA (0.09%) did not prevent growth of *L. monocytogenes* in the inoculated samples but resulted in slightly slower growth than that observed in the control samples. Growth in fish with 4.9% NaCl processed as the standard NaCl percentage was equal to growth in salted fish processed with reduced NaCl (3.8% NaCl), with *L. monocytogenes* exceeding 7.0 log CFU/g after 9 days of vacuum-packaged storage at 10°C (Fig. 4a).

Addition of antimicrobials to the formulation of brine injected into the cold-smoked salmon did not significantly increase the total aerobic bacterial count compared with that of the untreated control (Fig. 4b). Delayed growth of aerobic bacteria seen in samples supplemented with 2.1% PL + 0.12% SDA and other samples injected with PL plus SDA or PL plus SA was probably caused by the lower numbers of *L. monocytogenes* in the samples. Growth of lactic acid bacteria, which are the dominant microflora of cold-smoked salmon, was only slightly affected by incorporation of preservatives (results not shown).

To determine whether the addition of PL plus SDA or PL plus SA to cold-smoked salmon products could effectively limit the growth of *L. monocytogenes* during tem-

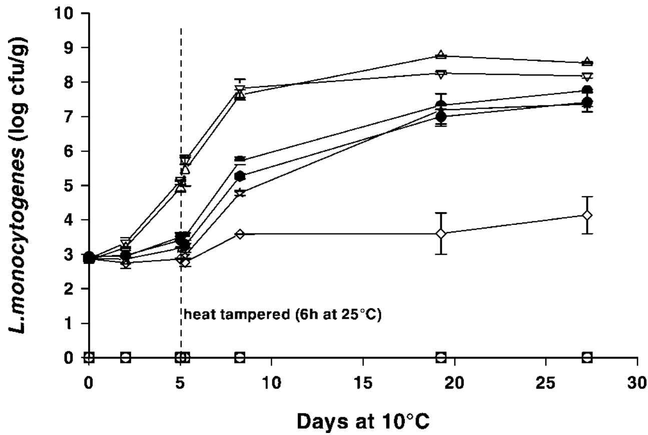

FIGURE 5. *Growth of* Listeria monocytogenes *(strain La22) inoculated in minced cold-smoked salmon. The salmon were brine injected with NaCl and a combination of potassium lactate (PL) and sodium acetate (SA) or sodium diacetate (SDA). Uninoculated control of standard NaCl percentage (□); inoculated control of standard NaCl percentage (△); uninoculated control of reduced NaCl percentage (○); inoculated control of reduced NaCl percentage (▽), supplemented with 1.9% PL and 0.09% SDA (◓), supplemented with 2.1% PL and 0.12% SDA (◇), supplemented with 2.0% PL and 0.12% SA (★), and supplemented with 2.3% PL and SDA, but SDA content was not determined (●). Cold-smoked fish were vacuum packed and stored at 10°C before and after temperature abuse at 25°C for 6 h. Points are averages of two determinations, and error bars are standard deviations. PL, SA, and SDA percentages are given in the water phase.*

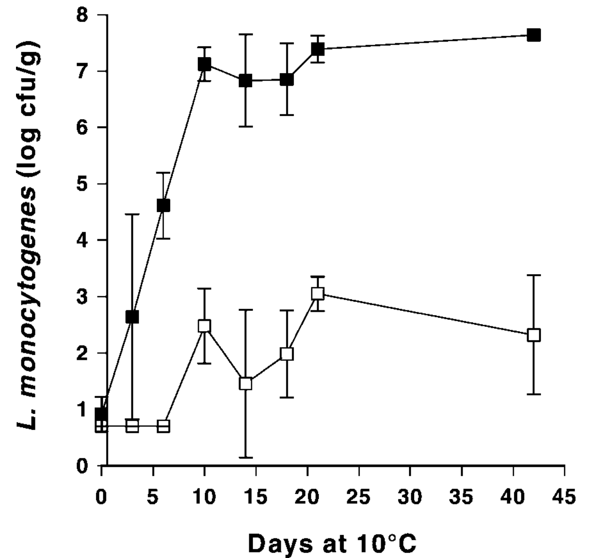

FIGURE 6. *Growth of* Listeria monocytogenes *(strain La22) inoculated between two slices of cold-smoked salmon. The salmon was brine injected with a reduced NaCl percentage (■) or with NaCl and a combination of potassium lactate (PL) and sodium diacetate (SDA) (□). The content in the product was 2.1% PL and 0.12% SDA. Cold-smoked fish were sliced, vacuum packed, and stored at 10°C. Points are averages of three determinations, and error bars are standard deviations. PL and SDA percentages are given in the water phase.*

perature abuse, samples were left at 25°C for 6 and 24 h after 5 and 13 days of storage, respectively. Holding at 25°C for 6 h after 5 days of storage at 10°C resulted in a 2-log increase in *L. monocytogenes* after 8 days for all samples except that containing 2.1% PL + 0.12% SDA (Fig 5). In this sample, temperature abuse resulted in only a minor increase in numbers of *L. monocytogenes.* Temperature abuse at 25°C for 6 h after 13 days of storage resulted in growth similar to that seen after 5 days of storage, whereas heat tempering at 25°C for 24 h after both 5 and 13 days of storage resulted in an increase of approximately 3 log CFU/g for all samples except those from treatment 3 (2.1% PL + 0.12% SDA) (results not shown). For treatment 3 samples, *L. monocytogenes* increased approximately 1 log CFU/g. Samples that were left at 25°C for 24 h were clearly spoiled and exhibited off-odors.

A lower inoculum concentration (initial population of *L. monocytogenes* on inoculated cold-smoked salmon was approximately 10 CFU/g for all samples) and inoculation between two slices of cold-smoked salmon (rather than homogenized product) were also evaluated to imitate contamination from equipment surfaces during processing. As expected, control samples supported *L. monocytogenes* growth with populations exceeding 7 log CFU/g after 10 to 20 days of storage at 10°C (Fig. 6). However, the combination of 2.1% PL plus 0.12% SDA (treatment 3) delayed growth of *L. monocytogenes* for up to 42 days.

DISCUSSION

In this study, we demonstrated that brine injection resulting in a combination of 2.1% (wp) PL plus 0.12% (wp) SDA (Figs. 4a and 6) can inhibit growth of *L. monocytogenes* in vacuum-packed cold-smoked salmon. Lower concentrations of PL and SDA or similar additions of PL plus SA resulted in an extended lag phase and in slower growth than that in nontreated fish (Fig. 4a). These findings are similar to those for pork bologna *(6)* or frankfurters *(5, 38),* in which *L. monocytogenes* was inhibited by similar combinations and concentrations of the two preservatives. In many studies, SL or PL in combination with SDA have been assessed as a postprocessing treatment for commercial bologna and ham slices *(13),* frankfurters *(5, 24),* and beef franks *(42).* In one study, PL and SDA were adsorbed to slices of cold-smoked salmon *(49).* Several of these treatments inhibited *L. monocytogenes*; however, the adsorption method of addition is not practical during fish processing in many smokehouses.

L. monocytogenes was completely inhibited in cold-smoked salmon homogenate and juice by a combination of 2% (wp) PL plus 0.14% (wp) SDA (Figs. 1 and 2). The increased antilisterial activity of SDA may be attributed to the compound itself, to its synergistic effect with PL, or both but not to pH per se. The addition of 3% (wp) PL did not completely inhibit *L. monocytogenes,* as previously reported *(42).* In agreement with other studies, combinations of PL and SDA exhibited greater antilisterial activity than either compound used alone. Schlyter et al. *(36)* suggested that antilisterial activity of SL was synergistically enhanced by the addition of SDA in turkey slurries, and a synergistic effect of SL and SDA was also reported for wieners and cooked bratwurst *(15).*

L. monocytogenes survived in cold-smoked salmon

during refrigerated storage even in the presence of the organic acid additives, in agreement with other studies *(5, 24, 49).* The pathogen grew in cold-smoked salmon with 3.8 or 4.9% (wp) NaCl, as previously reported *(8, 17, 23, 29).* Addition of 2.1% PL and 0.12% SDA decreased the pH from 6.2 to 6.1, but this marginal pH reduction alone cannot explain the antilisterial effect. The mechanism of antimicrobial activity and the specific mode of action on the cell of the combination of PL and SDA are not yet known. However, by using combinations of lactates with SDA three additional hurdles are incorporated into food processing. The combination lowers the water activity of the product and adds two antimicrobial ingredients: lactic acid and acetic acid *(38).*

In our study, the preservatives were incorporated into cold-smoked salmon by addition to the brine solution used for injecting the salmon before smoking. Addition of PL and SDA to the brine decreased the solubility of NaCl, and it is a challenge to ensure a minimum NaCl content of 3% (wp) in the finished product while incorporating as much preservative as possible. Three percent NaCl is the target concentration to prevent growth and toxin production by *Clostridium botulinum* in this product *(20).* However, it is not known whether growth of *C. botulinum* is inhibited by PL and SDA. The lactate (0.8% wp) detected in the untreated salmon was probably present at the time of slaughter; the natural lactate content of salmon is 3,700 to 15,400 ppm *(14, 18).* It may be possible to incorporate a higher concentration of preservatives into cold-smoked salmon, but it would require a combination of injection with brine containing preservatives and a dry salting step.

The preservatives in the brine-injected cold-smoked salmon did not adversely affect flavor or odor and did not introduce off-flavors (Fig. 3). One treatment produced a lower intensity oily flavor, which probably was caused by differences in the lipid content and not by the preservatives in the brine. This study is the first to assess the sensory effects of these preservatives in smoked fish, and our data are in agreement with those from similar studies on meat products, in which addition of SL and SA to servelat sausage and cooked ham *(7)* and SL and SDA to frankfurters *(5)* had no adverse sensory effects.

Cold-smoked salmon must be stored at 5°C in Denmark; however, trials were carried out at 10°C to simulate abusive temperatures. PL and SDA are probably more effective at 3 to 5°C. Yoon et al. *(49)* found that all tested concentrations of PL and SDA completely inhibited the growth of *L. monocytogenes* on salmon stored at 4°C for 32 days. This finding is consistent with those of challenge trials in meat products in which *L. monocytogenes* grew faster at 10°C than at 4°C *(5, 6).* In the present study, we included temperature abuse intervals to simulate placing of the product on a buffet. This abuse caused only a minor increase in *L. monocytogenes,* and growth ceased during subsequent refrigerated storage. To obtain a more stable product, it may be possible to supplement the brining solution with an even higher concentration of SDA, to about 0.24%. In other studies, 1.8% SL plus 0.25% SDA significantly reduced *L. monocytogenes* growth in comparison with 1.8% SL plus 0.125% SDA *(5, 6, 27).*

Trends in counts on L&H agar plates were similar to those on Palcam plates for all treatments, especially during the first days of storage, because most colonies found on L&H were *L. monocytogenes.* The higher counts obtained on L&H plates during the last days of storage may reflect growth of spoilage bacteria (Fig. 4b). Lactic acid bacteria, which predominate in cold-smoked salmon, were only slightly affected by the incorporation of preservatives into the product, in concordance with other studies *(5).* Therefore, incorporating PL and SDA into cold-smoked salmon may not result in extended shelf life, and longer storage and additional time for *L. monocytogenes* to grow may not be allowable.

The treatments in the present study did not reduce the initial population of *L. monocytogenes*; numbers remained stable during storage, even at abuse temperatures. Most studies such as ours have been carried out as challenge trials in which *L. monocytogenes* has been inoculated onto the product. As demonstrated by Dalgaard and Jørgensen *(8),* growth in naturally contaminated products may be significantly slower and the effect of preservatives may be more pronounced. In our challenge trials, variation between triplicate samples was larger when *L. monocytogenes* was inoculated between two slices of cold-smoked salmon (Fig. 6) than when the pathogen was mixed into the fish homogenate (Fig. 4a). Although care was taken to inoculate the slices evenly, differences in the microenvironment of the slices may influence growth. On-going trials have indicated that this variation may depend on the inoculum volume (unpublished data).

Cold-smoked salmon may vary considerably in NaCl percentage, pH, lactate, and smoke components, and all of these factors affect the growth of *L. monocytogenes.* Therefore, concentrations and conditions for lactate and diacetate use must be refined and validated for each type of cold-smoked salmon product and processing condition. Several other factors must be investigated to obtain a more complete view of the effectiveness of such treatments, including the antimicrobial effects of treatments on the indigenous microbial (nonpathogenic) flora of the product, which may affect product quality. The effectiveness of treatments also must be investigated under potential conditions of temperature abuse during distribution, retail, or consumer storage.

The data presented here could serve as a guide to the industry for deriving and validating their own formulations to fit their product specifications and expectations and to meet the requirements of new regulations.

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