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Inactivation of *Listeria monocytogenes* on Hot-smoked Salmon by the Interaction of Heat and Smoke or Liquid Smoke†

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ABSTRACT

L. monocytogenes was inoculated onto the surface of brined salmon steaks and heat processed in a commercial smokehouse to simulate a hot process for preparing smoked fish. The minimum temperature required for inactivation of *L. monocytogenes* was 153°F (67.2°C) when generated smoke was applied throughout the entire process. When generated smoke was added only during the last half of the process, *L. monocytogenes* was recovered from steaks heated to temperatures as high as 176°F (80.0°C). When smoke was not applied during the process, *L. monocytogenes* survived on steaks heated to internal temperatures between 131°F and 181°F (55.0 to 82.8°C) but was not isolated from steaks heated above 181°F (82.8°C). When liquid smoke CharSol C-10 was applied as a full-strength (100%) dip before processing, *L. monocytogenes* was inactivated in samples processed at temperatures as low as 138°F (58.9°C). When liquid smoke 10-Poly or CharSol C-10 was applied at a concentration of 50%, the lethal temperature was increased to the range of 145 to 150°F (62.8 to 65.6°C). With further dilution of C-10 to 25% and 10% the inactivation temperatures increased to 156°F (68.9°C) and 163°F (72.8°C). A full-strength dip of CharOil, the oil-soluble fraction of CharSol C-10, was less effective, and *L. monocytogenes* survived in salmon steaks processed to an internal temperature of 166°F (74.4°C), the highest temperature tested with this liquid smoke. This study provides evidence that heat alone is not reliable for inactivation of *L. monocytogenes* during the hot-smoking process. The proper stage and duration of smoke application or proper composition and concentration of liquid smoke in combination with heat are critical for inactivation of the organism.

Key words: *Listeria monocytogenes*, heat inactivation, smoke, liquid smoke

Listeria monocytogenes has caused a number of foodborne listeriosis outbreaks *(15, 35)*. It has also been frequently isolated from raw and ready-to-eat vegetables, meat, poultry, seafood, and dairy products *(7, 11, 13, 20, 38)*. Based on the ability of *L. monocytogenes* to grow at refrigeration temperatures *(22, 33, 34)*, the high mortality rate in the different outbreaks, and the lack of knowledge of the infectious dose for immunocompromised individuals and pregnant women, the Food and Drug Administration, the U.S. Department of Agriculture, and the Centers for Disease Control have agreed on a zero tolerance for *L. monocytogenes* in foods that do not receive further heat treatment before consumption *(28)*. For processors of ready-to-eat seafood, it is therefore most important to understand the growth characteristics of the bacterium and the processing conditions necessary for its inactivation in different product forms.

The thermal tolerance of *L. monocytogenes* in meat and poultry products has been reported *(18, 37)*. These studies show that the heat resistance of the organism is related to the composition of the product *(2, 16, 19, 36)*. For example, a processing temperature of 170.6°F (77°C) for 16 s is adequate for pasteurization of dairy products *(17)*, but the organism has been shown to survive an internal temperature of 172.4 to 185°F (78 to 85°C) for 15 min in grilled meatballs *(23)* and internal temperatures of 165 to 180°F (73.9 to 82.2°C) in poultry products *(4, 5)*.

To date, there have been no indications that *L. monocytogenes* survives the hot-smoked process and when *L. monocytogenes* is isolated from hot-smoked fish, it is generally assumed that the source of the bacterium is from post-process contamination *(21)*. Previous studies on survival of *L. monocytogenes* in hot-smoked fish, however, have not focused on defining the time and temperature or heat and smoke requirements to inactivate the organism. The purpose of the current study, therefore, was to determine the interaction of processing temperature and generated smoke or liquid smoke and to define the conditions necessary to inactivate *L. monocytogenes* inoculated onto the surfaces of salmon steaks during the hot-smoking process.

MATERIALS AND METHODS

Listeria *strains and inocula*

L. monocytogenes strains 4-121 and 1455, isolated at our laboratory from cold-smoked salmon, were used in all experiments.

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† Reference to a trade name does not constitute endorsement by the U.S. Department of Commerce over other similar products.

Cultures were grown in Trypticase soy broth (BBL 11771; Becton-Dickinson, Cockeysville, MD) with 0.6% yeast extract (Difco Laboratories, Detroit, MI) (TSBYE) for 24 h. Cultures were adjusted to a final absorbance of 0.30 at 525 nm as measured on a Spectronic 20 spectrophotometer (Bausch & Lomb). This resulted in a cell density of approximately 4×10^8 colony-forming units (CFU) per milliliter. These cells were diluted in sterile 0.1% peptone water so that 0.1 ml contained 330 to 350 cells, the desired inoculum per sample. This represented a level of 10 to 100 times that found in raw products *(11)*. Counts were verified by plating on Trypticase soy agar (BBL 11046) with 0.6% yeast extract (TSAYE) and 0.05% glucose.

Fish and brining

Eviscerated and frozen chum salmon (*Oncorhynchus keta*) were obtained from a local processor. Each fish was cut into steaks 1.25 in. (32 mm) thick weighing between 170 and 230 g each, vacuum-packaged in oxygen-impermeable bags (2-mil [50-μm] polyester, Kapak Corporation, Bloomington, MN) and stored at −15°F (−26.1°C).

Before brining, the steaks were thawed in plastic bags submerged in cold running water maintained at 60°F (15.6°C). The steaks were then brined in a 2.4% NaCl solution at 38°F (3.3°C) with a fish-to-brine ratio of 1:7 (wt/vol). After brining 2 days, the steaks were allowed to equilibrate one day at 38°F (3.3°C) to facilitate uniform salt penetration *(30)*.

Application of generated and liquid smoke

Generated smoke was produced from alderwood sawdust in an external smoke generator connected to the smokehouse. When steaks were processed with generated smoke, the smoke was usually added throughout the entire heating process. In some experiments smoke was added during the last half of the process to determine if the application of smoke added after the surface was dried and a pellicle was formed caused differences in the inactivation of *L. monocytogenes*.

Three different liquid smoke products were compared: CharSol C-10 and CharOil (Red Arrow Products, Manitowac, WI) and 10-Poly (Hickory Specialties, Crossville, TN). CharSol C-10 and 10-Poly are unfractionated liquid smoke. CharOil is an oil-soluble fraction of CharSol C-10. Liquid smoke was applied as a dip after brining. The steaks were dipped in either a 100% solution or a solution with a reduced concentration of 50%, 25%, or 10% for 1 min and allowed to drain on stainless steel mesh screens before inoculation and heat processing.

Inoculation and heat processing

Salmon steaks were placed on stainless steel mesh screens. Using a tuberculin syringe, 0.1 ml of inoculum was evenly distributed on the surface of each half of the steak. Then the steaks were heat-processed in a custom-built, electronically controlled, electrically heated smokehouse. During heat processing, the internal temperature of each half steak was monitored continuously by copper-constantan thermocouples inserted into the deepest part of the loin muscle and connected to a 24-point temperature-recording potentiometer. The temperature in the smokehouse was increased in 10°F (5.6°C) increments every 30 min, starting with 130°F (54.4°C), in a stepwise heating process, to simulate commercial processing conditions. When the internal temperature of all the steaks were between 131°F and 185°F (55.0 to 85.0°C), the house temperature was adjusted so that constant internal temperatures could be maintained for the last 30 minutes of processing. Final processing temperature for each half steak was calculated as the average internal temperature during the last 30 minutes of processing *(10, 30)*.

Enrichment and isolation of Listeria

A preenrichment technique was used to enhance the detection of *L. monocytogenes* that may have been injured or stressed by heating or by the smoke constituents *(3, 25, 26)*. After processing, the salmon steaks were aseptically cut in half and were transferred to sterile prechilled screw-cap glass jars and stored at 5°C for four days. Each half of the steak was then preenriched in 120 ml Trypticase soy broth with 0.6% yeast extract and 0.1% glucose (TSBYE+ G) for six hours at 30°C. At the end of this enrichment and recovery period, 120 ml of double-strength (2×) UVM selective enrichment broth and 160 ml single-strength (1×) UVM was added to each jar so that the final concentration of UVM was 1× and the sample-to-medium ratio was 1:10. Incubation was continued at 30°C. This served as the primary selective enrichment *(8)*. McLain and Lee's modification of UVM *(27)* was used as the secondary enrichment broth (UVM-2). In this procedure, after 24 h, a 0.1-ml portion of the UVM enrichment was inoculated into 10 ml of UVM-2, which has an increased concentration of acriflavin, and this secondary enrichment was incubated at 30°C for 1 day.

After 1, 6, and 9 days of enrichment in UVM and 1 day in UVM-2, 0.1-ml portions of the enrichments were surface plated on the selective agars. Oxford agar (OX; listeria selective agar base CM586 supplemented with SR140, Oxford Ltd., Basingstoke, Hampshire, England) and hemolytic ceftazidime listeria agar (HCLA) *(31)*. OX plates were incubated at 30°C, and HCLA plates were incubated at 35°C for 24 h.

Identification of Listeria *species*

Representative black colonies from OX and β-hemolytic colonies from HCLA were picked and streaked for isolation and purification on horse blood agar *(27)*. Isolates were identified and confirmed following schemes described by Lovett *(24)* and McLain and Lee *(27)*.

Chemical analyses

Representative samples from each experiment were analyzed for sodium chloride and moisture following methodology described previously *(30)*. The concentration of water-phase NaCl in salmon steaks after processing was between 2.0 and 3.0%. In samples processed with generated smoke, the top 4- to 5-mm layer of representative steaks was removed immediately after processing and analyzed for total phenols by methods previously described *(10)*.

RESULTS

Inactivation of L. monocytogenes *on salmon steaks hot processed without smoke*

The effect of heat, with and without generated smoke, on the inactivation of *L. monocytogenes* in hot-processed salmon samples is summarized in Table 1. When samples were processed without smoke, *L. monocytogenes* survived in most of the samples heated to internal temperatures as high as 181°F (82.8°C). *L. monocytogenes* was isolated from three samples processed at 181°F (82.8°C), but survivors were not found in any of the remaining samples processed at higher temperatures.

In the initial studies, 203 samples were processed without smoke in a commercial size smokehouse with air circulation. *L. monocytogenes* was detected in 111 (54.6%) of these samples. These results were confirmed in 12 additional experiments in which samples, also monitored with thermocouples, were processed in a smaller laboratory

TABLE 1. *Effect of heat and generated smoke on inactivation of* L. monocytogenes *(330 to 350 CFU/sample) in hot-processed salmon steaks*

Internal temperature of samples °F (°C)[a]	No smoke	Smoke added throughout process[b]	Smoke added last 3 h of process[c]
131–135 (55.0–57.2)	3/3[d]	4/8	
136–140 (57.8–60.0)	2/2	2/7	1/1
141–145 (60.6–62.8)	6/8	9/45	13/13
146–150 (63.3–65.6)	27/28	3/78	19/20
151–155 (66.1–68.3)	22/25	4/82	8/8
156–160 (68.9–71.1)	12/30	0/76	1/1
161–165 (71.7–73.9)	8/32	0/35	
166–170 (74.4–76.7)	9/36	0/23	0/14
171–175 (77.2–79.4)	9/19	0/4	2/15
176–180 (80.0–82.2)	10/13		1/13
181–185 (82.8–85.0)	3/7		0/1

[a] Samples were processed to the specified internal temperature and held at that temperature for 30 minutes.
[b] Smoke deposition was 0.23 to 0.30 mg total phenols/10 g.
[c] Smoke deposition was less than 0.05 mg total phenols/10 g.
[d] Number of samples positive for *Listeria monocytogenes*/number tested.

oven without air circulation. In these experiments, *L. monocytogenes* was detected in 28 out of 95 (29%) samples processed as high as 181°F (82.8°C), but were not recovered from any samples processed at higher temperatures (data not included in Table 1).

Effect of generated smoke on the inactivation of L. monocytogenes *in hot-processed salmon*

Generated smoke was added to the smokehouse during two different stages of processing to determine its lethal effect on *L. monocytogenes*. The smoke was either added during the entire process, or during the last 3 h of a 6-h process. Smoke deposition was quantified by measuring the total phenol concentration in the outermost 4- to 5-mm layer of the steaks after processing. The application of smoke throughout the entire heat process resulted in greater smoke deposition and total phenols were 0.23 to 0.30 mg/10 g. When smoke was applied only during the last 3 h of the process, there was very little smoke deposition and total phenols were less than 0.05 mg/10 g.

Addition of smoke throughout the entire process reduced the number of samples on which survivors were found, especially when processed at the higher temperatures. *L. monocytogenes* was not isolated from any of the 178 samples that were processed with generated smoke to an internal temperature of 153°F (67.2°C) or higher, but was isolated from 4 out of 42 samples processed at 151 and 152°F (66.1 and 66.7°C). One of the four positive samples was processed at 151°F (66.1°C) while the remaining three positive samples were processed at 152°F (66.7°C) (Table 1).

When smoke was added only during the last half of a 6-h process, *L. monocytogenes* was recovered from 45 samples processed at temperatures up to 176°F (80.0°C), but was not recovered from the remaining 10 samples processed at 177°F (80.6°C) or higher (Table 1).

Effect of liquid smoke on the inactivation of L. monocytogenes *in hot-processed salmon*

The liquid smoke products CharSol C-10 and CharOil (Red Arrow) and 10-Poly (Hickory Specialties) were compared for their lethal effect on *L. monocytogenes* inoculated onto the surface of salmon steaks (Table 2). The brined steaks were dipped for 1 minute in the liquid smoke and drained, then inoculated with *L. monocytogenes* and heat processed in the smokehouse.

CharOil, the oil-soluble fraction of CharSol C-10, when used at 100% concentration, was not as effective as the parent smoke, CharSol C-10, in inactivating *L. monocytogenes*. The organism was recovered from CharOil-treated samples that were processed up to 166°F (74.4°C), the highest temperature used for this treatment. *L. monocytogenes* was not recovered, however, from any of the samples dipped in 100% CharSol C-10 and processed at temperatures as low as 138°F (58.9°C).

When CharSol C-10 was used at a concentration of 50%, *L. monocytogenes* was not recovered from any of the 60 samples processed above 150°F (65.6°C), but was detected in 13 of the 48 samples processed between 131°F and 149°F (55.0 to 65.0°C).

As the concentration of CharSol C-10 was decreased further, the internal processing temperatures necessary to inactivate *L. monocytogenes* increased. When CharSol C-10 was used at a concentration of 25%, *L. monocytogenes* was isolated from two samples processed at 155°F (68.3°C) and from one sample processed at 152°F (66.7°C). With 10% CharSol C-10, *L. monocytogenes* survived at 161°F (71.7°C) and 162°F (72.2°C) (Table 2).

When salmon steaks were dipped in a 50% 10-Poly liquid smoke solution, *L. monocytogenes* was detected in samples processed up to 144°F (62.2°C) (Table 2).

TABLE 2. *Effect of liquid smoke, CharSol C-10, CharOil, and 10-Poly on the inactivation of* L. monocytogenes *(330 to 350 CFU/sample) in hot-processed salmon steaks*

Internal temperature of samples °F (°C)[a]	Liquid smoke					
			CharSol C-10			
	CharOil, 100%	10-Poly, 50%	100%	50%	25%	10%
131–135 (55.0–57.2)		1/5[b]		1/2		
136–140 (57.8–60.0)		2/4	0/2	8/16	1/2	
141–145 (60.6–62.8)	4/4	2/10	0/8	2/12	4/4	0/2
146–150 (63.3–65.6)	2/2	0/4	0/12	2/18	8/14	6/6
151–155 (66.1–68.3)	9/12	0/10	0/12	0/20	3/6	
156–160 (68.9–71.1)	2/7	0/2		0/15	0/8	7/16
161–165 (71.7–73.9)	0/6		0/4	0/16	0/2	2/12
166–170 (74.4–76.7)	1/1			0/5	0/8	0/4
171–175 (77.2–79.4)				0/4		

[a] Samples were processed to the specified internal temperature and held at that temperature for 30 minutes.
[b] Number positive/number tested.

TABLE 3. *Effect of incubation time in UVM broth on the recovery of* L. monocytogenes *from hot-processed salmon steaks*

Treatment	Total number of samples with *L. monocytogenes*	Incubation time when *L. monocytogenes* was first detected			
		UVM			UVM-2
		1 day	6 day	9 day	1 day
No smoke	111	96	9	5	1
Generated smoke, throughout process	22	5	14	1	2
Generated smoke, last 3 h	45	30	NT[a]	15	0
CharOil, 100%	18	16	0	1	1
10-Poly, 50%	5	3	1	1	0
CharSol C-10, 100%	0	0	0	0	0
CharSol C-10, 50%	13	5	1	7	0
CharSol C-10, 25%	16	14	NT	2	0
CharSol C-10, 10%	15	14	0	0	1

[a] NT = not tested.

Effect of extended incubation of samples in UVM on detection of L. monocytogenes

The UVM enrichments were tested for *L. monocytogenes* after 1, 6, and 9 days of incubation at 30°C. Results from this part of the study are summarized in Table 3. Of the 245 samples from which *L. monocytogenes* was recovered, 57 were negative after 1 day of incubation, but were positive after extended incubation of 6 and 9 days. When samples were treated with 50% CharSol C-10 or generated smoke during the last half of the process, the extended incubation time was necessary to recover *L. monocytogenes* from samples processed at higher temperatures. The remaining positive samples from the extended incubation were from the same temperature range in which other positive samples had already been detected after 1 day of incubation in UVM or UVM-2. Five of the enrichment samples were positive in UVM-2 that were not positive in UVM after 1 day of incubation. These same five samples, however, also were positive in UVM when the incubation time was increased to 6 and 9 days.

DISCUSSION

This study has identified the temperatures required to inactivate *L. monocytogenes* on the surface of salmon steaks processed with and without liquid or generated smoke in a hot-smoke process. When salmon steaks were processed with heat and in the absence of smoke, the product had to reach internal temperatures of 182°F (83.3°C) or higher before *L. monocytogenes* was inactivated. The interaction of heat and undiluted liquid smoke, CharSol C-10, lowered the lethal temperature to 138°F (58.9°C) when it was applied at the beginning of the process. As the concentration of liquid smoke decreased, the lethal temperatures for *L. monocytogenes* increased. When smoke, from an external smoke generator connected to the smokehouse, was applied throughout the entire process, the lethal temperature for this pathogen was reduced to 153°F (67.2°C). Generated smoke was less effective when it was applied during the later stages of the process after a dried pellicle had formed on the product. Applying smoke at this step in the process increased the lethal temperature by 25°F (14°C).

Previous research at our laboratory has shown that smoke was readily absorbed on the surface of the product before the outer layer of the salmon steak had dehydrated and formed a coagulated protein layer (pellicle) on the surface of the fish *(9)*. In the current study we have shown that for smoke to be listericidal, it is important that it be applied before the pellicle has been formed. Chan et al. *(6)* have also shown that the total smoke absorption is markedly reduced when it is applied to dried fish surfaces.

Jemmi and Keush *(21)* have reported that an internal temperature of 149°F (65°C) for 20 minutes was adequate to inactivate *L. monocytogenes* from artificially contaminated hot-smoked trout when smoke was added during the last 45 minutes of the process. The difference in recovery of *L. monocytogenes* from eviscerated trout in their study and salmon steaks in our study could possibly be attributed to the difference in product form and recovery methods used to determine the survival of *L. monocytogenes*.

In the current study with salmon steaks, the flesh was exposed and a solubilized protein layer formed on the surface following brining. During drying and heating this protein layer was denatured and a dried pellicle formed on the surface. *L. monocytogenes* inoculated on the surface of the steak, therefore, became embedded in this solubilized protein layer and the pellicle as it was being formed, thus providing protection to this bacterium from smoke and heat. In the study by Jemmi and Keusch *(21)* the skin and viscera cavity lining covered the flesh of the trout, minimizing the amount of solubilized protein on the surface. When *L. monocytogenes* was inoculated onto this surface, the inoculum remained on the skin, scales and viscera cavity lining, thus exposing this pathogen directly to the actions of smoke and heat. In addition, the differences in the recovery and enrichment media used, as well as the sample size analyzed may have also contributed to the differences in the results.

In other studies, the use of cold enrichments (extended incubation at 4°C), and enrichments in a nonselective medium followed by enrichment in selective media, have

promoted repair, growth, and recovery of heat-injured cells *(1, 3, 14, 25, 32)*. In an effort to increase the recovery of possible heat-injured cells in the current study, samples were stored for 4 days at 5°C, preenriched in nonselective TSBYE broth, enriched in UVM broth, and tested for *L. monocytogenes* after various incubation periods. The extended incubation time in UVM broth increased the number of samples with surviving *L. monocytogenes* by 23% and, in addition, it increased the detection of this pathogen from some samples processed at higher temperatures. In five cases, *L. monocytogenes* was recovered after 1 day in UVM-2 but not after 1 day in UVM. These same samples, however, were also positive when the incubation time in UVM was increased to 6 and 9 days. UVM-2 has been shown, in other studies at our laboratory and by McLain and Lee *(27)*, to increase the detection and isolation of *L. monocytogenes* in raw fish products containing large populations of other bacterial species *(11)*.

The results of this study are in agreement with those of other investigations on the survival of *L. monocytogenes* exposed to elevated temperatures in the absence of bactericidal agents such as smoke. This bacterium was shown to survive in chicken breasts *(4, 5)* cooked to an endpoint temperature of 180°F (82.2°C) and red meat products *(23)* heated to an internal temperature of 78 to 85°C for 15 min. This study also confirms results from earlier research at this laboratory *(9, 10)* and others *(12, 29)* on the bactericidal activity of liquid smoke.

In a previous study of cold-smoked salmon at this laboratory, *L. monocytogenes* was shown to be confined to the surface of raw salmon unless it was physically introduced into the interior by procedures such as the injection of recirculated brine *(11)*. Since the cold-smoking process does not include a lethal heating step, it was concluded that in order to control *L. monocytogenes* in this type of product the following three steps were necessary: (a) elimination or reduction of *L. monocytogenes* on the outside surfaces of frozen or fresh fish before they are filleted; (b) prevention of recontamination of product during all stages of processing; and (c) inhibition of any possible survivors or recontaminants which could grow during distribution and at the consumer level *(11)*.

In the control of *L. monocytogenes* in cold- and hot-smoking operations, the hot process has the advantage in that it includes a potential lethal heating step if it is used in combination with liquid or generated smoke. Therefore, in addition to the three essential steps listed to control *L. monocytogenes* in cold-smoked products we have added the following control step: (d) inactivation of *L. monocytogenes* by utilizing the interaction of smoke and heat. The generated smoke must be applied to products such as salmon steaks before the surface becomes dry and a pellicle has formed. Otherwise the smoke deposition decreases and the pellicle serves as a protective barrier for *L. monocytogenes* from smoke and heat. When liquid smoke is applied, products similar to the parent products, CharSol C-10 and 10-Poly, should be used. Oil soluble fractions are less effective.

Steps (a) and (b) recommended for the cold process for smoked fish should also be followed for the hot process. Step (a) reduces or eliminates *L. monocytogenes* on the raw product before brining and processing and can markedly increase the effectiveness of the entire process. This step could even reduce the temperatures required to inactivate any possible remaining cells of this pathogen. Step (b), the prevention of recontamination especially after the products are removed from the smokehouse and during packaging, is essential. Step (c), the inhibition of any possible survivors or recontaminants, is less critical for the hot process than for the cold process. However, if approved and acceptable inhibitors can be used, it would add additional safety to these types of products. If these recommendations are followed during the processing and packaging of hot-smoked fish, this should markedly reduce the chances that *L. monocytogenes* will be present on any of the finished products.

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