The Bacteriological Safety of Unpasteurized Cured Meat Products in Response to the Manufacturing Processes

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ABSTRACT. Unpasteurized cured hams were prepared from pork loins externally or internally inoculated with *Salmonella* serovar typhimurium, *Escherichia coli*, or *Staphylococcus aureus*. The fates of these pathogens during the manufacturing processes of curing, cool smoking or warm smoking and during storage at 15 or 27°C were examined. *Salmonella* and *E. coli* but not *S. aureus* decreased as the curing time progressed. During cool smoking for 60 h, all the pathogens inoculated externally were killed on the loins cured with nitrite at 0.02% and salt at 6% or a higher concentration. The raw meat must be handled hygienically since the internal inocula were not completely killed. During warm smoking, *Salmonella* vanished and staphylococci decreased. Staphylococci did not grow nor produced enterotoxins on slices of raw hams held for 14 days at 15°C.—**KEY WORDS:** *Salmonella* serovar typhimurium, *Staphylococcus aureus*, unpasteurized cured ham.

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Unpasteurized cured meat products (raw hams) have legally been approved to be marketed in Japan since 1982 [8], since the need for the manufacture and circulation of raw hams in this country became larger as more and more Japanese travellers to western countries got acquired the taste of raw hams.

Raw hams are cured, smoked and/or dried meat products without thermal treatment, and have been popular in European countries for centuries. There are varieties of raw hams in Europe.

The present study was designed to evaluate the bacteriological safety of unpasteurized cured meat products for the following reasons: (1) raw hams have scarcely been produced and consumed in this country, and (2) outbreaks of foodborne diseases have occasionally been caused by consumption of raw hams [2, 3, 12].

MATERIALS AND METHODS

Raw material: Boneless pork longissimus muscles (loins), with pH values lower than 5.8 [2], were obtained a day after slaughter and held overnight in a cold room (2–4°C). They were trimmed by removing excess back fat and shaped as in commercial operation (mean weight, 1.5 kg).

Inoculation: The loins were externally or internally inoculated with individual species of *Escherichia coli* (serovar 0:128), *Salmonella* serovar (ser.) typhimurium ATCC 14028, and *Staphylococcus aureus*; a composite of enterotoxin A (an isolate from a foodborne intoxication outbreak), B (FRI 243) and C (FRI 361) producers was employed. For each experiment, the cultures were grown overnight separately in brain heart infusion (BHI, Difco) at 37°C, and the

organisms of each species suspended in 10 liter water.

The external inoculation was achieved by soaking the loins in one of the bacterial suspensions for 5 min, and the internal inoculation by injecting the suspension into the loins through a multiple-needle injector. Excess water was drained and the loins were dried at an ambient temperature. The population of each organism inoculated externally or internally was 10^4 – 10^5 colony-forming units (CFU)/g.

Curing procedures: The loins were divided into four groups. On the basis of the fresh weights of the loins, salt (NaCl) was added at 3% (group 1), 4.5% (group 2), 6% (group 3), or 8% (group 4), and sodium nitrite at 0.02% to all groups. All the loins were rubbed with the cures and placed in a 3–4°C curing room.

Smoking/drying procedures: After curing for 10 days, the loins were rinsed in running water to remove the excess curing salts and hung in an air-conditioned smoke house. The smoking/drying experiments were conducted at 25–30°C (cool smoking) and at 45–55°C (warm smoking); the relative humidities were approximately 90 and 70%, respectively. Smoke was lightly generated from hardwood (Smoke wood, Shinsei Sangyo, Tokyo) and introduced into the smoke house.

Production of enterotoxins by staphylococci during storage at 15 or 27°C: To examine for production of enterotoxin by staphylococci, the unpasteurized cured hams were prepared from the loins without inoculation. The curing salt mixture of group 1 or 3 was added. After dry cure for 10 days, cool smoke was applied. The products were sliced (about 50 g). A 0.1-ml portion of the staphylococcal suspension diluted in sterile brine (4.5% or 6.96%) [10] was pipetted onto each slice and spread over the surface with a sterile L-shaped glass rod; the staphylococcal composite consisting of almost the same numbers of viable cells of the enterotoxin A (FRI 722), B and C producers. Each slice was then

placed on a polystyrene tray, wrapped with polyethylene film and held at 15 or 27°C.

Microbiological methods: A 25-g sample was taken from the surface (top about 5 mm) of the externally inoculated loin or from the center of the internally inoculated one. The sample was macerated for 2 min in a Stomacher (Colworth) bag with 225 ml of sterile saline. Appropriately diluted samples were each spread on a plate of EMB agar for *E. coli*, MLCB agar for *Salmonella* and egg yolk mannitol salt agar for *S. aureus*. The plates were incubated and examined for the typical colonies. Randomly selected colonies were confirmed as *E. coli* or *S. ser. typhimurium* by agglutination with the appropriate antisera (Denka Seiken, Tokyo). Randomly chosen isolates of *S. aureus* were tested for the coagulase activity.

The viable cells were counted also by the MPN method. EC broth, Hajna tetrathionate broth (both were the products of Nissui, Tokyo), and BHI broth supplemented with 7.5% salt were utilized.

Staphylococcal enterotoxins were assayed by the microslide gel diffusion technique as described by Casman *et al.* [1].

Chemical analyses: Samples for chemical analyses were prepared by the same methods as described above without any inoculation of the pathogens. Two samples were used for analyses. The lean meat was composited, and ground through a 3 mm plate. Moisture, salt, pH, residual nitrite, and phenols were determined by the standard method [9]. Water activity (a_w) was also determined [11].

RESULTS

Fates of pathogens and changes in compositions during the dry-cure period: *S. ser. typhimurium* was inactivated gradually during the 65-day curing period, with a 1- to 2-log decrease (Fig. 1). Such tendencies were shown in any test variables of the curing salts. The decrease in the viable cells de-

Fig. 1. Fate of *S. ser. typhimurium* during dry curing. On the basis of the fresh weights of the loins, salt was added at 3% (group 1), 4.5% (group 2), 6% (group 3), or 8% (group 4), and sodium nitrite at 0.02%. Closed circles represent viable cells of *Salmonella* on the surface (top about 5 mm) of the samples externally inoculated; open circles those in the center of the samples internally inoculated.

Fig. 2. Fate of *S. aureus* during dry curing. The curing salts added were as in the footnote of Fig. 1. Closed diamonds represent viable cells of *S. aureus* on the surface (top about 5 mm) of the samples externally inoculated; open diamonds those in the centers of the samples internally inoculated.

pended on the period of curing and the site of inoculation: the viable cells decreased more apparently on the surface than in the center of the loin and by prolonging the period of curing. Such may also be the case in the center of the loins of group 1, although no determination was made.

The fate of *E. coli* on the surface of the loins of group 4 was similar to that of *S. ser. typhimurium* (data not shown). On the contrary, *S. aureus* was not inactivated during dry cure regardless of the amount of curing salts or the inoculating site (Fig. 2). The curing salt mixture of group 1 may have caused the same phenomena in the center of the loins, although no determination was made.

Toward the end of the curing period, slime was formed on the surface of the meat of groups 1 and 2, but not on those of group 3 or 4.

The chemical compositions during the dry-cure period are presented in Table 1.

Within the first few days of dry cure, the loins lost the weights by about 2–4% due to the loss of meat juice. This resulted in local dehydration; the moisture content of the surface was lower than that of the center. The surface was then gradually rehydrated. The moisture content inside the meat decreased from approximately 75% to 67–71% also within the first 10 days, but not thereafter except for the loins of group 3 or 4.

The salt content in the external and the

Table 1. Chemical Compositions of the loins during dry curing

	Sampling position	Group ^{a)}	Period of curing (in days)		
			10	30	45
Moisture (%)	Exterior	1	60.1 ^{b)}	62.3	67.8
		2	61.9	62.6	66.3
		3	64.7	58.7	63.3
		4	59.3	61.1	69.1
	Interior	1	68.2	68.2	68.7
		2	67.0	67.4	70.3
		3	71.0	65.7	66.9
		4	67.9	66.5	64.5
NaCl (%)	Exterior	1	— ^{c)}	4.5	3.9
		2	4.1	5.2	5.3
		3	5.9	7.8	6.7
		4	6.5	8.3	7.9
	Interior	1	1.4	2.4	2.5
		2	2.5	4.0	3.5
		3	3.4	3.7	3.8
		4	4.2	5.5	3.9
Water activity	Exterior	1	0.98	0.97	0.96
		2	0.95	0.96	0.94
		3	0.94	0.93	0.92
		4	0.92	0.90	0.90
	Interior	1	0.98	—	0.97
		2	0.97	—	0.96
		3	0.96	—	0.95
		4	0.93	—	0.94
Residual nitrite (mg/kg)	Exterior	1	98.3	—	96.2
		2	75.1	—	94.9
		3	41.9	—	56.2
		4	72.0	—	44.7
	Interior	1	27.8	—	42.5
		2	31.6	—	38.7
		3	36.9	—	24.0
		4	33.2	—	18.9

a) On the basis of the fresh weights of the loins, salt was added at 3% (group 1), 4.5% (group 2), 6% (group 3), or 8% (group 4), and sodium nitrite at 0.02%.

b) Mean of duplicate samples.

c) not determined.

internal part of the loins increased appreciably during the same period; the increase was in parallel with the amounts of the cures added and with the period of curing.

The a_w values inside the loins markedly decreased also in the first 10 days. It took not more than 10 days for the a_w values of the

loins of groups 3 and 4 but about 45 days for those of groups 1 and 2 to reach 0.96 or lower; Hechelmann *et al.* [2] recommended and the current regulation [8] specifies a_w 0.96 for raw ham after completion of curing.

The levels of residual nitrite were generally lower in the center than on the surface of

Table 2. Fate of *S. ser. typhimurium* inoculated onto and into the loins during cool smoking

Site Inoculated ^{a)}	Group ^{b)}	Log viable cells of <i>Salmonella</i> after cool smoking ^{c)} (in hours)						
		0	10	20	30	40	50	60
Exterior	1	4.63 ^{d)}	2.67	1.76	0.63 ^{e)}	1.20	0.63	2.15
	2	4.54	2.45	2.56	0.60	0.54	0.36	(—) ^{f)}
		4.52		1.59		(—)		(—)
	3	4.68	0.36	1.04	0.36	(—)	(—)	(—)
		4.45		0.36		(—)		(—)
	4	4.36	2.20	0.36	(—)			
		4.30	1.00	(—)	(—)			
	2	4.65		2.85		3.11		2.51
	3	5.04		3.98		4.04		3.45
	4	4.78	3.81	3.52	2.46			

a) The loins were inoculated externally or internally with *S. ser. typhimurium* before dry cure.

b) On the basis of the fresh weights of the loins, salt was added at 3% (group 1), 4.5% (group 2), 6% (group 3) or 8% (group 4), and sodium nitrite at 0.02%.

c) After 10-day dry cure, the loins were smoked/dried in a 25-30°C smoke house, where smoke was lightly introduced.

d) Mean of duplicate samples.

e) The values lower than 1 log were obtained by the MPN method.

f) negative with a 3-g sample.

Fig. 3. Fate of *E. coli* inoculated on the surface of the loins during cool smoking. On the basis of the fresh weights of the loins externally inoculated with *E. coli*, salt and sodium nitrite were added to them at rates of 8% and 0.02%. After the completion of 10-day dry cure, cool smoking was applied.

loins. Although the same amount of sodium nitrite was added, the levels of residual nitrite were different among the test variables. No further study was performed to explain such differences.

To examine for the effects of the kind of muscle and the way of curing, other tests were made with the pork semimembranosus muscles, the size and the weight of which were adjusted similarly to those of the loins. Almost the same results as shown above were obtained by wet cure except for the weight gain (data not shown); the pickle consisted of 15% salt and 0.15% sodium nitrite as the regulation [8] specifies.

In the following studies, the period of curing was set at 10 days, since the a_w values reached 0.96 or lower within 10 days in at least two of the four test variables.

Fates of pathogens and changes in compositions during the cool-smoke period: Externally or internally inoculated *Salmonella*

Table 3. Fate of *S. aureus* inoculated onto and into the loins during cool smoking

Site Inoculated ^{a)}	Group ^{b)}	Log viable cells of <i>S. aureus</i> after cool smoking ^{c)} (in hours)						
		0	10	20	30	40	50	60
Exterior	1	5.18 ^{d)}	4.00	3.23	2.64	0.63 ^{e)}	0.36	2.26
	2	4.95	4.00	3.38	1.00	2.70	2.94	0.36
		4.59		3.18		0.36		(—) ^{f)}
	3	5.18	3.69	4.20	3.26	3.11	(—)	(—)
		4.81		2.81		(—)		(—)
	4	4.38	3.90	2.60	0.36			
		4.38	3.89	1.00	0.36			
Interior	2	4.68		3.23		4.18		3.56
	3	4.81		4.30		4.45		3.74
	4	4.72	4.28	3.81	3.72			

a) The loins were inoculated externally or internally with *S. aureus* before dry cure.

b) See the footnote (b) of Table 2.

c) See the footnote (c) of Table 2.

d) Mean of duplicate samples.

e) See the footnote (e) of Table 2.

f) See the footnote (f) of Table 2.

Table 4. Chemical compositions of the cured loins during cool smoking

	Group ^{b)}	Period of cool smoking ^{a)} (in hours)					
		10	20	30	40	50	60
Moisture (%)	1	67.1 ^{c)}	68.4	63.3	65.4	63.6	65.1
	2	66.9	63.4	65.3	64.2	64.8	63.5
	3	69.6	66.1	62.0	64.1	64.2	63.8
	4	68.9	64.9	61.2			
NaCl (%)	1	3.4	3.5	2.4	2.9	2.9	3.2
	2	4.1	5.6	4.8	5.0	4.6	4.4
	3	4.6	5.9	6.0	5.9	5.9	5.2
	4	6.1	7.5	7.1			
Water Activity	1	0.98	0.97	0.97	0.97	0.97	0.97
	2	0.96	0.95	0.96	0.96	0.96	0.95
	3	0.95	0.94	0.94	0.95	0.94	0.94
	4	0.94	0.92	0.92			
Phenols ^{d)} (ppm)	1	— ^{e)}	12.5	32.1	19.5	25.0	38.1
	3	—	7.1	15.6	18.1	30.1	35.8

a) See the footnote (c) of Table 2.

b) See the footnote (b) of Table 2.

c) Mean of duplicate samples.

d) The exterior sites (top ca. 5 mm) of the lean meat were analyzed.

e) not determined.

Table 5. Fate of *S. ser. typhimurium* and *S. aureus* inoculated onto the loins during warm smoking

Inoculated bacteria ^{a)}	Group ^{b)}	Log viable cells after warm smoking ^{c)} (in hours)						
		0	3	6	9	12	15	20
<i>Salmonella</i>	2	4.52 ^{d)}	5.20		2.30	1.00	0.36 ^{e)}	(—) ^{f)}
	3	4.81	4.52	2.49	2.51	1.00	(—)	
	4	4.36		0.36		(—)		
<i>S. aureus</i>	2	4.94	5.51		4.00	5.40	3.60	3.60
	3	5.20	4.36	5.00	5.45	4.49	3.70	
	4	4.80		3.36		4.20		

a) The loins were inoculated externally with *Salmonella* or *S. aureus* before dry cure.

b) See the footnote (b) of Table 2.

c) After 10-day dry cure, the loins were smoked/dried in a 45–55°C smoke house, where smoke was lightly introduced.

d) See the footnote (d) of Table 2.

e) See the footnote (e) of Table 2.

f) See the footnote (f) of Table 2.

organisms were killed considerably during the cool smoking (Table 2). The larger the amount of curing salts added and the longer the period of smoking, the more appreciable the reduction of the viable cells on the surface as compared with that in the center of the loins. It took less than 60, 40 and 30 h for the externally inoculated *S. ser. typhimurium* to vanish (MPN<0.3/g) on the surface of the loins of groups 2, 3 and 4, respectively. The viable cells on the surface of group 1 decreased, but were detected throughout the period. Internally inoculated *S. ser. typhimurium* was also inactivated during the period, with only about two-log reduction regardless of the group.

E. coli was also killed on the surface of the loins of group 4 during the period of cool smoking (Fig. 3). *S. aureus* was not easily killed, compared with the other two genera of pathogens (Table 3). To kill externally inoculated staphylococci, cool smoking for longer than 60, 40 and 30 h was required for groups 2, 3 and 4, respectively. Internally inoculated *S. aureus* reduced only to approximately 1/10.

The chemical compositions during cool

smoking are shown in Table 4. It was noticed that the moisture contents decreased and the salt contents increased, resulting in reduced a_w values, with increments of time duration. Such alteration, however, was not necessarily noticeable in other groups than 4. Phenol compounds became deposited as the smoking time progressed.

Fates of pathogens and changes in compositions during warm-smoke period: Externally inoculated *S. ser. typhimurium* was killed during warm smoking for 20 h (Table 5). It took less than 20, 15, and 12 h for *S. ser. typhimurium* externally inoculated on the loins of groups 2, 3 and 4 to reach MPN lower than 0.3/g.

Although staphylococci were killed during the same period, the extent of decreases ranged from only about 1/4 to 1/30.

The products became tough in 12–15 h of warm smoking, due probably to thermal denaturation of meat protein. The period of warm smoking was, therefore, set at 20 h to avoid deterioration of the meat quality.

The changes in the compositions during warm smoking were similar to those during cool smoking except for the time course (data

Table 6. Growth of *Staphylococcus aureus* and the Production of Enterotoxins A, B, and C on Unpasteurized Cured Meat Products Held at 15 or 27°C

Temperature (°C)	Group ^{a)}	Period of storage ^{b)} (in days)				
		1	2	4	7	14
15	1	Log CFU			6.40	8.43
		Enterotoxin (μg/g)			nd ^{b)}	nd
	3	Log CFU			3.20	4.00
		Enterotoxin (μg/g)			nd	nd
27	1	Log CFU		4.75	6.91	9.54
		Enterotoxin (μg/g)		nd	(B) ^{c)} .03-.05	(B).13-.27
					(C).03-.06	(C).03-.06
	3	Log CFU		4.11	3.60	3.34
		Enterotoxin (μg/g)		nd	nd	nd
					(B).06-.12	(C).03-.06

a) On the fresh-weight basis, salt and sodium nitrite were added at 3% and 0.02% (group 1), or 6% and 0.02% (group 3) to loins. The loins were cured for 10 days in a 3–4°C room, and smoked/dried. The products were sliced. A composite of approximately equal numbers of enterotoxin A, B and C producers was inoculated on the slices. The inoculum was 4.30–4.40 of Log (CFU/g).

b) No enterotoxin (<0.03 μg/g) was detected.

c) Enterotoxin types detected.

not shown).

Growth of S. aureus and production of enterotoxins during storage at 15 or 27°C: *S. aureus* grew and produced enterotoxins on the slices of unpasteurized cured ham prepared with a curing salt of group 1 ($a_w=0.97$) (Table 6). When the inoculated hams were held at 27°C, *S. aureus* exponentially multiplied in a day and then reached a plateau at higher than 9 log viable count in 4 days of storage, forming yellow slime. Staphylococcal enterotoxins of B and C were increasingly detected after 2 days of storage (0.03–0.80 μg/g), however, enterotoxin A was not detected for 7 days. During the storage at 15°C, *S. aureus* grew but never produced enterotoxins for 14 days.

On the other hand, growth of *S. aureus* and production of enterotoxins were considerably retarded on the hams of group 3 ($a_w=0.94$). When the inoculated hams were held at 27°C, *S. aureus* decreased in number during the first 4 days. Although *S. aureus* grew and produced enterotoxins thereafter, more than 4 but no more than 7 days were required for

small amounts of enterotoxins B and C to be demonstrated. No enterotoxin A was detected. During the storage at 15°C, neither growth nor enterotoxin production by *S. aureus* was observed for 14 days.

DISCUSSION

The purposes of curing are to impart the characteristic keepability, cured meat color, and aromatic taste to meat. To attain such purposes, salt and some or all of nitrite, nitrate, ascorbate, sugar, spices, seasonings and starter culture are added to meat [4], depending upon the products and the manufacturers. Unpasteurized cured hams are assorted into four based on the curing salts added: (1) salt without nitrite or nitrate as Parma ham; (2) salt and nitrate; (3) salt, nitrate and nitrite; and (4) salt and nitrite [2]. In the present study, curing was carried out by the method (4), since use of nitrate as a curing ingredient is becoming rare internationally [2].

During the curing period of 65 days at 3–

4°C, *Salmonella* and *E. coli* were inactivated to approximately 1/10 to 1/100 (Fig. 1). The cell reduction was supposedly caused by the complicated effects of salt, nitrite, low temperature and others, for the cells decreased more appreciably at the site, where these parameters were probably satisfied. The longer the period of curing, the more noticeable the reduction of the viable cells on the surface as compared with that in the center of the loins.

Langlois *et al.* [7] reported that coliforms were not detected in any dry-cured country hams after the salt equalization process. Kokubo *et al.* [5] studied the fate of experimentally inoculated *Salmonella* and spores of *Clostridium perfringens* and *Bacillus cereus* during the manufacturing processes of unpasteurized cured ham. They reported that these pathogens decreased during curing by any of such methods as brine injection, wet cure and dry cure. The present study showed that the changes in a_w , salt contents, and residual nitrite scarcely differed among the methods of curing nor the kinds of the muscle shaped similarly (data not shown).

In commercial operations, hams are re-stacked and re-rubbed with additional cure to achieve its uniform and thorough distribution in the meat block [4]. Such procedures were omitted in this study, because the lot size and the weights of the individual pieces of meat employed were rather limited and even distribution of the curing salts throughout the loins was not supposedly impeded. The burning step [4] or the salt equalization process [7] usually following the curing process was also omitted for the same reason.

During cool smoking for 60 h, externally inoculated *Salmonella*, *E. coli* and staphylococci were killed. To reduce these viable cells by more than 5 log CFU, it seems necessary to cure the loin with nitrite and salt at 6% or a higher concentration (Fig. 3, Tables 3 and 5). Internally inoculated *Salmonella* and *S. aureus* were also inactivated, but

with only about 1- to 2-log reduction. This suggests that raw meat should be handled under intensively hygienic conditions to prevent the entry of pathogens into the meat.

During warm smoking, *Salmonella* inoculated externally was also killed. Kokubo *et al.* [5] reported that *S. ser. typhimurium* and *C. perfringens* drastically decreased during smoking for 5 h at 50°C. They stated that clostridial spores decreased in the media, growth parameters of which were adjusted likewise to those of raw ham during the smoking process; temperature (25 or 50°C), pH (about 6) and salt (approximately 5%) [6]. On the contrary, externally inoculated staphylococci decreased only to approximately 1/4 to 1/30 during warm smoking.

Hechelmann *et al.* [3] studied growth of *S. aureus* (enterotoxin A and B producers) internally inoculated in bone-in raw ham, prepared by the combined method of dry and wet cure with 1% nitrate-added nitrite-pickling-salt (NPS). They reported that staphylococci did not multiply during curing for 3 weeks at 10°C, the burning step for 7 weeks at 10°C, smoking for 3 days at 20°C and storage for 11 weeks at 18°C.

While inoculated slices of an experimentally prepared product ($a_w=0.94$) were held at 27°C, staphylococcal enterotoxins B and C (0.03–0.80 µg/g) were produced in more than 4 but less than 7 days (Table 6). On the contrary, staphylococci did not grow nor produced enterotoxin in 14 days at 15°C. In this sense, the storage temperature lower than 10°C specified by the standards of storage of meat products [8] seem justified.

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要 約

非加熱食肉製品の微生物学的安全性に及ぼす製造工程の影響: 重久 保・山田良司・太治司郎・品川邦汎¹⁾・阪口玄二²⁾ (日本ハム株式会社中央研究所, ¹⁾岩手大学農学部獣医公衆衛生学教室, ²⁾大阪府立大学農学部獣医公衆衛生学教室)——サルモネラ, 大腸菌及びブドウ球菌を肉塊の表面または内部に接種した豚ロース肉を用いて非加熱食肉製品を製造し, 塩漬, 冷燻, 温燻と保存期間中のこれら食中毒細菌の消長を調べた。塩漬期間中にサルモネラと大腸菌は減少したが, ブドウ球菌は減少しなかった。亜硝酸ナトリウム 0.02% と 6% 以上の食塩を添加して塩漬した場合には, 60時間の冷燻中に肉塊の表面に接種した食中毒細菌は死滅した。肉塊内部に接種した食中毒細菌は生残するので, 原料肉の取扱いは衛生的に行う必要があると思われた。温燻期間中にはサルモネラは死滅し, ブドウ球菌は減少した。ブドウ球菌を接種した非加熱ハムを 15°C 14日間保存してもエンテロトキシンは産生されなかった。