Journal of Food Protection, Vol. 50, No. 6, Pages 494-501 (June 1987)

Thermal Inactivation of *Salmonella* Species in Fluid Milk[1]

J.-Y. D'AOUST[2*], D. B. EMMONS[3], R. McKELLAR[3], G. E. TIMBERS[4], E. C. D. TODD[2], A. M. SEWELL[2] and D. W. WARBURTON[2]

Bureau of Microbial Hazards, Health Protection Branch, Health and Welfare Canada, Sir Frederick G. Banting Research Centre, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2 and Food Research Centre and Engineering and Statistical Research Centre, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

(Received for publication October 27, 1986)

ABSTRACT

The thermal resistance of *Salmonella senftenberg* 775W, *Salmonella muenster* previously isolated from raw fluid milk, and two mixtures each consisting of ten *Salmonella* strains commonly isolated from human or non-human sources was tested. Cells were suspended in whole milk at a final concentration of 10^5 cells/ml. The inoculated milk was thermally processed at temperatures ranging from 60°C to 74°C using a pilot-scale plate pasteurizer unit. The mean and minimum residence time of milk in the holding tube of the pasteurizer was 17.6 and 16.2 s, respectively. The maximum temperature at which viable salmonellae were detected in the human (61.5°C) and non-human (64.5°C) mixtures was considerably lower than that obtained with *S. senftenberg* 775 W (67.5°C). *S. muenster* failed to show any milk-adapted response and could not be recovered at temperatures greater than 63.0°C. Treatment at 63°C produced a 4 $\log_{10}$ or greater reduction in the number of viable *Salmonella* including the heat resistant *S. senftenberg* 775 W, and a minimum 2 $\log_{10}$ decrease at 60°C. These findings warrant caution in the use of subpasteurizing temperatures for thermal processing of fluid milk.

Recent foodborne outbreaks of human salmonellosis have underlined the importance of milk and milk products as vehicles of infection. Cheddar cheese from pasteurized milk contaminated with *Salmonella heidelberg* resulted in an outbreak in Colorado (1976) involving an estimated 16,000 cases of illness *(10)*. In 1980-82, the province of Ontario noted large increases in the number of human cases of *Salmonella muenster* infections resulting from consumption of raw milk and non-pasteurized cheese *(2)*. More recently, Cheddar cheese manufactured in eastern Canada from improperly pasteurized and heat-treated milk was implicated in more than 1500 cases of salmonellosis from *Salmonella typhimurium* phage-type 10 *(8)*.

The potential hazard of salmonellae in raw milk is exemplified in the recent milkborne outbreak in Illinois involving more than 14,000 cases of illness *(20)*, and in the ability of these pathogens to survive in cheese for periods exceeding the mandatory 60-day refrigerated storage of cheese manufactured from non-pasteurized milk *(8,16,21)*.

A large number of plants in Canada manufacture cheese from raw milk or milk thermally processed at subpasteurizing temperatures. The present study examines the thermal inactivation of salmonellae from human and non-human sources in the temperature range of 60 to 74°C.

MATERIALS AND METHODS

Bacterial cultures

Test strains of *Salmonella* were obtained from the National Enteric Reference Center and from a collection held in the microbiology research laboratory (Health and Welfare Canada). Cultures were stored at room temperature in a semi-solid medium consisting of meat extract (5.0 g), peptone (10.0 g), NaCl (3.0 g), $Na_2HPO_4 \cdot 12H_2O$ (2.0 g), and agar (10.0 g) dissolved in 1 L of distilled water, final medium pH 7.4 (Institut Pasteur, Paris).

Inoculum

Salmonella test strains were grown under standardized conditions to obtain cultures of known cell density. Each of the 22 test organisms was subcultured twice in 9 ml of nutrient broth (NB) followed by inoculation (1 ml) into 2 L of NB incubated for 16-18 h at 35°C. Triplicate experiments showed that strains grown under these conditions reliably produced cultures of 10^8 cells/ml. Absorbance (OD_{600}) was also used to detect major changes in growth characteristics.

Four different inocula were used to artificially contaminate raw milk in this study. One inoculum (human mixture) consisted of an equal mixture of 10 serovars frequently encountered in humans in Canada. The selected test strains were not necessarily of human origin. These included *Salmonella typhimurium* PT10 (implicated in the 1984 cheese outbreak in eastern Canada), *Salmonella infantis, Salmonella hadar, Salmonella agona, Salmonella enteritidis, Salmonella heidelberg, Salmonella newport, Salmonella saint-paul, Salmonella thompson,* and *Salmonella schwarzengrund*. A second inoculum (non-

[1]*Contribution number I-882 from the Engineering and Statistical Research Centre and contribution number 718 from the Food Research Centre.*
[2]*Health Protection Branch, Health and Welfare Canada.*
[3]*Food Research Centre, Agriculture Canada.*
[4]*Engineering and Statistical Research Centre, Agriculture Canada.*

human mixture) consisted of *Salmonella muenster* (implicated in the 1980-82 episode of contaminated cheese in Ontario), *Salmonella kentucky* (lac$^+$ biotype), *Salmonella anatum, Salmonella montevideo, Salmonella mbandaka, Salmonella albany, Salmonella brandenburg, Salmonella senftenberg, Salmonella newington,* and *Salmonella california*. The two remaining preparations consisted of single strains of the heat resistant *S. senftenberg* 775 W (kindly provided by N. A. Cox, USDA, Athens, GA) and of *S. muenster* isolated from one lot of raw milk used in the present study.

Raw milk for thermal processing was inoculated at a final cell concentration of 10^5 salmonellae per ml where each of the 10 members of the human and non-human mixtures was added at a level of 10^4 cells/ml. To prepare the inoculum, an appropriate volume of each *Salmonella* NB culture containing the required number of cells was sedimented at 13,000 × *g* for 10 min. Each pellet was suspended in 10 ml of raw milk and added to 5 L of raw milk. The complete inoculum was stored overnight at 4°C to mimic commercial holding practices and to provide for cell equilibration before heat treatment. Preliminary studies ensured accuracy of cell numbers in the inoculum and absence of substantial cell death during overnight equilibration. In these studies, NB cultures of each test organism were inoculated at a level of 10^4 cells per ml in 99 ml of (non-heated) raw milk and in milk previously heated at 100°C for 1 min. The inoculated milk was stored overnight at 4°C. Viable salmonellae were then enumerated on MacConkey agar by plating 0.1 ml of appropriate serial dilutions prepared in peptone water and incubating the plates overnight at 35°C.

Bacteriological analyses

Aerobic plate counts (APC) of raw milk were determined by the pour-plate technique. One ml of 10^0 to 10^{-3} serial dilutions of raw milk prepared in peptone water was mixed with tempered plate count agar. Upon solidification, plates were incubated for 16-18 h at 35°C and counted.

The 3-tube Most Probable Number (MPN) technique and a standard cultural procedure *(12)* were used to enumerate *Salmonella* in thermally processed milk. The amounts of milk used to determine survival depended on treatment temperature. For example, triplicate volumes of 100, 10 and 1 ml of heat-treated milk (≥64.5°C) were preenriched overnight at 35°C in nine volumes of brilliant green water (BGW) for 16-18 h at 35°C. At lower treatment temperatures, triplicate 1-ml portions of selected serial dilutions ranging from 10^{-1} to 10^{-4} were preenriched in nine volumes of skim milk broth with added brilliant green *(12)*. This change in preenrichment medium was introduced because preliminary data showed an inability of BGW to consistently recover all test strains of salmonellae from 10^{-2} or greater dilutions. Such limitation in method sensitivity likely resulted from the toxicity of brilliant green and the growth-limiting amounts of nutrients in the volume of diluted milk analyzed. Portions (1 ml) of each preenrichment culture were then selectively enriched in nine volumes of tetrathionate brilliant green (TBG) and selenite cystine (SC) broths incubated for 16-18 h at 43 and 35°C, respectively. Each enrichment culture was streaked on bismuth sulfite (BSA) and brilliant green sulfa (BGS) agar and incubated for 16-18 h at 35°C. Suspect colonies were screened biochemically on triple sugar iron (TSI) and lysine iron (LI) agars and confirmed serologically using polyvalent and single grouping antisera. Levels of *Salmonella* survival at each test temperature was determined from an MPN conversion of positive results. Bacteriological media were obtained from Difco Laboratories (Detroit).

Pasteurizer

The pasteurizer (Fig. 1) was a regenerative plate unit (Junior Paraflow, APV-Crepaco, Toronto) with 24 passages for milk in each of the up- and down-sides of the regenerative section, and nine passages in the final heat section. Minimum residence times estimated with a methylene blue solution were 11.4 s and 4.3 s in the regeneration and final heating sections, respectively. A level of regeneration in the heat exchanger of 85% was estimated from the milk temperature in the final-heat and regeneration sections. The pasteurized milk section was not maintained under positive pressure with respect to the raw milk section. A back-pressure, spring-loaded compression valve (model D60TM, Ladish Co., Brantford, Ont.) was fitted at the pasteurizer outlet and used for fine adjustment of the flow rate at 363 kg (800 lb) of whole milk per hour.

The stainless steel holding tube of the pasteurizer was approximately 421 cm in length with an internal diameter of 2.2 cm, and insulated with 2-cm thick tubes of foamed plastic wrapped in aluminum foil. The Reynolds Number for milk at 72°C was estimated at 10,500, a value that is well above the minimum of 4,000 required for turbulent flow. Increased turbulence reduces plug flow of liquid in the holding tube and the length of tube required to ensure that all parts of milk are heated for the selected time with a concomitant reduction in the average holding time. The minimum holding time is the time required for the first particles of milk to pass through the holding tube, and is less than the average holding time. The minimum holding time was determined with electrodes (Milk Tester, model MTC 1000, Lamenite Electronic Co. Inc., Franklin Park, IL) as recommended by the American Public Health Association *(1)*. The mean minimum holding time derived from nine separate measurements was 16.2 s (standard deviation of ±0.5 s). An average holding time of 17.6 s was estimated from the flow rate and volume of water in the holding tube at 25°C.

The temperature of milk in the pasteurizer was controlled with an HTST Celsius Fulscope Controller (Model 352, Taylor Instrument Co., Toronto, Ont.) which operated an air-activated variable steam-injection valve in the hot-water circulation system. Temperatures were set using the variable "Set-Pointer Adjusting Knob" in the control panel. Thermocouples (Fig. 1) were used to monitor milk or water temperatures: raw milk entering the regeneration section (TC_1); untreated milk between the regeneration and final-heat sections (TC_2); heated milk entering (TC_3) and leaving (TC_4) the holding tube; treated milk leaving the regeneration section (TC_5); treated milk leaving the cooling section (TC_6); hot water entering (TC_7) and leaving (TC_8) the pasteurizer. The stainless sheath, grounded Type T thermocouples were connected to a Digistrip II recorder (model DR-1A, Kaye Instruments Inc., Bedford, MA) set to read the temperature at the inlet of the holding tube at 2-s intervals and to provide a print-out of all temperature measurements at 1-min intervals. A calibrated mercury thermometer graduated at 1.0°C intervals was placed at the inlet of the holding tube; readings were estimated within 0.1°C. The thermocouples and mercury thermometer were calibrated at the Heat and Thermometry Laboratory, Physics Division, National Research Council of Canada (NRC). All were accurate within ±0.2°C in the 60°C to 72°C range. Reported temperatures are the corrected values.

Milk flow

For each trial, 1200 L of raw, whole milk from local herds was pumped from a delivery truck to a water-cooled, 1670-L bulk tank (model FTS-18, Cherry-Burrel Corp., Cedar Rapids,

IA). Milk was held overnight in the bulk tank or occasionally received on the day of testing. The bulk tank was connected through a three-way valve to a variable speed, positive-displacement pump (model 10DO, Waukesha Foundry Corp. Inc, Waukesha, WI) and to an overflow reservoir used for start-up (Fig. 1). The heat-treated milk was channeled to a 1370-L vat (Alpha-Laval, Peterborough, Ont.) fitted with a three-way valve for in-line milk sampling and weigh-scale checking of flow rate. The estimated time for transfer of heat-treated milk from the holding tube of the pasteurizer to the sampling point was 40 s.

Upon completion of an experimental trial, the bulk tank was filled with hot water (85°C) and left standing for 60 min. The contents of the bulk tank were then passed through the pasteurizer operated at ≥85°C. The contents of the receiving vat were decontaminated at the conclusion of each trial by heating to 85°C and holding the milk overnight. Environmental sampling after each trial failed to uncover any cross-contamination of the premises and underlined the efficacy of thermal and chemical disinfection of equipment in 200-400 mg/L Roccal (National Laboratories, Sterling Drug Ltd., Toronto, Ont.).

Heat treatment

At the beginning of each trial, a 1-L raw milk sample was taken from the bulk tank for SPC and qualitative *Salmonella* determinations. An appropriate *Salmonella* inoculum prepared in 5 L of raw milk, as described previously, was then added to the bulk tank containing 1200 L of milk. The inoculated milk was mechanically stirred for 10 min, after which a 1-L sample was taken (t_o). Concomitantly, the pasteurizer was equilibrated at 74°C with water from the overflow reservoir. To initiate a trial, the pasteurizer was switched from water to inoculated milk and the flow rate determined by the weigh-scale technique at regular intervals; the back-pressure valve was used to make necessary adjustments. In the last four trials, an accumulating magnetic milk meter (model 7601, Emerson Electric Co., Statesboro, GA) installed between the pasteurizer outlet and receiving vat was also used to measure flow rate.

Thermal inactivation of salmonellae was studied in the range of 60-74°C where the pasteurizer temperature was first set at 74°C and then gradually decreased to the next lower test temperature through fine adjustment of the HTST steam-water heating system. Each temperature change required 15 to 20 min. The nature of the control system resulted in a drop of 0.5°C below the target temperature before stabilization. After equilibration of the pasteurizer at the target temperature for a minimum of 5 min, a 1-L sample of heat-treated milk was collected at the inlet of the receiving vat and immediately stored on ice pending analysis. This procedure was repeated for each test temperature. At the conclusion of the trial, a 1-L sample of inoculated raw milk was taken from the bulk tank for *Salmonella* analysis (t_f).

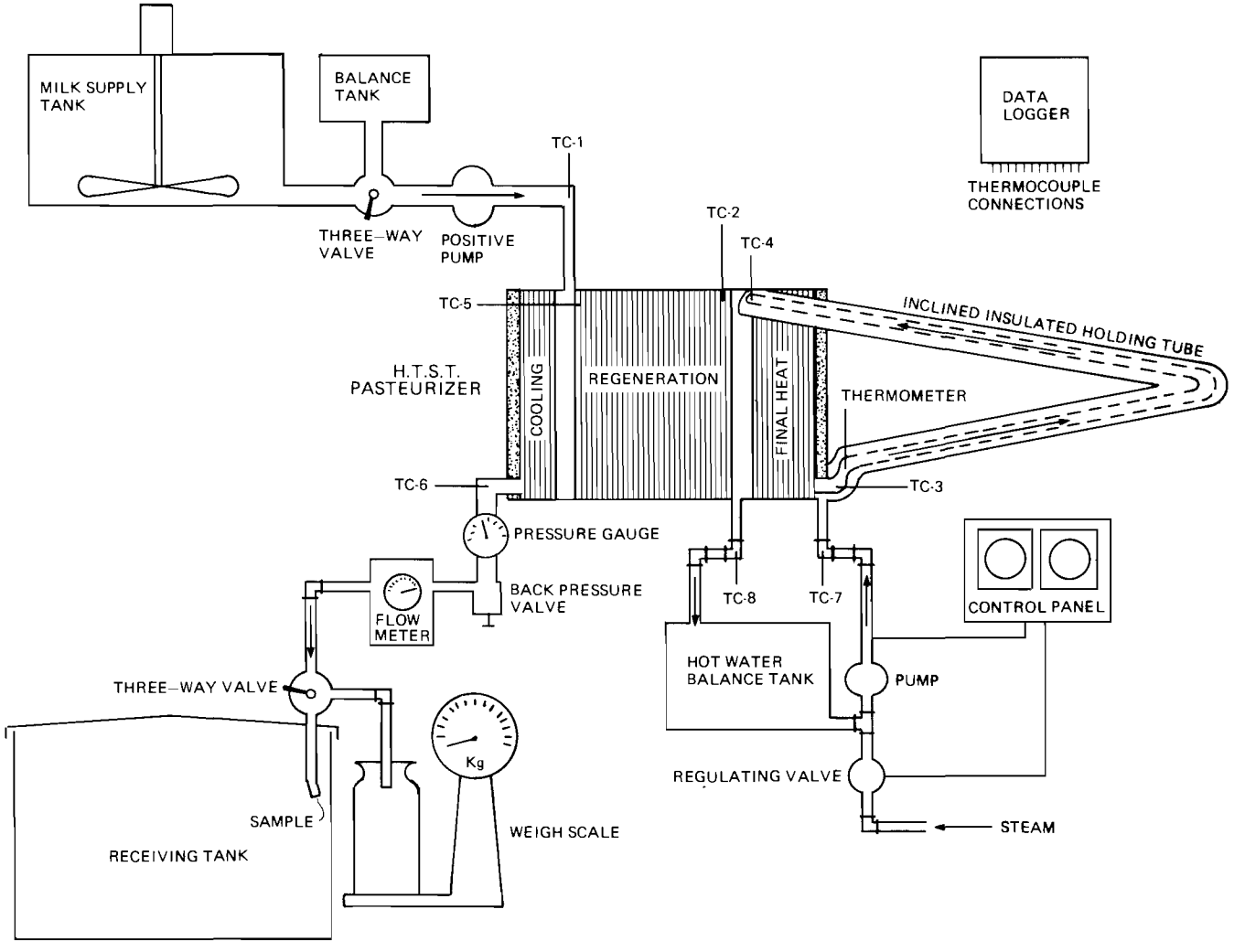

Figure 1. *System for thermal treatment of milk.*

Calculation of decimal reduction times (D)

D values were calculated using the following equation *(19)* based on first order reaction kinetics:

$$k = \frac{2.303}{t} \log \frac{a}{b}$$

where a = initial number of cells
b = number of surviving cells at time t
t = heating time (17.6 s)
D = time to destroy 90% of cells

and $D = \frac{2.303}{k}$

RESULTS AND DISCUSSION

A non-exhaustive survey of the 233 registered cheese manufacturing plants (A. R. Goll, Agriculture Canada, personal communication) showed wide variations in the thermal processing of cheese-milk (Tables 1 and 2). Of 90 plants surveyed by the Health Protection Branch, 31 produced Cheddar cheese from heat-treated (non-pasteurized) milk and 41 from pasteurized milk. Two plants in Ontario and one in Nova Scotia produce Cheddar from raw, non-heated milk. One plant in British Columbia also uses raw milk to manufacture Gouda cheese. Various regimens for milk pasteurization involving temperatures ranging from 71.6°C to 76.1°C and 13 to 22 s holding times were used in Cheddar production (data not shown). Thermal processes for manufacture of ethnic cheeses showed even greater disparity. A more detailed examination of subpasteurizing temperatures used in Cheddar production showed that 15 (54%) of the 28 plants treated milk at ≤64.5°C (Table 2). These data provided the bases for the range of test temperatures used in the present study.

The APC of raw milk ranged from 1.9×10^2 to 5.5×10^3 with a median count of 2.5×10^3 cells/ml (Table 3). These values are well within the norms for milk produced under good sanitary conditions *(18)*.

TABLE 2. *Subpasteurization of cheese-milk in Canadian Cheddar production.*

Treatment temperature (°C)	Number of plants
60.0-61.5	1
>61.5-63.0	11
>63.0-64.5	3
>64.5-66.0	6
>66.0-67.5	2
>67.5-69.0	5
TOTAL	28

No major problems were encountered in the preparation and equilibration of *Salmonella* inocula in refrigerated raw milk. Under standard growth conditions, triplicate sets of results showed that *Salmonella* test strains grew to comparable cell concentrations, ranging from 1.5 to 8.2×10^8 cells/ml with a median value of 3.3×10^8 cells/ml (data not shown). Ancillary work also showed minimal cell death during overnight refrigerated equilibration of inocula in heated and non-heated raw milk. In contrast to work reported with *Campylobacter jejuni (5)*, the milk lactoperoxidase system apparently did not adversely affect survival of salmonellae under the storage conditions used in the present study. High initial (t_o) levels of contamination were selected to enable determination of high decimal reductions. Although detailed studies on the number of *Salmonella* in raw milk have yet to be published, such high levels of contamination might result from abuse of raw milk on the farm, in transit to the cheese plant, or from active growth during a refrigeration breakdown in bulk receiving tanks.

Thermal processing at >64.5°C for a minimum holding time of 16.2 s was effective in reducing the numbers of all test strains to undetectable levels except *S. senftenberg* 775W which survived treatment at 67.5°C (Table 3). The

TABLE 1. *Thermal treatment of cheese-milk in Canada.*

	Number of plants				
		Subpasteurization		Pasteurization[b]	
Province	Total[a]	Cheddar	Others[c]	Cheddar	Others
British Columbia	7	1	2[d]	2	2
Alberta	10	3	0	5	9
Manitoba	5	5	0	5	3
Ontario	49	16[e]	2	14	39
Québec	15	2	0	13	11
Nova Scotia	2	2[e]	0	1	0
New Brunswick	1	1	0	0	1
Prince Edward Island	1	1	0	1	1
TOTAL	90	31	4	41	66

[a]Several plants manufactured a variety of cheeses from pasteurized and non-pasteurized milk.
[b]Pasteurization at ≥72°C for 16 s or at 60-63°C for ≥30 min. Higher temperatures and/or longer periods of heat treatment were applied for ricotta and other ethnic cheese.
[c]Includes Mozzarella, Gouda, Colby, ricotta, farmer's, brick, Camembert and other varieties.
[d]One plant manufactured Gouda from raw, non-heated milk.
[e]Two plants in Ontario and one in Nova Scotia manufactured Cheddar from raw, non-heated milk.

TABLE 3. *Thermal inactivation of* Salmonella *in fluid milk.*

	Bulk tank milk		Level of *Salmonella*[b]		Viable *Salmonella* in heat-treated milk[b] Treatment temperature (°C)									
Trial	APC[a]	Salmonella	t_o	t_f	60.0	61.5	63.0	64.5	66.0	67.5	69.0	70.5	72.0	74.0
Human mixture														
1	2.5×10^3	-	1.1×10^6	2.4×10^5	36	-[c]	-	-	-	-	-	-	-	-
2	1.9×10^2	-	4.6×10^5	2.4×10^5	3.9	0.21	-	-	-	-	-	-	-	-
3	2.9×10^3	-	2.4×10^5	1.1×10^6	240	2.3	-	-	-	-	-	-	-	-
4	5.5×10^3	-	4.6×10^5	4.6×10^5	24	0.015	-	-	-	-	-	-	-	-
Non-human mixture														
5	2.9×10^3	+[c,d]	2.4×10^5	2.4×10^5	9.3	2.3	0.003	-	-	-	-	-	-	-
6	3.9×10^3	-	2.4×10^6	1.5×10^5	>110	9.3	2.4	0.0073	-	-	-	-	-	-
7	3.3×10^3	-	2.3×10^5	2.3×10^5	2.3	0.04	-	-	-	-	-	-	-	-
S. senftenberg 775W														
8	2.4×10^3	+[c]	2.4×10^5	4.6×10^5	930	430	9.3	9.3	0.93	0.093	-	-	-	-
9	1.0×10^3	-	1.5×10^5	1.5×10^5	4600	43	15	2.3	0.21	0.0091	-	-	-	-
S. muenster														
10	9.2×10^2	-	2.4×10^5	1.1×10^6	230	0.29	-	-	-	-	-	-	-	-
11	8.4×10^2	-	$>1.1\times10^6$	4.6×10^5	9.3	2.3	0.023	-	-	-	-	-	-	-

[a]Acrobic plate count (APC) per ml.
[b]*Salmonella* per ml determined by the 3-tube most probable number (MPN) technique.
[c]*S. muenster* was isolated.
[d]Milk used to prepare the inoculum also contained *Salmonella muenster, Salmonella albany* and *Salmonella mbandaka.*
[e]Temperature of 61.1-61.3°C actually attained.

TABLE 4. *Decimal reduction times (D).*

Trial		D values (min) Treatment temperature (°C) 60.0	61.5	63.0	64.5	66.0	67.5
Human mixture							
1	t_o	0.065[a] (4.5)[b]					
	t_f	0.077 (3.8)					
2	t_o	0.058 (5.1)	0.046 (6.4)				
	t_f	0.061 (4.8)	0.048 (6.0)				
3	t_o	0.098 (3.0)	0.059 (4.9)				
	t_f	0.080 (3.7)	0.052 (5.6)				
4	$t_o = t_f$	0.069 (4.3)	0.039 (7.5)				
Non-human mixture							
5	$t_o = t_f$	0.067 (4.4)	0.059 (5.0)	0.037 (7.9)			
6	t_o	0.068 (4.3)	0.054 (5.4)	0.049 (6.0)	0.034 (8.5)		
	t_f	0.094 (3.1)	0.070 (4.2)	0.061 (4.8)	0.040 (7.3)		
7	$t_o = t_f$	0.059 (4.9)	0.043 (6.8)				
S. senftenberg 775 W							
8	t_o	0.122 (2.4)	0.107 (2.7)	0.067 (4.4)	0.067 (4.4)	0.054 (5.4)	0.046 (6.4)
	t_f	0.109 (2.7)	0.097 (3.0)	0.063 (4.7)	0.063 (4.7)	0.052 (5.6)	0.044 (6.7)
9	$t_o = t_f$	0.193 (1.5)	0.083 (3.5)	0.073 (4.0)	0.061 (4.8)	0.050 (5.9)	0.041 (7.2)
S. muenster							
10	t_o	0.097 (3.0)	0.050 (5.9)				
	t_f	0.080 (3.7)	0.045 (6.5)				
11	t_o	0.058 (5.1)	0.052 (5.6)	0.038 (7.7)			
	t_f	0.063 (4.7)	0.055 (5.3)	0.040 (7.3)			

[a]D-values (min.) calculated using t_o and t_f as the initial number of microorganisms.
[b]Equivalent $\log_{10}$ reductions.

thermal resistance of human and non-human mixtures was similar, exhibiting a minimum 3 $\log_{10}$ reduction in counts at 60°C. Notably, milk contaminated with the non-human mixture (trial 6) resulted in an unusually high heat resistance at 64.5°C (7.3 $\log_{10}$ reduction). The thermal behavior of *S. muenster* originally isolated from raw milk was unremarkable and failed to indicate any milk-adapted response (trials 10 and 11).

Decimal reduction times (D) in Table 4 were calculated from data presented in Table 3 *(13,19)*. Within the limitations imposed by calculation of D values from few data obtained at a fixed processing time, our D values compared favorably with published values. The D values at 60°C ranged from 0.058-0.098 min, and from 0.037-0.061 min at 63°C for all strains except *S. senftenberg* 775 W (Table 4). Similar values (0.060-0.095 min) were reported earlier with six strains of *Salmonella* heated at 62.8°C in sterile whole milk *(17)*. However, the $D_{66°C}$ = 0.050-0.054 for *S. senftenberg* 775 W in our study was substantially lower than the $D_{65.6°C}$ = 0.57-1.00 reported by other workers and may reflect strain-specific resistance to thermal inactivation *(17,19)*.

Ability to effect a 4 $\log_{10}$ reduction in viable salmonellae at lower processing temperatures should not lead to complacency (Table 4). Earlier studies showed that bacterial heat resistance is not only dependent on the water activity (a_w) of the heating menstruum but also on the nature of dissolved solutes *(3,6,11)*. The ability of non-autoclaved, whole milk to increase the heat resistance of heat-stressed *S. anatum* greatly exceeded that obtained with proteins, peptides, carbohydrates, gums and stabilizers *(15)*. Data further showed that this thermal stability in whole milk was neither casein- nor lactose-dependent, and was lost during autoclaving. Thermal resistance of bacterial pathogens does not appear to be a stable characteristic and may respond to changes in milk composition or physiological state of the contaminating microflora *(17)*. The unusual thermostability of *S. senftenberg* 775W at high a_w is well documented *(11,14,17,22)*, and use of this organism in our study does not necessarily correspond to an artificial laboratory situation. Standard *Salmonella* identification schemes are based on biochemical and serological evidence, and do not entail heat resistance profiles as diagnostic tools. Therefore, recognition of raw milk as a potential ecological niche of *S. senftenberg* 775W and other heat resistant strains such as *S. irumu* and *S. typhimurium (4,6)* is not unreasonable.

Mean temperatures for the 11 trials were ≤0.2°C (thermocouples) and ≤0.3°C (mercury thermometer) lower than target temperatures (Table 5). These observations indicate that nominal temperatures for data in Table 3 generally err on the low side and therefore tend to underestimate the lethality of the thermal process. It is further suggested that the effect of the ≤0.6°C difference between the minima and maxima thermocouple readings (Table 5) would be negligible given the wide variations in decimal reductions observed in replicate trials at a given temperature (Table 4). Flow rate fluctuations of

≤4% in trials 6 and 10, and of 5-10% in trials 2 and 4 were noted. An acute decrease to 61.5°C during adjustment of the pasteurizer to 66°C in trial 3 was also observed. In no instance did these operational discrepancies appear to influence final results.

Figure 2 shows approximate temperature profiles at two processing temperatures for our pilot-scale and commercial units (D. Voloshin, APV-Crepaco, personal communication). The slower temperature increases and decreases in the pilot-scale unit would result in greater heat effects compared to a commercial unit with the same holding-tube residence time. The length of the holding tube on the pilot-scale unit was measured from its junction with the plates of the final heat section. Commercial units are timed from points exterior to the plates and frame of the pasteurizer, and usually include an additional length of tube as a safety factor (G. Cavan, APV-Crepaco, personal communication). The greater heat effect and shorter holding tube in the pilot-scale unit would tend to counterbalance each other in terms of lethality but the quantitative interaction between these factors is unknown.

TABLE 5. *Inlet temperature of the pasteurizer holding tube.*

Treatment temperature (°C)	Thermocouple reading (°C)			Mercury thermometer reading (°C)
	Mean[a]	Maximum	Minimum	Mean[a]
74.0	73.8 ± 0.16	74.1	73.5	73.7
72.0	71.9 ± 0.13	72.1	71.7	71.7
70.5	70.5 ± 0.16	70.8	70.2	70.2
69.0	68.8 ± 0.21	69.1	68.5	68.8
67.5	67.4 ± 0.18	67.7	67.1	67.2
66.0	65.9 ± 0.14	66.1	65.7	65.8
64.5	64.3 ± 0.17	64.5	63.9	64.3
63.0	62.9 ± 0.16	63.0	62.6	62.8
61.5	61.4 ± 0.15	61.6	61.1	61.2
60.0	60.0 ± 0.12	60.1	59.8	59.8

[a]Mean of eleven trials with corresponding standard deviations. Readings were corrected on the basis of NRC calibrations.

The ability of salmonellae to survive for up to 10 months in Cheddar cheese is well documented *(8,16,21)*. Regulations requiring 60-d aging of cheese manufactured from heat-treated (non-pasteurized) milk are therefore ineffective for decontamination of *Salmonella*-infected cheese. Contrary to the general belief that ingestion of large numbers of salmonellae is required to cause human illness, recent evidence suggests that a single *Salmonella* cell in Cheddar cheese constitutes an infective dose *(7)*. Intermittent passage of few salmonellae into a finished product because of non-calibrated holding tubes or plates, inaccurate thermosensors or other mechanical malfunctions could have devastating effects on the economic via-

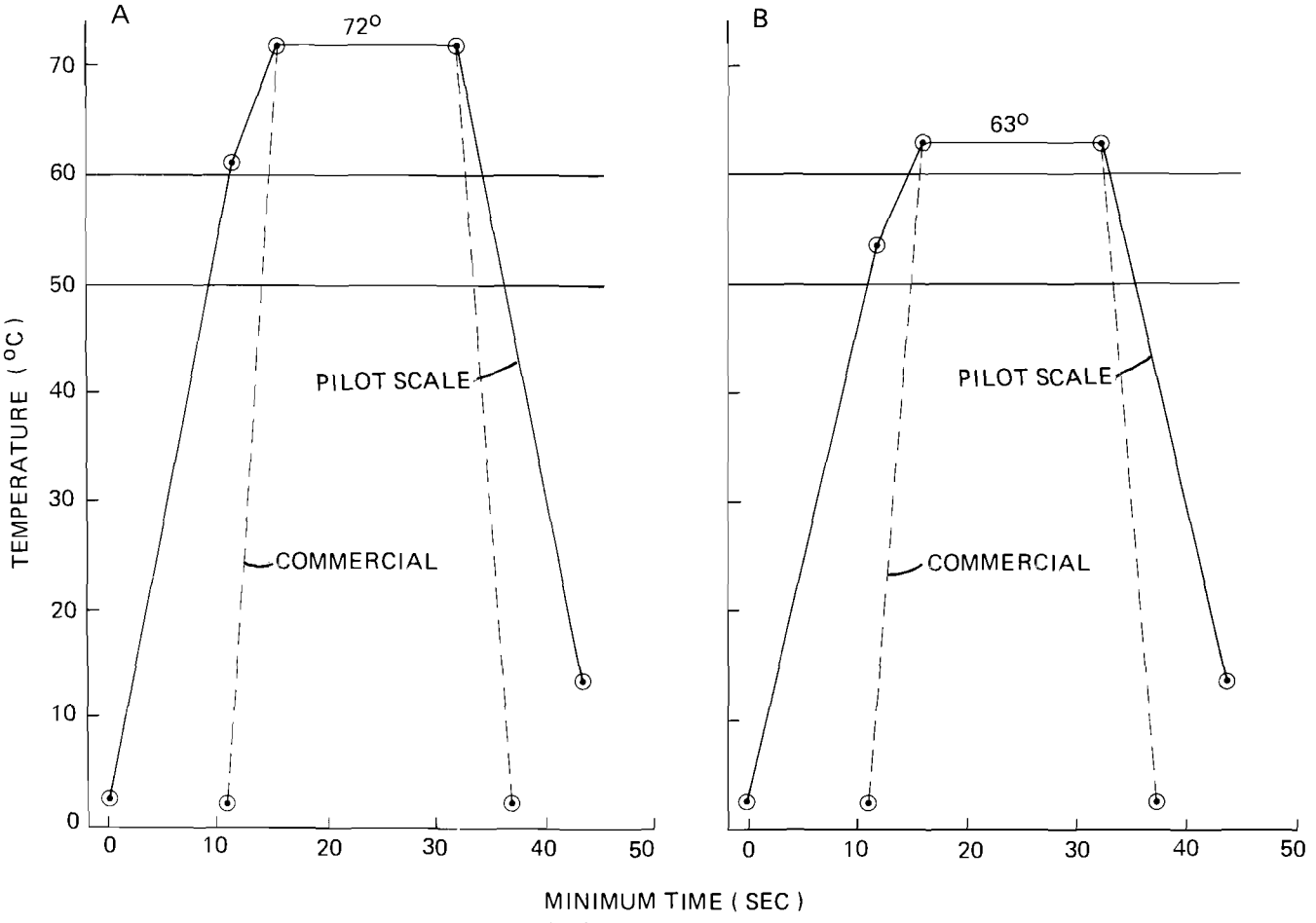

Figure 2. *Temperature profiles during heat treatment of milk.*

TABLE 6. *Temperatures at selected points in the milk processing line.*

Thermocouple measurement point	Thermal treatment					
	72°C			63°C		
	Mean[a]	Max	Min	Mean[a]	Max	Min
Raw milk entering HTST (TC_1)	3.0 ± 1.16	5.5	2.0	2.5 ± 1.59	5.9	1.5
Milk at the regeneration - final heat interface (TC_2)	61.5 ± 0.56	62.5	60.7	53.6 ± 0.46	54.2	52.8
Milk entering holding tube (TC_3)	71.9 ± 0.13	72.1	71.7	62.9 ± 0.16	63.0	62.6
Milk leaving holding tube (TC_4)	71.8 ± 0.17	72.0	71.5	62.8 ± 0.19	63.0	62.5

[a]Mean of 11 trials with corresponding standard deviation.

bility of a negligent manufacturer. Production of cheese from non-heated milk and sale of unpasteurized or certified raw milk *(9)* ignore the vast body of pertinent epidemiological data on foodborne outbreaks and available technology for the manufacture of safe and wholesome milk and milk products.

In addition to *Salmonella* spp., elimination of pathogens such as *Listeria, Yersinia, Campylobacter* and hemorrhagic *Escherichia coli* (0157:H7) at the level of the pasteurizer is germane to the marketing of safe milk and milk products. Studies on the heat survival of the latter organisms were recently completed in our laboratories and will be the subject of future reports.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the collaboration of R. Burke and inspectors of the Field Operations Directorate, Health Protection Branch, in providing data on thermal treatment of milk in Canadian cheese plants; the technical assistance of D. Beckett and R. Cooligan of the Food Research Centre of Agriculture Canada, and A. Jean, Food Directorate of the Health Protection Branch; the assistance of M. Gemme and G. Busque, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, in calibrating the holding tube.

REFERENCES

1. American Public Health Association. 1967. Accepted practices for the sanitary construction, installation, testing and operation of HTST pasteurizers. Standard 603-05, revised.
2. Anonymous. 1982. Presence of *Salmonella muenster* in Ontario cheese. Ont. Dis. Surveillance Rep. 3:139-143.
3. Baird-Parker, A. C., M. Boothroyd, and E. Jones. 1970. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of salmonellae. J. Appl. Bacteriol. 33:515-522.
4. Bersani, C., and C. Cantoni. 1983. Termoresistenza della *Salmonella irumu*. Arch. Vet. Ital. 34:118-121.
5. Beumer, R. R., A. Noomen, J. A. Marijs, and E. H. Kampelmacher. 1985. Antibacterial action of the lactoperoxidase system on *Campylobacter jejuni* in cow's milk. Neth. Milk Dairy J. 39:107-114.
6. Corry, J. E. L. 1974. The effect of sugars and polyols on the heat resistance of salmonellae. J. Appl. Bacteriol. 37:31-43.
7. D'Aoust, J.-Y. 1985. Infective dose of *Salmonella typhimurium* in Cheddar cheese. Am. J. Epidemiol. 122:717-720.
8. D'Aoust, J.-Y., D. W. Warburton, and A. M. Sewell. 1985. *Salmonella typhimurium* phage-type 10 from Cheddar cheese implicated in a major Canadian foodborne outbreak. J. Food Prot. 48:1062-1066.
9. Fierer, J. 1983. Invasive *Salmonella dublin* infections associated with drinking raw milk. West. J. Med. 138:665-669.
10. Fontaine, R. E., M. L. Cohen, W. T. Martin, and T. M. Vernon. 1980. Epidemic salmonellosis from Cheddar cheese: surveillance and prevention. Am. J. Epidemiol. 111:247-253.
11. Goepfert, J. M., I. K. Iskander, and C. H. Amundson. 1970. Relation of the heat resistance of salmonellae to the water activity of the environment. Appl. Microbiol. 19:429-433.
12. Health Protection Branch. 1978. Methods for the isolation and identification of *Salmonella* from foods. Acceptable (HPB) method MFA-20. Health and Welfare Canada, Ottawa.
13. Kessler, H. G. 1981. Food engineering and dairy technology. Verlag A. Kessler, Freising, West Germany, pp. 174-183.
14. McDonough, F. E., and R. E. Hargrove. 1968. Heat resistance of *Salmonella* in dried milk. J. Dairy Sci. 51:1587-1591.
15. Moats, W. A., R. Dabbah, and V. M. Edwards. 1971. Survival of *Salmonella anatum* heated in various media. Appl. Microbiol. 21:476-481.
16. Park, H. S., E. H. Marth, J. M. Goepfert, and N. F. Olson. 1970. The fate of *Salmonella typhimurium* in the manufacture and ripening of low-acid Cheddar cheese. J. Milk Food Technol. 32:280-284.
17. Read, R. B., Jr., J. G. Bradshaw, R. W. Dickerson, and J. T. Peeler. 1968. Thermal resistance of *Salmonella* isolated from dry milk. Appl. Microbiol. 16:998-1001.
18. Sims, J. E., D. C. Kelley, and V. D. Foltz. 1969. Effects of time and temperature on salmonellae in inoculated butter. J. Milk Food Technol. 32:485-488.
19. Stumbo, C. R. 1973. Thermobacteriology in food processing, 2nd ed. Academic Press, New York. pp. 70-92.
20. Sun, M. 1985. Desperately seeking *Salmonella* in Illinois. Science 228:829-830.
21. White, C. H., and E. W. Custer. 1976. Survival of *Salmonella* in Cheddar cheese. J. Milk Food Technol. 39:328-331.
22. Winter, A. R., G. F. Stewart, V.H. McFarlane, and M. Solowey. 1946. Pasteurization of liquid egg products III. Destruction of *Salmonella* in liquid whole egg. Am. J. Pub. Hlth. 36:451-460.