HEAT RESISTANCE OF *CLOSTRIDIUM PERFRINGENS* VEGETATIVE CELLS IN MEAT AS DEFINED BY D AND Z-VALUES

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INTRODUCTION

Clostridium perfringens is an important cause of food poisoning world-wide (Stringer et al. 1980). Outbreaks primarily involve meat and meat products; the illness occurs due to ingestion of approximately 10^6 to 10^7 viable cells per gram of food (Hauschild 1970). The ingested cells sporulate in the intestine and release a heat-labile enterotoxin known as *C. perfringens* enterotoxin (CPE), which is responsible for typical symptoms (Duncan 1975).

Juneja et al. (1994b) observed that *C. perfringens* spores germinated and grew from an inoculum of approximately 1.5 $\log_{10}$ to about 6.0 $\log_{10}$ cfu/g when the cooling time to achieve 7.2°C was extended to 18 h. These authors reported that pasteurized cooked beef must be cooled to 7.2°C in 15 h or less to prevent growth from spores of *C. perfringens*. If the product did not receive sufficient heat to kill spores, and the cooling rate after heating is too slow, vegetative cells produced due to growth from spores of *C. perfringens* must be killed by sufficient reheating before consumption to guard against food poisoning. Lack of data on the thermal death time values for *C. perfringens* vegetative cells in beef and turkey that included sodium pyrophosphate (SPP) in the formulation prompted us to define the heat treatment required to give a specified lethality for *C. perfringens* vegetative cells in these products. Accordingly, we quantitatively assessed the influence of SPP in beef and turkey on thermal inactivation of *C. perfringens* vegetative cells.

MATERIALS AND METHODS

Bacteria strains, sample preparation, and inoculation

Three strains of *Clostridium perfringens*, NCTC 8238, NCTC 8239, and NCTC 10240, were used in this study. Sodium pyrophosphate was mixed into autoclaved ground beef and turkey samples with a Hobart mixer to give a final concentration of 0.15 or 0.3% (w/w). Duplicate 3g ground beef or turkey samples were aseptically weighed into 15 × 22.9 cm sterile whirl-pak sampling bags (Model B736, NASCO Modesto, CA) and inoculated with 0.1 ml of an appropriate dilution of heat-shocked (75°C/20 min) *C. perfringens* cocktail so that the final concentration of cells was approximately 8 $\log_{10}$ cfu/g. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample, compressed into a thin layer (approximately 1-2 mm thick) by pressing against a flat surface, excluding most of the air, and then heat sealed.

Thermal inactivation and enumeration

Two replications were performed for each phosphate level in beef and turkey and the control samples (with no added SPP). Bags at room temperature were placed in a basket and then fully submerged in a temperature controlled water bath (Exacal, Model Ex-251HT, NESLAB Instruments, Inc., Newington, NH) stabilized at 55, 57.5, 60, 62.5 or 65°C. The temperature was continuously monitored by two copper-constantan thermocouples inserted, prior to heat sealing, at the center of two uninoculated bags. The thermocouple readings were measured and recorded using a Keithyl-Metrabyte data logger Model DDL 4100 (Tauton, MA) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the bag internal temperature. Two bags for each replicate were then removed at designated time intervals; sampling frequency was based on the heating temperature. After removal, bags were immediately plunged into an ice-water bath, then analyzed within 30 min. Surviving bacteria were determined by spiral plating appropriate dilutions in 0.1% peptone water onto agar dishes containing Tryptose-sulfite-cycloserine (TSC) agar without egg yolk enrichment (Hauschild and Hilsheimer 1974). The TSC agar plates were overlaid with an additional 10 ml of TSC agar. After overlaying, the agar was allowed to solidify before placing dishes into anaerobic jars. The D- and z-values were calculated by linear regression.

RESULTS AND DISCUSSION

Surviving *C. perfringens* cells/g of beef or turkey were determined and logarithms were plotted against exposure time at the test temperatures. *C. perfringens* cells heated at 55 to 62.5°C in beef or turkey exhibited log-linear decline in surviving cells with time. No obvious lag periods or shoulders and tailing were evident in any of the survivor curves. Linear survival curves indicate that the *C. perfringens* cell population was homogenous in heat resistance.

The D-values in beef that included no SPP were 21.6, 10.2, 5.3 and 1.6 min at 55, 57.5, 60 and 62.5°C, respectively (Table 1). Addition of 0.15% SPP in beef reduced heat resistance at all temperatures, and the effect was quantified as significantly decreased ($p<0.05$) D-values; the values ranged from 17.9 min at 55°C to 1.2 min at 62.5°C. The heat resistance was further significantly decreased ($p<0.05$) when SPP level in beef was increased to 0.3%. When *C. perfringens* cells were heated in turkey that included 0.15 or 0.3% SPP or with no SPP, D-values calculated were significantly less ($p<0.05$) when compared with the corresponding temperatures D-values in beef with and without SPP (Table 1). The only exception was the D-value of 1 min in beef or turkey supplemented with 0.3% SPP. As observed in beef, SPP rendered the cells sensitive to heat at all temperatures in turkey. Regression curves calculated for the four heating temperatures (55, 57.5, 60, and 62.5°C) yielded r^2 values of greater than 0.90.

Thermal death time curves were plotted from the D-values obtained in beef and turkey with and without SPP in order to calculate z-values. Z-values in beef, with and without added SPP, calculated from the curve between 55 and 62.5°C ranged from 6.22°C to 6.74°C (Figure 1). The values in turkey were very similar and ranged from 6.44 to 6.77°C (Figure 1).

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When *C. perfringens* cells inoculated in beef (8 $\log_{10}$ cfu/g) were heated at 65°C for 15 sec, as high as 6 $\log_{10}$ cfu/g of *C. perfringens* cells were recovered (Table 2). This could be overcome since heating at 65°C for 1 min completely inactivated 8 $\log_{10}$ cfu/g of *C. perfringens* cells. Since SPP increased the sensitivity of cells to heat, heating for 30 s at 65°C inactivated the same number of cells. In turkey, regardless of the presence of SPP, heating at 60°C for 30 s was adequate to inactivate a high number of cells. These results, with respect to reheating temperature for inactivation of large numbers of *C. perfringens* cells, are in agreement with our earlier data (Juneja et al. 1994a). In that study, we reported that reheating cooked beef to an internal temperature of 65°C before consumption would prevent food poisoning since the vegetative cells were killed.

Control measures for *C. perfringens* food poisoning must ensure that large numbers of vegetative cells are not consumed. Our study has shown a significant reduction in vegetative cells of *C. perfringens* by reheating precooked products to an internal temperature of 65°C for 1 min immediately before consumption of the product. The data presented in Table 1 can be used as an aid to predict the time required at specified temperatures to achieve a certain number of log-cycle reductions of *C. perfringens* when heated in precooked beef or turkey. Based on the thermal-death-time values determined in this study, contaminated cooked beef should be reheated to an internal temperature of 62.5°C for at least 9.6 min and turkey for 7.8 min; this is based on the argument that reheating temperature must destroy at least 6 $\log_{10}$ cfu/g of *C. perfringens* vegetative cells. Also, the present study suggests that in the presence of 0.3% SPP in beef or turkey, 6 min at 62.5°C would be sufficient to achieve a 6-D process. Thermal death time values from this study will assist restaurants and institutional food service settings in designing acceptance limits on critical control points that ensure safety against *C. perfringens* in cooked beef and turkey.

References

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Table 1. Heat resistance (expressed as D-values in min) of *Clostridium perfringens* three-strain mixture in precooked ground beef and turkey that contained 0, 0.15 or 0.30% sodium pyrophosphate at 55-62.5°C

Heating Menstruum	Temperature (C)			
	55	57.5	60	62.5
Beef + No SPP[a]	21.6 ± 0.1	10.2 ± 0.0	5.3 ± 0.0	1.6 ± 0.0
Beef + 0.15% SPP	17.9 ± 0.3	9.4 ± 0.0	3.5 ± 0.0	1.2 ± 0.0
Beef + 0.3% SPP	16.2 ± 0.2	7.8 ± 0.1	3.0 ± 0.0	1.0 ± 0.0
Turkey + No SPP	17.5 ± 0.1	9.1 ± 0.0	4.2 ± 0.1	1.3 ± 0.0
Turkey + 0.15% SPP	16.2 ± 0.1	6.9 ± 0.1	3.0 ± 0.1	1.1 ± 0.0
Turkey + 0.3% SPP	14.5 ± 0.1	6.4 ± 0.1	2.8 ± 0.0	1.0 ± 0.0

[a]Sodium pyrophosphate.

Table 2. Heat resistance of *Clostridium perfringens* three-strain mixture in precooked ground beef and turkey that contained 0, 0.15 or 0.30% sodium pyrophosphate at 65°C

Heating Menstruum	Heating time (Sec.)			
	0	15	30	60
Beef + No SPP[a]	8.1 ± 0.1	6.5 ± 0.1	1.4 ± 0.3	ND[b]
Beef + 0.15% SPP	8.1 ± 0.3	5.8 ± 0.2	ND	ND
Beef + 0.3% SPP	7.9 ± 0.2	5.2 ± 0.1	ND	ND
Turkey + No SPP	8.1 ± 0.0	5.8 ± 0.1	ND	ND
Turkey + 0.15% SPP	8.0 ± 0.4	4.1 ± 0.5	ND	ND
Turkey + 0.3% SPP	8.0 ± 0.2	4.0 ± 0.0	ND	ND

[a]Sodium pyrophosphate.
[b]Not detected.

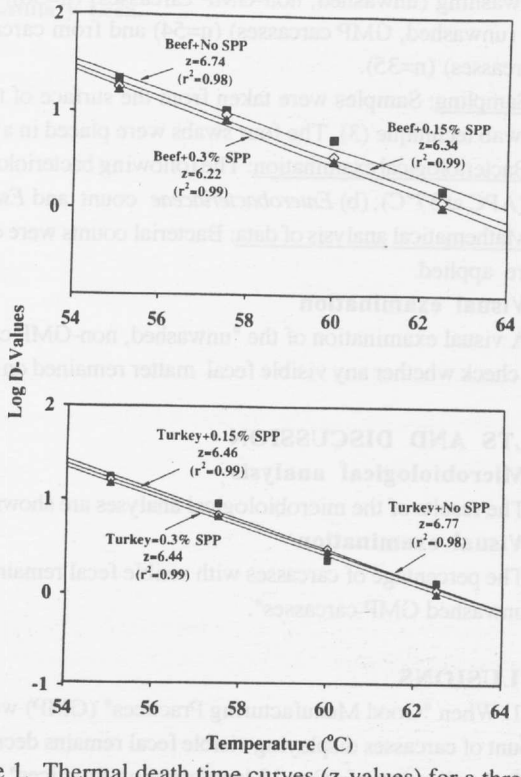

Figure 1. Thermal death time curves (z-values) for a three-strain mixture of *Clostridium perfringens* in precooked ground beef and turkey which contained 0, 0.15 or 0.30% sodium pyrophosphate, over the temperature range 55-62.5°C. D-values, used to determine the z-values, were the means of two replicates.