

The effect of temperature abuse on *Clostridium perfringens* in cooked turkey stored under air and vacuum

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The potential for growth of *Clostridium perfringens* in aerobic and anaerobic (vacuum) packaged cooked ground turkey was investigated. Samples of autoclaved ground turkey were inoculated with $\sim 3.0 \log_{10}$ cfu g^{-1} of *C. perfringens* strain NCTC 8238 or NCTC 8239, packaged and stored at various temperatures. Vegetative growth and heat-resistant spores were enumerated by plating unheated and heated (75°C for 20 min) samples, respectively, on tryptose-sulfite-cycloserine agar. The type of atmosphere influenced the growth of *C. perfringens* at 15 and 28°C. Both strains grew to about 7 logs within 9 h anaerobically and by 24 h aerobically at 28°C. While aerobic growth was slow at 15°C, mean \log_{10} cfu g^{-1} increased anaerobically by 4–4.5 logs by day 8 for both strains. Spores were not found at 4 and 15°C, but were detected as early as 24 h at 28°C under anaerobic conditions in both strains. *C. perfringens* population stabilized or slowly decreased at 4°C. Cyclic and static temperature abuse of refrigerated products for 5 h will not permit *C. perfringens* growth. However, temperature abuse of such products for relatively long periods may lead to high and dangerous numbers of organisms. Reheating such products to an internal temperature of 65°C before consumption would prevent food-poisoning since the vegetative cells were killed.

Introduction

Clostridium perfringens is an important cause of food-poisoning all over the world including the United States and Canada (Dische and Elek 1957, Stringer et al. 1980, CDC 1985). Illness occurs due to consumption of food contaminated with large number of vegetative cells, followed by intractestinal sporulation and enterotoxin formation (Hauschild 1970, Stark and Duncan 1971, Duncan

1975). In *C. perfringens* outbreaks reported for the years 1973–1987, one of the food vehicles implicated was turkey (Bean and Griffin 1990). Todd (1989a,b) estimated that 652 000 cases of food-borne *C. perfringens* illness occur each year in the US with 7.6 deaths and a yearly cost of 123 million dollars.

Precooked, vacuum-packaged, uncured refrigerated turkey products may contain *C. perfringens* spores due to the minimal thermal treatment given to such products. Also, these products may be subjected to post-heating contamination with spores or vegetative cells. *Clostridium perfringens* food-poisoning is often associated with institutional settings or large gatherings where food

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is prepared in large quantities a day or more in advance of the meal and the food is improperly stored (Bryan 1969, Duncan 1970, Zottola 1979).

Food products stored in refrigerated retail cabinets are commonly temperature abused for prolonged periods (Hall 1970). In a study by Naik and Duncan (1977), when moist cooked turkey was incubated anaerobically at 37°C, the vegetative cell count of *C. perfringens* increased from 6.20 to 8.45 log₁₀ cfu g⁻¹ and remained relatively constant at 8.0 log₁₀ cfu g⁻¹ even after 18 h of incubation. Strong and Ripp (1967) reported that the total viable counts increased from 4.0 to 6.6 log₁₀ cfu g⁻¹ within 4 to 6 h in turkey slices held at 24 or 37°C. While the previous studies investigated the fate of *C. perfringens* at higher temperatures, cyclic and static temperature abuse of refrigerated precooked turkey products, which may occur during transportation, distribution, storage or handling in supermarkets or by consumers, has not been reported in the scientific literature. *Clostridium perfringens* may grow to considerably high levels if refrigerated precooked meat products are poorly handled. Therefore, this study was designed to investigate (a) the effect of cyclic and static temperature abuse on the potential for growth and sporulation of *C. perfringens* in precooked, aerobic and vacuum packaged, refrigerated turkey, and (b) the effect of reheating of refrigerated and temperature abused turkey samples on the fate of *C. perfringens*.

Materials and Methods

Test organism

Clostridium perfringens strains NCTC 8238 (Hobbs serotype 2) and NCTC 8239 (Hobbs serotype 3) obtained from American Type Culture Collection were used in the study. Stock cultures of the organisms were main-

tained at 4°C in cooked-meat medium (Difco Laboratories, Detroit, MI, USA).

Preparation of inoculum

To prepare inocula, 0.1 ml from the stock cultures were inoculated into 10 ml of freshly prepared fluid thioglycollate medium (FTM), heat shocked at 75°C for 20 min and incubated at 37°C for 18 h. The cells were harvested by centrifugation at room temperature for 10 min at 7700 × g, the cell pellet washed twice and finally resuspended in sterile 0.1% peptone water (w/v).

Sample preparation and inoculation

Ground turkey, obtained from a local retail market, was placed in thin layers on plastic trays and autoclaved at 121°C for 15 min. The fat was poured off while the turkey was hot and the meat cooled at 4°C to an internal temperature of 25°C. The pH of the ground turkey was determined using a combination electrode (Sensorex, semi-micro, A.H. Thomas, Philadelphia, PA, USA) attached to an Orion model 601A pH meter. Duplicate 25 g ground turkey samples were aseptically weighed into filter stomacher bags (SFB-0410; Spiral Biotech., Bethesda, MD, USA) and inoculated with 1 ml of *C. perfringens* cell suspension of each strain separately so that the final concentration of cells was approximately 3 log₁₀ cfu g⁻¹. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample. Half of the bags were placed in 7" × 8" plastic barrier bags (Koch Model 01 46 09, Kansas City, MO, USA). The oxygen transmission rate of the nylon/polyethylene film was 3.5 cc/100 in² (1 in = 2.54 cm) in 24 h measured at 75°F and 75% relative humidity. The bags were evacuated to a negative pressure of 1000 millibars and heat sealed using a Multivac Model A300/16 gas packaging machine (W. Germany).

Storage, temperature abuse and sampling

The inoculated aerobic and vacuum-packaged samples were stored at 4, 15 and 28°C. Samples from each atmosphere stored at 28°C were analyzed at 3, 6, 9, 12, 24, 48, 72, 96 and 120 h. Samples stored at 15°C were analyzed on day 1, 2, 3, 4, 5 and 8; and those at 4°C on day 6, 12, 18, 24 and 30. To determine the effect of cyclic and static temperature abuse, samples stored at 4°C were moved 6 days before their scheduled sampling day (6, 12, 18, 24 and 30) to 28°C,

held at this temperature for 5 h, returned to 4°C and then plated on their scheduled sampling day. Some samples were transferred to 28°C for 5 or 18 h before plating on their scheduled sampling day.

Bacterial enumeration

On the scheduled sampling day, samples were removed and enumerated for vegetative growth and heat-resistant spores according to the procedure as described by Naik and Duncan (1977). *C. perfringens* colony forming units were determined following incubation of Tryptose-sulfite-cycloserine agar (TSC) plates for 48 h at 37°C in a GasPak system (Baltimore Biological Laboratory, Cockeysville, MD, USA).

Reheating and holding temperature

To determine the fate of *C. perfringens* vegetative cells and spores during reheating, 25 g of ground turkey samples inoculated with $3 \log_{10}$ cfu g^{-1} were vacuum-packaged and stored at 4°C for 7 days and at 28°C for 48 h. Thereafter, two bags from each incubation temperatures (4 and 28°C) were opened and a sterile copper-constantan thermocouple was placed at the center of the ground turkey. The bags were sealed under vacuum and submerged in a 65 or 98°C operating water bath (Exacal, Model EX-251HT, NESLAB Instruments, Inc., Newington, NH, USA) along with the experimental samples. The internal temperature of the samples was constantly monitored by the thermocouples. The readings were measured and recorded by a Keithly-Metrabyte data logger connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the sample temperature. Samples were removed when the internal temperature reached the target temperatures of 45, 55, 65, 75, 85 and 95°C. After reheating, packages were opened and incubated aerobically at room temperature. Samples were analyzed for *C. perfringens* vegetative cells and spores at 1.5, 3, 4.5, 6, 12 and 24 h.

Data processing

Bacterial growth curves were generated from the experimental data using the Gompertz equation (Gibson et al. 1987) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-based program was developed by W.C. Damert (US Department

of Agriculture, Eastern Regional Research Center, Philadelphia, PA, USA). The Gompertz parameter values were subsequently used to calculate generation times and lag times as described by Gibson et al. (1987).

Results and Discussion

The pH value of the cooked ground turkey used in the study was 6.46. *C. perfringens* grew rapidly in aerobic- and anaerobic (vacuum)-packaged precooked ground turkey during storage at 28°C (Fig. 1). For strains NCTC 8238, total viable counts after 24 h at 28°C increased from 1.80 to 7.23 \log_{10} cfu g^{-1} aerobically and to 7.20 \log_{10} cfu g^{-1} within 9 h anaerobically. In the case of strain NCTC 8239, mean \log_{10} cfu g^{-1} values after 24 h at 28°C increased from 1.72 to 6.39 \log_{10} cfu g^{-1} for air-storage samples and to 7.18 \log_{10} cfu g^{-1} within 9 h for vacuum-packaged samples. At 15°C, the type of atmosphere influenced the growth of *C. perfringens* with only 0.3 log increase in aerobic growth for strain NCTC 8238 and 1.58 log increase for strain NCTC 8239 by day 8 (Fig. 1). Under vacuum, growth of *C. perfringens* was rapid and mean cfu g^{-1} increased by 4–4.5 logs by day 8 for both strains.

Anaerobically, there was a decline in population densities from 2.91 \log_{10} cfu g^{-1} at day 0 to 1.02 \log_{10} cfu g^{-1} after 30 days at 4°C for strain NCTC 8238 (Fig. 2). Similar declines were seen in 4°C anaerobic storage for strain NCTC 8239 (data not shown). Also, *C. perfringens* vegetative cells of both strains were slowly destroyed during 30 days of aerobic storage at 4°C (Fig. 3).

The generation time was 22.8 min for strain NCTC 8238 and 29.4 min for strain NCTC 8239 at 28°C under aerobic conditions (Table 1). Anaerobically at 28°C, the growth was rapid and the generation times decreased to 19.2 min and 21.0 min for strains NCTC 8238

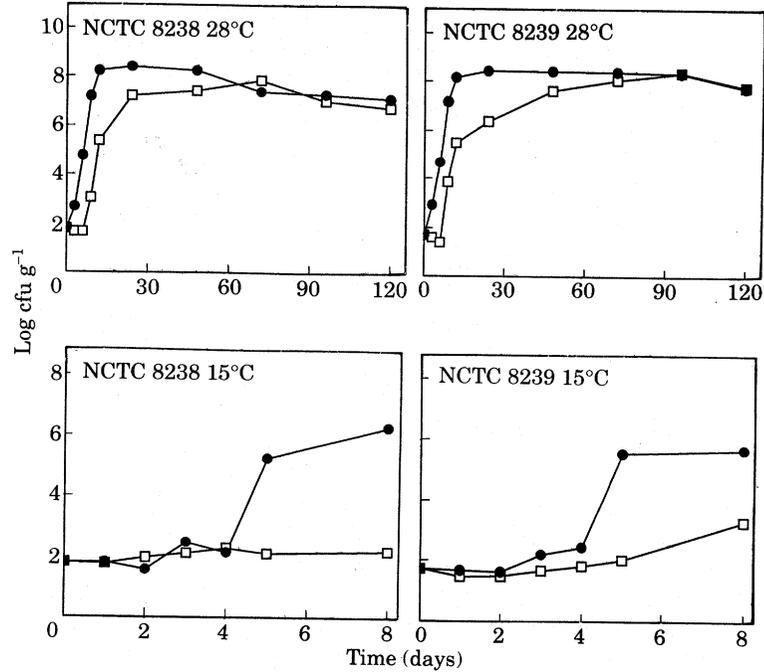


Fig. 1. The effect of temperature abuse (storage at 15 or 28°C) on growth of *Clostridium perfringens* in aerobically (□) and anaerobically (●) packaged cooked ground turkey.

and NCTC 8239, respectively. *C. perfringens* exhibited a 3× shorter lag time under vacuum at 28°C. Similarly, *C. perfringens* anaerobic growth was rapid

at 15°C (Table 1). The generation time was 43.2 min anaerobically vs about 11 h under aerobic conditions for both strains. However, both atmospheres exhibited

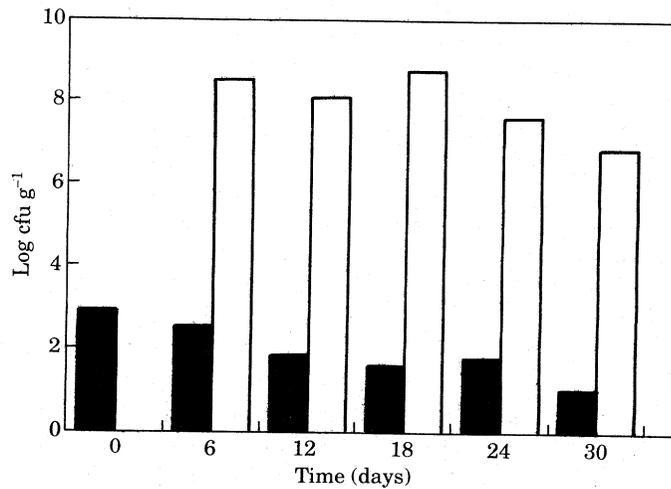


Fig. 2. The effect of static temperature abuse (28°C) on growth of *Clostridium perfringens* strain NCTC 8238 in anaerobically packaged cooked ground turkey stored at 4°C (■). The samples were moved to 28°C for 18 h before plating on their scheduled sampling day (□).

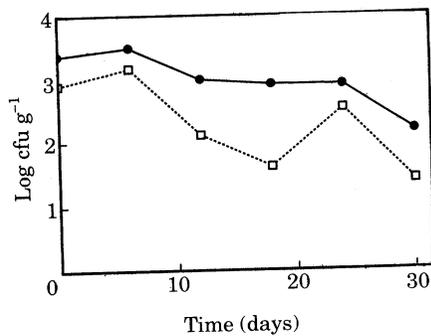


Fig. 3. Survival of *Clostridium perfringens* in aerobically packaged cooked ground turkey stored at 4°C. □, NCTC 8238; ●, NCTC 8239.

the same lag time of 3 to 4 days at 15°C. The only exception was the lag time of 1.5 days under aerobic conditions for strain NCTC 8238.

To simulate the cyclic temperature abuse of refrigerated precooked meat products which may occur in supermarkets, ground turkey samples were transferred to 28°C for 5 h, 6 days before their scheduled sampling day. On the scheduled sampling day, the total viable count in all meat samples were <3 logs regardless of the packaging or further length of storage at 4°C (data not shown). Thus, a short period of temperature abuse does not foster the growth of *C. perfringens*. When anaerobically packaged samples were temperature

abused at 28°C for 18 h before plating on their scheduled sampling day, the total viable count in all meat samples ranged from 6.86 to 8.75 log₁₀ cfu g⁻¹ (Fig. 2). Similar results were obtained when aerobically packaged samples were temperature abused at 28°C for 18 h before plating on their scheduled sampling day (data not shown). However, total viable count were <3 logs when meat samples were temperature abused for 5 h before plating (data not shown). Additional studies indicated that the cyclic and static temperature abuse of ≤10 h will not permit *C. perfringens* multiplication.

While *C. perfringens* did not sporulate at 4 and 15°C, 1.01–1.16 log₁₀ spores g⁻¹ were detected in both strains as early as day 1 at 28°C only in vacuum-packaged turkey. The type of atmosphere has little influence on sporulation at 28°C after day 1. Sporulation ranged from 4.49 to 6.41 log₁₀ cfu g⁻¹ regardless of packaging or length of storage from day 2 to day 5 (data not shown). Naik and Duncan (1977) reported 4.20 log₁₀ spores g⁻¹ within 4 h in moist cooked turkey inoculated with 6.20 log₁₀ cfu g⁻¹ and stored anaerobically at 37°C and the count was as high as 6 log₁₀ spores g⁻¹ at 18 h. Dework (1972) observed 1.94 to 3.39 log₁₀ spores g⁻¹ within 10 h in turkey rolls inoculated with 5.47 log₁₀ cfu g⁻¹ and stored anaerobically at 37°C.

Table 1. Mean generation times^{ab} of *Clostridium perfringens* in autoclaved ground turkey at various temperatures.

| Strain | Temperature | Aerobic | | Anaerobic | |
|-----------|-------------|----------------|-----------|----------------|-----------|
| | | G _t | Lag | G _t | Lag |
| NCTC 8238 | 28°C | 22.8 min | 7.47 h | 19.2 min | 2.57 h |
| | 15°C | 10.98 h | 1.55 days | 43.2 min | 3.97 days |
| NCTC 8239 | 28°C | 29.4 min | 6.02 h | 21.0 min | 2.07 h |
| | 15°C | 11.76 h | 3.82 days | 43.2 min | 3.94 days |

^aGeneration times (G_t) calculated from regression lines for exponential growth using the Gompertz equation.

^bMeans represent two replications.

In precooked, vacuum-packaged, uncured refrigerated turkey products, inadequate reheating temperatures may contribute to *C. perfringens* food-poisoning. In fact, reheating of precooked foods before consumption may kill the vegetative cells, but heat resistant spores may be heat shocked by the cooking temperature with resultant activation and germination. For example, Craven and Lillard (1974) observed that when commercially prepared, breaded and battered, precooked chicken inoculated with *C. perfringens* spores was heated in microwave to maximum internal temperatures ranging from 49 to 84°C, germination and outgrowth of spores were stimulated. In the present study, when vacuum-packaged samples were temperature abused at 28°C for 48 h (to induce spore formation) and then subsequently reheated in a water bath to internal temperatures of 65°C and above, vegetative cells were destroyed and about 2 log₁₀ spores g⁻¹ were recovered. The spore levels remained in the order of 2 logs even after 24 h of storage at room temperature but no spore germination occurred (data not shown). This may be attributed to the fact that *C. perfringens* spores were not heat shocked during reheating. Heat treatment for 20 min at 75°C is required to heat shock the spores with resultant germination and outgrowth (Johnson 1990). Craven (1980) reported that spores will germinate at a reduced rate without prior heat shock. Reheating the ground turkey to an internal temperature of ≤55°C did not reduce the total viable cell count and the cell number remained at 7 logs at all sampling times and there was a maximum of 1 log increase in number (initial count about 6.9 log₁₀ cfu g⁻¹). Reheating the samples stored at 4°C for 7 days to an internal temperature of 65°C killed all vegetative cells and no spores were detected. However, exposure of packages to an internal temperature of 45 or 55°C did not reduce the *C. perfringens* population (data not shown). Following the sub-lethal heat treatment which may have injured the vegetative cells, cell number increased by only < 1 log during storage at room temperature for 24 h (initial count about 2.7 log₁₀ cfu g⁻¹). Willardsen et al. (1978) reported that inactivation of *C. perfringens* began at approximately 55°C but a minimal exposure to temperatures near 60°C may be insufficient to inactivate vegetative cells of *C. perfringens*. Woodburn and Kim (1966) found that cooking stuffed turkeys to doneness in ovens at 94, 163 and 232°C did not destroy *C. perfringens* vegetative cells.

On the basis of these findings, it is concluded that the cyclic and static temperature abuse of precooked, vacuum-packaged, uncured, refrigerated turkey products for a relatively long period may allow *C. perfringens* to grow to possible infective levels of at least 5 log₁₀ cfu g⁻¹. Abused precooked foods, if not reheated adequately before consumption, will most likely cause food-poisoning. However, our results indicated that reheating to 65°C would be sufficient to kill *C. perfringens* vegetative cells in precooked turkey.

References

- Bean, N. H. and Griffin, P. M. (1990) Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles, and trends. *J. Food Protect.* **53**, 804–817.
- Bryan, F. L. (1969) What the sanitarian should know about *Clostridium perfringens* food-borne illness. *J. Milk Food Technol.* **32**, 381–389.
- CDC (1985) Foodborne disease outbreaks annual summary, 1982. Center for Disease Control, Atlanta, GA.

- Craven, S. E. and Lillard, H. S. (1974) Effect of microwave heating of precooked chicken on *Clostridium perfringens*. *J. Food Sci.* **39**, 211–212.
- Craven, S. E. (1980) Growth and sporulation of *Clostridium perfringens*. *Food Technol.* **34**, 80–87.
- Dework, F. M., Jr. (1972) Sporulation of *Clostridium perfringens* type A in vacuum-sealed meats. *Appl. Microbiol.* **24**, 834–836.
- Dische, F. E. and Elek, S. D. (1957) Experimental food poisoning by *Clostridium welchii*. *Lancet* **ii**, 71–74.
- Duncan, C. L. (1970) *Clostridium perfringens* food poisoning. *J. Milk Food Technol.* **33**, 35–41.
- Duncan, C. L. (1975) Role of clostridial toxins in pathogenesis. In *Microbiology* (Ed. Schlessinger, D.) pp. 283–291. Washington, DC, American Society for Microbiology.
- Gibson, A. M., Bratchell, N. and Roberts, T. A. (1987) The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol.* **62**, 479–490.
- Hall, L. P. (1970) A bacteriological survey of British produced frozen vegetables. In *Technical Bulletin*, No. 18. Chipping Campden, Gloucester: UK Campden Food Preservation Research Association.
- Hauschild, A. W. H. (1970) Erythematous activity of the cellular enteropathogenic factor of *Clostridium perfringens* type A. *Can. J. Microbiol.* **16**, 651–654.
- Johnson, E. A. (1990) *Clostridium perfringens* food poisoning. In *Foodborne diseases* (Ed. Clive, D.O.) pp. 229–240. California, Academic Press.
- Naik, H. S. and Duncan, C. L. (1977) Enterotoxin formation in foods by *Clostridium perfringens* type A. *J. Food Safety* **1**, 7–18.
- Stark, R. L. and Duncan, C. L. (1971) Biological characteristics of *Clostridium perfringens* type A: *in vitro* system for sporulation and enterotoxin synthesis. *J. Bacteriol.* **144**, 306–311.
- Stringer, M. F., Turnbull, P. C. B. and Gilbert, R. J. (1980) Application of serological typing to the investigation of outbreaks of *Clostridium perfringens* food poisoning 1970–1978. *J. Hyg. (Camb)* **84**, 443–456.
- Strong, D. H. and Ripp, N. M. (1967) Effect of cookery and holding on hams and turkey rolls contaminated with *Clostridium perfringens*. *Appl. Microbiol.* **15**, 1172–1177.
- Todd, E. C. D. (1989a) Cost of acute bacterial foodborne disease in Canada and the United States. *Int. J. Food Microbiol.* **9**, 313–326.
- Todd, E. C. D. (1989b) Preliminary estimates of costs of foodborne disease in the United States. *J. Food Protect.* **52**, 595–601.
- Willardsen, R. R., Busta, F. F., Allen, C. E. and Smith, L. B. (1978) Growth and survival of *Clostridium perfringens* during constantly rising temperatures. *J. Food Sci.* **43**, 470–475.
- Woodburn, M. and Kim, C. H. (1966) Survival of *Clostridium perfringens* during baking and holding of turkey stuffing. *Appl. Microbiol.* **14**, 914–920.
- Zottola, E. A. (1979) *Clostridium perfringens* food poisoning. Extension Bulletin 365. Agricultural Extension Service, University of Minnesota, St. Paul, MN.