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INFLUENCE OF MODIFIED ATMOSPHERE PACKAGING ON GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN COOKED TURKEY

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ABSTRACT

Clostridium perfringens containing samples of sterile ground turkey were studied to assess growth under modified atmosphere conditions. Samples were packaged under various atmospheres ($CO_2/O_2/N_2$: 75/5/20, 75/10/15, 75/20/5, 25/20/55, 50/20/30), stored at 4, 15 and 28C, and sampled periodically for growth. Diluted samples were plated on Shahidi Ferguson *perfringens* agar (Difco Laboratories, Detroit, MI) to determine vegetative cell counts. Temperature abuse (cyclic and static) of the turkey product was also investigated. The results showed that the growth of *C. perfringens* was slowest under 25-50% CO_2 /20% O_2 /balance N_2 at 15 and 28C. There was no growth at 4C for up to 28 days. Temperature abuse (28C storage) of refrigerated products for 8 h did not permit *C. perfringens* growth. Use of 25-50% CO_2 /20% O_2 /balance N_2 may extend the shelf-life of turkey, but in the absence of proper refrigeration, it cannot be relied upon to eliminate the risk of *C. perfringens* food poisoning.

INTRODUCTION

The demand for refrigerated, convenient, ready-to-eat foods has generated increased interest in modified atmosphere packaging (MAP) of foods to extend shelf-life and sustain visual appearance of foods. While there exists voluminous information on the technological applications of MAP (Farber 1991; Church and Parsons 1995), the microbiological safety of MAP has not been thoroughly investigated and continues to be questioned. A major food safety concern expressed for MAP products is the possible growth of anaerobic and psychrotro-

phic pathogens. These concerns are justified in view of (1) the stimulatory effect of CO₂ on spore germination (Foegeding and Busta 1983); (2) the inhibition of spoilage aerobic microflora which are indicators of incipient spoilage; and (3) the potential for temperature abuse. Additionally, concern for microbial hazards is pertinent in view of the increased evidence of growth of certain foodborne pathogens at common refrigeration temperatures (Palumbo 1986; Schofield 1992). Extended shelf-life of MAP foods may allow foodborne pathogens to grow to hazardous dose levels.

Clostridium perfringens disease outbreaks primarily involve meat and meat products, and illness develops after the consumption of food that is contaminated with large numbers of vegetative cells which sporulate in the intestine (Duncan 1975; Hauschild *et al.* 1970). The heat-labile enterotoxin known to be responsible for the pathological effects in humans is synthesized only during sporulation and is released from the sporangium after lysis (Duncan *et al.* 1972; Duncan 1975; Stark and Duncan 1971). The importance of *C. perfringens* is increased by reports that some strains can grow at 6C (Johnson 1990). Moreover, potential for *C. perfringens* growth is increased in view of the reports revealing that holding temperatures of retail cabinets and consumer refrigeration units is commonly > 10C (Anon. 1989; Bryan 1988; Daniels 1991; Wyatt and Guy 1980; Van Grade and Woodburn 1987). Bean and Griffin (1990) reported that food poisoning with *C. perfringens* is usually associated with temperature abuse during postprocess handling and storage.

In a study by Hintlian and Hotchkiss (1987), when the influence of modified atmosphere (MA) containing 75% CO₂ on growth of *C. perfringens* and *Pseudomonas fragi* in cooked roast beef was investigated, it was noted that the inclusion of 10% O₂ in the MA inhibited *C. perfringens*, as compared to those meat samples stored under CO₂ and N₂. While the study by Hintlian and Hotchkiss (1987) provided some characterization of *C. perfringens* in MAs of high CO₂ levels, there appears to be no work available on growth in cooked turkey in an atmosphere containing low CO₂ levels. Accordingly, the work described below was carried out to determine the effect of three levels of CO₂ (25, 50, and 75%), three levels of O₂ (5, 10, and 20%) with N₂ as the filler gas on the fate of *C. perfringens* in cooked turkey stored under refrigerated and temperature abuse (cyclic and static) conditions.

MATERIALS AND METHODS

Test Organism

Clostridium perfringens strains NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3) and NCTC 10240 (Hobbs serotype 13) were used in the study. Working cultures of the organisms were maintained at 4C in cooked-

meat medium as sporulated stock cultures (Difco Laboratories, Detroit, MI) and used through the course of the study.

Preparation of Inoculum

To prepare inocula, 0.1 mL from the stock cultures of each *C. perfringens* strain were inoculated separately into 10 mL of freshly prepared fluid thioglycolate medium, heat shocked at 75C for 20 min and incubated at 37C for 18 h. The cells were harvested by centrifugation at room temperature for 10 min at $7,700 \times g$, the cell pellet washed twice and finally resuspended in sterile 0.1% peptone water (w/v) to give approximately $7 \log_{10}$ cfu/mL. Equal cell numbers of individual strains were then pooled and the resulting "cocktail" (10^4 cfu/mL) was used in growth experiments.

Gas Mixtures

Five commercially obtained (BOC Gases, Riverton, NJ) different gas mixtures (99.99% pure, analyzed by the company) were evaluated as to their effect on fate of *C. perfringens* population inoculated in cooked turkey. Two replications were performed for each atmospheric treatment. The five atmospheres under investigation were: (a) 75% CO₂/5% O₂/20% N₂, (b) 75% CO₂/10% O₂/15% N₂, (c) 75% CO₂/20% O₂/5% N₂, (d) 25% CO₂/20% O₂/55% N₂, and (e) 50% CO₂/20% O₂/30% N₂. Nitrogen was used as the filler gas in the gas mixtures.

Sample Preparation and Inoculation

Ground turkey was obtained from a local super market and autoclaved at 121C for 15 min. The fat was poured off while the turkey was hot and then meat cooled at 4C to an internal temperature of 25C. The pH of the ground turkey was determined using a combination electrode (Sensorex, semi-micro, A.H. Thomas, Philadelphia, PA) 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) and inoculated with 1 mL of *C. perfringens* cell suspension cocktail so that the final concentration of cells was approximately $3 \log_{10}$ cfu/g. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample and placed in 7 in. \times 8 in. plastic barrier bags (Koch Model 01 46 09, Kansas City, MO). The oxygen transmission rate of the nylon/polyethylene film was 3.5 cc/100 in.² [1 in. = 2.54 cm] in 24 h measured at 75F and 75% relative humidity. The bags containing samples for MA storage were evacuated to a negative pressure of 1000 millibars, backflushed to positive pressure of 200 millibars with gas of the desired mixture and heat sealed using a Multivac Model

A300/16 gas packaging machine (W. Germany). The evacuation/flushing procedure was repeated two times to obtain thorough exchange of atmospheres. All packaging was done at room temperature. The gas to meat ratio in all packs was approximately 9:1.

Storage, Temperature Abuse, and Sampling

The inoculated turkey samples were stored at 4, 15, and 28C. Storage temperatures were monitored daily. Samples from each atmosphere stored at 28C were analyzed at 3, 6, 9, 12, and 24 h. Samples stored at 15C were analyzed on day 7, 14, 21, and 28; and those at 4C on day 7, 14, 21, and 28. To determine the effect of cyclic and static temperature abuse, refrigerated (4C storage) turkey samples stored under MA of 75% CO₂/20% O₂/ 5% N₂ were moved 7 days before their scheduled sampling day (7, 14, 21, and 28 days) to 28C, held at this temperature for 8 and 14 h, returned to 4C and then plated on their scheduled sampling day. Some samples were transferred to 28C for 8 or 12 h before plating on their scheduled sampling day.

Bacterial Enumeration Procedure

At the scheduled sampling time, samples were removed and the contents of each pack were homogenized in 0.1% peptone water (wt/vol) using a stomacher Lab-blender (Model 400, Tekmar Company, Cincinnati, OH). Additional decimal dilutions were made as needed, followed by spiral plating (Spiral Systems Model D plating instruments; Cincinnati, OH) on tryptose-sulfite-cycloserine agar without cycloserine, i.e., Shahidi-Ferguson perfringens (SFP) agar and egg yolk enrichment. After overlaying with an additional 10 mL of SFP agar, the plates were allowed to solidify before placing into anaerobic jars. The total *C. perfringens* population was determined after 48 h of incubation at 37C using a Gas Pak system (Baltimore Biological Laboratory, Cockeysville, MD).

Experimental Design

The experimental design used in the study was 3 × 5 factorial in a completely randomized design with two replicate experiments. Each replicate experiment consisted of 3 temperatures (4, 15, and 28C) and five atmospheres mentioned above. Since transformed bacterial counts data approximate a normal distribution (Javis 1989), log₁₀ cfu/g values were used to calculate mean log₁₀ cfu/g values. To determine the influence of MAs on cumulative change in log₁₀ cfu/g, the mean cfu/g at time 0 was subtracted from the mean cfu/g at each subsequent scheduled sampling time.

RESULTS AND DISCUSSION

The pH of the cooked ground turkey used in the study was 6.25. The cumulative changes in average \log_{10} cfu/g for *C. perfringens* at 28 and 15C are shown in Tables 1 and 2, respectively. By 3 h at 28C, *C. perfringens* grew under all MA's conditions studied (Table 1). Growth was slow and increased about 1 log under MA containing 25-50% CO₂ and 20% O₂ (balance N₂) compared to > 3 log increase under atmospheres containing 75% CO₂, 5, 10 or 20% O₂ (balance N₂). Growth of *C. perfringens* under MA containing 25, 50 or 75% CO₂ and 20% O₂ (balance N₂) was markedly less than under other atmospheres studied, with about 3.5 log increase in numbers during 9 h of storage, compared with an increase of about 5.8 log under atmospheres containing 75% CO₂/ 5 or 10% O₂/ balance N₂. Modified atmospheres containing 25-50% CO₂ and 20% O₂ relatively arrested growth of *C. perfringens* in cooked turkey after 24 h of storage. These findings differ from previous reports that *C. perfringens* grows well at an abuse temperature of 26.7C regardless of CO₂ or O₂ levels under MA (Hintlian and Hotchkiss 1987). Differences between the results from the present study and that of Hintlian and Hotchkiss (1987) may arise from using two different muscle foods and the later study co-inoculated *C. perfringens* with *P. fragi*. Juneja *et al.* (1994) reported on growth characteristics of *C. perfringens* in cooked turkey at 28C. They found that the organism began multiplying after a lag phase of 2.57 h (anaerobically stored samples) and 7.47 h (aerobic samples). Anaerobic growth reached 7 logs within 9 h and aerobic by 24 h. Parekh and Solberg (1970) found no significant difference in the growth rate between eight strains of *C. perfringens* growing in fluid thioglycollate broth whether in a 100% CO₂ or 100% N₂ atmosphere. However, these experiments were conducted at 43C, a temperature at which CO₂ might have much less biostatic activity because of its decreased solubility at high temperatures.

At 15C, growth under atmospheres containing 20% O₂ was less than 3 logs by day 7 (Table 2). However, *C. perfringens* growth was > 5 logs by day 14 regardless of the packaging. Inhibition in MA containing 20% O₂ was much greater at 15C than at 28C. In a study by Juneja *et al.* (1994), *C. perfringens* aerobic growth in cooked turkey was relatively slow at 15C and mean \log_{10} cfu/g increased anaerobically by 4 - 4.5 logs by day 8. In the present study, increase in *C. perfringens* growth was approximately 7 logs during 35 days storage under MA of 75% CO₂/5 or 10% O₂/balance N₂ (Table 2).

During storage at 4C, *C. perfringens* growth was not observed in turkey samples regardless of the CO₂ or O₂ levels (data not shown). Similar observations were observed by Juneja *et al.* (1994) and Hintlian and Hotchkiss (1987). Studies done at 4C in which cooked turkey was inoculated with *C. perfringens* and then stored either in air or vacuum, showed that the organism was unable

to grow either in air or vacuum (Juneja *et al.* 1994). In a study by Hintlian and Hotchkiss (1987), when cooked, roast beef co-inoculated with *Salmonella typhimurium*, *Staphylococcus aureus*, *C. perfringens* and *P. fragi* was packaged under air or MA (75% CO₂ /10% O₂/15% N₂) and stored at 4C, *C. perfringens* was unable to grow either in air or the MA.

TABLE 1.
CLOSTRIDIUM PERFRINGENS VEGETATIVE CELLS CUMULATIVE CHANGE^{1,2}
IN LOG₁₀CFU/G IN COOKED TURKEY STORED UNDER MODIFIED
ATMOSPHERES AT 28C

Time (h)	Change in log ₁₀ cfu/g in Samples Stored Under Modified Atmosphere CO ₂ :O ₂ :N ₂ (% Ratio)				
	75:20:5	75:5:20	75:10:15	50:20:30	25:20:55
3	3.57	3.41	3.60	1.16	1.16
6	3.81	4.10	4.01	2.52	2.21
9	3.78	5.88	5.72	3.37	2.98
12	6.63	6.80	7.58	5.73	6.23
24	8.65	9.10	9.29	6.87	7.28

¹cfu/g (hour X) - cfu/g (hour 0) at each sampling time.

²Data expressed in log₁₀ cfu/g.

TABLE 2.
CLOSTRIDIUM PERFRINGENS VEGETATIVE CELLS CUMULATIVE CHANGE^{1,2}
LOG₁₀CFU/G IN COOKED TURKEY STORED UNDER MODIFIED
ATMOSPHERES AT 15C

Time (d)	Change in log ₁₀ cfu/g in Samples Stored Under Modified Atmosphere CO ₂ :O ₂ :N ₂ (% Ratio)				
	75:20:5	75:5:20	75:10:5	50:20:30	25:20:55
7	2.68	3.85	3.78	2.80	2.76
14	5.28	5.54	5.85	5.38	5.29
21	5.79	5.75	6.08	5.85	5.80
28	5.99	6.02	6.39	6.28	6.07
35	6.51	6.74	6.90	6.15	6.07

¹ cfu/g (day X) - cfu/g(day 0) at each sampling time.

² Data expressed in log₁₀ cfu/g.

The National Food Processors Association (NFPA 1988) has indicated that manufacturers should assume that temperature abuse will occur at some point during the transportation, distribution, storage or handling in supermarkets or by consumers. To determine the effect of cyclic temperature abuse, refrigerated

turkey samples stored under MA of 75% CO₂/20% O₂/ 5% N₂ were moved to a 28C environment for 8 and 14 h, 7 days before their scheduled sampling day (7, 14, 21, and 28). While there was no increase in the number of organisms in the samples that were abused for 8 h, *C. perfringens* grew to > 7 log₁₀ cfu/g in samples that were abused for 14 h (Fig. 1). When samples were transferred 8 and 12 h before plating to 28C, *C. perfringens* grew to > 7 log₁₀ cfu/g only in 12 h temperature abused samples (Fig. 2). Hintlian and Hotchkiss (1987) found that *C. perfringens* could not be recovered after 21 days when refrigerated beef samples were transferred at various times during the 42 days sampling period to 12.8C for a final 7 days.

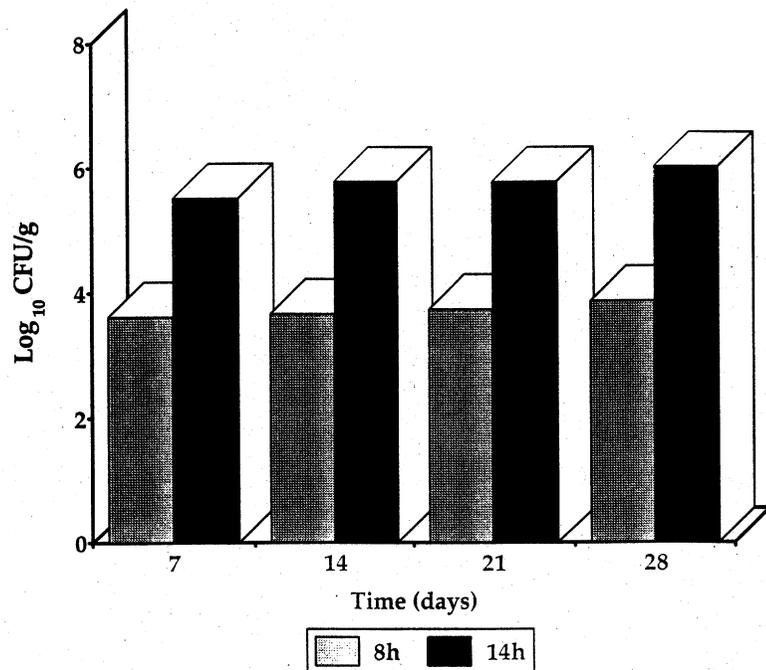


FIG. 1. THE EFFECT OF CYCLIC TEMPERATURE ABUSE (28C) ON GROWTH OF *CLOSTRIDIUM PERFRINGENS* STRAIN NCTC 8238 IN MODIFIED ATMOSPHERE (75% CO₂/5% O₂/20% N₂) PACKAGED COOKED TURKEY STORED AT 4C. The samples were moved to 28C for 8 h and 14 h, 7 days before plating on their scheduled sampling day (7, 14, 21, 28).

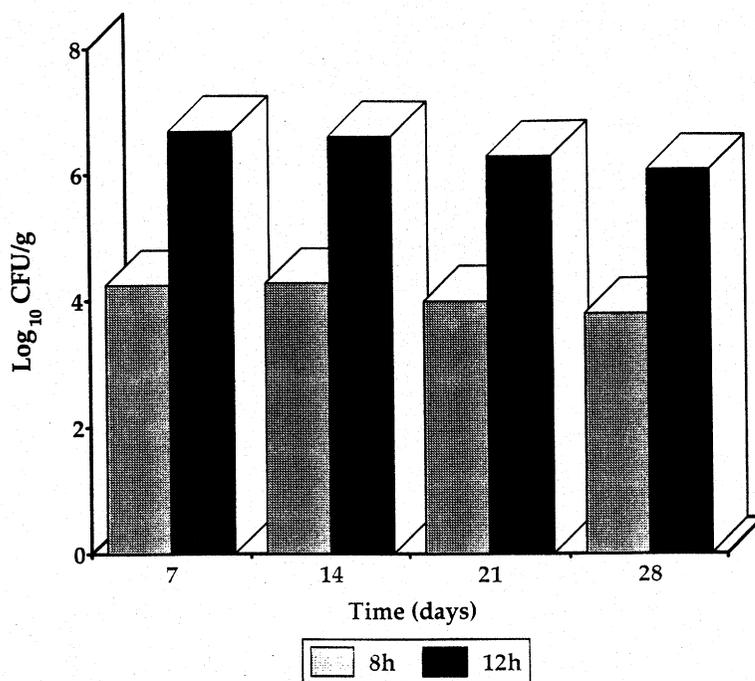


FIG. 2. THE EFFECT OF STATIC TEMPERATURE ABUSE (28C) ON GROWTH OF *CLOSTRIDIUM PERFRINGENS* STRAIN NCTC 8238 IN MODIFIED ATMOSPHERE (75% CO₂/5% O₂/20% N₂) PACKAGED COOKED TURKEY STORED AT 4C
The samples were moved to 28C for 8 h and 12 h before plating on their scheduled sampling day (7, 14, 21, 28).

The modified atmosphere packaging is a well established technology. The aspects that need to be studied include microbiological safety of refrigerated, ready-to-eat foods with extended shelf-life, product safety during temperature abuse, and failures in packaging systems (Farber 1991). Early workers who studied the use of MA for various commodities demonstrated that CO₂ or combination of CO₂ and O₂ could be effective in extending the shelf-life of meats (Clark and Lentz 1969).

Institutional foodservice settings, catering establishments and retail consumers who demand high quality, ready-to-eat, convenient food products may be attracted to MAP ready-to-eat turkey. Consequently, the ability of *C. perfringens* to grow under these conditions is critical to consumers. The results of our study indicate that *C. perfringens* growth is effectively inhibited in MA-storage using 25-50% CO₂/20% O₂/balance N₂, in conjunction with good refrigeration at 4C. Our study emphasizes the importance of storing foods at as

low a temperature as possible and MA cannot be used as a substitute for refrigeration (Genigeorgis 1985). Cyclic and static temperature abuse of refrigerated products for 8 h did not permit *C. perfringens* growth. However, temperature abuse of cooked turkey for periods longer than 12 h at 28C led to growth of *C. perfringens* to hazardous or infective dose levels. We recommend that the manufacturers, public health and food safety officials must monitor these products continually.

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