

34

F. A. Draughon
F. A. Draughon, Major Professor

Genevieve Christen
H. Dwight Lorday
John R Mount

Lew Minkel
Vice Provost
and Dean of the Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in her absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Randall K. Okebus

Date December 2, 1988

EFFECTS OF SEVEN MODIFIED GAS ATMOSPHERES
ON THE SURVIVAL OF CAMPYLOBACTER JEJUNI
IN PROCESSED TURKEY ROLL

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Randall Kent Phebus

December 1988

AG-VET-MED.

Thesis
88
.P448

DEDICATION

To my mother and father in hopes that this
thesis will in some way repay their love,
dedication and support of me throughout
my life and college career.

ACKNOWLEDGMENTS

I would like to extend my sincere appreciation to Dr. Ann Draughon for her expert guidance and patience throughout my graduate study. Without her inspiration, this investigation would still be in its beginning phase. My appreciation is also extended to Dr. John Mount, Dr. Dwight Loveday and Dr. Genevieve Christen for their assistance in serving as committee members and to Dr. Bill Sanders for his help in designing this experiment. An additional note of thanks is given to Dr. Mount and Craig Bacon for their expert help with SAS procedures.

A special thanks is extended to all of my coworkers in the laboratory who kept things light when they began to get heavy. I am grateful to Boyd Lee who covered for me periodically and who served as a lab partner and friend throughout this study.

Finally, my deepest appreciation and love is extended to Cindy who has kept my life together for the past few years allowing me to concentrate on my work.

ABSTRACT

The question of whether modified atmosphere packaging is a problem concerning the pathogen Campylobacter jejuni in turkey roll was addressed in this study. The survival rates of pure cultures of C. jejuni inoculated into turkey roll slices and stored under seven different atmospheric mixtures were observed. The first series of experiments involved turkey roll held at 4 C for 18 days. The second series of experiments concerned turkey roll stored at 21 C for 48 hours. The various atmospheric effects on aerobic plate count, psychrotrophic plate count and lactic acid bacterial count were also observed.

Increasing carbon dioxide concentration inside the package resulted in an increase in C. jejuni survival rate at both storage temperatures. The same increases in carbon dioxide provided greater inhibition to aerobic and psychrotrophic counts as compared to low carbon dioxide levels. The effect of carbon dioxide was more pronounced at 4 C on C. jejuni survival and on the growth of lactic acid bacteria, psychrotrophs, and aerobic bacteria. The importance of enrichment of food samples for the isolation of C. jejuni was clearly illustrated. Campylobacters were isolated from turkey roll held under all atmospheres by enrichment procedures on the eighteenth day and forty-eighth hour of storage at 4 and 21 C, respectively.

It was concluded that elevated carbon dioxide levels used as packaging atmospheres for turkey roll could be a possible risk when C. jejuni is concerned. This is a result of very low increases in aerobic and psychrotrophic spoilage bacteria with corresponding increases in C. jejuni survival.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. REVIEW OF THE LITERATURE	3
The Genus <u>Campylobacter</u>	3
<u>Campylobacter</u> Enteritis	9
<u>Campylobacter</u> Culturing Techniques	17
Modified Atmosphere Packaging of Poultry	22
III. MATERIALS AND METHODS	24
Experimental Design	24
Sample Preparation	24
Cultures	25
Preparation of Inoculum	25
Inoculation of Samples	26
Modified Atmosphere Storage	26
Microbial Analysis	27
Turkey Roll Composition	29
Statistical Analysis	29
IV. RESULTS AND DISCUSSION	31
Bacterial Growth/Survival at 4 C	31
Bacterial Growth/Survival at 21 C	48
V. CONCLUSIONS	65
LIST OF REFERENCES	67
APPENDIXES	79
APPENDIX A. CORRELATION TABLES	80
APPENDIX B. MEDIA FORMULATIONS EMPLOYED	84
APPENDIX C. PROXIMATE COMPOSITION OF TURKEY ROLL	86
VITA	88

LIST OF TABLES

TABLE	PAGE
1. Correlations Among Types of Bacterial Counts Tested Within Each of Seven Gas Packaging Atmospheres in Turkey Roll Stored at 4 C.	81
2. Correlations of Mean Slope Values Among Increasing Carbon Dioxide Levels (0-100%) Used in Packaging of Turkey Roll and Growth/Survival of Specific Types of Bacteria at 4 and 21 C	82
3. Correlations Among Types of Bacterial Counts Tested Within Each of Seven Gas Packaging Atmospheres in Turkey Roll Stored at 21 C	83
4. Proximate Composition of Turkey Roll (As-Received Basis).	87

LIST OF FIGURES

FIGURE	PAGE
1. Survival of <u>C. jejuni</u> inoculated into turkey roll and stored at 4 C under air or modified atmospheres (enumeration by direct plating).	32
2. Survival of <u>C. jejuni</u> inoculated into turkey roll and stored at 4 C under air or modified atmospheres (enumeration by plating after enrichment).	33
3. Mean slope values for <u>C. jejuni</u> survival curves shown in Figure 1.	35
4. Aerobic bacterial growth in turkey roll stored at 4 C under air or modified atmospheres	37
5. Mean slope values for aerobic plate count curves shown in Figure 4.	41
6. Psychrotrophic bacterial growth in turkey roll stored at 4 C under air or modified atmospheres	43
7. Mean slope values for psychrotrophic plate count curves shown in Figure 6.	45
8. Lactic acid bacterial growth in turkey roll stored at 4 C under air or modified atmospheres	47
9. Mean slope values for lactic acid bacterial growth curves shown in Figure 8	49
10. Survival of <u>C. jejuni</u> inoculated into turkey roll and stored at 21 C under air or modified atmospheres (enumeration by direct plating).	50
11. Mean slope values for <u>C. jejuni</u> survival curves shown in Figure 10	52
12. Survival of <u>C. jejuni</u> inoculated into turkey roll and stored at 21 C under air or modified atmospheres (enumeration by plating after enrichment).	54

13.	Aerobic bacterial growth in turkey roll stored at 21 C under air or modified atmospheres	56
14.	Mean slope values for aerobic plate count curves shown in Figure 13	57
15.	Psychrotrophic bacterial growth in turkey roll stored at 21 C under air or modified atmospheres	59
16.	Mean slope values for psychrotrophic plate count curves shown in Figure 15	61
17.	Lactic acid bacterial growth in turkey roll stored at 21 C under air or modified atmospheres	62
18.	Mean slope values for lactic acid bacterial growth curves shown in Figure 17.	63

CHAPTER I

INTRODUCTION

Campylobacter jejuni is classified as a gram-negative, microaerophilic bacterium and is a leading cause of acute gastroenteritis in humans, equalling or surpassing the incidence of enteritis caused by Shigella or Salmonella. The major foods implicated in campylobacter enteritis outbreaks are milk, red meats and poultry. Campylobacter jejuni has been isolated from meat at point-of-sale in retail supermarkets (114), which indicates the potential risk of human infection.

The meat industry continuously strives to increase the shelf-life of its products. This is especially true for poultry items which tend to spoil more readily. Many different approaches have been taken to accomplish this feat, with the most common being vacuum packaging. However, the techniques of controlled and modified atmosphere packaging are being employed to greater extents.

The use of various mixtures of carbon dioxide, nitrogen and oxygen changes the spoilage microflora of poultry from that of predominantly aerobic, namely Pseudomonas sp., to one of lactic acid producing bacteria. There is also a possibility that anaerobic or microaerophilic pathogens may be able to survive more readily in the absence of oxygen and competitive aerobic organisms. In the case of poultry,

C. jejuni is a major pathogen of concern due to its high frequency of isolation from raw products. Before modified atmosphere packaging can be widely used in the poultry industry, it is necessary to determine if pathogens can grow or survive in modified atmospheres and cause illness without the warning signs of spoilage to the consumer.

The objectives of this study were to determine: (a) the extent to which Campylobacter jejuni inoculated into processed turkey roll can survive at 4 and 21 C under various atmospheric mixtures of carbon dioxide, nitrogen and oxygen inoculated into processed turkey roll; (b) the effect of different gas atmospheres on the growth and type of spoilage microflora in turkey roll; and (c) if correlations exist between C. jejuni survival and the growth of competing spoilage microorganisms.

CHAPTER II

REVIEW OF THE LITERATURE

A. THE GENUS CAMPYLOBACTER

Classification and Taxonomy

Originally, Campylobacter was classified under the genus Vibrio. In 1963, it was determined that V. fetus and V. bubulus had a different mol% G + C of the DNA content from the other Vibrio members. With this difference, and since V. fetus was not fermentative, it was concluded that these two should form a new genus called Campylobacter, meaning curved rod (38, 51, 65, 76, 92). Campylobacter fetus contains three subspecies; C. fetus subsp. fetus, subsp. intestinalis and subsp. jejuni (76). Growth at 25 C and 42 C has been used as the major differential characteristic of the genus, with subsp. jejuni growing at 42 C but not at 25 C, whereas the other two subspecies grow at 25 C but not 42 C (38, 43, 44, 89, 92).

Until the "Approved Lists of Bacterial Names" was published in 1980, there was a French classification (110) and an American classification (91) of campylobacters which caused confusion. Almost all strains associated with acute enteritis belong to the group with thermophilic characteristics. In the American classification, all thermophilic strains are included in the single subspecies C. fetus

subsp. jejuni, but in the French classification, they are divided into the two species C. jejuni and C. coli (89).

Physical Characteristics

Campylobacters are curved, spiral rods 1.5 to 3.5 μm long by 0.2 to 0.4 μm wide. These organisms are gram negative, non-sporeforming microaerobes and are motile with a single polar flagellum at one or both ends of the cell (89, 92). Skirrow and Benjamin (89) reported C. fetus subsp. fetus to be predominantly monotrichate and organisms of the jejuni/coli group to be amphitrichate. Motility is by a characteristic corkscrew type of motion with cells moving quickly across a microscopic field (65, 92, 97). In older cultures (greater than 48 hours), cells may become coccoid due to loss of cell integrity (7, 18, 89, 107). This is a degenerate stage produced by unfavorable growth conditions (18, 76). Buck et al. (18) suggests that when research is conducted with this organism, it must be kept in mind that cells incubated on solid media for even 48 hours under crowded conditions may be in the lytic stage and may have antigenic and physiological characteristics profoundly different from those of normal cells.

Colonies of C. jejuni are typically flat, glossy and effuse on solid media with large islands of growth commonly being formed. In 18 to 24 hour cultures, colonies are almost transparent, but with continued incubation, growth

thickens and most strains take on a tan color (7, 76, 89, 97). Buck and Kelly (16) found that moisture content of the growth medium produced a profound effect on the colony morphology of C. jejuni and speculate that other factors, such as concentration of agar, the state of the nutrients, or the buildup of toxic products could have a similar effect. On selective blood agar plates, the colonies will be non-hemolytic (97). Wet mounts of cells from suspect colonies produced on selective agar media should be examined using a phase-contrast microscope (7, 65).

Biochemical Characteristics

Campylobacter jejuni is oxidase and catalase positive, reduces nitrate to nitrite, and does not produce hydrogen sulfide in triple sugar iron agar but does produce hydrogen sulfide as detected by the presence of a lead acetate strip over a medium containing 0.02% cysteine-HCl (7, 38, 44, 89, 92, 97). Campylobacter jejuni will grow in 1% glycine and 1% bile (92), will not oxidize or ferment glucose (7, 44, 97), and will reduce selenite (89). Campylobacters do not hydrolyze gelatin or urea and are methyl red and Vogues Proskauer negative (76). Campylobacter jejuni shows a wide range of sensitivity to 2, 3, 5-triphenyltetrazolium chloride (7, 89). Stern (97) states that an excellent basal medium for assessing biochemical characteristics of campylobacters is brucella broth containing 0.16% agar. Beuchat (7) also

found this to be a good medium for maintaining cultures or for biochemical tests and recommends that inoculation be 5 to 10 mm below the surface of the medium since Campylobacter growth will be restricted to that area.

Growth Requirements

Campylobacter fetus does not use carbohydrates since energy for growth comes from the tricarboxylic acid cycle (51, 92). Kreb's cycle intermediates and amino acids serve as primary energy sources (51). They grow in a defined growth medium with amino acids and vitamins. Strains of C. fetus vary considerably in their minimal nutritional requirements. The vitamin niacin is required by all, whereas other vitamins are merely stimulatory (57).

Most strains of C. jejuni are sensitive to sodium chloride. Skirrow and Benjamin (89) found only 12 of 100 representative strains grew on 1.5% NaCl agar. Mehlman and Romero (57) found C. jejuni grew in the presence of 0.7% NaCl or slightly higher at a pH of 7.0 to 7.5. Doyle and Roman (26) found strains of C. jejuni could grow at 42 C in the presence of 1.5% NaCl, but not 2% NaCl. Campylobacter jejuni grew poorly in the absence of added NaCl and grew best in the presence of 0.5% NaCl at 42 C. As the incubation temperature dropped from 42 C to 4 C, the tolerance of C. jejuni to NaCl was diminished. They advise the use of a growth medium containing 0.5% NaCl for

recovering or enumerating this organism, but not 1% or more.

Temperature Effects

Sanitary preparation and cooking to a temperature that will destroy other gram negative foodborne pathogens should remove the public health hazards associated with C. jejuni indigenous foods (8, 32, 33, 101). Few survivors can be expected in meats that have been heated and/or kept at 60 C for several minutes (21, 101, 103). At lower temperatures, survival will depend upon the combination of heating time and temperature employed (21). Hanna et al. (40) found almost identical results with Yersinia enterocolitica, an organism of similar nature. Acuff et al. (3) revealed that roasting, braising and stewing were effective in destruction of C. jejuni on contaminated turkey thighs when the internal temperature reached 55 C. Palumbo (66) found that heat-injured C. jejuni would repair at 42 C within 4 hours in brucella broth with FBP (ferrous sulfate, sodium metabisulfite, sodium pyruvate) under microaerophilic conditions.

Koidis and Doyle (52) found that C. jejuni survived well in refrigerated ground beef containing large numbers of indigenous bacteria. Blaser et al. (12) reported that C. jejuni inoculated into milk survived better at 4 C than at 25 C; at 4 C remaining viable for as long as 22 days. Others have shown that refrigeration (21, 103) and frozen temperatures (21, 32, 101, 103) decrease viable numbers of

C. jejuni significantly, although survival does occur for extended periods of time. Campylobacter jejuni will remain viable on naturally contaminated poultry carcasses stored at -20 C for three months and at 4 C in excess of seven days (103). It should be recognized that processes such as freezing/thawing can cause sublethal injury and that the detection-enumeration procedures may lead to low recovery rates for such cells (4).

pH Effects

Although the effect of pH on the survival of C. jejuni undoubtedly depends upon the characteristics of the individual food, the rapid destruction in brucella broth at pH 5 suggests poor survival in acid or acidified foods (21). Gill and Harris (33) found for most strains of C. jejuni, the minimum pH for growth was 5.8. The growth requirements are such that the organism could not grow on normal (5.5 to 5.8) pH meat. Christopher et al. (21) indicate that C. jejuni strains 29428 and Par 6 could not survive in brucella broth adjusted to pH 5. At pH 9, counts decreased rapidly and could not be detected after three days. Counts increased rapidly at pH 7 with similar increases at pH 6 and 8. The production of acid from carbohydrates or of amines from the decarboxylation of amino acids by other microbial populations may inhibit the growth of campylobacters (57).

Antibiotic Sensitivity

Campylobacter jejuni is sensitive to high concentration disks (30 µg) of naladixic acid, whereas the other two subspecies are resistant (38, 43, 59, 89, 92). Erythromycin, gentamicin, tobramycin, amikacin, furozolidone (109) and kanamycin (59) are usually considered the most active antibiotics against C. jejuni. Also, chloramphenicol and clindamycin have shown good activity (59, 109). The tetracyclines (59, 72, 109), along with thiamphenicol (109) have shown fairly good activity. Penicillin (38, 72, 109), ampicillin (38, 76, 109) and cephalothin (38) have shown a wide range of results depending on strain. Bacitracin, polymyxin B (72), amoxycillin and carbenicillin (109) have shown very poor activity against C. jejuni. Ray and Johnson (74) found that C. jejuni became sensitive to polymyxin B after freezing and thawing. Erythromycin seems to be the antibiotic of choice when treating campylobacter enteritis (38, 51), although, Michel et al. (59) found 12.5% of C. jejuni strains resistant to erythromycin. Studies suggest that animals can act as reservoirs of bacteria harboring antibiotic resistance plasmids (54, 82, 108).

B. CAMPYLOBACTER ENTERITIS

Occurrence

Campylobacter fetus has classically been an infectious disease of ungulates and birds (38, 97). Infections in man

have been recognized only since 1947 and are associated with subspecies intestinalis and jejuni (38, 76, 97).

Campylobacter jejuni is now recognized as a leading cause of acute bacterial gastroenteritis in humans (27, 52, 55, 81, 95, 101, 108) and may exceed Salmonella and Shigella in frequency (10, 68, 76, 88, 97, 101). In the past several years, there has been an increasing number of reports implicating campylobacters as the causative agent of approximately 5% of cases of acute gastroenteritis (76). However, the mode and vehicles of transmission of infection are still inadequately understood (14, 113). It was not until 1973 that C. jejuni was shown to be commonly associated with gastroenteritis (19). Because the organism is a newly recognized agent of foodborne disease, researchers have only recently begun to identify how campylobacters respond to conditions relevant to the composition, processing and storage of foods (27).

In the last several years, this increased awareness of human disease linked to C. fetus has arisen mainly as the result of more sophisticated microbiological methods (76, 81, 101). In 1977, Skirrow (87) defined a medium for these thermophilic organisms that simplified techniques and led to an increased frequency of isolation (13, 44, 51). Considerable difficulty has been encountered in defining a pathogenicity test for C. jejuni. Consequently, the public health significance of the presence of C. jejuni in foods

cannot be evaluated until it has been determined whether equivalent virulence is characteristic of all strains or is limited to certain ones (99).

Symptoms

Campylobacter enteritis can affect all ages, but the peak incidence appears to be in the 1- to 5-year-old age group (76). Symptoms commonly consist of diarrhea, abdominal pain, vomiting, anorexia, nausea, headache and bloody stool (14, 51, 76). Diarrhea usually lasts only three to five days, but recurrent or relapsing diarrhea for periods as long as nine months have been reported (76). Human subjects infected with this organism may present a wide variety of clinical syndromes which include enteritis, bacteremia, phlebitis, arthritis, septic abortion and meningitis (38, 105). Subspecies jejuni occurs predominantly among infants in whom diarrhea is very common and fever is less constant. The patient's age and underlying diseases in part determine the severity of infection. The prognosis is generally good except in very young and severely debilitated patients (38). The incubation period seems to be two to five days (51). A rapid presumptive laboratory diagnosis may be made during the acute phase of the illness by direct phase-contrast microscopy of stools. The organism persists in the stools

of untreated patients for up to seven weeks following the onset of symptoms (51).

Sources of Infection

The real mechanism of transmission and infection of campylobacteriosis is not known in detail. The infection can be transmitted from person to person (90, 108), from animals to humans (108) and from consumption of contaminated foods (90). The organism can be carried in the intestines of apparently healthy young animals and birds as part of the normal gut microflora (10, 31, 38, 76, 88, 92, 103). In older animals, only a small number of C. jejuni are found in the intestinal flora (76, 92). This organism causes several diseases in animals including avian hepatitis (39, 45, 76) and bluecomb disease (39, 45), bovine winter scours, and ovine abortion (76). Other domestic animals such as dogs and cats have been reported to be sources of infection of campylobacteriosis (11, 36, 38, 69, 108). Ingestion of infected or contaminated foodstuffs probably accounts for most cases of infections in humans; a smaller proportion is probably due to close contact with domestic animals (2, 38, 51).

Campylobacter jejuni so far has been associated with pork, ground beef, chicken and milk. Raw milk is the most frequently implicated vehicle of campylobacteriosis (15, 49, 56, 57, 73, 77, 78, 97, 105, 111). Pasteurization of milk

gives complete protection against the spread of campylobacter enteritis even when large numbers of organisms are present (35, 111, 113). Campylobacter jejuni has been isolated from lamb, pork and beef carcasses, although most research has found low rates and low levels of contamination (95, 98, 108).

The veterinary and public health significance of C. jejuni has been documented extensively (11, 24) with specific consideration given to the role of poultry as a source of C. jejuni enterocolitis in humans (36, 60, 93). Many studies have shown chicken carcasses and parts from slaughter houses and retail outlets to be contaminated with C. jejuni. Smeltzer (90) isolated C. jejuni from 94% of fresh, commercially dressed, poultry carcasses. Park et al. (68) recovered the organism from fresh, eviscerated market chickens at an average of 58% and stated that C. jejuni may survive in whole chickens through the entire marketing process. Isolation of the organism from poultry carcasses and portions at point-of-sale indicates the potential for human infection (114). In a study by Shanker et al. (85) of Australian poultry processing plants, Campylobacter sp. was isolated from 41% of cloacal swabs and 45% of the processed carcasses. Also, 82% of chicken isolates and 98% of human enteric isolates from the same locale were of an identical biotype suggesting an epidemiological link. Munroe et al. (61) found 93% of the chickens checked at a slaughter house

positive for C. jejuni. Of these positive chickens, 96% of the isolates belonged to eleven C. jejuni serotypes that occur most frequently in human cases of enteritis. Almost identical results have been shown in turkey processing. Rayes et al. (75) found 64.1% of fresh packaged turkey wings and 55.6% of frozen turkey wings to contain C. jejuni. Leuchtefeld and Wang (53) found 100% of cecal cultures from 600 turkeys over a one year period at a poultry processing plant positive for C. jejuni. Yusufu et al. (115) reported that C. jejuni could not be isolated from further processed raw turkey products, including mechanically deboned meat. Heat-treated, further processed products are not a likely source of C. jejuni (4).

The incidence of poultry carcass contamination is related to the incidence of flock infection, and both have been reported to vary widely (85, 115). Smitherman et al. (93) found that when a chicken house became positive for C. jejuni, virtually all samples from that house were positive within a week indicating that C. jejuni spreads rapidly among birds in the house. Genigeorgis et al. (30) found 81.8% of birds from 24 houses (15,000 to 20,000 birds per house) on four ranches to be infected with C. jejuni at slaughter time. Shane et al. (84) demonstrated the potential role of houseflies (Musca domestica) in the transmission of C. jejuni infection in poultry flocks. Acuff et al. (1) have identified litter and drinking water as

likely sources of C. jejuni for turkeys raised in commercial brooder facilities. Studies indicate a high level of C. jejuni in the intestinal tracts of chickens and turkeys (20, 36, 64, 115). Oosterom et al. (64) found large contamination with C. jejuni can exist on birds, equipment, hands of processing line workers and in air samples from processing facilities. Scalding water temperatures have a detrimental effect on the agent (115) but heavy cross-contamination occurs during the defeathering and evisceration processes (64). Acuff et al. (4) found almost identical results at a turkey processing plant. Campylobacter jejuni levels as high as log 3.4 have been reported from feather picker drip (30).

The propensity of the organism for wild and domestic birds, in general, may be related to the high body temperature (42 C) of birds, which corresponds to the optimal temperature for growth of C. jejuni in laboratories (53). Isolation rates have been shown to be higher from fresh poultry compared to frozen, but a portion of the bacterial population will survive frozen temperatures (7, 41, 63, 99). The data on wholesale frozen turkeys indicate that the drip from thawed turkeys is the most likely location of C. jejuni (4). Campylobacter jejuni is capable of surviving storage temperatures commonly associated with perishable foods (41, 113). Meats properly cooked and not subjected to cross-contamination from raw food and held at proper temperatures

should not be a health hazard (113). Recontamination of cooked meats would be the most likely mechanism leading to consumption of campylobacters with chicken dishes (32). The widespread occurrence of C. jejuni on raw poultry emphasizes the need for proper food handling practices in food service establishments and in the home (20). Hopkins and Scott (46) revealed that there is a statistically significant association between handling and preparation of raw chicken and illness caused by C. jejuni. Gill and Harris (32) state that the limited circumstances under which cooked poultry meat is likely to carry C. jejuni in significant numbers suggest a need for caution in ascribing outbreaks of campylobacter enteritis to consumption of poultry. Still, with the high contamination rates of poultry and the accepted level of 500 organisms to cause illness in humans (77), much concern has been shown by the poultry industry over this organism.

The recent development of serotyping systems has allowed some local outbreaks of campylobacter enteritis to be traced to their sources (23, 70). At present, there is no widely accepted or reproducible animal test model, and so it is not possible to identify which strains may be virulent. Therefore, the presence of C. jejuni in food must be considered a potential hazard (23).

C. CAMPYLOBACTER CULTURING TECHNIQUES

Media Employed

The two major methods for isolating C. jejuni are by direct plating of sample onto a selective medium or by selective enrichment of the sample prior to plating. Direct plating is more applicable for recovery from fecal specimens than from food samples due to the high numbers of organisms in the fecal specimen (2, 7). When isolating campylobacters from foods, a relatively large sample size or surface area should be analyzed and the use of selective enrichment broths, microaerobic conditions and selective isolation or filtration techniques is essential (7).

Several enrichment methods may be used to select for C. jejuni from specimens containing other indigenous flora, however, most of these methods were developed in clinical laboratories and do not have the sensitivity and selectivity needed for detection in foods where small numbers of organisms may occur (97). Resistance to antibiotics is frequently employed in selective enrichment. Blaser et al. (13) described a Campy-thio enrichment broth containing vancomycin, trimethoprim, polymyxin B and amphotericin B that allowed for 33% greater recovery of C. jejuni than did the use of selective medium alone. The specimens were inoculated into Campy-thio and held in the refrigerator for eight hours before plating onto selective agar medium. The

low temperature inhibits the growth of Campylobacter but the indigenous bacteria are diminished by the antimicrobials. Rubin and Woodard (81) also found increased isolation rates by using cold enrichment in Campy-thio broth (4 C for 24 hours) before plating on Campy-BAP plates. Acuff et al. (2), working with turkey eggs and meat, employed an enrichment procedure using brucella broth supplemented with FBP, equine blood and five antimicrobial agents to recover initial cell numbers of 0.3 to 3.3 per milliliter of broth. Park et al. (67) found enrichment in vancomycin, trimethoprim, polymyxin B (VTP)-FBP broth incubated at 42 C for 48 hours at pH 7.0 to be the optimum procedure for recovery of C. jejuni from poultry. Recovery rates as low as one cell per ten grams in the presence of 10^5 to 10^7 competitive bacteria per gram of sample were recorded. Beuchat (7) describes a similar static enrichment procedure. The enrichment procedure of Doyle and Roman (25) is sensitive for recovering C. jejuni from foods such as raw milk and hamburger, but they found that the background flora of chicken skin hampered recovery of campylobacters. Wesley et al. (112) developed an enrichment broth containing hematin and three antibiotics specifically aimed at recovering C. jejuni from poultry products. Stern (102) states that supplementing enrichment broths with 7% lysed horse blood yielded a significantly ($P < 0.05$) higher

number of C. jejuni after enrichment than did non-blood broths with poultry.

Butzler's, Skirrow's and Campy-BAP selective media use several antibiotics to which C. jejuni is resistant. The plates are supplemented with horse or sheep blood, depending upon the specific formulation (97). Stern (96) found that of these three media, Campy-BAP medium was the most sensitive and Butzler's medium the most selective. He further suggests the combination of these two media in the recovery of C. jejuni from foods. The plating media reported by Blaser et al. (13), Butzler et al. (19), Skirrow (89) and Patton et al. (69) consist primarily of brucella agar or thioglycollate agar base, blood, and a number of antimicrobial agents such as vancomycin, polymyxin, trimethoprim, amphotericin, cephalothin, bacitracin, novobiocin, actidione and colistin (2). Others have used similar combinations of blood agar and antibiotics (20, 87, 89, 109). Campylobacters will grow on brucella agar, Mueller-Hinton agar and thio-blood agar when incubated under the proper atmospheric conditions (57, 76). Other selective agars, such as MacConkey's agar, will support light growth but Salmonella-Shigella agar will not (76).

Culturing and Identification

Patton et al. (69) reported that by holding primary isolation plates for 72 hours, the percentage of

Campylobacter positive cultures was increased by 9%. Rettig (76) found that visible growth in blood cultures may not be evident until five to fourteen days after inoculation. Optimum incubation time depends on the type of culture conditions; it may be shortened by agitating the culture (25) or by the use of a biphasic medium (79), and it can be further shortened by increasing the ratio of inoculum size to the volume of enrichment medium (67). Stern (102) used a method of rapid cultivation of Campylobacter developed by Rollins (79) to prepare and enumerate inocula. Strains were maintained in 10 ml of fluid thioglycollate at 5 C, transferred to a fresh medium and incubated at 42 C for 48 hours. A 1 ml sample was inoculated into a biphasic medium consisting of brucella agar overlayed with 25 ml of brucella broth which was supplemented with FBP. Strains were grown at 42 C at 40 oscillations per minute for 17 hours before use.

Swab, rinse and excision sampling methods are commonly used for detection of microorganisms on poultry carcasses. Isolation rates of C. jejuni are essentially equivalent with all three techniques (9). Many researchers have found the most probable number procedure to be unacceptable for enumerating campylobacters in foods (7, 102).

Harvey (43) suggests the hippurate hydrolysis test as a means of differentiating C. jejuni and C. intestinalis. Strains of C. jejuni hydrolyze hippurate in the two hour

rapid test described by Hwang and Ederer (48), whereas C. intestinalis do not. Herbert et al. (44) found 81% of the C. jejuni strains hydrolyze sodium hippurate but other Campylobacter strains did not.

Campylobacter jejuni is sensitive to air, surviving only one to two days in solid media, two to four days in liquid media, and ten to twenty days in semisolid media at room temperature. Survival can be enhanced by holding cultures at 4 C and by reducing oxygen tension (7). The aerotolerance of Campylobacter has been shown to be improved by the addition of specific quantities of ferrous sulfate, sodium metabisulfite and sodium pyruvate (FBP) to broth and agar and also, colonies appeared one to two days sooner than with unsupplemented agar (7, 29, 66, 89, 92, 97). It was concluded that FBP acts by quenching superoxide anions and other free radicals in the medium and not by any action on cellular metabolism (89, 97).

Methods for Obtaining Proper Atmospheric Conditions

One of the earliest and perhaps most established methods of producing modified atmospheric conditions is evacuation of the air from an anaerobic-type jar and replacement with a gas mixture (17). Stern (97) describes a method of exchanging the atmosphere of a container used to culture C. jejuni with 5% oxygen, 10% carbon dioxide and 85% nitrogen. Blood agar plates containing the samples are

inverted and placed in a modified anaerobe jar, the air is evacuated with a standard laboratory vacuum line and the gas mixture is introduced into the jar until a positive pressure is detected coming out of the jar. The jar is then sealed and placed in an appropriate incubator. A system called Campy-Pak II (BBL Microbiology Systems, Cockeysville, Md.) is designed specifically for use with microaerophilic organisms. Buck et al. (17) found this system virtually identical to the standard method although more expensive. The Fortner principle is also used where a rapidly growing, facultative anaerobe is incubated along with the Campylobacter plate to reduce the oxygen concentration (17, 50).

D. MODIFIED ATMOSPHERE PACKAGING OF POULTRY

In the food industry, modified gas atmosphere packaging is often used, in addition to cold temperature, to increase the shelf-life of meat. Studies have shown that vacuum-packaging reduces the growth of aerobic bacteria, especially Pseudomonas sp., but has minimal effect on the growth of anaerobic bacteria (62, 80). Packaging with carbon dioxide has been found to inhibit aerobes (P. fluorescens in particular), reduce anaerobic growth and have little effect on the growth of Lactobacillus sp. (28, 34, 47). Nitrogen gas packaging has been observed to have little effect on Lactobacillus growth (28, 47). Carbon dioxide

inhibits or retards growth of bacteria by extending the lag phase and generation time of bacteria (106). Tan and Gill (104) demonstrated that this inhibition by carbon dioxide was due to the inhibition of substrate uptake by the cell and not to inhibition of intercellular enzymes.

Modified atmospheres may also be beneficial in reducing the rate of growth of certain pathogenic bacteria. Sillicker and Wolfe (86) demonstrated that a carbon dioxide atmosphere had an inhibitory effect on Salmonella sp. and Staphylococcus aureus. This effect was also observed by Gray et al. (37).

CHAPTER III

MATERIALS AND METHODS

A. EXPERIMENTAL DESIGN

Seven modified gas atmospheres were analyzed as to their abilities to restrict the survival of Campylobacter jejuni in processed turkey roll. Turkey roll slices inoculated with two strains of C. jejuni and uninoculated control slices were stored under each atmosphere at 4 and 21 C for 0, 1, 3, 6, 12 and 18 days and 0, 6, 12, 24 and 48 hours, respectively. Three replications were performed for each treatment. The seven atmospheres under investigation included:

- a) 100% carbon dioxide
- b) 80% carbon dioxide / 20% nitrogen
- c) 60% carbon dioxide / 40% nitrogen
- d) 40% carbon dioxide / 60% nitrogen
- e) 100% nitrogen
- f) 100% air
- g) 10% carbon dioxide / 85% nitrogen / 5% oxygen

B. SAMPLE PREPARATION

Frozen processed turkey roll (North American Provision Co., Phoenix, AR) was purchased from a local retail outlet. Turkey roll with the same manufacturer's lot number was

utilized for the entire investigation. The turkey rolls were thawed at 4 C and sliced into 10 g samples. These turkey roll slices were immediately wrapped in butcher's paper and refrozen at -20 C until needed.

C. CULTURES

Two strains of C. jejuni, ATCC 29428 (American Type Culture Collection, Rockville, MD) and CJ-B4086 (obtained from N. A. Cox, Richard B. Russell Agriculture Research Center, Athens, GA) were used in this study. Cultures were maintained in test tubes containing 7 ml of brucella broth medium with 0.1% agar added to lower oxygen tension in the medium. The tubes were incubated at 42 C for 48 hours under an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen (OCN), and then stored at 4 C. The cultures were transferred weekly with strains being inoculated into fresh brucella broth tubes and again incubated at 42 C for 48 hours under OCN.

D. PREPARATION OF INOCULUM

One test tube containing 7 ml of a 48 hour C. jejuni culture in brucella broth (0.1% agar) was emptied into a 250 ml Erlenmeyer flask containing 100 ml of brucella broth (0.1% agar) supplemented with Blaser-Wang antibiotics (Oxoid U.S.A., Inc., Columbia, Maryland). These enrichment flasks were fitted with one-hole rubber stoppers equipped

with rubber hoses to allow an exchange of atmosphere by pulling a vacuum and then flushing with OCN gas. The enrichment cultures were then incubated for 24 hours at 42 C before being used for inoculation. Before inoculation of the turkey roll slices, each inoculum culture was observed under a phase contrast microscope for viability of C. jejuni cells and absence of contamination. These characteristics were confirmed by the appearance of small, curved rods exhibiting a cork-screw type of motility and very rapid movement across the microscopic field.

E. INOCULATION OF SAMPLES

Turkey roll slices were thawed at 4 C overnight for inoculation. Prior to inoculation, 10 g slices were placed into sterile petri plates in a folded manner. Samples were inoculated by pipetting 0.1 ml of the C. jejuni enrichment cultures into the fold of the turkey slices. Inoculation levels of C. jejuni averaged log 6.0 CFU/g of meat. Controls of uninoculated slices were also analyzed.

F. MODIFIED ATMOSPHERE STORAGE

The petri plates containing inoculated samples and uninoculated control samples for each of the seven atmospheres were placed into glass anaerobe jars modified to allow evacuation of air and flushing with a specified gas atmosphere. The jars were evacuated of air by a standard

laboratory vacuum line and flushed with gas of the desired mixture. When flushing the gas mixtures into the anaerobe jars, the jars were filled until a slight positive pressure could be detected at the hose inlet. This evacuation/flushing procedure was repeated three times to ensure thorough exchange of atmospheres. The jars containing samples for the 18 day study were reflushed every two days to ensure that the desired gas atmospheres were being maintained. The seven different gas mixtures used in this investigation were mixed to specifications and guaranteed to be 99.999% pure (MG Industries, Valley Forge, PA).

G. MICROBIAL ANALYSIS

The following microbial tests were performed on the turkey roll slices at each sampling time:

- a) aerobic plate count (APC) used procedure 4.51 outlined in "Compendium of Methods for the Microbiological Examination of Foods" (6), with a modification in the diluent. Based on preliminary studies, Cary Blair diluent (0.1% agar) was used since it gave improved recovery of C. jejuni. Plates were incubated at 32 C for 48 hours.
- b) lactic acid bacterial count (LAC) used procedure 16.422 of the "Compendium" (6). The samples were plated from Cary Blair diluent (0.1% agar) and

plates were incubated at 35 C for 48 hours.

- c) psychrotrophic plate count used procedure 9.51 of the "Compendium" (6) and used Cary Blair diluent (0.1% agar). Plates were incubated at 7 C for 10 days.
- d) direct C. jejuni count used bloodfree agar base containing cefoperazone selective supplement (Oxoid) incubated at 42 C for 72 hours under an atmosphere of OCN produced by gas generating kits (Oxoid). Bloodfree agar plates were dried for approximately four minutes under ultraviolet light in a laminar flow hood immediately prior to use. Drying was necessary to prevent spreading of C. jejuni colonies to aid counting of plates. Also included in the agar was 1 ml of 2, 3, 5-triphenyl-tetrazolium chloride (1% solution) per 100 ml of agar to assist in differentiating C. jejuni colonies from turkey roll particles (71).
- e) enrichment C. jejuni count used the same procedure outlined in (d) after enriching a 10 g turkey roll slice in the same manner as described in section D, "Preparation of Inoculum".

Decimal dilutions were made of turkey roll samples after mixing in a Stomacher 400 Lab-Blender for two minutes. Cary Blair medium containing only 0.1% agar was used as the diluent. This medium was chosen following a preliminary

investigation of seven commonly used diluents on the basis of its ability to protect C. jejuni throughout the dilution procedure and give consistent recovery counts (71).

Presumptive colonies of C. jejuni appearing on bloodfree agar as 1 mm in diameter, glossy, milky-white and raised were confirmed by observation of morphology and motility under a phase-contrast microscope.

H. TURKEY ROLL COMPOSITION

Turkey rolls used in this study were analyzed by proximate analysis using AOAC procedures 24.027 for nitrogen, 24.005 for fat, 24.002 for moisture, and 24.009 for ash (5). The frozen turkey roll sample was prepared by grinding in a Waring blender.

I. STATISTICAL ANALYSIS

Statistical analysis was performed from an incomplete block fractional factorial design with data being collected from three replications (83). Analysis of variance was used to evaluate comparative data. Data presented are the means of all replications of each study. Slope values of survival/growth curves for each atmosphere were generated by linear regression using the mean log growth values for each sampling time. Significant differences ($P < 0.05$) among means were separated using the least square means method.

Correlations were determined by use of general linear models employed by SAS. Correlations of mean slope values were determined among carbon dioxide levels used in packaging and growth/survival of specific types of bacteria. These correlations only included the five atmospheres containing varying levels of carbon dioxide and nitrogen. Also, correlations were determined among the five types of microbiological tests performed within each of the seven atmospheres by using actual growth/survival counts at each sampling time. This was done to observe competition effects.

CHAPTER IV

RESULTS AND DISCUSSION

A. BACTERIAL GROWTH/SURVIVAL AT 4 C

Survival of Campylobacter jejuni

The first objective of this study was to determine the effects of various levels of carbon dioxide and nitrogen used as packaging atmospheres on the survival of C. jejuni inoculated into processed turkey roll. Both survival curves and mean slope values of survival curves representing changes in C. jejuni survival over time will be presented to facilitate discussion of the data.

Campylobacter jejuni survival in processed turkey roll stored at 4 C was evaluated by both direct (Figure 1) and enrichment (Figure 2) plating procedures. Campylobacter jejuni was not detected in uninoculated control samples by either plating method. The level of detection for this study was approximately ten campylobacters per gram of turkey roll.

Direct plate counts showed that initial levels of C. jejuni inoculated into turkey roll slices were approximately log 6.0 to log 7.0 CFU/g under all atmospheres (Figure 1). Atmospheres containing elevated levels of carbon dioxide (40-100%) and 100% nitrogen had greater than log 4.8 CFU/g after six days of storage at 4 C as compared to the 10% carbon dioxide/85% nitrogen/5% oxygen (OCN) atmosphere. No

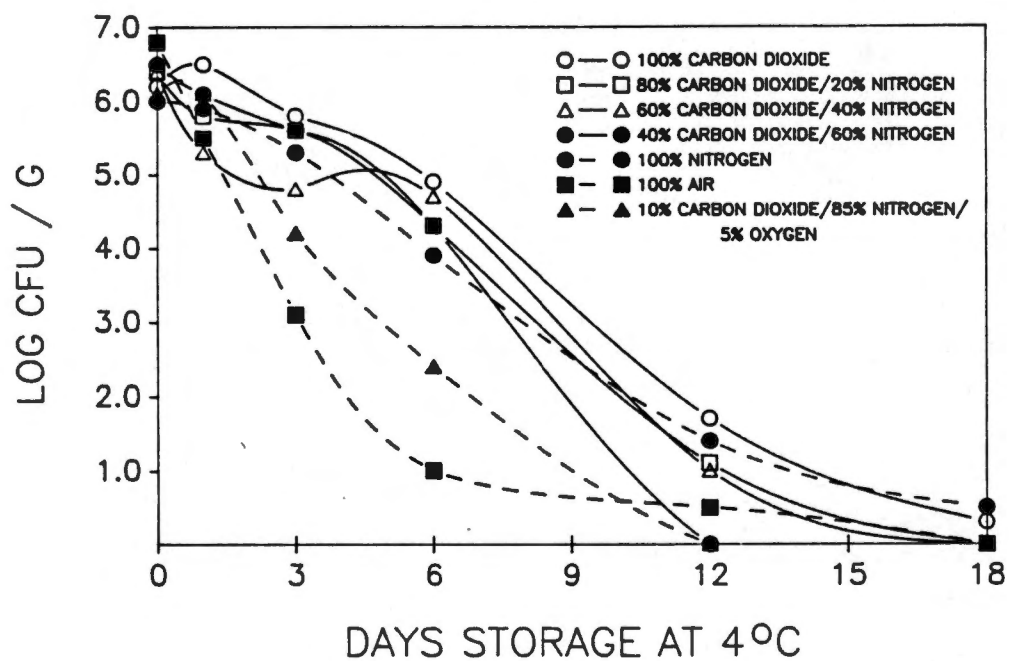


FIGURE 1. Survival of *C. jejuni* inoculated into turkey roll and stored at 4 C under air or modified atmospheres (enumeration by direct plating).

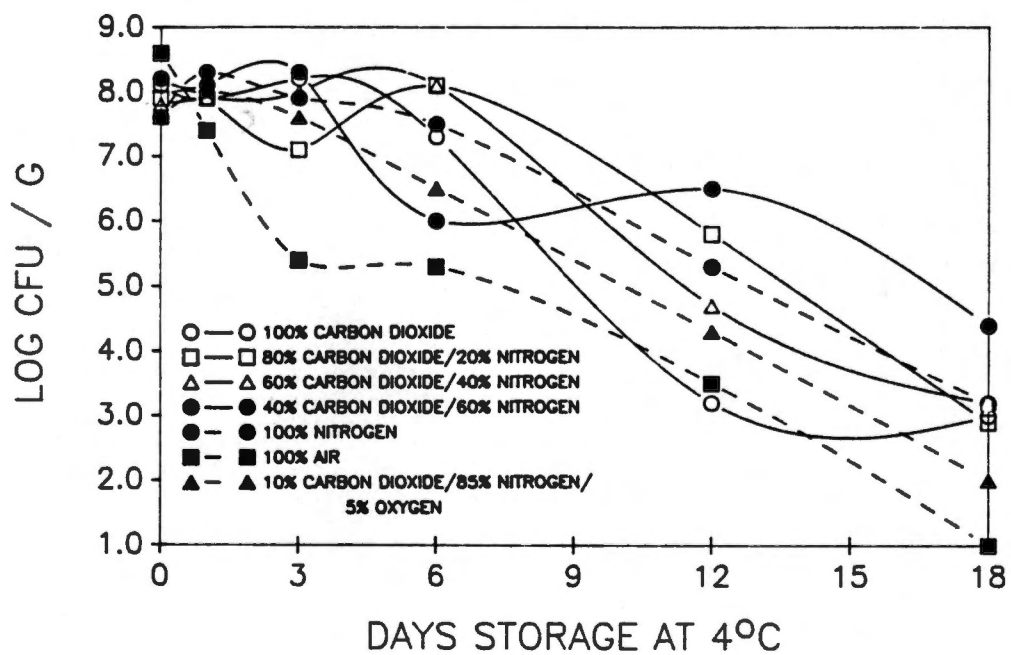


FIGURE 2. Survival of *C. jejuni* inoculated into turkey roll and stored at 4 C under air or modified atmospheres (enumeration by plating after enrichment).

campylobacters were detected in turkey roll held under 100% air after six days of storage (Figure 1). The 40% carbon dioxide/60% nitrogen mixture seemed to be more injurious to C. jejuni than the other three atmospheres containing elevated carbon dioxide concentrations (Figure 1). A sharp decline in viable C. jejuni numbers in turkey roll resulted between the sixth and twelfth days of storage at 4 C under this atmosphere. Counts decreased from approximately log 4.5 CFU/g to below detectable levels over this six day period. Campylobacters could not be detected in turkey roll stored under any of the atmospheres tested by the eighteenth day of sampling (Figure 1) by direct plating procedures.

Slope values in Figure 3 provide a means of statistically comparing C. jejuni survival rates in processed turkey roll over time under the various atmospheres. These slope values were obtained by linear regression and represent changes in C. jejuni survival from the day of inoculation until the sampling time at which counts were below the level of detection (approximately 10 CFU/g). A negative slope indicated that the number of viable C. jejuni cells decreased over time. As the negative slope values became greater, the injury or destruction to C. jejuni had increased. For example, the 100% carbon dioxide atmosphere resulted in a mean slope value of -0.31 and indicated a slower loss in viability of C. jejuni than in

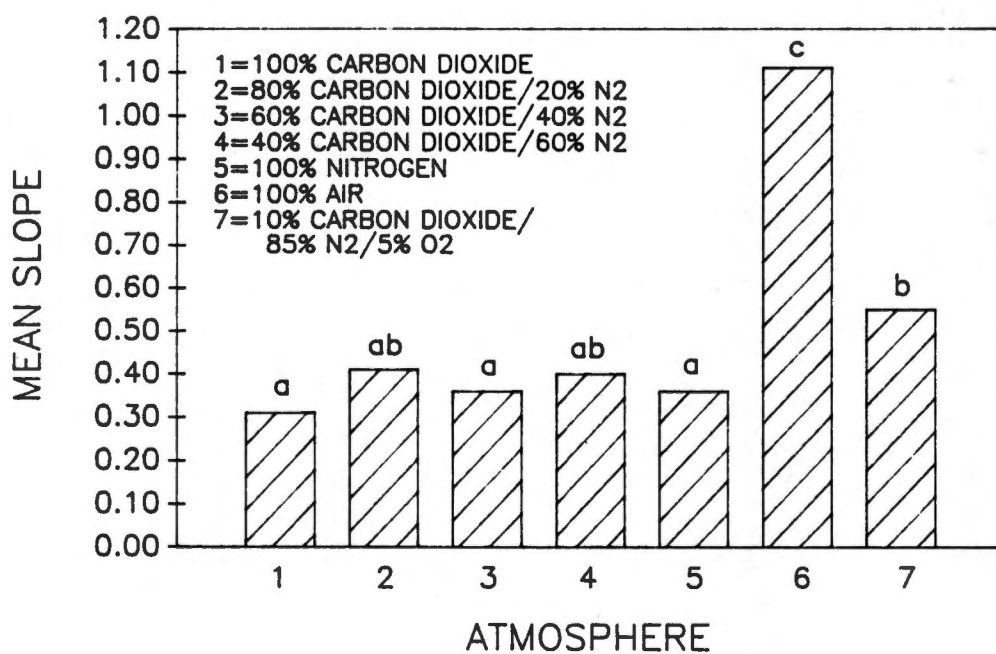


FIGURE 3. Mean slope values for *C. jejuni* survival curves shown in Figure 1. Mean slope is represented as the absolute value of the negative slope. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c).

the OCN atmosphere which resulted in a slope of -0.55 (Figure 3).

The atmospheres containing elevated carbon dioxide levels (40-100%) and 100% nitrogen resulted in significantly ($P < 0.05$) lower negative slope values than 100% air (Figure 3). This finding supports observations concerning the survival curves presented in Figure 1. The OCN atmosphere (Figure 3) was significantly ($P < 0.05$) more inhibitory than the atmospheres of 100% carbon dioxide, 60% carbon dioxide and 100% nitrogen which contained no oxygen. It should be pointed out that the OCN atmosphere is recommended for isolation and cultivation of C. jejuni at 42 C (97).

Therefore, the optimal atmosphere for survival of C. jejuni might differ at 4 C as compared to 42 C. Stern et al. (100) found similar results with this atmosphere at 4 C. It could also be hypothesized that dissolved oxygen is greater at 4 C than at 42 C and thus may be toxic to C. jejuni. This hypothesis would be supported by our findings that the 100% air (which contains approximately 21% oxygen) was the most toxic atmosphere to C. jejuni at 4 C (Figure 3).

For all atmospheres except 100% carbon dioxide, a significant ($P < 0.05$) negative correlation was found between aerobic plate count and C. jejuni direct counts over storage (Table 1). Since aerobic plate counts increased in all treatments (Figure 4) as C. jejuni decreased, with the exception of 100% carbon dioxide, the decreases in C. jejuni

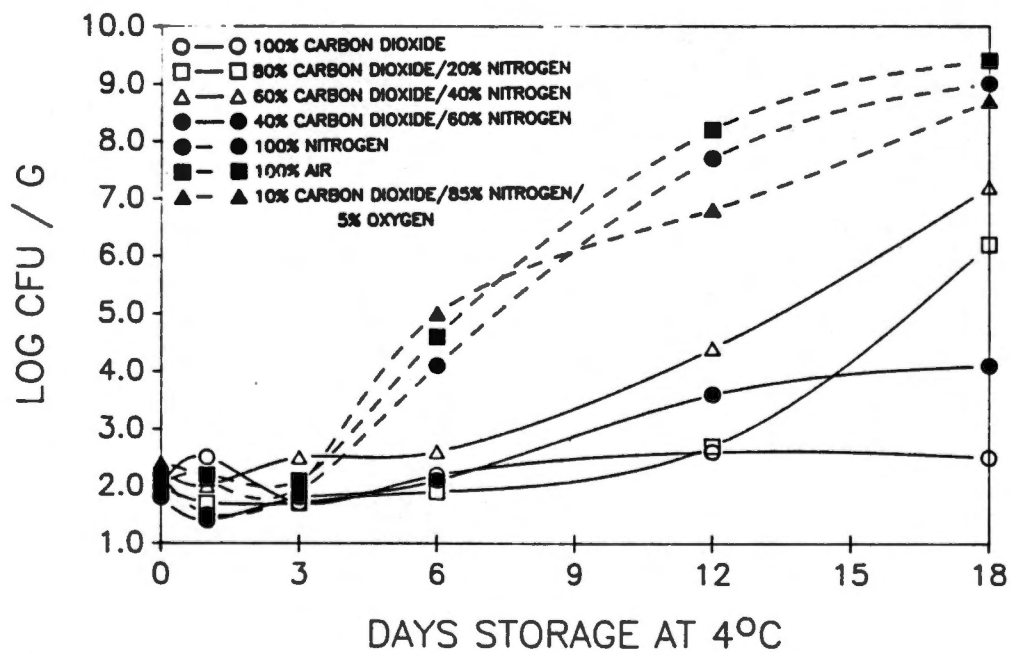


FIGURE 4. Aerobic bacterial growth in turkey roll stored at 4 C under air or modified atmospheres.

may be associated in some way with competitive aerobic or facultatively anaerobic bacteria. No significant ($P < 0.05$) correlation was found to indicate that increasing carbon dioxide levels from 0 to 100% positively or negatively affected C. jejuni survival at 4 C.

Enrichment procedures are generally used before selective plating for the detection and recovery of C. jejuni from food products (25). In research studies, however, data on surviving numbers of campylobacters are complicated by the use of enrichment steps. Since bacterial numbers increase during enrichment, the researcher cannot detect the actual number of cells in the product at the time of sampling. This has led to the omission of the enrichment step by some researchers doing survival studies of C. jejuni (25). The importance of enrichment for the detection of campylobacters from turkey roll was illustrated by the data in Figure 2. Campylobacter jejuni was detected from turkey roll stored under all seven packaging atmospheres on the eighteenth day at 4 C when enrichment procedures were employed. Enrichment survival curves of C. jejuni (Figure 2) were generally 1.0 to 3.0 logs higher than direct plating survival curves (Figure 1). The atmospheres of 100% air, OCN and 40% carbon dioxide were the most injurious to C. jejuni by the direct plating method. By enrichment, however, these atmospheres had recoveries 4.0 to 5.0 logs higher (Figure 2). These larger differences in enrichment

versus direct counts indicated that 100% air, OCN and 40% carbon dioxide caused severe injury to C. jejuni cells but did not completely destroy them. The enrichment procedure acts by repairing these injured cells, thus allowing recovery by selective plating. This observation indicates the necessity of enriching food samples for the detection of C. jejuni since levels as low as 500 cells have been shown to cause human illness when ingested (77).

Aerobic Bacterial Growth

Another objective of this study was to observe the effect of various levels of carbon dioxide on the growth of spoilage microflora of processed turkey roll. Correlations were determined between C. jejuni survival and aerobic bacterial growth to observe the effect of competition on C. jejuni survival at 4 C.

Aerobic plate counts showed an initial level of approximately log 2.0 CFU/g of turkey roll. The packaging atmospheres containing elevated carbon dioxide levels (40-100%) were the most inhibitory to aerobic bacterial growth in turkey roll stored at 4 C (Figure 4). The 100% carbon dioxide atmosphere was the most inhibitory over the eighteen days of storage. Virtually no aerobic growth occurred under this atmosphere. The 40% carbon dioxide atmosphere also caused aerobic bacterial inhibition with counts increasing by only log 1.5 CFU/g over the eighteen days of storage

(Figure 4). The three atmospheres containing 0-10% carbon dioxide (OCN, 100% air and 100% nitrogen) resulted in substantial increases in aerobic counts in turkey roll held at 4 C. These three atmospheres allowed an increase of approximately log 7.0 CFU/g by the eighteenth day sampling (Figure 4).

This study clearly demonstrated that high carbon dioxide levels were effective in controlling aerobic bacterial growth in turkey roll stored at 4 C. Thomas et al. (106) demonstrated that carbon dioxide inhibits or retards bacterial growth by extending the lag phase. The same effect was observed in this study. The aerobic bacterial growth curves (Figure 4) of the elevated carbon dioxide atmospheres caused the lag phase of the bacteria to be extended as compared to the atmospheres of 100% air, 100% nitrogen and OCN. These latter three atmospheres resulted in a lag phase of three days at 4 C, whereas, the three atmospheres comprised of carbon dioxide and nitrogen remained in lag phase from six to twelve days. The 100% carbon dioxide atmosphere held aerobic bacteria in lag phase throughout the eighteen days of storage (Figure 4).

Slope values of aerobic bacterial growth curves of turkey roll stored at 4 C were used to compare the seven atmospheres over the eighteen days of storage (Figure 5). These slope values, once again, support observations discussed from the actual growth curves (Figure 4). The

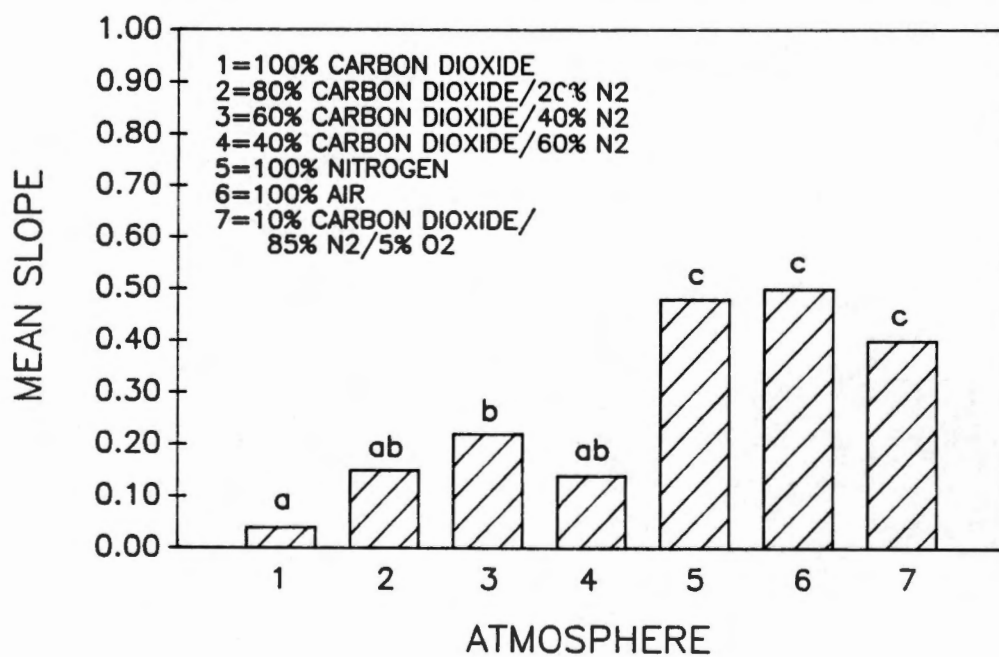


FIGURE 5. Mean slope values for aerobic plate count curves shown in Figure 4. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c).

four atmospheres containing high carbon dioxide levels were significantly ($P < 0.05$) more inhibitory to aerobic bacterial growth than those of OCN, 100% air and 100% nitrogen (Figure 5). This can be seen by the much lower slope values for the high carbon dioxide atmospheres.

A significant ($P = 0.0001$) negative correlation was found to exist between increasing carbon dioxide levels used for packaging and the growth of aerobic bacteria (Table 2) on processed turkey roll stored at 4 C. A significant ($P < 0.05$) positive correlation was also found between aerobic plate count and psychrotrophic plate count within each atmosphere (Table 1). This indicated that the predominant spoilage microflora of processed turkey roll stored at 4 C were psychrotrophs. As stated in discussion of C. jejuni survival in turkey roll at 4 C, a significant ($P < 0.05$) negative correlation existed between C. jejuni survival and the growth of aerobic bacteria. Hanninen et al. (42) concluded that aerobic competition did not effect the survival of C. jejuni at 4 C when inoculated into fresh beef.

Psychrotrophic Bacterial Growth

The psychrotrophic bacterial growth curves (Figure 6) were very similar to those of aerobic growth (Figure 4) for turkey roll stored at 4 C. Elevated carbon dioxide atmospheres were considerably superior in psychrotrophic

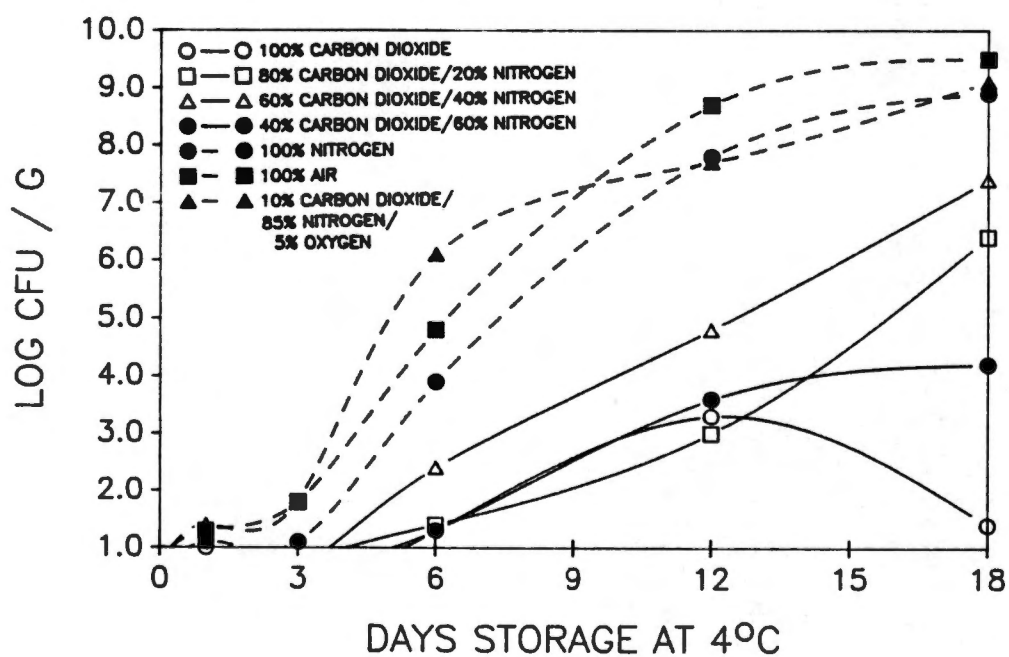


FIGURE 6. Psychrotrophic bacterial growth in turkey roll stored at 4 C under air or modified atmospheres.

bacterial inhibition as compared to 100% air, 100% nitrogen and OCN. Psychrotrophic numbers remained very low (less than log 1.5 CFU/g) over the first three days of storage at 4 C. Psychrotrophic bacterial counts rose sharply between the third and sixth days of storage for turkey roll held under 100% air, 100% nitrogen and OCN. These three packaging atmospheres resulted in a 3.0 to 4.5 log increase in psychrotrophic numbers over this three day period (Figure 6). Psychrotrophic counts were not detectable (less than log 1.0 CFU/g) from turkey roll held under elevated carbon dioxide levels before the sixth day of sampling. By comparing aerobic plate counts (Figure 4) and psychrotrophic plate counts (Figure 6) of turkey roll at 4 C, it can be seen that aerobic counts were approximately 1.0 log higher over the initial three days of storage. The psychrotrophic counts increased more rapidly between the third and sixth days than aerobic counts and were approximately the same by the sixth storage day. This observation indicated that mesophilic bacteria survived at 4 C in turkey roll over the first three days at which time psychrotrophs began to dominate.

The slope values of the psychrotrophic growth curves (Figure 7) show the same results as the curves themselves (Figure 6). The 100% carbon dioxide atmosphere was significantly ($P < 0.05$) more inhibitory to psychrotrophic growth than all other atmospheres. The remaining three

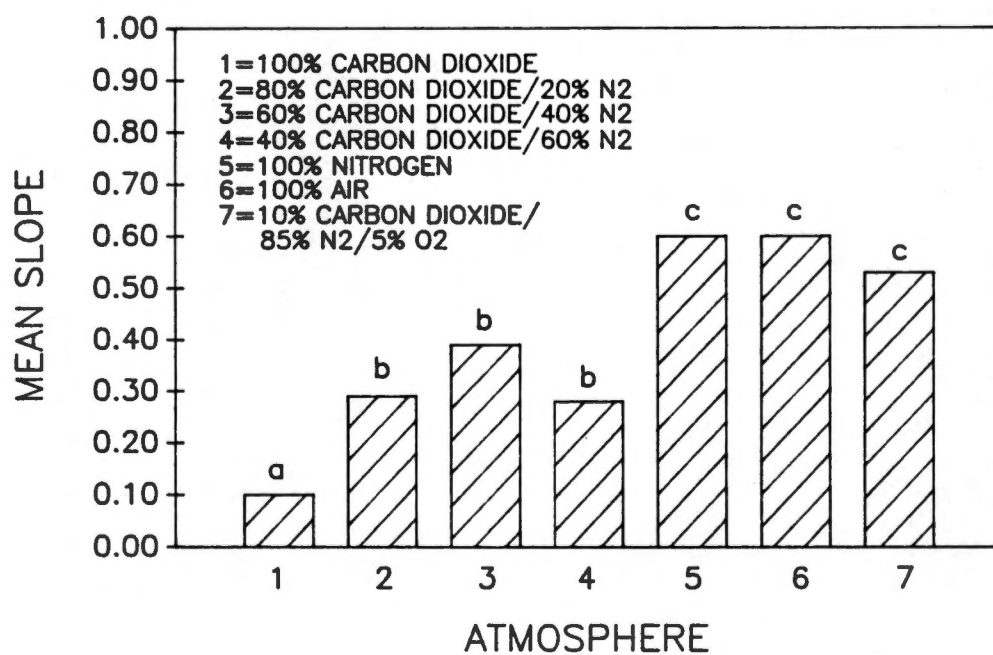


FIGURE 7. Mean slope values for psychrotrophic plate count curves shown in Figure 6. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c).

elevated carbon dioxide atmospheres were significantly ($P < 0.05$) more inhibitory than 100% air, 100% nitrogen and OCN (Figure 7). A significant ($P = 0.0001$) negative correlation was found to exist between increasing carbon dioxide level and psychrotrophic bacterial growth in turkey roll stored at 4 C (Table 2).

Lactic Acid Bacterial Growth

Growth of lactic acid bacteria remained fairly low in processed turkey roll at 4 C under all seven atmospheres (Figure 8) throughout the eighteen days of storage. Initially, counts were less than log 1.0 CFU/g under all atmospheres. The OCN atmosphere resulted in the highest level of lactic acid bacterial growth with counts reaching log 3.7 CFU/g by day eighteen. The atmospheres containing high levels of carbon dioxide were very inhibitory to lactics. These atmospheres, with the exception of 60% carbon dioxide, resulted in less than a log 0.5 CFU/g increase in lactics from turkey roll stored at 4 C for eighteen days (Figure 8). The 100% air, 100% nitrogen and 60% carbon dioxide allowed approximately a log 1.2 CFU/g increase over the same time period.

Low initial counts of lactic acid bacteria were also observed by Mercuri et al. (58). They found that 83% of precooked turkey roll samples had levels of lactic acid bacteria equal to or below log 2.0/g. Spahl et al. (94)

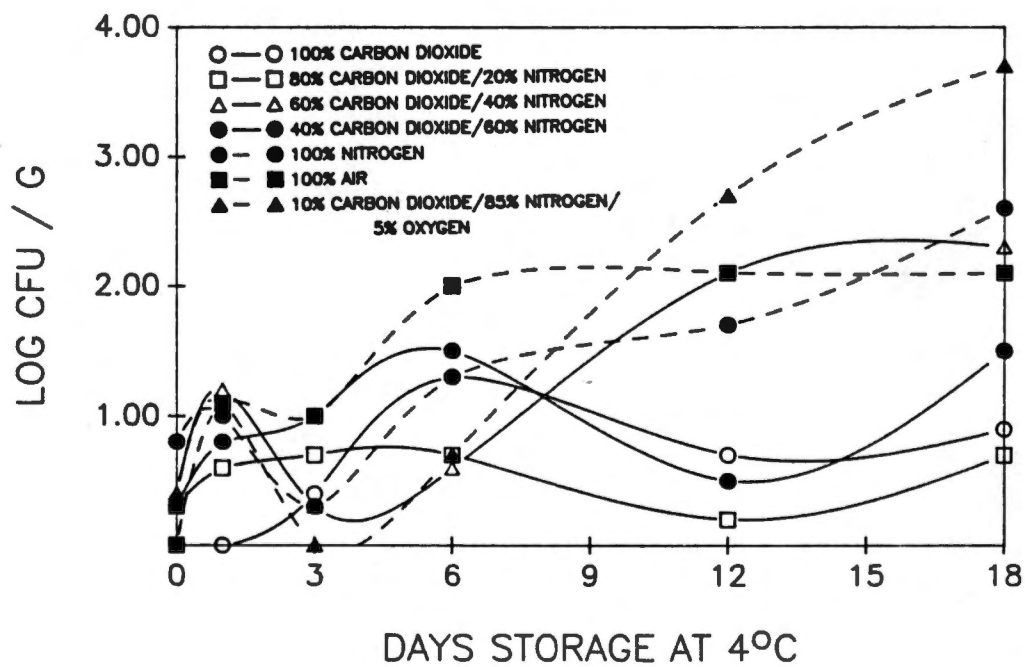


FIGURE 8. Lactic acid bacterial growth in turkey roll stored at 4 C under air or modified atmospheres.

found that lactic acid bacterial numbers never became significant in a study of pork chops stored at 2 C under various levels of carbon dioxide, nitrogen and oxygen. Christopher et al. (22) noted that the number of lactobacilli in pork packaged under various modified atmospheres remained low during early storage but increased rapidly between 21 and 35 days of storage. Our study was not long enough in duration to determine if this would have occurred in processed turkey roll.

When slope values of the lactic acid bacteria growth curves were statistically analyzed, few differences in counts were observed (Figure 9). The 80% carbon dioxide mixture was significantly ($P < 0.05$) more inhibitory than the 60% carbon dioxide and the OCN atmospheres. The OCN atmosphere allowed the highest level of lactic growth and was significantly ($P < 0.05$) inferior in lactic inhibition than all atmospheres except 100% nitrogen and 40% carbon dioxide (Figure 9).

B. BACTERIAL GROWTH/SURVIVAL AT 21 C

Survival of Campylobacter jejuni

Direct and enrichment plating procedures were employed to evaluate C. jejuni survival in turkey roll stored at 21 C. By direct plating, the initial inoculum of C. jejuni was approximately log 5.0 to 6.5 CFU/g (Figure 10).

Campylobacter jejuni numbers decreased noticeably under all

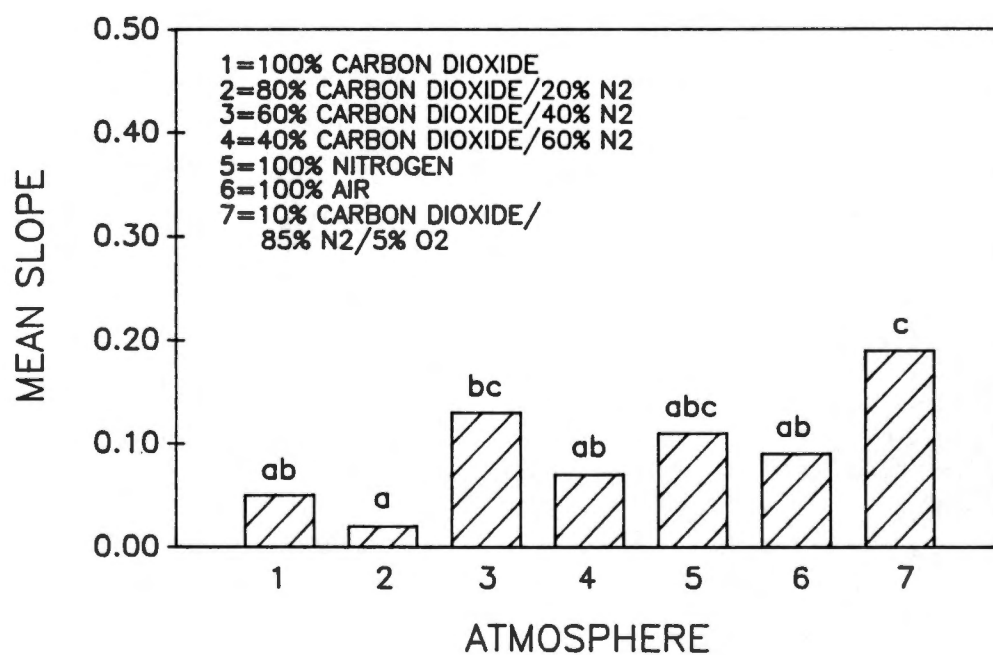


FIGURE 9. Mean slope values for lactic acid bacterial growth curves shown in Figure 8. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c).

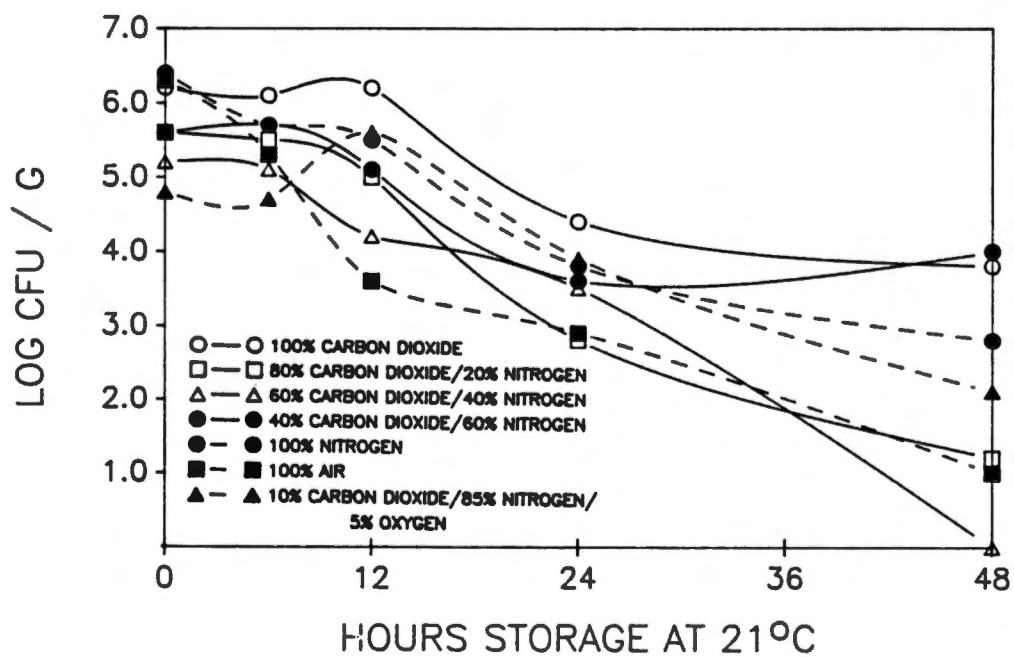


FIGURE 10. Survival of *C. jejuni* inoculated into turkey roll and stored at 21 C under air or modified atmospheres (enumeration by direct plating).

atmospheres after the twelfth hour of storage. The 100% carbon dioxide atmosphere provided the most protection to C. jejuni with counts decreasing by log 2.0 CFU/g over the 48 hour storage period. Only slight differences in levels of C. jejuni were noted at the 24 hour sampling period among the atmospheres of OCN, 60% carbon dioxide, 100% nitrogen and 40% carbon dioxide (Figure 10). At this point in sampling, the 100% air and 80% carbon dioxide atmospheres resulted in lower C. jejuni recoveries than the other five atmospheres tested. Campylobacters were detected at the 48 hour sampling from turkey roll stored under all atmospheres except 60% carbon dioxide (detection limit of approximately 10 campylobacters/g) (Figure 10).

The use of slope values of C. jejuni survival curves to determine differences in survival under various atmospheres over 48 hours of storage indicated that the differences between atmospheres were not great (Figure 11). The 100% carbon dioxide atmosphere, which appeared the least injurious to C. jejuni as seen from the survival curves (Figure 10), was only statistically ($P < 0.05$) more protective than 100% nitrogen and 100% air (Figure 11). The lack of differences in C. jejuni survival between the seven gas atmospheres indicated that the effect of carbon dioxide on the survival of C. jejuni in turkey roll was diminished at 21 C (Figure 11) as compared to 4 C (Figure 3). This was probably due to decreased solubility of carbon dioxide at the 21 C storage

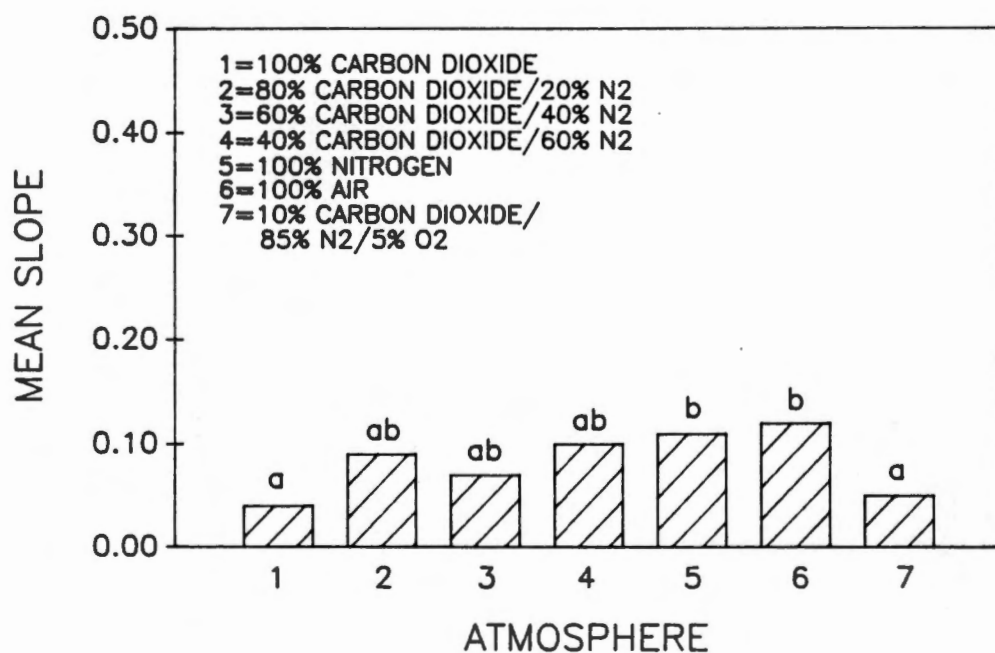


FIGURE 11. Mean slope values for *C. jejuni* survival curves shown in Figure 10. Mean slope is represented as the absolute value of the negative slope. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b).

temperature. No significant ($P < 0.05$) correlation was found among carbon dioxide levels and C. jejuni survival at 21 C. A significant ($P < 0.05$) negative correlation was found between C. jejuni survival and the growth of aerobic bacteria within each atmosphere at 21 C (Table 3).

Enrichment plating (Figure 12) of C. jejuni from turkey roll samples held at 21 C provided recoveries that were approximately log 2.0 CFU/g higher than direct plating (Figure 10). These enrichment counts did not seem to indicate that certain atmospheres resulted in higher injury to C. jejuni as opposed to death than others. All atmospheres resulted in similar differences between enrichment and direct plating counts at 21 C, whereas, at 4 C these differences were greater for certain atmospheres (Figures 1 and 2). This observation further supports the conclusion that carbon dioxide used in high levels as a packaging atmosphere for turkey roll does not exert the same protective effect on C. jejuni at 21 C as seen at 4 C. Once again, this was probably due to decreased solubility of carbon dioxide at 21 C. Campylobacters were detected from turkey roll samples held under all atmospheres at the 48 hour sampling by enrichment (Figure 12).

Aerobic Bacterial Growth

The initial aerobic bacterial counts for turkey roll stored at 21 C were between log 1.0 and log 2.0 CFU/g for

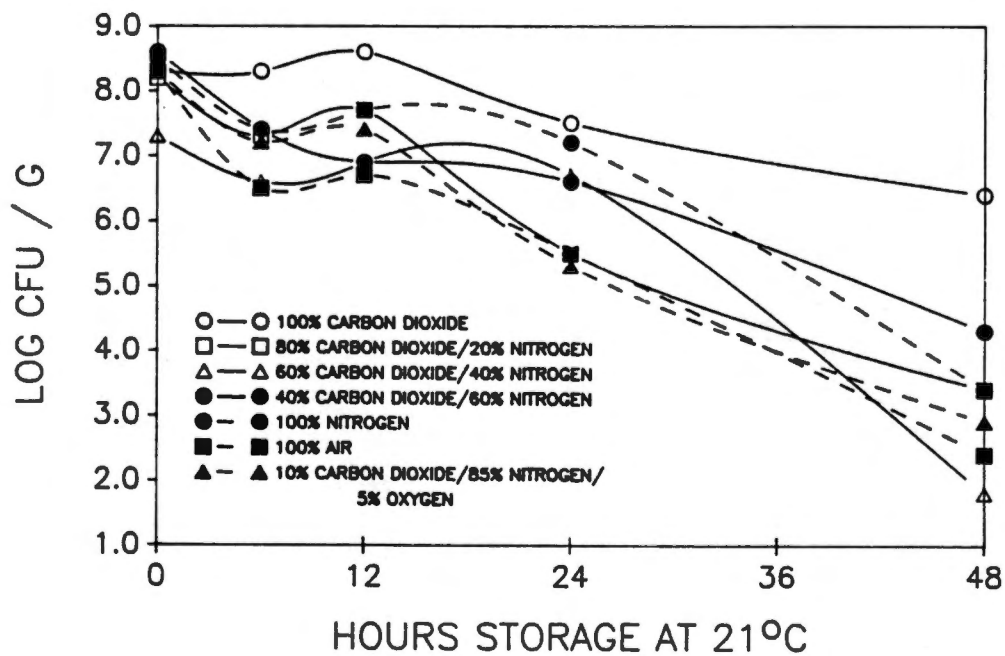


FIGURE 12. Survival of *C. jejuni* inoculated into turkey roll and stored at 21 C under air or modified atmospheres (enumeration by plating after enrichment).

all atmospheres (Figure 13). The 100% air atmosphere resulted in the highest aerobic growth with counts increasing to log 9.0 CFU/g by the 48 hour sampling. All other atmospheres, with the exception of 100% carbon dioxide, allowed similar increases in aerobic bacterial growth to approximately log 8.0 CFU/g. The 100% carbon dioxide atmosphere was the most inhibitory and resulted in an increase in aerobic growth to log 5.5 CFU/g at the 48 hour sampling (Figure 13). Carbon dioxide inhibits or retards bacterial growth by extending the lag phase (106). As seen in Figure 13, the lag phases of the growth curves were very short for all atmospheres (approximately six hours). The inhibitory action of carbon dioxide on aerobic bacterial growth was greatly diminished at 21 C as compared to 4 C (Figure 5).

The aerobic bacterial growth curve slopes (Figure 14) indicated that 100% carbon dioxide was significantly ($P < 0.05$) more inhibitory to aerobic growth in turkey roll than all atmospheres except 40% carbon dioxide. The 100% nitrogen atmosphere was significantly ($P < 0.05$) less inhibitory than all atmospheres containing elevated carbon dioxide levels (Figure 14). These slope values support the observations discussed from the actual aerobic bacterial growth curves in which few differences were found between atmospheres for aerobic growth, with the exception of 100% carbon dioxide being noticeably more inhibitory.

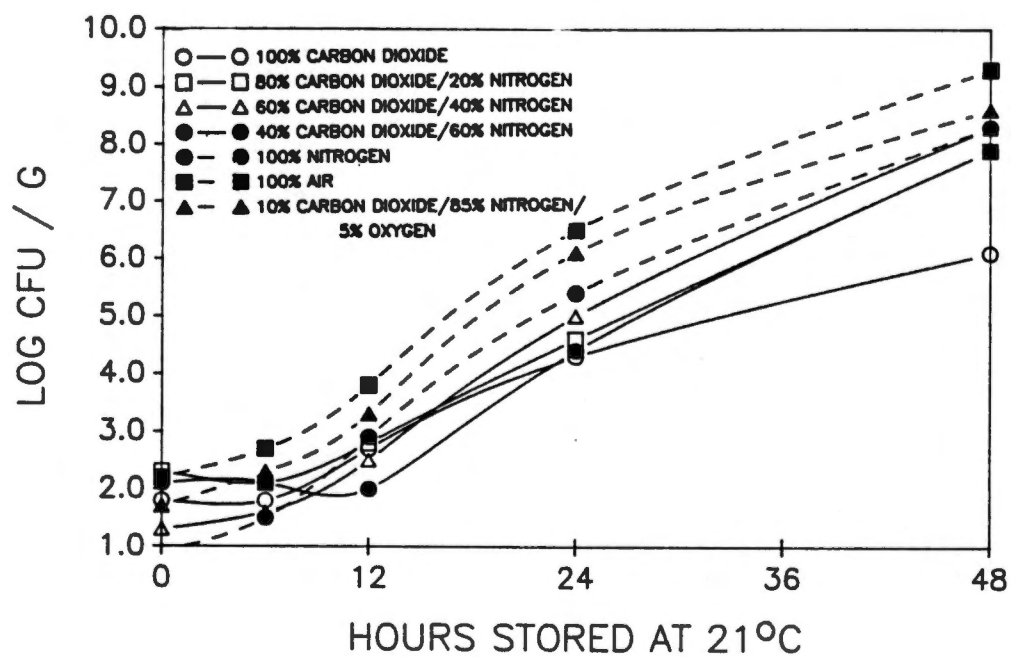


FIGURE 13. Aerobic bacterial growth in turkey roll stored at 21 C under air or modified atmospheres.

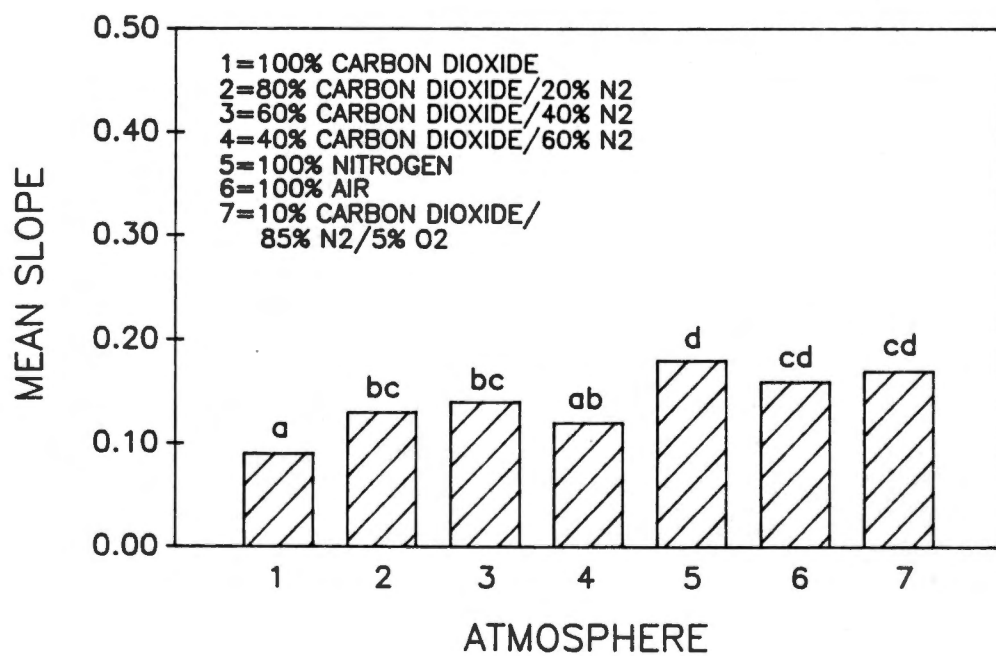


FIGURE 14. Mean slope values for aerobic plate count curves shown in Figure 13. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c,d).

Psychrotrophic Bacterial Growth

The psychrotrophic bacterial growth curves (Figure 15) indicated that the level of psychrotrophic bacteria present in turkey roll stored at 21 C was very low initially. The 100% carbon dioxide atmosphere was the most inhibitory to psychrotrophic growth with counts only reaching log 4.0 CFU/g after 48 hours of storage at 21 C. The 40% carbon dioxide mixture was slightly inhibitory to psychrotrophs and resulted in a count of log 6.5 CFU/g after 48 hours. The remaining five atmospheres allowed somewhat higher psychrotrophic growth, specifically between the sixth and twenty-fourth hours of storage (Figure 15). The final 48 hour psychrotrophic counts for these five atmospheres were between log 7.0 and log 8.5 CFU/g.

At 21 C, the psychrotrophic growth curves (Figure 15) were approximately log 1.0 CFU/g lower than the corresponding aerobic plate count curves (Figure 13) for all atmospheres. This indicated that the spoilage microflora present on the turkey roll at 21 C included both mesophilic and psychrotrophic bacteria throughout the 48 hours of storage. At the 4 C storage temperature, the aerobic plate count curves (Figure 4) and the psychrotrophic growth curves (Figure 6) resulted in almost identical counts over the eighteen days of storage. This indicated that the spoilage microflora of turkey roll held at refrigeration temperature was almost exclusively psychrotrophic.

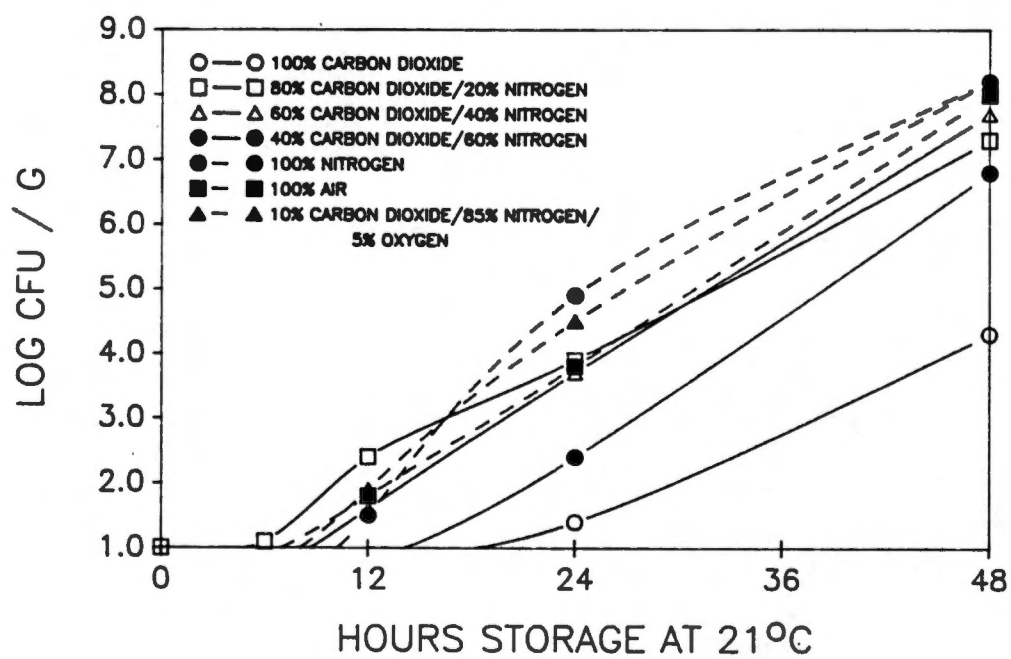


FIGURE 15. Psychrotrophic bacterial growth in turkey roll stored at 21 C under air or modified atmospheres.

The slope values for the psychrotrophic growth curves at 21 C substantiate the observation that carbon dioxide effects on bacteria were not as pronounced at 21 C as compared to 4 C. The 100% carbon dioxide atmosphere was significantly ($P < 0.05$) more inhibitory to psychrotrophs at 21 C than all other atmospheres except 40% carbon dioxide (Figure 16). The 40% carbon dioxide mixture was significantly ($P < 0.05$) more inhibitory than 100% nitrogen and the OCN atmosphere.

Lactic Acid Bacterial Growth

Unlike lactic acid bacterial growth at 4 C (Figure 8), growth of lactics at 21 C in turkey roll was substantial under all atmospheres (Figure 17). Initial levels of lactics present in the turkey roll were approximately log 1.0 to log 2.0 CFU/g at 21 C. Lactic acid bacterial counts increased markedly between the twelfth and twenty-fourth hours of storage under all atmospheres (Figure 17). The 100% air atmosphere resulted in the highest growth of lactics with counts increasing to approximately log 9.0 CFU/g by the 48 hour sampling. The 100% carbon dioxide atmosphere resulted in the slowest increase in lactics with counts increasing by log 4.0 CFU/g over 48 hours of storage at 21 C (Figure 17).

The slope values of lactic acid bacterial growth curves (Figure 18) indicated that only small differences existed

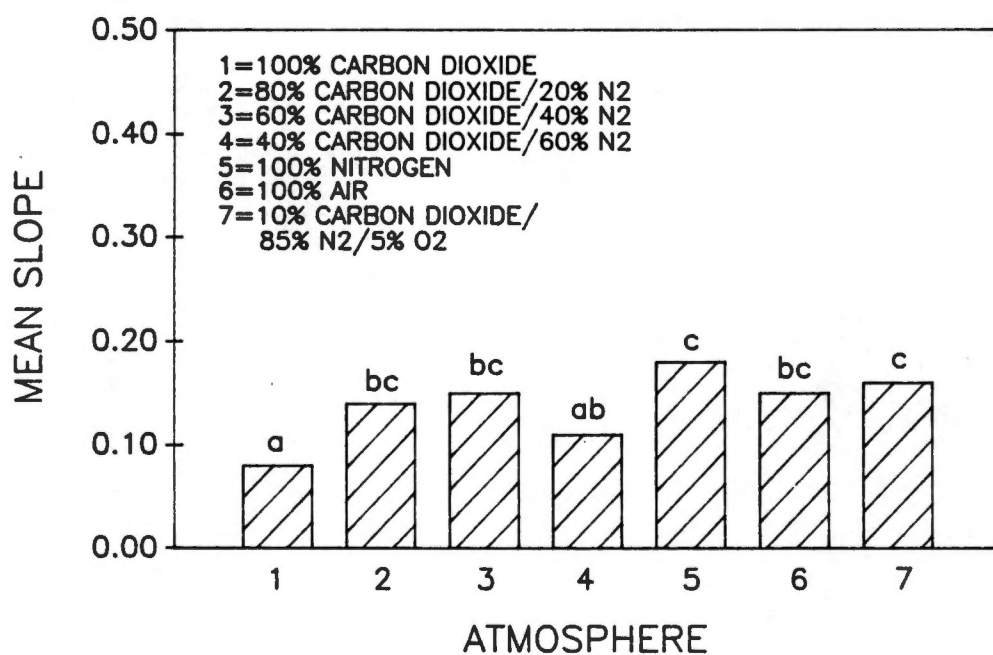


FIGURE 16. Mean slope values for psychrotrophic plate count curves shown in Figure 15. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c).

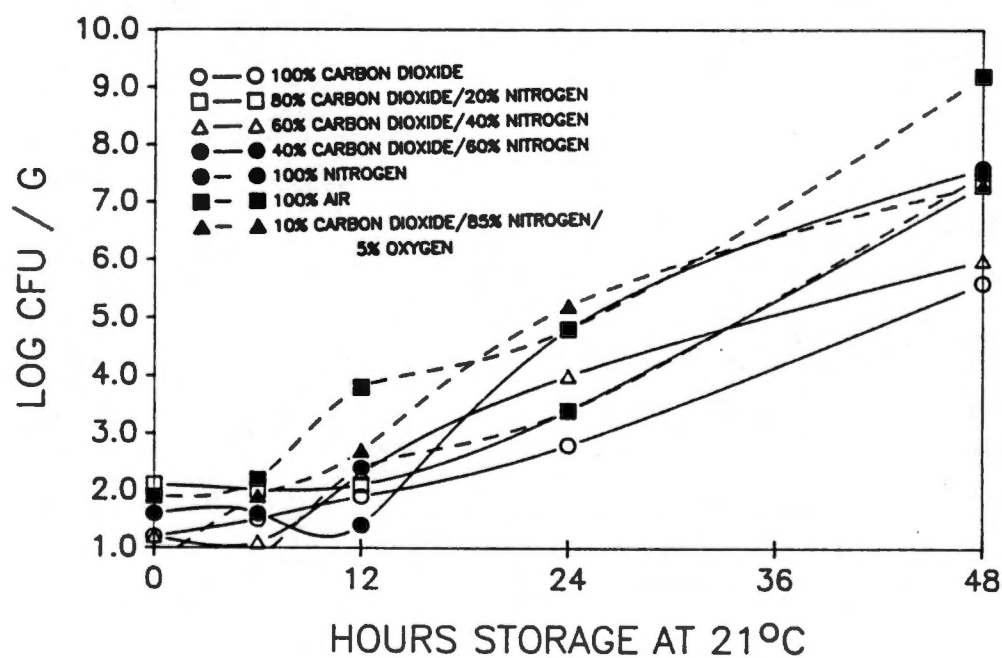


FIGURE 17. Lactic acid bacterial growth in turkey roll stored at 21 C under air or modified atmospheres.

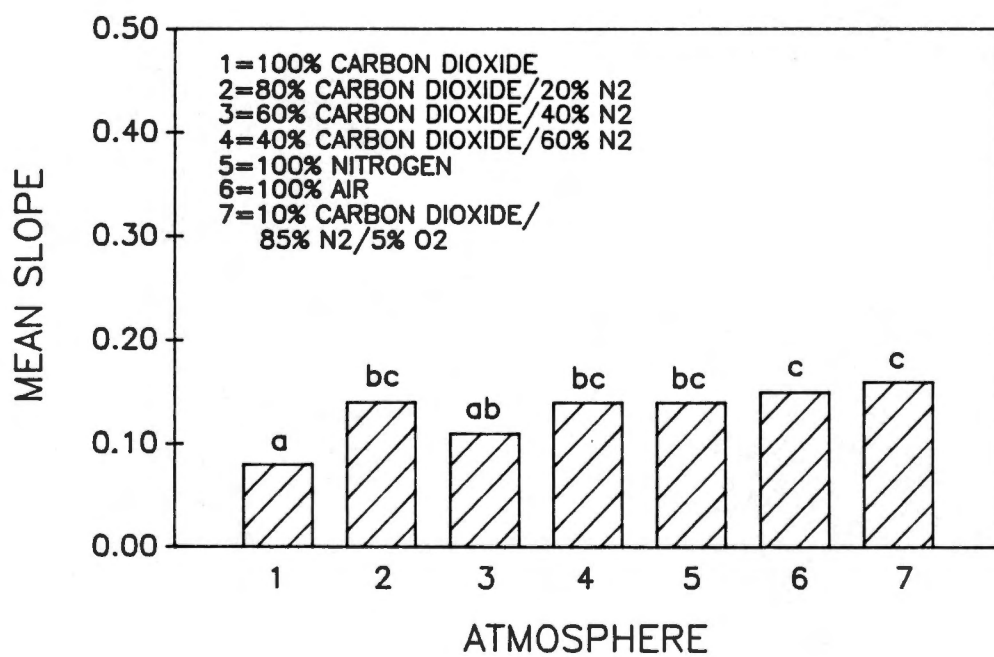


FIGURE 18. Mean slope values for lactic acid bacterial growth curves shown in Figure 17. Statistically significant differences ($P < 0.05$) are indicated with different letters (a,b,c).

among most of the atmospheres tested. The 100% carbon dioxide atmosphere was significantly ($P < 0.05$) more inhibitory to lactic acid bacteria than all atmospheres except 60% carbon dioxide (Figure 18).

CHAPTER V

CONCLUSIONS

The first objective of this study was to determine the extent to which Campylobacter jejuni inoculated into processed turkey roll could survive at 4 and 21 C under various atmospheric mixtures of carbon dioxide, nitrogen and oxygen. Campylobacter jejuni numbers declined steadily under all atmospheres at both storage temperatures. The atmospheres comprised of elevated levels of carbon dioxide (40-100%), generally, were the most protective to C. jejuni in turkey roll over storage. However, this protection was more pronounced at 4 C and is expected to be the result of increased carbon dioxide solubility at the lower temperature. The 100% nitrogen atmosphere was also protective to C. jejuni in turkey roll at 4 and 21 C. The two atmospheres containing oxygen were the most inhibitory to C. jejuni even though the OCN atmosphere is normally considered the optimal atmospheric mixture for laboratory cultivation of C. jejuni. Competition from indigenous spoilage microflora of the turkey roll seemed to adversely affect the survival of C. jejuni as seen by significant ($P < 0.05$) negative correlations between aerobic bacterial growth and C. jejuni survival at both temperatures.

The importance of selectively enriching food samples for the detection of C. jejuni was obvious from this study.

Campylobacters were recovered from turkey roll stored under all atmospheres on the eighteenth day at 4 C and on the forty-eighth hour at 21 C. Since very low levels of C. jejuni contamination have been shown to cause illness, the importance of detecting low numbers in foods is emphasized.

High levels of carbon dioxide were inhibitory to aerobic and psychrotrophic bacterial growth in the turkey roll. Once again, the inhibitory action of carbon dioxide was more noticeable at 4 C and resulted in an extended lag phase of bacterial growth. Campylobacter jejuni numbers remained higher in the elevated carbon dioxide atmospheres, while at the same time, spoilage bacterial numbers were suppressed considerably. With this being the case, a potential risk seems to be involved with the use of high carbon dioxide concentrations for packaging atmospheres for turkey roll when C. jejuni is being considered. It must be kept in mind, however, that C. jejuni could be a problem in turkey roll packaged under any atmosphere if cross-contamination were to occur before consumption. Our study indicates that C. jejuni has the ability to survive at 4 C in turkey roll up to eighteen days even under aerobic packaging (as seen by enrichment plating procedures).

LIST OF REFERENCES

LIST OF REFERENCES

1. Acuff, G. R., C. Vanderzant, and F. A. Golan. 1982. Examination of turkey eggs, poults and broiler house facilities for Campylobacter jejuni. J. Food Prot. 45: 1279-1281.
2. Acuff, G. R., C. Vanderzant, F. A. Gardner, and F. A. Golan. 1982. Evaluation of an enrichment-plating procedure for the recovery of Campylobacter jejuni from turkey eggs and meat. J. Food Prot. 45: 1276-1278.
3. Acuff, G. R., C. Vanderzant, M. O. Hanna, J. G. Ehlers, F. A. Gardner. 1986. Effects of handling and preparation of turkey products on the survival of Campylobacter jejuni. J. Food Prot. 49: 627-631.
4. Acuff, G. R., C. Vanderzant, M. O. Hanna, J. G. Ehlers, F. A. Golan, and F. A. Gardner. 1986. Prevalence of Campylobacter jejuni in turkey carcass processing and further processing of turkey products. J. Food Prot. 49: 712-717.
5. A.O.A.C. 1984. "Official Methods of Analysis," 14 ed. (Ed.) S. Williams, Association of Official Analytical Chemists, Washington, DC.
6. APHA. 1984. "Compendium of Methods for the Microbiological Examination of Foods." Am. Pub. Health Assoc., Washington, DC.
7. Beuchat, L. R. 1986. Methods for detecting and enumerating Campylobacter jejuni and Campylobacter coli in poultry. Poultry Sci. 65: 2192-2198.
8. Blankenship, L. C. and S. E. Craven. 1982. Campylobacter jejuni survival in chicken meat as a function of temperature. Appl. Environ. Microbiol. 44: 88-92.
9. Blankenship, L. C., S. E. Craven, J. Y. Chiu, and G. W. Krumm. 1983. Sampling methods and frozen storage of samples for detection of Campylobacter jejuni on freshly processed broiler carcasses. J. Food Prot. 46: 510-513.
10. Blaser, M. J. 1982. Campylobacter jejuni and food. Food Technol. 36: 89-92.

11. Blaser, M. J., F. M. LaForce, N. A. Wilson, and W. L. L. Wang. 1980. Reservoir for human campylobacteriosis. *J. Infect. Dis.* 141: 665-669.
12. Blaser, M. J., H. L. Hardesty, B. Powers, and W. L. L. Wang. 1980. Survival of Campylobacter fetus subsp. jejuni in biological milieus. *J. Clin. Microbiol.* 11: 309-313.
13. Blaser, M. J., I. D. Berkowitz, F. M. LaForce, J. Cravens, L. B. Reller, and W. L. L. Wang. 1979. Campylobacter enteritis: clinical and epidemiological features. *Ann. Intern. Med.* 91: 179-185.
14. Blaser, M. J., P. Checko, C. Bopp, A. Bruce, and J. M. Hughes. 1982. Campylobacter enteritis associated with foodborne transmission. *Amer. J. Epidemiol.* 116: 886-894.
15. Blaser, M. J., J. Cravens, B. W. Powers, F. M. LaForce, and W. W. L. Wang. 1979. Campylobacter enteritis associated with unpasteurized milk. *Amer. J. Med.* 67: 715-718.
16. Buck, G. E. and M. T. Kelly. 1981. Effect of moisture content of the medium on colony morphology of Campylobacter fetus subsp. jejuni. *J. Clin. Microbiol.* 14: 585-586.
17. Buck, G. E., C. Fojtasek, K. Calvert, and M. T. Kelly. 1982. Evaluation of the Campy Pak II gas generator system for isolation of Campylobacter fetus subsp. jejuni. *J. Clin. Microbiol.* 15: 41-42.
18. Buck, G. E., K. A. Parshall, and C. P. Davis. 1983. Electron microscopy of the coccoid form of Campylobacter jejuni. *J. Clin. Microbiol.* 18: 420-421.
19. Butzler, J. P., P. Dekeyser, M. Detrain, and F. Dehaen. 1973. Related Vibrio in stools. *J. Pediatr.* 82: 493-495.
20. Christopher, F. M., G. C. Smith, and C. Vanderzant. 1982. Examination of poultry giblets, raw milk and meat for Campylobacter fetus subsp. jejuni. *J. Food Prot.* 45: 260-262.
21. Christopher, F. M., G. C. Smith, and C. Vanderzant. 1982. Effect of temperature and pH on the survival of Campylobacter fetus. *J. Food Prot.* 45: 253-259.

22. Christopher, F. M., C. Vanderzant, Z. L. Carpenter, and G. C. Smith. 1979. Microbiology of pork packaged in various gas atmospheres. J. Food Prot. 42: 323-327.
23. Clark, A. G. and D. H. Bueschgens. 1985. Laboratory infection of chicken eggs with Campylobacter jejuni by using temperature or pressure differentials. Appl. Environ. Microbiol. 49: 1467-1471.
24. Doyle, M. P. 1981. Campylobacter fetus subsp. jejuni: an old pathogen of new concern. J. Food Prot. 44: 480-488.
25. Doyle, M. P. and D. J. Roman. 1982. Recovery of Campylobacter jejuni/coli by selective enrichment. Appl. Environ. Microbiol. 43: 1343-1353.
26. Doyle, M. P. and D. J. Roman. 1982. Response of Campylobacter jejuni to sodium chloride. Appl. Environ. Microbiol. 43: 561-565.
27. Doyle, M. P. and D. J. Roman. 1982. Sensitivity of Campylobacter jejuni to drying. J. Food Prot. 45: 507-510.
28. Enfors, S. O., G. Molin, and A. Ternstrom. 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4 C. J. Appl. Bacteriol. 47: 197-208.
29. George, H. A., P. S. Hoffman, R. M. Smibert, and N. R. Krieg. 1978. Improved media for growth and aerotolerance of Campylobacter fetus. J. Clin. Microbiol. 8: 36-41.
30. Genigeorgis, C., M. Hassuneh, and P. Collins. 1986. Campylobacter jejuni infection on poultry farms and its effect on poultry meat contamination during slaughtering. J. Food Prot. 49: 895-903.
31. Gill, C. O. and L. M. Harris. 1982. Contamination of red meat carcasses by Campylobacter fetus subsp. jejuni. Appl. Environ. Microbiol. 43: 977-980.
32. Gill, C. O. and L. M. Harris. 1984. Hamburgers and broiler chickens as potential sources of human campylobacter enteritis. J. Food Prot. 47: 96-99.

33. Gill, C. O. and L. M. Harris. 1982. Survival and growth of Campylobacter fetus subsp. jejuni on meat and in cooked foods. Appl. Environ. Microbiol. 44: 259-263.
34. Gill, C. O. and K. H. Tan. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. Appl. Environ. Microbiol. 39: 317-319.
35. Gill, K. P. W., P. G. Bates, and K. P. Lander. 1981. The effect of pasteurization on the survival of Campylobacter species in milk. Brit. Veterinary J. 137: 578-584.
36. Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser. 1980. Broiler chickens as potential source of campylobacter infections in humans. J. Clin. Microbiol. 11: 508-510.
37. Gray, R. J. H., P. H. Elliott, and R. I. Tomlins. 1984. Control of two major pathogens on fresh poultry using a combination potassium sorbate/carbon dioxide packaging treatment. J. Food Sci. 49: 142-145, 179.
38. Guerrant, R. L., R. G. Lahita, W. C. Winn, Jr., and R. B. Roberts. 1978. Campylobacteriosis in man: pathogenic mechanisms and review of 91 bloodstream infections. Amer. J. Med. 65: 584-592.
39. Hagan, J. R. 1964. Diagnostic techniques in avian vibronic hepatitis. Avian Dis. 8: 428-437.
40. Hanna, M. O., J. C. Stewart, Z. L. Carpenter, and C. Vanderzant. 1977. Effect of heating, freezing, and pH on Yersinia enterocolitica-like organisms from meat. J. Food Prot. 40: 689-692.
41. Hanninen, M.-L. 1981. Survival of Campylobacter jejuni/coli in ground refrigerated and in ground frozen beef liver and in frozen broiler carcasses. Acta Vet. Scand. 22: 566-577.
42. Hanninen, M.-L., H. Korkeala, and P. Pakkala. 1984. Effect of various gas atmospheres on the growth and survival of Campylobacter jejuni on beef. J. Appl. Bacteriol. 57: 89-94.
43. Harvey, S. M. 1980. Hippurate hydrolysis by Campylobacter fetus. J. Clin. Microbiol. 11: 435-437.

44. Herbert, G. A., D. G. Hollis, R. E. Weaver, M. A. Lambert, M. J. Blaser, and C. W. Moss. 1982. 30 year of campylobacters: biochemical characteristics and a biotyping proposal for Campylobacter jejuni. J. Clin. Microbiol. 15: 1065-1073.
45. Hofstad, M. S., E. H. McGehee, P. C. Bennett. 1958. Avian infectious hepatitis. Avian Dis. 2: 358-364.
46. Hopkins, R. S. and A. S. Scott. 1983. Handling raw chicken as a source for sporadic Campylobacter jejuni infections. J. Infect. Dis. 148: 770.
47. Huffman, D. L. 1974. Effect of gas atmosphere on microbial quality of pork. J. Food Sci. 3: 723-725.
48. Hwang, M. N. and G. M. Ederer. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. J. Clin. Microbiol. 1: 114-115.
49. Jones, P. H., A. T. Willis, D. A. Robinson, M. B. Skirrow, and D. S. Josephs. 1981. Campylobacter enteritis associated with the consumption of free school milk. J. Hyg. Camb. 87: 155-162.
50. Karmali, M. A. and P. C. Fleming. 1979. Application of the Fortner principle to isolation of Campylobacter from stools. J. Clin. Microbiol. 10: 245-247.
51. Karmali, M. A. and P. C. Fleming. 1979. Campylobacter enteritis. CMA Journal. 120: 1525-1532.
52. Koidis, P. and M. P. Doyle. 1983. Survival of Campylobacter jejuni in fresh and heated red meat. J. Food Prot. 46: 771-774.
53. Luechtefeld, N. W. and W. L. L. Wang. 1981. Campylobacter fetus subsp. jejuni in a turkey processing plant. J. Clin. Microbiol. 13: 266-268.
54. Marsik, F. J., J. T. Parisi, and D. C. Blenden. 1975. Transmissible drug resistance of Escherichia coli and Salmonella from humans, animals and their rural environments. J. Infect. Dis. 132: 296-302.
55. Martin, W. T., C. M. Patton, G. K. Morris, M. E. Potter, and N. D. Puhr. 1983. Selective enrichment broth for isolation of Campylobacter jejuni. J. Clin. Microbiol. 17: 853-855.

56. McNaughton, R. D., R. Leyland, and L. Mueller. 1982. Outbreak of campylobacter enteritis due to consumption of raw milk. CMA Journal. 126: 657-658.
57. Mehlman, I. J. and A. Romero. 1982. Improved growth medium for Campylobacter species. Appl. Environ. Microbiol. 43: 615-618.
58. Mercuri, A. J., G. J. Banwart, J. A. Kinner, and A. R. Sessoms. 1970. Bacteriological examination of commercial precooked Eastern-type turkey rolls. Appl. Microbiol. 19: 768-771.
59. Michel, J., M. Rogol, and D. Dickman. 1983. Susceptibility of clinical isolates of Campylobacter jejuni to sixteen antimicrobial agents. Antimicrobial Agents and Chemotherapy. 23: 796-797.
60. Montrose, M. S., S. M. Shane, and K. S. Harrington. 1984. Roll of litter in the transmission of Campylobacter jejuni. Avian Dis. 29: 392-398.
61. Monroe, D. L., J. F. Prescott, and J. L. Penner. 1983. Campylobacter jejuni and Campylobacter coli serotypes isolated from chickens, cattle, and pigs. J. Clin. Microbiol. 18: 877-881.
62. Newton, K. G. and W. J. Rigg. 1979. The effect of film permeability on the storage life and microbiology of vacuum-packed meat. J. Appl. Bacteriol. 47: 433-441.
63. Oosterom, J., G. J. A. DeWilde, E. DeBoer, L. H. DeBlaauw, and H. Karman. 1983. Survival of Campylobacter jejuni during poultry processing and pig slaughtering. J. Food Prot. 46: 702-706.
64. Oosterom, J., S. Notermans, H. Karman, and G. B. Engels. 1983. Origin and prevalence of Campylobacter jejuni in poultry processing. J. Food Prot. 46: 339-344.
65. Palumbo, S. A. 1986. Campylobacter jejuni in foods: its occurrence, isolation from foods, and injury. J. Food Prot. 49: 161-166.
66. Palumbo, S. A. 1984. Heat injury and repair in Campylobacter jejuni. Appl. Environ. Microbiol. 48: 477-480.

67. Park, C. E., Z. K. Stankiewicz, J. Lovett, J. Hunt, and D. W. Francis. 1983. Effect of temperature, duration of incubation, and pH of enrichment culture on the recovery of Campylobacter jejuni from eviscerated market chickens. Cana. J. Microbiol. 29: 803-806.
68. Park, C. E., Z. K. Stankiewicz, J. Lovett, and J. Hunt. 1981. Incidence of Campylobacter jejuni in fresh eviscerated whole market chickens. Cana. J. Microbiol. 27: 841-842.
69. Patton, C. M., S. W. Mitchell, M. E. Potter, and A. F. Kaufmann. 1981. Comparison of selective media for primary isolation of Campylobacter fetus subsp. jejuni. J. Clin. Microbiol. 13: 326-330.
70. Penner, J. L., J. N. Hennessy, and R. V. Congi. 1983. Serotyping of Campylobacter jejuni and Campylobacter coli on the basis of thermostable antigens. European J. Microbiol. 2: 378-383.
71. Phebus, R. K., B. J. Lee, and F. A. Draughon. 1988. Effects of diluents on recovery of Campylobacter jejuni in turkey rolls. Presented at the 49th Institute of Food Technologists Annual Meeting. New Orleans, LA. Abstract #348.
72. Plastringe, W. N., L. F. Williams, D. E. Trowbridge. 1964. Antibiotic sensitivity of physiologic groups of microaerophilic vibrios. Amer. J. Vet. Res. 25: 1295-1299.
73. Potter, M. E., M. J. Blaser, R. K. Sikes, A. F. Kaufmann, and J. G. Wells. 1983. Human campylobacter infection associated with certified raw milk. Amer. J. Epidemiology. 117: 475-483.
74. Ray, B. and C. Johnson. 1984. Survival and growth of freeze-stressed Campylobacter jejuni in selective media. J. Food Safety. 6: 183-195.
75. Rayes, H. M., C. A. Genigeorgis, and T. B. Farver. 1983. Prevalence of Campylobacter jejuni on turkey wings at the supermarket level. J. Food Prot. 46: 292-294.
76. Rettig, P. J. 1979. Campylobacter infections in human beings. J. Pediatrics. 94: 855-864.

77. Robinson, D. A. 1981. Infective dose of Campylobacter jejuni in milk. Brit. Med. J. 282: 1584.
78. Robinson, D. A. and D. M. Jones. 1981. Milk-borne campylobacter infection. Brit. Med. J. 282: 1374-1376.
79. Rollins, D. M., J. C. Coolbough, R. I. Walker, E. Weiss. 1983. Biphasic culture system for rapid Campylobacter cultivation. Appl. Environ. Microbiol. 45: 284-289.
80. Roth, L. A. and D. S. Clark. 1972. Studies on the bacterial flora of vacuum-packaged fresh beef. Cana. J. Microbiol. 18: 1761-1766.
81. Rubin, S. J. and M. Woodard. 1983. Enhanced isolation of Campylobacter jejuni by cold enrichment in campy-thio broth. J. Clin. Microbiol. 18: 1008-1010.
82. Saida, K., Y. Ike, and S. Mitsuhashi. 1981. Drug resistance and R plasmids of Escherichia coli strains isolated from pigs, slaughterers, and breeders of pigs in Japan. Antimicrobial Agents and Chemotherapy. 19: 1032-1036.
83. Sanders, W. L. 1988. Personal correspondence. University of Tennessee, Agricultural Experiment Station, Knoxville, TN.
84. Shane, S. M., M. S. Montrose, and K. S. Harrington. 1984. Transmission of Campylobacter jejuni by the housefly (Musca domestica). Avian Dis. 29: 384-391.
85. Shanker, S., J. A. Rosenfield, G. R. Davey, and T. C. Sorrell. 1982. Campylobacter jejuni: incidence in processed broilers and biotype distribution in human and broiler isolates. Appl. Environ. Microbiol. 43: 1219-1220.
86. Silliker, J. H. and S. K. Wolfe. 1980. Microbiological safety considerations in controlled atmosphere storage of meats. Food Technol. 34: 59-63.
87. Skirrow, M. B. 1977. Campylobacter enteritis: a "new" disease. Brit. Med. J. 2: 9.
88. Skirrow, M. B. 1982. Campylobacter enteritis--the first five years. J. Hygiene. 89: 175-184.

89. Skirrow, M. B. and J. Benjamin. 1980. "1001" campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J. Hyg. Camb. 85: 427-442.
90. Smeltzer, T. I. 1981. Isolation of Campylobacter jejuni from poultry carcasses. Australian Veterinary J. 57: 511-512.
91. Smibert, R. M. 1974. In Bergey's Manual of Determinative Bacteriology. 8th ed. Williams and Wilkins. Baltimore, Maryland. pp. 207-211.
92. Smibert, R. M. 1978. The genus Campylobacter. Ann. Rev. Microbiol. 32: 673-709.
93. Smitherman, R. E., C. A. Genigeorgis, and T. B. Farver. 1984. Preliminary observations on the occurrence of Campylobacter jejuni at four California chicken ranches. J. Food Prot. 47: 293-298.
94. Spahl, A., G. Reineccius, and S. Tatini. 1981. Storage life of pork chops in carbon dioxide-containing atmospheres. J. Food Prot. 44: 670-673.
95. Stern, N. J. 1981. Campylobacter fetus subsp. jejuni: recovery methodology and isolation from lamb carcasses. J. Food Sci. 46: 660-663.
96. Stern, N. J. 1982. Selectivity and sensitivity of three media for recovering Campylobacter fetus subsp. jejuni from ground beef. J. Food Safety. 4: 169-175.
97. Stern, N. J. 1982. Methods for recovering Campylobacter jejuni from foods. J. Food Prot. 45: 1332-1337.
98. Stern, N. J. 1981. Recovery rate of Campylobacter fetus ssp. jejuni on eviscerated pork, lamb, and beef carcasses. J. Food Sci. 46: 1291-1293.
99. Stern, N. J., S. S. Green, N. Thaker, D. J. Krout, and J. Chiu. 1984. Recovery of Campylobacter jejuni from fresh and frozen meat and poultry collected at slaughter. J. Food Prot. 47: 372-374.
100. Stern, N. J., M. D. Greenberg, and D. M. Kinsman. 1986. Survival of Campylobacter jejuni in selected gaseous environments. J. Food Sci. 51: 652-654.

101. Stern, N. J. and A. W. Kotula. 1982. Survival of Campylobacter jejuni inoculated into ground beef. Appl. Environ. Microbiol. 44: 1150-1153.
102. Stern, N. J., P. J. Rothenberg, and J. M. Stone. 1985. Enumeration and reduction of Campylobacter jejuni in poultry and red meats. J. Food Prot. 48: 606-610.
103. Svedhem, A., B. Kaijser, and E. Sjogren. 1981. The occurrence of Campylobacter jejuni in fresh food and survival under different conditions. J. Hyg. Camb. 87: 421-425.
104. Tan, K. H. and C. O. Gill. 1982. Physiological basis of carbon dioxide inhibition of a meat spoilage bacterium, Pseudomonas fluorescens. Meat Sci. 7: 9-17.
105. Taylor, P. R., W. M. Weinstein, and J. H. Bryner. 1979. Campylobacter fetus infection in human subjects: association with raw milk. Amer. J. Med. 66: 779-783.
106. Thomas, Y. O., A. A. Kraft, R. E. Rust, and D. K. Hotchkiss. 1984. Effect of carbon dioxide flushing and packaging methods on the microbiology of packaged chicken. J. Food Sci. 49: 1367-1371.
107. Tritz, G. J. and J. E. Ogg. 1967. Physical and chemical conditions inducing the coccoid form of ovine Vibrio fetus. Amer. J. Vet. Res. 28: 123-129.
108. Vanhoof, R., H. Goossens, H. Coignau, G. Stas, and J. P. Butzler. 1982. Susceptibility pattern of Campylobacter jejuni from human and animal origins to different antimicrobial agents. Antimicrobial Agents and Chemotherapy. 21: 990-992.
109. Vanhoof, R., B. Gordts, R. Dierickx, H. Coignau, and J. P. Butzler. 1980. Bacteriostatic and bactericidal activities of 24 antimicrobial agents against Campylobacter fetus subsp. jejuni. Antimicrobial Agents and Chemotherapy. 18: 118-121.
110. Veron, M. and R. Chatelain. 1973. Taxonomic study of the genus Campylobacter Sebald and Veron and designation of the neotype strain for the type species Campylobacter fetus (Smith and Taylor) Sebald and Veron. International J. Systemic Bacteriology. 23: 122-134.

111. Waterman, S. C. 1982. The heat-sensitivity of Campylobacter jejuni in milk. J. Hyg. Camb. 88: 529-533.
112. Wesley, R. D., B. Swaminathan, and W. J. Stadelman. 1983. Isolation and enumeration of Campylobacter jejuni from poultry products by a selective enrichment method. Appl. Environ. Microbiol. 46: 1097-1102.
113. Wyatt, C. J. and E. M. Timm. 1982. Occurrence and survival of Campylobacter jejuni in milk and turkey. J. Food Prot. 45: 1218-1220.
114. Yogasundram, K. and S. M. Shane. 1986. The viability of Campylobacter jejuni on refrigerated drumsticks. Veterinary Research Communications. 10: 479-486.
115. Yusufu, H. I., C. Genigeorgis, T. B. Farver, and J. M. Wempe. 1983. Prevalence of Campylobacter jejuni at different sampling sites in two California turkey processing plants. J. Food Prot. 46: 868-872.

APPENDIXES

APPENDIX A

CORRELATION TABLES

TABLE 1. Correlations Among Types of Bacterial Counts
Tested Within Each of Seven Gas Packaging
Atmospheres in Turkey Roll Stored at 4 C.

Atmosphere	r values	
	APC vs C. jejuni (direct)	APC vs PPC
100% CO ₂	-0.59	0.71
80% CO ₂ / 20% N ₂	-0.87	0.95
60% CO ₂ / 40% N ₂	-0.92	0.96
40% CO ₂ / 60% N ₂	-0.97	0.99
100% N ₂	-0.99	0.99
100% Air	-0.84	0.99
10% CO ₂ / 85% N ₂ / 5% O ₂	-0.94	0.99

TABLE 2. Correlations of Mean Slope Values Among Increasing Carbon Dioxide Levels (0-100%) Used in Packaging of Turkey Roll and Growth/Survival of Specific Types of Bacteria at 4 and 21 C.

<u>Type of Microbial Test vs CO₂ Level</u>	<u>r values</u>	
	<u>4 C</u>	<u>21 C</u>
<u>C. jejuni</u> (direct plating)	0.10	0.18
Aerobic Plate Count	-0.71	-0.50
Psychrotrophic Plate Count	-0.67	-0.37
Lactic Acid Bacterial Count	-0.22	-0.41

**TABLE 3. Correlations Among Types of Bacterial Counts
Tested Within Each of Seven Gas Packaging
Atmospheres in Turkey Roll Stored at 21 C.**

<u>Atmosphere</u>	<u>r values</u>	
	<u>APC vs C. jejuni (direct)</u>	<u>APC vs PPC</u>
100% CO ₂	-0.96	0.96
80% CO ₂ / 20% N ₂	-0.98	0.99
60% CO ₂ / 40% N ₂	-0.98	1.00
40% CO ₂ / 60% N ₂	-0.76	0.99
100% N ₂	-0.99	0.99
100% Air	-0.96	0.98
10% CO ₂ / 85% N ₂ / 5% O ₂	-0.88	0.99

APPENDIX B

MEDIA FORMULATIONS EMPLOYED

CAMPYLOBACTER BLOOD-FREE SELECTIVE AGAR BASE
(OXOID)

Formula: (grams per liter)

nutrient broth no. 2	25.0
bacteriological charcoal	4.0
casein hydrolysate	3.0
sodium deoxycholate	1.0
ferrous sulphate	0.25
sodium pyruvate	0.25
agar	12.0

To be used in conjunction with cefoperazone selective supplement (OXOID).

Note: This agar was preferred to blood agar formulas due to the ease of preparation and more consistent results provided.

CAMPYLOBACTER ENRICHMENT BROTH

Formula: (grams per liter)

dehydrated brucella broth	29.0
agar	1.0

Used in conjunction with Blaser-Wang antibiotic supplement (OXOID) at recommended dosage.

CARY-BLAIR DILUENT

Formula: (grams per liter)

calcium chloride (1% solution)	9 ml
sodium thioglycollate	1.5
disodium phosphate	1.1
sodium chloride	5.0
agar	1.0
distilled water	991 ml

Note: pH was not adjusted before use.

APPENDIX C

PROXIMATE COMPOSITION OF TURKEY ROLL

TABLE 4. Proximate Composition of Turkey Roll (As-Received Basis).

Component	Percentage
Fat	22.0
Protein	12.5
Moisture	58.7
Ash	2.9
Carbohydrate ^a	4.8

^a Carbohydrate content was determined by subtracting the percent fat, protein, moisture, and ash from 100%.

VITA

Randall Kent Phebus was born November 3, 1962 in Waverly, Tennessee to Patsy and Kent Phebus. He was graduated from Waverly Central High School in May 1981, after which he entered the University of Tennessee at Knoxville. In June 1986, he was awarded a Bachelor of Science degree in Agriculture majoring in Animal Science. In the fall of 1986, he began working toward a Master of Science degree in Food Technology and Science at the University of Tennessee, Knoxville as a graduate research assistant. He was awarded the Master's degree in December 1988.

The author is a member of the Institute of Food Technologists, Phi Kappa Phi and Gamma Sigma Delta.