Survival of Campylobacter jejuni in Modified Atmosphere Packaged Turkey Roll

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ABSTRACT

Survival of Campylobacter jejuni, inoculated into turkey roll slices and stored under seven different atmospheric mixtures, was determined. Turkey roll samples were stored at 4°C for 18 d and at 21°C for 48 h. The effects of various atmospheric mixtures on aerobic, psychrotrophic, and lactic acid bacterial populations were also determined throughout storage. Campylobacter jejuni was inactivated under all atmospheric gas mixtures tested throughout storage. Increasing CO₂ concentration inside the package from 0% to 100% CO₂ resulted in a lower rate of inactivation of C. jejuni at both storage temperatures. Increases in CO₂ concentrations provided greater inhibition of aerobic and psychrotrophic populations as compared to low CO₂ levels. The effect of CO₂ on survival of C. jejuni and growth rate of aerobic, psychrotrophic, and lactic acid bacteria was more pronounced at 4°C. Campylobacters were isolated from inoculated turkey roll held under all atmospheres by enrichment procedures on the 18th day and 48th hour of storage at 4 and 21°C, respectively, with an initial population of log 6.0 campylobacters/g. However, no campylobacters were isolated by 18 d of storage at 4°C by direct plating.

Campylobacter jejuni is classified as a gram-negative, microaerophilic bacterium and is a leading cause of gastroenteritis in humans (4,6,14). The number of cases of Campylobacter enteritis may equal or surpass that of Salmonella or Shigella in frequency (9,11). An increasing number of reports implicates campylobacters as the causative agent of approximately 5% of cases of acute gastroenteritis (11). Campylobacter jejuni has been isolated from lamb, pork, and beef carcasses at low rates and levels of contamination (16). The organism has a high propensity for wild and domestic birds, probably as the result of their high body temperature (42°C) which is considered optimal for growth of C. jejuni (7). Very high isolation rates of C. jejuni have been reported from fresh poultry carcasses (7,13) and isolation of the pathogen at point-of-sale in retail markets indicates the potential risk of human infection (19).

A substantial amount of research has been performed on the advantages and disadvantages of modified atmosphere packaging of meats. Shelf life can be improved by extending the lag phase and generation time of spoilage bacteria (18). Modified atmospheres may also be beneficial in reducing the rate of growth of certain pathogenic bacteria (5,12).

However, certain anaerobic and microaerophilic pathogens may be able to survive in the absence of O₂ and competition from aerobic spoilage microorganisms. Infection of the consumer without the warning signs of spoilage could be the consequence. The objectives of this study were to determine: a) the extent to which C. jejuni inoculated into processed turkey roll could survive at 4 and 21°C under various atmospheric mixtures of N₂, CO₂, and O₂ and b) the effect of different gas atmospheres on the growth rate and type of spoilage microflora in turkey roll.

MATERIALS AND METHODS

Experimental design

Seven different gas atmospheres were evaluated as to their effect on populations of C. jejuni inoculated into processed turkey roll. Turkey roll slices inoculated with two strains of C. jejuni and uninoculated control slices were stored under each atmosphere at 4°C for 0, 1, 3, 6, 12, and 18 d in study 1 and at 21°C for 0, 6, 12, 24, and 48 h in study 2. Three replications were performed in duplicate for all atmospheric treatments. The seven atmospheres under investigation were:

- a) 100% CO₂
- b) 80% CO₂/20% N₂
- c) 60% CO₂/40% N₂
- d) 40% CO₂/60% N₂
- e) 100% N₂
- f) 100% air
- g) 5% O₂/10% CO₂/85% N₂ (OCN)

Turkey roll preparation

Three 8-lb frozen processed turkey rolls (North American Provision Co., Phoenix, AZ) were purchased from a local retail outlet. Label statements indicated that turkey rolls consisted of turkey meat, turkey skins, water, modified food starch, salt, sugar, sodium phosphate, and flavorings. Turkey rolls are typically heated to an internal temperature of 71°C during processing. Turkey rolls with the same manufacturer's lot number were utilized throughout all experiments. Frozen turkey rolls were received in vacuum packaged films and were thawed for approximately 24 h at 4°C to an internal temperature of -2°C. Thawed turkey rolls (12.5 cm in diameter) were sliced into approximately 2 mm thick portions and immediately wrapped in waxed freezer paper (20 slices/package) and refrozen at -20°C until needed for testing (<3 months).

Proximate composition and pH

Turkey rolls 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 (2). Carbohydrate content was determined by difference. The initial pH of turkey roll samples was determined using an Orion Research Digital Ionometer 501 pH meter (Orion Research Inc., Boston, MA).
Inoculation of turkey roll

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 brucella broth with 0.1% agar added to lower oxygen tension in the medium. Tubes were incubated at 42°C for 48 h under an atmosphere of OCN and then stored at 4°C. Cultures were transferred weekly with strains being inoculated into fresh brucella broth tubes and again incubated at 42°C for 48 h under OCN. Cultures were inoculated into fresh broth and incubated for 24 h at 42°C for use as the inoculum.

Statistical analysis of data

Statistical analysis was performed from an incomplete block fractional factorial design with data being collected from three replications performed in duplicate for each treatment. Atmospheric treatments were assigned randomized numbers within each replication to determine order of inoculation and plating of samples. Analysis of variance was used to evaluate comparative data. Data presented are the means of the three replications for each study. Significant differences (P<0.05) among means were separated using the least squares method.

RESULTS AND DISCUSSION

Proximate composition of turkey rolls

Proximate analysis results for processed turkey rolls used in this investigation are described in Table 1. The initial pH of turkey roll samples was 6.50+0.2.

Survival of C. jejuni at 4°C

Campylobacter jejuni was not detected in uninoculated control samples by either direct or enrichment plating (level of detection was approximately 10 CFU/g of turkey roll). The enrichment method increases the numbers of C. jejuni present in the sample to facilitate detection, thus making it difficult to determine the actual population of C. jejuni at the time of sampling. Therefore, much of the following C. jejuni data and discussion is based on results of the direct plating procedure. Analysis of variance showed that survival of C. jejuni strains CJ-B4086 and ATCC 29428 was not significantly (P>0.05) different under all packaging atmospheres. Therefore, data are presented as the mean of the two strains.

Atmospheres containing elevated levels of CO₂ (40% to 100%) and 100% N₂ allowed the highest levels of C. jejuni to survive with greater than log 4.0 CFU/g being detected after 6 d of storage at 4°C (Fig. 1). The 100% air atmosphere resulted in significantly (P<0.05) less survival of C. jejuni than all other atmospheres tested as no viable campylobacters were detected in turkey rolls after 6 d of storage.
storage. The 40% CO₂ mixture caused a higher inactivation rate of C. jejuni than the other three atmospheres containing elevated CO₂ concentrations (Fig. 1). A significant (P<0.05) decline in viable C. jejuni population in turkey rolls resulted between the 6th and 12th days of storage at 4°C under this atmosphere. Campylobacters could not be detected in turkey rolls stored under any of the atmospheres tested by the 18th day of sampling by direct plating procedures (Fig. 1).

A significantly (P<0.05) greater rate of inactivation of C. jejuni was observed in the OCN atmosphere than in the atmospheres of 100% CO₂, 60% CO₂, and 100% N₂ which contained no O₂. The OCN atmosphere is recommended for laboratory isolation and cultivation of C. jejuni at 42°C (15). Therefore, the optimal atmosphere for survival of C. jejuni might differ at 6°C as compared to 42°C. Stern et al. (17) found similar results with this atmosphere at 4°C. It could be hypothesized that dissolved oxygen levels are higher 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 (containing approximately 21%O₂) was the most toxic atmosphere to C. jejuni at 4°C (Fig. 1).

Survival of C. jejuni at 21°C
Campylobacter jejuni populations decreased rapidly under all atmospheres tested throughout the initial 24 h of storage at 21°C (Fig. 2). The 100% air atmosphere caused the greatest degree of C. jejuni inactivation during this period with populations declining by log 3.6 CFU/g. The 100% CO₂ atmosphere maintained a significantly (P<0.05) higher population of viable C. jejuni throughout the 48-h storage period. Under this atmosphere, populations declined by only log 2.3 CFU/g over 48 h. The increase in C. jejuni population detected between the 24 and 48 h sampling for the 40% CO₂ atmosphere (Fig. 2) could not be explained by these investigators. Inactivation of C. jejuni by the OCN

Enrichment procedures are generally used before selective plating for the detection and recovery of C. jejuni from food products (3). The importance of enrichment was clearly evident for the detection of C. jejuni from turkey rolls in this investigation. C. jejuni was detected from samples stored under all seven atmospheres on the 18th day at 4°C when enrichment procedures were employed. However, no C. jejuni could be detected by the 18th day by direct plating.

Survival of C. jejuni inoculated into turkey roll and stored at 4°C under modified atmospheres containing 40 to 100% carbon dioxide (A) and nitrogen alone or in combination with oxygen (B) enumerated by direct plating.

Figure 1. Survival of C. jejuni inoculated into turkey roll and stored at 4°C under modified atmospheres containing 40 to 100% carbon dioxide (A) and nitrogen alone or in combination with oxygen (B) enumerated by direct plating.

Figure 2. Survival of C. jejuni inoculated into turkey roll and stored at 21°C under modified atmospheres containing 40 to 100% carbon dioxide (A) and nitrogen alone or in combination with oxygen (B) enumerated by direct plating.
survival of campylobacter jejuni

atmosphere was not as noticeable at 21°C (Fig. 2) as at 4°C (Fig. 1). Campylobacters were detected at the 48 h sampling from inoculated turkey roll stored under all atmospheres tested except 60% CO₂ by direct plating (Fig. 2). Campylobacters were detected from inoculated samples held under all seven atmospheres at the 48 h sampling by enrichment.

The lack of difference in C. jejuni inactivation at 21°C among the seven atmospheres tested indicated that the atmospheric effects of CO₂ and O₂ on C. jejuni survival and/or inactivation in turkey rolls was diminished at 21°C (Fig. 2) as compared to 4°C (Fig. 1). This was probably due to decreased solubility of CO₂ and O₂ at 21°C since gases are more soluble at lower temperatures.

growth of spoilage microorganisms at 4°C

Aerobic plate counts showed an initial population of approximately log 2.0 CFU/g of turkey rolls (Fig. 3). The packaging atmospheres containing elevated CO₂ levels (40 to 100%) were the most inhibitory to aerobic bacterial growth in a turkey roll at 4°C. Virtually no increase in aerobic bacterial populations occurred under the 100% CO₂ atmosphere throughout 18 d of storage. The OCN, 100% air, and 100% N₂ atmospheres allowed substantial increases in aerobic bacterial populations at 4°C. Populations increased by approximately log 7.0 CFU/g over the 18-d study under these atmospheres.

Thomas et al. (18) demonstrated that CO₂ inhibits or retards bacterial growth by extending the lag phase. The same effect was observed in this study. High CO₂ atmospheres (40 to 100%) held aerobic bacteria in lag phase from 6 to 12 d, whereas 100% N₂, 100% air, and OCN atmospheres resulted in only a 3-d lag phase (Fig. 3).

The growth rates of psychrotrophic bacteria at 4°C in turkey roll were also suppressed by high CO₂ atmospheres. Psychrotrophic bacterial populations remained low under all atmospheres (<log 1.5 CFU/g) over the first 3 d of storage (Fig. 4). Psychrotrophic bacterial populations increased noticeably between days 3 and 6 for turkey held under 100% air, 100% N₂, and OCN. The 100% CO₂ atmosphere was the most inhibitory to psychrotrophic bacteria at 4°C with populations only reaching log 1.5 CFU/g over 18 d of storage (Fig. 4).

Growth rate of lactic bacteria remained slow in turkey roll stored at 4°C under all seven atmospheres throughout the 18-d study (Fig. 5). Initially, lactic acid bacterial populations were <log 1.0 CFU/g under all atmospheres. The OCN mixture resulted in the highest rate of lactic acid bacterial growth with populations reaching log 3.7 CFU/g by day 18. The higher CO₂ atmospheres (80% and 100%) were the most inhibitory to lactic acid bacteria (Fig. 5). Atmospheres containing 40% and 60% CO₂ inhibited lactic acid bacterial populations through the sixth day of storage but were not significantly (P>0.05) different from air after 18 d. Low initial populations of lactic acid bacteria were also observed by Mercuri et al. (8) in precooked turkey roll samples.

growth of spoilage microorganisms at 21°C

The initial aerobic bacterial populations for turkey rolls stored at 21°C were between log 1.0 and 2.0 CFU/g (Fig. 6). The 100% air atmosphere resulted in the largest increase in populations of aerobic bacteria with populations increasing to log 9.0 CFU/g within 48 h (Fig. 6). All other atmospheres, with the exception of 100% CO₂, allowed similar increases in aerobic bacterial populations to approximately log 8.0 CFU/g over 48 h of storage. The 100% CO₂ atmosphere was the most inhibitory to aerobes at 21°C with populations only reaching log 5.5 CFU/g in 48 h (Fig. 6). The lag phases for the aerobic bacterial growth curves were <6 h, indicating that the inhibitory action of CO₂ was greatly diminished at 21°C (Fig. 6) as compared to 4°C (Fig. 3).

The initial psychrotrophic bacterial populations were very low in a turkey roll at 21°C. However, these populations increased rapidly for all atmospheres except 100% CO₂ and reached greater than log 6.0 CFU/g by the 48-h sampling (Fig. 7). The 100% CO₂ atmosphere exerted moderate inhibitory action on psychrotrophic bacterial growth with populations being held to log 4.0 CFU/g over 48 h of storage.

Unlike lactic acid bacterial growth at 4°C (Fig. 5), in-

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A major concern with the use of modified atmosphere packaging of food products is that pathogens may be able to survive and proliferate to populations that may be infec-

Figure 7. Psychrotrophic bacterial populations in turkey roll stored at 21°C under air and modified atmospheres.

h under this atmosphere (Fig. 8). The 100% CO₂ atmosphere allowed the least lactic acid bacterial growth, reaching log 4.0 CFU/g after 48 h at 21°C.

Figure 8. Lactic acid bacterial populations in turkey roll stored at 21°C under modified atmospheres containing 40 to 100% carbon dioxide (A) and nitrogen alone or in combination with oxygen (B).

creases in populations of lactic acid bacteria at 21°C were substantial under all atmospheres (Fig. 8). Lactic acid bacterial populations increased markedly between hours 12 and 24 of storage. The 100% air atmosphere resulted in the largest increase in populations of lactic acid bacteria. Populations increased to approximately log 9.0 CFU/g within 48

Figure 6. Aerobic bacterial populations in turkey roll stored at 21°C under air and modified atmospheres.
vative for humans without a concomitant increase in population of spoilage bacteria to warn the consumer of the potential microbiological hazard. This study indicates that a potential risk may exist with storage of turkey roll contaminated with *C. jejuni* in modified atmospheres containing high levels of CO₂. The atmospheres that were the most protective to *C. jejuni* were also the most inhibitory to the natural spoilage organisms of turkey roll. It must, however, be stressed that *C. jejuni* numbers declined rapidly under all seven atmospheres at both storage temperatures. Therefore, the initial level of contamination with *C. jejuni* seems to be a more critical concern than is survival of the pathogen, regardless of the packaging atmosphere utilized. Postprocess slicing and packaging operations could be important sources of *C. jejuni* in turkey rolls and, as such, critical control points for controlling *C. jejuni* contamination should be determined for the production of turkey rolls.

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REFERENCES