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GROWTH AND SURVIVAL OF SALMONELLA AND CAMPYLOBACTER
IN CHICKEN NUGGETS AS A FUNCTION OF TEMPERATURE

A Thesis

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DEDICATION

This thesis is dedicated with love to my parents. It is their love, encouragement, and never ending support which have inspired me to achieve my academic endeavors. I hope that someday I can give to them what they have given to me.

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ABSTRACT

The effect of freezing, refrigeration, and temperature abuse on growth and survival of Salmonella and Campylobacter in chicken nuggets was studied. Storage temperature effects on aerobic plate counts, psychrotrophic plate counts and coliforms were also observed.

Salmonella and Campylobacter were able to survive freezing at -18C over a 6 week storage period, however, significant decreases ($P < 0.05$) were observed for all strains, especially S. infantis. Enrichment techniques aided in recovering cells that otherwise would have gone undetected.

Storage at 4C for 6 days was not as detrimental to Salmonella and Campylobacter as was -18C. Survival was observed for both pathogens. Samples inoculated with 1×10^5 salmonellae/g showed better survival than did samples inoculated with 1×10^3 or 1×10 salmonellae/g. Campylobacter survival was advantageous at 4C.

Survival of Salmonella and Campylobacter was also observed at 21C for 24 hours. Growth of Salmonella was observed in nuggets inoculated with 1×10 salmonellae/g (especially S. blockley). Aerobic plate counts increased significantly ($P > 0.05$) when nuggets were stored at 21C for 24 hours.

It was concluded that storage of chicken nuggets at

-18C and 4C offered little risk to humans from the pathogens Salmonella and Campylobacter. It is advised that chicken nuggets stored at 21C for an extended period of time not be consumed due to the possibility that if a pathogen were present, that it may grow if conditions are favorable.

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CHAPTER I

INTRODUCTION

Both Salmonella and Campylobacter jejuni cause acute bacterial gastroenteritis and are commonly implicated in foodborne disease outbreaks. In recent years, isolation of C. jejuni from patients with diarrheal illness have surpassed both Salmonella and Shigella (10). The number of Salmonellosis cases reported each year have been increasing at a steady rate since 1969 (28). Both organisms are common intestinal inhabitants of animals, especially poultry. The consumption of poultry has increased since World War II (26) and in 1987, poultry consumption surpassed beef consumption for the first time.

Over the past decade, the poultry industry has brought many forms of restructured meat products to market (105). The poultry industry has primarily focused on fast-food markets and products that would find immediate acceptance by consumers. One product that has met the demands of consumers is chicken nuggets.

Surprisingly, very little is known about the microbiology of restructured poultry products and no publications were found on the fate of pathogens such as Salmonella or Campylobacter in chicken nuggets or on the typical microflora of such products. These products are such an important part of the market that information on

the microbiological character is essential to assure their continued acceptance.

The objectives of this study were: (1) to investigate the growth and survival of Salmonella and Campylobacter during storage of chicken nuggets under freezing, refrigeration, and abuse conditions, and (2) to investigate the growth of spoilage microorganisms during storage of chicken nuggets under freezing, refrigeration, and abuse conditions.

CHAPTER II

REVIEW OF LITERATURE

A. SALMONELLA

1. Clinical Characteristics

Acute gastroenteritis caused by Salmonella is the result of ingesting food or water contaminated with salmonellae. The number of cells required to cause infection varies from as low as 100 cells/gram of food to as many as 10^9 cells/gram, however 10^6 - 10^9 cells/gram have been found to cause infection in more than 50% of healthy volunteers who ingested the organism (83). The incubation period may range from 6 - 72 hours but is usually 12 - 48 hours (73,100,108). Symptoms include nausea and vomiting followed by abdominal cramps and diarrhea. A slightly elevated temperature (38 - 39C) is common. The disease is self-limiting and can last 1 - 7 days. Clinical manifestations range from mild to very severe, resulting in dehydration, extreme pain, bloody, slimy stools, convulsions and even death (100). Severe cases may mimic cholecystitis and acute appendicitis. The average mortality rate is 4.1% (93) and is more likely to occur in infants, the elderly and debilitated patients. The organism may be excreted asymptomatically for several weeks and up to 5% of patients become carriers following recovery (67).

2. Microbiological Characteristics

Salmonellae are gram negative, nonsporulating, nonencapsulated rods (0.7 - 1.5 X 2.0 - 5.0 um) belonging to the family Enterobacteriaceae (74). They are facultative anaerobes and most are motile via peritrichous flagella. They are able to use citrate as a sole source of carbon. Nitrates are reduced to nitrites. Decarboxylation of lysine, ornithine and arginine occurs. Production of acid, gas and hydrogen sulfide occurs in Jordan's tartrate medium, glucose and triple sugar iron agar, respectively (74,108). Dulcitol may be fermented by numerous strains, however sucrose, salicin, raffinose and lactose are not fermented. Utilization of inositol is common of numerous strains, however sodium malonate is not (108). Production of urease, indole, lipase, and deoxyribonuclease does not occur. Phenylalanine and tryptophan are not oxidatively deaminated, gelatin is not liquefied and growth does not occur in mediums containing potassium cyanide (74,108).

Serological analysis is based on the Kauffman-White scheme which identifies and detects the O (somatic), Vi (capsular) and H (flagellar) antigens (74). This method is used diagnostically and is utilized by the Salmonella Surveillance program performed by the Centers for Disease Control in epidemiological investigations (108).

3. Epidemiology

Human salmonellosis is a significant public health and economic problem. The number of cases reported annually is increasing at a steady rate (18,20,28,129). The Salmonella Committee of the National Research Council estimated approximately 2 million cases of salmonellosis per year (28). In the United States, the number of human salmonellae isolates increased more than 60% between 1963 and 1979 (20). This increase may be the result of several factors including increased production of animal feeds and processed foods, and distribution of these products both internationally and nationally (18).

It has been estimated that more than 2000 serotypes of Salmonella exist (74). All of these are potential pathogens to man, animals or both (20,28). The majority of salmonellae are non-host specific and may inhabit the intestinal tracts of warm and cold-blooded animals (18). Epidemiological studies have shown that serotypes isolated from humans are usually the same serotypes isolated from animals (20). This is possible since foods of animal origin are the most important vehicles in the transmission of Salmonella (20,107). Of all the serotypes isolated every year, S. typhimurium is the most frequent (20). Other frequently isolated serotypes in the United States and Canada include S. heidelberg, S. enteritidis, S. infantis, and S. newport (125,127).

Todd (125) compared foodborne disease in six countries. Salmonella was found to be the most prevalent foodborne disease agent (86.6%) in England and Wales, and second in Canada and the United States behind Staphylococcus aureus. Bryan (20) observed Salmonella trends in the United States and Canada during the mid 1970's. Salmonella was responsible for 40% of all reported cases of known etiology in the United States and 39% in Canada. Horwitz and Gangarosa (65) reported 65% of all foodborne outbreaks in the early 1970's in the United States were attributed to Salmonella.

Outbreaks of salmonellosis are commonly associated with poultry (125). After World War II, the consumption of poultry increased due to year round availability (126). This increase in consumption led to an increase in salmonellosis. Poultry ranks as the number one or number two cause of foodborne salmonellosis in Australia, Canada, England, and Wales and it is fourth in the United States (126). In outbreaks traced to poultry between 1973 and 1975, Salmonella was the dominant agent in Canada, the United States, England, and Wales. Turkey was implicated more frequently than chicken (126). Although poultry is a primary vehicle in the transmission of salmonellosis outbreaks, several secondary factors contribute to the problem. Bryan (20) reported that between 1973 - 1976, the majority of outbreaks in the United States were the result of

mishandling in food service establishments and in the home. Other factors contributing to foodborne salmonellosis include improper holding temperature, inadequate cooking or thermal processing, contaminated raw ingredients, poor personal hygiene, contaminated equipment, and cross contamination (19,20,125,126).

The economic impact of salmonellosis is staggering. Substantial costs in medical care and loss of income due to absence from work must be considered. In 1969, the annual cost of salmonellosis was estimated at \$300 million (28). However, in 1978, medical costs alone were estimated at \$1.2 billion / 2.5 million cases in the United States (126). In Canada, costs were estimated between \$25 - \$100 million per year in 1978 (126). There is also a great expense associated with the recall of products contaminated with Salmonella. In 1969, the cost of recalling contaminated chocolate was estimated at \$5 million (28).

4. Incidence

Salmonellae are distributed world-wide and are considered to be the most ubiquitous pathogens in nature (100). They affect not only humans, but also mammals, birds, reptiles and insects. Poultry has been responsible for a large number of salmonellosis outbreaks. In 1939, Edwards (41) regarded fowl as the greatest reservoir of Salmonella infection in the United States. In 1948, Edwards et al. (42)

reported that serotypes found in man were the same as those found in fowl.

Salmonella infection in poultry usually begins at the farm. Several studies have been done to determine the source of infection and the incidence of Salmonella on poultry farms. It has been determined that feed ingredients may play a major role in the transmission of Salmonella to poultry (21,33). Many of these ingredients are rendered animal by-products, such as processed meat, and bone and fish meal which harbor salmonellae. Feed may also become contaminated via birds, rodents and insects which are known carriers of Salmonella (20). Other sources of contamination include infected chicks entering poultry houses, contaminated litter, fecal droppings, contaminated drinking water and cross contamination among the birds (21,33). McBride et al. (81) studied 13 turkey flocks to determine the level of infection. Cecae samples were analyzed from all flocks and 0 - 72% of the birds were contaminated. The average incidence was 11.6% with one - third of the flocks containing greater than 10% infection. McBride et al. reported there was no difference in the infection levels between hens, toms and broilers. However, there was considerable flock to flock variation. Dougherty (33) studied the infection rate on broilers from the time they arrived on the farm until slaughter. Cloacal swab samples were taken of chicks entering the poultry houses and 37.5%

were positive. Dougherty further reported that a reduction in the infection level occurred between the third and fifth weeks of age. Morris et al. (85) reported similar results in breeder chicks between 3 and 9 weeks of age.

Although contamination of poultry with Salmonella begins at the farm (21), the actual numbers of birds entering the processing plant carrying Salmonella is low (33). Such contamination results in the dissemination of salmonellae to meat and equipment during processing and may lead to the contamination of the final product (21,48,54,130,134).

Particular areas of the processing plant have been shown to contribute to the incidence of salmonellae in poultry. The scalding and plucking operations have been shown to contribute to cross-contamination of chicken carcasses. Mulder et al (88) reported a higher incidence of cross-contamination during scalding and plucking when lower temperatures (52 - 54C) were used than when a higher temperature (60C) are utilized. Morris and Ayres (87) reported that they were unable to recover any salmonella from scald tank water. Therefore, the plucking operation is probably a more significant source of salmonellae contamination than the scald water.

Several studies have reported the highest percentage of positive Salmonella cultures following defeathering (21,25,33,48,87). Dougherty et al. (33) reported an incidence

of 65% positive Salmonella samples following defeathering of chicken carcasses. Bryan et al. (21) reported 63% of turkey carcasses were contaminated following defeathering and that 76% of the samples taken from the picking equipment were positive for salmonella. Galton et al. (48) reported the incidence of Salmonella in ducks following defeathering to be 24.8%. This lower incidence has been attributed to a hot wax treatment of the birds following defeathering. However, experimental conditions may have been dissimilar in the two studies. The effects of a hot wax treatment on Salmonella in turkeys would be a useful study.

Evisceration has been implicated as a major source of carcass and equipment contamination (25,54,120). McBride et al. (82) reported an incidence of 0 - 69% following evisceration of turkey carcasses. Saddler and Corstvet (101) analyzed cloacal swabs from turkeys, chicken fryers and chicken hens and samples yielded 5.3%, 1.1% and 0.5% positive Salmonella, respectively. Surkiewicz et al. (120) surveyed nine federally inspected processing plants. Salmonellae were isolated from 20.5% of eviscerated chickens. Campbell et al. (25) also surveyed nine federally inspected plants 10 years after Surkiewicz et al. and compared the results. Campbell et al. (25) reported an incidence of 11.6% following evisceration, which is a 45% reduction in the results reported by Surkiewicz et al (120). Edible viscera has been shown to contain Salmonella (48,54).

However, Morris and Ayres (87) were unable to isolate Salmonella from any edible viscera sampled.

Several studies have reported that carcass contamination is reduced by spray washing (21,25,86). Bryan et al.(21) reported a decrease in the incidence of Salmonella on carcasses following spray washing. Morris and Wells (86) and Campbell et al. (25) reported that spray washing was effective in removing Salmonella and that recontamination occurred in areas of extensive handling and areas in which there was contact between the carcasses.

The chilling operation has also been implicated as a source of cross-contamination of poultry with Salmonella (21,48,82,120). Galton et al. (48) reported that chill water was an effective vehicle in the spread of Salmonella to carcasses. McBride et al. (82) determined the incidence of Salmonella contamination in 11 flocks of chickens after chilling. The incidence ranged from 0 - 96% with an average of 22% positive samples. Surkiewicz et al. (120) reported that there was, on the average, no change in the incidence of Salmonella contamination when carcasses entered and exited the chill tanks. Morris and Ayres (87) were unable to recover Salmonella from chill tank water.

Several studies have suggested that the incidence of Salmonella on poultry carcasses and in the poultry processing plants corresponds to the incidence of Salmonella among flocks (82,86). McBride et al. (82) found that the

variability in the incidence of Salmonella contamination among flocks was significant and that flocks with high incidences prior to processing usually had a high incidence of contamination after processing.

With the exception of the study by Morris and Ayres (87), it appears that there is a build up of Salmonella in the processing plant. This results in a high level of contamination throughout the operation and possibly at the retail level.

Several studies have been performed to determine the prevalence of Salmonella on retail poultry. Felsenfeld et al. (44) reported that 5% of the poultry that they purchased in Chicago markets from 1943 - 1949 were positive for Salmonella. In a study of dressed broiler chickens from retail stores in three Canadian cities, Thatcher and Loit (122) isolated salmonellae from 14% of fresh birds that had not been treated with chlortetracycline. Duitschaeffer (40) reported an incidence of 34.8% in packages of raw chicken pieces purchased in Ontario. A survey done by Ladiges and Foster (72) in Denver yielded an incidence of 8.3% in whole chicken fryers. Woodburn (135) purchased dressed broiler - fryer chickens from retail stores in Indiana. Salmonellae were isolated from 72 out of 263 samples taken and a higher number of positive samples were observed during the fall season than in winter, spring and summer. Wilson et al. (134)

reported an incidence of 17% in market poultry purchased in Cincinnati. However, little seasonal variation in the recovery of salmonellae was observed.

5. Environmental Factors

Salmonellae have simple growth requirements and can multiply in a wide variety of laboratory media provided that the pH, temperature, and water activity are within the ranges for growth. In a food environment, these factors, in association with food additives, are employed to inhibit the growth and survival of Salmonella.

The optimum temperature for growth of Salmonella is 35 - 37C, however this can be affected by pH, water activity and competing microflora. Matches and Liston (77) determined the minimum growth temperatures of 3 strains of Salmonella in agar and broth. S. heidelberg grew at 5.9C after 12 days of incubation. S. typhimurium and S. derby grew at 6.7C and 7.5C respectively after 19 days incubation. Angelotti et al. (6) inoculated custard, chicken a la King, and ham salad with Salmonella and monitored growth between 4.4C and 10C. Salmonella was only able to grow in chicken a la King at 6.7C and above over a 5 day period. Farrell and Upton (43) studied the effect of low temperatures on the growth of S. typhimurium inoculated onto bacon. At 5C, there was a significant increase in cell numbers during the first 2 days of storage followed by a significant decrease

in numbers after day 13. At 16C, there was a significant increase up to day 7 followed by a significant decrease after day 18. Goepfert and Chung (55) studied the growth and survival of S. typhimurium and S. anatum in vacuum packaged sliced luncheon meat stored at 5C for 6 weeks. A 30 - 90% reduction in viable cells was observed within the first 2 weeks followed by a levelling off effect. Goepfert and Chung (55) concluded that although Salmonella numbers decline over refrigerated storage, they are able to persist at a reduced level throughout the shelf life of the product. Goepfert and Kim (56) inoculated several foodborne pathogens into raw ground beef and stored it for 2 weeks at temperatures ranging from 1 to 12.5C. Salmonella was able to grow in ground beef at 12.5C however, Goepfert and Kim noted that the normal microflora of the ground beef had increased to such levels that the meat was rendered unacceptable.

Several studies have shown the effect of freezing on Salmonella in different foods. Farrell and Upton (43) inoculated bacon with S. typhimurium and stored it for 32 days at -22C. A significant decrease in cells was observed and by day 32, only 10.4% of the initial Salmonella were still viable. Gunaratne and Spencer (59) studied the effects of several cryogenic freezing methods on broiler thighs that had been dipped in a solution inoculated with Salmonella. Liquid nitrogen accounted for the greatest

reduction in salmonellae followed by air blast freezing. Mates (80) performed a microbiological survey of frozen ground poultry meat in Israel. A total of 172 samples were analyzed and 33% were found to be contaminated with Salmonella. Mates concluded that ground poultry meat should not be sold to the public due to the high isolation rate of Salmonella.

Several studies have reported on the effects of temperature abuse on Salmonella. Angelotti et al. (6) inoculated custard and chicken a la King with Salmonella and monitored growth and survival at temperatures between 44 - 49C for 24 hours. In custard, Salmonella grew at 46C but decreased in numbers at 47C and above. A similar result was observed for chicken a la King, however salmonellae were killed at 47C and above. Goepfert and Chung (55) studied the effects of temperature abuse on salmonellae in luncheon meats. Vacuum packaged bologna and liver cheese were contaminated with S. anatum and S. typhimurium and were left unopened at 23C for 48 hours. During the first 24 hours, S. anatum grew in the bologna. During the second 24 hours, the number of viable S. typhimurium and S. anatum increased 25 - 30 fold in both luncheon meats.

Salmonellae are heat sensitive and ordinary pasteurizing or cooking conditions are usually sufficient in destroying salmonellae in high moisture foods. In a study done by Seward et al. (106), they found that the use of 1%

polyphosphates, particularly sodium polyphosphate or sodium tripolyphosphate, enhanced the heat - sensitization of Salmonella in plate count agar and in the presence of food ingredients over a range of pH values. They concluded that heat - sensitization is occurring in heat processed products that contain polyphosphates and that this effect may contribute to the efficacy of lower heat - processing temperatures.

Thermal inactivation times of salmonella in foods have been studied. Goodfellow and Brown (57) determined D - values of salmonella in ground beef. A D - value of 61 - 62 minutes was observed at 51.6C, 3.8 - 4.2 minutes at 57.2C, and 0.6 - 0.7 minutes at 62.7C. Angelotti et al. (7) studied time temperature effects on S. senftenberg and S. manhattan in custard and chicken a la King. In custard, D₁₄₀ was 11.32 minutes for S. senftenberg and 2.44 minutes for S. manhattan. In chicken a la King, D₁₄₀ was 9.61 minutes for S. senftenberg and 0.40 for S. manhattan. Craven and Blankenship (29) studied the effect of added soy protein on the heat resistance of Salmonella in beef. A significant difference was found between the D - values of beef alone and the beef - soy mix. Salmonella were found to be more heat resistant with at least 30% added soy protein to meat.

Many poultry products are a result of further processing systems. Several studies have reported on the

effects of further processing on Salmonella. Wilkinson et al. (133) inoculated turkey rolls with 1×10^8 cells/gram of S. typhimurium, S. enteritidis, and S. thompson. The turkey rolls were placed in a 121C oven and the internal temperature was monitored. When the internal temperature reached 60C, S. typhimurium was detected in one roll. At 67C (3.6 hours), all salmonellae were destroyed. Bryan et al. (22) also studied the destruction of salmonellae during thermal processing of turkey rolls. Turkey rolls were inoculated with 1×10^6 cells/gram of S. senftenberg 775. They reported that thermal processing temperatures of 73.9C for 5 1/2 hours and 85C for 4 1/2 hours were sufficient in destroying S. senftenberg 775. Denton and Gardner (31) reported that even though the number of salmonellae in mechanically deboned turkey meat was relatively high, various cooking treatments (oven roasting, immersion water treatments, and smoke house cooking) were all effective in significantly reducing the number of salmonellae in the final product.

The optimum pH for the growth of Salmonella is between 6.5 and 7.5 with values above 9.0 and below 4.0 being bacteriocidal (27,93). Chung and Goepfert (27) determined the minimum pH value and the type of acidulant that would permit initiation of growth by Salmonella in trypticase soy broth. They found that within an initial inoculum level of 10^4 - 10^6 cells/ml salmonellae were able to initiate growth

in broth acidified with citric acid, hydrochloric acid, or tartaric acid at a pH of 4.05 ± 0.05 . Matches and Liston (79) studied the effect of pH on low temperature growth of 3 strains of Salmonella. S. heidelberg was able to grow at 5.9C at a pH value of 7.0. The minimum growth temperature for S. typhimurium was 6.4C and occurred at a pH value of 8.0. A higher minimum growth temperature (9C) was observed for S. derby, however, this occurred at a pH value of 6.0.

The minimum water activity (A_w) at which Salmonella can grow is in the range of 0.940 - 0.959 (51,116). This value is similar to that found in poultry (67). Ingredients such as salt and nitrites can be used to lower the A_w of foods and act as preservatives and antimicrobials. Gibson and Roberts (51) studied the effect of pH, A_w , sodium nitrite, and temperature on the growth of Salmonella in brain heart infusion broth. Growth was evident at pH 5.8 - 6.8 and any combination of salt (0 - 4%) and nitrite (0 - 400 ug/ml) within one week of storage at temperatures ranging from 20 - 35C. Growth was evident at pH 6.8 and 10C in 2% salt and 400 ug/ml NaNO_2 . However, as the pH was decreased to 5.6, no growth was evident after incubation for 10 weeks. Thayer et al. (123) studied the effects of salt, pH, and temperature on growth of S. typhimurium in glucose - mineral salts medium. They reported that maximum growth of S. typhimurium occurred at a pH value of 7.0, 0.05% NaCl at 19C under aerobic conditions. However, under anaerobic

conditions, growth, compared to aerobic conditions was suppressed. Alford and Palumbo (5) studied the interaction of salt, pH, and temperature on the growth and survival of Salmonella in ground pork. In a broth environment, growth was inhibited at a pH of 5.0 and 2% NaCl at 10C. At a pH of 5.8 (which is similar to meats), 5% NaCl was required to inhibit growth at 10C. In ground pork, Salmonella would not grow at 4C but would grow in pork containing 3.5% NaCl stored at 10C. Salmonella was able to compete with the natural microflora of the ground pork at 10C even when the salmonellae consisted of less than 5% of the initial microflora. The results of this study showed that decreasing temperatures increased the inhibitory effects of pH and NaCl, but decreased the lethal effects.

B. CAMPYLOBACTER

1. History

The knowledge of Campylobacter as a human pathogen has greatly increased over the last decade. Campylobacter has been recognized as a veterinary pathogen for more the 40 years. It was first discovered in 1909 by two veterinarians, McFaydean and Stockman, in association with epizootic abortion in sheep (84). In 1913, they demonstrated that the organism was present in the aborted material, however they did not name the causative bacterium (84). In 1919, while Smith and Taylor

were investigating infectious abortions in cattle, a spirillum was isolated from aborted bovine fetus fluids and subsequently named Vibrio fetus (115) based on morphology and origin.

Microaerophilic vibrios were implicated as the etiologic agent of winter dysentery in calves by Jones et al. in 1931 (68). These researchers (68) called this organism Vibrio jejuni since the organism was determined to first cause infection in the jejunum. In 1944, Doyle (34) described a similar organism as the cause of swine dysentery and named it Vibrio coli.

The first reported incidence of microaerophilic vibrios associated with human diarrheal disease was in 1946 by Levy (75) in which milk was considered the cause of the outbreak. Organisms resembling Vibrio jejuni were isolated from blood cultures from several afflicted patients. It was not until 1957 that human strains of microaerophilic vibrios were extensively studied. King (71) observed that Vibrio fetus isolates from human blood could be divided into two groups based on temperature characteristics. Those which grew at 25C and 37C but not at 42C were characteristic of Vibrio fetus. However, a second group of closely related organisms grew at 37C and 42C but not at 25C. This latter group was termed "related vibrios" and was isolated from the blood of patients with gastroenteritis. In 1963, Sebald and Veron

(104) discovered that the two groups of vibrios differed in their DNA nucleotide base-pair ratio (Guanine + Cytosine mol. %) from other vibrios. As a result of these findings, V. fetus and V. bubulus were removed from the genus Vibrio and placed in a new genus, Campylobacter, meaning curved rod.

Campylobacter was reclassified in 1973 by Veron and Chatelain (128) who decided to name the species based on the description of Vibrio fetus by Smith and Taylor. They found that V. fetus var. intestinalis most closely resembled V. fetus and renamed that species C. fetus subsp. fetus and named the strains as C. jejuni and C. coli. However, in 1974, Smibert (112) claimed that the original strain of V. fetus closely resembled V. fetus var. venerealis and renamed it C. fetus subsp. jejuni. The strains C. fetus subsp. jejuni were named C. fetus subsp. intestinalis. The officially recognized nomenclature for campylobacters published in the "Approved List" of bacterial names (109) is that of Veron and Chatelain (128) which are C. jejuni and C. coli.

2. Clinical Characteristics

Campylobacter jejuni is now well recognized as a common cause of acute bacterial gastroenteritis in humans. It is isolated from patients with diarrhea more frequently than both Salmonella and Shigella (11). Infection is usually

caused by the ingestion of contaminated food and the minimum infective dose is 500 cells (97). The illness can last 2 to 7 days with an incubation period of 1 to 7 days (131). Common symptoms include fever, bloody diarrhea, and abdominal pain. Malaise, headache, musculoskeletal pain and sometimes rigors and delirium may also be present (110). Vomiting is not prevalent. Profuse diarrhea may cause sufficient dehydration and electrolyte imbalance to result in hospitalization (76). Diarrhea and abdominal pain may be severe enough to mimic acute enterocolitis and acute appendicitis (10). Erythromycin and tetracycline may be used to treat severe cases (11). The organism may be excreted asymptotically up to 7 weeks following illness (76,131). The illness is usually self-limiting (69) and death is rare (111). Campylobacter jejuni infection occurs more frequently in children. Seasonal incidence tends to be higher in the summer and fall months (24). C. jejuni has also been reported to cause enteric infection in animals (114), abortion in sheep and cattle (10), and infectious hepatitis in poultry (113).

3. Microbial Classification

Campylobacters are gram negative, curved, spiral rods 0.2 - 0.5 μ m wide and 0.5 - 5.0 μ m long belonging to the family Spirallaceae. Older cultures (72 hours) tend to form coccoid or spherical morphologies which are considered to be

a degenerative state (23). They are microaerophilic (requiring 5% O₂, 10% CO₂, and 85% N₂ for growth) and non-spore forming. Motility is via a single, polar flagellum at one or both ends of the cell. Campylobacters are highly motile exhibiting a characteristic rapid darting, corkscrew-like motility (113). Campylobacters are unable to utilize carbohydrates, therefore their primary energy sources are Kreb's cycle intermediates and amino acids (113). They are oxidase and catalase positive and are able to reduce nitrates to nitrites. Methyl red and Vogues Proskauer reactions are negative. Gelatin, urea, casein, ribonucleic acid, deoxyribonucleic acid and esculin are not hydrolyzed. They exhibit no lipase activity and they are unable to produce pigments. C. jejuni is able to grow in a semisolid medium with 0.1% added NaCl (35).

4. Origin and Prevalence

Campylobacter jejuni is distributed world-wide and is part of the normal intestinal microflora of many wild and domestic animals including sheep (110), cattle (92,113), swine (113), goats (112), poultry (58), ducks (70), and pets including dogs and cats (11,14).

Campylobacter enteritis is often considered to be a foodborne infection although the source of transmission in the majority of cases is unknown (62). Confirmed sources of infection include the consumption of unpasteurized milk (98)

and contaminated drinking water (124). Circumstantial evidence has implicated raw or undercooked poultry (17,63, 66), cake icing (12), mushrooms (62) and pork (136) as vehicles of transmission.

Poultry and meat are considered to be potential sources of Campylobacter infection for humans due to the ubiquity of this organism in the intestinal contents and carcasses of these animals. Studies have been performed to determine the origin and prevalence of C. jejuni in poultry and ducks at the farm. Acuff et al. (2) were unable to isolate C. jejuni from fertile turkey eggs or newly hatched poults. However, C. jejuni was present in 76% of fecal swabs of 19 day old turkeys. Kasrazadeh and Genigeorgis (70) isolated C. jejuni from ducklings as early as day 4 and all birds were positive by day 11. Campylobacter free environments have been reported in broiler chicks up to 2 weeks of age (89). Eventually though, the majority of birds become infected as determined by Genigeorgis et al. (50) who noted an increase in infection rates from 2.3% on day 10 to 81.8% by slaughter. C. jejuni is suspected to originate in wet litter and drinking water via rodents, flies, and wild birds (2,50,89) but this has not been proven microbiologically.

Slaughtering and processing of poultry carcasses have a profound effect on C. jejuni contamination. The percentage of birds containing C. jejuni upon entering the processing plant varies from 0 - 100% (4,50,90). A number of studies

have reported that C. jejuni is frequently found in the intestinal tract of birds arriving at the slaughterhouse (50,70,90) and that this is the most probable source of contamination. Oosterom et al. (90) reported an isolation rate of 10^7 C. jejuni per gram in the intestinal contents of birds to be processed. Investigations have been done to determine the effects of various processing stages on C. jejuni. Acuff et al. (4) reported that large numbers of C. jejuni were destroyed during scalding of turkey carcasses. Oosterom et al. (90) found that C. jejuni contamination of poultry was reduced at 58C but not always at 51.8C. However, Wempe et al. (133) and Genigeorgis et al. (50) did not find a significant decrease in C. jejuni numbers following scalding. Recontamination of carcasses seems to occur following defeathering (50,70,90,133) with isolation of C. jejuni from feather - picker - drip water samples at rates as high as 94% at a level of log 3.4 (50). Acuff et al. (4) recovered few or no C. jejuni from turkey carcasses following chill tank immersion. However, Wempe et al. (133) found the chill tank (chlorinated or unchlorinated) to represent a major area of cross contamination.

Many researchers have reported that C. jejuni is present in commercially processed poultry and meat at the retail level. As many as 68% of ready for market poultry products in the U.S. may be contaminated with the organism at the processing level (133). Lammerding et al. (73) found

that as many as 73.7% of turkey and 38.2% of chicken carcasses tested positive for C. jejuni in a study done to determine the prevalence of C. jejuni across Canada. At the supermarket level, as many as 64.1% of packaged fresh turkey wings (95) and 30% of fresh retail chickens (118) have been reported to test positive for C. jejuni. Isolation of C. jejuni from red meats tends to be considerably lower than poultry at the retail level (62,118). Although C. jejuni can survive frozen storage on poultry carcasses (4,53,99), the effect of freezing is deleterious to the organism and results in a reduction of the number of cells present (53,117). Acuff et al. (4) studied the effects of further processing on turkey products and although C. jejuni was recovered from turkey meat during initial stages of further processing, no C. jejuni were recovered from heat treated, further processed products. Therefore, it would seem that foodborne illness caused by C. jejuni in cooked poultry products would most likely be caused by cross-contamination (53,4).

5. Environmental Factors

Food processors commonly utilize environmental factors to control or inactivate pathogens and undesirable microorganisms in foods. Many of these factors such as temperature, sodium chloride, pH, and atmosphere affect the growth and survival of C. jejuni.

In laboratory culture media, growth of C. jejuni is restricted to temperatures greater than 30C and less than 47C (36). The optimum temperature for growth ranges between 42C and 45C. Several studies have shown that C. jejuni does not grow well in food at this temperature range.

Blankenship and Craven (9) studied the response of C. jejuni in sterile ground chicken meat. At 37C, there was a 1 - 2 log increase in growth over a 4 day storage period with a gradual decline by 17 days. However, there was no growth reported at 43C. Christopher et al. (26) inoculated C. jejuni into skim milk and beef, both of which were held at 40C. Although survival of C. jejuni in beef was superior to that in skim milk, levels of the organism were undetectable by 72 and 24 hours, respectively. Svedhem et al. (121) reported a 2 log increase in 8 hours for C. jejuni held at 42C in chicken, followed by a rapid decrease to undetectable levels after the second day. When C. jejuni was inoculated into minced pork and beef held at 42C, no growth occurred and there was a 6 log reduction in cells by day 2. Gill and Harris (52) inoculated meat pies with over 10^5 C. jejuni cells / gram and stored them at 37C and 43C. After 3 days of incubation, no C. jejuni cells could be detected at either temperature. However, after a 2 - 3 day lag phase, growth of C. jejuni did occur in cooked meat medium at 37C suggesting that C. jejuni may be unable to compete with other organisms present in foods (52).

Numerous studies have reported that low temperature storage is advantageous for C. jejuni survival (9,13,26, 37,60). Hanninen (60) reported that when ground beef liver was inoculated with C. jejuni and stored at 4C there was no effect on the survival of C. jejuni and that there was a very slight logarithmic reduction. Blankenship and Craven (9) reported no growth of C. jejuni inoculated into sterile chicken meat stored at 4C and that over a 17 day storage period there was a gradual decline in cell numbers (<2 log). Blaser et al. (13) reported a higher survival rate of C. jejuni in pasturized milk at 4C than 25C. At 4C, C. jejuni cells remained viable up to 22 days whereas no viable organisms were detected after 3 days at 25C. Doyle and Roman (37) reported that there was great variation among strains of C. jejuni stored at 4C in unpasteurized milk. The most tolerant strain had less than a 2 log decrease in viable cells after 14 days, whereas the most sensitive strain had greater than a 6 log decrease by day 7. Doyle and Roman (37) also reported that cell death correlated with an increase in spoilage microflora and a decrease in pH. Christopher et al. (26) reported survival of C. jejuni in skim milk was best at 1C and 10C, with viable cells detected after 12 and 11 days, respectively.

C. jejuni is sensitive to freezing. However it has been recovered from frozen meat and poultry at the retail level (95,99,117). Christopher et al. (26) reported

better survival of C. jejuni in solid foods than liquid at -20C. After 8 days of storage in skim milk at -20C, no viable cells were detected. Survival in beef stored at -20C was detected after 30 days at a level of 5.6×10^5 C. jejuni cells / gram. Stern and Kotula (119) reported a 3 log reduction of C. jejuni inoculated into ground beef held 14 days at -15C. Hanninen (60) reported a 2 - 3 log reduction of C. jejuni in ground beef liver stored at -20C for 3 months. The majority of cells (about 90%) lost viability during the initial days of storage. The influence of thawing and storage at 4C resulted in a 60 - 70% increase in viability. On frozen broiler carcasses, Hanninen (60) reported a 0.5 - 2.0 log decrease in C. jejuni during a 12 week storage period at -20C. Survival was determined to be better in the drip than on the skin. Rosef et al. (99) reported C. jejuni may survive 4 weeks at -25C on frozen poultry carcasses.

Thermal inactivation of C. jejuni in foods is necessary to assure that viable cells are destroyed during processing and prior to consumption. This is especially important for raw milk, poultry and beef since these foods have been implicated as vehicles of human Campylobacter infections. Doyle and Roman (36) determined the time - temperature treatment necessary to destroy 90% of C. jejuni cells in skim milk. The D_{55} values for 5 strains ranged from 0.74 to 1.00 minute. Christopher et al. (26) reported that there was

no survival of C. jejuni ($10^6 - 10^7$ cells / ml) in skim milk when heated at 60C for one minute. The results of these studies show that proper pasteurization of milk should be sufficient in destroying viable C. jejuni cells (26,36). Several studies have been conducted to determine the effect of internal heating temperatures on the survival of C. jejuni in meat and poultry. Stern and Kotula (119) studied the heat resistance of C. jejuni in ground beef. An initial inoculum of 10^7 cells / gram was inoculated into ground beef. They reported that all C. jejuni cells were inactivated in less than 10 minutes when an internal temperature of 70C was reached. Christopher et al. (26) inoculated 6.6×10^6 C. jejuni cells / gram into the center of beef roasts. When heated to an internal temperature of 53C, one strain was detected at a level of 10 cells / gram. However, when heated to 57C no viable cells were detected. Blankenship and Craven (9) investigated the thermal inactivation of C. jejuni in chicken meat. D - values at 55C and 57C were 2.12 and 0.79 minutes, respectively. Proper cooking techniques used to destroy salmonellae should be sufficient in eliminating C. jejuni.

Sodium chloride is used primarily as a food preservative. It functions as a growth inhibitor for microbial cells since it affects the water activity of the food. Campylobacter grows best in the presence of 0.5% NaCl added to brucella broth at 42C (21). It can grow in NaCl

concentrations up to 1.5% (38,61) at or near its optimum temperature. However, concentrations of NaCl exceeding 2.0% are bacteriocidal to C. jejuni (38,61). Several studies have demonstrated the effect of temperature and NaCl concentration on C. jejuni. Doyle and Roman (38) reported that decreasing the temperature from 42C to 25C increased the NaCl tolerance of C. jejuni to 4.5%. The rate of death was reduced from 1 log₁₀ per 1.5 - 2.0 hours at 42C to 1 log₁₀ per 14 hours at 25C. Decreasing the temperature to 4C increased the NaCl tolerance to a concentration of 6.5%. A substantial number of viable cells were still detected after 3 weeks. Hanninen (61) and Abram and Potter (1) found similar results at refrigeration temperatures using 2.0 and 3.0% NaCl, respectively. Abram and Potter (1) investigated the effect of salt concentration on C. jejuni in foods. Survival of C. jejuni in ground chicken, beef and cod with 0.0, 1.0 and 2.0% added NaCl was determined at -18, 6 and 10C over a 5 day period. At -18C, the survival of C. jejuni was not affected by the level of NaCl. At 6 and 10C, there was less than a 1 log decrease in the survival of C. jejuni with increasing levels of NaCl in raw and cooked chicken and beef. These findings suggest that NaCl may not be an effective antimicrobial in refrigerated foods but at higher temperatures it should be more than sufficient.

The response of C. jejuni to pH varies with the strain and is influenced by temperature. Doyle and Roman (36)

studied the effect of pH on the growth on C. jejuni in Brucella broth. The best growth occurred at a pH range of 6.0 - 8.0 with an optimum pH range of 6.5 - 7.5. Doyle and Roman (36) also studied the effects of pH and temperature on survival of C. jejuni. They reported that at a pH range of 3.0 - 4.5, the rate of cell death was dependent upon temperature with the most rapid death occurring at 42C and the slowest at 4C. They also reported that when C. jejuni was inoculated into Brucella broth adjusted to a pH of 5.0 and stored 2 weeks at 4C, there was less than a 3 log₁₀ decrease in viable cells. This suggests that C. jejuni may be able to survive in low acid foods held at refrigeration temperatures for several weeks. Christopher et al. (26) reported that at 37C, C. jejuni counts increased rapidly in Brucella broth adjusted to a pH range of 6.0 - 8.0 over a 2 day storage period. At pH 5.0, there was a rapid decrease in cell numbers and by 24 hours there were no survivors. Gill and Harris (51) observed the effects of pH and temperature on the survival of C. jejuni inoculated onto meat. High (6.8) pH meat decreased by 1 log and normal (5.8) pH meat decreased by 2 log over a 2 week period at -18C. When chilled at -1C, there was a gradual decline in C. jejuni numbers in high pH meat but in normal pH meat, no survivors were detected by day 8. At 25C, there was a rapid decrease in cell numbers in both high and normal pH meats.

The effect of drying on C. jejuni is dependent on

strain, relative humidity (RH) and medium used to suspend the organism (38). Doyle and Roman (38) studied the effect of drying on 4 strains of C. jejuni in skim milk and Brucella broth on glass slides. At 25C, only 2 strains survived a <1% RH for more than 24 hours. At 4C, all strains were able to survive <1% RH for an extended period. The most tolerant strain exhibited a 2.5 log decrease over 29 days. C. jejuni was more sensitive to drying in milk than Brucella broth. Doyle and Roman (38) also reported that C. jejuni can survive best in environments containing less than 15% RH and least in environments containing 59% RH. Bracewell et al. (16) studied the effects of drying on C. jejuni inoculated onto pork skin. The pork skin was stored at 4C with 15% RH. Forced ventilation was also used so as to imitate a chill room environment. Within the first 24 hours, there was a $2.5 \log / \text{cm}^2$ decline followed by a further decline of $2.0 \log / \text{cm}^2$ after 48 hours. This method appears to be beneficial to reducing C. jejuni on carcasses, however it is contradictory to Doyle and Roman's findings.

Campylobacter jejuni is classified as a microaerophilic bacterium meaning that it can only tolerate low amounts of oxygen. Smibert (112) reported that normal atmospheric concentrations of oxygen are toxic to C. jejuni. Fletcher and Pastridge (45) studied the effects of gaseous environments on C. jejuni. They found that the optimum

concentrations of oxygen and carbon dioxide for growth were 5% and 10%, respectively.

Several studies have been performed to determine the effect of ingredients in media on the growth of C. jejuni. George et al. (49) reported that the addition of ferrous sulfate, sodium metabisulfate and sodium pyruvate to both Brucella broth and Brucella agar increased the aerotolerance of 98% of C. jejuni strains tested to 21% oxygen. Bowdre et al. (15) reported that the addition of dihydroxyphenyl compounds to Brucella agar greatly increased the aerotolerance of C. jejuni. Similar findings were reported by Hoffman (63) who also noted a decrease in both catalase and oxidase activities as a result of these additions. Blankenship and Craven (9) studied the effect of ambient atmosphere and carbon dioxide atmospheres on the storage of raw chicken drumsticks at 4C. C. jejuni survived in both atmospheres for up to 21 days with a decline of 1.5 log in CO₂ and 2.0 log in ambient atmosphere.

2

CHAPTER III

MATERIALS AND METHODS

A. TEST ORGANISMS

The test organisms used were three strains of Salmonella and two strains of Campylobacter jejuni. Salmonella blockley 762-55, S. infantis, and S. typhimurium 4066 were obtained from Dr. B. Swaminathan of the Centers for Disease Control in Atlanta, GA. Stock cultures were maintained on Trypticase Soy Agar (TSA) and stored at room temperature. Working cultures were maintained in Trypticase Soy Broth (TSB) incubated at 35C, and stored at 4C. Cultures were transferred weekly.

Campylobacter jejuni 29428 was purchased from the American Type Culture Collection, Rockville, MD. C. jejuni CJ4086 was obtained from Dr. M.P. Doyle at the Food Research Institute, Madison, WI. Both were received as lyophilized cultures. All cultures were maintained in Brucella Broth supplemented with 0.1% agar and stored at 4C. Cultures were transferred weekly.

B. PREPARATION OF INOCULUM

The Salmonella inoculum was prepared by growing an 18 hour culture in TSB at 35C and then serially diluting the culture in 0.1% peptone water to give the following levels

of inoculum: 1×10^5 cells/ml, 1×10^3 cells/ml, and 1×10^1 cells/ml.

The C. jejuni inoculum was prepared by growing a 24 hour culture in Brucella Broth supplemented with 0.1% agar and storing under a microaerophilic atmosphere of 5% O_2 : 10% CO_2 : 85% N_2 at 42C. The atmosphere was provided by pulling a vacuum on an anaerobe jar and replacing it with the pre-mixed gases. The atmosphere was flushed 2 - 3 times. The C. jejuni cultures contained approximately 1×10^7 cells/ml.

C. PREPARATION OF CHICKEN NUGGETS

Two commercial brands of breaded, fully cooked, frozen nuggets were used. The main ingredients in Brand A consisted of chicken, water, salt, and sodium phosphate. Brand B ingredients were chicken breast with rib meat, water, isolated soy protein, salt, and sodium phosphates. Both brands were battered and breaded. The products were frozen (-18C) upon receipt. While frozen, nuggets were weighed and inoculum (0.1 ml) was injected into the center of the nugget. Nuggets were labeled as to brand, strain, and level of inoculum. Nuggets were stored in a plastic Stomacher bag for the designated storage time and temperature. An inoculated sample for each strain and level of inoculum and a control sample with no inoculum were prepared for each brand and storage period. The experiment

was designed as a completely randomized block for each temperature and genus.

D. STORAGE PERIODS

The effect of freezing on survival was evaluated at -18C with sampling at time intervals of 0, 2, 4, and 6 weeks. The effect of refrigerated storage was evaluated at 4C with sampling time intervals of 0, 2, 4, and 6 days. A model for temperature abuse was evaluated at 21C with sampling at time intervals of 0, 6, 12, and 24 hours.

E. METHODS FOR MICROBIAL ANALYSES

At each sampling, both brands of chicken nuggets were assayed for aerobic plate count (APC), coliform plate count (CPC), and psychrotrophic plate count (PPC). Surviving salmonellae and campylobacters were enumerated via direct and enriched plating techniques using the methods outlined by the Bacteriological Analytical Manual (47).

1. Salmonella

Direct enumeration of Salmonella consisted of diluting (1:5) the sample in 0.1% peptone water, homogenizing the sample by stomaching for 2 minutes (Model 400, Seward Laboratory, London) and spread plating 0.1 ml aliquots onto Bismuth Sulfite Agar (BSA). Plates were incubated at 35C for 24 hours. Enriched enumeration of Salmonella consisted of

enriching the sample in tetrathionate broth (1:10) and incubating at 35C for 24 hours. Brilliant Green Agar (BGA) was streaked with the broth to determine if Salmonella could be recovered and 0.1 ml aliquots were spread plated onto BSA. Both media were incubated at 35C for 24 hours. Colonies typical of Salmonella on BSA were counted. Representative colonies were inoculated into Triple Sugar Iron Agar slants and incubated at 35C for 24 hours and confirmed.

2. Campylobacter

Direct enumeration of Campylobacter consisted of diluting (1:5) the sample in Cary Blair transport medium (Appendix A), homogenizing the sample by stomaching for 2 minutes, and spread plating 0.1 ml aliquots onto Blood - Free Campylobacter Agar with cefoperazone selective supplement. Plates were incubated in an anaerobe jar for 72 hours at 42C in an atmosphere of 5% O₂ :10% CO₂ :85% N₂. Enriched enumeration of campylobacter consisted of static broth enrichment of the sample in 100 ml of Brucella Broth supplemented with 0.10% agar and Blaser - Wang antibiotics (91). The sample was homogenized for 2 minutes in a stomacher and then aseptically transferred into a 500 ml Erlenmeyer flask fitted with a number 10 one - hole rubber stopper which was fitted with a 1 cc plastic syringe. Attached to the plastic syringe was Tygon tubing (7.6 cm

in length and 1.5 cm in diameter) and a metal clamp. This apparatus served as an anaerobe flask for culturing the enriched campylobacters. The enrichment flasks were evacuated and flushed 2 - 3 times with an atmosphere of 5% O_2 :10% CO_2 :85% N_2 and incubated at 42C for 24 hours. Samples undergoing frozen storage underwent static enrichment in Brucella Broth supplemented with 0.1% agar, 0.3% sodium succinate, and 0.01% cysteine - HCl (94). The enrichment flasks were flushed 2 - 3 times with the appropriate atmosphere and incubated at 35C for 6 hours and then transferred to 42C for 18 hours (94). Blood - Free Campylobacter Agar with cefoperazone selective supplement was spread plated with 0.1 ml aliquots, stored in an anaerobe jar under the appropriate atmosphere and incubated at 42C for 72 hours. Colonies typical of C. jejuni were counted. Phase - contrast microscopy was used to confirm representative colonies.

F. SAMPLE COMPOSITION

The composition of each brand of nugget was determined using the methods outlined by the Association of Official Analytical Chemists (8): moisture (24.002), ash (24.009), protein (24.007), fat (24.005), and salt (24.010). Carbohydrates were determined by difference. Narrow range pH paper was used to estimate the pH of the samples.

G. STATISTICAL ANALYSES

Data were analyzed by analysis of variance and means were separated when significant difference ($P < 0.05$) were detected by Duncan's New Multiple Range Test or Least Square Means (103). Two replication were performed on all experiments. Salmonella and Campylobacter were analyzed as separate experimental designs.

CHAPTER IV

RESULTS AND DISCUSSION

A. CHEMICAL ANALYSES

1. Proximate Composition

The proximate composition of both brands of chicken nuggets are listed in Table 1. Brand B chicken nuggets had a higher percentage of water than Brand A chicken nuggets, however, Brand A chicken nuggets had 10% more fat than Brand B chicken nuggets. Protein, ash, and carbohydrate-by-difference, were not significantly different ($P>0.05$).

2. Salt

The percent of salt in Brand A chicken nuggets and Brand B chicken nuggets was 2.10% and 2.92%, respectively, and these were significantly different from one another (Table 1).

3. pH

The average pH (Table 1) of both brands of chicken nuggets was 6.4 which is in the range reported for poultry.

B. MICROBIAL PROFILE OF CHICKEN NUGGETS

Table 2 shows the microbial profile of uninoculated chicken nuggets over storage. Aerobic plate counts (APC's)

Table 1. Proximate composition, initial pH, and NaCl analysis of Brand A and Brand B chicken nuggets.

Analysis	Brand A	Brand B
% Moisture	45.44x	54.79y
% Fat	26.39y	16.47x
% Protein	15.09x	14.41x
% Ash	1.61x	1.25x
% Carbohydrate	11.47x	13.08x
% NaCl	2.10x	2.92y
pH	6.40x	6.40x

x,y

Means within the same row followed by different letters are significantly different ($P < 0.05$).

Table 2. Microbial profile of uninoculated chicken nuggets over storage.

Brand	a		APC Log	PPC	Coliform	b <u>Campylobacter</u> / <u>Salmonella</u>
	Sampling time					
A	0		1.24x	0.20x	<1.0	<1.0
B	0		1.60x	0.42x	<1.0	<1.0
A	6w (-18C)		1.80x	0.57x	<1.0	<1.0
B	6w (-18C)		2.52x	0.97x	<1.0	<1.0
A	6d (4C)		1.63x	0.10x	<1.0	<1.0
B	6d (4C)		2.05x	0.70x	<1.0	<1.0
A	24h (21C)		4.18y	0.90x	<1.0	<1.0
B	24h (21C)		4.68y	1.60x	<1.0	<1.0

a. Sampling times and temperature combinations were:
-18C for 6 weeks,
4C for 6 days,
21C for 24 hours.

b. Counts after enrichment.

x,y

Means within the same column within the same sampling time and temperature followed by different letters are significantly different ($P < 0.05$).

for Brand A and Brand B chicken nuggets remained the same when stored at -18C for 6 weeks and 4C for 6 days. Brand B chicken nuggets had a slightly higher initial APC than did Brand A chicken nuggets at time 0, however, this difference was not significant ($P < 0.05$). The APC's for both brands of chicken nuggets increased $\log_{10} 3.0$ ($P < 0.05$) over the 24 hour storage period at 21C.

Psychrotrophic plate counts (PPC's) for both brands of nuggets were similar over all storage periods and temperatures. Brand B chicken nuggets had higher PPC's than did Brand A chicken nuggets, however, no significant differences were observed ($P > 0.05$).

Coliforms were not detected in any of the uninoculated samples for either brand of nuggets. The only time coliforms were detected in any of the samples was when the nugget was inoculated with Salmonella, suggesting a false positive reaction for coliforms.

C. AEROBIC PLATE COUNTS

Aerobic plate counts (APC's) of samples stored at -18C are shown in Figure 1. Initial APC of Salmonella inoculated nuggets ranged from $\log_{10} 1.2$ to $\log_{10} 2.9$ CFU/g (Figure 1A). Nuggets inoculated with higher levels of Salmonella had the highest APC. There was not a significant decrease ($P > 0.05$) in CFU/g over time in samples inoculated with Salmonella and held at -18C. The two brands of nuggets did not differ

FIGURE 1. Aerobic plate counts of chicken nuggets stored for six weeks at -18C.

A. Nuggets inoculated with Salmonella.

B. Nuggets inoculated with Campylobacter.

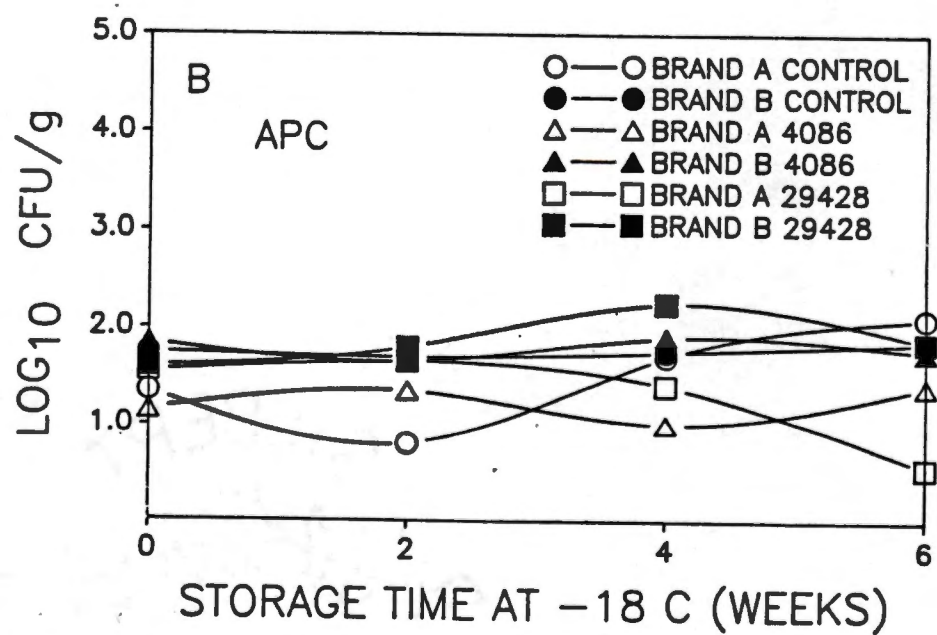
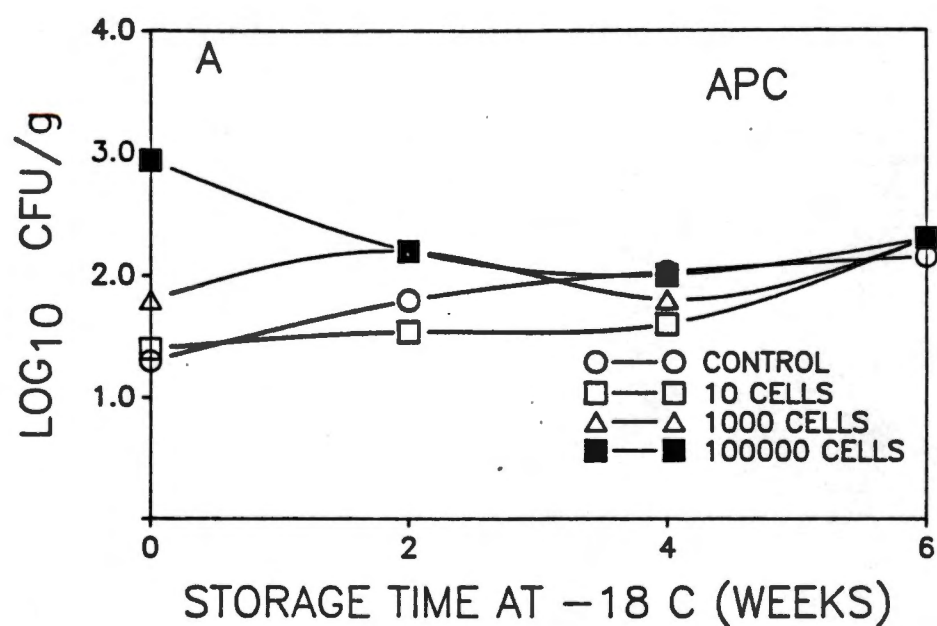


FIGURE 1.

FIGURE 2. Aerobic plate counts of chicken nuggets stored for six days at 4C.

- A. Nuggets inoculated with Salmonella.
- B. Nuggets inoculated with Campylobacter

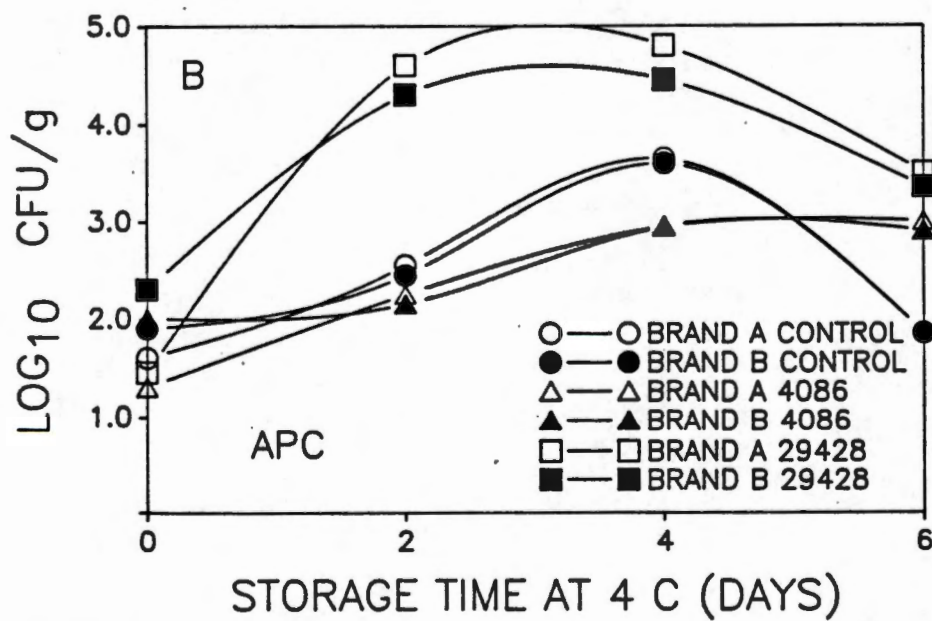
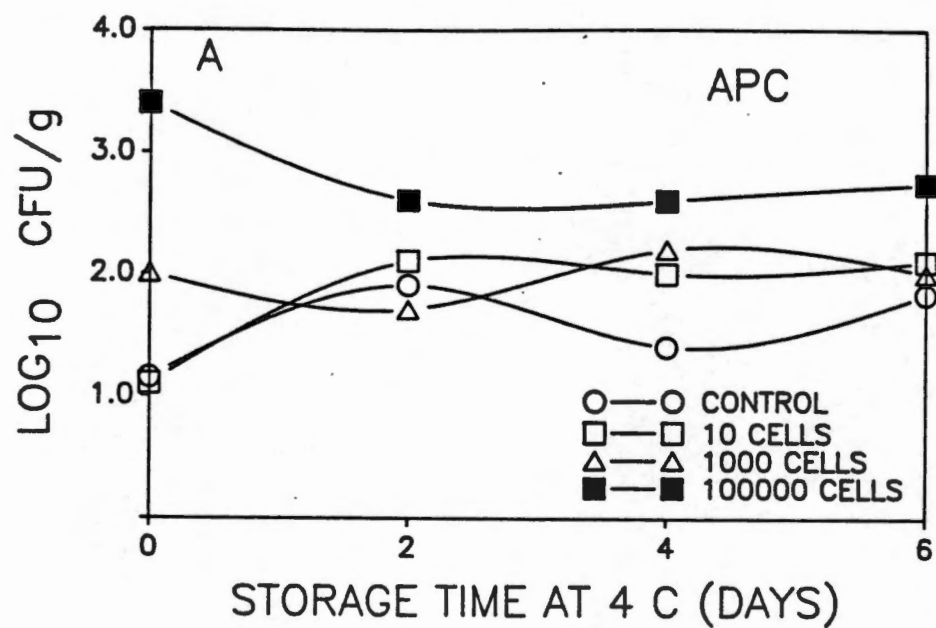


FIGURE 2.

significantly ($P>0.05$) with respect to recovery of Salmonella. Therefore, all APC data for samples inoculated with Salmonella is the mean of data for the two brands of nuggets. In the nuggets inoculated with 1×10^5 salmonellae/g, APC/g was only $\log 2.9$ CFU/g. Since viable Salmonella would have grown on the APC, this indicated approximately a 2 log destruction in Salmonella due to freezing.

Initial APC of Campylobacter inoculated nuggets stored at -18°C (Figure 1B) were between $\log 1.0$ and $\log 2.0$ CFU/g. Since initial levels of C. jejuni were approximately $\log 6.0$ CFU/g and APC's were not different ($P>0.05$) in inoculated and uninoculated nuggets, C. jejuni did not appear to be detected on the APC. This is not surprising due to its microaerophilic and thermophilic nature. APC did not change significantly over the six week storage period and did not differ significantly overall in nuggets ($P>0.05$).

Aerobic plate counts of nuggets stored at 4°C are shown in Figure 2. There were no significant ($P>0.05$) increases or decreases in APC CFU/g over time in samples inoculated with Salmonella (Figure 2A). Nuggets inoculated with 10^5 salmonellae/g had initial APC's of 10^5 CFU/g and increased $\log 1.0$ CFU/g over the first 2 days of storage. Nuggets inoculated with 1×10^5 salmonellae/g had a $\log 1.0$ decrease in APC/g during the first 2 days of storage.

FIGURE 3. Aerobic plate counts of chicken nuggets stored for 24 hours at 21C.

- A. Nuggets inoculated with Salmonella.
- B. Nuggets inoculated with Campylobacter.

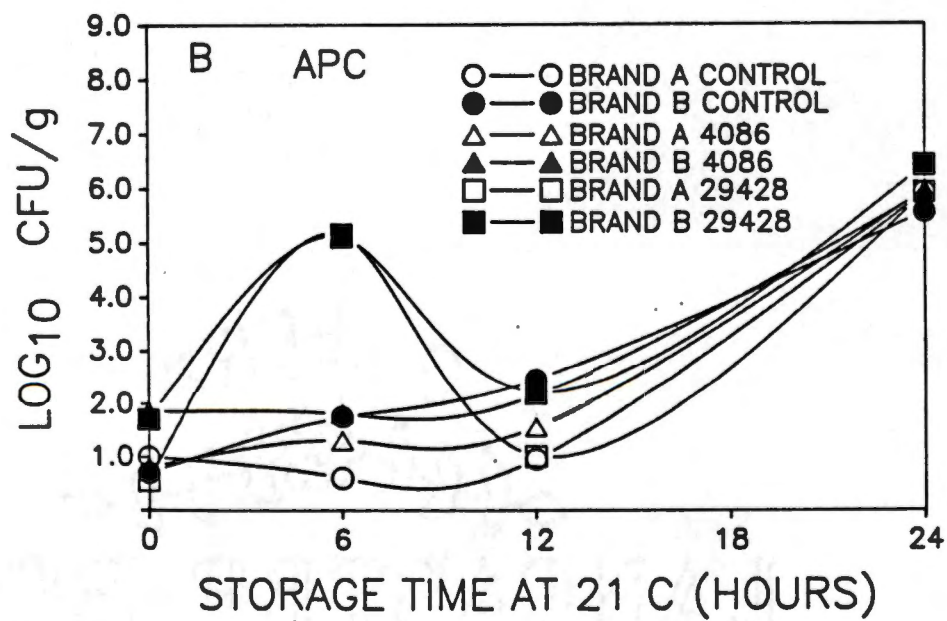
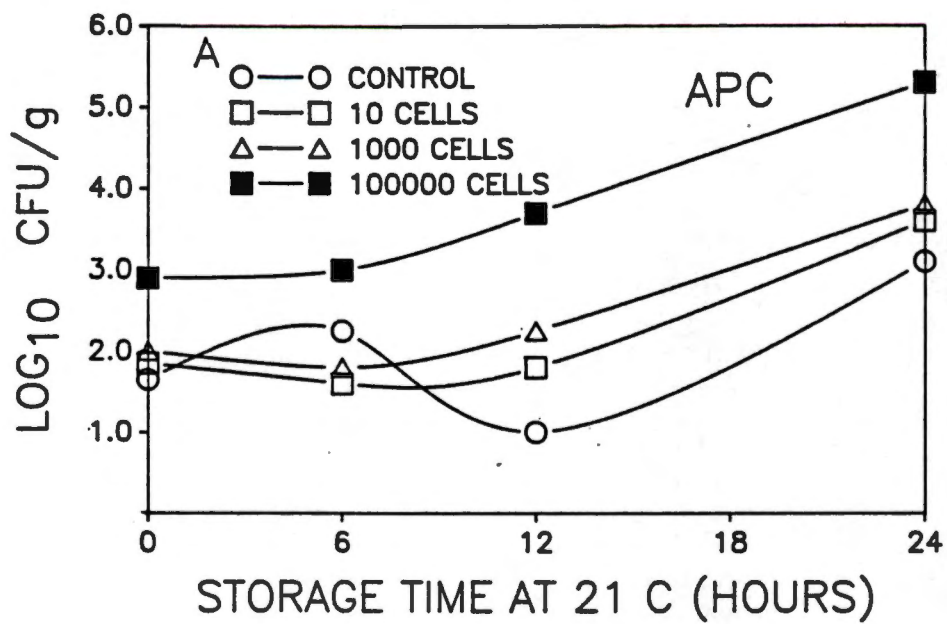


FIGURE 3.

Nuggets inoculated with Campylobacter (Figure 2B) had approximately a 2 log increase in APC's/g over the first 4 days of storage followed by a slight decrease over the final 2 days of storage. Inoculated nuggets had a significantly ($P>0.05$) higher APC/g than nuggets not inoculated with C. jejuni. It is not likely that C. jejuni could grow on the APC, therefore, increases in APC's of the inoculated nuggets may be due to the slight increase in moisture provided by the inoculum. There were no significant differences between brands ($P>0.05$), although numerical differences were observed.

Aerobic plate counts of samples stored at 21C are shown in Figure 3. APC of nuggets stored at 21C increased significantly ($P<0.05$) over time (Figures 3A and 3B). Final APC's of approximately $\log_{10} 5.3$ CFU/g were found for samples inoculated with 1×10^5 salmonellae/g (Figure 3A) and all samples in the Campylobacter study (Figure 3B). Nuggets inoculated with higher levels of Salmonella (1×10^5 /g) also had significantly higher APC/g after 24 hours than uninoculated nuggets. Salmonella grows well on standard methods agar and would be included in the APC (47). Aerobic plate counts of samples inoculated with Campylobacter (Figure 3B) increased significantly ($P<0.05$) over the final 12 hours of storage at 21C. APC of samples inoculated with strain 4086 showed a lag period for 12 hours

FIGURE 4. The effect of frozen storage (-18C) on recovery of Salmonella.

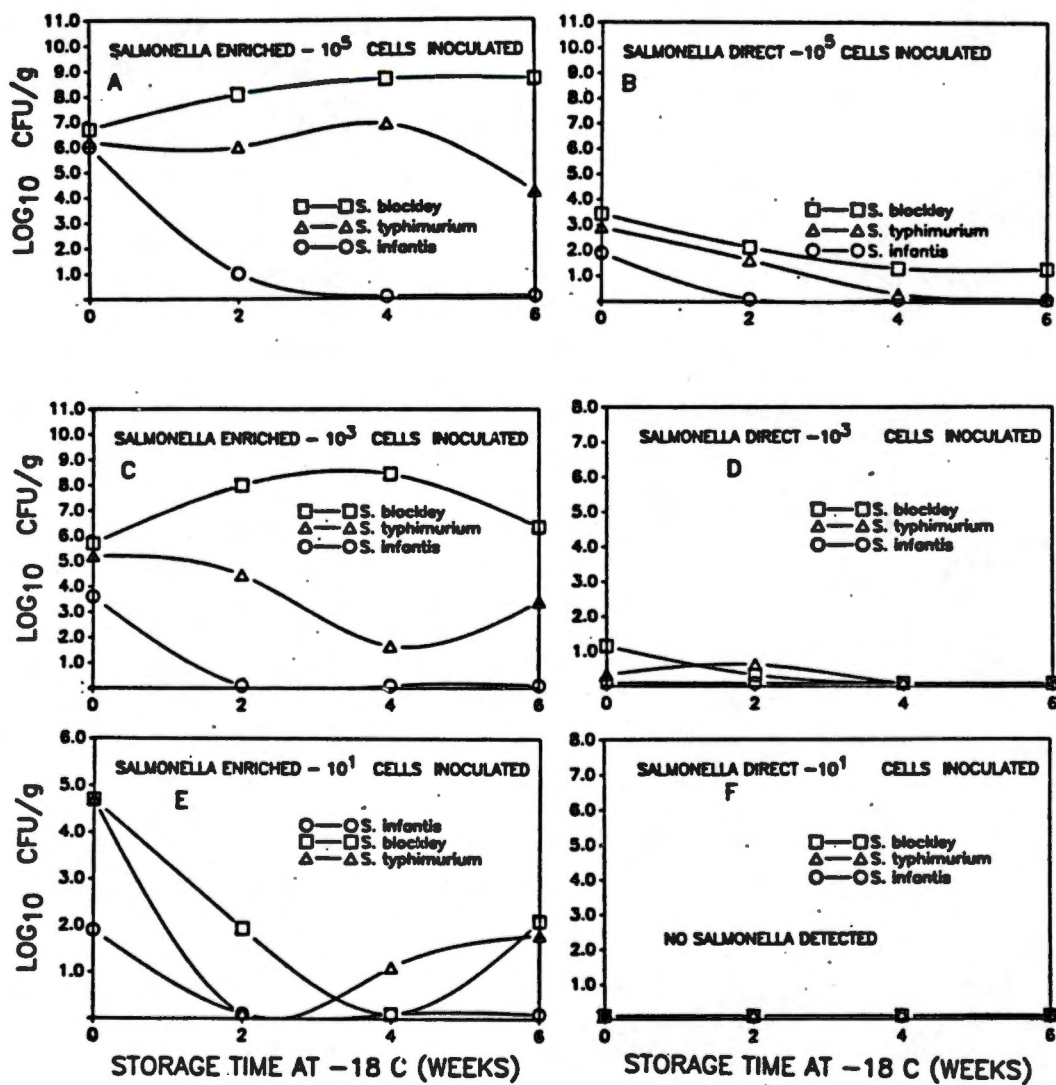


FIGURE 4.

at 21C followed by a geometric increase in growth. APC of nuggets inoculated with C. jejuni increased approximately log 3.5 CFU/g ($P < 0.05$) then decreased by log 3.5 ($P < 0.05$) over the first 12 hours of storage, followed by a logarithmic increase in the last 12 hours of storage at 21C. This increase/decrease in early storage may reflect variable recovery of strain 29428 on the APC. C. jejuni is not an organism which would normally be detected on an APC. There was no significant difference ($P > 0.05$) between brands inoculated with the same strain.

D. THE EFFECT OF STORAGE AT -18C

1. Salmonella

The effect of frozen storage on recovery of Salmonella inoculated into chicken nuggets is shown in Figure 4. Enrichment of samples inoculated with 1×10^5 salmonellae/g provided initial counts of log 6.0 to log 7.0 CFU/g. Enumeration of Salmonella by enrichment gave counts that were significantly greater ($P < 0.05$) than the level inoculated (Figure 4A). In enriched samples inoculated with 1×10^5 S. infantis/g, S. infantis decreased rapidly and was not detected at the 4th week of storage at -18C. A 5.0 log/g decrease in cell numbers was observed during the first 2 weeks of storage with S. infantis. Levels of S. blockley and S. typhimurium remained stable over the first 4 weeks of storage. S. typhimurium experienced a log 3.0 ($P < 0.05$)

reduction between the 4th and 6th weeks of storage at -18C.

S. blockley did not decrease during storage at -18C.

Direct plating of nuggets inoculated with 1×10^5 salmonellae/g gave Salmonella counts of between $\log 2.0$ to $\log 3.5$ CFU/g initially (Figure 4B). This was significantly lower than the $\log 6.0$ to $\log 7.0$ CFU/g recovered initially by enrichment (Figure 4A). Direct S. infantis counts decreased from an initial level of $\log 2.0$ CFU/g to undetectable levels ($P < 0.05$) over the first 2 weeks of storage. S. infantis could not be detected throughout the remainder of the six week storage period. S. typhimurium also decreased to nondetectable levels over the first 4 weeks of storage at -18C. S. typhimurium could not be detected after 6 weeks of storage at -18C by direct plating. S. blockley decreased ($P < 0.05$) from $\log 3.5$ to $\log 2.0$ CFU/g over the six week storage period at -18C. S. infantis was not recovered by either direct plating or enrichment after two weeks at -18C even when inoculated at 1×10^5 CFU/g. Thus, this particular serotype of Salmonella was extremely susceptible to the effects of freezing. S. blockley was recovered at high levels (greater than $\log 7.0$ CFU/g) by enrichment. However, only $\log 2.0$ CFU/g was recovered by direct plating after six weeks. These findings point to the necessity of using enrichment for detection of some Salmonella, even in research using high initial inocula.

3

Enrichment of samples inoculated with 1×10^3 salmonellae/g recovered levels of Salmonella ranging from log 3.5 for S. infantis to log 5.8 for S. blockley (Figure 4C). S. infantis numbers decreased significantly ($P < 0.05$) to undetectable levels over the first 2 weeks of storage at -18C and could not be detected throughout the remainder of the storage period. A log 3.0 CFU/g decrease ($P < 0.05$) in cells was observed for S. typhimurium over the first 4 weeks of storage at -18C. Recovery increased slightly over the final 2 weeks of storage at -18C giving a final count of approximately log 4.0 CFU/g. A significant increase ($P < 0.05$) in cell recovery was observed for S. blockley over the first 4 weeks of storage at -18C. Counts of S. blockley were stable over the six week storage period at -18C. Counts appeared to increase significantly in early storage ($P < 0.05$). However, the increased counts were probably due to recovery variation.

S. blockley and S. typhimurium inoculated into samples at the 1×10^3 salmonellae/g level gradually declined to undetectable levels during the first 4 weeks of storage (Figure 4D). S. infantis could not be detected throughout the storage period.

Enrichment of samples inoculated with 10 salmonellae/g recovered an initial count of Salmonella of log 4.7 CFU/g for both S. blockley and S. typhimurium (Figure 4E).

S. typhimurium decreased ($P < 0.05$) to undetectable levels after 2 weeks. S. blockley decreased to undetectable levels after 4 weeks of storage. A 2.0 log/g increase in recovery was observed for both S. typhimurium and S. blockley for the remainder of the storage period. Since Salmonella cannot grow at -18°C , any increases were due to improved recovery rather than growth. S. infantis gradually decreased to undetectable levels by week two and remained undetectable throughout the remainder of the storage period. An interesting finding was that S. infantis did not increase from 10 cells/g even with enrichment (Figure 4E). Freezing is apparently so injurious to this particular serotype that it cannot recover during selective enrichment. These findings raise two questions: 1. should non-selective enrichment be routinely used for frozen meat samples to detect Salmonella as a first step prior to enrichment, and 2. considering the diversity among the 2000 serotypes of Salmonella (only three of which were evaluated in this study), will the non-selective enrichment media customarily employed for detection of Salmonella in dried foods be sufficient to recover some of the more "fragile" Salmonella?

Direct plating of samples inoculated with 1×10^6 salmonellae/g yielded undetectable levels over time at -18°C for all 3 strains of Salmonella (Figure 4F).

Although Salmonella was able to survive freezing at

-18C, there were variations in survival among strains and levels of inoculum. Freezing was detrimental to Salmonella cells during the first 2 weeks of storage, since the largest decreases in cell numbers occurred in the first two weeks. S. infantis was the most sensitive strain to storage at -18C at all levels of inoculum. S. blockley survived much better than S. typhimurium and S. infantis at -18C over six weeks of storage. These results are in agreement with Farrell and Upton (43) who inoculated bacon with S. typhimurium. They found a significant decrease in S. typhimurium counts over storage at -22C, but that the cells remained viable throughout storage.

2. Campylobacter

Enrichment of chicken nuggets inoculated with 1×10^6 C. jejuni/g yielded significant decreases ($P < 0.05$) in cell numbers over storage (Figure 5A). Initial counts ranged from $\log_{10} 6.3$ for C. jejuni 4086 to $\log_{10} 8.1$ for C. jejuni 29428. Although there were no significant differences between brands inoculated with the same strain, survival of C. jejuni 29428 was significantly greater ($P < 0.05$) than C. jejuni 4086 over storage. Strain variability is common with C. jejuni and has been shown to occur in other food products at -20 and 4C (36,119). Since some strains of C. jejuni are more resistant to cold stress than others, it is important to evaluate at least two strains in studies of this type.

FIGURE 5. The effect of frozen storage (-18C) on recovery of Campylobacter.

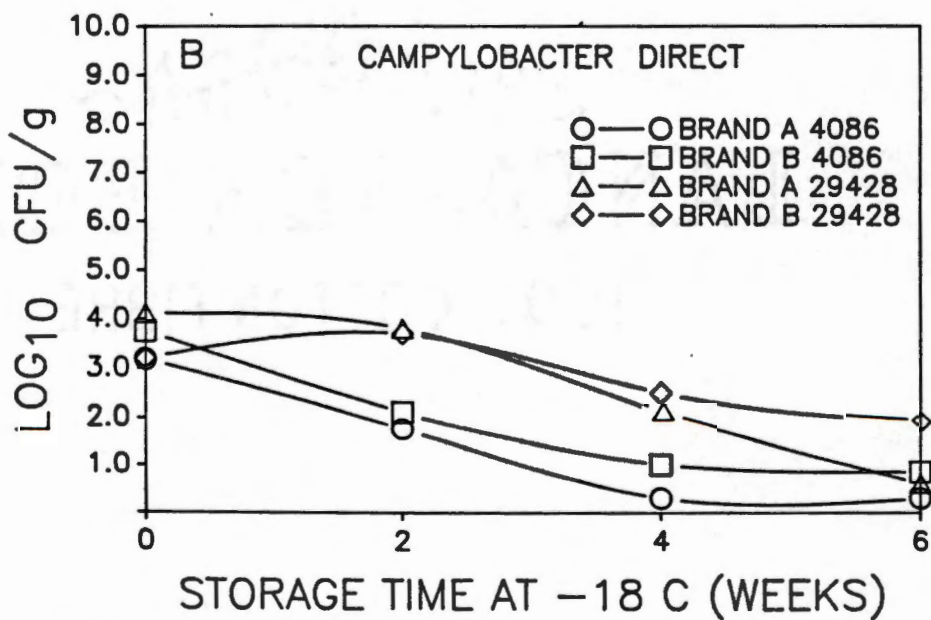
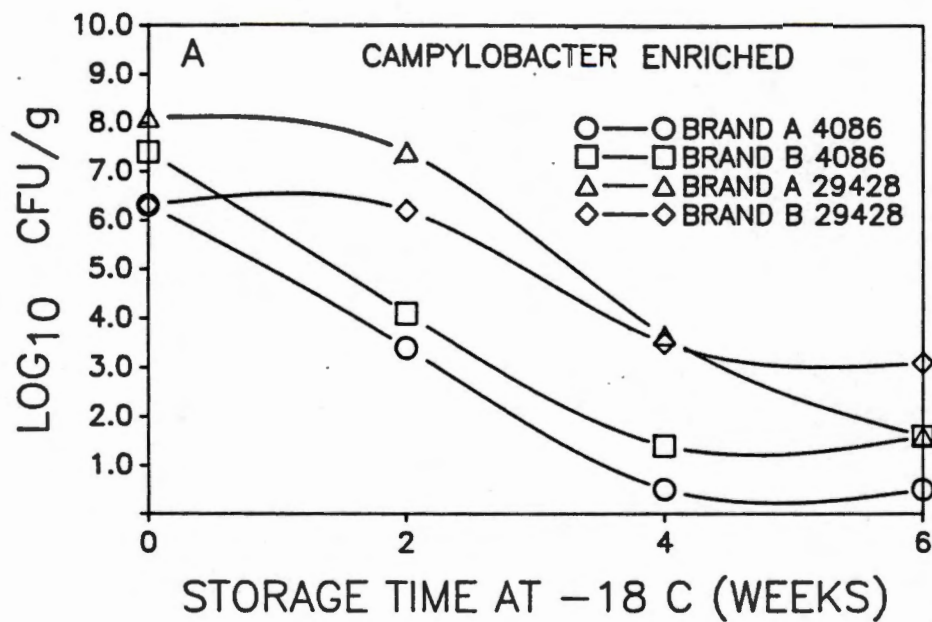


FIGURE 5.

Ideally, a large number of strains should be evaluated. However, this is not usually feasible due to the large number of samples involved.

Direct plating of chicken nuggets inoculated with C. jejuni yielded log 3.0 to log 4.0 initially (Figure 5B). Direct plating recovered levels of C. jejuni which ranged from log 4.0×10^5 to log 5.0×10^5 CFU/g less than the initial counts recovered by enrichment and plating of chicken nuggets (Figure 6B).

These results support previous findings that C. jejuni is sensitive to freezing (119). A significant ($P < 0.05$) loss in viability of cells occurred during the first 4 weeks of storage. The reduction of cell numbers may also be attributed to the NaCl content of the chicken nuggets. Several studies have shown that NaCl concentrations of 2.0% and above are deleterious to C. jejuni survival (38,60). Both brands of chicken nuggets had NaCl concentrations of at least 2.0%.

E. THE EFFECT OF STORAGE AT 4C

1. Salmonella

The effect of refrigerated storage on recovery of Salmonella inoculated into chicken nuggets is shown in Figure 6. Enrichment of samples inoculated with 10^5 salmonellae/g recovered initial counts of log 2.9 to log 8.8 CFU/g. Enumeration of Salmonella by enrichment gave

FIGURE 6. The effect of refrigerated storage (4C) on recovery of Salmonella.

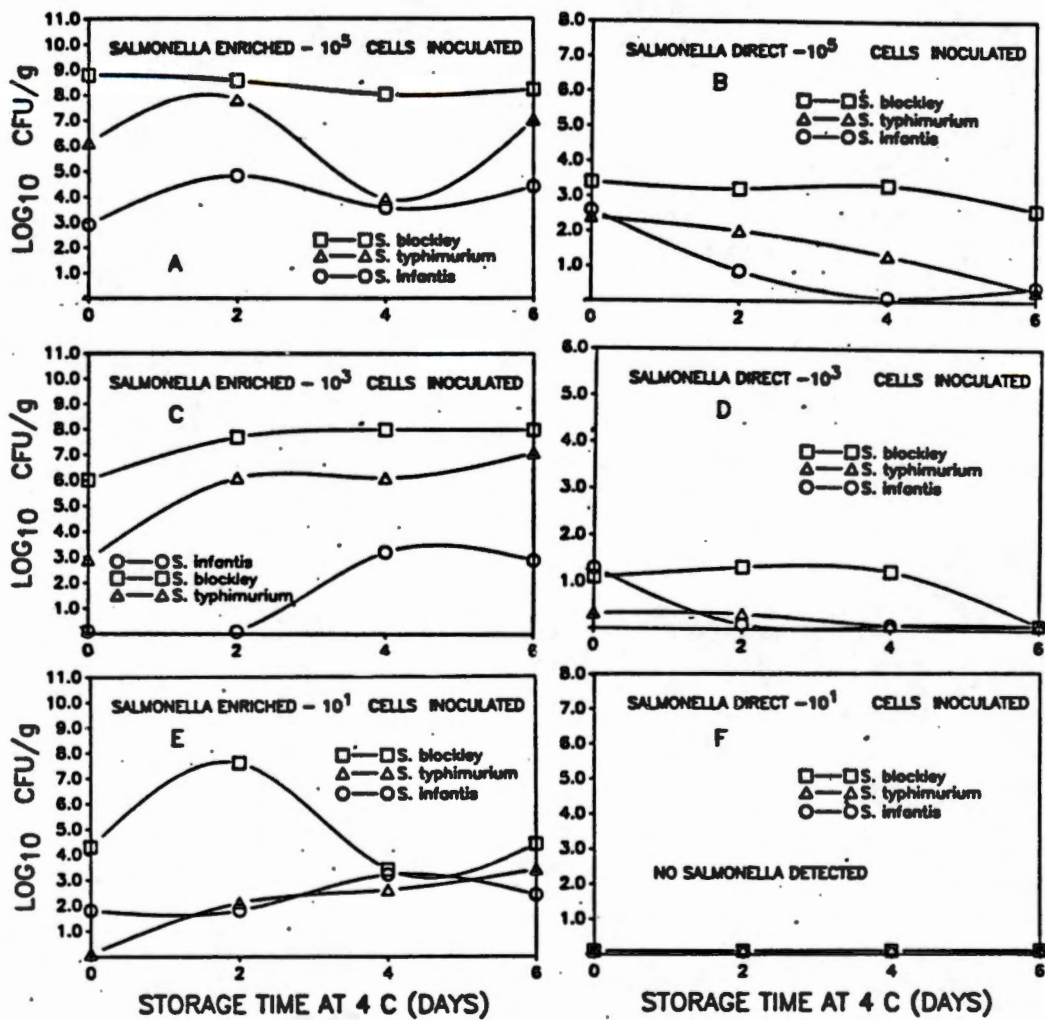


FIGURE 6.

counts that were significantly greater ($P < 0.05$) than the level inoculated (Figure 6A). Recovery of S. blockley and S. typhimurium were significantly greater ($P < 0.05$) initially (day 0) than S. infantis. Recovery of S. infantis increased log 2.0 CFU/g during the first 2 days of storage.

¹⁰
S. infantis levels remained stable over the remainder of the storage period. S. typhimurium recovery also increased by about log 2.0 during the first 2 days of storage, however, a log ¹⁰ 4.0 decrease followed by a log ¹⁰ 3.0 increase was observed over the 4th and 6th days of storage, respectively. These results were probably due to recovery variation since Salmonella does not grow at 4C. Recovery of S. blockley decreased slightly over the six day storage period.

⁵
Direct plating of nuggets inoculated with 1×10^5 salmonella/g yielded initial counts ranging from log 2.4 ¹⁰ for S. typhimurium to log 3.4 for S. blockley (Figure 6B). Both S. infantis and S. typhimurium decreased to ¹⁰ undetectable levels by the 4th and 6th days of storage, respectively. S. blockley decreased slightly over time.

³
Enrichment of samples inoculated with 1×10^3 salmonellae/g recovered levels of Salmonella ranging from undetectable for S. infantis to log 6.0 CFU/g for ¹⁰ S. blockley (Figure 6C). Recovery of all 3 strains of Salmonella increased over time at 4C. Both S. blockley and S. typhimurium were recovered at significantly greater levels ($P < 0.05$) than S. infantis during the first 2 days of

storage and counts continued to be log 3.0 to log 4.0
greater over the remainder of the storage period. These
findings show that variation among strains is possible
during recovery.

Direct plating of nuggets inoculated with 1×10^3
salmonellae/g yielded initial counts ranging from log 0.3
for S. typhimurium to log 1.2 for S. infantis (Figure 6D).
S. typhimurium counts decreased to undetectable levels by
the 4th day of storage at 4C. S. infantis decreased
significantly ($P < 0.05$) to undetectable levels by the second day
of storage at 4C. S. blockley counts were significantly
greater ($P < 0.05$) than both S. typhimurium and S. infantis by
the second day of storage. S. blockley levels remained
constant throughout the first four days of storage. There
was, however, a log 1.2 ($P < 0.05$) decrease to undetectable
levels by the sixth day of storage at 4C.

Following enrichment, nuggets inoculated with 10
salmonellae/g had initial counts ranging from undetectable
levels for S. typhimurium to log 4.1 for S. blockley
(Figure 6E). Recovery of S. typhimurium increased log 3.5
over the six day storage period ($P < 0.05$). S. infantis
recovery increased gradually up to the fourth day of storage
followed by a slight decrease by the sixth day of storage.
Recovery of S. blockley was significantly greater ($P < 0.05$)
during the first 2 days of storage. However, S. blockley

counts returned to initial levels after six days of storage at 4C. No Salmonella were recovered by direct plating of nuggets inoculated with 10 salmonellae/g (Figure 6F).

Enrichment of chicken nuggets inoculated with Salmonella stored at 4C showed a proliferation of Salmonella over the first 2 - 4 days of storage. S. blockley dominated over S. typhimurium and S. infantis in CFU/g at all levels of inoculum. S. infantis was significantly lower ($P < 0.05$) than both S. blockley and S. typhimurium. Farrel and Upton (43) who monitored growth and survival of S. typhimurium in bacon at 5C, found that direct plating of nuggets inoculated with Salmonella showed a decrease in CFU/g over time for all strains at the two highest levels of inoculum. The direct counts are in agreement with results found by Goepfert and Chung (55). Although cell numbers declined over storage for direct plating, it is possible that cells may have been injured during storage since refrigeration is bacteriostatic and not bacteriocidal, and the enrichment procedure was able to detect the injured cells resulting in higher counts.

It is evident from this research that if a product contains 1×10^3 salmonellae/g or more, it is highly probable that although cell numbers decline with storage, Salmonella are able to survive refrigerated storage for at least six days. However, it is unlikely that a fully cooked product such as chicken nuggets would contain salmonellae at

a level above 1×10^3 cells/g unless there were major sanitation problems.

2. Campylobacter

Enrichment of chicken nuggets inoculated with C. jejuni yielded initial counts averaging $\log_{10}$ 8.5 (Figure 7A). Both strains decreased with storage. A significant decrease ($P < 0.05$) of $\log_{10}$ 2.5 was observed for both strains in Brand B chicken nuggets with time. After 6 days at 4C, strains inoculated into Brand A chicken nuggets had higher CFU/g than did strains inoculated into Brand B chicken nuggets possibly due to the higher salt content of the Brand B nuggets.

Direct plating of chicken nuggets inoculated with C. jejuni yielded initial counts averaging $\log_{10}$ 5.5 (Figure 7B). No significant difference ($P > 0.05$) was observed between brands inoculated with the same strain. Both strains decreased with storage at 4C. However, a significant decrease ($P < 0.05$) of $\log_{10}$ 3.5 was observed for strain 29428 inoculated into the Brand B chicken nugget. After 6 days, C. jejuni 4086 (Brand B) was significantly greater ($P < 0.05$) than C. jejuni 29428 (Brand B).

Survival of Campylobacter at 4C has been reported in previous studies (26,37). It appears from the number of campylobacters that were recovered over time that both strains of C. jejuni were able to survive in the presence of

FIGURE 7. The effect of refrigerated storage (4C) on recovery of Campylobacter.

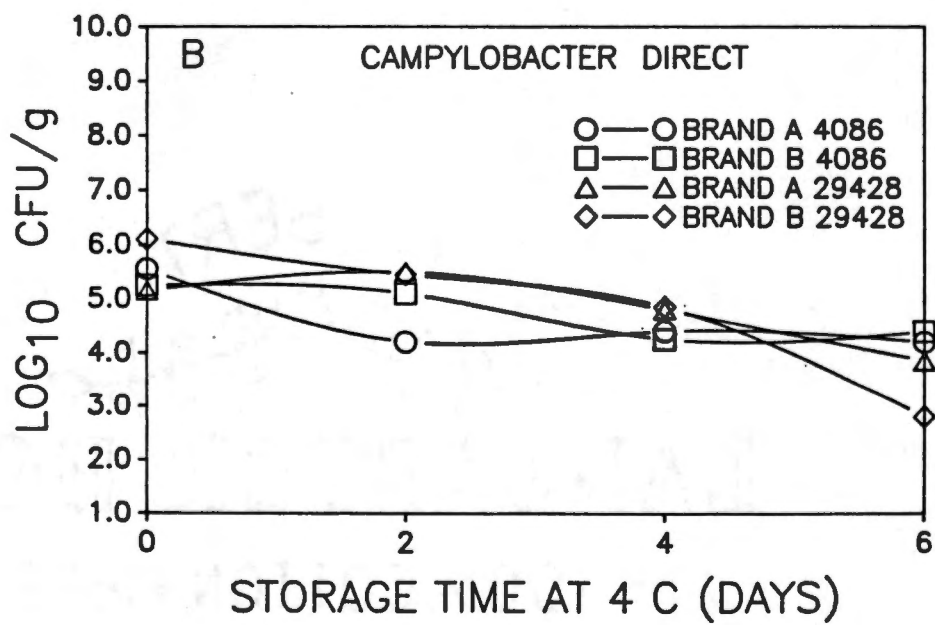
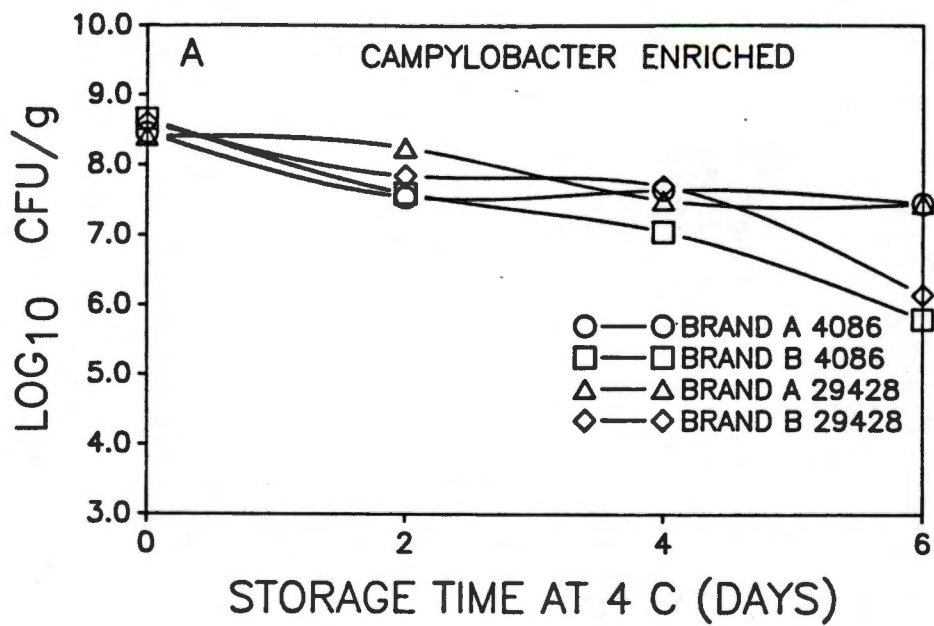


FIGURE 7.

indigenous microflora of the chicken nuggets. Therefore, it is possible to assume that contamination of chicken nuggets with large numbers of C. jejuni stored at 4C may be a potential health hazard. However, proper cooking methods should be sufficient in destroying all campylobacters.

F. THE EFFECT OF STORAGE AT 21C

1. Salmonella

The effect of temperature abuse (21C) on recovery of Salmonella inoculated into chicken nuggets is shown in Figure 8. Enrichment of nuggets inoculated with 1×10^5 salmonellae/g showed strain variation (Figure 8A) Both S. blockley and S. typhimurium were significantly greater ($P < 0.05$) than S. infantis throughout the storage period. Counts of all three strains of Salmonella did not change significantly over 24 hours of storage as detected through enrichment. These data indicate that Salmonella did not compete with aerobic spoilage microflora in nuggets.

Direct plating of nuggets inoculated with 1×10^5 showed that salmonellae recoveries increased significantly ($P < 0.05$) over time (Figure 8B). S. blockley counts at time 24 hours were $\log_{10} 1.5$ ($P < 0.05$) and $\log_{10} 2.5$ ($P < 0.05$) greater than S. typhimurium and S. infantis, respectively. S. typhimurium was $\log_{10} 1.5$ ($P < 0.05$) greater than S. infantis after 24 hours of storage. However, direct counts showed that Salmonella did not ever increase above the

FIGURE 8. The effect of temperature abuse (21C) on recovery of Salmonella.

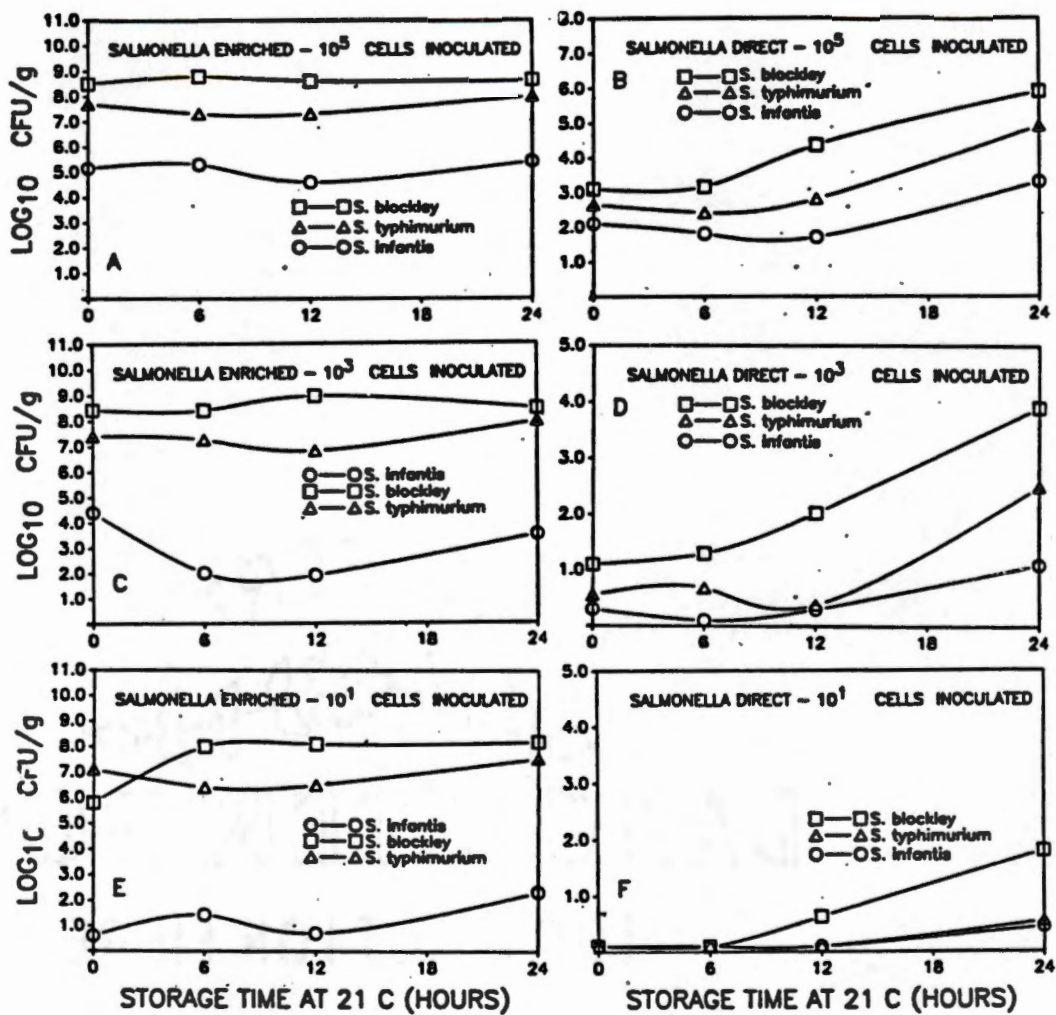


FIGURE 8.

initial ⁵10⁵ inoculum, except for S. blockley (log10⁵5.8) during 24 hours of storage at 21C. Since nuggets are a highly nutritious environment for Salmonella, these data suggest that there was initial injury to Salmonella, followed by repair and recovery.

Enrichment of nuggets inoculated with 1 x 10³ salmonellae/g showed strain variation over storage (Figure 8C). Both S. blockley and S. typhimurium had counts which were significantly greater (P<0.05) than S. infantis throughout the storage period. S. infantis had an initial count of log ¹⁰4.3 which decreased log ¹⁰2.3 (P<0.05) within the first 6 hours of storage. An increase of log ¹⁰1.5 (P<0.05) was observed during the final 12 hours of storage. Recovery of S. blockley increased slightly over the first 12 hours of storage and S. typhimurium decreased slightly over the first 12 hours of storage. However, there was not an increase in counts in any of the strains during storage.

Direct plating of nuggets inoculated with 1 x 10³ salmonellae/g showed an increase in cell numbers over time for all 3 strains (Figure 8D). Both S. blockley and typhimurium had significantly higher (P<0.05) counts than S. infantis after 24 hours of storage. As discussed above, the increased numbers observed over 24 hours at 21C by direct plating appear to represent increased recovery rather than growth, since counts did not change in enriched samples

during storage and final counts were not over 10^3 except with S. typhimurium.

Enrichment of nuggets inoculated with 10^3 salmonellae/g had initial counts ranging from log 1.0 for S. infantis to log 7.0^{10} for S. typhimurium (Figure 8E). Recovery of S. infantis was significantly less ($P < 0.05$) than both S. blockley and S. typhimurium over time. S. infantis recovery increased log 1.5 ($P < 0.05$) over a 24 hour storage period at 21C. Recovery of S. typhimurium remained stable at 21C over the 24 hour storage period. Recovery of S. blockley increased from log 5.8^{10} to log 8.0^{10} ($P < 0.05$) in the first 6 hours of storage and remained constant throughout the storage period.

Direct plating of nuggets inoculated with 10^3 salmonellae/g yielded undetectable levels for S. blockley up to 6 hours of storage and 12 hours of storage for both S. infantis and S. typhimurium (Figure 8F). S. blockley increased log 1.8 ($P < 0.05$) over the final 18 hours of storage. S. blockley recovery was significantly greater ($P < 0.05$) than recovery of S. infantis and S. typhimurium after 24 hours of storage at 21C.

Recovery of Salmonella increased significantly ($P < 0.05$) at all levels of inoculum and all strains at 21C (Figures 8B, 8D, 8F). The most significant increase was observed during the final 18 hours of storage. However, actual

increases in cell numbers were only observed for the 10^1 level of inoculum. The moisture content of the chicken nuggets provides an excellent environment for growth of bacteria at 21C. These results are similar to those of Goepfert and Chung (55). They studied luncheon meats inoculated with Salmonella at 23C and found that Salmonella were able to compete with the natural microflora of luncheon meat. Salmonellae inoculated onto luncheon meat did not grow significantly during the first 24 hours of storage at 23C. However, a 25 - 30 fold increase was observed during the second 24 hours at 23C.

2. Campylobacter

Enrichment of nuggets inoculated with C. jejuni yielded initial counts averaging $\log 8.6$ (Figure 9A). There was a significant reduction ($P < 0.05$) in 10^1 CFU/g over time. Brand B chicken nuggets inoculated with strain 4086 exhibited significantly lower ($P < 0.05$) C. jejuni counts than other treatments at 21C. Nuggets inoculated with strain 29428 had higher CFU/g than nuggets inoculated with strain 4086 after 24 hours at 21C.

Direct plating of nuggets inoculated with C. jejuni showed a significant decrease ($P < 0.05$) in CFU/g over time (Figure 9B). There was no significant difference between nuggets inoculated with the same strain. Nuggets inoculated

FIGURE 9. The effect of temperature abuse (21C) on recovery of Campylobacter.

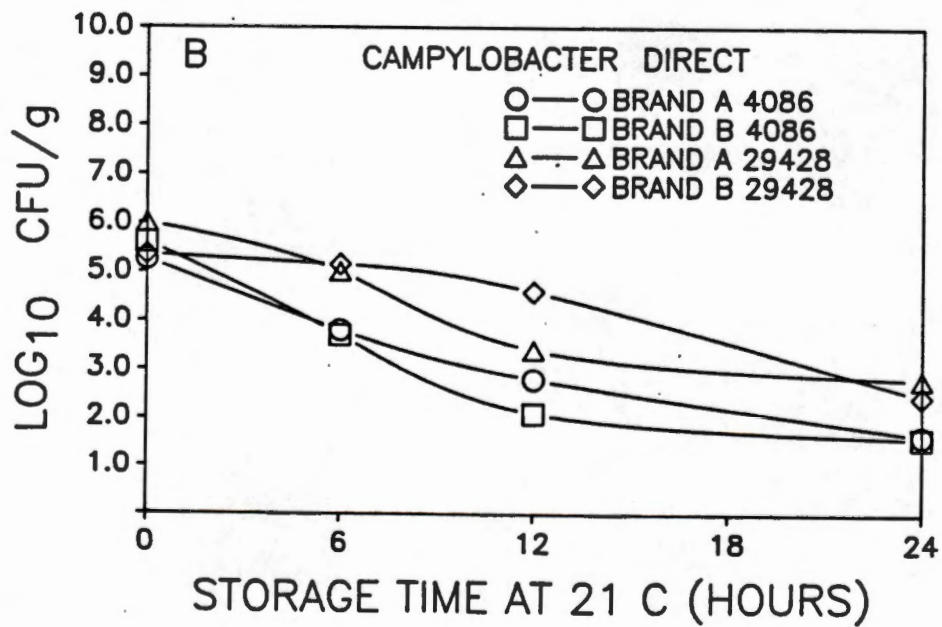
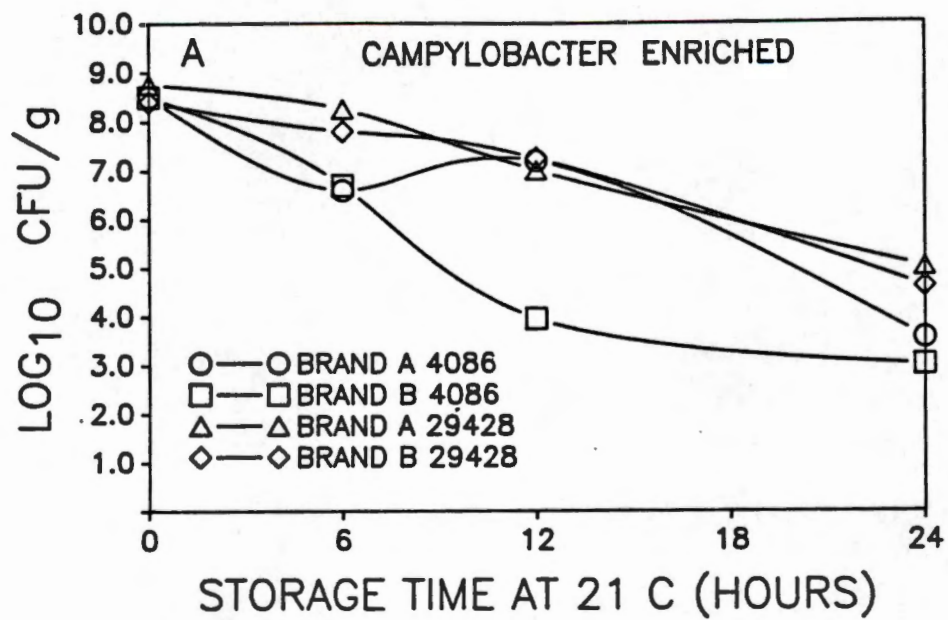


FIGURE 9.

with strain 29428 had higher CFU/g than nuggets inoculated with strain 4086.

Previous studies have reported that C. jejuni does not survive well in food at temperatures above 10C (13,26). Christopher et al. (26) inoculated beef cubes with 5.6×10^5 cells/g and held them at 20C for 72 hours. C. jejuni strain 29428 decreased from $\log_{10} 6.0$ at time 0 to $\log_{10} 3.3$ after 24 hours of storage. The results of the present study are very similar to Christopher et al., except that a $\log_{10} 4.0$ decrease was observed over a 24 hour storage period. Chicken nuggets consist of comminuted poultry meat. Comminution increases the microbial load of the chicken nuggets. Rapid increases in APC were apparent in our study (Figure 3B). Campylobacter was not able to compete with the natural microflora of the chicken nuggets at 21C. The greater than 2.0% NaCl content of the nuggets and the fact that Campylobacter has a temperature optimum of 42C support this conclusion. Data from the Campylobacter study show that abuse of nuggets does not encourage growth of C. jejuni in nuggets.

CHAPTER V

CONCLUSION

Salmonella and Campylobacter were able to survive storage at -18C, 4C, and 21C. S. blockley and C. jejuni 29428 were able to withstand these storage conditions better than S. typhimurium, S. infantis, and C. jejuni 4086. S. infantis was very sensitive to freezing (-18C) conditions and refrigerated (4C) storage.

Chicken nuggets are a fully cooked product which are usually stored frozen until they are heated by the consumer. It is very unlikely that if either Salmonella or Campylobacter were present on poultry prior to the production of chicken nuggets, that they would be able to survive the heat treatments involved in the processing of these products. However, since poultry is the main ingredient of this product and Salmonella and Campylobacter are common inhabitants of poultry, the possibility of post - processing contamination should not be ruled out.

Mishandling of the product by the retailer or consumer must also be considered. Chicken nuggets are stored either in open freezers or enclosed freezers. Both of these kinds of freezers can experience extreme temperature fluctuations which could have a serious effect on potential pathogens in frozen poultry products such as chicken nuggets. Also, retailers tend to leave frozen products in unfrozen

environments while re-shelving products. This allows the product to undergo a freeze - thaw cycle which could also be a potential health hazard. Consumers tend to ignore manufacturer's instructions and may decide to store chicken nuggets in the refrigerator prior to use or perhaps the product was left at room temperature by mistake for several hours and then placed in the freezer. These too are potential health hazards, especially if pathogens are present.

In conclusion, Salmonella and C. jejuni do not appear to be potential health hazards in chicken nuggets. However, it is advised that this product be fully re-heated prior to consumption to destroy any potential pathogens that may exist.

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LITERATURE CITED

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APPENDIXES

LAKECASTER FOND
100% COTTON 1892
C/BERD

APPENDIX A

MEDIA FORMULATION

MODIFIED CARY BLAIR TRANSPORT MEDIA

Sodium thioglycollate	1.5g
Disodium phosphate	1.1g
Sodium chloride	5.0g
Agar	1.0g
Distilled water	991 ml
Calcium chloride (1% solution)	9 ml

APPENDIX B

STATISTICAL DATA

TABLE 3. Analysis of variance of Salmonella at -18C

Source	df	MS	F-value
Salmonella (S)	3	3.88	26.35
Time (T)	3	5.56	37.68
Inoculum (I)	2	19.51	132.20
S * T	9	0.45	3.06
S * T * I	22	1.59	10.80
Error	120	0.15	

P<0.0001

TABLE 4. Analysis of variance of Campylobacter at -18C

Source	df	MS	F-value
Campylobacter (C)	2	57.25	11.13 ***
Time (T)	3	14.86	2.89 *
Brand (B)	1	1.02	0.20
C * B	2	0.62	0.12
T * B	3	0.41	0.08
C * T * B	12	2.73	0.53
Error	72	5.14	

P<0.0001

*P<0.05 but >0.01

TABLE 5. Analysis of variance of Salmonella at 4C

Source	df	MS	F-value
Salmonella (S)	3	10.41	59.46
Time (T)	3	3.99	23.56
Inoculum (I)	2	41.14	242.63
S * T	9	0.78	4.65
S * T * I	22	1.68	9.95
Error	120	0.17	

P<0.0001

TABLE 6. Analysis of variance of Campylobacter at 4C

Source	df	MS	F-value
Campylobacter (C)	2	114.93	225.09
Time (T)	3	2.82	5.53
Brand (B)	1	0.02	0.05
C * B	2	0.04	0.08
T * B	3	0.20	0.39
C * T * B	12	0.84	1.66
Error	24	0.51	

P<0.0001

*

P<0.05 but >0.01

TABLE 7. Analysis of variance of Salmonella at 21C

Source	df	MS	F-value
Salmonella (S)	3	24.51	62.11
Time (T)	3	19.18	48.60
Inoculum (I)	2	100.25	254.04
S * T	9	1.91	4.85
S * T * I	22	0.91	2.33
Error	120	47.36	

P<0.0001

TABLE 8. Analysis of variance of Campylobacter at 21C

Source	df	MS	F-value
Campylobacter (C)	2	78.13	47.38
Time (T)	3	11.68	7.08
Brand (B)	1	0.00	0.00
C * B	2	0.05	0.03
T * B	3	0.05	0.03
C * T * B	12	1.82	1.11
Error	24	9.28	

P<0.0001

*

P<0.05 but >0.01

VITA

Martha Mary Walline was born on July 25, 1964 in Boston, Massachusetts to James and Marjorie Walline. After graduating from Williamsville South High School, Williamsville, New York in June, 1982, she entered Canisius College. In September, 1983, the author transferred to The University of Tennessee, Knoxville and majored in Animal Science. A Bachelor's Degree in Agriculture was awarded in June, 1986. In September 1986, the author was given the opportunity to pursue a Master of Science degree in Food Technology and Science. This degree was awarded in December 1988.

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