

Microbial Properties of Color-Modified Turkey

by

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ABSTRACT

Studies were performed to determine the effect of color modification procedures on the microbial characteristics of turkey thigh meat. Turkey thighs were flaked and then color-modified successively with three sodium phosphate buffers (pH 5.8, 7.4, and 8.0). At selected time intervals, flaked unwashed turkey (FUT; control) and color-modified turkey (CMT) stored at 3°C were analyzed for aerobic, psychrotrophic, and coliform bacterial counts. Aerobic and psychrotrophic numbers also were estimated in raw tissues held at -20°C. Cooked FUT and CMT were inoculated with two-strain composites of either Salmonella or Listeria monocytogenes and held at 4 and 20°C. Salmonella enteritidis, Salmonella typhimurium, L. monocytogenes Scott A, and a L. monocytogenes meat isolate were organisms used in the inoculation studies. Aerobic and psychrotrophic counts were not different ($p > 0.05$) at any sampling interval when numbers in raw FUT were compared to those in raw CMT. Coliform counts in raw FUT did not differ from those in raw CMT ($p > 0.05$) after 1 day at 3°C. In inoculation studies,

numbers of either pathogen generally did not differ ($p > 0.05$) between cooked FUT and CMT at selected sampling intervals. Salmonella counts declined gradually in cooked samples held at 4°C. By day 3, Salmonella levels increased more than 6 logs in tissues held at 20°C. L. monocytogenes counts increased approximately 5 logs in cooked FUT and CMT held at 4°C for 14 days. Numbers increased more than 5 logs in samples stored at 20°C for 48 hr. Although a significant ($p < 0.05$) factor in some studies, color modification was not considered to have a major effect on microbial growth in altered thigh.

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1.0 INTRODUCTION

1.1 Background

Greater consumer demand for nutritional and flavorful poultry meat products has led to the development of methods to increase utilization of muscle tissue raw materials. Such methods include the washing of meat with various extraction media to enhance its color and functional properties. This procedure provides processors with a technique for altering dark meat raw materials which then may be used to formulate intermediate value, further processed poultry products.

Washing with water to remove chromoproteins and other components from mammalian or fish muscle tissue has been reported by Fleming et al., 1960; Richansrud and Henrickson, 1967; Corning and Fronish, 1974; Suzuki, 1981; and Adu et al., 1983. Other investigators have employed buffers or chemical mixtures to extract pigments from meat (DeDuve, 1948; Hornsey, 1956; Fleming et al., 1960; Warris, 1979). These methods have provided the basis for poultry washing techniques developed by Ball and Montejano (1984), Hernandez et al. (1986), Dawson et al. (1988), and Elkalifa et al. (1988).

Washed muscle tissue has been shown to contain lower concentrations of water-soluble compounds and fat than corresponding unaltered tissues (Ball and Montejano, 1984; Adu et al. 1983; Hernandez et al., 1986; Dawson et al. 1988;

Elkalifa et al., 1988; Phelps et al., 1990). Reductions in certain minerals, vitamins, proteins, and/or lipids could potentially have a limiting effect on the growth of some microorganisms. Furthermore, washing may dislodge bacteria from meat surfaces and therefore lower microbial numbers in extracted tissues (Licciardello and Hill, 1978; Dickson, 1988). On the other hand, enhanced microbial proliferation could occur due to increased availability of some growth factors brought about by the extraction of muscle tissues.

Information exists on the microbial characteristics of washed, minced fish (surimi) and the restructured products formulated from this raw material. Licciardello and Hill (1978) compared the microbial quality of surimi with that of unwashed minced fish. These workers found that surimi contained lower aerobic and coliform numbers than did minced fish. Lower bacterial counts in surimi were attributable to the reduction of the initial microbial load by washing.

Yoon et al. (1988) performed shelf-life studies on crabmeat analogs (heat-processed, surimi-based products). These researchers reported a greater than 6 log increase in the aerobic bacterial counts of flaked crab after 2-weeks storage at 5°C. Heat processing of surimi and the addition of antidenaturants (i.e., sucrose) to this raw material probably influenced the growth of bacteria in crabmeat analogs.

In a separate study, the fate of pathogenic bacteria in imitation crab was observed (Yoon and Matches, 1988).

Aeromonas, Salmonella, and Staphylococcus numbers were found to increase at 10 and 15°C, but not at 0 and 5°C. Only Yersinia was able to grow at all four temperatures.

The effects of new technologies on the microbial safety and quality of novel food products should be determined. To date, no results have been published on the microbiology of washed poultry. Therefore, the microbial characteristics of color-modified turkey (CMT), a washed poultry raw material, were evaluated in this study.

1.2 Objectives

The objectives of this research were to:

- (1) assess the microbial quality of CMT by estimating aerobic and psychrotrophic bacterial counts present during refrigerated and frozen storage,
- (2) indicate the hygienic level of the color modification procedure by monitoring the concentrations of coliform bacteria,
- (3) determine the potential of CMT as a growth medium for pathogenic bacteria following post-processing contamination.

2.0 REVIEW OF LITERATURE

2.1 Description of CMT

Color-modified turkey (CMT) is a washed raw material prepared from flaked thigh meat. It is lighter in color, lower in fat, and higher in moisture than unwashed thigh tissue (Elkalifa et al., 1988; Phelps et al., 1990).

2.2 Washing of Muscle Tissue

Procedures to remove water-soluble components and/or fat from muscle tissues have been researched in red meat, poultry, and seafood. However, washing techniques have reached commercial utilization only in the seafood industry. Surimi, a washed fish mince, was commercialized as a seafood raw material in the early 1960's.

Before its wide use as a white fish source for surimi, Alaskan pollack was harvested solely for its roe (Suzuki, 1981). The flesh of the fish underwent rapid freeze denaturation and therefore was not employed as a food material. This large waste of a potential food source prompted scientists to search for ways to impede the rapid loss of acceptability due to freezing. A technology was developed where minced fish was water-washed, treated with an antidenaturant, and frozen in a block form. The removal of blood, fat, pigments, and odorous compounds led to a more organoleptically stable product with a moisture content of 77

to 80% (Suzuki, 1981). In addition, the color and texture of the minced fish has been shown to be improved by the washing process (Miyachi et al., 1973).

Evaluating surimi prepared from rockfish, Adu et al. (1983) found that washing reduced ash and lipid concentrations in extracted fish by 80 and 65%, respectively. In addition, these workers observed that most of the sarcoplasmic protein fraction was removed by extraction. There was no difference, however, in the amino acid content of washed fish as compared to that of unwashed fish.

A number of workers have applied washing procedures to red meats, primarily for the purpose of pigment isolation and quantitation. Since the quantities and reactivities of both myoglobin and hemoglobin are essentially responsible for the color of meat (Fox, 1966), most methods have concentrated on the extraction of these heme pigments.

Previous investigators have used water as an extraction medium for mammalian muscle tissue. Fleming et al. (1960) used cold distilled water to wash ground beef muscle and subsequently determine the concentrations of hemoglobin and myoglobin. Cornish and Froning (1974) refroze and thawed water-washed, blended ground meat slurries to remove sarcoplasmic proteins. Water also was used by Richansrud and Henrickson (1967) to extract pigments from bovine muscle.

Researchers attempted to improve chromoprotein extraction methods by using buffers or chemical mixtures. DeDuve (1948) reported that a 0.01 N acetate buffer solution (pH 4.5) removed 70 to 95% of the heme pigments from human muscle. Hornsey (1956) used a 4:1 acetone to water mixture to remove nitroso-heme proteins from cooked cured pork. Following the work of DeDuve (1948), Fleming et al. (1960) incorporated an acetate buffer (pH 4.5) in his extraction procedures to clear centrifugation slurries. Warris (1979) tested different extraction media and found acidified 80% acetone and 0.04 M phosphate buffer (pH 6.8) to be the most effective mixture for extracting hemoglobin and myoglobin from fresh meat.

A number of workers have modified previous washing methodologies to remove tissue components from poultry. Incorporating a surimi-type process, Ball and Montejano (1984) tested three different washing media - tap water, sodium bicarbonate (pH 8.45) and sodium acetate (pH 5.25) - for their effectiveness in removing pigments from deboned thigh meat of broilers. Using a 4:1 ratio of washing medium to product, extraction procedures reduced pigment contents by 73 to 88%. The washed thigh meat gained moisture (2 to 4%) leading to less fat and protein. Niacin and thiamine concentrations were decreased by the washes, resulting in reductions which ranged from 37 to 68% and 32 to 50% in these

vitamins, respectively. These authors concluded that washing was somewhat selective in removing muscle compounds.

Phosphate buffers (0.04 M) of pH 6.4, 6.8, 7.2, or 8.0 were analyzed for their ability to extract pigments and improve the textural properties of mechanically deboned turkey meat (MDTM) (Hernandez et al., 1986). The phosphate buffer of pH 8.0 was found to be the best extracting medium. Washing procedures reduced protein (from 16 to 11%) and fat (from 8 to 4%) and increased moisture (72 to 85%). These workers indicated that the loss of protein was probably due to the removal of water-soluble proteins. Because fat has a lower density than water, fat was removed by floating off during the extraction process.

Dawson et al. (1988) tested the effectiveness of three washing solutions - tap water (pH 6.8), 0.5% sodium bicarbonate solution (pH 8.5), and 0.1% acetate buffer (pH 5.1) - in reducing color and fat in mechanically deboned chicken meat (MDCM). All extraction procedures resulted in greater moisture and lower fat than that observed in unaltered MDCM. The moisture content of washed MDCM was shown to decrease as the pH of the extraction media and the final product decreased.

Two washing media were used by Elkalifa et al. (1988) to color modify turkey dark meat by pigment extraction. After

reducing thigh meat to flakes, either a series of 0.03 M potassium phosphate buffers (pH 5.8, 7.4, and 8.0) or a 0.02 M sodium acetate buffer (pH 5.2) and 0.03 potassium phosphate buffer (pH 7.4) mixture was used to wash flaked thigh tissue. Proximate analysis revealed that both extracting media were effective in reducing ($p < 0.05$) fat and protein and increasing ($p < 0.05$) moisture in treated tissues. Protein was reduced from 18.5% to 11.7% using the phosphate wash and to 11.0% using the acetate/phosphate wash. From an initial content of 5.6%, fat was decreased to 2.4% by the phosphate wash and to 2.2% by the acetate/phosphate treatment. Reasons indicated by these researchers for the reduction of protein and fat portions by washing were similar to those proposed by Hernandez et al. (1986).

2.3 Potential Influence of Washing Procedures on the Microbiology of Poultry Meat

Although studies have been performed to ascertain the color, compositional, and functional properties of washed poultry tissue, no information exists concerning its microbial properties. Nevertheless, some data are available on the microbial characteristics of surimi and surimi-based products (Licciardello and Hill, 1978; Yoon and Matches, 1988; Yoon et al., 1988).

The washing of muscle tissues drastically alters the physical and chemical characteristics of the final product. Removal of water-soluble components and fat may affect the microbial flora that ultimately predominates by favoring the growth of organisms with diverse metabolic abilities. On the other hand, extraction procedures could potentially increase the bioavailability of some nutrients by leaching metabolites or by altering tissue structure. This condition could enhance microbial growth.

Many microbes have the capability of growing on a vast variety of substrates; thus, it would be expected that these organisms would not be significantly influenced by a reduction in certain nutrients. Pseudomonas, a common psychrotroph, may grow on a variety of media because of its lipolytic and proteolytic abilities (Fung, 1987). However, some microorganisms are more restrictive in their growth requirements than others. The lactic acid bacteria require preformed amino acids, B vitamins, and purine and pyrimidine bases for growth (Gottschalk, 1986). Generally, pathogens are more nutritionally fastidious than non-pathogenic organisms.

The washing procedures performed on muscle tissues may remove organisms from meat surfaces. Immersion and washing treatments have been shown to be effective in removing bacteria from meat surfaces (Thomas and McMeekin, 1981;

Morrison and Fleet, 1985; Dickson, 1988). Conversely, some bacteria have demonstrated attachment mechanisms and may have a greater tendency to remain on tissues despite washing (Thomas and McMeekin, 1981; Farber and Idziak, 1984; Dickson and Koohmaraie, 1989).

2.4 Microbiology of Poultry and Poultry Products

2.41 Poultry Meat as a Microbial Growth Medium

Water, a source of energy, nitrogen, vitamins, and minerals must be present for microorganisms which are important in foods to grow and maintain physiological processes (Jay, 1986a). Because of its composition, poultry tissue is an excellent growth substrate for a variety of microorganisms. Poultry is rich in protein, contains a substantial amount of fat, and a variety of vitamins and minerals which microbes may utilize. The water activity of poultry meat is about .98 to .99, thus it is ideal for most microorganisms (Bryan, 1980). Furthermore, the muscle foods are highly buffered due to their protein content, therefore resisting pH changes more effectively than other foods such as vegetables (Jay, 1986a). Most microorganisms grow optimally at pH values around 7.0. The pH of breast meat is between 5.7 to 5.9, whereas that of dark muscle is between 6.4 to 6.7 (Bryan, 1980).

2.42 Microbiology of Carcasses and Intact Parts

Soil, litter, dust, feces and air contain microorganisms which may be transmitted to poultry flocks while on the farm. Incoming birds are the primary source of microorganisms found on poultry carcasses in processing plants. Genera of bacteria commonly associated with the feathers, feet, and bodies of birds are Acinetobacter, Moraxella, Pseudomonas, Corynebacterium, Micrococcus, Staphylococcus, and Flavobacterium (Barnes, 1960 and 1975).

Once in the processing plant, contaminated carcasses may contribute to the dissemination of certain microbes throughout the plant. Carcasses previously free of given genera or types of bacteria may be contaminated with "new" bacteria through cross-contamination with equipment or other carcasses carrying these microbes. Equipment surfaces of chutes, tables, knives, scissors, and conveyers are contamination points which may transfer microorganisms to processed birds. Most undesirable is the spread of pathogenic bacteria from relatively few birds to those previously free of these organisms.

Processes such as scalding, picking, washing, eviscerating, and chilling may serve to disseminate microbes from carcass to carcass. In some cases, scalding, washing, and chilling have reduced the bacterial load on surfaces of birds (Gardner and Golan, 1976). Evisceration creates an

avenue by which intestinal microorganisms can invade processed poultry. Bailey et al. (1987) cited several authors when reporting that members of the genera Lactobacillus, Corynebacterium, Escherichia, Streptococcus, and Clostridium were commonly found in the intestinal tracts of healthy birds.

Bryan (1980) stated that factors which influenced bacterial growth on eviscerated carcasses are the (a) numbers and types of psychrotrophic spoilage organisms present immediately after processing, (b) storage time and temperature, (c) type of tissue (skin or muscle), (d) pH, (e) redox potential, (f) type of packaging, (g) and the presence or absence of carbon dioxide.

May et al. (1962) reported that the primary region of bacterial contamination is the skin of freshly processed carcasses, with substantially lower levels of bacteria being found on muscle. May (1962) found that the uncut surface of chicken broilers purchased from processing plants and retail stores had mean counts of 3.32 and 3.18 log CFU/cm², respectively. Counts increased approximately sixfold in plants and eightfold in stores during cutting and packaging of broiler parts. Increased numbers were attributable to contamination on work surfaces, with some probably coming from manual handling.

Denton and Gardner (1981) observed a total mesophilic count of 2.04 log CFU/cm² on the skin surface of incoming carcasses in a turkey further-processing plant. Mean mesophilic counts for breast and thigh portions prepared from these carcasses were 2.66 and 2.84 log CFU/cm², respectively.

Generally, deep muscle tissue of poultry is free of microbes, or contains relatively few organisms, before processing (Baran et al., 1973). However, as storage time progresses, bacteria located on the surfaces of intact tissue can infiltrate deeper muscle. Gill and Penny (1977) demonstrated that proteolytic organisms can disrupt muscle structure to allow penetration of microbes into deep tissue. These workers claimed that the necessary proteolytic enzymes are not produced until the spoilage flora reaches maximum cell density.

2.43 Microbiology of Further Processing

In 1987, the poultry industry produced 7.15 billion pounds of further processed product (USDA, 1988). According to Froning (1987), the turkey industry marketed more than 50% of its finished product in the further processed form. Turkey bologna, salami, roasts, rolls, breaded steaks, and ham are items made by restructuring turkey raw materials. Chicken, as well, has been incorporated into a number of further processed products with success. Because of the

nutritional value of poultry and the demand for convenient and flavorful food products by contemporary consumers, further processed poultry items have become an important food alternative in retail markets. However, the additional handling required in the production of such items creates new microbial problems.

The cutting, mixing, formulation, deboning, and other activities of poultry further processing increase the likelihood of microbial contamination. Several researchers have studied the effects that additional processing has on the microbial quality of processed poultry.

Microbial counts ranged from 4.87 to 9.48 log CFU/g with a mean number of 7.92 log CFU/g for 74 samples of comminuted turkey meat obtained from retail markets (Guthertz et al., 1976). Coliform plate counts from these samples varied from 0.70 to 5.68 log CFU/g with a mean of 4.30 log CFU/g. Frozen comminuted turkey was found to have total counts similar to that of the fresh product with numbers ranging from 4.08 to 9.66 log CFU/g and a mean of 7.97 log CFU/g (Guthertz et al., 1977). These researchers stated that comminution of poultry greatly increases the surface area of and distributes bacteria throughout tissues, and oxygenates the product. It provides further opportunity for contamination by exposing the product to the surfaces of counters, knives, machines, and hands of workers. Guthertz (1976) concluded that

comminuted turkey meat purchased at the retail level is a product which has a high degree of bacterial contamination, a limited shelf-life, and the potential to harbor organisms of public health significance.

Aerobic counts ranging from 5.00 to 6.00 log CFU/g were reported by Maxcy et al. (1973) for fresh ground poultry. Similar bacterial concentrations were also observed for frozen product. Samples analyzed in this research were prepared by grinding hand deboned meat or by mechanically deboning. These researchers indicated that ground turkey contained a diverse microbial flora, with members of the genus Bacillus predominating in the fresh product.

Ostovar et al. (1971) reported total plate counts of 5.52 log CFU/g for immediate (conventional) processed mechanically deboned poultry meat (MDPM) formulated from broiler backs and necks. Counts for delayed processed (holding poultry parts 5 days at 3-5°C before processing) MDPM were 5.85 log CFU/g. Fecal coliform numbers for both processes were 2.66 log MPN/g of MDPM. Holding MDPM for 6 days at 3°C increased the total count to 5.82 log CFU/g in conventional processed samples and to 6.92 log CFU/g in delayed processed samples. Frozen MDPM held for 90 days at -15°C had total counts of 4.26 log CFU/g for the conventional process and 5.99 log CFU/g for the delayed process. Further frozen storage (up to 270 days) at -15°C reduced microbial

counts by approximately 2 logs in both conventionally and delayed processed MDPM.

Denton and Gardner (1981) reported that mechanical deboning led to increases of bacterial counts on poultry meat ($p < 0.05$). These workers reported a mean mesophilic count of 4.34 log CFU/g for MDTM (mechanically deboned turkey meat) versus a mean count of 3.47 log CFU/g for poultry before deboning. Also, MDTM had higher levels of coliforms (2.44 log CFU/g) as compared to meat before deboning (1.72 log CFU/g). The rise in bacterial numbers was attributable to elevated product temperature during deboning and to increased nutrient availability and surface area manifested by smaller particle sizes. Furthermore, infrequent sanitation during equipment inactivity could have contributed to bacterial contamination.

Previous investigators have studied the microbiology of products formulated from poultry raw materials. Zottola and Busta (1971) analyzed further processed items such as uncooked rolled roasts and pan roasts for microbial quality. Aerobic numbers ranged from 3.00 to 6.48 log CFU/g in these turkey products. These workers stated that aerobic numbers greater than 6.00 log CFU/g in raw products might be considered excessive or indicative of poor sanitation. Denton and Gardner (1981) reported mesophilic counts of 4.84 log CFU/g for uncooked comminuted rolls and 3.96 log CFU/g

for a raw emulsion wiener product. Cooking of these products by roasting, water immersion, or conventional smokehouse procedures was effective in lowering ($p < 0.05$) bacterial numbers.

2.44 Microbial Growth on Refrigerated Poultry

Total bacterial counts give an indication of the overall quality of poultry and poultry products, whereas coliform counts indicate the level of sanitation. However, microorganisms primarily responsible for the deterioration of refrigerated poultry are psychrotrophic bacteria. The psychrotrophs are classified as those microbes being able to grow at 5°C and below without regard to optimum temperature (Eddy, 1960).

Psychrotrophic bacteria enter poultry processing plants on the feet and feathers of birds and in small numbers in water and ice supplies (Bryan, 1980). These organisms, once in the plant, may multiply on soiled surfaces of equipment, in chill-water tanks, and on the surfaces of birds.

Guthertz et al. (1977) reported a psychrotrophic count of 6.38 log CFU/g for frozen comminuted turkey meat following thawing. Bacterial counts were shown to increase rapidly in fresh ground turkey and chicken stored at refrigeration temperatures (Maxcy et al., 1973). These workers reported that psychrotrophic counts increased from 3.5 log CFU/g to

approximately 8.0 log CFU/g on product stored at 5°C for 4 days. Analyzing samples from a turkey processing plant, Denton and Gardner (1981) observed psychrotrophic counts of 2.75 and 2.89 log CFU/cm² on the pre-cut surfaces of breast and thigh samples, respectively. These investigators reported a psychrotrophic count of 4.37 log CFU/g for MDTM prepared in this study.

Members of the genera Pseudomonas, Aeromonas, Acinetobacter, Moraxella, Flavobacterium, Alcaligenes, and Alteromonas are psychrotrophs commonly associated with the spoilage of processed poultry (Banwart, 1989). However, as low-temperature storage proceeds, pseudomonads dominate the microbial flora (Nagel et al., 1960; Barnes and Impey, 1968; McMeekin, 1977). Pseudomonads are mainly responsible for the spoilage characteristics of refrigerated poultry meat stored aerobically.

McMeekin (1977) demonstrated a shift in the proportion of psychrotrophic isolates in chicken leg muscle held at 2°C. As storage time increased, the number of Flavobacterium and Acinetobacter-Moraxella isolates decreased, while those classified as Pseudomonas group I and II increased.

Barnes and Impey (1968) found, as time progressed, that pseudomonads comprised an increasingly greater proportion of the psychrotrophic spoilage flora in minced poultry held at 1°C. Fluorescent pseudomonads decreased (from 34% to 16%) as

storage time led to the development of strong off-odors, while nonpigmented pseudomonads increased (from 11% to 58%) during this period. Pseudomonas, Acinetobacter-Moraxella, and Flavobacterium were among the psychrotrophic genera most frequently isolated from MDPM in two commercial plants, with Pseudomonas being the most prevalent genus (Ostovar et al., 1971).

Microbial spoilage of meat products is delayed, but not halted, at storage temperatures between -1 and 5°C (Gill and Newton, 1978). Slime formation and off-odors are characteristic of low-temperature bacterial spoilage of fresh poultry. As spoilage is manifested, off-odors are detected first followed by the coalescing of bacterial colonies (largely pseudomonads). Off-odors may emerge at a bacterial load between 7.0 and 8.0 log CFU/cm². Sliminess is usually evident at bacterial counts near 8.0 log CFU/cm² (Ayres, 1960). Soon after the onset of sliminess, growth of spoilage organisms slows as a result of limited oxygen diffusion through the slime layer (Gill, 1976).

Spoilage organisms utilize soluble, low molecular weight components of meat. Glucose is utilized initially, followed by the degradation of amino acids (Gill, 1976; Gill and Newton, 1977). The microbial breakdown of free amino acids and related compounds leads to the predominance of off-odors associated with spoiled meat. Volatile compounds such as

H₂S, methyl mercaptan, dimethyl sulfide, methyl acetate, and ethyl acetate are compounds produced as a result of poultry spoilage (Freeman et al., 1976).

2.45 Pathogenic Microbiology

Poultry meat products have been shown to harbor a variety of pathogenic bacteria. Among the pathogens which have been associated with poultry are Salmonella, Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni, and Yersinia enterocolitica (Cox and Bailey, 1987). In regard to poultry and its products, Salmonella has received the most attention as a causative agent of foodborne illness.

2.451 Salmonella

Members of the genus Salmonella are short, gram-negative, facultatively anaerobic, asporogenous rods which are usually motile by peritrichous flagella. They produce gas and acid from glucose and maltose, but generally not from lactose. Nearly 2000 serotypes of Salmonella can be distinguished according to their biochemical and serological characteristics. Salmonellae may be divided into three groups according to host preference: 1) those primarily adapted to humans (i.e., typhoid and paratyphoid agents), 2) those primarily adapted to a particular animal host (i.e., S. dublin), and 3) those unadapted to a host (Committee on

Salmonella, 1969). Foodborne gastroenteritis is caused most often by members of the unadapted group.

The primary habitat of salmonellae are the intestinal tracts of animals and humans. Although their main reservoir is the intestinal tract, they may be found in other parts of the body (Jay, 1986b). Because salmonellae are intestinal bacteria, these organisms are excreted in feces and may be disseminated by insects, rodents, and other living creatures. Furthermore, salmonellae may be transferred to sewage and water through fecal material. Water and foods contaminated by Salmonella are ingested by humans and animals leading to illness or asymptomatic conditions where the organism is shed and re-enters the environment. This produces a cycle which enables widespread distribution of this pathogen.

Clinical manifestations of salmonellosis may occur as acute gastroenteritis, enteric fever (typhoidal syndrome), bacteremia, or an asymptomatic state in which a healthy individual harbors the organism without showing signs of the disease (Dowell, 1982). Of the above conditions, acute gastroenteritis is the most common while enteric fever is the most severe. The food poisoning syndrome (acute gastroenteritis) generally occurs 12-36 hr after ingestion of contaminated food or water. Symptoms include diarrhea, abdominal cramps, vomiting, and fever which generally lasts from 1 to 7 days (Flowers, 1988). Raw foods, particularly

those of animal origin, have long been known to be primary vehicles of foodborne salmonellosis. Foods of animal origin often incriminated as being Salmonella sources are poultry, beef, and pork (Cox and Bailey, 1987). Consumption of raw milk and eggs contaminated with salmonellae have led to several outbreaks of gastroenteritis (Flowers, 1988).

The primary source of Salmonella for poultry is contaminated feed or feed ingredients (Cox and Bailey, 1987). Although commercial feed is frequently contaminated, percentages of turkeys and chickens entering the processing plant with salmonellae in their intestinal tracts are low. Approximately 2% of the chickens and 2-5% of the turkeys arriving at processing plants were shown to contain salmonellae in their intestines (Sadler et al., 1961; Sadler and Corstvet, 1965). However, once birds are inside the plant, there is a widespread dissemination of Salmonella due to processing (Dougherty, 1974; Cox and Bailey, 1987). Poultry carcasses once free of salmonellae are now infected with this organism resulting in a much higher percentage of contaminated carcasses than before the inception of processing.

The occurrence of Salmonella on processed poultry carcasses is well-documented. Morris and Ayres (1960) found 0-9% of the turkey and 7-14% of the chicken carcass samples in commercial plants to be contaminated with salmonellae. Of

279 post-chilled carcasses evaluated in a processing plant, McBride et al. (1980) reported 21.5% to be Salmonella-positive. Dougherty (1974) observed that 47% of the final product broiler carcasses from a processing plant were infected with salmonellae.

Because of the additional handling and equipment surface contact involved in the cut-up and further processing of poultry, there is an increased opportunity for the contamination of this muscle food with Salmonella. Several workers have evaluated the effects of additional processing on Salmonella levels in poultry.

Analyzing poultry samples from three commercial plants, Hagberg et al. (1973) isolated salmonellae in 7% and 8% of the deboned breasts and thighs, respectively. Among the Salmonella serotypes identified in the plants were S. saint-paul, S. newport, and S. heidelberg. Duitschaever (1977) found 34.5% of raw, retail cut-up chicken samples to be contaminated with salmonellae. Denton and Gardner (1981) observed an increase of Salmonella counts on both breast and thigh samples as a result of hand deboning. Numbers of salmonellae increased ($p < 0.05$) from incoming carcass counts of 0.49 log MPN/1000 cm² to 2.58 log MPN/100 g in breast samples and to 2.49 log MPN/100 g in thigh samples.

Twenty-one of 75 comminuted turkey samples obtained from retail outlets were found to be Salmonella-positive (Guthertz

et al., 1976.) Most isolates recovered from the processed turkey were identified as S. pullorum. Mechanical deboning of poultry produced substantial increases in the Salmonella numbers of MDTM (Denton and Gardner, 1981). Counts increased from 1.29 to 1.70 log MPN/100 g in poultry tissues as a result of deboning. Ostovar et al. (1971) isolated salmonellae in 11% of the MDPM samples obtained from two commercial plants. Zottola and Busta (1971) found salmonellae in 3 of 35 raw further processed samples and no salmonellae in 38 cooked further processed samples.

Morad et al. (1982) showed little change in Salmonella typhimurium counts estimated in inoculated cooked turkey meat stored at 4°C. Numbers remained at approximately 5 log CFU/g in samples held up to 8 days. White and Hall (1984) reported that salmonellae increased approximately 3 logs in fresh chicken meat held at 20°C for 24 hr. Aerobic plate counts increased by more than 2 logs throughout the storage period.

2.252 Listeria monocytogenes

In recent years, L. monocytogenes has emerged as a pathogen of significant public health interest. Once thought to be primarily a infectious agent of livestock, L. monocytogenes has been implicated in a number of human foodborne outbreaks (CDC, 1985; Ho et al., 1986; Fleming et al., 1985; Schlech, et al., 1983).

L. monocytogenes is a gram-positive, facultatively anaerobic, asporogenous rod which is capable of growth at psychrotrophic temperatures. It may grow from 2.5 to 45°C and has an optimum growth temperature between 30 and 37°C. (Petran and Zottola, 1989). The organism is ubiquitous in the environment and has been isolated from silage, vegetation, soil, sewage, mud, streams, and slaughter-house waste. Gray and Killinger (1966) reported that L. monocytogenes was isolated from more than 40 mammalian species and at least 17 different avian species including domesticated chickens and turkeys.

L. monocytogenes is a unique foodborne pathogen, causing diseases which affect organs other than the gastrointestinal tract. Primary manifestations of listeriosis include meningitis, spontaneous abortion, encephalitis and septicemia, while conditions such as cutaneous lesions, conjunctivitis, endocarditis, and peritonitis have been known to occur (Gray and Killinger, 1966; Nieman and Lorbor, 1980). Subpopulations most susceptible to listeriosis are pregnant women, newborns and infants, and immunocompromised persons (Seeliger and Finger, 1976).

Initially a concern in dairy and vegetable products, L. monocytogenes is being studied with greater frequency in muscle foods. A recent outbreak linked to poultry (Barnes et al., 1989) suggests that L. monocytogenes may be a potential

etiologic agent to humans when it is associated with meat or poultry products.

Previous studies have evaluated the fate of L. monocytogenes in red meats. The results of these experiments have been variable in respect to these muscle foods supporting the growth or survival of L. monocytogenes. Kahn et al. (1973) analyzed the growth of L. monocytogenes in lamb, minced meat, and sausage. These workers found that numbers declined in sterile lamb held at at 0°C, but increased by more than 3 logs in sterile lamb stored at 8°C. Organisms could be isolated and potentially increased in numbers for 15-20 days at 4°C and 8°C in minced meats and sausage; nevertheless, an estimate of counts could not be obtained due to competing microbial flora.

Numbers of listeriae declined 1 log initially in sterile ground beef held at 8°C and then generally stayed constant throughout the rest of a 17 day storage period (Gouet et al., 1978). Johnson et al. (1988) reported that L. monocytogenes counts in ground beef remained constant for 14 days at 4°C. Survival of listeriae in these experiments was not influenced by the packaging permeability to O₂.

Buchanan et al. (1987) observed slight growth of L. monocytogenes in irradiated ground beef. These authors suggested that L. monocytogenes growth in meat occurred as a result of reduced microbial competition. Shelef (1989) noted

survival but no proliferation of L. monocytogenes in fresh ground beef or liver stored until spoilage onset at 4 and 25°C.

Recent studies have analyzed the growth potential of L. monocytogenes in poultry. Harrison and Carpenter (1989) studied the fate of L. monocytogenes populations surviving moist heat treatment in inoculated chicken breast. These authors found that cells which survived thermal processing could grow in breast meat held at 4 and 10°C. Growth after thermal processing was dependent upon cooking temperature and packaging (film overwrap or vacuum-packaged). Except in those breast samples receiving the most lethal cook (82.2°C), L. monocytogenes counts increased ($p < 0.05$) by 4 weeks in samples held at 4°C. Numbers of listeriae increased ($p < 0.05$) by 10 days in samples stored at 10°C .

In a separate study, the fate of L. monocytogenes was evaluated in inoculated chicken breasts cooked with dry heat and subsequently stored at 4 and 10°C (Carpenter and Harrison, 1989). Except in the breasts processed at 71.1°C, listeriae increased ($p < 0.05$) in numbers by week 4 in samples which were overwrapped and held at 4°C. Increases ($p < 0.05$) in L. monocytogenes numbers by week 1 were seen in chicken breasts which were overwrapped or vacuum-packaged and held at 10°C.

In summary, washing methods may be used to improve the utilization of poultry muscle raw materials. Tissues treated by washing have been shown to be altered in chemical composition. As a result, microbial characteristics of these modified tissues may be affected. Because there is no data available on the microbiology of washed poultry, information is needed to ensure proper handling of this raw material in regard to microbial quality and safety.

3.0 MATERIALS AND METHODS

3.1 Source, Handling, and Processing of Thighs

For each of the first two replications of color modification, 9.1 kg batches of raw, skinless, boneless turkey thighs were transported in ice from a commercial turkey processing plant to the Muscle Foods Research Laboratory at VPI&SU. An 18.2 kg batch of thigh meat was transferred to the laboratory for the third and final replication of color modification. Upon arrival from the processing plant, thighs were quartered, placed in 33 cm x 66 cm type L348 Cryovac bags (W.R. Grace and Co., Duncan, S.C.), spread into a monolayer on a mesh storage rack, and stored overnight in a -20°C freezer before being processed on the following day.

To minimize microbial contamination from processing surfaces, equipment was thoroughly cleaned and sanitized on the day of flaking and color modification. Surfaces were cleaned with an alkaline detergent (Fisher Biodegradable), rinsed with cold tap water, and sanitized with a 200 ppm chlorine solution. The chlorine solution was left as a residual on equipment surfaces for 3-4 hr and rinsed off with cold tap water minutes before processing.

Processing of thighs occurred on November 10, 1988; January 26, 1989; and March 15, 1989. Thighs stored overnight at -20°C were tempered at 2°C for approximately 2

hr (or for a sufficient time to allow thigh separation). Employing blade openings of 8 mm, the Unicom 1000 Flaker (Ross Industries Inc., Midland, Va.) was used to reduce quartered thigh meat to 7 mm x 4 mm x 30 mm flakes. Flakes were collected in clean plastic lugs during processing and mixed with a large sterile spoon. Mixing of flaked unwashed turkey (FUT) was performed to obtain representative FUT samples in respect to microbial load. A portion of FUT (4 kg) was retained for color modification, while the remaining FUT was stored in a 2°C cooler until packaging.

3.2 Color Modification Procedure

Color modification of flaked tissue was accomplished using the method outlined by Elkhailifa et al. (1988) and modified by Phelps et al. (1990). The procedure incorporated successive washings with chilled (2°C) 0.03 M sodium phosphate buffers of pH 5.8, 7.4, and 8.0.

Four kilograms of flaked thigh were mixed with 12 L of the pH 5.8 sodium phosphate buffer (1:3 ratio of tissue to buffer). The mixture was blended for 5 min. at a speed setting of 2 in a CSE mixer (Model CDB-06150-V, Custom Stainless Equipment Co., Inc., Santa Rosa, Ca.). The resulting homogenate was permitted to rest 15 min before the spent buffer was decanted from the blender.

The remaining meat residue was pressed through a double layer of 40 grade cheesecloth (American Fiber and Finishing, Inc., Burlington, Mass.) to remove excess moisture. Afterwards, the residue was placed back into the blender and the above procedure was repeated twice, once with the pH 7.4 sodium phosphate buffer followed by a final wash with the pH 8.0 buffer.

The final washed residue, color-modified turkey (CMT), and the previously described FUT were aseptically transferred in 150-175 g portions (using a sterile spoon) to sterile 18 oz. Whirl-Pak bags (NABSCO). To simulate potential storage conditions used in commercial poultry processing plants, these tissues were stored up to 5 days at 3°C and for 30 days at -20°C.

3.3 Microbial Analysis

3.31 Microbial Evaluation of Intact Thigh Samples

To establish initial microbial loads, intact thighs were analyzed for aerobic, psychrotrophic, and coliform bacterial numbers. Three thighs were randomly selected from each lot received for flaking and color modification. From each thigh selected, a 25 g portion was aseptically excised and transferred to a sterile Stomacher 400 bag (Seward Medical, London). Thigh samples in Stomacher bags were homogenized with 225 mL of 0.1% peptone water for 1 min in a Stomacher

Lab Blender 400 (Tekmar Co., Cincinnati, Ohio). From the resulting mixture (1:10 dilution), appropriate serial dilutions were made in 99 mL peptone water blanks.

Estimates of aerobic and psychrotrophic bacterial counts were obtained by pouring duplicate plates of each dilution with trypticase soy agar (TSA; BBL, Coockeysville, Md.). Aerobic plates were incubated at 35°C for 48 hr, and plates for the determination of psychrotrophic numbers were held at 7°C for 10 days (APHA, 1984).

Presumptive coliform counts were obtained following the method outlined by Hartman (1979). The procedure involved pouring duplicate plates of appropriate dilutions with 8-10 mL of TSA, allowing this basal medium to solidify, and adding an 8-10 mL overlay of double-strength violet red bile agar (VRBA) (Difco; Detroit, Mi.). After incubating plates for 24 hr at 35°C, dark red colonies with typical diameters (0.5 mm or greater) were recorded as being presumptive colonies of coliform bacteria.

Three representative presumptive colonies from coliform plates of each thigh sample were transferred to tubes of 2% brilliant green bile broth (BGBB, Difco). BGBB tubes were incubated at 35°C and observed at 24 and 48 hr. Growth and gas production from presumptive colonies in BGBB confirmed the presence of coliforms.

3.32 Microbial Evaluation of Raw FUT and CMT

At selected intervals, three Whirl-Pak bags each of CMT and FUT were mixed by hand kneading and subsampled. A 25 g subsample was removed from each bag, diluted with 225 mL of 0.1% peptone water, and stomached for 30 sec. Tissues held at -20°C for 30 days were thawed at 4°C for approximately 36 hr before mixing and subsampling.

Using methods described above, aerobic and psychrotrophic counts of samples stored at 3°C were determined after 1, 3, and 5 days. Meat samples held at -20°C were evaluated for aerobic and psychrotrophic loads in the same manner. Because coliform counts generally indicate adequacy of sanitation rather than information about keeping quality, analysis of these bacteria were accomplished only on 1-day, 3°C samples.

3.33 Inoculation Studies with Pathogenic Bacteria

3.331 Preparation of Inoculum

Two-strain composites of both Salmonella and Listeria monocytogenes were prepared for inoculation. Strains used in this study were nalidixic acid-resistant mutants of Salmonella typhimurium and Salmonella enteritidis, L. monocytogenes strain Scott A, and a L. monocytogenes meat isolate. Salmonella strains were obtained from N. A. Cox, USDA, Agricultural Research Service, Athens, Ga. L.

monocytogenes Scott A was acquired from R. E. Brackett, University of Georgia, Athens, Ga. and a L. monocytogenes meat isolate was obtained from J. L. Johnson, Food Research Institute, University of Wisconsin-Madison, Madison, Wi.

Stock cultures of Salmonella strains were maintained on double strength nutrient agar stabs until inoculation into meat portions. In preparation for inoculation, the two strains of Salmonella were inoculated into individual tubes of trypticase soy broth (TSB; Difco) and incubated for 24 hr at 37°C.

From TSB tubes of each Salmonella strain, 8 mL of broth were transferred to a 15 mL centrifuge tube, and cells were harvested according to methods suggested by Schnaitman (1981). The tubes were spun at 3000 x g and 10°C for 15 min in a Sorval RC-5B Refrigerated Superspeed Centrifuge (Dupont Industries, Wilmington, Del.). Following centrifugation, the supernatant was decanted from each tube, and 10 mL of 0.01 M (pH 7.6) phosphate buffered saline (PBS) were added to each pellet. The pellets were washed by centrifugation for 5 min (3000 g, 10°C); after which, the wash solution was decanted, and the pellets were resuspended by vortexing in 5 mL of PBS.

The suspensions of each strain were diluted in spectrophotometric tubes containing PBS, and the optical densities of the dilutions were measured in a Spectronic 20 spectrophotometer (Bauch and Lomb, Rochester, N.Y.) at a

maximum absorbance of 600 nm. Optical density readings were interpolated on plots (optical density vs. log CFU/mL) of each strain to obtain an estimate of bacterial number. Inoculum solutions were adjusted and pooled to obtain a 1:1 strain ratio.

L. monocytogenes strains, maintained on TSA slants at 4°C, were inoculated into TSB with 0.6% yeast extract and incubated for 24 hr at 37°C. Inoculum preparation from L. monocytogenes cultures was the same as that described for Salmonella.

3.332 Preparation of Cooked Meat for Inoculation

Portions of CMT and FUT (200 g) were aseptically weighed into 250-mL sterile metal beakers, covered with aluminum foil, and placed in a 93.3°C water bath. The tissues were cooked for approximately 25 min or until an internal temperature of 71.1°C was achieved. After cooking, CMT and FUT portions were allowed to cool to room temperature before being transferred to stomacher bags.

Cooked CMT and FUT were stomached for 30 sec before being aseptically divided into 11-g quantities. Using methods previously described, aerobic numbers were determined in cooked tissue portions (11-g) on the day of heat processing. Eleven-gram samples in sterile petri dishes were spiked with 0.11 mL of inoculum from either of the two-strain

composites formulated for each pathogen. The resulting inoculum level was approximately 3.5 log CFU/g for Salmonella-inoculated samples and 3.0 log CFU/g for meat spiked with L. monocytogenes.

Following inoculation, the meat samples were transferred from petri dishes to sterile Stomacher bags. Bags with samples were folded three times and clamped with a paper clip. Tissues were then hand kneaded for 15 sec to disperse the inoculum and incubated at 4°C (typical refrigeration temperature) or 20°C (abuse temperature).

Uninoculated controls of CMT and FUT were stored with L. monocytogenes-inoculated samples to ensure that L. monocytogenes did not survive cooking. This was not necessary for the inoculation studies with Salmonella since only nalidixic acid mutants of this pathogen should be recovered on the selective medium.

3.333 Recovery of Pathogens from CMT and FUT

Inoculated tissues containing Salmonella strains and incubated at 20°C were analyzed at 0, 1, 2, 3, 4, and 5 days. Samples inoculated with L. monocytogenes and held at 20°C were tested at 0, 12, 24, 36, and 48 hr. Portions spiked with either Salmonella or L. monocytogenes and stored at 4°C were evaluated at days 0, 1, 4, 7, 14, and 21.

At appropriate time intervals, 99 mL of 0.1% peptone water were added to inoculated meat portions. The resulting mixture was homogenized in a stomacher for 1 min and serially diluted in 99 mL peptone water blanks for plating.

Salmonella numbers were determined by the procedure used by To and Robach (1980). For each dilution, 0.1 mL portions were spread onto duplicate plates of Brilliant Green agar (BGA; Difco) supplemented with 100 ppm nalidixic acid (Sigma, St. Louis, Mo.). Plates for the recovery of salmonellae were incubated at 37°C for 24 hr.

Estimates of L. monocytogenes counts were obtained by spread-plating 0.1 mL portions of appropriate dilutions onto duplicate plates of Modified Vogel Johnson (MVJ; Difco) agar (Buchanan et al., 1987). MVJ plates were incubated at 37°C for 48 hr.

Typical isolates of Salmonella from BGA were transferred to triple sugar iron agar (TSI; Difco) slants to confirm recovery of this pathogen. After incubation for 24 hr at 37°C, TSI slants were observed for typical reactions of Salmonella: acid butt, alkaline slant, H₂S production, and gas. Further characterization of Salmonella isolates was accomplished by analyzing a fraction of the TSI positive cultures with polyvalent O antisera (Difco) and API20E strips (Analytab Products, Plainview, N.Y.).

Typical L. monocytogenes isolates (tellurite positive/mannitol negative) from MVJ agar were confirmed by the following tests: gram stain, motility in brain-heart infusion broth (25°C), umbrella-like growth in motility medium, MR/VP, catalase, oxidase, nitrate reduction, beta hemolysis, urea hydrolysis, TSI agar, and fermentation in glucose, xylose, rhamnose, and maltose broths (Seeliger and Jones, 1986).

3.4 Moisture and pH Analysis

Moisture and pH determinations were made on raw and cooked CMT and FUT samples following AOAC (1984) methods. However, in the case of the inoculated samples, pH measurement was performed directly on the 1:10 homogenate used for plating. This was done to monitor the effect that pathogenic bacterial growth had on muscle pH.

3.5 Statistical Analysis

Microbial counts were converted to logarithms and analyzed by the General Linear Models procedure of the Statistical Analysis System (SAS, 1984). Differences among replications of bacterial populations in intact thighs were evaluated using analysis of variance (ANOVA). When the F-test was significant ($p < 0.05$), differences between means were determined using least significant difference (LSD).

ANOVA was used to ascertain differences in aerobic and psychrotrophic numbers between intact thighs and 1-day tissues and frozen and 1-day tissues. Differences in coliform counts between 1-day CMT and FUT were also determined using ANOVA.

Data from the evaluation of aerobic and psychrotrophic counts in tissue raw materials (three replications) were analyzed by a split-plot design of a 2 X 3 ANOVA. Results obtained from the inoculation studies (four replications) were evaluated using a split-plot design of a 2 X 5 or 2 X 4 ANOVA. When significant ($p < 0.05$) interactions (time by treatment) were obtained in the split-plot ANOVA designs, differences between means of tissues within sampling intervals were determined using least significant difference (LSD). The effects of color modification and time, as well as the interaction of these factors were included in the model (Zar, 1986).

4.0 RESULTS AND DISCUSSION

4.1 Compositional Analysis

Moisture and pH data of this study are similar to those reported by Elkalifa et al. (1988) and Phelps et al. (1990) for raw FUT and CMT. Mean moisture values for raw FUT and CMT were 73.6 and 83.5%, respectively. Cooking of raw muscle tissues tended to decrease moisture. The mean moisture value for cooked FUT was 70.1% and that for cooked CMT was 77.3%. Because the moisture content of raw and cooked tissues was at or above that of fresh poultry, which has a water activity of .98 to .99 (Bryan, 1980), it was not considered a factor limiting microbial proliferation in these tissues.

pH values in raw FUT and CMT portions were almost constant throughout 5 days of storage at 3°C (results not shown). pH remained near 7.0 for CMT and 6.5 for FUT. Since pH stayed close to neutrality in raw samples, it also was not regarded as a limiting factor to the growth of microorganisms.

4.2 Microbial Evaluation of Intact Thigh Samples

Counts for aerobic, psychrotrophic, and coliform bacterial populations were obtained from intact thigh samples of each batch (replication) before processing (Fig. 1). Mean aerobic counts were lower in batch 3 ($p < 0.05$) than in batch 1 or 2. Psychrotrophic bacterial numbers were higher (p

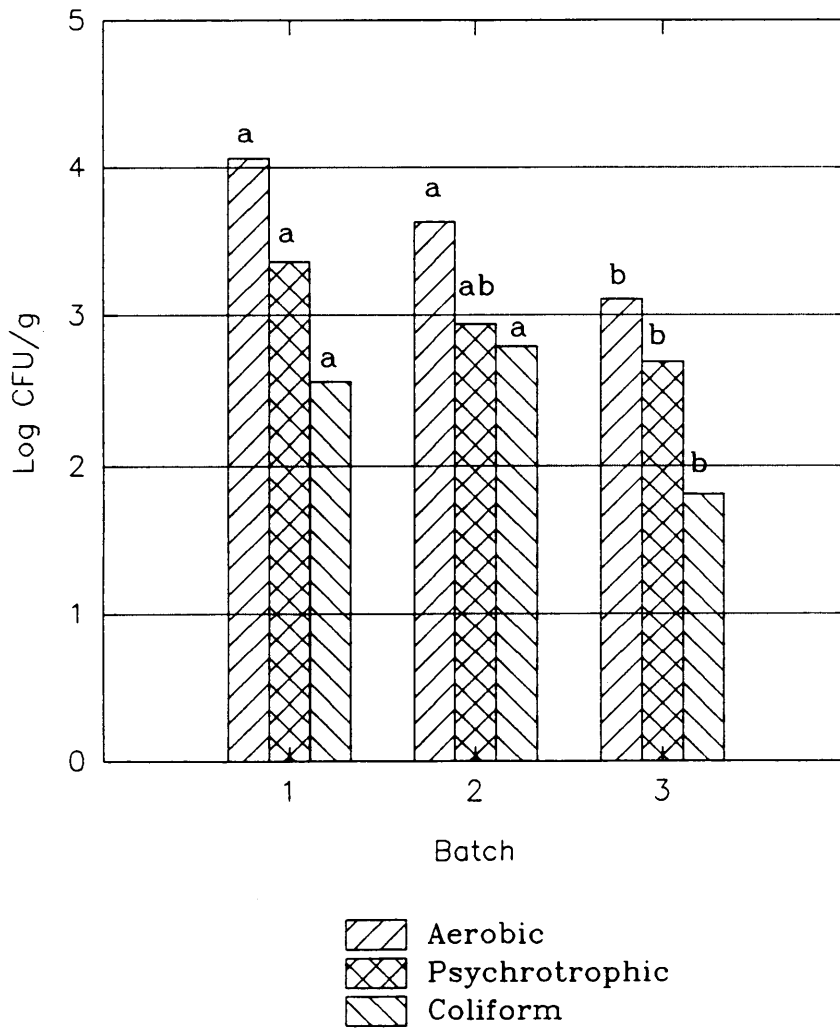


Figure 1. Bacterial levels in thigh samples obtained from a commercial turkey processing plant. Bar types with identical letters are not different ($p > 0.05$). Each bar represents the mean of three samples.

<0.05) in batch 1 than in batch 3. However, psychrotrophic counts in batch 2 were not different ($p > 0.05$) from those in either batch 1 or 3.

The aerobic and psychrotrophic counts of thigh meat estimated in this research generally are not atypical when compared to microbial numbers of intact poultry in other studies. May et al. (1962) reported total counts of 3.32 log CFU/cm² on the surface of chicken broilers in a processing plant. However, Denton and Gardner (1981) reported mean counts of 1.63 and 1.57 log CFU/cm² on skinless thigh tissue for aerobic and psychrotrophic populations, respectively.

As with the aerobic counts, numbers of coliforms in the third batch of thigh meat were lower ($p < 0.05$) than counts obtained from the first two replications. No coliforms were detected by Denton and Gardner (1981) on skinless thighs.

The fluctuation in bacterial numbers among batches could be attributable to the varying conditions of handling, processing, and/or sanitation in the plant during the preparation of a thigh lot for a given replication of the microbial quality study. In addition, the time of year in which a thigh batch was procured may reflect the microbial flora associated with a given flock and the conditions on the farm from which a flock was transported.

4.3 Microbial Evaluation of Raw FUT and CMT

The aerobic count of 1-day FUT was not greater ($p > 0.05$) than that in intact thigh (Fig. 2; Table 1). Also, particle reduction did not lead to higher numbers ($p > 0.05$) of psychrotrophic (Fig. 2; Table 2) and coliform bacteria (Fig. 2; Fig. 3) in 1-day FUT.

Denton and Gardner (1981) reported that further processing by mechanical deboning increased ($p < 0.05$) bacterial counts in poultry. These authors reported an approximate 1 log/g increase in the mean mesophilic count of poultry tissue after deboning. They suggested that infrequent sanitation, increased surface area due to particulation, and elevated product temperature during deboning were factors inducing higher microbial counts in the final product. In the present research, conditions such as these did not seem to play a major role in influencing bacterial concentrations on thigh meat processed by flaking.

Since thighs were partially frozen, proliferation of microorganisms during flaking may have been negligible. The temperature of the thigh tissue was probably low enough to prevent substantial microbial growth during processing.

The effect of color modification on aerobic bacterial counts in thigh tissue raw materials is presented in Table 1. Overall, the color modification procedure yielded higher ($p < 0.05$) aerobic numbers; however, counts did not differ (p

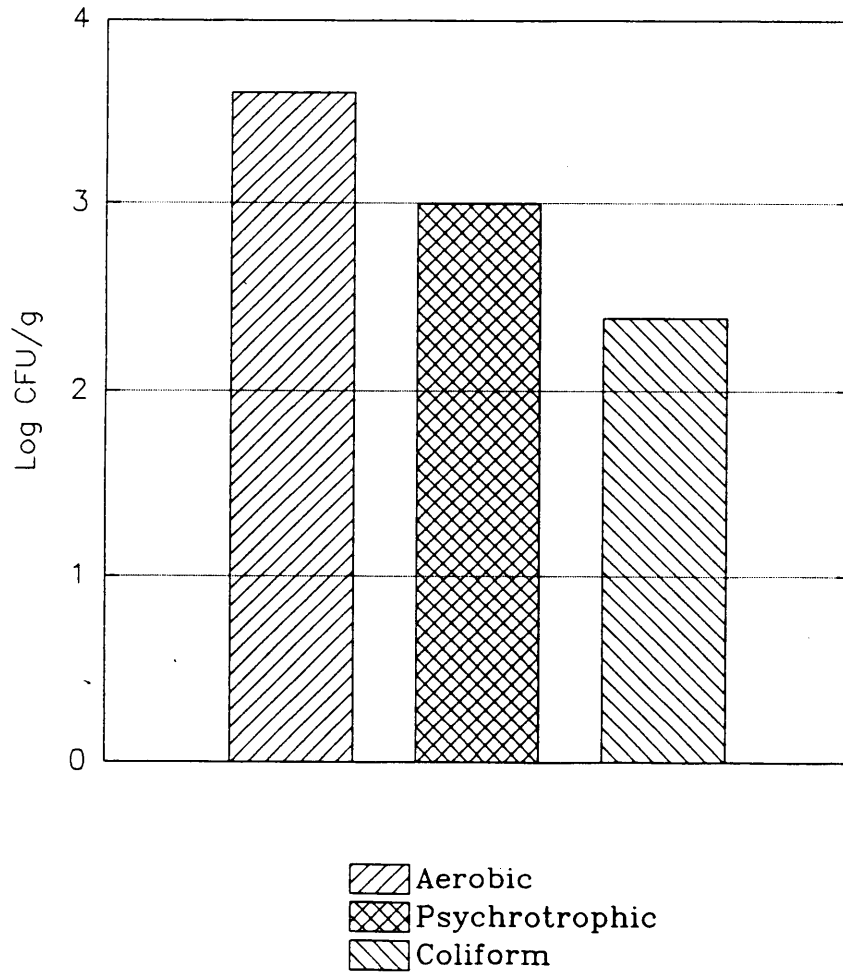


Figure 2. Overall bacterial levels in thigh samples obtained from a commercial turkey processing plant. Each bar represents the average of three means.

Table 1 - Aerobic bacterial counts^a in FUT and CMT stored at 3 and -20°C*.

Time (days)	Log CFU/g ± S.E.	
	FUT	CMT
3°C		
1	3.91 ± .13a	4.54 ± .32a
3	3.95 ± .14a	4.65 ± .19a
5	4.68 ± .56a	5.61 ± .37b
-20°C		
30	3.74 ± .19	3.82 ± .25

^aCounts are the average of three means.

^{a, b}Means within columns with identical letters are not different ($p > 0.05$).

*Means within rows are not different from each other. Day 1 tissue means of 3°C did not differ from those of -20°C tissues.

>0.05) between FUT and CMT at any sampling interval. At day 1, the numerically higher aerobic count in CMT as compared to that in FUT could be due to the additional contact of meat particles with equipment surfaces and extracting fluids during color modification. The larger initial count in 1-day CMT suggests why bacterial levels observed throughout the 5-day storage period in this tissue tended to be higher than those estimated in FUT during the same holding time (Table 1).

Aerobic counts in FUT (3.91 log CFU/g) and CMT (4.54 log CFU/g) held at 3°C for 1 day (Table 1) approximate microbial concentrations estimated in other types of further processed poultry raw materials. Ostovar et al. (1971) reported a total count of 5.52 log CFU/g in immediately processed MDPM, and Maxcy et al. (1973) observed a similar concentration in fresh ground poultry. A mean mesophilic count of 4.34 log CFU/g in MDTM was reported by Denton and Gardner (1981).

Aerobic numbers in FUT and CMT increased 0.77 log CFU/g and 1.07 log CFU/g, respectively, after holding samples 5 days at 3°C. The bacterial count in CMT was higher ($p < 0.05$) than the initial concentration by day 5. Although not statistically significant, the aerobic count tended to increase in FUT throughout storage.

After storing samples for 6 days at 3°C, Ostovar et al. (1971) observed an increase of 0.30 log CFU/g in the aerobic

count of MDPM. In the present study, increased bioavailability of some nutrients may have occurred due to the leaching out of these components from tissue surfaces during color modification. This may explain the larger increase in the aerobic count of CMT as compared to that reported by Ostovar et al. (1971) for MDPM.

Freezing at -20°C for 30 days appeared to decrease aerobic counts in both tissues when compared to 1-day samples (Table 1). Nevertheless, reduction of counts in FUT or CMT by frozen storage was not statistically significant ($p > 0.05$). Freezing (especially slow freezing) has been shown to injure or even kill microbes by dehydrating cells and physically damaging cell walls (Fung, 1987). Maxcy et al. (1973) found that the total bacterial concentration remained constant in ground poultry stored for 7 weeks at -20°C . However, Ostovar et al. (1971) indicated that freezing MDPM for 90 days at -15°C reduced the total count by approximately 1 log in immediately processed MDPM.

The color modification procedure did not influence ($p > 0.05$) psychrotrophic bacterial counts (Table 2). As with the analysis of aerobic numbers, no differences ($p > 0.05$) in psychrotrophic concentrations were found between FUT and CMT, although counts tended to be higher in 3-day and 5-day CMT than in 3-day and 5-day FUT, respectively.

Table 2 - Psychrotrophic bacterial counts^a in FUT and CMT stored at 3 and -20°C*.

Time (days)	Log CFU/g ± S.E.	
	FUT	CMT
3°C		
1	3.00 ± .38aA	2.97 ± .29aA
3	3.90 ± .31b	4.56 ± .35b
5	5.38 ± .36c	6.29 ± .31c
-20°C		
30	2.72 ± .41B	2.40 ± .16A

^aCounts are the average of three means.

a,b,c; A,B Means within columns with identical letters are not different (p >0.05).

*Means within rows are not different from each other.

Psychrotrophic numbers increased ($p < 0.05$) in both tissues by day 3 (Table 2). The increase was 2.38 log CFU/g in FUT and 3.32 log CFU/g in CMT by day 5. Psychrotrophic counts were shown to increase rapidly in fresh ground poultry held at 5°C (Maxcy et al., 1973). These researchers indicated that levels increased about 4.5 logs in samples stored at 5°C for 4 days. The smaller increase in psychrotrophic numbers of FUT and CMT as compared to that observed by Maxcy et al. (1973) for ground poultry held at 5°C could be due to the lower storage temperature (3°C) used in the present study.

Frozen FUT had a lower psychrotrophic count ($p < 0.05$) than 1-day product had (Table 2). The number in frozen CMT, nevertheless, was not lower ($p > 0.05$) than that in 1-day CMT. This is odd, since the difference between frozen and 1-day CMT counts was greater than that between frozen and 1-day FUT levels (Table 2). Perhaps the variability (among replications) observed in mean psychrotrophic numbers of CMT could be responsible for the finding of no difference ($p > 0.05$) when comparing frozen and 1-day tissue counts. However, the deviations in this case would not seem to be of practical importance from the standpoint of product deterioration and food safety.

Mean counts of aerobic and psychrotrophic bacteria in day-5 CMT were 5.61 and 6.29 log CFU/g, respectively (Tables

1 and 2). Spoilage of poultry is generally observed when microbial concentrations reach 7.0 to 8.0 log CFU/cm² (Ayers et al., 1960). Although the 5-day CMT was not considered spoiled, bacterial numbers were quite high. Therefore, in respect to microbial deterioration, CMT should be converted to further processed products as soon as possible, especially when this raw material is held above freezing.

When coliform counts of FUT and CMT portions were analyzed within each replication, inconsistent results were observed, and no trend in coliform levels could be determined when comparing the two processed tissues (Fig 3). Overall, the mean coliform count in 1-day CMT (2.63 log CFU/g) tended to be higher than that in 1-day FUT (2.24 log CFU); however, the difference was not statistically significant ($p > 0.05$). In replication 2, tissue exposure to contaminated washing buffers and/or equipment surfaces could have led to the 1.5-log increase in coliform numbers. However, deviations were much smaller between the coliform concentrations of FUT and CMT in the other two replications.

Since coliform counts did not increase ($p > 0.05$) in processed tissues as a result of flaking or color modification, it is suggested that these procedures, if performed properly, do not profoundly violate hygienic conditions required in quality meat processing.

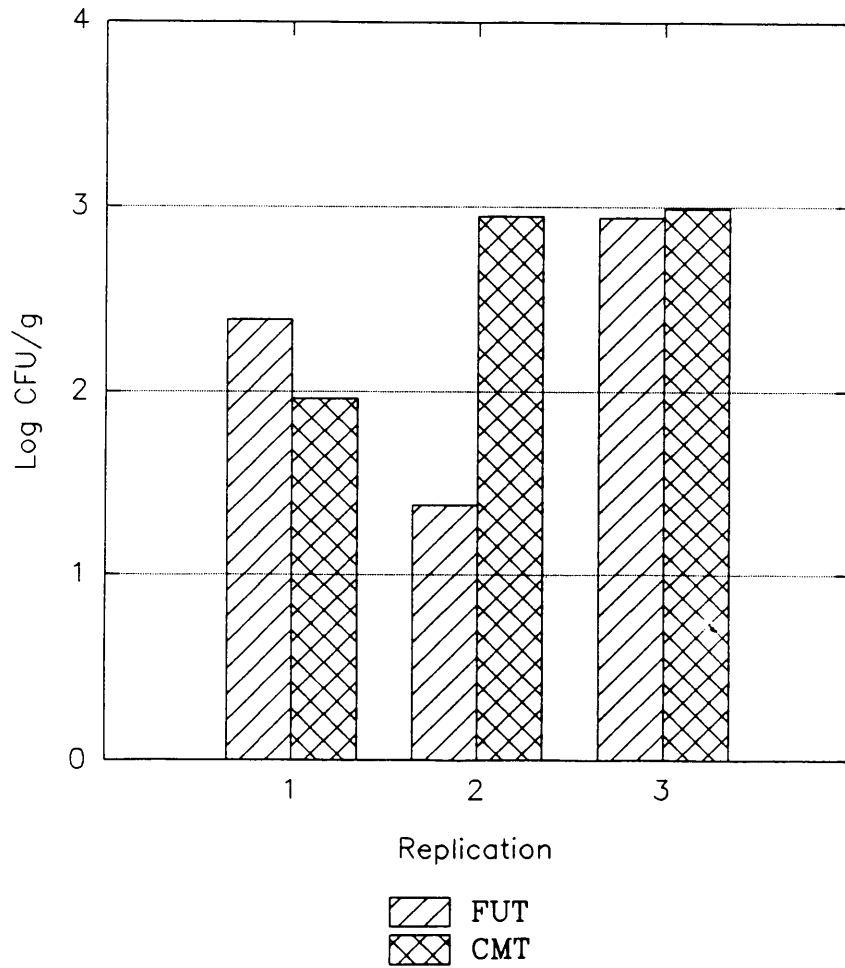


Figure 3. Coliform bacterial levels in FUT and CMT from each replication of color modification. Each bar represents the mean of three observations.

Mean coliform levels obtained from 1-day FUT and CMT are intermediate in number when compared to coliform concentrations estimated in other studies of further processed poultry. Guthertz et al. (1976) reported a coliform count of 4.3 log CFU/g in retail comminuted turkey meat. MDTM from a further processing plant had a coliform concentration of 2.44 log CFU/g (Denton and Gardner, 1981).

4.4 Inoculation Studies with Pathogenic Bacteria

4.41 Cooked Meat Microbiology

Aerobic counts were <2.0 log CFU/g in cooked FUT and CMT following thermal processing. Therefore, in respect to the number of competing microorganisms, cooked meat was a more favorable growth environment for pathogenic bacteria.

4.32 Salmonella Growth in Cooked Meats

To mimic post-processing contamination, the fate of Salmonella was evaluated in cooked FUT and CMT. Overall, color modification affected ($p < 0.05$) the growth of salmonellae in FUT and CMT stored at 4°C (Fig. 4). Nevertheless, no differences ($p > 0.05$) existed between Salmonella counts of FUT and CMT at any sampling interval.

Salmonella numbers gradually declined in FUT and CMT inoculated portions. Concentrations in both meat types were similar until day 9 when counts in CMT began to fall more

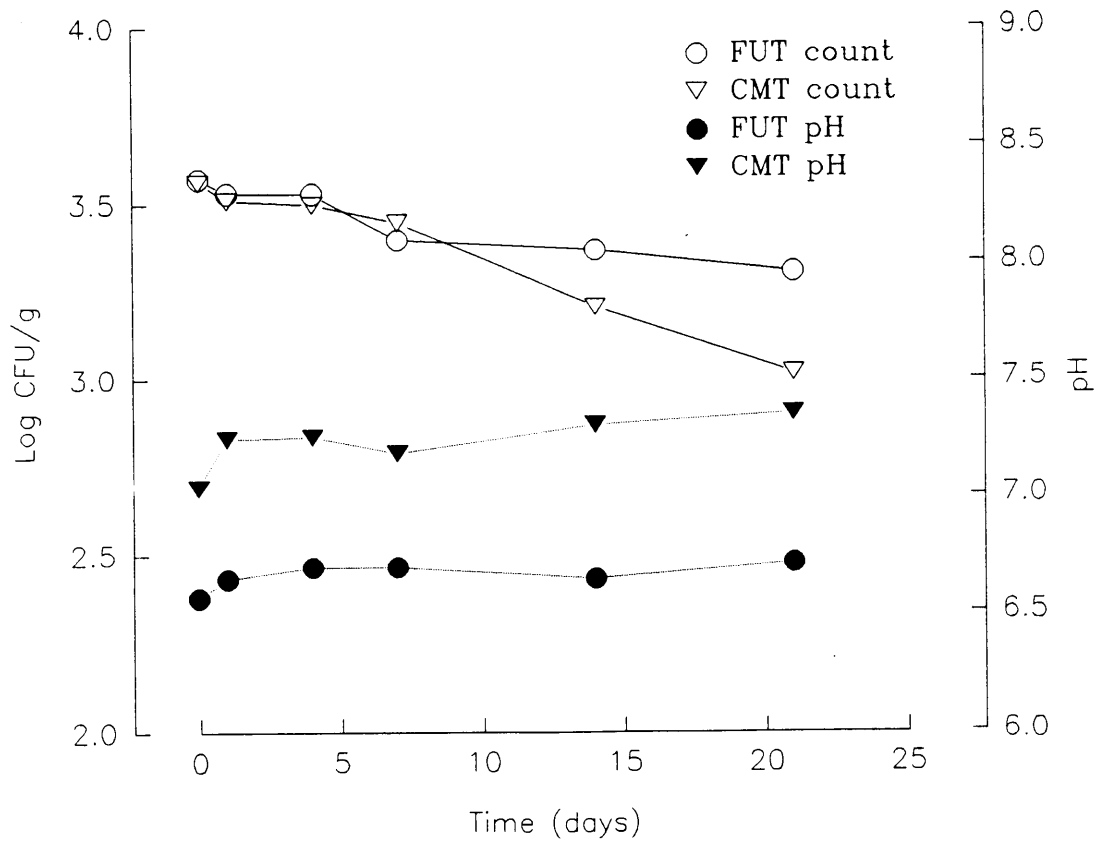


Figure 4. Survival of Salmonella and pH profile during storage of FUT and CMT at 4°C. Each data point is the mean of four observations.

rapidly than those in FUT (Fig. 4). Therefore, the combination of refrigerated storage and color modification may have had a potential limiting effect on the survival of salmonellae in tissues as opposed to cold storage at 4°C alone. Perhaps storage beyond 21 days would have yielded a difference ($p < 0.05$) between CMT and FUT Salmonella numbers.

The minimum growth temperature has been reported to be 5.3°C for S. heidelberg and 6.2°C for S. typhimurium (Matches and Liston, 1968). In the present study, a possible underestimation of Salmonella numbers in tissues held at 4°C may have occurred as a result of cell injury. Direct plating on the Salmonella-selective medium (Brilliant Green agar with nalidixic acid) could have prevented the recovery of cells potentially injured by refrigeration.

Previous research has shown Salmonella counts to remain constant or decline in refrigerated poultry and surimi-based (derived from washed fish muscle tissue) products. Morad et al. (1982) reported little change in Salmonella typhimurium levels of inoculated cooked turkey meat stored at 4°C. Counts stayed at approximately 5.0 log CFU/g throughout an 8-day storage period. In imitation crab held at 5°C, Salmonella counts declined rapidly (approximately 2 logs) during the first 7 days of storage; after which, numbers declined gradually until day 50 (Yoon and Matches, 1988).

The change in pH of Salmonella-inoculated samples held at 4°C was slightly alkaline; however, values remained near neutrality in both tissues (Fig. 4). Therefore, the growth of salmonellae probably was not influenced by pH.

The growth of Salmonella at 20°C was evaluated in the two cooked tissues over a 5-day storage period (Fig. 5). As in the 4°C study, the main effect of color modification influenced ($p < 0.05$) the growth of salmonellae. However, no differences ($p > 0.05$) in Salmonella counts were determined between FUT and CMT at any sampling time.

Both tissues at 20°C were excellent growth media for salmonellae. Numbers in FUT and CMT increased rapidly (approximately 6 logs) from 0 to 2 days. Counts peaked at approximately 10 log CFU/g by day 3 and remained at that level until day 5 (Fig. 5). Exhaustion of nutrients and/or the buildup of waste products may explain the leveling of counts after 3 days of storage. After 1 day at 20°C, counts tended to be slightly lower in CMT than in FUT throughout the storage period. However, washing of turkey thigh meat was considered to have little practical consequence on the growth of Salmonella at the abuse temperature.

Previous researchers have demonstrated rapid proliferation of salmonellae in poultry and surimi-based products held at abuse temperatures. White and Hall (1984) found that Salmonella numbers increased approximately 3 logs

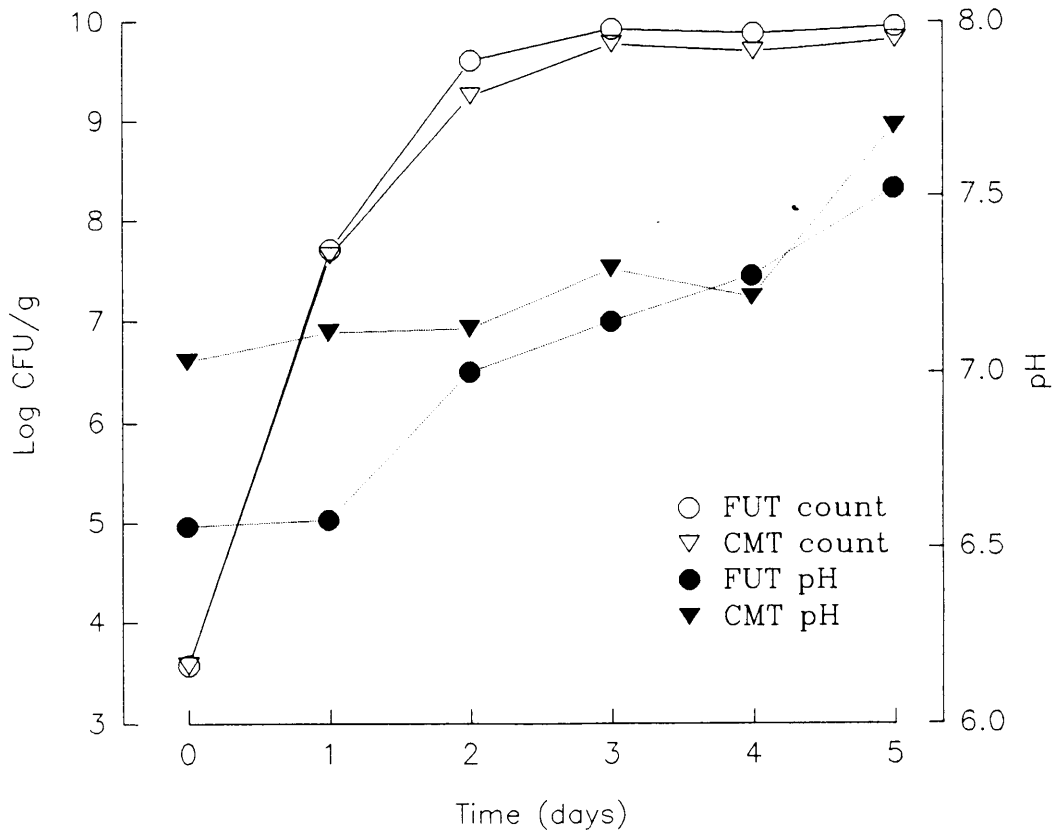


Figure 5. Growth of *Salmonella* and pH profile during storage of FUT and CMT at 20°C. Each data point is the mean of four observations.

in fresh chicken meat controls held at 20°C for 24 hr. In their research, salmonellae were able to compete well with the background microbial flora in the unprocessed chicken. Salmonella counts increased by approximately 5 logs in control samples of vacuum-packaged turkey slices and breasts stored at 15°C (To and Robach, 1980). Within 4 days, numbers of salmonellae increased 6 logs in imitation crab held at 15°C (Yoon and Matches, 1988).

The shift in pH was greater in Salmonella-inoculated samples held at 20°C than in those stored at 4°C (Fig. 4). pH increased approximately 1.0 unit in FUT and 0.70 units in CMT. Since values remained near neutrality, the change in pH probably did not have a major influence on the growth of Salmonella.

The notable alkaline shift in pH was probably due to the metabolism of amino acids after exhaustion of sugar substrates. Gill (1976) and Gill and Newton (1977) observed that amino acids were utilized by microbes in meat after the exhaustion of glucose.

4.33 L. monocytogenes Growth in Cooked Meats

L. monocytogenes was inoculated into cooked FUT and CMT, and its growth potential was evaluated in these tissues. Overall, the color modification procedure was not a factor ($p > 0.05$) affecting the proliferation of listeriae at 4°C (Fig.

6). However, the interaction of color modification and time was significant ($p < 0.05$).

L. monocytogenes concentrations increased approximately 5 logs in cooked tissues stored at 4°C for 14 days; after which, numbers increased only slightly until day 21 (Fig. 6). Slower multiplication in samples after 14 days may be attributable to nutrient depletion and/or buildup of waste products.

Listeriae grew at approximately the same rate in FUT and CMT until day 4. On day 7, L. monocytogenes numbers in CMT were higher ($p < 0.05$) than those in FUT. This finding was responsible for the significant interaction between color modification and time. At other sampling intervals, however, no differences ($p > 0.05$) in L. monocytogenes counts occurred between the two tissues. After approximately 12 days, numbers in FUT tended to be slightly higher than those in CMT throughout the rest of the study.

Generally, it does not appear that the growth of L. monocytogenes was influenced by the color modification procedure. Multiplication of this pathogen at 4°C was not surprising since prior research has shown L. monocytogenes to grow at a minimum temperature of 2.5°C (Petran and Zottola, 1989).

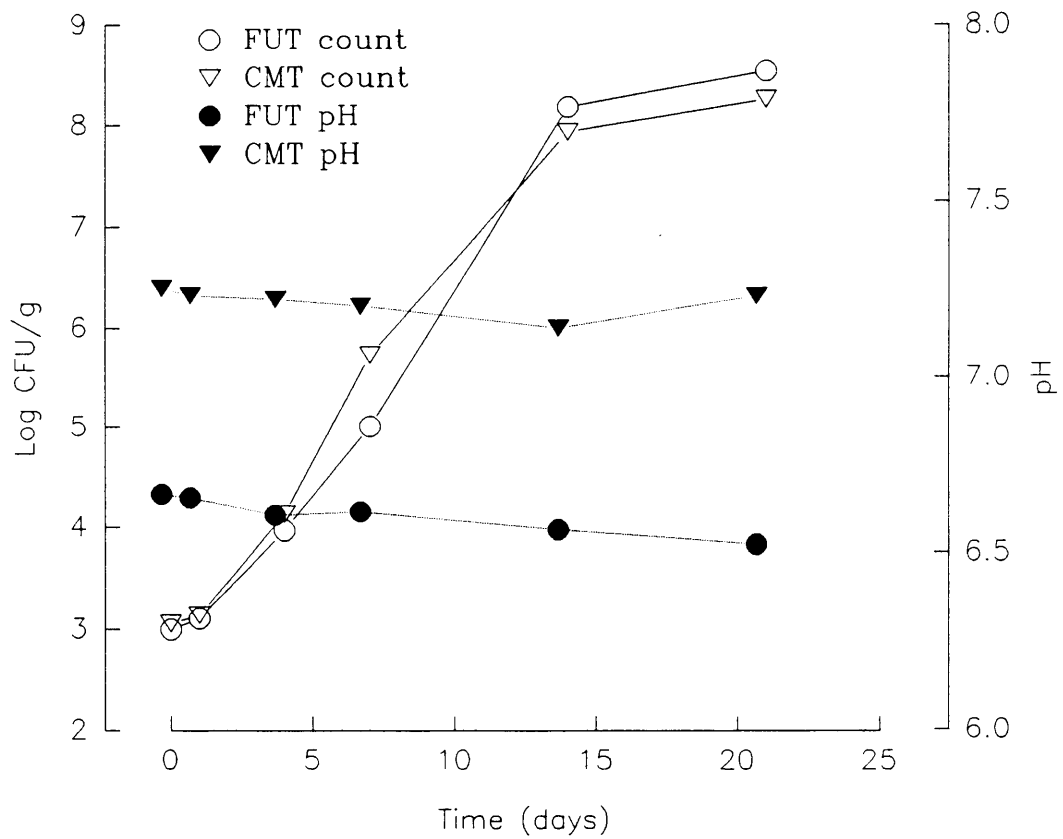


Figure 6. Growth of *L. monocytogenes* and pH profile during storage of FUT and CMT at 4°C. Each data point is the mean of four observations.

Changes in the pH of tissues held at 4°C and inoculated with L. monocytogenes tended to be slight (Fig 6). Johnson et al. (1988) observed only a small change in the pH (5.6 to 5.9) of ground beef inoculated with L. monocytogenes and held at 4°C for 2 weeks.

Cooked FUT and CMT inoculated with L. monocytogenes were stored at 20°C for up to 48 hr (Fig. 7). As in the 4°C study with L. monocytogenes, color modification did not influence ($p > 0.05$) the growth of this pathogen. Furthermore, numbers of listeriae did not differ between FUT and CMT ($p > 0.05$) at any sampling time.

Trends of the L. monocytogenes growth curves indicate that this pathogen proliferated at virtually the same rate in both tissues at 20°C (Fig. 7). Cultures in FUT and CMT increased to above 8.0 log CFU/g by 48 hr from an initial inoculum of 3.0 log CFU/g. A 4-log increase was seen from 0 to 24 hr in both tissues, before growth slowed from 24 to 48 hr.

pH values decreased initially in FUT and CMT before rising to approximately pH 7.0 after 36 hr in both tissues (Fig. 7). The breakdown of amino acids by L. monocytogenes after 36 hr is probably not a suitable explanation for this observation since phenylalanine deaminase; ornithine, lysine, and arginine decarboxylase; and arginine dihydrolase are not

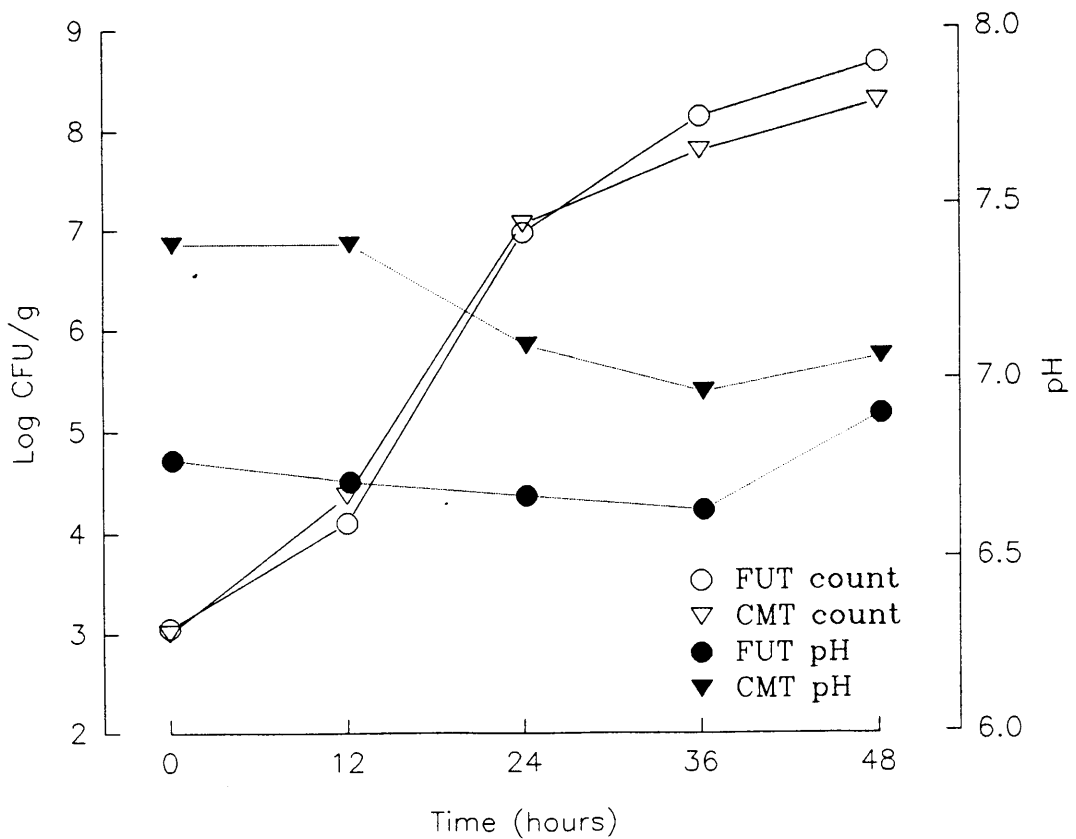


Figure 7. Growth of *L. monocytogenes* and pH profile during storage of FUT and CMT at 20°C. Each data point is the mean of four observations.

produced by this organism (Wetzler, 1968). The growth of an apparent Bacillus spp. (gram-, catalase-, and oxidase-positive, aerobic sporeformer) could possibly explain the rise in pH. This organism was isolated after 36 hr from samples of FUT and CMT inoculated with L. monocytogenes.

Carpenter and Harrison (1989) and Harrison and Carpenter (1989) demonstrated that L. monocytogenes populations which survived cooking in chicken breasts could proliferate at typical refrigeration (4°C) and at slightly abusive temperatures (10°C). However, a slower growth rate would be expected for cells injured by thermal processing. As a result, comparing the growth characteristics of L. monocytogenes in the above studies (pre-processing contamination) to those of the present research (post-processing contamination) would be difficult. Nevertheless, L. monocytogenes was shown to grow in cooked poultry at 4°C in the current and cited studies. Perhaps the interaction of such factors as pH, temperature, microbial flora, and nutrient content dictates the ability of L. monocytogenes to grow in meats.

5.0 SUMMARY AND CONCLUSIONS

The effect of color modification on the microbial properties of turkey thigh was investigated. Microbial quality of raw CMT was evaluated by estimating aerobic and psychrotrophic bacterial counts in samples held under refrigerated (3°C) and frozen (-20°C) storage. Sanitary conditions of raw material processing were assessed by monitoring coliform levels. To simulate post-processing contamination with pathogenic bacteria, the fate of Salmonella and L. monocytogenes was evaluated in cooked CMT stored at 4°C and 20°C. In all studies, pH and moisture were not considered major factors influencing microbial growth.

Aerobic and psychrotrophic bacterial counts in FUT and CMT did not differ ($p > 0.05$) at any sampling interval during 5 days of refrigerated storage. Aerobic numbers in FUT and CMT increased 0.77 logs and 1.07 logs, respectively. The increase in psychrotrophic counts was 2.38 logs in FUT and 3.32 logs in CMT. Coliform numbers in 1-day FUT and CMT were not different ($p > 0.05$).

Generally, counts of pathogens in FUT and CMT did not differ ($p > 0.05$) at selected sampling intervals of the inoculation studies. Salmonella concentrations declined gradually in cooked raw materials held at 4°C. Counts in CMT tended to be lower than those in FUT after day 9. By day 21, numbers dropped only slightly in FUT, but by 0.5 logs in CMT.

A difference in Salmonella levels ($p < 0.05$) might have been observed if the study would have been extended beyond 21 days of storage.

Salmonella proliferated essentially at the same rate in inoculated, cooked samples of FUT and CMT held at 20°C. Counts in both tissues increased rapidly (approximately 6 logs) from 0 to 2 days before peaking around 10 log CFU/g at 3 days. Although no difference ($p > 0.05$) in Salmonella numbers existed between FUT and CMT at any sampling time, Salmonella counts in CMT were slightly lower than those in FUT at all evaluation intervals following day 2.

L. monocytogenes grew rapidly in cooked tissues held at 4°C and 20°C. Counts increased approximately 5 logs in samples held at 4°C for 14 days before L. monocytogenes multiplication slowed. On day 7, numbers in cooked CMT were higher ($p < 0.05$) than those in FUT. When considering all studies (microbial quality of raw tissues and inoculation studies), this was the only time a difference ($p < 0.05$) in microbial concentrations was observed between FUT and CMT.

By 48 hrs at 20°C, numbers of listeriae in CMT and FUT increased more than 5 logs. A 4 log increase was seen from 0 to 24 hrs in both tissues; after which, the growth rate slowed.

Although it was found to be significant ($p < 0.05$) in some studies, color modification was not considered a major

factor affecting microbial growth in thigh tissues. Most importantly, CMT as compared to FUT, was not regarded as a more favorable growth substrate for pathogenic bacteria evaluated in this research.

Factors which potentially influence microbial properties of food products created by new processes should be identified by scientists and processors. This measure allows development of handling and processing conditions which ensure the safety and quality of modified foods. From a microbial standpoint, results from this research may aid processors who wish to utilize CMT as a raw material for further processed poultry products.

REFERENCES

- Adu, G. A., Babbit, J. D., and Crawford, D. L. 1983. Effect of washing on the nutritional and quality characteristics of dried minced rockfish flesh. *J. Food Sci.* 48:1053.
- AOAC. 1984. "Official Methods of Analysis," 14th ed. Association of Official Analytical Chemists, Washington, D.C.
- APHA. 1984. "Compendium of Methods for the Microbiological Examination of Foods," 2nd ed. American Public Health Association, Washington, D.C.
- Ayers, J. C. 1960. The relationship of organisms of the genus Pseudomonas to the spoilage of meat, poultry, meat. *J. Appl. Bacteriol.* 23:471.
- Bailey, J. S., Thomson, J. E., and Cox, N. A. 1987. Contamination of poultry during processing. In "The Microbiology of Poultry Meat Products". F. E. Cummingham and N. A. Cox (Ed.), p. 293. Academic Press, Inc., Orlando.
- Ball, H. R. and J. C. Montejano. 1984. Composition of washed broiler thigh meat. *Poul. Sci.* 63 (Suppl.1):60.
- Banwart, G. J. 1989. Food spoilage. In "Basic Food Microbiology," G. J. Banwart (Ed.), 2nd ed., p 393. Van Nostrand Reinhold, New York.
- Baran, W. L., Dawsin, L. E., and Lechowich, R. V. 1973. Influence of chlorine dioxide water treatment on numbers of bacteria associated with processed turkey. *Poul. Sci.* 52:1053.
- Barnes, E. M. 1960. The sources of the different psychrophilic organisms on chilled eviscerated poultry. *Proc. Int. Congr. Refrig.*, 10th, Copenhagen 3:97.
- Barnes, E. M. 1975. The microbiological problems of sampling poultry carcasses. *Qual. Poul. Meat, Proc. Eur. Symp. Poul. Meat Qual.*, 2nd, Ousterbech, Neth 23:1.
- Barnes, E. M. and Impey, C. S. 1968. Psychrophilic spoilage bacteria of poultry. *J. Appl. Bacteriol.* 31:97.

- Barnes, R., Archer, P., and Stack, J. 1989. Listeriosis associated with consumption of turkey franks. *Morbid. Mortal. Weekly Rpt.* 38 (15):267.
- Bryan, F. L. 1980. Poultry and Poultry Meat Products. In "Microbial Ecology of Foods," Vol 2, p. 410. Academic Press, New York.
- Bryan, F. L., Ayres, J. C., and Kraft, A. A. 1968. Contributory sources of salmonellae on turkey products. *Am. J. Epidemiol.* 87:578.
- Buchanan, R. C., H. G. Stahl, and D. L. Archer. 1987. Improved plating media for simplified, quantitative detection of Listeria monocytogenes in foods. *Food Microbiol.* 4:269.
- Carpenter, S. L. and Harrison, M. A. 1989. Survival of Listeria monocytogenes on processed poultry. *J. Food Sci.* 54:556.
- CDC. 1985. Listeriosis outbreak associated with Mexican-style cheese-California. *Morbid. Mortal. Wkly. Rept.* 34:357. Centers for Disease Control, Atlanta, Ga.
- Code of Federal Regulations. 1988. Title 9, Section 381.150.
- Committee on Salmonella. 1969. "An Evaluation of the Salmonella Problem," Publ. #1683, National Acad. Sci. Washington, D.C.
- Cornish, D. G. and Froning, G. W. 1974. Isolation and purification of turkey heme proteins. *Poul. Sci.* 53:365.
- Cox, N. A. and Bailey, J. S. 1987. Pathogens associated with processed poultry. In "The Microbiology of Poultry Meat Products". F. E. Cummingham and N. A. Cox (Ed.), p. 293. Academic Press, Inc., Orlando.
- Dawson, P. L., Sheldon, B. W. and Ball, H. R., Jr. 1988. Extration of lipid and pigment components from mechanically deboned chicken meat. *J. Food Sci.* 53:1615.
- DeDuve, C. A. 1948. Spectrophotometric method for simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. *Acta Chem. Scand.* 2:264.

- Denton, J. H. and Gardner, F.H. 1981. Effect of further processing systems on selected microbiological attributes of turkey meat products. J. Food Sci. 47:214.
- Dickson, J. S. 1988. Reduction of bacteria attached to meat surfaces by washing with selected compounds. J. Food Prot. 51:869.
- Dickson, J. S. and Koohmaraie, M. 1989. Cell charge characteristics and their relationship to bacterial attachment to meat surfaces. J. Appl. Microbiol. 55:832.
- Dougherty, T. J. 1974. Salmonella contamination in a commercial poultry (broiler) processing operation. Poul. Sci. 53:814.
- Dowell, V. R. 1982. Salmonellosis associated with the ingestion of meats in the United States. In "Control of the Microbial Contamination of Foods and Foods in International Trade; Microbial Standards and Specifications," H. Kurata and S. W. Hesselstine (Ed.), p. 95. Saikon Publishing Co., Ltd., Tokoyo.
- Duitschaeffer, C. L. 1977. Incidence of salmonellae in retailed raw cut-up chicken. J. Food Prot. 40:191.
- Eddy, B. P. 1960. The use and meaning of the term 'psychrophilic'. J. Appl. Bacteriol. 23:189.
- Elkhalifa, E. A., Graham, P. P., Marriott, N. G., and Phelps, S. K. 1988. Color and functional properties of dark turkey meat as influenced by washing treatments. J. Food Sci. 53:1068.
- Farber, J. M. and Idziak, E. S. 1984. Attachment of psychrotrophic meat spoilage bacteria to muscle surfaces. J. Food Prot. 47:92.
- Fleming, D. W., Cochi, S. L., MacDonald, K. L., Brondum, J., Hayes, P. S., Plikaytis, B. D., Holmes, M. B., Audurier, A., Broome C. V., and Reingold, A. L. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. New Engl. J. Med. 312:404.

- Fleming, H. P., Blumer, T. N., and Craig, H. B. 1960. Quantitative estimation of myoglobin and hemoglobin in beef muscle extracts. *J. Animal Sci.* 19:1164.
- Flowers, R. S. 1988. Salmonella: Bacteria associated with foodborne disease. *Food Technol.* 42:181.
- Fox, J. B., Jr. 1966. The chemistry of meat pigments. *J. Agric. Food Chem.* 14:207.
- Freeman, L. R., Silverman, G. J., Angelini, P., Merritt, J. C., and Esselen, W. B. 1976. Volatiles produced by microorganisms isolated from refrigerated chicken at spoilage. *Appl. Environ. Microbiol.* 32:222.
- Froning, G. W. 1987. Further-processed poultry meat products. In "The Microbiology of Poultry Meat Products," F. E. Cunningham and N. A. Cox (Ed.), p. 317. Academic Press Inc., New York.
- Froning, G. W., Daddario, J., and Hartung, T. E. 1968. Color and myoglobin concentration in turkey meat as affected by age, sex and strain. *Poul. Sci.* 47:1827.
- Fung, D. Y. C. 1987. Types of microorganisms. In "The Microbiology of Poultry Meat Products," F. E. Cunningham and N. A. Cox (Ed.), p. 5. Academic Press, Inc., New York.
- Gardner, F. A. and Golan, E. A. 1974. Carcass and equipment microbiological levels associated with processing turkeys. *Poul. Sci.* 53:1926.
- Gill, C. O. 1976. Substrate limitation of bacterial growth at meat surfaces. *J. Appl. Bacteriol.* 41:401.
- Gill, C. O. 1986. The control of microbial spoilage in fresh meats. In "Advances in Meat Research," A. M. Pearson and T. R. Dutson (Ed.), Vol. 2, p. 49. The Avi Publishing Co., Inc., Westport, Conn.
- Gill, C. O. and Newton, K. G. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. *J. Appl. Bacteriol.* 43:189.
- Gill, C. O. and Newton, K. G. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. *Meat Sci.* 2:207.

- Gill, C. O. and Penny, N. 1977. Penetration of bacteria into meat. *Appl. Environ. Microbiol.* 33:1284.
- Gottschalk, G. 1986. Nutrition of bacteria. In "Bacterial Metabolism," G. Gottschalk (Ed.), 2nd ed., p.1. Springer-Verlag, New York.
- Gouet, P. Labadie, J., and Serratore, C. 1978. Development of L. monocytogenes in monoxenic and polyxenic beef minces. *Zentrabl. Bakteriolog. Hyg., I. Abt. Orig. B* 166:87.
- Gray, M. L. and Killinger, A. H. 1966. Listeria monocytogenes and listeric infections. *Bacteriol. Rev.* 30:309.
- Guthertz, L. S., Fruin, F. T., Spicer, D., and Spicer, J. C. 1976. Microbiology of fresh comminuted turkey meat. *J. Milk Food Technol.* 39:823.
- Guthertz, L. S., Fruin, F. T., Okoluk, R. L., and Fowler, J. L. 1977. Microbial quality of frozen comminuted turkey meat. *J. Food Sci.* 42:1344.
- Hagberg, M. M., Busta, F. F., Zottola, E. A., and Arnold, E. A. 1973. Incidence of potentially pathogenic microorganisms in further-processed turkey products. *J. Milk Food Technol.* 36:625.
- Harrison, M. A. and Carpenter, S. L. 1989. Survival of large populations of Listeria monocytogenes on chicken breasts processed using moist heat. *J. Food Prot.* 52:376.
- Hartman, P. L. 1979. Modification of conventional methods for recovery of injured coliforms and salmonellae. *J. Food Prot.* 42:356-361.
- Hernandez, A., Baker, R. C., and Hotchiss, J. H. 1986. Extraction of pigments from mechanically deboned turkey meat. *J. Food Sci.* 51:865.
- Ho, J. L., Shands, K. N., Friedland, G., Eckind, P., and Fraser, D. W. 1986. An outbreak of type 4b Listeria monocytogenes infection involving patients from eight Boston hospitals. *Arch. Intern. Med.* 146:520.
- Hornsey, H. C. 1956. The color of cured pork. 1. Estimation of the nitric oxide heme pigments. *J. Sci. Food Agric.* 7:534.

- Jay, J. M. 1986a. Intrinsic and extrinsic parameters of foods that affect microbial growth. In "Modern Food Microbiology," J. M. Jay (Ed.), 3rd ed., p. 33. Van Nostrand Reinbold Co., Inc., New York.
- Jay, J. M. 1986b. Food-borne gastroenteritis caused by Salmonella and Escherichia. In "Modern Food Microbiology," J. M. Jay (Ed.), 3rd ed., p. 489. Van Nostrand Reinbold Co., Inc., New York.
- Johnson, J. L., Doyle, M. P. and Casseas, R. G. 1988. Survival of L. monocytogenes in ground beef. J. Food Microbiol. 6:243.
- Kahn, M. A., Palmas, C. V., Seaman, A., and Woodbine, M. 1973. Survival vs. growth of a facultative psychrotroph: meat and products of meat. Zentrabl. Bacteriol. Hyg., I. Abt. Orig. B. 157:277.
- Licciardello, J. J. and Hill, W. S. 1978. Microbiological quality of commercial frozen minced fish blocks. J. Food Prot. 41:948.
- Matches, J. R. and Liston, J. 1969. Low temperature growth of Salmonella. J. Food Sci. 33:641.
- Maxcy, R. B., Froning G.W., Hartung, T.E. 1973. Microbial quality of ground poultry meat. Poul. Sci. 52:486.
- May, K. N. 1962. Bacterial contamination during cutting and packaging chicken in processing plants and retail stores. Food Technol. 16:89.
- May, K. N. 1974. Changes in microbial numbers during final washing and chilling of commercially slaughtered broilers. Poult. Sci. 53:1282.
- May, K. N., Irby, J. D., and Carman, J. L. 1962. Shelf-life and bacterial counts of excised poultry tissue. Food Technol. 16:66.
- McBride, G. B., Skura, B. J., Yada, R. Y. and Bowmer, E. J. 1980. Relationship between incidence of Salmonella contamination among pre-scalded eviscerated and post-chilled chicken in a poultry processing plant. J. Food Prot. 43:538.

- McMeekin, T. A. 1977. Spoilage association of chicken leg muscle. *Appl. Environ. Microbiol.* 33:1244.
- Miyauchi, D., Kudo, G., and Patashnik, M. 1973. Surimi - A semi-processed wet fish protein. *Mar. Fish Rev.* 35(12):7.
- Morad, M. A., Branen, A. L., and Brekke. 1982. Antimicrobial activity of butylated hydroxyanisole and potassium sorbate against natural microflora in raw turkey meat and Salmonella typhimurium in cooked turkey meat. *J. Food Prot.* 45:1038.
- Morris, G. K., and Ayres, J. C. 1960. Incidence of salmonellae on commercial processed poultry. *Poul. Sci.* 39:1131.
- Morrison, G. J., and Fleet, G. H. 1985. Reduction of Salmonella on chicken carcasses by immersion treatments. *J. Food Prot.* 48:939.
- Nagel, C. W., Simpson, K. L., Ng, H., Vaughn, R. H., and Stewart, G. F. 1960. Microorganisms associated with spoilage of refrigerated poultry. *Food Technol.* 14:21.
- Nieman, R. E. and Lorber, B. 1980. Listeriosis in adults: a changing pattern. Report of eight cases and review of the literature, 1968-1978. *Rev. Infect. Dis.* 2:207.
- Newsome, R. L., Seman, D. L., Langlois, B. E. Moody, W. G., Fox, J. D., and Gay, N. 1987. Microbiological quality of restuctured and conventional steaks manufactured from electrically stimulated hot- and cold-boned carcasses. *J. Food Sci.* 52:861.
- Ostovar, K., MacNeil, J.H., and O'Donnel, K. 1971. Poultry product quality. 5. Microbiological evaluation of mechanically deboned poultry meat. *J. Food Sci.* 36:1005.
- Petron, R. L., and Zottola, E. A. 1989. A study of factors affecting growth and recovery of L. monocytogenes Scott A. *J. Food Sci.* 54:458.
- Phelps, S. K., Marriott, N. G., Graham, P. P., Barbeau, W. E. 1990. Nutrient characterization of color modified and unaltered turkey thigh meat. *J. Muscle Foods* (In Press).

- Richansrud, D. A. and Henrickson, R. L. 1967. Total pigments and myoglobin concentration in four bovine muscles. *J. Food Sci.* 32:57.
- Sadler, W. W., and Corstvet, R. E. 1965. Second survey of market poultry for Salmonella infection. *Appl. Microbiol.* 13:348.
- Sadler, W. W., Yamamoto, R., Adler, H. E., and Stewart, G. F. 1961. Survey of market poultry for Salmonella infection. *Appl. Microbiol.* 9:72.
- SAS. 1984. "SAS User Guide Statistics," A. A. Ray (Ed.), SAS Inst., Inc., Cary, N. C.
- Schlech, W. F. III, Lavigne, P. M., Bortolussi, R. A., Allen, A. C., Haldane, E. V., Wort, A. J., Hightower, A. W., Johnson, S. E., King, S. H., Nicholls, E. S., and Broome, C. V. 1983. Epidemic listeriosis-Evidence for transmission by food. *New Eng. J. Med.* 308:203.
- Schnaitman, C. A. 1981. Cell fractionation. In "Manual of Methods for General Bacteriology," P. Gerhardt, R. E. Murray, R. N. Costilow, W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (Ed.), p.56. ASM, Washington, D.C.
- Seeliger, H. P. R. and Finger, H. 1976. Listeriosis. In "Infectious Diseases of the Fetus and Newborn Infant," J. S. Remington and J. O. Klein (Ed.), p. 333. W. B. Saunders Co., Philadelphia.
- Seeliger, H. P. R., and Jones, D. 1986. Listeria. In "Bergey's Manual of Systematic Bacteriology," P. H. A. Sneath (Ed.), Vol. 2, p. 1235.
- Shelef, L. A. 1989. Survival of Listeria monocytogenes in ground beef or liver during storage at 4 and 25°C. *J. Food Prot.* 52:379.
- Suzuki, T. 1981. "Fish and Krill Protein: Processing Technology," Applied Science Publishers, Ltd., London.
- Thomas, C. J. and McMeekin, T. A. 1981. Attachment of Salmonella spp. to chicken muscle surfaces. 42:130.

- To, E. C. and Robach, M. C. 1980. Inhibition of food poisoning microorganisms by sorbic acid in cooked, uncured, vacuum packaged turkey products. J. Food Technol. 15:543.
- USDA. 1988. Poultry slaughter. Pou 2-1, p.3. Agricultural Statistics Board.
- Warris, P. D. 1979. The extraction of heme pigments from fresh meat. J. Food Technol. 14:75.
- Wetzler, T. F., Freeman, N. R., French, M. L. V., Renkowski, L. A., Eveland, W. C., and Carver, O. J. 1968. Biological characterization of Listeria monocytogenes. Health Lab Sci. 5:46.
- White, C. A. and Hall, L. P. 1984. The effect of temperature abuse on Staphylococcus aureus and salmonellae in raw beef and chicken substrates during frozen storage. Food Microbiol. 1:29.
- Yoon, I. H., Matches, J. R., and Rasco, B. 1988. Microbiological and chemical changes of surimi-based imitation crab during storage. J. Food Sci. 53:1343.
- Yoon, I. H. and Matches, J. R. 1988. Growth of pathogenic bacteria on imitation crab. J. Food Sci. 53:688.
- Zar, J. H. 1984. "Biostatistical Analysis," 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N. J.
- Zottola, E. A., and Busta, F. F. 1971. Microbiological quality of further-processed turkey products. J. Food Sci. 36:1971.

APPENDICES

Appendix A - Salmonella numbers estimated in inoculated FUT
and CMT stored at 4 and 20°C.

Time (days)	Log CFU/g \pm S.D.	
	FUT	CMT
4°C		
0	3.57 \pm .12	3.56 \pm .10
1	3.53 \pm .17	3.51 \pm .14
4	3.53 \pm .12	3.50 \pm .07
7	3.40 \pm .07	3.45 \pm .10
14	3.37 \pm .12	3.21 \pm .34
21	3.31 \pm .35	3.02 \pm .28
20°C		
0	3.57 \pm .12	3.56 \pm .10
1	7.71 \pm .23	7.65 \pm .46
2	9.62 \pm .28	9.26 \pm .17
3	9.93 \pm .07	9.78 \pm .08
4	9.89 \pm .14	9.71 \pm .19
5	9.96 \pm .06	9.83 \pm .07

Appendix B - L. monocytogenes numbers estimated in inoculated FUT and CMT stored at 4 and 20°C.

Time (days)	Log CFU/g \pm S.D.	
	FUT	CMT
4°C		
0	3.00 \pm .06	3.06 \pm .13
1	3.11 \pm .03	3.14 \pm .11
4	3.97 \pm .12	4.13 \pm .19
7	5.01 \pm .67	5.73 \pm .77
14	8.19 \pm .29	7.94 \pm .41
21	8.68 \pm .08	8.27 \pm .28
20°C		
0	3.06 \pm .13	3.01 \pm .05
12	4.10 \pm .05	4.37 \pm .10
24	6.97 \pm .73	7.06 \pm .44
36	8.14 \pm .14	7.79 \pm .10
48	8.68 \pm .47	8.29 \pm .90

Appendix C - ANOVA of aerobic counts on FUT and CMT samples held at 3°C.

Source	df	Mean Square	F Value	p
Replication	2	.6209	4.04	.1983
Treatment (T)	1	2.5538	16.62	.0552
Wholeplot error	2	.1536	0.57	.5884
Days (D)	2	1.5543	5.74	.0285
D*T	2	.0377	0.14	.8721
Subplot error	8	.2709		

Appendix D - ANOVA of psychrotrophic counts on FUT and CMT samples held at 3°C.

Source	df	Mean Square	F Value	p
Replication	2	1.3753	5.37	.1571
Treatment (T)	1	1.1961	4.67	.1633
Wholeplot error	2	.2563	2.68	.1289
Days (D)	2	12.2361	127.73	.0001
D*T	2	.3561	3.72	.0722
Subplot error	8	.0958		

Appendix E - ANOVA of Salmonella counts estimated in inoculated FUT and CMT held at 4°C.

Source	df	Mean Square	F Value	p
Replication	3	.1662	25.05	.0126
Treatment (T)	1	.0856	12.89	.0370
Wholeplot error	3	.0066	0.22	.8727
Days (D)	4	.1827	6.19	.0014
D*T	4	.0364	1.23	.3230
Subplot error	24	.0295		

Appendix F - ANOVA of Salmonella counts estimated in inoculated FUT and CMT held at 20°C.

Source	df	Mean Square	F Value	p
Replication	3	.0676	2.35	.2511
Treatment (T)	1	.3168	10.99	.0452
Wholeplot error	3	.0288	0.66	.5863
Days (D)	4	7.1050	162.01	.0001
D*T	4	.0258	0.59	.6735
Subplot error	24	.0439		

Appendix G - ANOVA of L. monocytogenes counts estimated in inoculated FUT and CMT samples held at 4°C.

Source	df	Mean Square	F Value	p
Replication	3	.9609	1.46	.3809
Treatment (T)	1	.0585	0.09	.7847
Wholeplot error	3	.6564	20.30	.0001
Days (D)	4	44.6705	1381.23	.0001
D*T	4	.3249	10.04	.0001
Subplot error	24	.0323		

Appendix G - ANOVA of L. monocytogenes counts estimated in inoculated FUT and CMT held at 20°C.

Source	df	Mean Square	F Value	p
Replication	3	.6351	14.73	.0267
Treatment (T)	1	.0741	1.72	.2812
Wholeplot error	3	.0431	0.31	.8210
Days (D)	3	28.7386	203.69	.0001
D*T	3	.2095	1.48	.2523
Subplot error	18	.1410		

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