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EFFECT OF METHOD OF FREEZING, PROCESSING AND PACKAGING VARIABLES ON MICROBIOLOGICAL AND OTHER QUALITY CHARACTERISTICS OF BEEF AND POULTRY

Iowa State University PH.D. 1981

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Effect of method of freezing, processing and packaging variables on microbiological and other quality characteristics of beef and poultry

by

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INTRODUCTION

INTRODUCTION

All organic substances such as meat, fish and vegetables contain enzymes and microorganisms which activate the decomposition process unless retarded or arrested by some means such as freezing, processing and/or packaging. There has been considerable success in improving the quality and storage life through these procedures over the past few decades.

In the early days of frozen foods, a sacrifice of quality for convenience was accepted with drastic results for the processor and it is unfortunate that this motive occasionally is still apparent in the attitude of both buyer and supplier when alternatives are available. It is generally accepted that faster freezing rates produce higher quality products than do slower freezing rates, yet some findings of experimental studies disagree with this especially in the area of tenderness measurements on meats (Cunningham, 1973; Streeter and Spencer, 1973). However, the beneficial effects of using cryogens have been demonstrated for various food products by several investigators (Novak and Rao, 1966; Khan and Van Den Berg, 1967; Li et al., 1969 and Kuschfeldt and Thiel, 1970; Reddy, 1978).

Recently, a great deal of interest has been focused on the beef industry since fast food restaurants use different proportions of frozen and fresh beef for hamburger patties which

are subsequently cryogenically frozen. Ground beef consumption is expected to become larger in the near future. Fast food chains may use frozen imported beef, as high as 50%, with available fresh beef in their hamburgers. Unfortunately, there is no information available on how this practice might affect microbiological quality of beef patties.

A rapid expansion in commercial processing of eviscerated, ready-to-cook poultry in the United States has resulted in centralizing the operations in order to reduce labor costs and improve sanitation at the packer level because the latter has better equipment and better control procedures than the retailer. Quite some time ago, Goresline et al. (1951) recommended the chlorination of water supplies used in poultry processing plants. Also, the early work of Clarke (1954) has established that in-plant chlorination reduces the total number of viable bacteria on the surface of eviscerated poultry. The greater bacterial activity of free as opposed to combined chlorine is well illustrated by the data given by Allen (1961). The recent U.S.D.A. proposal (Federal Register, 1978) to use a chlorine dip or spray at a concentration of 20 ppm free chlorine is being given consideration on the basis of its effectiveness in poultry processing.

Modern marketing techniques involve the prepackaging of many kinds of foods in order to facilitate distribution.

The influence of packaging films on the storage life of chicken has been demonstrated by Kish, 1953; Spencer et al., 1956; and Shrimpton and Barnes, 1960. Stretchable and shrinkable polyvinyl chloride films having low oxygen permeabilities have been developed to inhibit the growth of aerobic spoilage bacteria. However, such packaging films were not sufficient to allow significant oxidative deamination by bacteria since a considerable amount of oxygen is still present in the package (Debevere and Voets, 1973). Further investigations are to be considered to improve packaging materials and methods in order to retain vacuum and thereby extend the shelf life of broilers.

This work has been designed to evaluate and improve certain facets of freezing, processing and packaging that are of concern to both the industry and the consumer at the present time. Various aspects of freezing preservation and packaging were studied for feasibility in practical applications, but detailed consideration was also given to microorganisms involved. Specifically, the following objectives were considered.

1. To determine the effects of combining fresh and frozen beef in different proportions on the microbial flora of ground beef patties. Numerical taxonomy was applied to detect changes in type of bacterial flora due to cryogenic

freezing (liquid nitrogen (LN_2) or liquid carbon dioxide (LCO_2) and subsequent frozen storage.

2. In line with investigations of frozen meat, to compare the effects of cryogenic and conventional freezing methods on tenderness and other quality characteristics of turkey meat.

3. Packaging methods and materials were studied by evaluating the performance of a high barrier film to oxygen for suitability for vacuum packaging of chicken. In addition, this work was combined with an investigation of the effects of a chlorine dip on shelf life of eviscerated broilers.

LITERATURE REVIEW

Freezing of Foods

Fast freezing may cause damage to many foods (Morphew, 1969); but, it has also been observed that increasing the rate of freezing within certain limits results in less structural damage, better taste, and lower drip loss for many food products. This has been reported for meats (Gray, 1967; Bengtsson and Jakabsson, 1969), fish (Piskarev and Bomovalova, 1969), baked goods (Breyer et al., 1966), peppers, green beans, cauliflower (Monzini et al., 1969), mushrooms (Åström and Löndahl, 1969), tomatoes (Anonymous, 1964) and many other foods.

Average freezing times for poultry with conventional type refrigeration have been reported to range from as much as 30 hours for still air to 5 hours for air blast freezing (Ryan, 1966). Research for improvement of quality has led to the development of faster freezing methods. Carbon dioxide sublimes at -78.5C. The use of carbon dioxide as a refrigerant started to develop in the early 1930s. Freezing systems with solid carbon dioxide require mechanical action such as tumbling to increase contact between refrigerant and food (Anonymous, 1975).

The first attempt at freezing foods with liquids of extremely low boiling points were made in the decade of the 1940s

by immersion of foods in nitrous oxide (Breyer et al., 1966). The demand for liquid oxygen due to developments in aerospace, chemical and steel industries, resulted in the production of enormous amounts of liquid nitrogen (LN_2) as a by-product. LN_2 boils at -196C. When boiling in contact with food, it absorbs 86 BTU per pound (heat of vaporization). The gas produced continues to absorb heat at the rate of 0.45 BTU/pound/degree centigrade until the temperature of the food and the temperature of the gas equilibrate. From this comes the tremendous potential of LN_2 as a refrigerant. Immersion techniques for freezing with LN_2 have not been satisfactory (Breyer et al., 1966). The thermal shock results in cracking and peeling of many products, the cold gases are wasted through inefficient use of refrigerant, and violent boiling at the surface of the product prevents efficient contact between the refrigerant and the food. Most LN_2 freezers employ spray methods (Novak and Rao, 1966). Precooling of the product by nitrogen gas conditions it for the low temperature of the spray zone, diminishing the effect of thermal shock. The aim of these processes is to supercool the outer shell which freezes while the core temperature is still warm. After spraying, the products are stored under conventional refrigeration where the core and the surface temperature reach equilibrium - this depends on the depth at which the outer shell is frozen as well as on the temperature at which the

product is stored.

Cryogenic Meat Processing in the United States

An estimate of the present cryogenic fluid market for food freezing and its growth potential through 1987 was given by Harr and Minard, 1977 (Figures 1 and 2). In a 15-year period, cryogenic food freezing has progressed from the experimental stage to an accepted process. At the present, 98% of the ground beef patties used by fast food restaurants are cryogenically frozen (Ellis, 1979). Increased tendencies to have meals away from home indicate that Americans now eat one in five meals away from home and this frequency will increase to one in three meals by 1983 if the present trend continues, which will further affect the market for cryogenic materials (Ernst, 1979).

Since hamburger is by far the leading meat type consumed in commercial restaurants, followed by chicken, hot dogs and steak (Table 1), an expected 47% of new growth is expected to come from the red meat area. The increase in demand for hamburger meat has resulted in an increase in imports from 1640 million pounds of beef in the early 1970s to 2000 million pounds at the present (Harr and Minard, 1977). The frozen imported beef is combined with fresh meat for the hamburger patties which are subsequently frozen with LN_2 or LCO_2.

Figure 1. Estimated combined nitrogen and carbon dioxide market for freezing from 1971 through 1987 (1000's tons/year equivalent CO_2 by year)

Figure 2. Estimated growth in CO_2 chilling market from 1979 through 1982

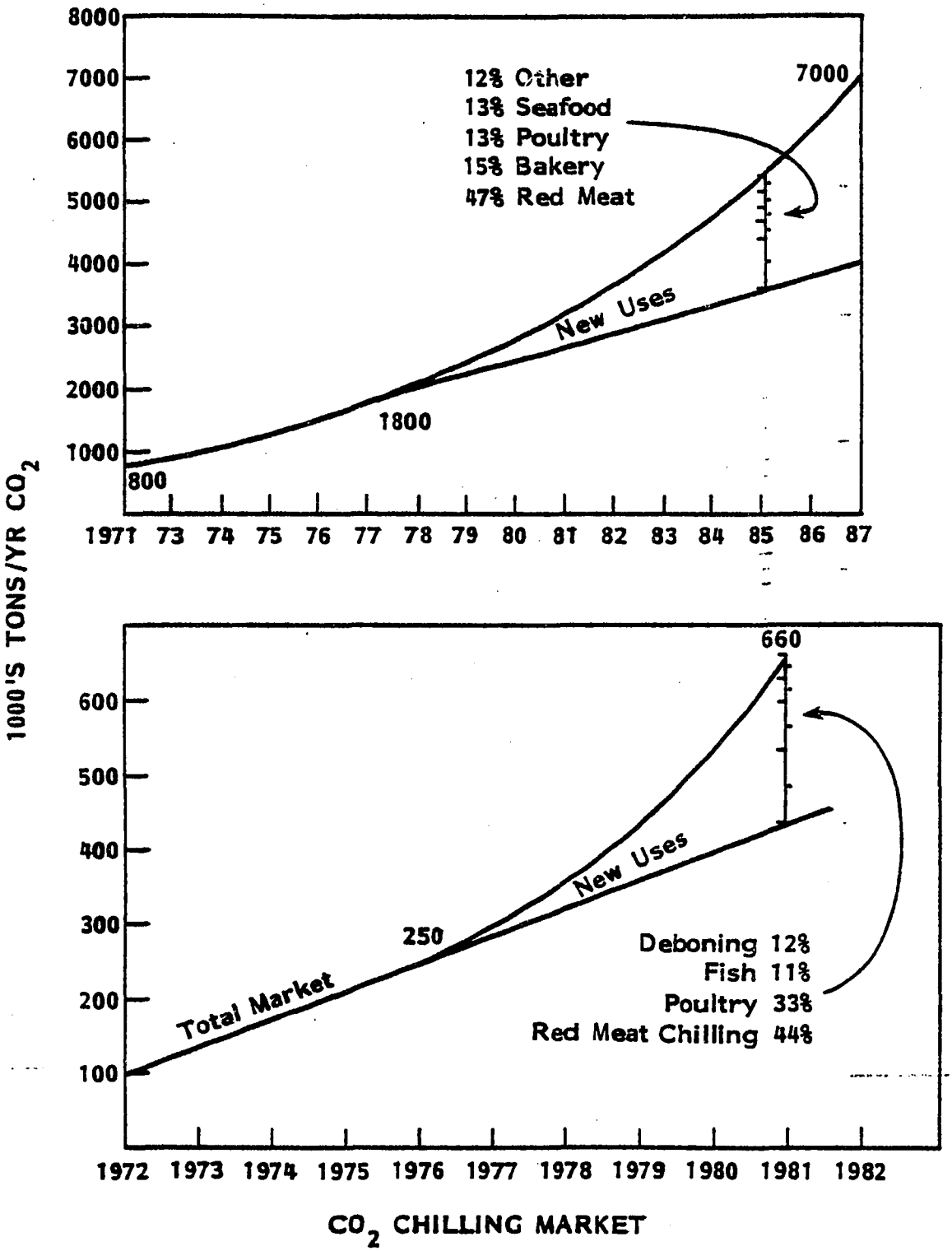

8000
7000
6000
5000
4000
3000
2000
1000
800
1800
7000
12% Other
13% Seafood
13% Poultry
15% Bakery
47% Red Meat
New Uses
1971 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87
1000'S TONS/YR CO_2
660
600
500
400
300
200
100
250
Total Market
New Uses
Deboning 12%
Fish 11%
Poultry 33%
Red Meat Chilling 44%
1972 1973 1974 1975 1976 1977 1978 1979 1980 1981 1982
CO_2 CHILLING MARKET

Table 1. Primary restaurant sources for leading meat products

	Estimated annual eater occasions (millions)	Prime restaurant sources	Share
Hamburgers	5,580	Hamburger restaurants	83%
		Full menu restaurants	13%
Fried chicken	1,619	Chicken restaurants	62%
		Full menu restaurants	16%
		Hamburger restaurants	5%
Hot dogs	875	Full menu restaurants	28%
		Other sandwich restaurants	25%
		Hamburger restaurants	18%
		Department stores	7%
		Ice cream restaurants	6%
Steak	815	Budget steak restaurants	34%
		Full menu restaurants	31%
		Other steak restaurants	18%
		Hotel restaurants	7%

Extensive research and field trials showed that carbon dioxide at a temperature of -109F offers or makes available more expendable units of BTU refrigeration than any other expendable refrigerant. A comparison of the costs of fluids, LN_2 and liquid carbon dioxide (LCO_2), for refrigeration value, is given in Figure 3. The actual refrigeration that can be extracted from each depends to some extent upon the freezing process but, for most purposes, it is adequate to assume that 1.2 pounds of LCO_2 will provide the same refrigeration that one pound of nitrogen will provide (Harr and Minard, 1977).

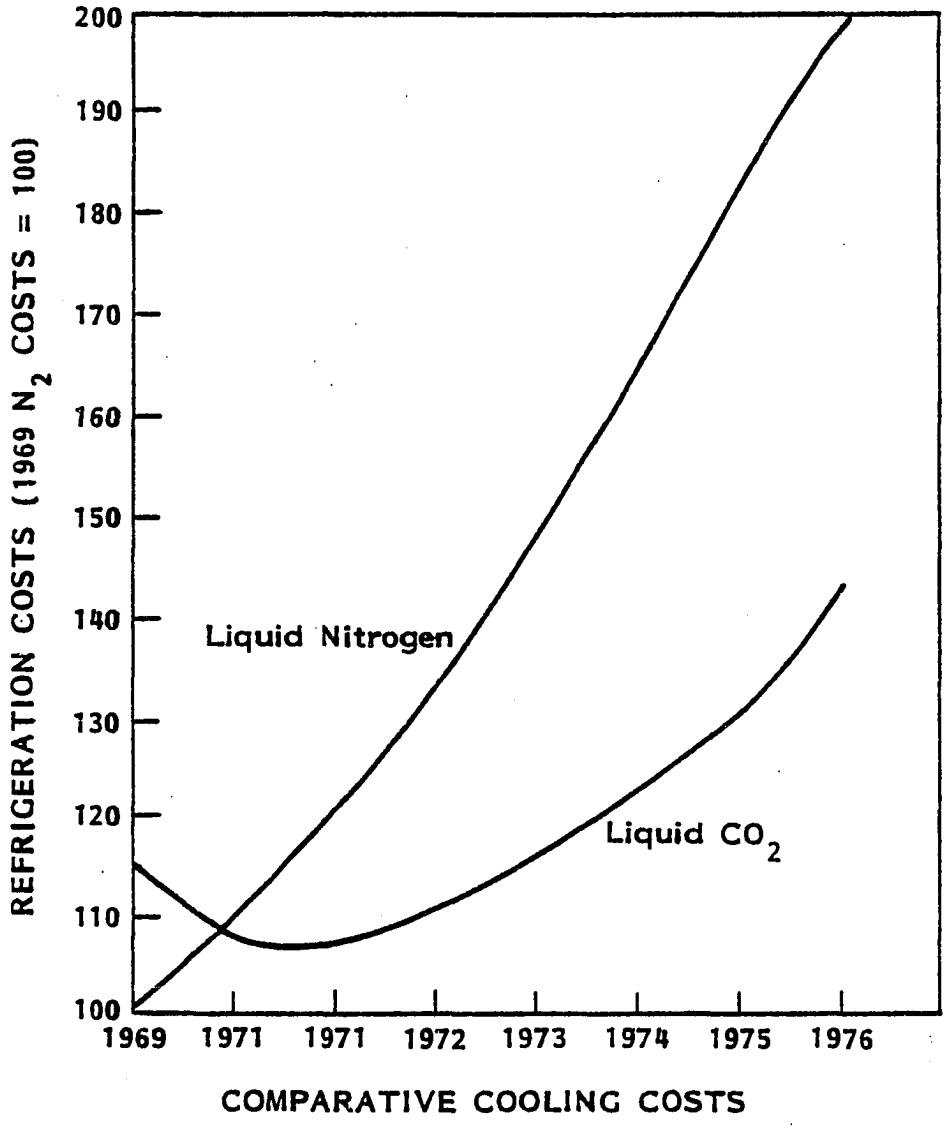


Figure 3. A comparison of the costs of liquid nitrogen and liquid CO_2 (based on equivalent refrigeration value) from 1969 through 1976

MICROBIOLOGY

Only a few pertinent review articles will be cited because of the great amount of literature on the subject.

Bacteria Associated with Fresh Poultry

Many types of microorganisms are present on poultry products as a result of contamination from feathers, feet and intestinal contents of the birds. Equipment and personnel on processing lines also contribute to the spread of bacteria. Bacterial types and numbers vary with different plant practices and the adequacy of plant sanitation. The species of bacteria encountered by several investigators belonged to the genera of Pseudomonas, Alcaligenes, Achromobacter, Escherichia, Aerobacter, Proteus, Salmonella, Micrococcus, Staphylococcus, Sarcina, Corynebacterium, Bacillus, Clostridium, Flavobacterium, Paracolobactrum, Microbacterium, Streptomyces, Haemophilus, Gaffkya, Neisseria, Actinomyces, Streptococcus, Brevibacterium, Arthrobacter and Lactobacillus (Kraft, 1971; Rey, 1975).

Bacterial Types Associated with with Raw Ground Beef

As a category of meats, comminuted meats such as ground beef invariably have higher numbers of microorganisms than noncomminuted meats such as steaks. This is true due to the fact that ground meats generally consist of trimmings from various cuts and thus represent pieces that have been handled excessively. Secondly, ground beef provides a greater surface area which itself accounts in part for the increased flora. The bacterial types associated with ground beef were found to be Achromobacter, Bacillus, Micrococcus, Streptococcus, Flavobacterium, Aeromonas and Moraxella-Acinetobacter (Ayres et al., 1950; Corlett et al., 1965; Jay, 1970; Welch and Maxey, 1975; Siedman et al., 1976; Reddy, 1978).

Common Food-borne Pathogens Associated with Poultry and Meats

Clostridium botulinum is the most harmful of the bacterial pathogens causing food-borne illness. But botulism from ingestion of chicken or beef is quite rare in the United States (U.S. Department of Health, Education and Welfare, 1979). However, endospores are capable of surviving prolonged frozen storage. In fact, two outbreaks reported by U.S. Department of Health, Education and Welfare (1968) were caused by chicken stew and liver paste, both prepared from

frozen chicken.

Minimum growth temperature for C. botulinum type A and type B is 10C (Rieman, 1969). C. botulinum type E, which has a fairly low minimal temperature, 3C, produces spores with very low heat resistance. A history of gross temperature abuse was reported for the outbreaks of botulism from poultry. Adequate refrigeration is a good safety measure in preventing botulism. According to the reports on food-borne outbreaks (U.S. Department of Health, Education and Welfare, 1979), several meat products that have been packed in air impermeable films with or without vacuum inside the package did not result in the occurrence of botulism poisoning.

C. perfringens is an ubiquitous organism. Spores are widely distributed in feces, soil and dust (Breed et al., 1957). The vegetative cells are microaerophilic and have a temperature growth minimal of 6.5C as reported by Hobbs, 1969). Vegetative cells do not survive well under refrigeration but the endospores can survive prolonged frozen storage (Despaul, 1964). Cooking eliminates competition from the natural saprophytic bacteria that might be present in the food since, in general, they are more sensitive to heat than are the spores of C. perfringens. Heating also drives off gases, lowering the oxygen tension in the food and activates the spores. The process of cooking large quantities of food in

large containers results in poor heat penetration and a slow cooling rate. These conditions, which are ideal for growth of C. perfringens, are more frequently found in institutional kitchens than in home cooking (U.S. Department of Health, Education and Welfare, 1979).

Some strains of Staphylococcus aureus produce a toxin that when ingested results in acute self-limiting gastroenteritis. The disease is characterized by a short incubation period after which nausea, vomiting, abdominal cramps, and diarrhea appear (Michener and Elliott, 1969). Minimal temperature for growth and toxin production by S. aureus is 6.7C (Angelotti et al., 1961). The organism, however, survives well during frozen storage (Reddy, 1978). For formation of sufficient toxin in foods to cause illness, the organism has to multiply to high numbers. Peterson et al. (1962) demonstrated that this organism is capable of multiplication to high numbers in foods even with the competition of a mixed saprophytic flora.

Staphylococcal toxin involved in food poisoning is fairly stable to heat; therefore, cooking does not necessarily render the food safe. The bacterial cells are easily killed by heat and the presence of viable forms in the cooked foods generally indicate contamination after cooking. Contamination after cooking is a frequent mode of entry of the organism in foods. Habitats of S. aureus are primarily the mucous

membranes of the nasal passages and the skin. Poor personal hygiene of food handlers was recognized as the contributing factor in food-borne disease outbreaks (U.S. Department of Health, Education and Welfare, 1979).

Any of several species (more than 2000) of Salmonella, when ingested in large numbers, can multiply within the small intestine, causing illness. The illness is characterized by a long incubation time of 26 to 48 hours and long recovery. Vomiting, diarrhea, abdominal pain, and fever are common symptoms. Fatalities are not uncommon in children, elderly people or otherwise debilitated patients; these individuals are susceptible to infection from ingestion of fewer cells than normal healthy adults. The affected individuals continue to excrete the infective organism during convalescence for a few days or weeks after recovery. In some cases they became asymptomatic carriers (Taylor and McCoy, 1969).

Salmonella from animal fecal sources are frequently present in animal products. High incidence of Salmonella is reported for animal feeds, unpasteurized liquid and dried eggs, red meats and poultry meat (Taylor and McCoy, 1969).

Temperatures below 6.7C control growth of Salmonella (Michener and Elliott, 1964). Oblinger and Kraft (1973) observed that Salmonella can grow to high numbers in competition with psychrotrophic species of Pseudomonas at 15C. This indicates a high level of probability of salmonellosis from food

containing Salmonella, and the need for a careful control of the temperature of storage of foods.

According to the U.S. Department of Health, Education and Welfare (1979) in 1978, 9 deaths were reported as associated with food borne outbreaks (Table 2). Botulism and staphylococcal intoxication each accounted for three deaths, while 1 was caused by salmonellosis and two were caused by diseases of unknown etiology. Out of 457 outbreaks of food-borne disease involving 11,490 cases, 114 and 217 were accounted for food eaten in the home and restaurants, respectively. Bacterial agents were the most common causes (70.1%) of the food-borne outbreaks of confirmed etiology. Salmonellae accounted for 31.8% of the cases followed by 15.9% for Staphylococcus aureus. The other food pathogens which were implicated in fewer cases are also present in Table 2.

The five most common factors contributing to food-borne disease outbreaks in the United States in order of frequency of occurrence include: 1) inadequate cooling of foods, (2) lapse of a day or more between preparing and serving, (3) infected persons handling foods which are not subsequently heat processed, (4) inadequate time or temperature or both during heat processing of foods, and (5) insufficiently high temperature during storage of hot foods.

Table 2. Confirmed foodborne disease outbreaks and cases, and percents of known etiology, 1978

Etiology	Number of outbreaks	%	Number of cases	%	Number of deaths
Bacterial					
B. cereus	6	3.8	248	4.9	
Brucella	-	0.0	0	0.0	
C. botulinum	12	7.6	58	1.1	3
C. perfringens	9	5.7	617	12.1	
E. coli	1	0.6	35	0.7	
Salmonella	50	31.8	1883	36.9	1
Shigella	3	1.9	163	3.2	
S. aureus	25	15.9	1493	29.3	3
Enterococci	1	0.6	58	1.1	
Streptococcus Group A	-	0.0	-	0.0	
V. cholerae 01	1	0.6	11	0.2	
V. parahaemolyticus	1	0.6	82	1.6	
Other bacteria	1	0.6	8	0.2	2
TOTAL	110	70.1	4656	91.3	

Effects of Freezing on Survival and Growth of Microorganisms in Foods

Microorganisms are broadly classified as thermophiles, mesophiles and psychrotrophs according to their ability to grow at high, intermediate or low temperatures. Minimum temperature for growth of most mesophiles is between 10 and 15C, while some thermophiles have minimal growth temperatures as high as 40 to 45C (Stanier et al., 1970). Psychrotrophs have considerably lower minimal temperatures than mesophiles and are the major problem with refrigerated foods.

Minimal growth has been reported at temperatures as low as -18C (Sulzbacher, 1950); but it is generally agreed that for practical purposes freezing at -10C controls bacterial growth, -12C controls yeasts and -18C controls fungi (Institut International du Froid, 1972). Microorganisms, however, can contribute to deterioration of frozen foods even at temperatures at which growth is restricted. Some extracellular enzymes, if released prior to freezing, can remain active at temperatures as low as -18C (Rey et al., 1969). Such enzymes would continue their catalytic action in the frozen food even though at a reduced rate (Institute International du Froid, 1972).

Studies of Mazur (1966) regarding bacteriological media and of Michener and Elliott (1969) for foods show that 90 to 99 percent of the bacterial populations die at, or near, the

freezing point. In these studies, when the bacterial suspension or the food was stored frozen for considerable periods of time, bacterial numbers continued to decrease but at a reduced rate. Also, it has been observed that bacterial numbers levelled off after one month of frozen storage, remaining stationary for several months (Reddy, 1978).

Effects of Freezing on Microflora on Poultry

Average numbers of total aerobic bacteria on poultry right after slaughter were reported to be 35,000 per cm^2 by Ayres et al. (1956) and 60,000 by Gunderson et al. (1954). To bring the effect of freezing into perspective, if birds are frozen immediately after slaughter and a reduction in numbers of 99 percent takes place upon freezing, still 350 to 600 aerobic bacteria per cm^2 might remain viable. Consequently, there would be enough bacteria present in the frozen poultry to resume growth after thawing. Decrease in bacterial numbers was greater during the freezing process than during subsequent frozen storage (Rey, 1975). Experiments with CO_2 snow as a cryogen and preservative for poultry showed an inhibitory effect particularly on the potential spoilage group of organisms, fluorescent pseudomonads (Reddy and Kraft, 1980).

It has been found that the reduction in numbers taking

place during freezing and frozen storage has little effect on subsequent spoilage after defrosting. There would be enough bacteria present in the frozen meat to resume growth after thawing and spoil the meat as fast as unfrozen meat becomes spoiled (Rey and Kraft, 1971; Sulzbacher, 1950; Spencer et al., 1956; Kitchell and Ingram, 1956).

Effects of freezing on Microflora on Beef

Studies on the bacteriological quality of retail cuts of beef demonstrated that loins shell-frozen with LN_2 could be held at room temperature in insulated boxes with no further refrigeration for 2 or 3 days without undue health hazard. Although the LN_2 freezing did not decrease the bacterial numbers as much as solidly freezing by conventional blast freezing, both methods caused extension of the lag phase of bacterial growth (Kraft and Rey, 1979). A significant reduction in viable cell counts of ground beef patties by LN_2 of LCO_2 by freezing rather than by mechanical freezing was observed by Reddy (1978). The survival rate of organisms was influenced by the composition of beef patties. The predominant flora that survived 5 months frozen storage was found to be of Moraxella-Acinetobacter group (Kraft et al., 1979).

Effects of Freezing on Specific Microorganisms

Exposure to freezing temperatures caused impairment in the ability of Pseudomonas to produce fluorescent pigment but did not affect the activity of their extracellular proteolytic and lipolytic enzymes (Rey et al., 1969). The sensitivity may be due to the cells' exposure to concentrated media salts during cooling and warming (Ruwart and Hang, 1974).

Staphylococcus aureus cultures when frozen and stored for long periods of time did not show changes in rate of growth or enterotoxin A production but showed an increase in sensitivity to NaCl and also an increase in nutritional requirements (Kueck, 1974; Jackson, 1974).

Metabolic injury due to freezing and thawing was observed by Janssen and Busta (1973), but not differences in virulence between injured and uninjured cells of Salmonella serotypes (Sorrells et al., 1970). As observed with other bacterial species, freezing reduces viable counts of Salmonella, but thereafter, the survivors die at a low rate (Committee on Salmonella, 1969).

The viability of Clostridium perfringens decreased during frozen storage (Traci and Duncan, 1974; Trakulchang and Kraft, 1977). Addition of soy to beef patties appeared to protect the C. perfringens and other flora from the lethal

effects of freezing (Reddy, 1978).

Freezing and thawing reduced the viability of E. coli (O'Hara, 1954; Warseck, 1973). Alur and Grecz (1975) attributed the possible cause of cell death to the DNA fragmentation as a result of freezing.

The survival rates as percentage of original counts after 27 months of frozen storage of chicken were 0.46, 0.9 and 1.7, respectively, for S. aureus, Salmonella and vegetative cells of C. perfringens (Davey et al., 1976).

Farber (1977) reported that Streptococcus faecium survives better than S. faecalis under frozen conditions and suggested that exclusive enumeration of S. faecalis from frozen vegetables is more closely associated with public health significance. The significance of enterococci as indicators for frozen poultry was evaluated by Kraft (1969). These organisms survive well during frozen storage and cannot be regarded as indicators of recent pollution in frozen foods. However, they would be more significant than E. coli in trying to establish the sanitary aspects of frozen meat prior to freezing. Rey and Kraft (1971) observed that numbers of enterococci remain constant during refrigerated storage of meat.

No correlation has been documented in the literature between the presence of either E. coli or of coliforms and the

presence of Salmonella in raw beef (American Meat Science Association National Livestock and Meat Board, 1976). Newton et al. (1977) suggested that meat can be contaminated with coliforms from hides or sources other than fecal pollution.

Processing of Poultry in the United States

Processing plants are highly mechanized and line operated. According to Childs et al. (1970), a schematic flow of poultry processing operations is as follows:

Antemortem inspection

Unloading and hanging

Slaughter: Shackle and bleeding

Defeathering: Scalding
Mechanical picking
Singeing
Pinning
Washing

Automatic feet cutting

Evisceration: Removal of uropygial gland, cut around vent, abdominal cut and pull out viscera, post mortem inspection

Removal of liver, gizzard, heart, head and alimentary canal

Slitting of neck and removal of lungs by vacuum

Cut off neck

Washing

Chilling: Whole birds can be chilled in ice slush tanks with air agitation but in most instances chillers operated on continuous flow of ice water or ice water with slush ice have replaced the bulk type chiller

In-plant chlorination

Chlorination of the spray washer or chiller washes has been investigated as a means to reduce microbial counts or to prevent spreading of contamination from bird to bird. In-plant chlorination, significantly improves sanitation in processing plants (Barnes, 1972; Ranken, 1973). Hypochlorite solutions at 40 to 60 ppm chlorine in the spray washer were effective in reducing microbial counts on the carcasses (Sanders and Blackshear, 1971). On the contrary, Kotula et al. (1967) did not find extension of shelf life with chlorine levels up to 50 ppm in post chill spray water. But Ziegler and Stadelman (1955) reported a significant increase in shelf life of carcasses with the use of 20 ppm chlorine water dip for 5 minutes.

Effect of chlorination on salmonellae and other food poisoning microorganisms on poultry carcasses has been studied with variable results. Dixon and Pooley (1961) found that 200 ppm chlorine treatment for ten minutes was effective when fewer than 1000 salmonellae organisms had been inoculated on the carcass. Use of 200 ppm chlorine in wash water could hardly reduce fecal organisms on poultry carcasses, but did

prevent cross contamination and prevented multiplication of spoilage organisms (Barnes, 1965). At the level of 20 ppm chlorine in chill water, Patterson (1968) did not find a significant reduction in total aerobic counts and incidence of fecal streptococci and Staphylococcus aureus. Dye and Mead (1972) found that chlorine treatment during processing probably would not eliminate clostridial spores from poultry carcasses, but even low levels of free available chlorine (0.5 ppm) are more effective in destroying spores in chill water than high levels of combined available chlorine. Similar results were obtained for Escherichia coli by Allen (1961) at different pH and temperature levels.

The effectiveness of chlorine as a bactericidal agent depends upon the conditions under which it is used in the poultry processing plants including the concentration of chlorine, the contact time, the temperature, pH value, and chemical composition of water. The U.S. Department of Agriculture has proposed the use of 20 ppm free chlorine dip or spray in poultry processing (Federal Register, 1978).

Other measures to improve microbiological quality of processed poultry

An increase in refrigerated shelf life of cut-up broilers after a one minute dip in one percent solution of ascorbic acid was reported by Arafa and Chen (1978).

Additional studies employing phosphoric acid and hydrochloric acid solutions at pH 2.75 indicated that the pH reduction was not the only factor responsible for the microbial reduction observed when broiler parts were dipped in one percent ascorbic acid solution.

Dipping fresh poultry breasts in 2.5 to 10 percent potassium sorbate solutions for one minute has resulted in significant reduction in total plate counts (Robach and Ivey, 1978).

Use of glutaraldehyde as a poultry processing equipment sanitizer was studied by Mast and MacNeil (1978). They observed no effect in reducing bacterial numbers at a concentration of 100 ppm glutaraldehyde. At the same concentration, chlorine consistently destroyed more bacteria than glutaraldehyde.

A comparative study of hot water dipping at 70C for 2 minutes with other chemical solutions has indicated that the former treatment yielded best quality chicken than the others (Patterson, 1975). Clostridium perfringens and Bacillus spp. survived hot water treatment and multiplied during storage. Dipping in succinic acid and 200 ppm free chlorine solutions resulted in taints after roasting.

During commercial processing of broiler chickens, injection of polyphosphates has resulted in microorganisms being added to the deep breast muscle. But this had no effect on

the shelf life of fresh chilled carcasses held at 1 or 10C (Mead and Adams, 1979). Addition of polyphosphate increased the death rate of salmonellae in breast muscle held at -2C and to a lesser extent at -20C but had little or no effect in leg muscle or in breast muscle held at 1C or -5C (Foster and Mead, 1976).

The quantities and kinds of chemicals that are to be used to improve the quality of poultry during processing should be within the limits of government regulations. Most of these chemicals have a very limited application due to reactions they bring about which result in discolorations and off-odors.

Packaging of Poultry

Introductory comments

Since a change in some factor in an environment should affect the microflora under the influence of such an environment, it seemed worthwhile to explore the important factor of packaging conditions. More specifically the primary purpose was to determine the effect on spoilage organisms of packaging films which differed in their permeability to gases and water. Plastic films are, however, generally several times more permeable to carbon dioxide than to oxygen. Thus, a film of sufficient permeability to maintain aerobic conditions inside a pack should usually allow the carbon dioxide produced

to escape. If, on the other hand, a film of low permeability is chosen to exclude oxygen, carbon dioxide may continue to be produced; and, if the permeability were too low it would be unable to escape quickly enough, gradually destroying the vacuum and with it, that close fit of the wrapper which is usually a desired feature in vacuum pack.

Poultry may be sold whole or cut-up as halves, quarters, or parts or it can be further processed into a large variety of special products. In the past, most cutting-up and tray packaging was done at the retail level. Nineteen percent of the chicken produced in the United States in 1965 was cut-up at plant level. This proportion has steadily increased for the last 15 years. The actual trend is then for centralized cutting operations at the plant level (U.S. Department of Agriculture, 1974).

Conventionally, the whole or cut-up birds may be trayed and overwrapped with pliofilm (stretch wrap). With trayed birds blotters can be used to soak up the liquid. Also, the birds can be wrapped in a heat shrinkable film such as Saran and shrunk by means of hot water (Sacharow and Griffin, 1970).

The influence of packaging materials on bacterial multiplication and shelf life of poultry was reviewed by Ayres (1959). Since typical spoilage organisms are highly aerobic, the permeability of materials to oxygen and exclusion of air from the packages affect their growth and

biochemical activity (Spencer et al., 1956; Stewart, 1953; Ingram, 1962; Ayres, 1959; Rey and Kraft, 1971).

Packaging materials

Polyethylene, a single component material is being used on a large scale. This is available as low and high density material and has differences in permeabilities. Another material in this polyolefin family is polypropylene, which is similar in many respects to a high density polyethylene. Another important single component material in rubber hydrochloride (pliofilm) and extensively used for stretch wrapping poultry (Sacharow and Griffin, 1970).

Multicomponent packaging materials which are relatively more impermeable than the films previously described. Coatings of nitrocellulose are commonly applied to cellulose films to control moisture and gas permeabilities. The typical examples of these are LSAD and MSAD films from Dupont Company. A fairly new development that came from Cryovac Company is the popular Saran, a copolymer of vinylidine chloride and vinyl chloride and one of the best transparent films with low permeabilities to both oxygen and water vapor. As a broad generalization, the permeability of most films to carbon dioxide usually parallels that to oxygen and is several times greater. The ratio appears to

be greatest with hydrophilic films such as wet cellulose and is least with hydrophobic materials. In practice, this means that most common materials, with the exception of polyvinylidine chloride copolymer (Saran), are appreciably permeable to carbon dioxide evolved from microbial degradation of the products (Ingram, 1962).

Vacuum packaging

Packaging of product in an impermeable film with the exclusion of air can be designated as vacuum packaging. Vacuum packaging has been used with a variety of meat products in order to control the growth of the aerobic spoilage organisms, such as Pseudomonas (Sutherland, 1975).

Shrimpton and Barnes (1960) have compared a permeable film (polythylene) with a heat shrinkable evacuated impermeable film (Saran). Storage life of eviscerated poultry at 1C was extended in the impermeable film packages. Lower oxygen content and a greater CO_2 build-up occurred in the impermeable film packages.

The effect on the microflora of chickens of packaging films with differing permeability to oxygen was studied by Debevere and Voets (1973). Fresh chicken packaged in shrinkable polyvinyl chloride had a lower aerobic count than the stretchable polyvinyl chloride. Chicken stored in polyethylene film had the highest aerobic and proteolytic

counts. Proteolytic and lipolytic spoilage of chicken stored at 5C was directly related to the oxygen transmitted by the packaging materials (Rey and Kraft, 1971). Thomson (1970) also reported similar results after a study on the influence of gas permeabilities of packaging materials. Members of the genus Enterobacter were reported as the predominant flora in vacuum-packaged cut-up fresh broilers stored at 2 to 4C while the air samples contained members of Pseudomonas (Arafa and Chen, 1978).

Several attempts have been made by various investigators to prolong the shelf life of vacuum packaged chicken to the maximum possible extent using a variety of copolymers. A cryovac super L showed an improvement of 100 percent in shelf life at 1C over its counterparts stored in wooden boxes (Cantoni and Bolther, 1975). Mulder and Garrits (1974) used a nylon-polyethylene laminate for vacuum packaging of smoked chicken. No salmonella or staphylococci were detected in any sample. Some factors that are to be considered in vacuum packaging include vacuum retention, possible hazards due to the growth of anaerobic and facultative anaerobic food pathogens, color retention and odor and CO_2 build-up. An ideal vacuum packaging material and method should overcome these problems in addition to prolonging the shelf life.

Classification of Bacteria

In any study of microbiology of food products, interest is centered on types of organisms and their activities in or on the products. Classification is the ordering of organisms into groups on the basis of their relationships. For purpose of this work, relationship is the resemblance or overall similarity as judged by the characters of the organisms without any implication to their relationship by ancestry.

The phenologic model has been used in recent approaches to classification. In phenologic classifications as many characters of the organisms as possible are compared but without giving any weight to any particular character. The groups are formed by sorting the organisms with maximum characteristics in common. According to Sneath and Sokal (1973), the sorting of characters when handling large numbers of organisms and features is not a simple task and requires the use of mathematical methods (Numerical Taxonomy).

Sneath (1962) has described the application of computers to the mathematical analysis of data related to the observed features of bacteria. According to his methods, the coefficient of similarities between a pair of organisms can be calculated by the formula

$$S = \frac{NS}{NS + ND}$$

where NS is the number of similar characters between the pair, ND the number of characters not shared by the two organisms and S is the coefficient of similarities. From these coefficient of similarities, a similarity matrix builds up by arranging the similarity coefficients obtained by comparison of all possible pairs of organisms. The process continues by comparing individuals to pairs already formed and grouping them on the basis of maximum common level of similarities. By repetition of the procedure, distinctive clusters of individuals can be formed. This agglomerative method of Sneath (1962) has been applied and recommended by several bacteriologists for classification of microorganisms (Corlett et al., 1965; Shiflett et al., 1966; Lee and Wolfe, 1967; Thornley, 1967; Rey, 1975).

Application of Numerical Taxonomy to Food Microbiology

Several factors must be considered regarding microorganisms which produce the spoilage of foods after the foods are subjected to the deleterious affects of processing such as heating, freezing, radiation or addition of chemicals. These factors include the relative resistance of the mixed population, and which of the surviving types actually are

capable of producing spoilage. A group or groups must not only be reasonably resistant to a particular processing operation but must also be present in large enough numbers so that the process under consideration will not eliminate them completely; above all, they must be able to multiply under the given storage conditions.

Lee and Wolfe (1967) and Splittstoesser et al. (1968) reported the use of computer analysis applied to isolates from different foods. Corlett et al. (1965) applied a computer program according to a code, which grouped their fresh isolates from Dover sole and ground beef into 10 microbial groups or genera. Shewan and Hobbs (1965) pointed out the difficulties that exist in comparing the numerous microbial classification systems and described a scheme to overcome these problems for classification of microorganisms isolated from fish by numerical methods. Replica plating and computer analysis of microorganisms from aged beef was applied by Valland (1969) for classification into genera. Rey (1975) studied the changes in the bacterial flora of chicken due to the effects of freezing by applying numerical methods.

Simple analytical procedures and computerized calculation of the results, as employed for numerical taxonomy, allow the investigator to use a more representative sample by increasing the sample size. Multiple inoculation procedures

(Hartman and Pattee, 1968) and microtechniques for syncronized testing of many individuals from a bacterial population are of value in handling large numbers of bacteria for taxonomic classification of bacteria isolated from foods.

Application of Numerical Taxonomy for Bacterial Classification

Numerical taxonomy is the grouping by numerical methods of taxonomic units into taxa on the basis of their character states (Sneath and Sokal, 1973). The term includes the drawing of phylogenetic inferences from the data by statistical or other mathematical methods to the greatest extent possible. The views of Michael Adanson (1727-1806) were modified and summarized by Sneath and Sokal (1973).

Criticism and Comments on Numerical Taxonomy

Sneath's (1957) acceptance of Adanson's earlier suggestion that equal taxonomic weight should be given to all characteristics of an organism was not enthusiastically received by several bacteriologists. Mistrust of this principle, coupled with a suspicion that computing machines were to be substituted for human judgement, resulted in strong opposition to the method when numerical taxonomy was introduced and developed within the various biological disciplines. Krieg and Lockhart (1966) did not find agreement between clusters

of enterobacteria formed by numerical taxonomy and groups formed by any of the schemes currently used for the classification. They concluded that according to the principles for natural grouping, some tribes or genera of enterobacteria formed by classical taxonomy have no justification and that some species separate distinctively from the tribe or genera to which they have been assigned.

Sneath (1962) had indicated that the concept of natural classifications may have its roots in the structure of the genes which will determine the phenotypes.

Chemical Constitution of Muscle

Since a portion of this research involved changes in muscle, the following review is considered pertinent.

The principal constituents of muscles are water, protein, and lipid, Proteins in muscle can be categorized as sarcoplasmic proteins (soluble in water or dilute salt solution), myofibrillar proteins (soluble in solutions of 0.4-1.5 ionic strength), and connective tissue proteins (collagen, elastin, and reticulum which are not soluble in neutral aqueous solutions) (Fennema et al., 1973).

The majority of the sarcoplasmic proteins function as glycolytic enzymes. Proteolytic enzymes such as cathepsins also have been isolated from the sarcoplasmic fractions of

postmortem muscle. Most of the sarcoplasmic proteins reside in extrafibrillar spaces in the fiber. Myofibrillar proteins are responsible for the textural quality and water binding capacity of muscle. Myosin constitutes the major part of myofibrillar proteins followed by actin, tropomyosin, troponins and actins (Fennema et al., 1973).

Also, muscle contains hydrolases such as cathepsins and β-galactosidases in cellular organelles known as lysosomes. Cathepsins have proteolytic activity and may be involved in tenderization of muscle during postmortem aging (Lawrie, 1979). Cathepsins A, B, C, and D have been isolated from bovine and chicken muscles (Bodwell and Pearson, 1964; Martins and Whitaker, 1968). Cathepsins may be liberated from muscle lysosomes when their lipoprotein membranes rupture as the pH decreases during postmortem glycolysis (Hamdy et al., 1961). A protease called calcium activated factor which is endogenous to the muscle cell has been shown to cause myofibril fragmentation or z-line degradation Busch et al. (1972). Although myofibrillar proteins are hydrolyzed only slightly by cathepsins, a sufficient number of peptide bonds may be broken in the z-filaments to cause an appreciable increase in muscle tenderness (Bodwell and Pearson (1964).

Water is the most abundant constituent of muscle tissue. According to Hamm (1960), the major portion of water, excluding

the water bound to the surfaces of proteins, is retained in skeletal muscle by capillary forces. Scopes (1970) estimated that myofibrils in situ contain approximately 84 percent water and occupy approximately 75 percent of the volume of a fiber. The remainder of the fiber volume must exist as interfibril spaces which are filled with sarcoplasm. Scopes (1970) considered that sarcoplasmic proteins are limited to spaces outside of the myofibrils since they would restrict the efficiency of the contractile process. The triglyceride content of intramuscular lipid is highly variable in most species of animals and is generally much greater than the phospholipid content (Hornstein et al., 1961).

Inorganic ions in muscle are important from the standpoint of water binding capacity, texture, color, and flavor. The most common inorganic ions (adsorbed and dissolved) in muscle are K (38%), Na (7.3)%, Mg (2.6%), Ca (1.3%), Fe (0.6%), and Zn (0.2%). The divalent ions such as Ca^{2+} and Mg^{2+} may be bound to myofibrillar proteins and to components of sarcoplasmic reticulum (Assaf and Bratzler, 1966).

Chemical Changes that Occur in the Muscles after Death

During a period of about 24 hours postmortem, glycogen is converted to lactic acid through anaerobic glycolysis, phosphocreatin is depleted in the first few hours, the adenosine triphosphate (ATP) content decreases following depletion of phosphocreatin, chemical bonds form between actin and myosin when the ATP content decreases to a critical level which is dependent on the species, and sarcoplasmic proteins precipitate. As the number of bonds between the actin and myosin filaments increase, extensibility of the muscle is reduced. When muscle is aged, the proteins in the z-line are altered and the bonds between actin and myosin are weakened (Goll et al., 1970).

The accumulation of lactic acid in muscle during postmortem glycolysis is responsible for the drop in pH to about 5.0 to 6.0 for mammals, between 5.7 and 5.9 for poultry and between 5.5 to 6.6 for fish. Muscle binds the minimum amount of water at about pH 5.5, the isoelectric point of actomysin (Newbold, 1966).

The chemical composition of a myosystem has a profound influence on the quality attributes of the frozen product. The amount and types of lipid in a muscle will govern the extent of rancidity during frozen storage. The exudation of fluid from thawed muscle is dependent on initial pH and

concentration of inorganic ions. The toughness and juiciness of cooked meat previously stored at freezing temperatures is related to the interaction of myosin and actin (Fennema et al., 1973).

Changes in Muscle During Freezing

Cryodamage to the sarcolemma by conventional slow freezing was observed in beef muscle (Hiner, 1951), poultry (Dubois et al., 1942), and fish (Tanaka, 1965). According to Love (1955 and 1966), cryodamage was presumably caused by expansion of large ice crystals near the sarcolemma. He considered that the rupturing of cell walls was caused by endosmosis brought about by extracellular freezing as a result of slow freezing. He also suggested that the DNA as an intracellular component migrated through cryodamaged cell walls. Therefore, determination of the amount of deoxyribonucleic acid in fluid pressed out of muscle would give the degree of cell breakdown. Khan (1964) demonstrated slight decreases in ribonucleic acid amount of chicken breast after storage for 45 weeks at -5C.

The reduction in water content by freezing will cause an increase in ionic strength. During frozen storage the conformation of myofibrillar proteins as filaments would be changed and proteins would interact to form insoluble complexes

and the enzymatic activity of myosin and sarcoplasmic proteins would be reduced. These changes would result in a reduction in total extractable protein. Protein alterations responsible for the decrease in solubility are collectively designated as denaturation (Fennema et al., 1973). Khan (1964) suggested that enzymes such as cathepsins might be released as a result of damage during freezing and storage, causing limited proteolysis. Proteolysis may affect the solubility and ion binding properties of the protein.

During frozen storage, the polyunsaturated fatty acids are autoxidized in the presence of oxygen to hydroperoxides, which in turn decompose to volatile aldehydes, ketones, acids, the constituents responsible for rancid odor. A colorimetric method for measuring a secondary decomposition product (malonaldehyde) of lipid autooxidation has been developed. Thiobarbituric acid (TBA) interacts with malonaldehyde to form a red color condensation product (Tarladgis et al., 1962). Autoxidation of lipids in frozen muscle can be minimized by restricting the oxygen content in the microenvironment of the product. Such restriction can be attained by using packaging materials with low moisture and oxygen permeabilities. For example, the storage life of frozen chicken packed in an oxygen-permeable film was only one-third of that of frozen in an oxygen-impermeable film (Klose et al., 1959).

Many investigators have shown that free fatty acids (FFA) accumulate during frozen storage of muscles. Generally speaking, the rate of FFA accumulation increases as the frozen storage temperature is raised to the freezing point of muscle. Formation of FFA in stored muscle is caused mainly by enzymatic hydrolysis of phospholipids and partly by enzymic hydrolysis of triglycerides (Anderson and Ravesi, 1970; and Davidkova and Khan, 1967). Free fatty acids (FFA) liberated during frozen storage of muscle may be involved in the insolubilization of myofibrillar proteins (King et al., 1962).

Effect of Freezing on Water Holding Capacity of Meat

According to Khan and Lentz (1965), factors responsible for drip losses such as freezing and thawing, pH, rapid rate of depletion of ATP and the size of the ice crystals formed as a result of slow freezing, are the same regardless of the state of rigor mortis at which the muscle was frozen. Since rigor begins when the ATP content of the muscle is depleted and a steady pH is attained neither lower pH of the muscle nor a rapid rate of depletion of ATP during thawing appears to be responsible for quantitative differences in drip obtained from poultry frozen during rigor and after rigor (de Fremery and Pool, 1960). Berg (1964) observed small losses of drip from poultry frozen before rigor and attributed this to the

elevated pH.

The fact that the water holding capacity of muscle frozen during rigor was minimum may be helpful in understanding the cause of drip and protein denaturation. A decrease in water holding capacity may increase the amount of water available for freezing during rigor and give rise to higher solute concentrations in muscle during and after freezing. These higher concentrations of solute may cause damage by affecting the solubility of muscle proteins and the ability of cell constituents and proteins to reabsorb water on thawing. Since the amount of bound water also governs the migration of free water and the size of ice crystals formed during freezing and subsequent storage (Fennema et al., 1973), the possibility of increased damage due to large ice crystals in muscle due to slow freezing cannot be excluded. Love (1968) observed the formation of very small ice crystals in the cod muscles that were frozen with liquid nitrogen or carbon dioxide. Moisture binding capacity of the liquid nitrogen frozen samples was superior to that of air frozen meat samples (Guslyannikov and Koreskov, 1976; Sebranek, 1977).

Effect of Freezing on Meat Tenderness

Attempts to determine the effect of freezing rate on the tenderness of frozen-thawed muscle have not resulted in complete agreement because other variables such as depth of freezing, length of storage, and pre-freezing history often influence the results. Some examples are in order and are given in the following reports. Hiner (1951) reported that beef tenderness was affected more by the ultimate freezing temperature than by freezing rate. He drew this conclusion because all beef muscle samples cooled to -78.5C by carbon dioxide had uniformly low shear values regardless of freezing rate and those cooled to -40C by air blast exhibited higher shear values. According to him, the rate of freezing must, however, be rapid enough to achieve intracellular ice formation if acceptable tenderness is desired. Other investigators who claimed that rapid freezing rates would yield better textured products include Barrie et al. (1964) for turkey meat, Novak and Rao (1966) for chicken parts, Dubois et al. (1942) for beef and poultry muscles, Li et al. (1969) for chicken thighs, MacCallum et al. (1965), Cunningham (1973) for fish, Arafa and Chen (1978) for broiler chickens.

Several other investigators have been unable to detect any relationship between freezing rate and tenderness.

Stewart et al. (1945) failed to detect a significant difference in the palatability of chicken muscle frozen rapidly in moving air at either -68°C as compared to chicken muscle frozen slowly in air at -21C. Marion and Stadelman (1958) and Miller and May (1965) reported that poultry muscle frozen at various rates did not differ significantly in tenderness. Since the samples frozen in the above mentioned studies were stored at freezing temperatures prior to examination, the effects of freezing rate may have been obscured.

Studies of the effect of frozen storage on tenderness of animal muscle have led to conflicting results. Some investigators have reported that post-rigor muscles of poultry, beef, lamb, and fish decrease in tenderness during frozen storage (Smith et al., 1969; Miller and May, 1965; and Connell and Howgate, 1969).

Contrary to the above findings, Field et al. (1966), and Smith et al. (1969) for beef, reported a reduction in shear values after frozen storage, indicating possible increased tenderization.

Since factors such as antemortem history, breed, pattern of postmortem glycolysis, and aging can influence the behavior of muscle during frozen storage, it is not surprising that the research results are not in perfect accord. Temperature of frozen storage is a major factor influencing the rate of textural deterioration. As the frozen storage temperature is

lowered, the rate of tenderness loss is decreased in chicken and turkey muscles (Klose et al., 1950) and in cod (Connell and Howgate, 1969).

Myofibril Fragmentation Index as a Measure of Tenderness of Meat

The decline in postmortem isometric tension (resolution of rigor mortis), has been proposed to be a result of the weakening of cross-bridges between the thick and thin filaments and the degradation of z-line structure (Goll, 1968; Goll et al., 1970). Degradation of the z-line would cause myofibrils to break into shorter segments; and as a result, a reduction would occur in isometric tension. In addition, it has been found that tensile strength measured by stretching the sample until rupture is related to Warner-Bratzler shear tenderness values (Bouton and Harris, 1972).

These studies indicate that the degradation of the z-line was possibly related to the reduction of fiber tensile strength and these in turn are correlated with meat tenderness. Because degradation, or weakening, of the z-line represents the amount of fragmentation of myofibrils, their preparation in a blender could correlate with meat tenderness. Parrish et al. (1973) observed that smaller myofibril fragments originated from the more tender meat samples and suggested that myofibril fragmentation was a much more important factor in meat tenderness

than was muscle shortening. Moller et al. (1973) found a high correlation coefficient (-0.75) between myofibril fragmentation and W-B shear values in bovine longissimus.

Davey and Gilbert (1969) found that the light absorbance of a myofibril suspension represented the extent of myofibril fragmentation. This method was used by Moller et al. (1973), who showed that fragmentation, as measured by percent of emission of a myofibril suspension on a spectrophotometer, gave a correlation coefficient of -0.78 with W-B shear force values for bovine longissimus at 7 days postmortem. These results were supported by the findings of Olson and Parrish (1977) and Culler et al. (1978).

Contrary to the results of the above mentioned studies in beef muscles, Hay et al. (1973) reported that postmortem aging of chicken carcasses to bring about changes in the interaction of action and myosin is not an essential prerequisite in the process of post rigor tenderness development. After further studies in 1973, they concluded that postmortem aging had no affect on the contraction and relaxation phenomena as evidenced by the uniformity of sarcomere lengths in breast muscle. Sayre (1970) studied chicken myofibril fragmentation in relation to factors influencing tenderness and concluded that fragmentation was not an accurate index of tenderness. Sarcomeres did not lengthen during the aging period after rigor mortis developed.

Rapid Chemical Tests as Indices of Meat Quality During Storage

Breakdown of proteins into peptides and amino acids, and oxidation or hydrolysis of lipids occur in fresh poultry and other meats (Jay, 1964; Shelef and Jay, 1970; Pearson, 1968; Kuzmin and Miltsyna, 1972; Matsumoto et al., 1976). Detectable unfavorable organoleptic changes appear when the number of bacteria exceed at least a million per cm^2 area of meat (Ayres et al., 1950; Peirson et al., 1970). Although bacterial count is generally regarded as a reliable criterion of spoilage, its determination requires a minimum of 48 hours of incubation for an accurate count. But the chemical changes caused by high bacterial populations can be measured more rapidly and could be a convenient alternative.

Shelef and Jay (1970) reported that the pH of beef rose with increasing bacterial population, and described a rapid method to detect spoilage by measuring pH following addition of a standard volume of hydrochloric acid to a filtered homogenate of the meat. Initially, the electromotive force (EMV) of fresh high quality meat is high. Pearson (1968) reported that EMF of spoiled meat may fall below -250 mv. The initial high EMF may prolong the initial lag phase in the growth curve of microorganisms. Jay (1964) recommended an extract release volume (ERV) technique to predict the refrigerated shelf life of beef. He believed that during

microbial spoilage ERV decreases due to an increase in hydration capacity of meat proteins by some unknown mechanism.

Pearson (1968) demonstrated that the "tyrosine" value of meat increased with storage time along with total volatile nitrogen until amino and deamination by the aerobic metabolism of microorganisms limited formation of free amino acids. The tyrosine value so measured included other reductants soluble in trichloroacetic acid such as tryptophan, cysteine, phenolics, sulfhydryls, etc. Khan (1964) determined the extent of proteolysis caused by cathepsins released as a result of damage during freezing and storage by measuring tyrosine value of the nonprotein nitrogenous extract of trichloroacetic acid.

A new extraction method for determining lipid oxidation during storage of beef and pork was described by Witte et al. (1970) using nonprotein nitrogen fraction obtained after precipitation of proteins with trichloroacetic acid.

The above mentioned tests were evaluated by Strange et al. (1977) for effectiveness in monitoring microbial quality during storage of intact meat. They concluded that tyrosine value indicated the spoilage of meat during storage more effectively than TBA while other chemical tests have a limited value in predicting the microbial quality. Information is not available on the usefulness of these tests as indicators of storage quality of poultry meat.

MATERIALS AND METHODS

Sources and Treatment of Samples

1. Beef patties: Ground beef patties were made from 600-800 lbs (270-360 Kg) "A" maturity steer carcasses that were chilled to about 5C for 24 to 72 hours before use. During boning, lean and fat tissues were combined into batches composed of about 20% fat which was confirmed by Anyl Ray Analysis (Anyl Ray Corp., Davenport, IA). Adjustments were made so that each batch had a fat content of 20%. Meat to be used for frozen beef addition was packed in plastic lined boxes and frozen in a blast freezer at about -29C (air velocity 4040 cfm). This beef was kept frozen for one week before combining with fresh beef. Flaking of frozen beef was performed with a Butcher Boy flaker (Model OMF, Lasar Manufacturing Company, Los Angeles, Calif.), after which the flaked trim was ground through a 0.32 cm plate. Fresh and frozen meat combinations were as follows:

A. 100% fresh

B. 80% fresh, 20% frozen

C. 50% fresh, 50% frozen

D. 100% frozen

All products were blender chilled until internal temperatures reached 0.5 to 1.0C. All products were made into patties in a commercial patty forming machine (Hollymatic Model 500A), with

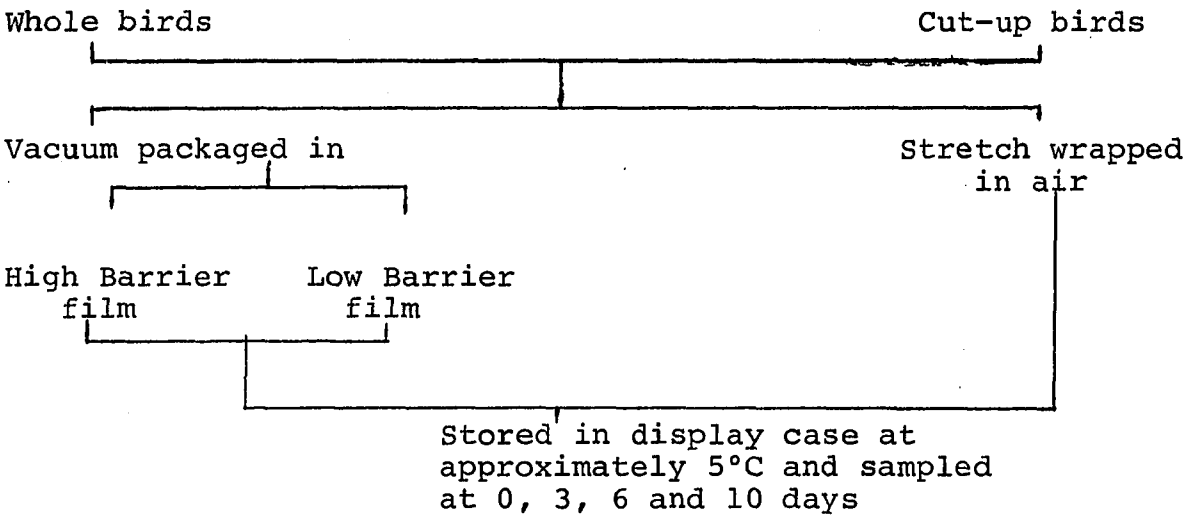
four patties per pound of meat. The patties were frozen cryogenically in a 20 ft Kwikfreeze tunnel (Airco Industrial Gases, Inc.) by liquid nitrogen (LN_2) or liquid carbon dioxide (LCO_2).

2. Turkey samples: Whole turkey samples were obtained from a turkey processing plant either after chilling (12 samples), or after brine freezing (9 samples). The former samples were subjected to cryogenic freezing in the freezer tunnel for nine to eighteen minutes using LN_2 or LCO_2 and then stored in a freezer at -29C for periodic quality evaluations along with the brine-frozen samples which were kept frozen continuously in the same freezer. Also, four unfrozen cut-up breast and thigh samples were tested as controls in order to compare the effects of freezing methods employed in this study. To obviate bird to bird variability, a total of 6 turkeys were tested for quality characteristics and comparisons were made between samples from left and right halves of the same bird.

3. Chicken samples: Eviscerated dry pack whole and cut-up broilers, from commercial processing plants were obtained from a nearby distributor for studies on the effects of vacuum packaging. The whole and cut-up broilers were divided into three groups each. Two groups were vacuum packed in a highly impermeable film (Surlyn and Saran) or in a material providing a lower barrier to oxygen (Surlyn without Saran) and the third

group was tray-packaged in air in a stretch wrap film. The flow chart diagram illustrates procedures used.

Flow Chart



The permeabilities to oxygen of these three packaging films are as follows:

High Barrier (HB): 18 cc/M^2/24 hrs at 73°F and 0% R.H.

Low Barrier (LB): 2000 cc/M^2/24 hrs at 73°F and 0% R.H.

Stretch Wrap (SW): 6500 cc/M^2/24 hrs at 73°F and 0% R.H.

Films were provided by the American Can Company, Neenah, Wisconsin.

Vacuum packaging was performed in a Bivac machine at a commercial meat specialty plant and attained a maximum vacuum of 28-29 inches of Hg.

A second study was conducted to determine the effects of chlorine treatment on the storage quality of packaged chicken. Chicken was obtained as described previously. The chicken was dipped in 25 Kg of water containing 20 ppm available chlorine in plastic trays at a rate of two birds per dip and four birds per tray. Chlorine concentration was measured by a N,N-Diethyl-p-phenylenediamine (DPD) colorimetric method after each dip with a Chlorine Kit (Hach Chemical Company, Ames, Iowa). Control broilers were dipped in chilled water with no added chlorine. All carcasses were drained before packaging. Chlorine and control treatments in the second study therefore differed from the previous work with dry pack poultry receiving no terminal "wetting" treatment. However, packaging treatments were similar in both studies.

The packaged samples were stored at 5C in a display case and evaluations were made on 0, 3, 6 and 10 days. Three replications were conducted for each study.

Bacteriological Methods

The analytical procedures employed to determine numbers or incidence of various types of bacteria are summarized in Table 3.

Sampling of beef patties: 30-gm samples were weighed into 270 ml of sterile 0.1% Bacto peptone (Difco Laboratories) in an Osterizer jar and blended for 2 minutes. Samples were

Table 3. Bacteriological procedures employed to determine bacterial densities or incidence of organisms

Types of microorganisms	Growth media	Plating technique	Incubation	Confirmatory tests
Mesophiles	Trypticase soy agar (BBL)[a]	Pour plates	30°C/24-36 hrs	
Psychrotrophs			5°C/7-8 days	
Fluorescent *Pseudomonas*	Medium B of King *et al.* (1954)	Surface plating	15°C/6 days	Fluorescence under UV light Flagellar staining
Enterococci	KF *Streptococcus* medium (Difco)	Pour plates	37°C/24 hrs	According to Barnes, 1956
Lactobacilli	LBS agar (BBL)	Pour plates	37°C/48 hrs	Gram stain
Coliforms	Violet Red Bile agar (Difco)[b]	Pour plates	37°C/24-36 hrs	Levine EMB agar (Difco)[b]
Staphylococcus	Staph 110 medium with egg yolk (Herman and Morelli, 1960)	Surface plating	37°C/48 hrs	Tube coagulase test
Salmonella	Procedures for meats (Galton *et al.* 1968)	Procedures for meats (Galton *et al.* 1968)	37°C/24 hrs	TSI and agglutination (Galton *et al.* 1968)
C. perfringens	SPS agar (Angelotti *et al.* 1962)	Anaerobic pouches (Bladel and Greenberg, 1965)	37°C/24 hrs	Motility & H_2S (Angelotti *et al.* 1962)

[a]BBL division of Bio Quest, Cockeysville, Maryland.

[b]Difco Laboratories, Detroit, Michigan.

plated after making appropriate dilutions for quantitative determination of mesophiles, psychrotrophs, coliforms and staphylococci. Sampling for Salmonella and C. perfringens was performed by swabbing areas of 50 cm^2. Evaluations were made before freezing, after freezing and during frozen storage for a period of 6 minutes.

Sampling of chicken: An area of 2 cm^2 each on each side of the breast surface was swabbed for enumeration of mesophiles, psychrotrophs, enterococci, coliforms and lactobacilli. The remainder of the breast areas was swabbed on either side for determining the incidence of Salmonella, C. perfringens and coagulase positive staphylococci.

Sampling of turkeys: Chilled or frozen turkey samples were inoculated with Streptococcus faecalis, S. faecium and Pseudomonas fluorescens cultures before cryogenic freezing (using LCO_2) and blast freezing at -29C. Areas of 10 cm^2 were swabbed to determine the effects of freezing and frozen storage on survival of inoculated cultures. Potassium tellurite was added to KF Streptococcus medium after 4 hr incubation to provide a concentration of 0.04% in the medium for selective enumeration of S. faecalis (Whittenbury, 1965).

Taxonomic studies of bacterial cultures isolated from beef patties:

Isolation of cultures: Plates incubated at 7°C for psychrotrophic counts having less than 100 colonies were selected from the following samples:

1. Fresh unground beef
2. Formulated 50% fresh and 50% frozen beef patties
3. Formulated 100% frozen beef patties
4. Formulated 100% fresh beef patties

Five to 10 colonies were selected at random from the plates and each colony was streaked twice onto Trypticase Soy Agar (TSA), BioQuest Division of BBL), and incubated at about 22C for 2 days for purification. Gram stain preparations of each culture were examined with a light microscope to determine if cultures were morphologically homogeneous. Bacterial cultures that had not been purified after two successive streak plating operations were discarded. The number of cultures purified for each treatment is given in Table 4.

A total of 418 cultures were characterized in this study along with 14 known cultures as references (Table 5), and grouped into different genera using Numerical Taxonomy as a tool of identification.

Table 4. Number of cultures characterized according to product and treatment

Product	Treatment	Number of cultures	
Fresh beef	Before freezing	61	
		After freezing	After 3 months in frozen storage
100% fresh beef patties	Frozen by LN_2	33	27
	Frozen by LCO_2	32	28
50% fresh: 50% frozen	Frozen by LN_2	30	28
	Frozen by LCO_2	32	29
100% frozen beef patties	Frozen by LN_2	32	28
	Frozen by LCO_2	32	26

Table 5. Reference cultures used for taxonomic classification

Name	Designation/source
Moraxella - Acinetobacter	MX36 Frozen beef, Food Technology Dept., ISU
Moraxella - Acinetobacter	MX40 Frozen beef, Food Technology Dept., ISU
Moraxella - Acinetobacter	MX19 Frozen beef, Food Technology Dept., ISU
Pseudomonas aeruginosa	Department of Bacteriology, ISU
Pseudomonas sp (Non-fluorescent)	PS6 Frozen beef, Food Technology Dept., ISU
Flavobacterium suavevelens	FS, Department of Bacteriology, ISU
Escherichia coli	EC1, Department of Food Technology, ISU
Bacillus subtilis	Department of Food Technology, ISU
Bacillus megaterium	Department of Food Technology, ISU
Micrococcus luteus	Department of Bacteriology, ISU
Staphylococcus aureus	SA-1 Department of Food Technology, ISU
Enterobacter aerogenes	ATCC 13048
Aeromonas liquifaciens	Department of Bacteriology, ISU
Pseudomonas fragi	Department of Food Technology, ISU

Tests used for classification: Characters used in this study are presented in Table 6. Fifty-nine characters with a total of 188 states were tested for every culture. States can be defined as the various responses that can be observed with a character; for example, with nitrate utilization as the character the states could be: 1. Nitrate not utilized, 2. Nitrate utilized and being reduced to nitrite, or 3. Nitrate utilized and yielding end products past the reduction stage of nitrite.

All the tests requiring surface inoculation of solid media were made with the replicator instrument described by Hartman and Pattee (1968). The cultures to be tested were grown in Trypticase Soy Broth (BBL) in medicine dropper bottles (standard 1/2 oz. from Arthur H. Thomas Co., Philadelphia, Pennsylvania). The bottles were incubated at 30C for 24 to 48 hrs. The broth cultures were transferred with the droppers into the wells of microliter plates (Cooke Engineering Co., Alexandria, Virginia) in a predetermined sequence to charge the capillary tubes of the inoculator. Square plastic petri plates (Lab Tek Products, Westmont, Illinois) were prepared with all the testing media. The media were allowed to solidify and dried in a 30C incubator to avoid any exudation on the surface of the agar by the time of inoculation. Every plate was inoculated simultaneously with 68 different cultures by touching the surface of the agar with the precharged

Table 6. Characters used for numerical taxonomy

Description	Number of features	Number of states
Cell morphology, gram reaction	2	6
Cell arrangement, chromogenesis	2	12
Form, elevation and margin of colonies	3	16
Form of growth on agar slants	1	6
Type of growth on thioglycolate agar	1	3
Optical features and consistency on agar plates	2	6
IMViC tests	4	8
Motility and nitrate reduction	2	4
Reaction on TSI	1	4
Growth at 5, 15, 30, 37 and 45C	5	15
Growth in the presence of NaCl (2.5%, 4.0%, 6.5% and 12%)	4	12
Growth at pH 4.0, 5.5, 6.0, 8.0 and 9.0	5	15
Growth and reaction on SS, EMB, KF, VRB, BG at 37 and 30C	10	30
Growth and reaction on Staph 110 with egg yolk medium, tellurite glycine, mannitol salt and Chapman stone media	6	19

Table 6 (Continued)

Description	Number of features	Number of states
Hydrolysis of starch, gelatin, casein, beef fat, tributyrin and arginine	6	18
Production of fluorescence	1	2
Production of catalase and cytochrome oxidase	2	6
Utilization of glucose and lactose	2	6

capillaries of the inoculator.

TSA was used in general as the basal medium for replica plating. The medium was modified as follows for individual tests: the pH was adjusted to 8.0 and 9.0 with 6 N NaOH; additions of the following ingredients: 5% egg yolk, 0.2% soluble starch, 1.5% skim milk, or 0.1% cellulose; 0.5% tributyrin and 0.5% tragacanth gum (Mathison, Coleman and Bell, Norwood, Cincinnati, Ohio) were added with homogenization for hydrolysis of tributyrin.

The medium of Goldman and Rayman (1952) with the modification of Reddy (1978) was used for hydrolysis of beef fat. Gelatinase activity was tested on the medium of Smith and Goodner (1958). Growth at pH 4.0 was tested on Potato Dextrose Agar (Difco) after adjusting the pH with a 10% solution of tartaric acid. Growth at pH 5.5 was tested on Potato Dextrose Agar. Growth at 6.0 was tested on TSA. Medium B of King et al. (1954) was used for production of fluorescent pigment. Acid from glucose and lactose was tested by stab inoculation of OF medium (Difco) containing 1% of glucose or lactose in open and sealed tubes, incubated for 2 weeks (Hugh and Leifson, 1953). Motility and reduction of nitrate were assayed as described by Angelotti et al. (1962). The method of Kovacs (1956) was employed for detection of cytochrome oxidase activity. Catalase was determined by flooding the surface of the bacterial colonies with 3% hydrogen peroxide.

Incubation time for the various tests was 24 hr at 45 or 37C, 48 hr at 30C or room temperature (about 25 ± 2°C), 4 days at 15C or 2 weeks at 5C. To test for hydrolysis of beef fat, the plates were incubated for 10 to 15 days regardless of incubation temperature used.

Taxonomic classification by computer program: Differences between the microflora isolated from samples from various treatments were determined by cluster analysis by the technique of Sneath and Sokal (1973). The computer program was written by Ms. Deborah Shank (Department of Statistics, Iowa State University). Affinity between the organisms was the basis for clustering. The degree of similarity was calculated for every possible pair of organisms according to the number of morphological and physiological characters shared by each pair. The formula of Sneath and Sokal (1973) was used for calculation of similarities in percent:

$$S = 100 \times \frac{NS}{NS + ND}$$

where S = percent similarities, NS = number of similar characters and ND = number of characters not shared by the pair.

To calculate the percent similarities, equal weight was given to positive and negative characters. Characters such as growth on a selective medium (primary) and type of reaction

manifested in the medium (secondary) were also coded so as to contribute equal weight to the percent similarity. Similarly, the responses of dichotomous characters such as motility (+ or -), multivalued qualitative characters such as colony consistency (smooth, rough or mucoid) and semi-quantitative multivalued characters such as growth (absent, scant, moderate or abundant) were given equal scores for the calculation of affinities between organisms.

A similarity matrix was computed for the bacteria recovered from each treatment using the single linkage method of Sneath and Sokal (1973). From the similarity matrix, dendograms were printed showing phenotypic relationships between organisms. For graphical presentation of the results, simplified versions of these dendograms were made.

Homogeneity chi-square analysis was used to evaluate differences in morphology and physiology of bacterial populations isolated from the beef samples subjected to various treatments. For example, the proportion of organisms utilizing glucose among those isolated before freezing was compared to the proportion of organisms utilizing glucose among those isolated after freezing. The characters used for these evaluations are listed in Table 6. The comparisons made were: unfrozen versus immediately frozen beef patties, and immediately after freezing versus 3 months under frozen

conditions.

Differences in numbers of different types of bacteria recovered from the beef and chicken samples were compared by analysis of variance after logarithmic transformation of the data (Snedecor and Cochran, 1967).

Tests to Determine the Quality of Turkey Meat

Myofibril fragmentation index (MFI)

MFI was determined by modification of the procedure of Olson et al. (1976). Three 1.27 cm cores from a frozen (after thawing for 24 hrs at 5C) or fresh turkey breast or thigh muscle were finely scissored and minced, and readily apparent pieces of fat and connective tissue were removed. Four grams of the finely scissor-minced muscle were homogenized for 30 seconds in 10 volumes (V/W) of a 2°C isolating medium consisting of 100 mM Kcl, 20 mM K phosphate, 1 mM EDTA, 1 mM $MgCl_2$ and 1 mM sodium azide. The homogenate was sedimented in a centrifuge (Sorvall, RC2B, Ivan Sorvall Inc., Norwalk, Conn.) at 1000 x g for 15 minutes and then the supernatant was decanted. The sediment was next suspended in 10 volumes (V/W) of the same isolating medium using a stirring rod, sedimented again at 1000 x g for 15 minutes and the supernatant decanted. The retained sediment was resuspended in 2.5

volumes (V/W) of isolating medium and passed through a polyethylene strainer to remove the connective tissue and debris. To facilitate passage through the strainer, 2.5 additional volumes (V/W) of isolating medium were used. Protein concentration of the suspension of myofibrils was determined by the biuret method of Gornall et al. (1949).

An aliquot of the myofibril suspension was diluted with the same isolating medium to a protein concentration of 0.5 ± 0.05 mg/ml and confirmed by the biuret method of Gornall et al. (1949). The diluted myofibril suspension was stirred, dispensed into a cuvette and absorbance of this suspension was measured immediately at 540 nm with a Spectronic 20 Bausch and Lomb colorimeter. Absorbance units were multiplied by 200 to give a myofibril fragmentation index for that particular sample.

Sarcomere Length Determination

Determinations of sarcomere length were done on the diluted myofibril suspensions (0.5 ± 0.05 mg/ml). The microscope slide was partitioned into imaginary quadrants and 24 myofibrils per sample were measured with a Vicker's image splitter attached to a Zeiss (Carl Zeiss Photomicroscope I, Germany) photomicroscope. The photomicroscope was adapted with a Neofluar 100 x phase objective. Average sarcomere lengths in μm were determined by:

$$\frac{\text{The sum of the lengths measured}}{\text{The sum of the number of sarcomeres}} \times 1.33 = \text{correction factor for optovar}$$

Analysis of myofibrillar proteins

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed according to the method of Olson et al. (1976) with assistance from the Muscle Biology Group, Iowa State University.

Determination of Tyrosine Value and Thiobarbituric Acid (TBA) Number

Twenty grams of meat were blended with 50 ml of cold 20% trichloroacetic acid (TCA) for 2 minutes. The blender contents were rinsed with 50 ml of water, mixed together, and filtered through a Whatman #1 filter. This filtrate was termed the TCA extract and was used in the tyrosine and TBA tests. Two and one-half ml of the TCA extract were diluted with 2.5 ml of water. To this, 10 ml of 0.5 N NaOH were added followed by 3 ml of Folin's Reagent (diluted 1 Folin's: 2 water). After mixing, the color was developed for 15 minutes at room temperature (approximately 25°C) before reading at 660 nm. The tyrosine value was reported as mg of tyrosine/g of meat by determining the values from a tyrosine standard graph. The TBA number was determined using a variation of the method described by Witte et al. (1970). A 5-ml aliquot

of the TCA extract was mixed with 5 ml of 0.01 M 2-thiobarbituric acid (Eastman Kodak Co., Rochester, New York). Either of two procedures was used for TBA color development. One method involved storage for 14 hr at room temperature and the other for 30 min at 100C. Color development, measured as absorbance at 532 nm, was identical when either procedure was used with standard solutions of tetraethoxy propane or with TCA extracts of meat. Absorbance at 532 nm was reported as the TBA number.

Determination of Guanidino Compounds, Essentially Creatine and Creatinine

Creatine and creatinine were estimated according to the method of Dahl (1963). Fifteen milliliters of TCA extract was taken in a 50-ml volumetric flask and volume adjusted to 25-ml with distilled water. Then 10 ml of 2N HCl was added and autoclaved at 121C for 20 minutes to convert creatine to creatinine. The flask was cooled to room temperature and 9 ml of 10% NaOH and 3 ml of saturated picric acid solution were added. Then the volume was adjusted to 50 ml with water. The mixture was allowed to stand for 1 hr at room temperature. Then the volume was adjusted to 50 ml with water the mixture allowed to stand for 1 hr at room temperature before measuring absorbance at 532 nm. Standard solutions containing 100, 200, 300 and 400 µg of creatine were determined simultaneously.

Determination of Ribonucleic Acid Derivatives

Concentration changes in ribonucleic acid derivatives were determined by measuring ultraviolet absorption of the TCA extract at 260 nm in accordance with the method described by Khan (1964).

Determination of Expressible Juice

The property of breast and thick muscles of turkey samples subjected to cryogenic and blast freezing methods to retain muscle fluid (or juice) was determined by the method of Matsushima and Topel (1969). 0.3 gm-samples were weighed to 0.3 g on 9 cm filter paper (Whatman No. 1), placed between sheets of plexiglass and subjected to 350.5 Kg/cm^2 pressure by means of a Carver Press for 3 minutes (Fred S. Carver, Carver Laboratory Press, N.J.)

Determination of Cooking Losses

Cooking losses were measured for breast muscles of turkeys that were subjected to cryogenic and blast freezing methods. Samples weighing 25 gm were cooked in a $2\frac{1}{2} \times 11\frac{1}{2}$ cm glass tube at 90C for 10 minutes in a constant temperature water bath (Miller et al. 1968). After cooking, the tubes were cooled in an ice water bath rapidly and the cooked meat was separated from the liquid by centrifuging for 15 minutes at 4000 x 15 g. The volume of liquid collected was measured as cooking loss.

Determination of Drip Losses

Drip losses from the samples frozen by cryogenic and blast freezing methods were determined by allowing the samples to thaw in their plastic bags at 5C for 3 days. Free liquid was then measured.

RESULTS AND DISCUSSION

Bacterial Populations on Beef Samples

Before freezing

The initial contamination of ground beef is a variable factor and can serve as an index of the sanitary conditions observed during processing into the ground product. The average total aerobic counts (mesophiles and psychrotrophs) before freezing as shown in Figure 4 are within the limits of proposed guidelines for ground beef (Wehr, 1978). The presence of coliforms in raw meat has been emphasized because of the meaningful role they play with regard to sanitary quality of water or milk. However, it is difficult to make a similar association with raw meat. A search of the literature has failed to show any documented correlation between the presence of E. coli or of coliforms and the presence of Salmonella in raw beef (American Meat Science Association, Proc. 29th Reciprocal Meat Conference, 1976 and 1979 Annual Summary Report of Communicable Disease Center, Atlanta, Georgia). In the present study, although coliforms were present in fairly large numbers (10^3 to 10^4 organisms/gm), no Salmonella were recovered from these samples. This suggests that the majority of coliforms contaminated the meat from hides or sources other than fecal pollution (Nottingham et al., 1974; Newton et al., 1977; Reddy, 1978).

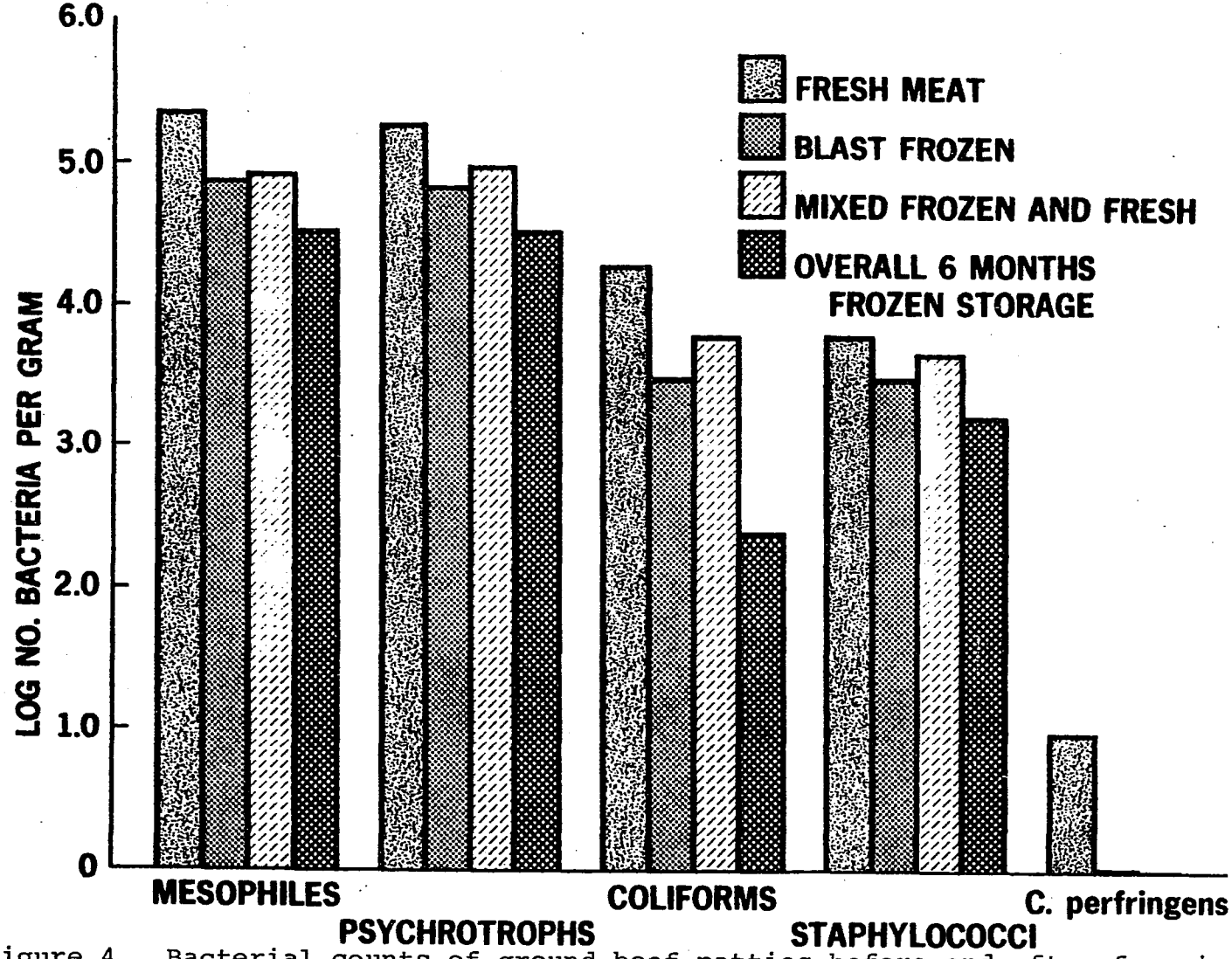


Figure 4. Bacterial counts of ground beef patties before and after freezing and frozen storage

Clostridium perfringens was present in low numbers in beef before freezing ranging from less than 1 to 10 per gram. The low contamination of meat with organisms that could be traced to probable fecal contamination was taken as an indication that good processing practices had been applied to these samples.

The staphylococcal counts ranged from 10^3 to 10^4 per gram (Figure 4). All the samples tested before freezing yielded coagulase positive Staphylococcus. These organisms are commonly present on the human skin and nasal passages. The high frequency of isolation of coagulase positive Staphylococcus from these samples reflected the high degree of manipulation to which the ground beef is subjected.

Blast frozen and frozen storage of fresh mixed frozen meat after LN_2 or LCO_2 freezing

The bar graphs in Figure 4 show that upon freezing, the average numbers of mesophiles and psychrotrophs decreased. Coliforms were reduced to a much greater extent than other types, a trend observed throughout frozen storage. Freeze injury in coliforms has been demonstrated by several authors in the literature (Ray and Speck, 1973; Alur and Grecz, 1975; Speck et al., 1975). Kraft et al. (1979) also observed a reduction in bacterial numbers on ground beef patties subjected to blast or cryogenic freezing. Recovery of

Clostridium perfringens was reduced considerably after freezing; only 9 of the 112 samples tested contained C. perfringens. No salmonellae were recovered from any of the samples tested either before or after freezing. Populations of staphylococci remained relatively constant, even throughout the six month holding period, similar to results obtained in our previous study on composition of ground beef patties (Kraft et al., 1979). The frequency of isolation of coagulase positive Staphylococcus varied very little after freezing and during frozen storage.

The overall effect of the freezing process can be summarized as follows: 1) all bacterial types decreased in number, 2) the effect was not uniform for all types present on ground beef and was more detrimental to certain types of bacteria than to others. Sulzbacher (1950) reported growth of bacteria in pork stored at -4 and -18C. Enough information exists in the literature, however, to support the contention that bacterial growth does not take place in most foods during storage under adequate frozen conditions (at or below -10C) although large numbers of microorganisms present in food retain viability for long periods of time (Stanier et al., 1970). The results of this study are in general agreement with this view.

Combining frozen meat with fresh meat resulted in increased bacterial counts when compared with fresh beef

alone (Figure 4). Bacterial counts were subjected to a "t" test by pooled variance and differences were expressed by calculating least significant differences (Table 7). Numbers of organisms in fresh beef were significantly lower throughout storage than in any of the mixtures of fresh and frozen beef (Tables 8, 9, 10 and 11). Combinations of fresh and frozen meat in a 50:50 ratio were conducive to higher counts of spoilage organisms than observed when lower percentages of frozen meat were used for the patties initially (0 or 20%). Differences in numbers with this ratio were highly significant ($P<.01$) for mesophiles, psychrotrophs, and coliforms but significant at the 5% level for staphylococci. The combination of 20% frozen to 80% fresh beef more closely resembled 100% frozen beef in bacterial levels than it did fresh beef only. Possibly, greater availability of nutrients occurred when a relatively small amount of frozen beef was combined with fresh meat because of availability of tissue fluids as a result of freezing. However, destruction of some bacterial cells might similarly occur, as indicated by Elliott and Michener (1965). Both effects may have been produced to the extent that numbers of organisms surviving frozen storage were very similar with both meat combinations (20% frozen: 80% fresh or 100% frozen). With the 50:50 combination, sufficient tissue damaged beef may have been provided to allow for maximum nutrient availability; hence, bacterial numbers

Table 7. Effect of proportions of frozen and fresh beef on bacterial counts of stored patties

Percent frozen beef[1]	n	Log number bacteria per gram			
		Mesophiles	Psychotrophs	Coliforms	Staphylococci
0	28	4.31^{a}	4.24^{a}	2.24^{a}	3.16^{a}
20	28	4.57^{b}	4.56^{b}	2.42^{b}	3.29^{b}
50	28	4.72^{c}	4.72^{c}	2.58^{c}	3.38^{bd}
100	28	4.58^{b}	4.60^{b}	2.43^{b}	3.26^{abe}

[1]For each column for the percent frozen beef, counts having different letter superscripts are significantly ($P<.01$) different from each other.
Superscript "bd" differs from "b" significantly at the 5% level but not at the 1% level,
superscript "abe" differs from "a" significantly at the 5% level but not at the 1% level,
superscript "b" does not differ significantly from "abe".

Table 8. Mesophilic counts during 6 months of frozen storage in log numbers per gram of beef patty

Days	n	Percent frozen beef			
		0	20	50	120
1	4	4.31	4.52	4.63	4.77
30	4	4.28	4.57	4.68	4.78
60	4	4.16	4.26	4.47	4.38
90	4	4.24	4.55	4.50	4.43
120	4	4.38	4.78	4.89	4.62
150	4	4.30	4.67	4.86	4.62
180	4	4.48	4.65	4.98	4.56

Table 9. Psychrotrophic counts during 6 months of frozen storage in log numbers per gram of beef patty

Days	n	Percent frozen beef			
		0	20	50	100
1	4	4.48	4.70	4.82	4.88
30	4	4.45	4.75	4.84	4.95
60	4	4.66	4.64	4.69	4.69
90	4	4.30	4.62	6.63	4.44
120	4	4.36	4.71	4.91	4.66
150	4	3.37	4.25	4.49	4.25
180	4	4.04	4.24	4.70	4.27

Table 10. Coliform counts during 6 months of frozen beef storage in log numbers per gram of beef patty

Days	n	Percent frozen beef			
		0	20	50	100
1	4	2.26	2.50	2.72	2.71
30	4	2.28	2.48	2.65	2.69
60	4	2.53	2.55	2.55	2.44
90	4	2.24	2.43	2.48	2.29
120	4	1.96	2.20	2.50	2.12
150	4	2.16	2.31	2.58	2.21
180	4	2.23	2.48	2.59	2.48

Table 11. Staphylococcal counts during 6 months of frozen beef storage in log numbers per gram of beef patty

Days	n	Percent frozen beef			
		0	20	50	100
1	4	3.11	3.16	3.26	3.36
30	4	3.13	3.25	3.37	3.37
60	4	3.25	3.18	3.31	3.26
120	4	3.23	3.39	3.42	3.28
150	4	3.21	3.31	3.43	3.20
180	4	3.28	3.41	3.56	3.30

were greatest with this combination. While no actual proof is available from this work to conclusively explain the results obtained, the possibilities described should be considered. It is also possible that breaking of clumps of organisms as a result of freezing may have added to the counts in samples containing frozen meat in combination with fresh beef. In the original unfrozen meat, fewer injured cells might be expected to be present than in the frozen meat. These organisms, when given the available nutrients resulting from tissue damage in the frozen meat, might have readily grown to increase populations in fresh-frozen combinations as compared with meat that was not initially frozen, or with 100% frozen beef containing more injured organisms. It should also be recognized that plating procedures may produce variability in counts. However, these same procedures were used consistently for all treatments and statistical treatment of the data would also take inherent variation into account.

Figure 5 demonstrates the similarity of effects of cryogenic freezing methods on numbers of organisms examined. Counts shown in Figure 5 represent average populations after freezing and frozen storage of patties. From these data, equal results might be expected when either LN_2 or LCO_2 are used for freezing ground beef patties with subsequent holding at freezing temperatures, regardless of amount of previously

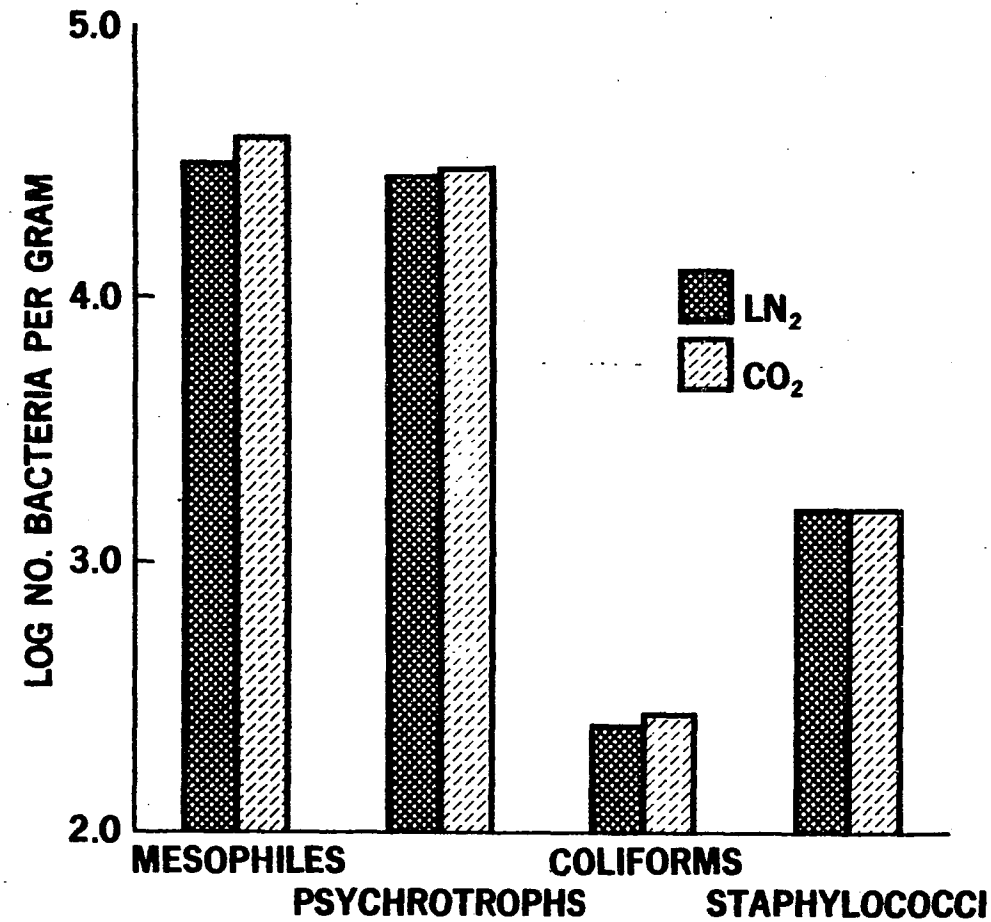


Figure 5. Effect of liquid nitrogen and liquid carbon dioxide freezing on bacterial types during subsequent frozen storage

frozen beef mixed with fresh meat before cryogenic freezing.

Figure 6 shows the incidence of coagulase positive Staphylococcus as percentage of total staphylococcal counts on Staph 110 medium. The 50:50 combination resulted in higher recoveries than any other combinations, regardless of freezing methods (LN_2 or LCO_2). This may be due to greater recovery of the freeze injured organisms as a result of nutrients provided by damaged tissues or related to higher incidence of organisms initially.

Taxonomic Classification of Bacterial Flora on Beef Patties

Explanation of numerical taxonomy, interpretation and presentation of results

Taxonomic grouping of the organisms isolated from the beef patties was done for the purpose of detecting changes in the type of microflora during freezing and during frozen storage.

Independent analysis was made for every group of isolates according to its source of isolation; 1) fresh, unfrozen beef, 2) beef patties after freezing by LN_2 or LCO_2, and 3) beef patties subsequently held in frozen storage for 3 months at -20C after cryogenic freezing. When calculating coefficient of similarities for each of these treatments, the set of 14 known cultures was included with the cultures isolated from the beef patties for comparative purposes.

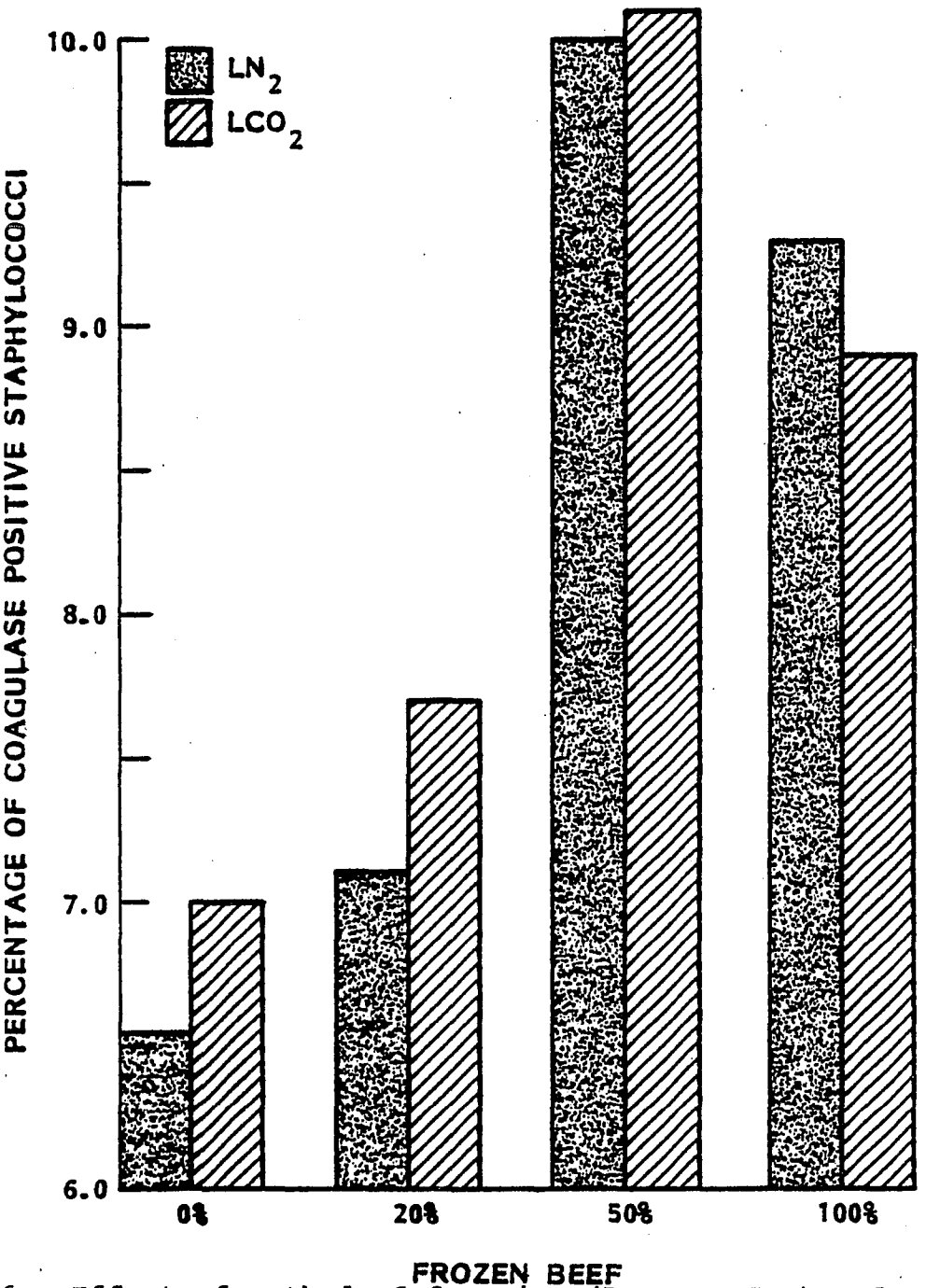


Figure 6. Effect of method of freezing (LN_2 or LCO_2) and properties of frozen beef in combination with fresh beef on the incidence of coagulase positive staphylococci

The dendrograms printed by the computer were branched tree diagrams. Figure 7 shows a hypothetical dendrogram as printed by the computer. Each branch or vertical line represented a different culture. At the top of each branch a code number was printed for identification of the organisms represented by each line. The ordinate had a scale in percent to measure the level of similarities. The branches were connected by horizontal lines similar to the top of a bar in a bar graph and showed the level of similarities between the organisms so connected by reading the scale on the ordinate. For example, cultures 2 and 3 in Figure 7 would have had 99% similar responses or shared 99% of the characters tested. These dendrograms simply showed phenotypic relationships between microorganisms. The computer dendrograms linked the isolates from beef patties (represented in Figure 7 by numbers) among themselves as well as with the known cultures which were represented by letters in Figure 7. The closeness between the microorganisms isolated from beef and the known cultures was determined by the highest linkage clustering method.

For the construction of taxonomic groups, Sneath (1962) recommended as the criterion for admission to a group either

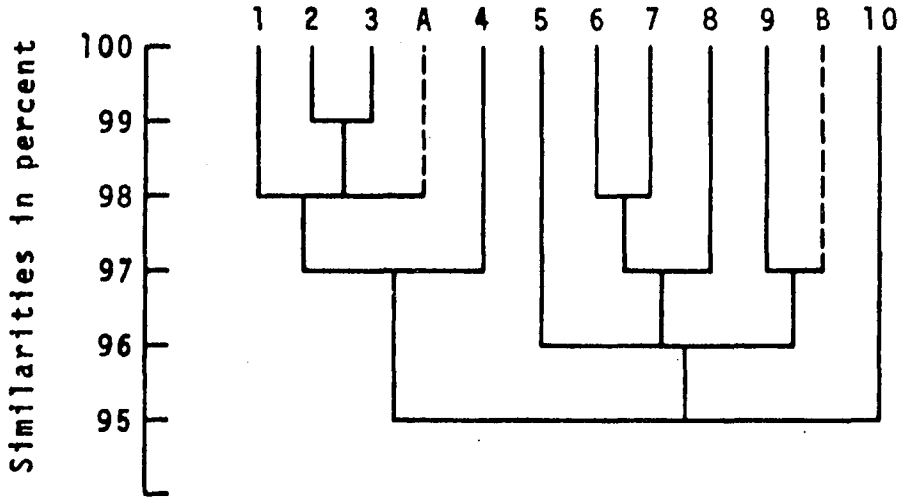


Figure 7. Hypothetical dendrogram as printed by the computer

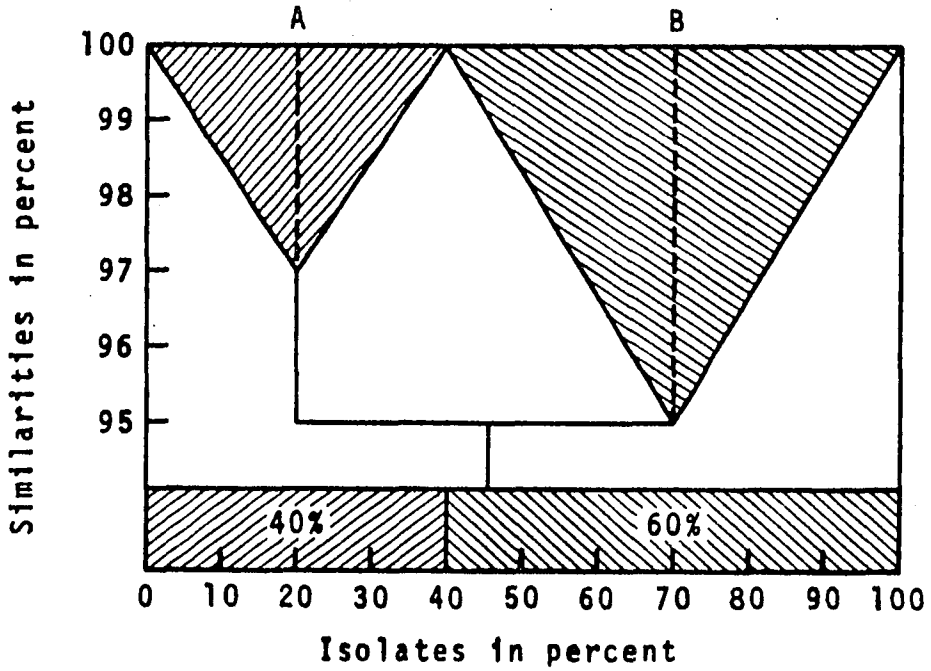


Figure 8. Simplified version of the dendrogram in Figure 7

the highest similarity value between an entrant and the existing members of the group or the use of the mean similarity values. In calculating mean similarity values, each strain of the group has equal weight in determining the similarity level for the group. A nucleus of a group is formed by the pair of strains having the highest similarity. Strains which have the highest mean similarity with the members of the nucleus are added sequentially. After incorporation of each new strain to the nucleus, the mean similarity for the group is recalculated. The mean similarity of the group is taken to represent the similarity level at which the cluster forms. Two groups fuse at a similarity level which is equal to the mean of the intergroup similarity value. This method gives very defined clusters. Thornley (1967) used mean similarity in a taxonomic study of Acinetobacter. To make simplified versions of the computer dendrograms, the phenetic groups formed by Thornley were prefixed by choosing 72.5, 82.5, and 92.5% similarity at limits or boundaries to form 4 phenons. Therefore, defined groups formed above 92.5% similarity were considered more closely related than groups formed between 82.5 and 92.5% even though some groups formed close to the 92.5% level, either above or below this boundary. Such groups may be closely related but separated from each other arbitrarily because of the chosen limits.

Another approach is to select differential characters to form groups (Colwell, 1964). Characteristics are chosen according to their significance as a diagnostic key. This method assumes the existence of a hierarchy of characters and the groups are formed on the same basis used for phylogenetic taxa. In the present investigation, where a known set of cultures was used, the highest linkage method of Sneath (1962) was considered the most adequate. However, in preparing the simplified dendrograms the highest linkage of the isolates from beef patties with the known cultures was the criterion for group formation. The groups were formed at the highest common level of similarities between the unknown and the known cultures. This system assumes that the known cultures used were typical representatives of their taxa and that differences as small as a 1% similarity for intergroup linkage were acceptable variability. These two assumptions were made with the recognition that aberrant forms of a class might have been misplaced.

In the hypothetical dendogram in Figure 7, cultures 1, 2, 3 and 4 (unknown) would have been considered to form a phylogenetic cluster with A (a known culture) rather than with B since they linked at a higher level of similarities with A (97%) than with B (95%). The same consideration would have led to cluster cultures 5, 6, 7, 8, and 9 with culture B at

96% similarity rather than with A at 95%. In cases similar to that of culture 10 in Figure 7, which linked at 95% with either A or B the highest linkage could not be used in determining its placement into either cluster and other judgements would have to be made. To be able to include culture 10 within the cluster formed by 1, 2, 3, A and 4, the highest common level of similarities of the cluster would have had to be lowered from 97 to 95% or 2 units on the similarity scale. By including culture 10 within the cluster formed by 5, 6, 7, 8, 9 and B the level of similarities of the cluster would have to be lowered only one unit on the similarity scale (from 96 to 95%). Therefore, culture 10 would have been placed in the cluster with B with the least alteration of the phenotypic level of similarities for the clusters printed by the computer.

The tree diagram in Figure 7 could be represented as shown in Figure 8. The triangles represent clusters of microorganisms that grouped at equivalent levels of similarities: cluster A at 97% and cluster B at 95%. The computer dendrograms in this work ranged from 75 cultures for classification of microorganisms isolated before freezing (61 unknown plus 14 known) to 205 after freezing (191 unknown plus 14 known) and 180 (166 unknown plus 14 known) after frozen storage at -29C for 3 months.

To prepare graphs for presentation of results the tree diagrams had to be simplified and adapted to fit into conventional size graphs. To keep the size of the clusters in perspective, the base of the triangles were drawn proportional to the numbers of isolates or unknown cultures included into each cluster as a percent of the total number of microorganisms isolated from the particular treatment. For example, cluster A in Figure 8 which was formed by 4 of the 10 unknown cultures (1, 2, 3, and 4) was represented by triangle A in Figure 8. The size of the base of this triangle is 40 units on the scale in percent on the abscicca indicating that the cluster includes 40%, or 4 of the 10 isolates in the graph. The known cultures A and B were represented by discontinuous lines in Figure 7. On the simplified dendrograms to follow (Figures 9 through 11) the reference cultures were identified by names of genus or the taxonomic family.

Microflora present on the beef before freezing

About 97% of the isolates (Figure 9) were formed into eight clusters according to the location of the set of known cultures in the dendrograms. Moraxella-Acinetobacter cultures represented 28% of the total flora and formed into 3 groups at a mean similarity level of 88.5% with the reference cultures (Table 12). The second large group was formed by

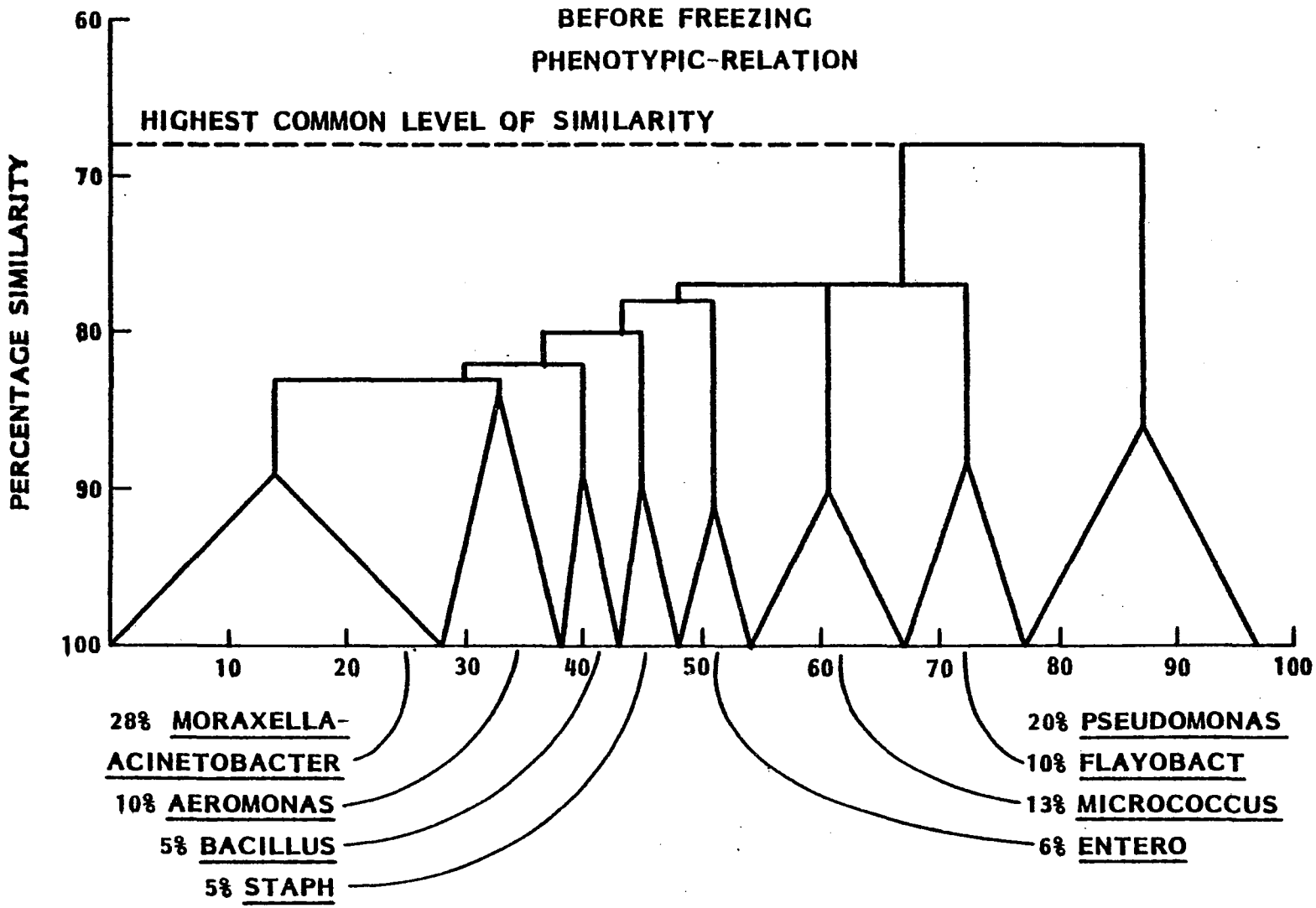


Figure 9. Dendrogram of clusters of organisms before freezing

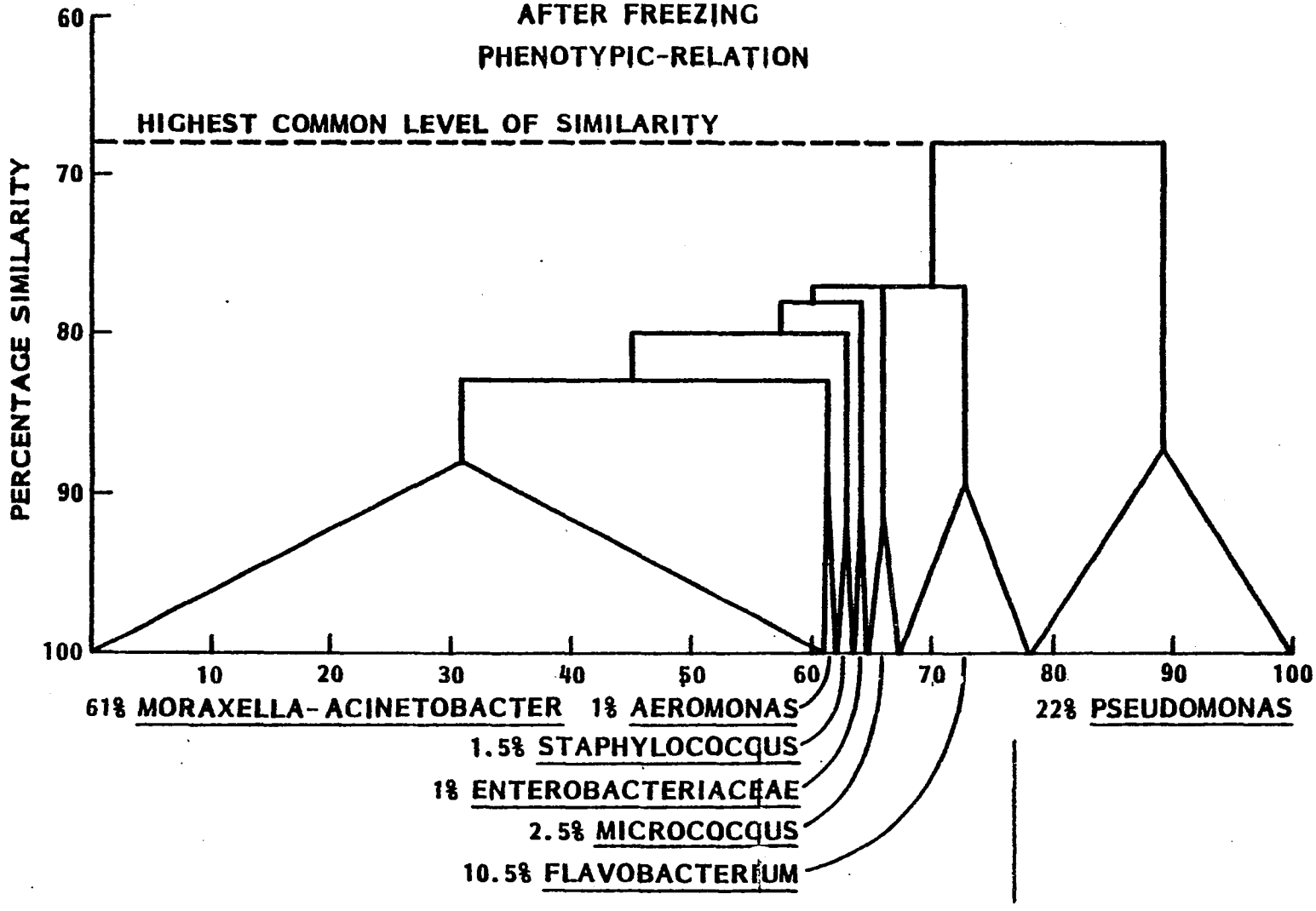


Figure 10. Dendrogram of clusters of organisms after freezing

Table 12. Taxonomic relationship of microorganisms isolated from beef before freezing

No. of isolates	% of Isolates	Proportion of isolates forming distinctive clusters with known cultures	Known cultures	Average level of similarity at which cluster formed with known clusters
18	28%	59.0%	*Moraxella-Acinetobacter* sps.	88.5%
		6.0%		
		35.0%		
6	10%	16.0%	*Flavobacterium suaveolens*	88.0%
		14.0%		
6	10%	33.0%	*Aeromonas liquifaciens*	84.0%
		67.0%		
7	13%	100.0%	*Micrococcus luteus*	90.0%
4	6%	50.0%	*Enterobacter aerogenes*	91.0%
		50.0%	*Escherichia coli*	
3	5%	67.0%	*Staphylococcus aureus*	89.5%
		33.0%		
3	5%	100.0%	*Bacillus subtilis* *Bacillus megaterium*	89.0%
12	20%	8.5%	*Pseudomonas* sp. (PS6)	86.0%
		50.0%	*Pseudomonas aeruginosa*	
		25.0%		
		16.5%		
2	3%	Unidentified		

Pseudomonas-like organisms which constituted 20% of the total isolates and formed into 2 clusters at a mean similarity level of 86% with the reference cultures (Table 12). The other members represented the genera Micrococcus, Flavobacterium, Aeromonas, Staphylococcus, Bacillus, or members of the family Enterobacteriaceae, in smaller proportions. Three percent of the cultures did not fall in any of the clusters and were labelled as a miscellaneous group (Table 12).

Microflora present on beef after freezing

After freezing, the organisms grouped into 7 genera (Figure 10) in the dendrogram. Two taxonomic relationships of microorganisms are presented in Table 13. Moraxella-Acinetobacter and Pseudomonas-like organisms represented 61% and 22% of the total flora, respectively. As expected, a decrease in Pseudomonas members was not observed as a result of freezing in this study. This may be due to the presence of surviving Pseudomonas in the frozen flaked ground beef used for the formulation. Those Pseudomonas numbers that survived freezing must have remained constant during further freezing. The Flavobacterium genus represented the third predominant group after freezing and was followed by other minor groups which included members of Micrococcus, Staphylococcus, Aeromonas and Enterobacteriaceae (Table 13).

Figure 11. Dendrogram of clusters of organisms after 3 months frozen storage at -29C

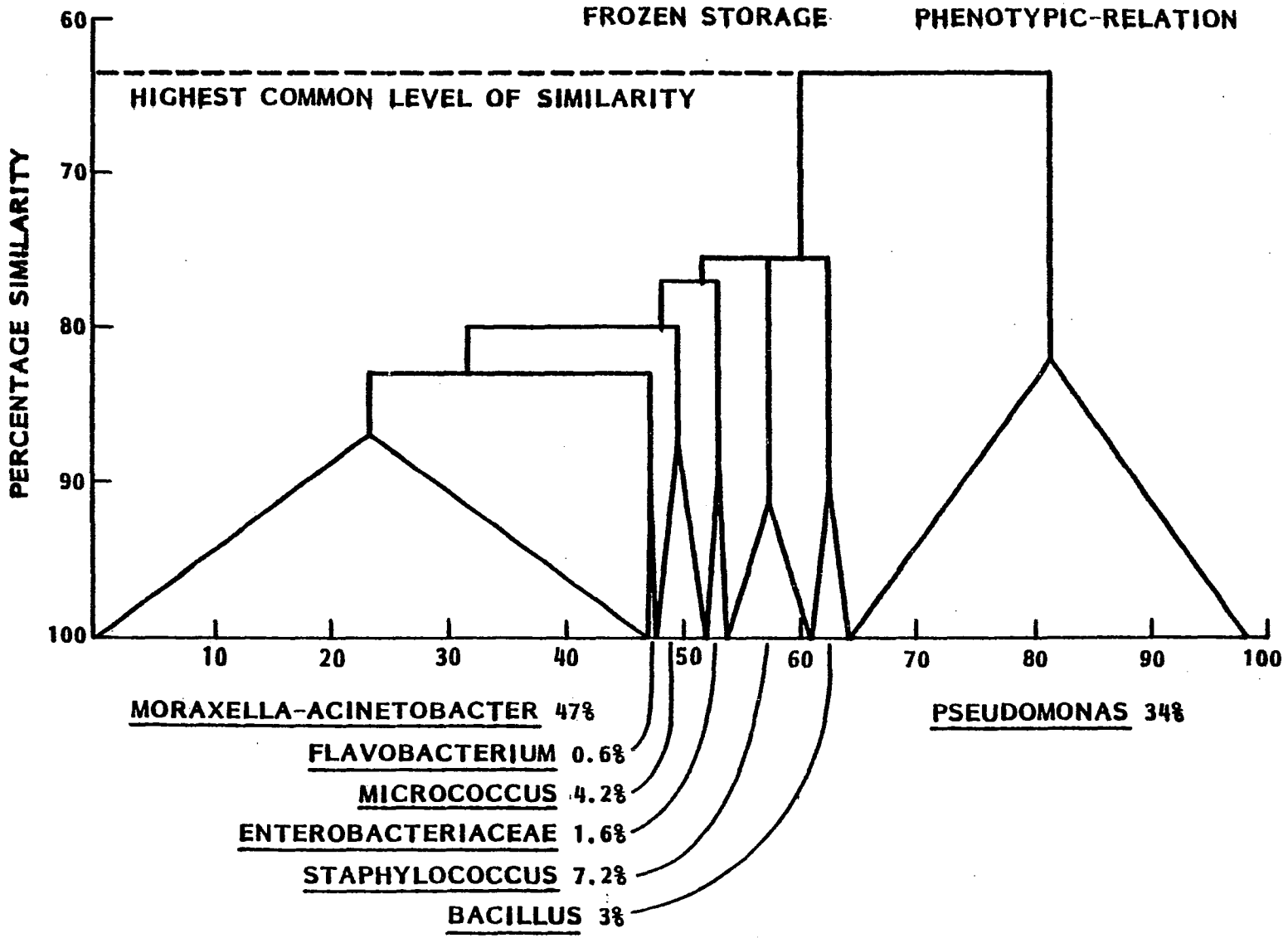

FROZEN STORAGE PHENOTYPIC-RELATION
HIGHEST COMMON LEVEL OF SIMILARITY
PERCENTAGE SIMILARITY
60
70
80
90
100
10
20
30
40
50
60
70
80
90
100
MORAXELLA-ACINETOBACTER 47%
PSEUDOMONAS 34%
FLAVOBACTERIUM 0.6%
MICROCOCCUS 4.2%
ENTEROBACTERIACEAE 1.6%
STAPHYLOCOCCUS 7.2%
BACILLUS 3%

Table 13. Taxonomic relationships of microorganisms isolated from beef patties after freezing

No. of isolates	% of Isolates	Proportion of isolates forming distinctive clusters with known cultures	Known cultures	Average level of similarity at which cluster formed with known clusters
117	61.3%	25.0% 21.0% 42.0% 12.0%	*Moraxella-Acinetobacter* sps.	88.5%
20	10.5%	60.0% 40.0%	*Flavobacterium suaveolens*	88.0%
2	1.0%	100.0%	*Aeromonas liquifaciens*	85.0%
5	2.6%	100.0%	*Micrococcus luteus*	90.0%
2	1.0%	50.0%	*Enterobacter aerogenes* *Escherichia coli*	91.0%
3	1.6%	100.0%	*Staphylococcus aureus*	91.0%
42	22.0%	40.0% 28.5% 31.5%	*Pseudomonas aeruginosa* *Pseudomonas* sp. (PS6)	87.0

Microflora present on beef after frozen storage

Figure 11 also shows that the predominant genera after 3 months of frozen storage were Moraxella-Acinetobacter and Pseudomonas. The former group was formed into two clusters at a mean similarity level of 87% with the reference cultures, representing a more homogeneous nature than for the other treatments. The Pseudomonas group also showed the same tendency and represented 34.6% of the total isolates. Members of the genus Staphylococcus showed an increase more than other minor groups during frozen storage at -29C (Table 14).

Comparison of the effects of freezing and frozen storage

The reduction in microflora after freezing seemed to be due to a greater reduction in numbers of Aeromonas, Bacillus, Enterobacteriaceae, Micrococcus and Staphylococcus than Pseudomonas and Moraxella-Acinetobacter groups. As expected, a great decrease in the later groups after freezing did not show in this study because the samples that were tested after freezing contained mixtures of fresh and previously frozen beef. Frozen storage produced an increase in the proportion of Pseudomonas members with a simultaneous decrease in Moraxella-Acinetobacter and members of other genera. These results indicate that the Pseudomonas-like organisms that survived initial freezing must have remained

Table 14. Taxonomic relationship between microorganisms isolated from beef patties after 3 months frozen storage

No. of isolates	% of Isolates	Proportion of isolates forming distinctive clusters with known cultures	Known cultures	Average level of similarity at which cluster formed with known clusters
78	48.0%	86.0% 14.0%	Moraxella-Acinetobacter spp.	87.0%
1	0.6%	100.0%	Flavobacterium suaveolens	90.0%
7	4.2%	100.0%	Micrococcus luteus	87.0%
3	1.9%	67.0% 33.0%	Enterobacter aerogenes Escherichia coli	88.0%
12	7.4%	75.0% 25.0%	Staphylococcus aureus	91.0%
5	3.1%	100.0%	Bacillus spp.	90.0%
56	34.6%	36.0% 64.0%	Pseudomonas aeruginosa Pseudomonas sp. (PS6)	87.0%

at constant densities during frozen storage whereas as Moraxella-Acinetobacter members were more resistant to initial freezing but gradually lost viability during frozen storage.

Variations, both quantitative and qualitative, in bacterial genera predominating in meat, poultry and other foods, may be expected due to the influence of a number of factors such as season, environment and plant sanitation. The hypothesis that freezing might affect some predominant organisms more than others was considered. The predominance of Moraxella-Acinetobacter and Pseudomonas members has been observed in a previous study (Kraft et al., 1979) on beef patties frozen for five months at -29C. The higher survival (48%) of Moraxella-Acinetobacter and Pseudomonas (34%) groups than others suggest that the differences are due to either higher incidence in fresh ground beef (Siedman et al., 1976) or differences in resistance to freeze damage. The survival of the Moraxella-Acinetobacter group of organisms to stress conditions such as low water activity (Juven and Gertshovki, 1976), radiation (Welch and Maxey, 1975) and prolonged frozen storage at -29C for 5 months (Kraft et al., 1979) suggests that these are relatively hardy types of organisms. Rey (1975) reported that Pseudomonas survived well during frozen storage of chicken, although initially their counts were reduced.

Significance of the properties used for classification

Many of the characters used to determine similarities between organisms were enzymatic functions. Environmental factors such as freezing can block many normal pathways (Koepsell, 1950; Alford, 1960) or inducible enzyme systems of psychrotrophic bacteria (Hegeman, 1965). The experimental procedures used for these investigations involved three successive transfers onto trypticase soy agar for isolation and purification. Trypticase, an enzymatic digest of casein, is present in this medium and promotes quick recovery of bacterial cells metabolically injured by freezing (Straka and Stokes, 1959). It was assumed that the properties of the microflora used for classification were normal responses rather than erratic behavior of cells injured by freezing.

Stanier et al. (1966) have indicated that identical responses to some tests by different bacteria is not necessarily indicative of a high degree of relationship between two species since some substrates might be degraded to the same end product by biochemically different pathways. For classification of organisms important in food spoilage or possible hazard, differences of this kind would not have the same weight as for phylogenetic taxonomy. The contribution to food spoilage by degradation of food components to identical end products would be equivalent, regardless of the

metabolic pathway involved. Therefore, even when some of the characters used in this classification might not be valid for a typological approach, they were considered valid for the association of microorganisms contributing to food spoilage. These diagnostic characteristics indicate the behavior of the isolates from beef under defined conditions. Chronological and environmental variability were minimized since the responses were obtained at the same age of organisms and in the same environment for isolates within each group. With the simplicity of the methodology it was possible to compare many characters for large groups of microorganisms. The drawing of large samples from the populations made them more representative than if smaller samples were used. This, in turn, allowed for increased application of the probabilities to make interpretations of results.

Effects of freezing and frozen storage on some properties of the microflora

Very few of the characters tested were absolutely present or absent for the microflora of beef patties. Ability to grow at 30C and at pH 6.0 were common characters, while none of the isolates was capable of growth at pH 4.0 nor on 12% NaCl media. All other responses varied among isolates and changed to some degree with the various treatments.

Results of chi-square tests for significance of the changes in characters of the microflora with treatments are

presented in Table 15. The change in proportions of isolates with treatment for the various characters is presented in Figures 12 to 14. Figure 12 shows the psychrotrophic nature of the microflora isolated from the samples. About 75% grew well at 5C before freezing, with an increase to about 80% after freezing and frozen storage. Eight percent of the organisms isolated after freezing and 16% of those isolated after frozen storage were able to grow well at 37C also. The percentage of isolates capable of hydrolyzing beef fat increased from about 18% before freezing to 35% after frozen storage, probably due to the increase in proportion of Pseudomonas (Table 14). This finding is further evidenced by the fall and rise in the proportion of gelatin and casein hydrolyzing isolates (Figure 13) after freezing and frozen storage, respectively. Although the Moraxella-Acinetobacter group is capable of hydrolyzing tributyrin, it is considered as a group of metabolically inert organisms since none of the isolates could utilize even simple sugars like glucose (Reddy, 1978). Changes in the proportions of organisms with characters such as oxidase activity, glucose utilization (Figure 14), gelatin and beef fat hydrolysis (Figure 13) were associated with the changes in Pseudomonas group. These observations reinforce the assumption that the Pseudomonas group is more susceptible

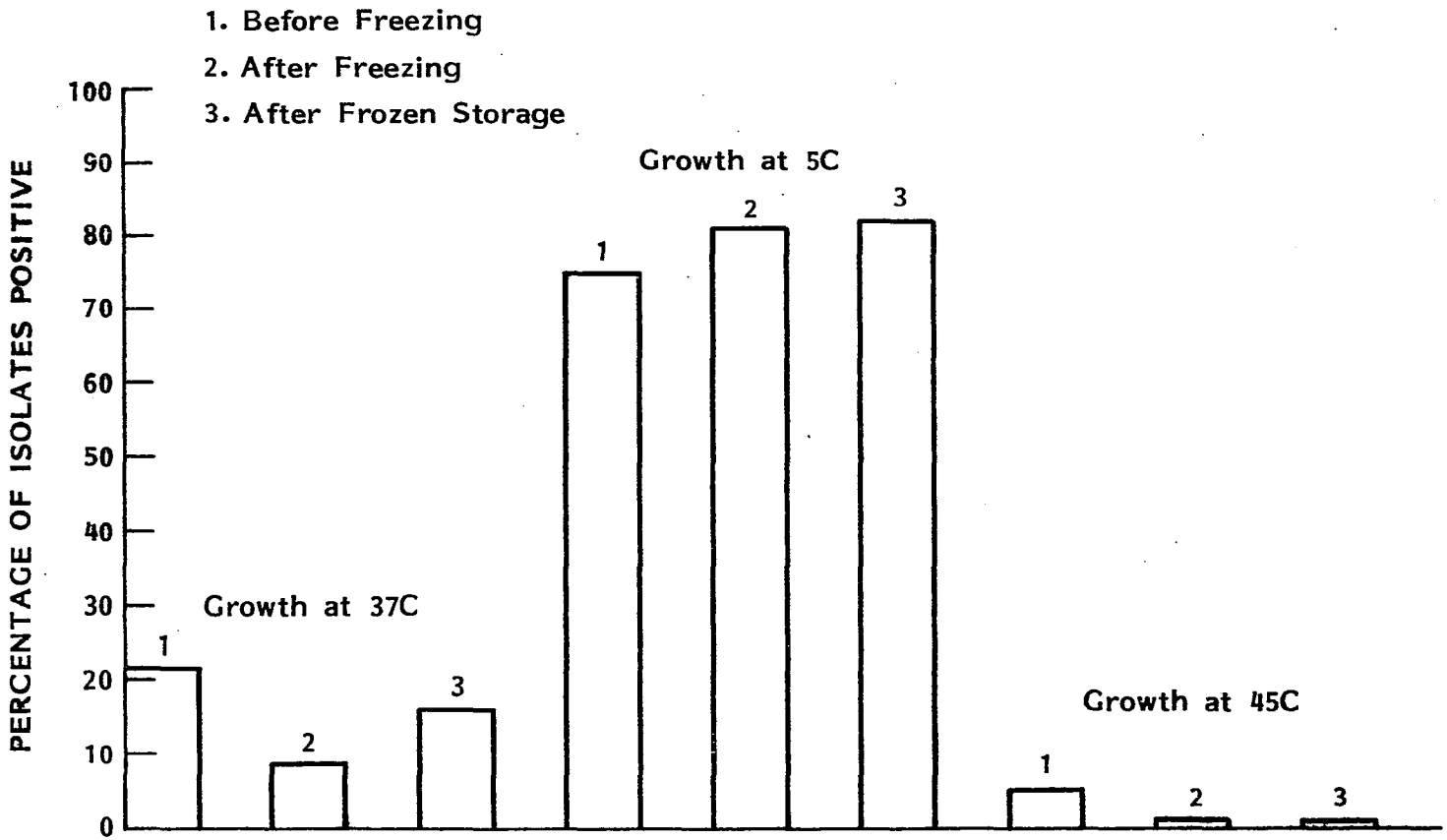


Figure 12. Changes in the proportions of microorganisms growing abundantly at 5, 37, and 45C before freezing, after freezing and after frozen storage

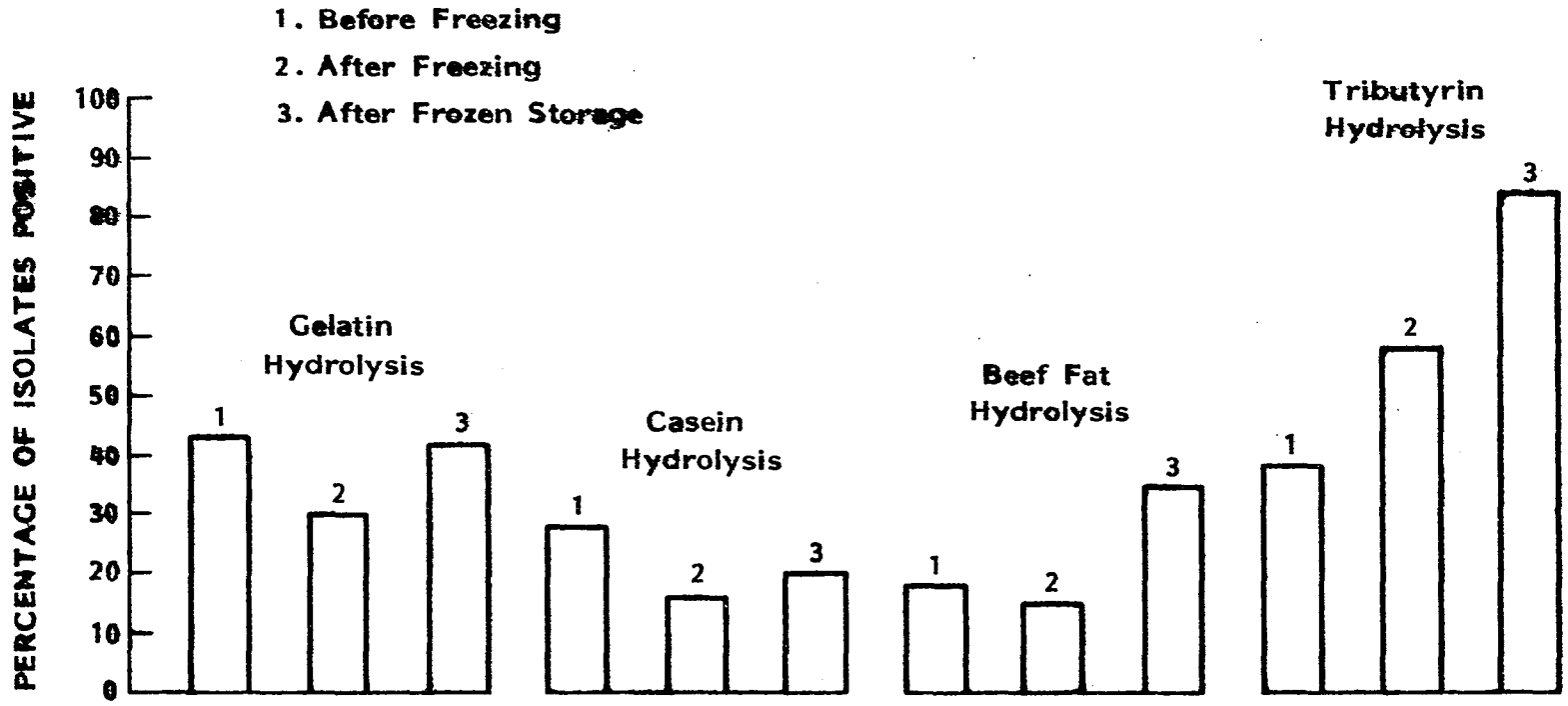


Figure 13. Changes in the proportion of isolates having different biochemical characteristics before freezing, after freezing and after frozen storage

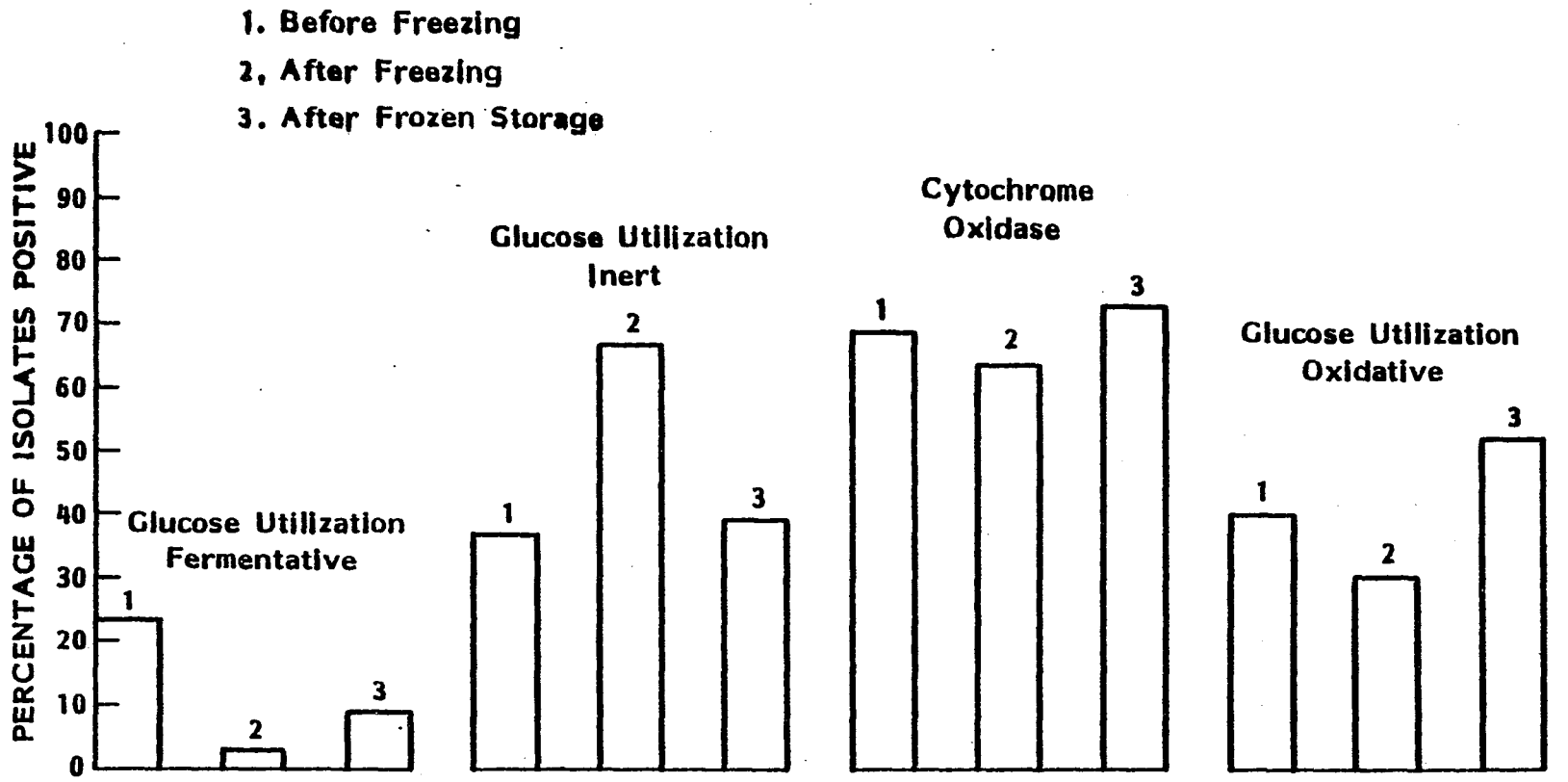


Figure 14. Changes in the proportion of isolates having different biochemical characteristics before freezing, after freezing and after frozen storage

Table 15. Chi-square tests for significance of the changes in proportions of isolates with treatments for different characters of the microflora

Characters	Before versus after freezing	After freezing versus frozen storage
Growth at 5C	*	N.S.[a]
Growth at 37C	**	**
Growth at 45C	**	N.S.[a]
Gelatin hydrolysis	**	**
Casein hydrolysis	**	*
Beef fat hydrolysis	*	**
Tributyrin hydrolysis	**	**
Cytochrome oxidase	*	*
Glucose utilization (oxidative)	**	**
Glucose utilization (fermentative)	**	**
Glucose utilization (inert)	**	**

[a]N.S. = not significant.

*Significant at 5% level of probability.

**Significant at 1% level of probability.

to the cold shock of the freezing process, but more stable than the Moraxella-Acinetobacter group to prolonged frozen storage (Tables 12 to 14).

Kitchell and Ingram (1956) reported that frozen defrosted meat spoils under refrigeration at the same rate as unfrozen controls. Rey and Kraft (1971) observed a selective effect of frozen storage on the natural bacterial flora of chicken which resulted in increased proportions of metabolically active psychrophiles. In the present study, organisms with properties related to spoilage such as lipolysis of beef fat, casein and gelatin hydrolysis (protein breakdown), also increased in proportions during frozen storage (Figure 13).

From the foregoing discussion it is evident that most bacterial species remain viable for long periods of time under freezing temperatures. But freezing would selectively modify the bacterial flora that is present on beef under commercial conditions since rate of death upon freezing and frozen storage is not equal for all members of the bacterial population. The bacterial flora present in frozen beef patties will be determined then by the type of bacteria present before freezing. The bacteria surviving freezing, being those cells with greatest resistance to injury at freezing temperatures, will be able to resume active growth upon thawing, if conditions are favorable. Therefore, it is important to

minimize contamination of the fresh product, especially with potential pathogens, regardless of whether or not freezing is to act as a means of preservation.

Microbiological Quality of Dry Packed Chicken (First Study)

Effect of packaging materials on mesophilic and psychrotrophic bacteria

In Figures 15 to 18, it can be noted that tray-packed stretch-wrapped poultry had the most rapid increases in the mesophilic and psychrotrophic bacterial flora and to the highest levels, regardless of whether the chicken was whole (Figure 15) or cut-up (Figure 16). Spoilage as determined by the type of odor revealed that stretch-wrapped chicken had the shortest shelf life, and the bacterial numbers reached levels of 10 million per cm^2 on whole birds and 100 million per cm^2 on cut-up birds within 6 days at 5C. A putrefactive type of odor typical of the growth of Pseudomonas was noticed on these birds. Ayres et al. (1950) described this kind of odor at the time of spoilage of chicken and attributed the spoilage to the Pseudomonas group of organisms. In contrast, in the present work, vacuum-packaged chicken showed only gradual increase in bacterial numbers. Bacterial counts reached about 1 million for the low barrier (LB) whole carcasses and between 100

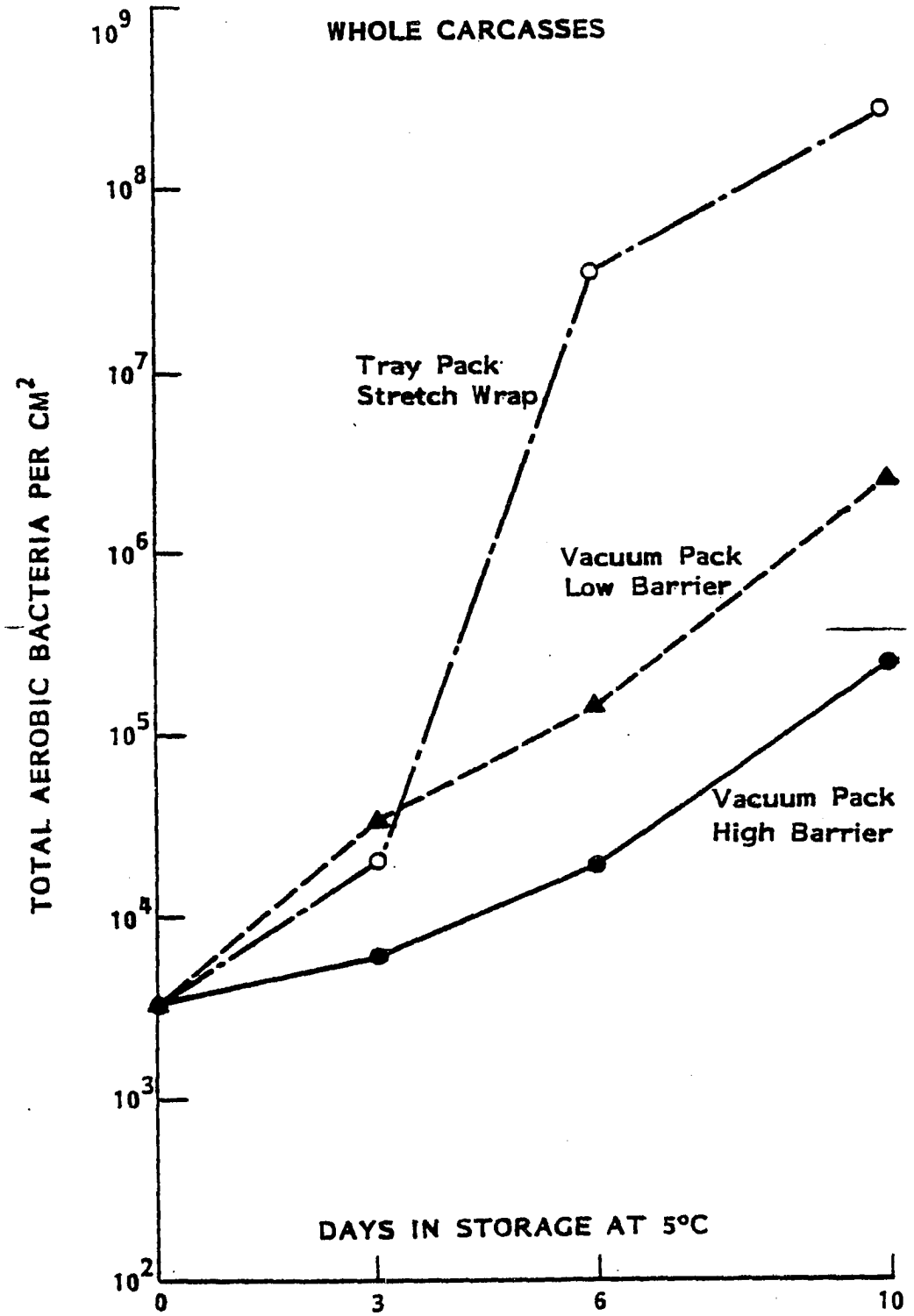


Figure 15. Effect of packaging materials and methods on total aerobic bacteria (mesophiles) on whole chicken during storage at 5C in a display case

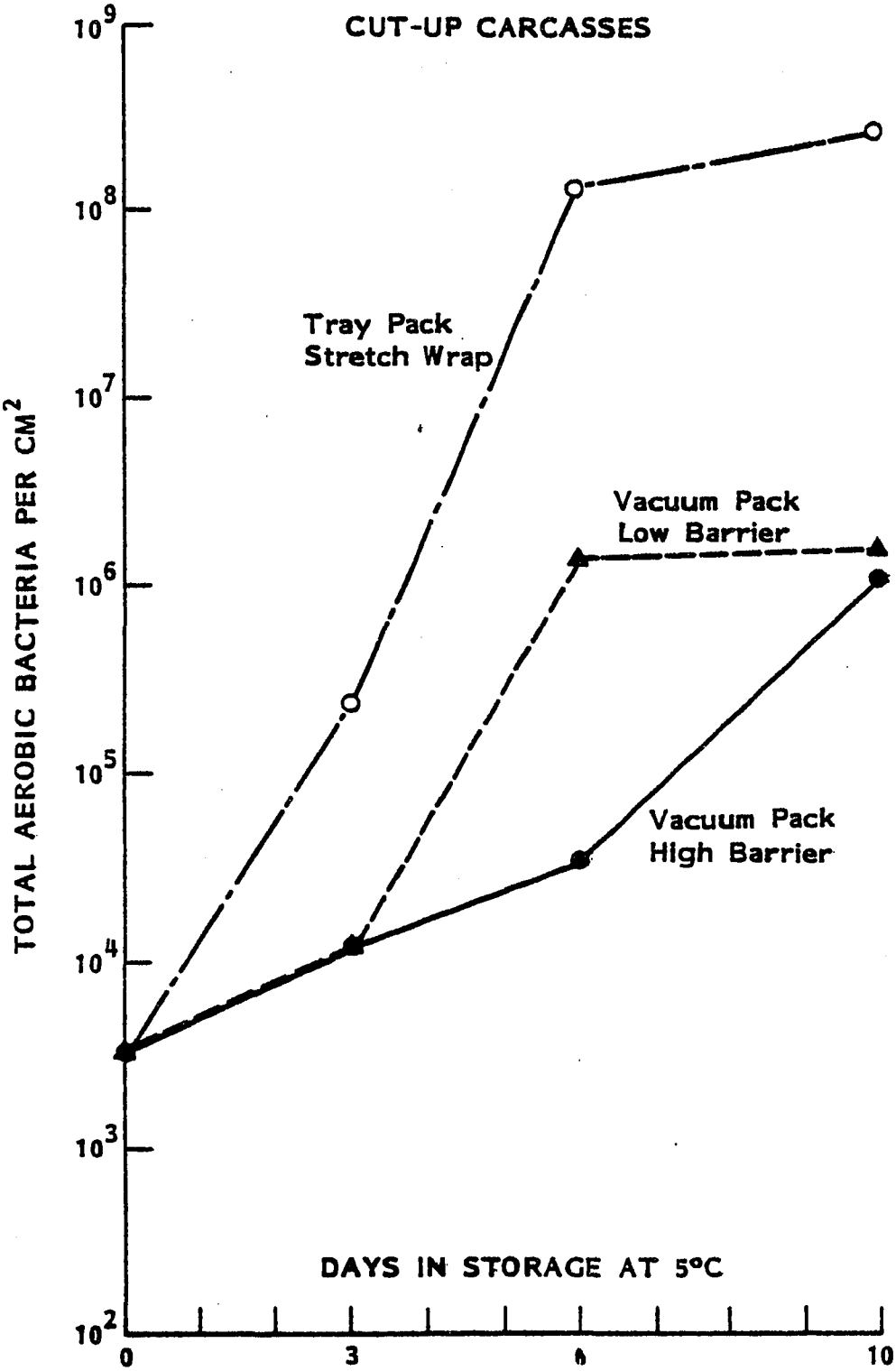


Figure 16. Effect of packaging materials and methods on total aerobic bacteria (mesophiles) on cut-up chicken during storage at 5C in a display case

thousand and 1 million per cm^2 for high barrier (HB) whole birds at 10 days. Cut-up carcasses showed higher bacterial populations by comparison with whole birds. According to Baran et al. (1970), growth of aerobes is slower in vacuum-packaged meat as compared with meat packed in air. High-barrier packaging film and high vacuum levels are responsible for prohibiting the transfer and increase in oxygen needed for aerobic bacterial growth. The slow growth noted in vacuum-packed chicken in Figures 15 to 18 may, therefore, be accounted for by the impermeability of the Saran in the film, as well as low oxygen level created by the vacuum.

Longest shelf life and acceptability were provided by the HB film with whole carcasses and only slightly greater counts were observed for HB cut-up poultry. This increase in contamination may be due to more handling of cut-up birds during the cutting process and also to the possible spread of microorganisms from contaminated surfaces to the freshly cut surfaces. All cut-up poultry spoiled faster than whole birds. With the LB film, bacterial counts increased rather sharply between 3 and 6 days for cut-up birds (Figures 16 and 18) as compared with whole birds (Figures 15 and 17).

Psychrotrophic bacteria (Figures 17 and 18) were favored by the LB film in comparison with the HB material. This may

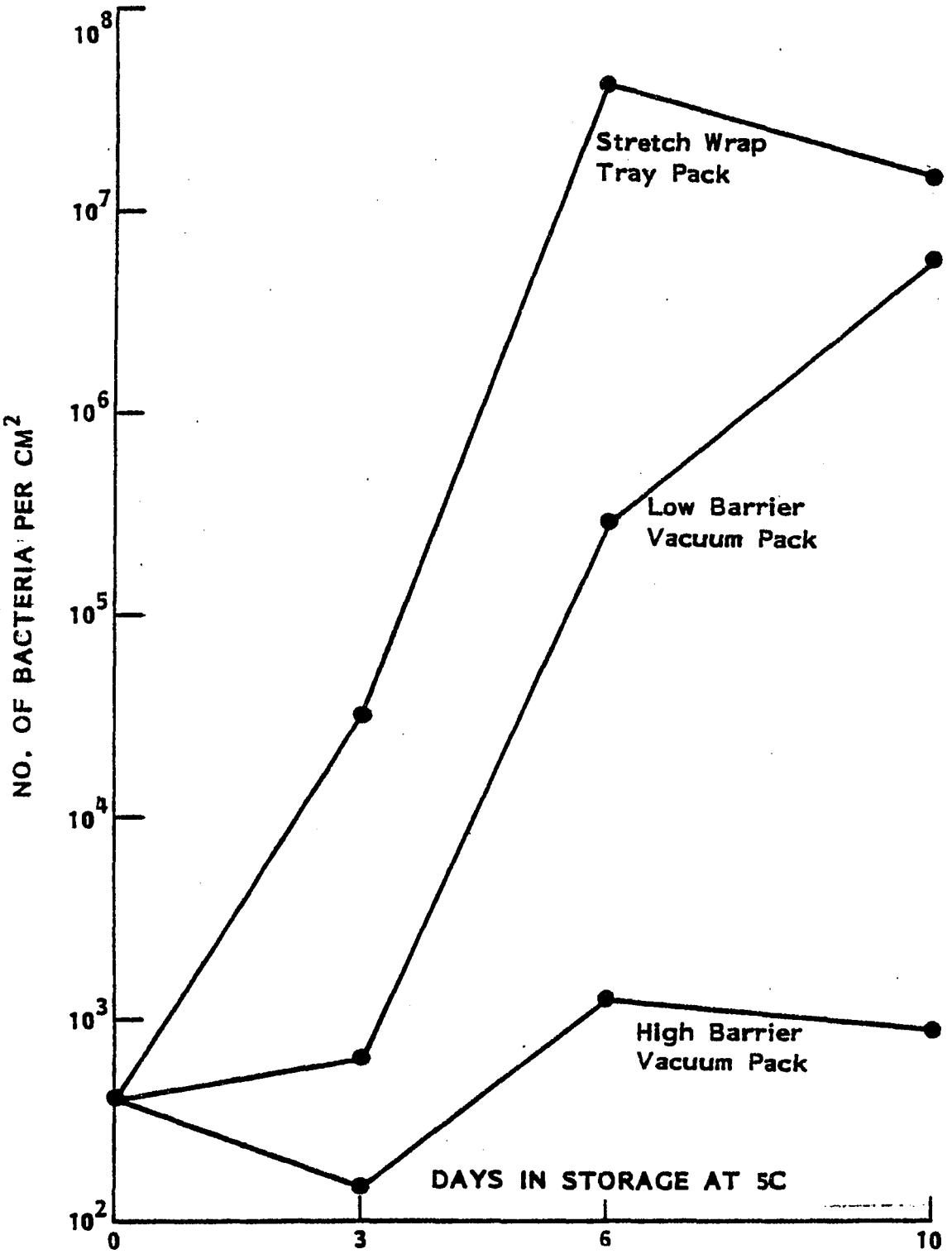


Figure 17. Effect of packaging materials and methods on psychrotrophic bacteria on whole chicken during storage at 5C in a display case

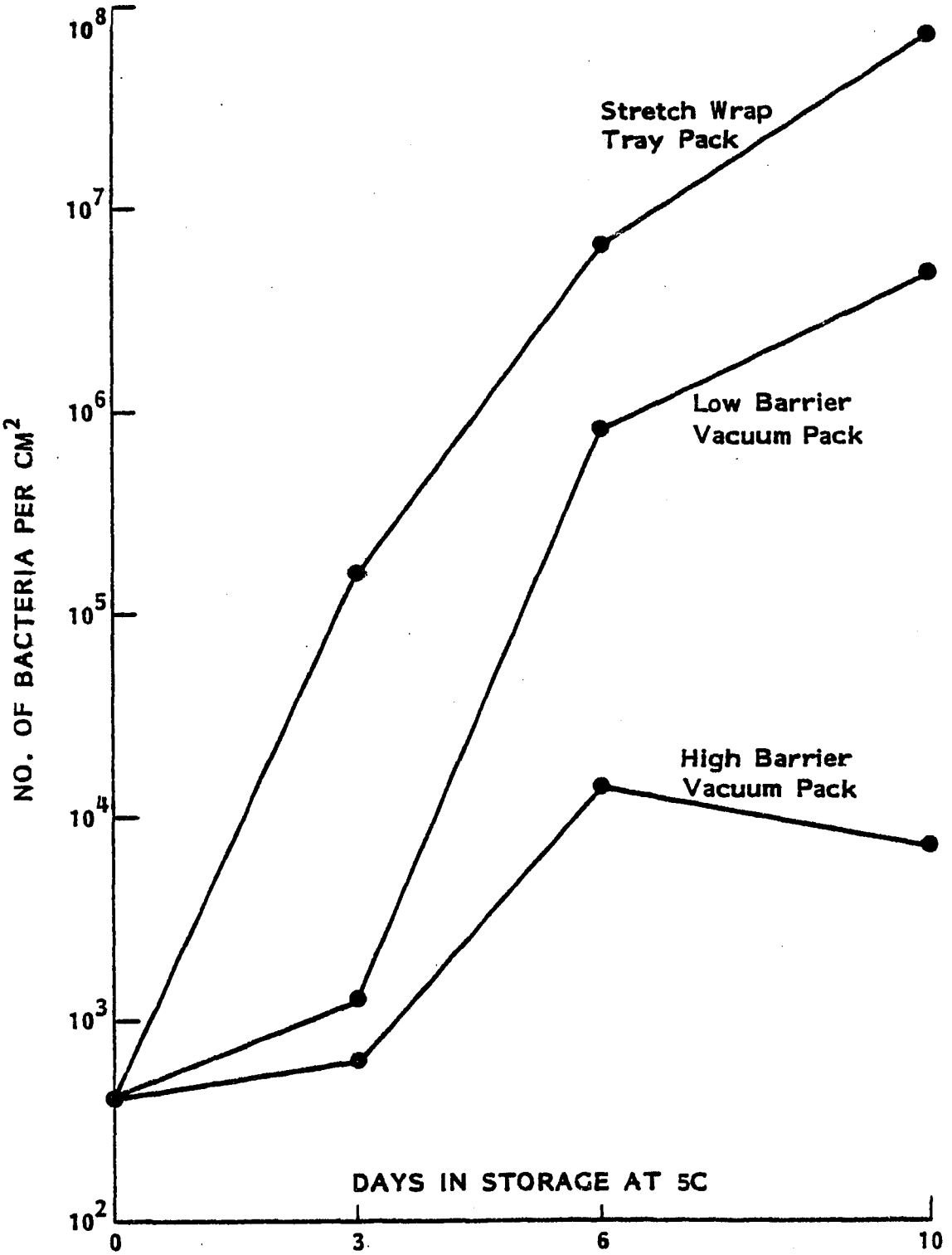


Figure 18. Effect of packaging materials and methods on psychrotrophic bacteria on cut-up chicken during storage at 5C in a display case

have been due to the greater oxygen permeability of the LB film which resulted in proliferation of typical aerobic spoilage types. However, vacuum packaging retarded development of psychrotrophs in comparison with stretch wrapping (SW), regardless of materials used for vacuum packaging. Under the conditions presented in this research, for HB whole poultry, it is possible keeping time could be extended beyond the 10 day limit in these trials to about 12 to 13 days. This condition probably would not apply to LB whole birds or cut-up birds, which showed incipient spoilage and had populations of about 1 million per cm^2 by the tenth day of storage. Stretch-wrapped chicken demonstrated sufficient coalescence of bacterial colonies to produce slime, but no slime formation occurred with either HB or LB vacuum-packaged chicken, whether whole or cut-up. In other work (Debevere and Voets, 1973), stretching the film increased its oxygen permeability and resulted in a higher aerobic bacterial growth rate on the carcasses that were packed in the stretchable polyvinyl chloride film than that observed on those packed in the shrinkable polyvinylchloride film (Debevere and Voets, 1973). The LB film used in the present study did not appear to provide sufficient vacuum to inhibit bacterial growth as much as HB film. By using such a packaging film a considerable amount of oxygen can be transferred into the package and can destroy the vacuum required

for inhibition of aerobic spoilage bacteria.

Effect of packaging materials on lactobacilli and enterococci

Figures 19 and 20 show that HB and LB films allowed the growth of lactobacilli to a significantly greater extent than SW film. This effect was not pronounced for the growth of enterococci (Figures 21 and 22). In a vacuum package, the initial low residual oxygen content, and the subsequent utilization of oxygen and production of CO_2 from the respiration of natural flora sustain the anaerobic conditions which favor growth of anaerobes and facultative organisms such as lactobacilli, enterococci and members of genus Clostridium (Solberg, 1967). Under the conditions present in this research, vacuum packaging favored the growth of lactobacilli and enterococci irrespective of types of poultry (i.e. whole or cut-up). These organisms produced an atypical odor, not characteristic of definite spoilage, nor as pronounced as the typical odors formed by Pseudomonas on LB chicken. Sour odor was associated with lactobacilli and did not become evident on HB packaged chicken until 10 days. At that time, the odor was only moderate. HB wrapped whole chicken was still judged to be acceptable at that time.

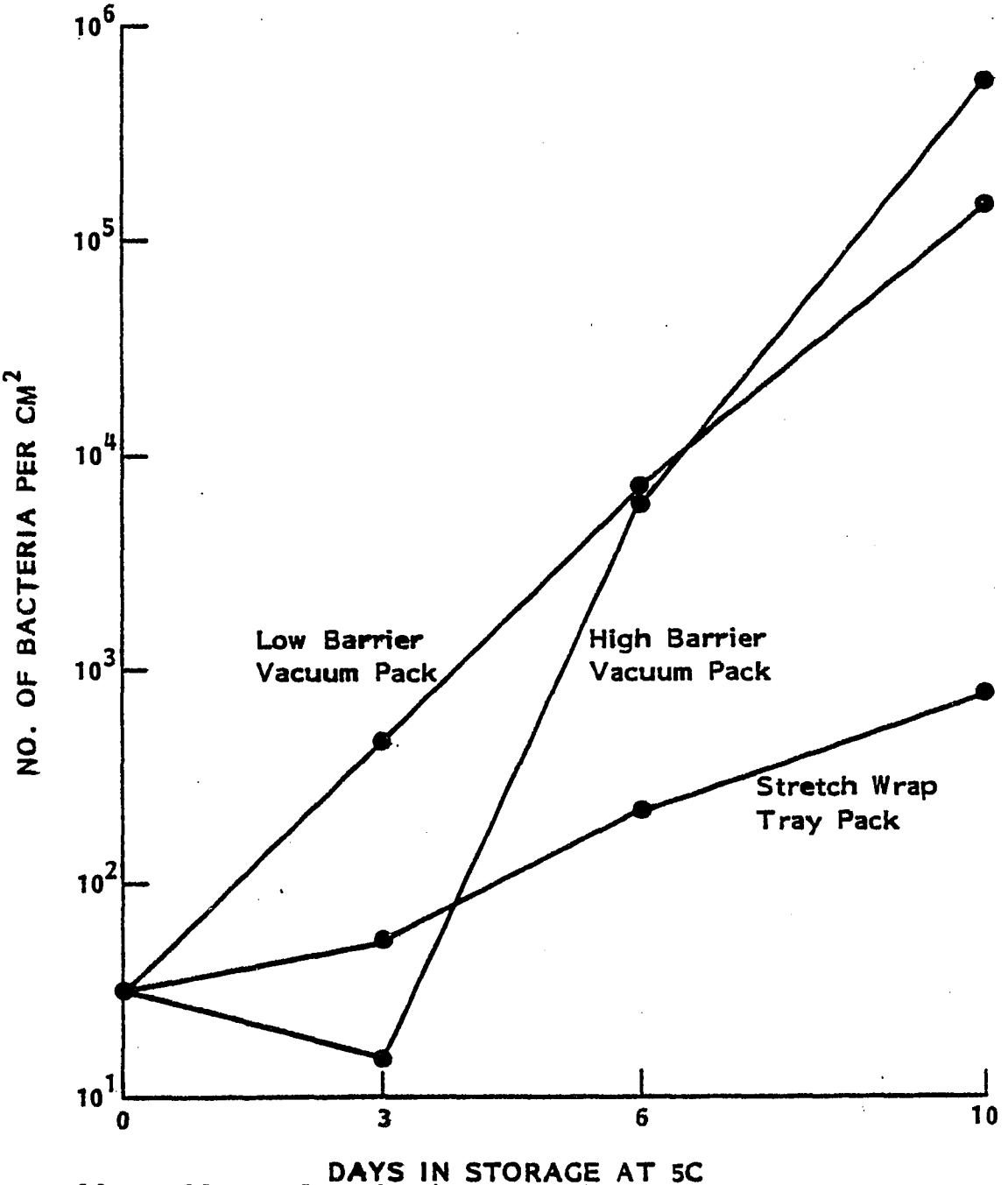


Figure 19. Effect of packaging materials and methods on lactobacilli on whole chicken during storage at 5C in a display case

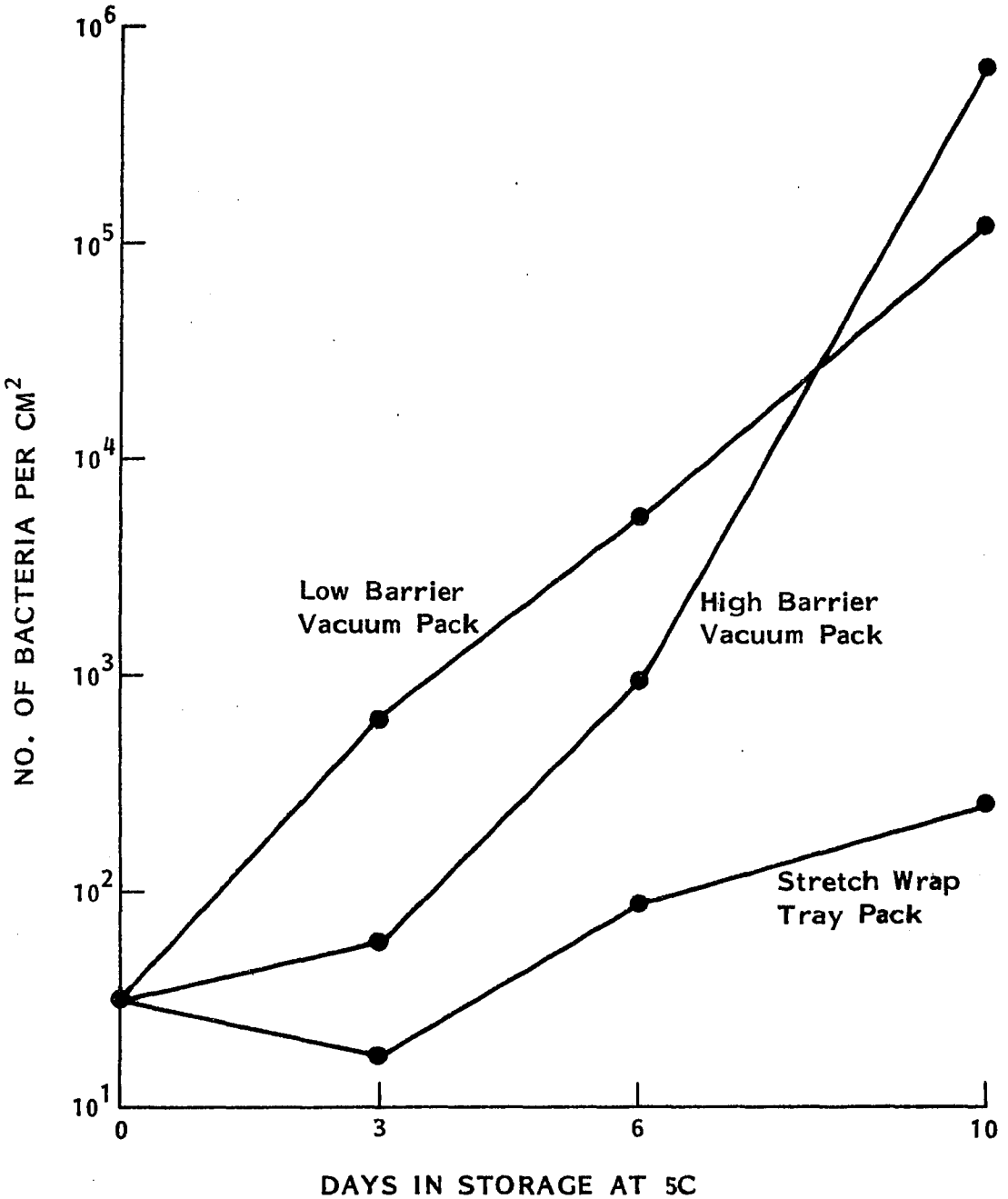


Figure 20. Effect of packaging materials and methods on lactobacilli on cut-up chicken during storage at 5C in a display case

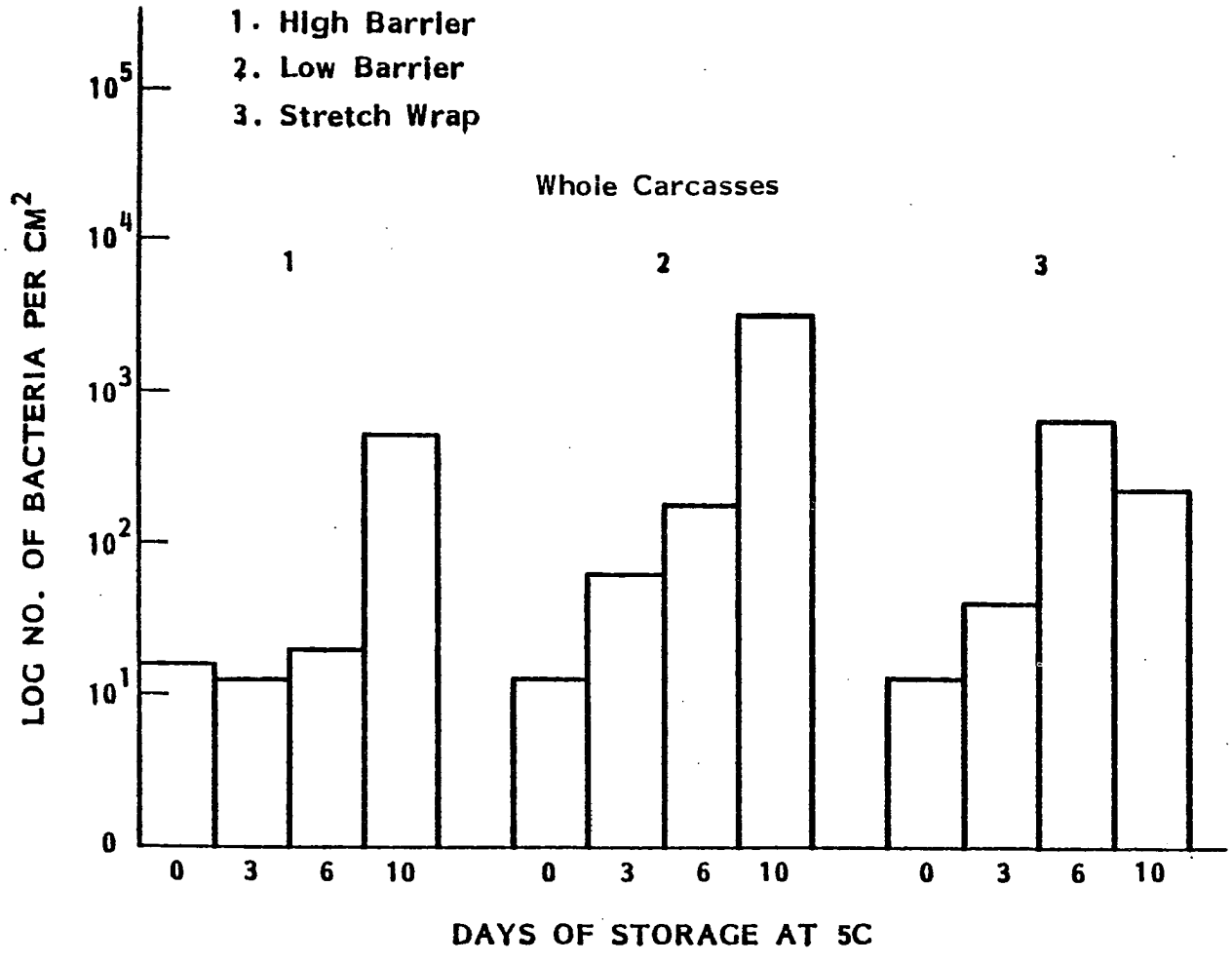


Figure 21. Effect of packaging materials and methods on enterococci on whole chicken during storage at 5C in a display case

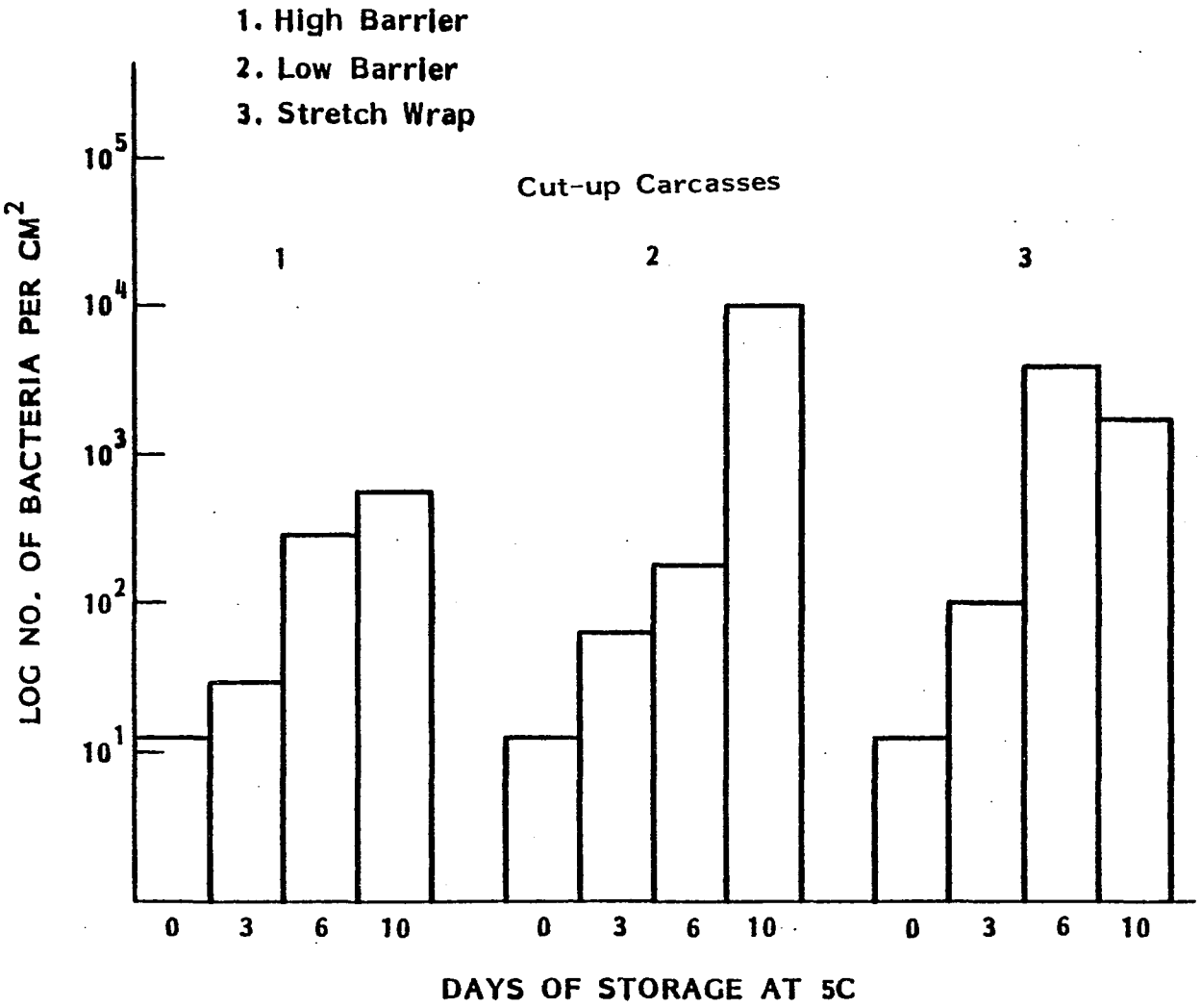


Figure 22. Effect of packaging materials and methods on enterococci on cut-up chicken during storage at 5C in a display case

Statistical analysis of the results

Table 16 presents a statistical analysis for this study. As may be expected, increase in storage time produced significant increases in levels of all types of microorganisms with the exception of psychrotrophs. Psychrotrophic bacteria reached about as high numbers after 6 days as after 10 days. However, it is also possible that the actual peak in psychrotroph populations occurred between 6 and 10 days, and the 10 day count may have been associated with a downward trend. Nevertheless, growth of spoilage types over time was related to development of off-odors as mentioned previously. Packaging materials and methods significantly affected bacterial growth, although the HB and LB films were not highly different from each other in effect for mesophilic organisms, lactobacilli or enterococci. The principal significant difference in bacterial growth was between HB or LB vacuum packaged chicken and stretch wrapped birds; with regard to differences between populations on whole or cut-up poultry, only mesophilic spoilage organisms differed significantly.

Incidence of food-borne pathogens on chicken samples

Incidence of coagulase positive staphylococci and Salmonella spp. is presented in Table 17. Sixteen of 27 (59.3%) of whole birds demonstrated presence of coagulase-

Table 16. Effect of packaging, cutting and storage on bacterial counts of chicken[1]

Treatment		No. of samples	Log number bacteria per cm^2			
			Mesophiles	Psychrotrophs	Lactobacilli	Enterococci
Days in storage	3	18	4.23^{a}	3.33^{a}	1.75^{a}	1.64^{a}
	6	18	5.85^{b}	5.33^{b}	2.99^{b}	2.63^{b}
	10	18	6.45^{c}	5.65^{b}	4.66^{c}	3.18^{bc}
Packaging material[2]	HB	18	4.75^{a}	3.12^{a}	3.40^{a}	1.99^{a}
	LB	18	5.22^{ab}	4.80^{b}	3.75^{a}	2.65^{ab}
	SW	18	6.57^{c}	6.40^{c}	2.04^{b}	2.82^{bc}
Type of poultry	Whole	27	5.26^{a}	4.60^{a}	3.12^{a}	2.31^{a}
	Cut-up	27	5.76^{b}	4.95^{a}	3.01^{a}	2.66^{a}

[1]For each column within a treatment, counts having different letter superscripts are significantly different from each other at the 1% level. Counts that share a common letter superscript are significantly different from each other at the 5% level.

[2]HB = High Barrier; LB = Low Barrier; SW = Stretch Wrapped (tray).

Table 17. Incidence of coagulase positive staphylococci and *Salmonella* on chicken samples (first study)

	Packaging material	Days of storage	N	Positive for coagulase Staphylococci	Positive for *Salmonella*
Whole birds	HB	3	3	2	-
		6	3	2	-
		10	3	2	-
	LB	3	3	2	-
		6	3	2	-
		10	3	1	-
	SW	3	3	2	-
		6	3	2	-
		10	3	1	-
Cut-up birds	HB	3	3	3	1
		6	3	3	2
		10	3	3	-
	LB	3	3	3	-
		6	3	3	2
		10	3	3	-
	SW	3	3	3	3
		6	3	3	2
		10	3	2	1

positive staphylococci, and almost all (25 of 27, or 92.6%) of cut-up carcasses were positive for the organisms. The high incidence of staphylococci in cut-up poultry may be due to contamination resulting from cutting and handling operations. These results suggest that coagulase-positive staphylococci can be found frequently on commercially processed chicken and type of packaging material has little effect on the frequency of incidence. Incidence of coagulase positive staphylococci on commercially processed poultry has been reported by several investigators (Walker and Ayres, 1956; Barnes and Shrimpton, 1959; Rey and Kraft, 1971; Reddy, 1978).

Incidence of salmonellae differed considerably between whole and cut-up birds (Table 17), but not among chickens packaged by vacuum with HB or LB film. Salmonella spp. that were recovered from eleven of 27 cut-up birds (40.7%) gave positive agglutination reaction with Bacto Salmonella "O" antiserum and also were confirmed by typing for "O" and "H" antigens by the State Hygienic Laboratory (University of Iowa, Iowa City, Iowa). The serotypes were S. litchfield (4 of 11) and S. heidelberg (7 of 11). Duitschaever (1977) reported an incidence of 35% for Salmonella serotypes on retail raw chicken. Cutting apparently caused contamination by salmonellae. Of the two serotypes isolated, S. litchfield has been relatively uncommon on poultry, but its significance

in this work is not known.

No Clostridium perfringens was recovered from any samples of packaged poultry or fresh chicken. This organism has been incriminated several times and regarded as a potent food poisoning causative agent (Hall and Angelotti, 1965; Kraft, 1971). No recoveries of this organism in this study suggest that their incidence was low initially on fresh chicken and/or related to the good sanitary practices at the processing plant.

Microbiological Quality of "Wet" Packed Chicken With or Without Chlorine Dip (Second Study)

Effect of 20 ppm chlorine water dip on microbial flora of chicken

Figure 23 represents the effect of 20 ppm chlorine in water on growth of mesophilic bacteria on stored packaged chicken. From the figure, it can be noted that chlorine in a water solution as a dip provided lower counts than for control chicken dipped in water with no chlorine added. In preliminary work, the chlorine concentration was found to decrease to 11 ppm after dipping the first two birds and at the end of a second dip of two birds, it further decreased to 6 ppm available chlorine. In the present study, four birds were dipped at the rate of two per dip. Although the initial 20 ppm available chlorine in the water was not

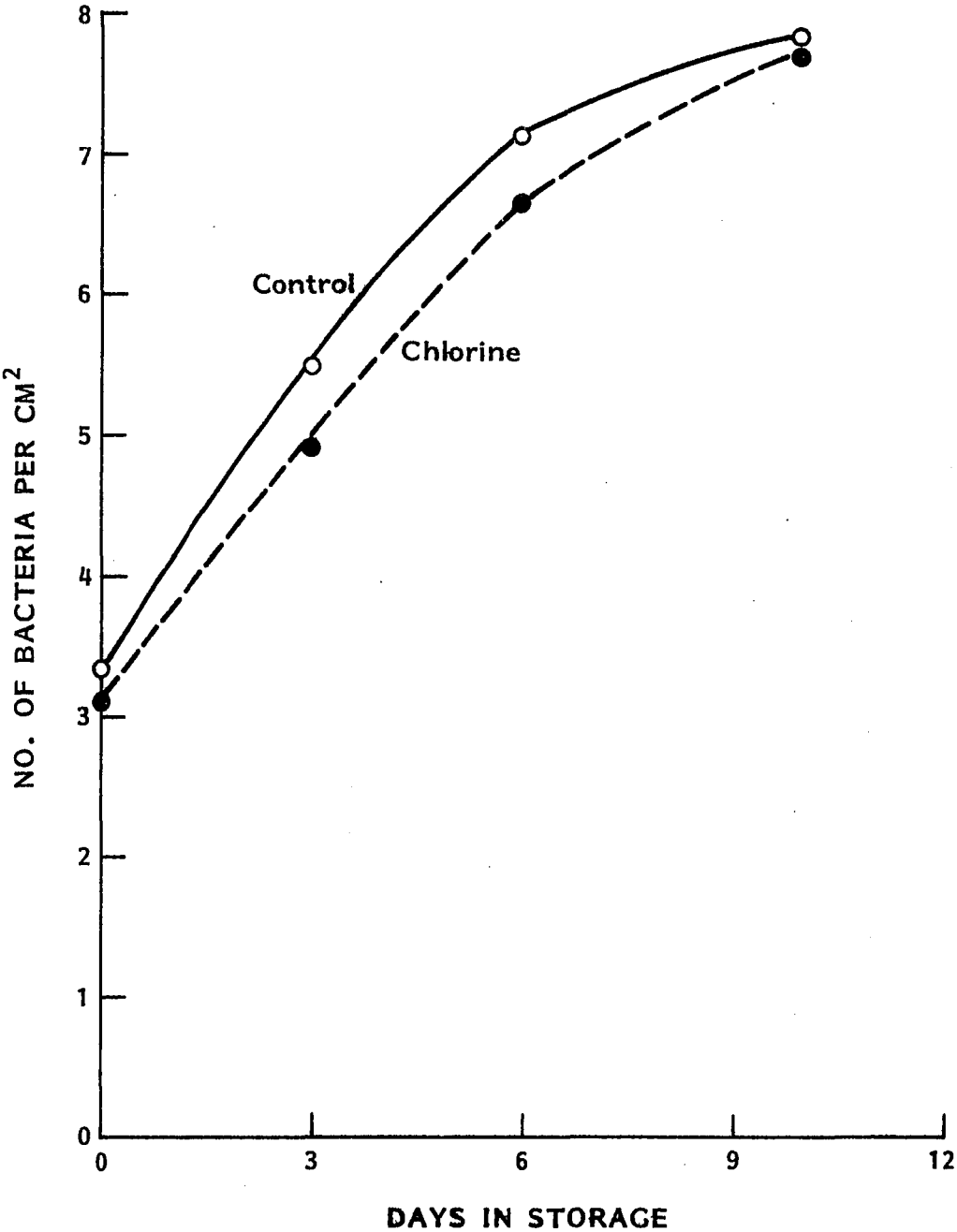


Figure 23. Effect of 20 ppm available chlorine in dip water on growth of mesophilic bacteria on chicken during storage at 5C in a display case

maintained absolutely at that level, differences in bacterial counts were highly significant (Table 18) for chlorine treatment and no chlorine treatment for mesophiles and psychrotrophs on chicken. Keeping time was extended about 2 days by the use of chlorine in water (to about 8 days compared with about 6 days for controls). The effect of in-plant chlorination on microbial flora on poultry was reported by Barnes (1972) and Ranken (1973). Further, Ziegler and Stadelman (1955) demonstrated an extended shelf life of chicken by using 10 or 20 ppm chlorinated water as a post chill dip for five minutes. Significant resistance of certain bacterial types, particularly fecal streptococci and staphylococci, was reported by Patterson (1968) when he used 20 ppm chlorine water in continuous immersion chillers in poultry processing plants. The bactericidal effect of chlorine depends upon certain important factors such as concentration, contact time, temperature, etc. Under the experimental conditions presented in this work, chlorine at 20 ppm concentrations did not significantly reduce lactobacilli, enterococci or coliforms. However, decreases were noted for these organisms for chlorine-treated samples compared with controls (Table 18).

Table 18. Effect of packaging, cutting, chlorine treatment and storage on bacterial counts of chicken[1]

Treatment		No. of samples	Log number bacteria per cm^2				
			Mesophiles	Psychrotrophs	Lactobacilli	Enterococci	Coliforms
Days in storage	3	36	5.22^{a}	4.73^{a}	2.60^{a}	1.65^{a}	1.55^{a}
	6	36	6.88^{b}	6.66^{b}	3.92^{b}	2.39^{b}	2.37^{b}
	10	36	7.76^{c}	7.60^{c}	4.42^{bc}	2.73^{b}	2.74^{b}
Control	0 ppm	54	6.81^{a}	6.54^{a}	3.67^{a}	2.35^{a}	2.02^{a}
Chlorine	20 ppm	54	6.43^{b}	6.12^{b}	3.62^{a}	2.16^{a}	2.24^{a}
Packaging material[2]	HB	36	6.30^{a}	5.68^{a}	4.59^{a}	2.47^{a}	2.10^{c}
	LB	36	6.68^{ab}	6.45^{b}	4.67^{a}	2.56^{a}	2.62^{a}
	SW	36	6.89^{bc}	6.86^{b}	1.68^{b}	1.73^{b}	1.93^{b}
Type of poultry	Whole	54	6.48^{a}	6.21^{a}	3.54^{a}	2.17^{a}	2.13^{a}
	Cut-up	6.76^{ab}	6.45^{b}	3.76^{a}	2.34^{a}	2.31^{a}	

[1]For each column within a treatment, counts having different letter superscripts are significantly different from each other at the 1% level. Counts that share a common letter superscript are significantly different from each other at the 5% level.

[2]Packaging material: HB = High Barrier; LB = Low Barrier; SW = Stretch Wrapped (tray).

Effect of packaging materials on microbial flora of chicken

Of the three packaging materials, the HB film provided greatest reduction of mesophilic growth, the low barrier film (LB) was next, and the stretch wrap (SW) least effective (Figure 24), similar to the previous study (Figures 15 and 16). Also, it can be noted from the above figures that packaging materials and methods did not have as great an effect on bacterial numbers or shelf life for carcasses treated with chlorine in a dip or for controls dipped in water as they did for dry packed chicken evaluated in the previous study.

For mesophiles, differences were significant at the 5% level between HB and LB films and between LB and SW films, and at the 1% level (highly significant) between HB and SW films (Table 18). Psychrotrophs were significantly inhibited in growth by the HB film, with no significant difference between LB and SW packaging. For other organisms (lactobacilli, enterococci or coliforms), LB and HB were comparable in effect, but both allowed significantly lower microbial growth than the stretch wrap material. From the previous study, inhibition of aerobic spoilage from growth of psychrotrophs would be expected by the use of HB film.

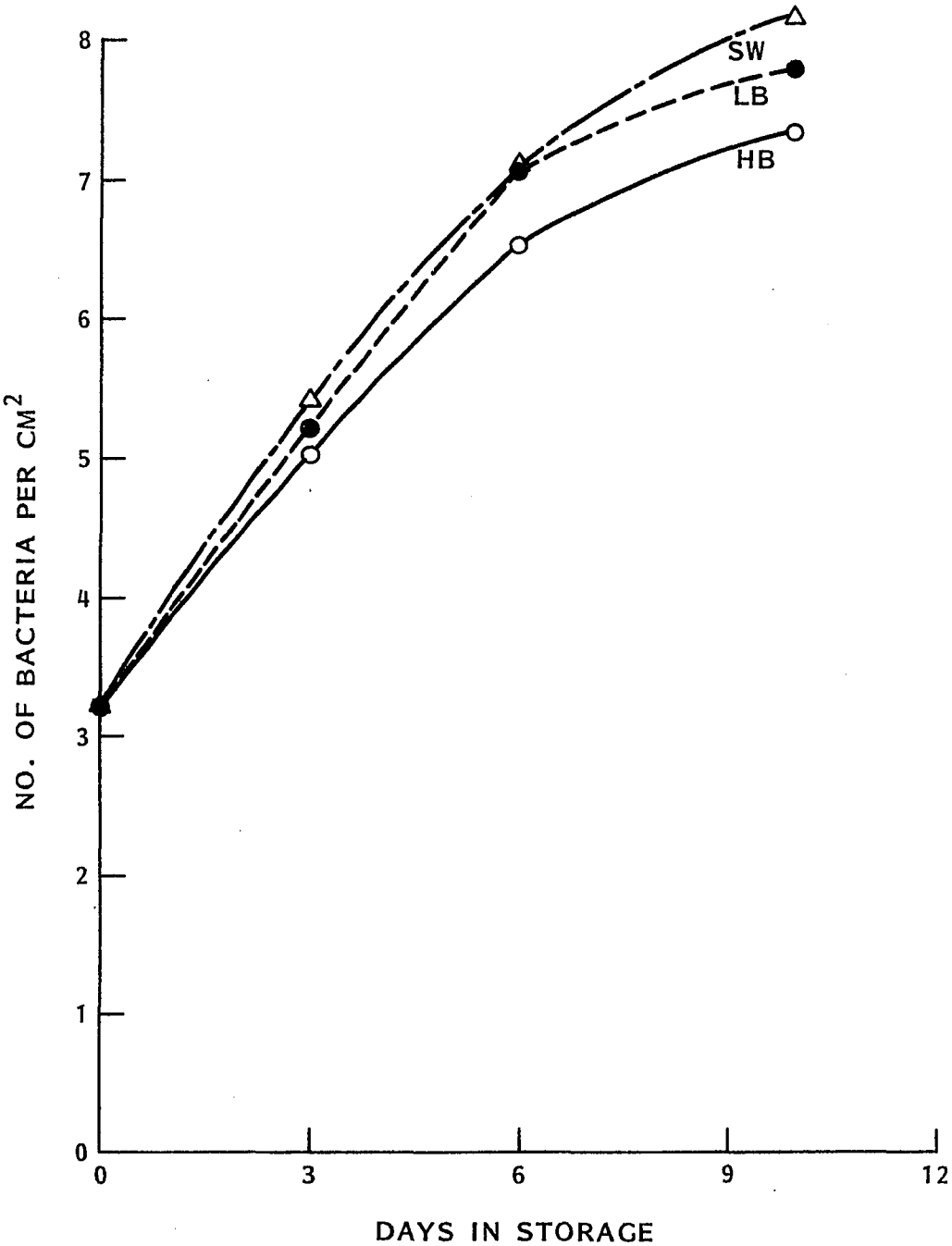


Figure 24. Effect of packaging materials on growth of mesophilic bacteria on chicken dipped in chlorine and storated at 5C in a display case

Effect of cutting on the microbial flora of chicken

Similar to earlier results with dry pack chicken (Figures 15 through 18), cut-up poultry generally spoiled at a faster rate than whole birds (Figure 25). However, an important observation to be noted here is that cavity odors in whole carcasses became pronounced during storage (from 3 to 6 days). This was not detected on dry packed poultry in the earlier work reported. Use of HB or LB films did not produce consistent differences regarding cavity odors. In later stages of storage, odor was more marked from cavities of whole carcasses than from cut-up chicken. Reasons for these differences are unknown, but odors were likely to be caused by spread of contamination. However, in all cases, the SW chicken was least acceptable, as previously observed for dry packed birds maintained in the dry state.

Incidence of food-borne pathogens on chicken samples

Tables 19 and 20 show the occurrence of coagulase positive staphylococci and Salmonella spp. on the chicken samples. Incidence of both food poisoning types was decreased by the use of the chlorine treatment. Nineteen of 54 samples treated with chlorine showed coagulase positive staphylococci compared with forty-five of 54 control samples dipped in water with no chlorine. Salmonellae were present on only four of 54 chlorine treated samples compared with eleven of 54

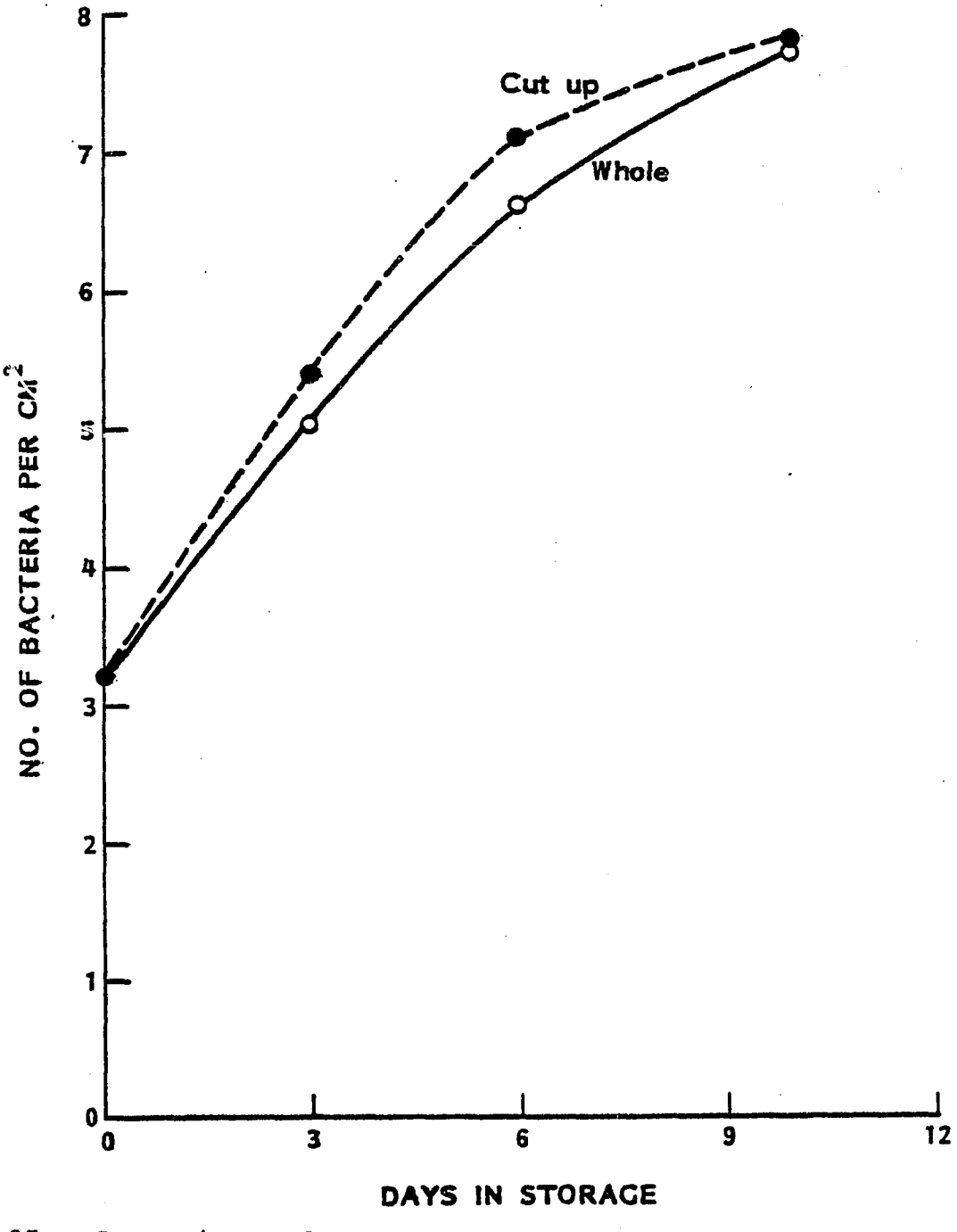


Figure 25. Comparison of cut-up and whole treated chicken treated with a chlorine dip on the growth of mesophilic bacteria during storage at 5C in a display case

Table 19. Incidence of coagulase positive staphylococci and *Salmonella* on chicken samples (second study)

	Packaging material	Days of storage	No. of samples	No. of samples positive for coagulase staphylococci	No. of samples positive for *Salmonella*
Control whole birds	HB	3	3	3	-
		6	3	2	-
		10	3	2	-
	LB	3	3	3	1
		6	3	3	1
		10	3	2	-
	SW	3	3	3	-
		6	3	2	-
		10	3	1	-
Cut-up birds	HB	3	3	3	2
		6	3	3	-
		10	3	3	-
	LB	3	3	3	1
		6	3	3	1
		10	3	3	-
	SW	3	3	2	2
		6	3	2	3
		10	3	2	-

Table 20. Incidence of coagulase positive staphylococcus and Salmonella on chicken samples (second study) treated with 20 ppm chlorine

			N	Coagulase staphylococci	Salmonella positive samples
Chlorine whole birds	HB	3	3	1	1
		6	3	1	-
		10	3	0	-
	LB	3	3	1	-
		6	3	1	-
		10	3	1	-
	SW	3	3	1	1
		6	3	1	-
		10	3	0	-
Cut-up birds	HB	3	3	1	-
		6	3	2	-
		10	3	2	-
	LB	3	3	2	1
		6	3	1	-
		10	3	1	-
	SW	3	3	1	1
		6	3	1	-
		10	3	1	-

control samples. Contamination by these organisms was favored by cutting up the chicken in comparison with whole chicken, as in the earlier study. Although highly sanitary techniques were practical, cutting of birds resulted in spread of contamination by the potential pathogens. Chlorine treatment was effective in reducing salmonellae growth on processed poultry. The bactericidal effect of chlorine on salmonellae was demonstrated by Thomson et al. (1967) and by Dixon and Pooley (1961) using 100 to 200 ppm chlorine on artificially inoculated poultry carcasses. The results of this study suggest that 20 ppm chlorine is also effective in reducing salmonellae on chicken carcasses. The serotypes isolated were S. braenderup (7 of 15) and S. heidelberg (8 of 15). These two serotypes may be somewhat more resistant to chlorine than other types, or they may have had a higher incidence initially on chicken samples in this study.

Clostridium perfringens was recovered from only two samples before wet dip treatments and none were isolated after either of the treatments. If these organisms are considered as an index of the effect of vacuum packaging on growth of anaerobes on poultry, little or no hazard may be anticipated.

From these studies, it may be noted that use of a wet dip, with or without chlorine, did not provide as good

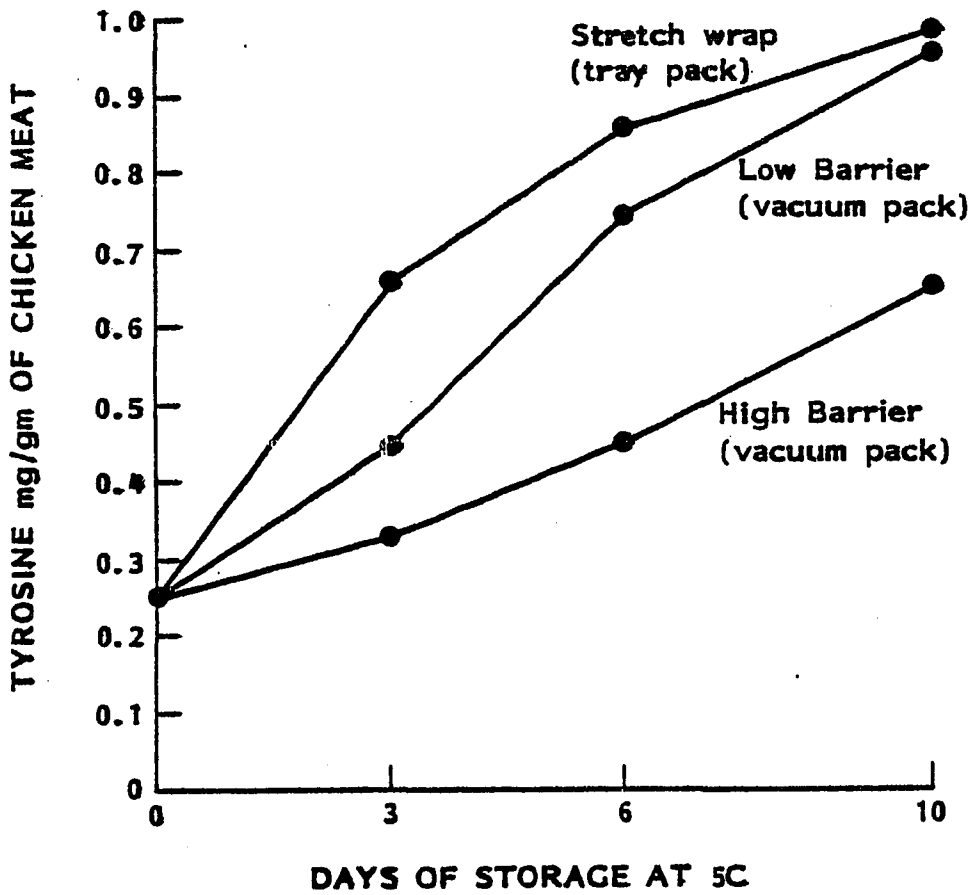


Figure 26. Effect of packaging materials and methods on tyrosine content of chicken during storage at 5C in a display case

a microbiological quality or shelf life as the dry pack did. In no instance were dipped birds acceptable after 10 days. Cutting up chicken increased the spread of potential pathogens such as Salmonella and coagulase positive staphylococci. Chlorine treatment was effective in reducing the incidence of these pathogens. Packaging materials seemed to have an important effect on bacterial counts or shelf life for carcasses treated with chlorine in a dip or for controls dipped in water as they did for dry packed chicken. Of the three films and packaging methods, the HB and vacuum pack provided the greatest inhibition of psychrotrophic growth (spoilage flora), the LB and vacuum pack was next, and the SW and air pack was least effective.

Rapid Chemical Tests as Indices of Storage Quality of Chicken Packaged in Three Types of Films

Measurements of proteolysis and lipid oxidation in chicken during storage in a display case at 5C, are presented as tyrosine values and TBA numbers in Tables 21 and 22. From Figures 26 and 27 it can be noted that an increase in tyrosine and TBA values occurred as storage time progressed for all the samples. An interesting observation in this study is the trend of the tyrosine curve (Figure 26), similar to that of the growth of psychrotrophs (Figure 17). The TBA curve (Figure 27) was related to the oxygen transfer

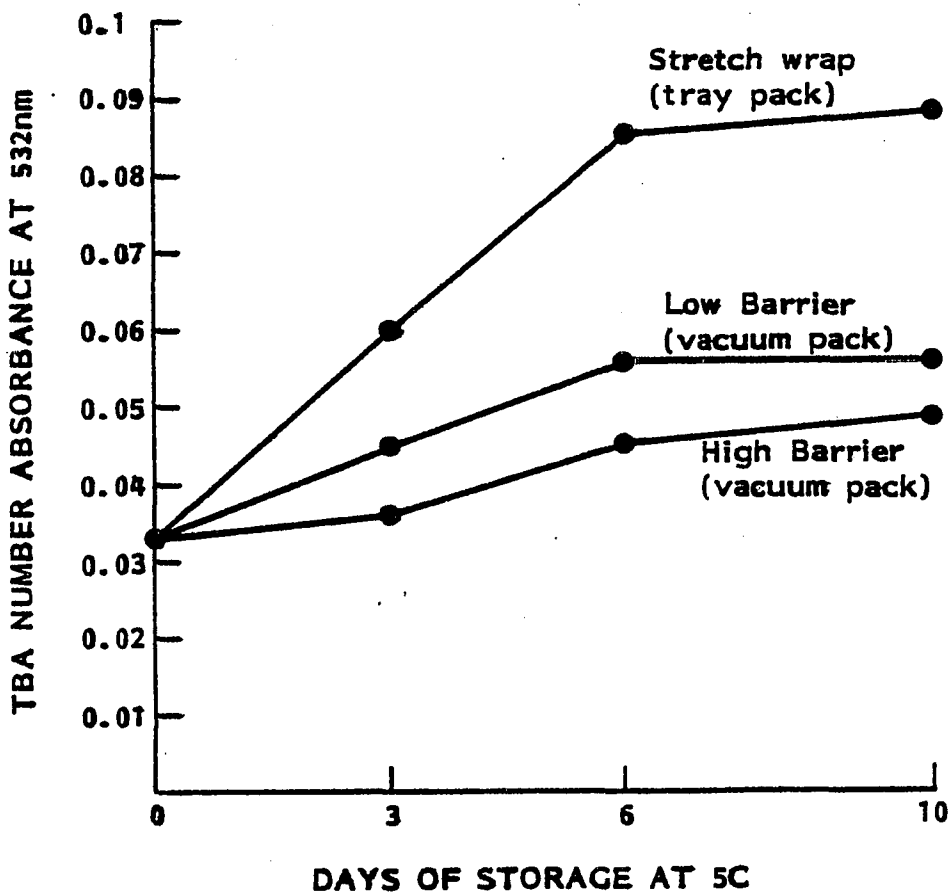


Figure 27. Effect of packaging materials and methods on 2-thiobarbituric acid (TBA) number during storage of chicken at 5C in a display case

Table 21. Effect of packaging materials on tyrosine (mg/gm) values during storage of chicken samples at 5C

Packaging material	N	Days of storage at 5C			
		0	3	6	10
		(values are means of three samples)			
High barrier (whole)	3	0.252	0.312	0.456	0.655
Standard deviation			0.104	0.115	0.125
Low barrier (whole)	3	0.252	0.448	0.744	0.958
Standard deviation			0.115	0.109	0.135
Stretch wrap (whole)	3	0.252	0.645	0.855	0.995
Standard deviation		0.054	0.112	0.118	0.142

Table 22. Effect of packaging materials on TBA number during storage of chicken samples at 5C

Packaging material	N	Days of storage at 5C			
		0	3	6	10
		(values are means of three samples)			
High barrier (whole)	3	0.033	0.036	0.045	0.048
Standard deviation			0.014	0.016	0.019
Low barrier (whole)	3	0.033	0.045	0.056	0.055
Standard deviation			0.012	0.008	0.018
Stretch wrap (whole)	3	0.033	0.060	0.086	0.089
Standard deviation		0.004	0.020	0.018	0.015

permeabilities of the three films. These results suggest that tyrosine value would be a good indicator of the proteolytic activity of the microflora and TBA number is indeed a useful tool to measure lipid oxidation. Strange et al. (1977) have applied seven rapid tests for monitoring alterations in beef quality during storage. Of the tests used, only tyrosine value and TBA number correlated well with total bacterial counts and time of storage. In this study, tyrosine values approached 0.780 mg/gm of chicken meat at the time of spoilage. Although the TBA numbers were of similar magnitude for high and low barrier packaged chicken, the latter samples spoiled earlier (6 days) than did the high barrier packaged chicken (10 days). For the stretch wrapped chicken, TBA numbers were higher than for other samples and ranged about from 0.080 to 0.090 at the time of spoilage.

Effect of cryogenic (LCO_2) and conventional freezing (air blast at -29C) on the microbial flora of turkey samples

Table 23 indicates that both freezing methods reduced numbers of mesophiles and fluorescent pseudomonads. Freezing with LCO_2 prior to storage at -29C had a more detrimental effect on the viability of the microflora than by air blast freezing alone, but the differences were negligible during later frozen storage. In our previous studies (Kraft

Table 23. Effect of LCO_2 and air blast freezing on mesophiles and fluorescent pseudomonads on turkey

	Days in frozen storage at -29C	Counts per cm^2	
		Mesophiles	Fluorescent pseudomonads
Before freezing		5.1×10^5	8.0×10^4
Storage at -29C (air blast)	16	2.0×10^5	1.1×10^4
	78	1.8×10^4	7.5×10^3
	110	1.1×10^4	6.8×10^3
Before freezing		2.8×10^5	6.0×10^4
LCO_2 freezing prior to storage at -29C (air blast)	16	2.1×10^4	9.0×10^3
	78	3.2×10^4	3.4×10^3
	110	8.9×10^3	8.2×10^3

et al., 1979) on the microflora of ground beef patties, similar results were obtained.

Table 24 represents the differences between the Streptococcus agar without potassium tellurite (a) and Streptococcus agar with potassium tellurite (b) on the recovery of Streptococcus faecalis and Streptococcus faecium inoculated on turkey samples. Potassium tellurite has been used to examine heat damage in S. faecalis by Payne and Morley (1976), who have also observed that sublethally damaged cells could recover their resistance to tellurite. The use of potassium tellurite as a selective agent supplementing the KF Streptococcus agar medium was found to be effective (Figure 28) for the enumeration of S. faecalis. The 20 colonies isolated from KF Streptococcus agar medium with potassium tellurite were found to be S. faecalis when screened by fermentation tests as indicated (Barnes, 1956):

Fermentation tests	S. faecalis	S. faecium
Arabinose	-	+
Citrate	+	-
Gluconate	+	-

Only seven of 20 colonies isolated from KF Streptococcus medium without potassium tellurite were positive for the above screening tests. Barnes (1956) found a higher correlation (0.8) between coliform counts and S. faecalis counts than between the coliform counts and enterococci counts

Figure 28. Use of potassium tellurite in KF Streptococcus agar media for selective enumeration of *S. faecalis*

A. KF Streptococcus agar with potassium tellurite

B. KF Streptococcus agar without potassium tellurite

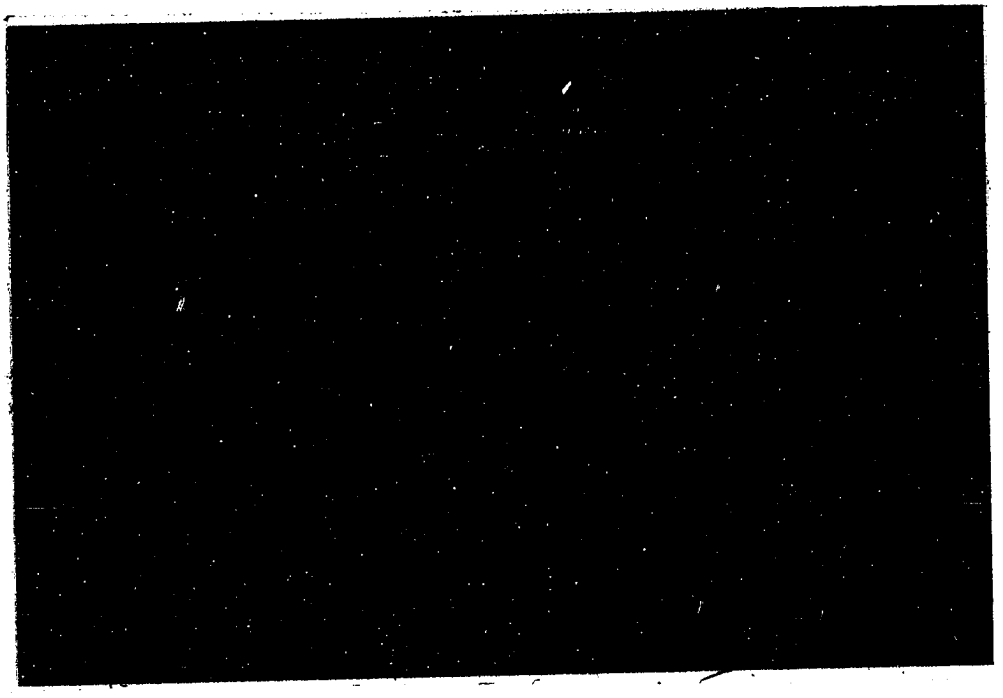


Table 24. Effect of potassium tellurite in the medium for recovery of Streptococcus faecalis and Streptococcus faecium inoculated on turkey samples frozen by LCO_2 or air blast

	Days in frozen storage at -29C	Counts per cm^2	
		Streptococcus agar[a]	Streptococcus agar[b]
Before freezing		3.8×10^5	1.8×10^5
Storage at -29C (air blast)	16	2.6×10^5	1.5×10^5
	78	2.0×10^5	3.2×10^3
	110	1.1×10^5	2.7×10^3
Before freezing		2.2×10^5	7.0×10^4
	16	1.8×10^4	9.5×10^3
	78	1.6×10^4	2.5×10^3
	110	1.6×10^4	2.1×10^3

[a]Contains no potassium tellurite.

[b]Contains potassium tellurite.

(0.67). For poultry meat it would appear that enumeration of S. faecalis would enhance the significance of public health tests for bacterial indication of contamination, rather than the enumeration of enterococci, as has been claimed by several investigators. In the present work, use of potassium tellurite did serve to differentiate between S. faecalis and other enterococci.

Effect of LCO_2 and conventional air blast freezing (029C) on the Myofibril Fragmentation Index (MFI) of breast and thigh muscles of turkey

MFI of the samples (Tables 25 and 26) increased during frozen storage more uniformly with LCO_2 initial freezing than with blast freezing alone; the fragmentation scores were higher for breast muscles, suggesting that they were more tender than the thighs. In fact, some increase was noted for LCO_2 frozen breast and thigh muscles during frozen storage. Since neither sensory nor objective assessments were made for tenderization, the data obtained in this study cannot be completely substantiated for tenderness evaluation. However, Rose and Lentz (1956) stated that wide variations could occur in taste panel evaluations and objective scores, which necessitates the use of large numbers of birds in relating processing variables to sensory tests. The current literature on the MFI relationship with the tenderization process (Moller et al., 1973; Olson and Parrish, 1977;

Table 25. Effect of LN_2 and conventional air blast freezing on MFI of turkey meat

	Days of storage at -29C	N	MFI	
			Breast	Thigh
Fresh turkey meat	0	4	27.6	17.4
LN_2 freezing with subsequent storage at -29C	16	2	57.5	30.0
	24	1	71.0	42.2
	32	1	83.0	40.5
Blast freezing and continued storage at -29C	16	1	72.0	50.5
	24	1	82.0	38.0
	32	2	58.2	46.2

Table 26. Effect of LCO_2 and conventional air blast freezing on myofibril fragmentation index of turkey meat

	Days of storage at -29C	N	MFI	
			Breast	Thigh
LCO_2 freezing with subsequent storage at -29C	16	1	74.4	38.0
	46	1	74.4	56.4
	78	1	68.0	48.0
	110	1	67.2	39.6
Blast freezing and continued storage at -29C	16	1	70.0	33.0
	46	1	68.0	55.0
	78	1	74.8	42.0
	110	1	76.2	39.0

Culler et al., 1978) has given strong evidence that MFI determination provides a reliable estimate of tenderness.

The MFI results obtained in this study indicate that LN_2 or LCO_2 freezing compares favorably with conventional air blast freezing with regard to tenderness. Trends appear to be similar during frozen storage for LN_2 frozen turkey compared with air blast frozen (Table 25) or for LCO_2 frozen and air blast frozen (Table 26). Sayre (1970) pointed out that areas of weakening or widely distributed breaks in myofibrils might be responsible for tenderness during the post mortem aging of chicken breast muscle. Freezing and frozen storage might have caused the structural weakening of myofibrils in this study and produced more fragmentation than found with the fresh meat samples (Table 25). In an investigation on the effects of freezing, Klose et al. (1959) reported that tenderization could be accomplished in hard frozen turkeys while thawing by adjusting the frozen storage temperature upward (25F) for a short, controlled period. Thawing for 24 to 30 hours at 5C in this study might have raised the internal temperature of the frozen turkeys closer to the optimum level and could have increased the tenderness. Also, Bouton et al. (1958) showed substantial improvement in tenderness of frozen beef quarters after slow thawing. A significant decrease in shear force for meat samples frozen at -3C for 28 days compared with aging meat

at 15C for 3 days was observed by Winger and Fennema (1976).

The results of a second study (Table 27) wherein the fresh and their counterpart frozen halves were tested for MFI, clearly demonstrate that cryogenic freezing by LN_2 with subsequent frozen storage at -29C increases MFI comparable to that of conventional storage at -29C. Freezing with LN_2 without further frozen storage increased MFI very slightly.

Effect of LN_2 freezing and air blast freezing on sarcomere lengths of breast and thigh muscles of turkey

The relationship of sarcomere lengths to tenderness has been of interest since the report of Locker and Hagyard (1963). However, no apparent differences in the length of sarcomere were observed either between methods of freezing or types of muscles in this study (Table 28). No significant relationship was observed between the sarcomere length and tenderness scores by Culler et al. (1978) in loin steaks of conventionally aged beef carcasses.

Effect of method of freezing on myofibrillar proteins of breast and thigh muscles of turkey

The analysis of myofibrillar proteins by SDS -10% Polyacrylamide Gel electrophoresis did not show differences in protein bands between LN_2 or air blast frozen samples (Figure 29). However, an intense protein band (probably

Figure 29. Analysis of myofibrillar proteins of LN_2 or air blast frozen samples of turkey breast and thigh muscles by SDS-10% polyacrylamide gel electrophoresis

Left to right:

1. LN_2 thigh
2. LN_2 thigh
3. LN_2 breast
4. LN_2 breast
5. Blast frozen thigh
6. Blast frozen thigh
7. Blast frozen breast
8. Blast frozen breast
9. LN_2 thigh
10. LN_2 thigh
11. LN_2 breast
12. LN_2 breast
13. Standard

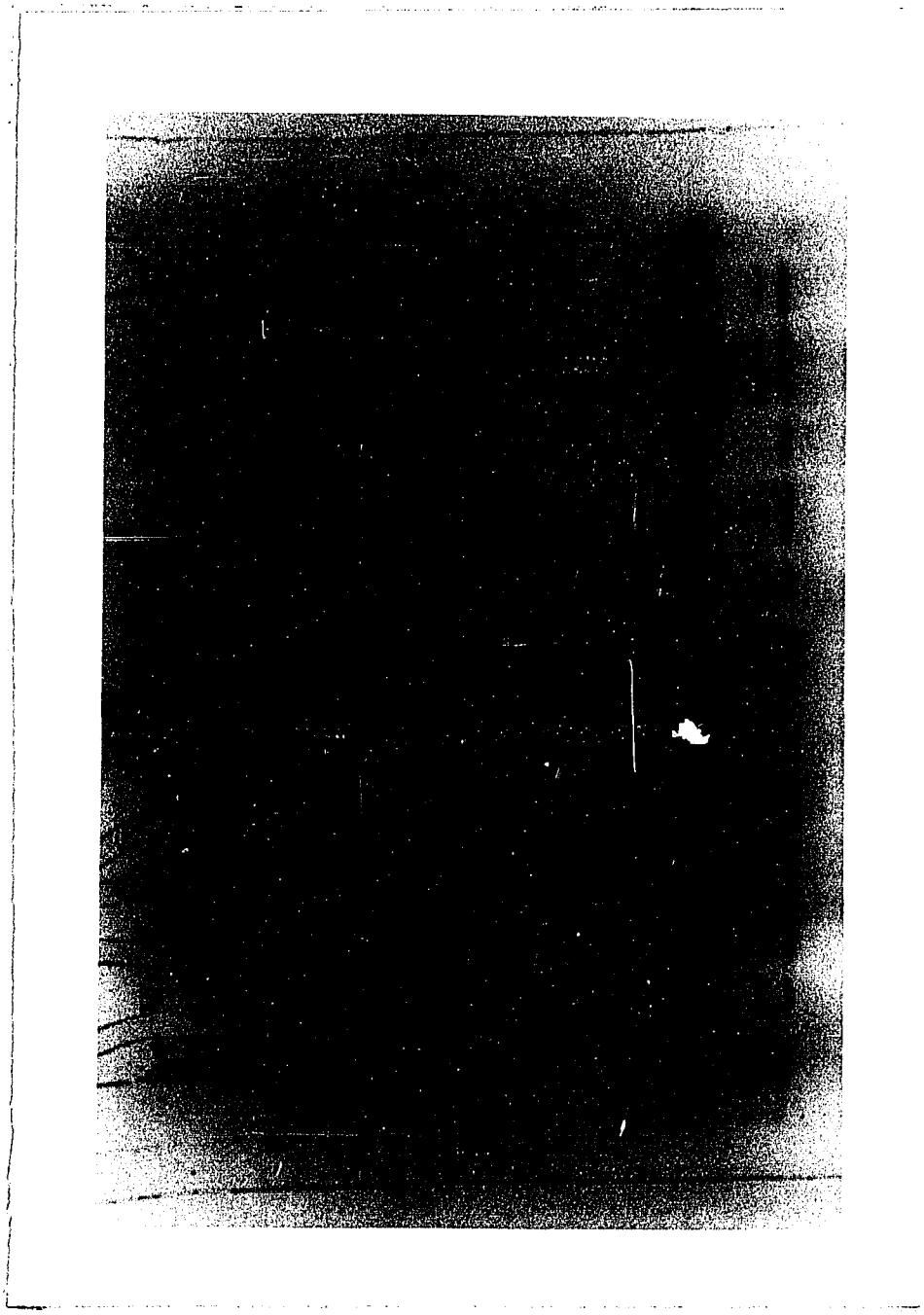


Table 27. Effect of method of freezing on MFI of turkey meat (second study)

Samples[a]	Method of freezing	MFI	
		Breast	Thigh
1A	Fresh	37.0	31.0
2A	Fresh	32.6	27.8
3A	Fresh	41.2	32.6
4A	Fresh	39.4	31.6
5A	Fresh	38.8	32.2
6A	Fresh	37.4	30.6
Std. deviation	Fresh halves	3.55	2.56
4B	LN_2 ten minutes	52.0	39.2
6B	LN_2 ten minutes	51.0	29.2
1B	LN_2 and -29C 8 - 8 weeks	70.4	56.6
3B	"	78.6	60.2
2B	-29C for 8 weeks	74.4	54.8
5B	"	71.2	56.6
Std. deviation	Frozen halves	11.50	12.01

[a]A - fresh half of turkey, B - frozen counterpart.

Table 28. Effect of method of freezing on sarcomere lengths[a] of breast and thigh muscles

Samples	Liquid nitrogen frozen		Blast frozen	
	Muscle type		Muscle type	
	White	Red	White	Red
1	1.87 ± 0.23	1.87 ± 0.19	1.72 ± 0.12	1.95 ± 0.19
2	1.95 ± 0.09	2.11 ± 0.30	1.97 ± 0.11	1.98 ± 0.16

[a]The sarcomeres were measured with a Vicker's image splitter-phase contrast microscope at a total magnification of X500.

Table 29. Effect of LN_2 or LCO_2 and air blast freezing at -29C on TBA number of turkey meat

	Days of frozen storage (-29C)	TBA number
Fresh turkey meat	0	0.026
LN_2 freezing and storage at -29C	16	0.011
	24	0.021
	32	0.020
Blast freezing and continued storage at -29C	16	0.009
	24	0.042
	32	0.041
LCO_2 freezing and storage at -29C	16	0.012
	46	0.046
	78	0.035
	110	0.052
Blast freezing and continued storage at -29C	16	0.018
	46	0.055
	78	0.056
	110	0.068

troponin-T or Tropolyosin) was observed directly below actin in thigh muscle samples (Figure 30). High myofibrillar fragmentation index and beef tenderness in relation to the degradation of myofibrillar proteins (absence of troponin-T and presence of a 30,000 dalton component) was reported by Olson and Parrish (1977). This phenomenon was not observed in this study for turkeys. This may be related to the differences in myofibrillar proteins between beef and turkey muscles.

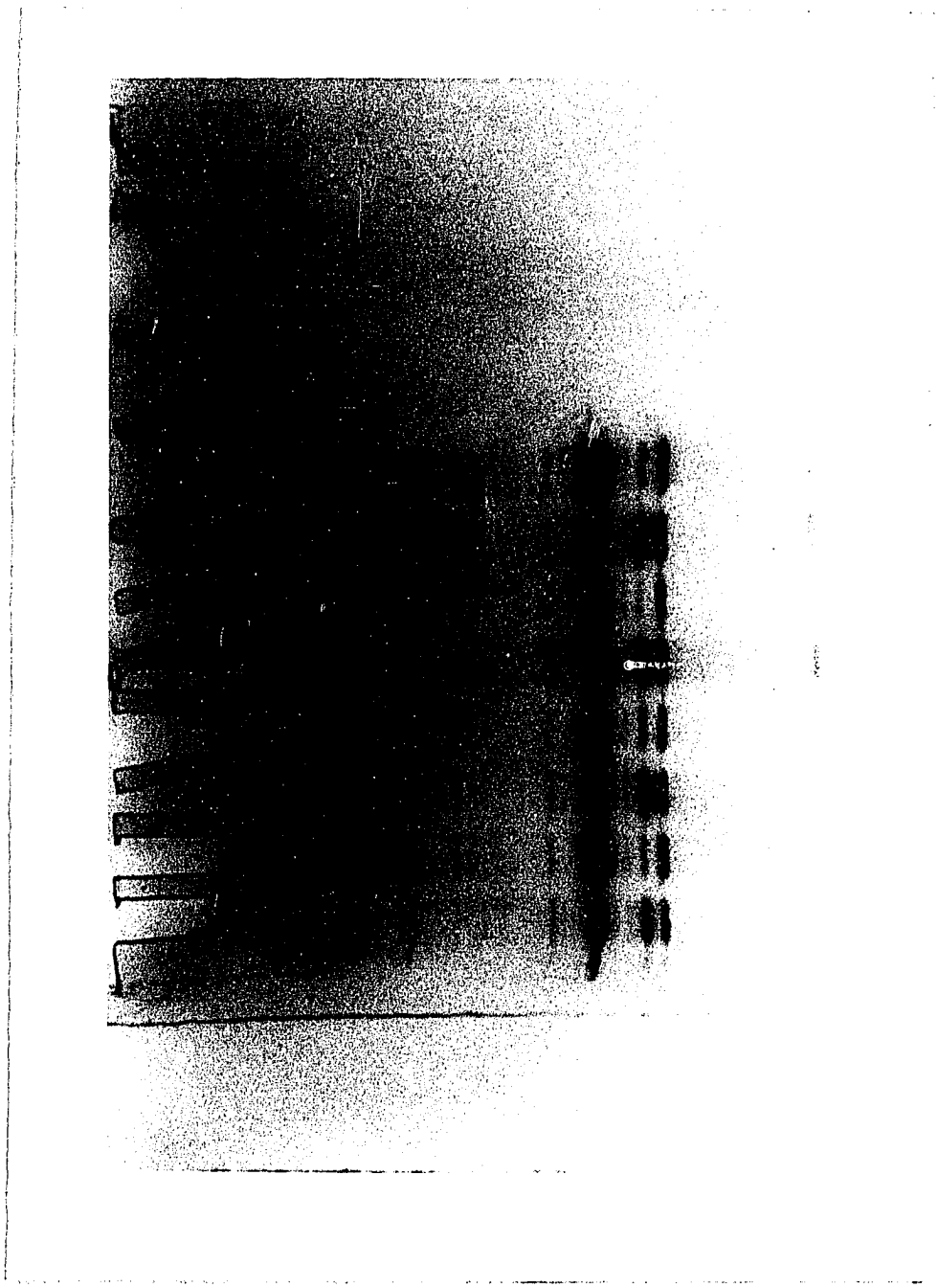
Effect of method of freezing on TBA number of turkey muscles

Table 29 shows the oxidative changes in fat measured as TBA number. The effect of frozen storage on lipid oxidation has been documented by several workers in the past. A striking observation in this study was the occurrence of low TBA values for cryogenically frozen samples during storage compared with the samples frozen continuously by air blast alone. Wyche et al. (1972) observed higher TBA values with chicken parts with skin attached frozen by the air blast method than the samples frozen by liquid freon. From these results, it appears that slower oxidative changes occur during storage when turkey is frozen more rapidly cryogenically than by the slower air blast method. Possibly, very rapid initial freezing retarded enzyme activity during later

Figure 30. Analysis of myofibrillar proteins of fresh (unfrozen) breast and thigh muscles of turkey by SDS-10% polyacrylamide gel electrophoresis

Left to right:

1. Fresh thigh
2. Fresh breast
3. Fresh thigh
4. Fresh breast
5. Fresh thigh
6. Fresh breast
7. Fresh thigh
8. Fresh breast



storage more than slower blast freezing.

Effect of LN_2 and air blast freezing on the concentration of guanidino compounds (creatine and creatinine), nucleic acid derivatives and tyrosine

Table 30 shows that freezing methods applied to turkey samples did not affect the enzymes creatine phosphatase or RNase appreciably and consequently did not produce appreciable concentration changes in creatine and ribonucleic acid derivatives. Khan (1964) reported minute changes in these compounds in frozen chicken only after 10 months of storage at -20C. Folin-ciocalten reagent-positive materials such as free amino acids, peptides, phenols, tryptophan, cysteine, sulfhydryl compounds including H_2S and other reducing materials expressed as tyrosine showed slight increases after 8 weeks of frozen storage. These results indicate that enzymes such as cathepsins were probably released as a result of tissue damage during frozen storage, causing limited proteolysis. These changes were more pronounced in chicken after 45 weeks of frozen storage (Khan, 1964).

Effect of method of freezing on water holding capacity, drip and cooking losses of turkey meat

Table 31 shows the effects of LN_2 freezing, LN_2 freezing with subsequent storage at -29C and continuous air blast freezing at -29C on water holding capacity expressed as expressible juice, drip and cooking losses. A decreased ratio

Table 30. Effect of method of freezing on the concentration of quanidino compounds (creatine and creatinine), nucleic acid derivatives and Folin-ciocalten positive materials (tyrosine) in turkey meat

Samples[a]	Method of freezing	Weight of turkey halves in Kg	Meat	Guanidino compounds mg/gm of meat	Nucleic acid derivatives (at 260 mu)	Tyrosine mg/gm of meat
1A	None (fresh)	2.55	Breast	1.38	0.925	0.232
			Thigh	0.98	0.836	0.184
2A	"	2.35	Breast	1.44	0.934	0.226
			Thigh	1.16	0.812	0.182
3A	"	2.25	Breast	1.40	0.966	0.228
			Thigh	1.14	0.845	0.186
4A	"	2.15	Breast	1.36	0.964	0.235
			Thigh	1.20	0.858	0.200
5A	"	2.15	Breast	1.46	0.943	0.224
			Thigh	1.24	0.816	0.210
6A	"	2.20	Breast	1.44	0.654	0.220
			Thigh	1.16	0.822	0.178
4B	LN_2 ten minutes	2.10	Breast	1.37	0.944	0.241
			Thigh	1.20	0.845	0.202
6B	"	2.10	Breast	1.43	0.945	0.230
			Thigh	1.15	0.812	0.184

[a] A - fresh turkey parts, B - frozen counterpart of same turkey.

Table 30 (Continued)

Samples[a]	Method of freezing	Weight of turkey halves in Kg	Meat	Guanidino compounds mg/gm of meat	Nucleic acid derivatives (at 260 mu)	Tyrosine mg/gm of meat
1B	LN_2 ten minutes and -29C for 8 weeks	2.45	Breast	1.38	0.906	0.248
			Thigh	1.02	0.824	0.195
		2.25	Breast	1.42	0.925	0.241
			Thigh	1.15	0.816	0.206
3B	"	2.25	Breast	1.42	0.925	0.241
			Thigh	1.15	0.816	0.206
2B	-29C for 8 weeks	2.40	Breast	1.45	0.892	0.262
			Thigh	1.14	0.815	0.215
5B	"	2.10	Breast	1.53	0.906	0.253
			Thigh	1.27	0.798	0.226

Table 31. Effect of method of freezing on water holding capacity (expressible juice), drip and cooking losses

Samples[a]	Method of freezing	Weight of turkey halves in Kg	Meat type	Expressible juice ($\frac{\text{meat area}}{\text{juice area}}$)	Drip (ml/100 gms)	Cooking losses (ml/25 gms)
1A	None (fresh)	2.55	Breast Thigh	0.587 0.660		5.25
2A	"	2.35	Breast Thigh	0.731 0.716		4.90
3A	"	2.25	Breast Thigh	0.698 0.774		5.25
4A	"	2.15	Breast Thigh	0.516 0.699		5.56
5A	"	2.15	Breast Thigh	0.595 0.918		5.20
6A	"	2.20	Breast Thigh	0.580 0.764		5.45
4B	LN_2 ten minutes	2.10	Breast Thigh	0.547 0.670	1.25	8.15
6B	"	2.10	Breast Thigh	0.616 0.802	1.29	7.60

[a] A - fresh half of turkey, B - frozen counterpart of turkey.

Table 31 (Continued)

Samples[a]	Method of freezing	Weight of turkey halves in Kg	Meat type	Expressible juice ($\frac{\text{meat area}}{\text{juice area}}$)	Drip (ml/100 gms)	Cooking losses (ml/25 gms)
1B	LN_2 ten minutes and -29C weeks	2.45	Breast Thigh	0.659 0.674	1.15	9.44
3B	"	2.25	Breast Thigh	0.679 0.765	1.22	9.85
2B	Blast and -29C, 8 weeks	2.40	Breast Thigh	0.922 0.753	1.73	10.35
5B	"	2.10	Breast Thigh	0.902 0.884	1.77	10.80

of meat to juice area, higher drip and cooking losses after 8 weeks frozen storage suggest proteolysis caused by the release of cathepsins might have affected the solubility and ion binding properties of the proteins (Fennema et al., 1973; Khan, 1964). The high cooking losses observed may be due to the high temperature specified by Miller et al. (1968) and does not necessarily represent practical procedures.

Results from this part of the study indicated that all the freezing methods cause an increase in Myofibril Fragmentation Index measurements. The relationship of MFI to tenderness of meat has been reported by several investigators in the past although the reliability of the test may be questioned by others, since methods must be highly standardized. However, the present study cannot corroborate this relationship since no sensory evaluations were made to correlate the MFI measurements with tenderness or palatability of meat samples.

No perceptible changes occurred in the amounts of guanidino compounds and nucleic acid derivatives as a result of the freezing methods used. This suggests that these freezing methods do not produce drastic damage to muscle tissues. However, higher tyrosine values were observed for slow frozen (air blast at -29C) samples as compared with LN_2 or LCO_2 frozen samples. This might be due to the possible occurrence of proteolysis resulting from the release of cathepsins from damaged tissue cells. Also, higher drip and

cooking losses for the slowly frozen samples might be due to initiation of deteriorative changes in muscle proteins as suggested by Khan and Van Den Berg (1967).

SUMMARY

As a general description of this work, various aspects of freezing preservation and packaging were studied for feasibility of use for beef and poultry in practical applications. With detailed consideration also given to microorganisms involved, specific studies are described in the following summary statements.

Beef patties composed of fresh, frozen beef, or combinations of fresh and frozen beef were frozen by liquid nitrogen (LN_2) or liquid carbon dioxide (LCO_2) and stored at -29C for six months. The purpose of this work was to determine the influence of frozen meat in combination with fresh meat (before cryogenic freezing) on the microbial flora and keeping quality of ground beef patties. The results were expected to influence possible use of frozen beef when fresh beef was not immediately available or expedient to use. Analyses for various bacteria were made before and after freezing by cryogens and at monthly intervals during frozen storage to evaluate effects of originally combining fresh and frozen beef on the subsequent microbial flora. Samples were examined for mesophiles, psychrotrophs, coliforms, Salmonella, coagulase positive staphylococci and Clostridium perfringens. Changes in bacterial flora as a result of freezing and frozen storage were studied by classifying the isolates by numerical

taxonomy. Replica plating and computerized cluster analysis were tools used to compare the isolates. A set of known cultures representing various genera and species was used as reference to classify the unknown isolates that were formed in clusters in the computer dendrograms. Reference cultures included Moraxella-Acinetobacter, Pseudomonas, Flavobacterium, Escherichia coli, Enterobacter aerogenenous, Bacillus, Aeromonas, Micrococcus and Staphylococcus. From the evaluation of the clusters formed by the isolates from beef patties and the association of clusters with known cultures similarities and differences between bacterial population on samples from the various treatments were observed.

Freezing caused reduction of all types of bacteria tested before frozen meat was mixed with fresh beef (i.e., fresh meat compared with blast frozen meat). No distinct differences were found in survival or growth of different organisms because of method of cryogenic freezing. The mixture of frozen and fresh beef in a 50:50 ratio produced highest bacterial numbers than when different proportions of fresh and frozen beef were combined and held in frozen storage. Lowest bacterial counts resulted from use of fresh beef frozen cryogenically with LN_2 or LCO_2. With the 50:50 combination, sufficient tissue damaged beef may have been provided to allow for maximum nutrient availability, hence bacterial numbers were greatest with this combination. Populations of staphylococci remained

relatively constant throughout the period of frozen storage. Numbers of coliforms decreased markedly during frozen storage. Clostridium perfringens was recovered from very few samples (nine out of 112). No salmonellae were recovered regardless of type of meat used or method of cryogenic freezing.

Taxonomic evaluation of the microflora showed that Moraxella-Acinetobacter increased considerably in percentage of total isolates after freezing, with a decline during frozen storage. Pseudomonas were not greatly affected in regard to proportion of the representative population, but they showed an increase as the Moraxella-Acinetobacter group decreased in percentage during frozen storage. The various types were more uniformly distributed in proportion in fresh beef, but after freezing, Moraxella-Acinetobacter and Pseudomonas dominated the bacterial population. With regard to the spoilage potential of the flora, the percentage of isolates capable of hydrolyzing beef fat increased after frozen storage. Proteolysis of casein and gelatin was not greatly changed as a result of freezing and frozen storage. These changes generally followed the pattern of alterations in the proportions of Pseudomonas. No health hazard was evident from any of the combinations of fresh or frozen meat or LN_2 or LCO_2 freezing and frozen storage. The practical implications of these observations should be considered if fast food restaurants are

to use frozen meat in combination with fresh beef in formulation of ground beef patties.

Effects of packaging materials and methods (vacuum packaging vs. tray pack) on keeping quality and microbial numbers and types associated with fresh whole and cut-up broilers were determined. Also, the effect of available chlorine at a level of 20 ppm as a dip before packaging was determined with the above packaging materials and methods on whole and cut-up poultry.

In general, use of a wet dip, with or without chlorine, did not provide as good microbiological quality or shelf life as the dry pack. However, when considering poultry subjected to water dip treatment, chlorine provided lower bacterial counts than observed for control chicken dipped in water with no chlorine added. Differences were highly significant for mesophiles and psychrotrophs, but chlorine treatment did not significantly affect numbers of lactobacilli, enterococci or coliforms. In no instance were dipped birds acceptable after 10 days. Of the three films and packaging methods, the high barrier (vacuum pack) material provided greatest restriction of bacterial growth, the low barrier film (vacuum pack) was next and the stretch-wrap (air pack) least effective with any packed poultry or poultry subjected to a wet dip application. Psychrotrophs were significantly inhibited in growth by the high barrier film, with no significant difference between low

barrier and stretch wrap packaging. All cut-up poultry spoiled faster than any of the whole birds. Incidence of salmonellae and coagulase positive staphylococci was greatest on cut-up chicken. The cutting process apparently spread the organisms and was responsible for higher microbial counts. No Clostridium perfringens was found on any samples.

Rapid chemical tests such as determination of proteolytic activity (tyrosine value) and lipolytic activity (TBA number) were found to be useful tools to assess the quality of chicken during refrigerated storage.

The effect of cryogenic and conventional freezing methods on quality related factors such as Myofibril Fragmentation Index, expressible juice (retention of meat fluids), protein denaturation (accumulation of nonprotein nitrogenous end products and free amino acids as tyrosine), lipid oxidation (2 thio barbituric acid number), drip and cooling losses were determined for turkey.

All the freezing methods caused an increase in Myofibril Fragmentation Index which has been linked to the process of tenderization by several workers in the past. These increases were more marked in breast muscle than in the thigh muscle.

Conventional blast frozen samples showed higher losses of expressible juice, drip and loss of fluid during cooking than LN_2 frozen samples. These higher losses might be related

to the protein denaturation during slow freezing (reduction in water content with a corresponding increase in ionic strength resulting in loss of soluble proteins).

Higher TBA (thiobarbituric acid) numbers were observed for slowly frozen turkey meat during later frozen storage than for the samples previously frozen with cryogens. This might be due to instant inactivation of enzymatic activity by cryogenic freezing as compared with irreversible acceleration of enzymatic activity during early stages of slow freezing (Winger and Fennema, 1976).

CONCLUSIONS

1. Fresh ground beef contains a heterogeneous microflora distributed uniformly in the meat. Although the freezing process causes a decrease in total bacterial numbers, the proportion of psychrotrophic bacteria increases, particularly the Moraxella-Acinetobacter group.

2. Members of the genus Pseudomonas that survive freezing are more stable to prolonged frozen storage than the surviving Moraxella-Acinetobacter and mesophiles on ground beef.

3. Mixing fresh beef with frozen beef for ground beef patties produces higher bacterial numbers than either fresh or frozen meat alone during later frozen storage.

4. Frozen storage of ground beef causes an increase in the proportion of spoilage types such as Pseudomonas compared with the percentage immediately after freezing. In general, freezing increases the percent of organisms capable of hydrolyzing beef fat.

5. A high barrier film and vacuum packaging for poultry provide highly inhibitory packaging conditions for psychrotrophic and mesophilic organisms and thus increase shelf life during refrigerated storage. Conversely, use of a low barrier material with vacuum or hand wrapping in a stretch wrap film with an environment of air in the package provides more favorable conditions for the growth of spoilage types.

6. Use of available chlorine at a level of 20 ppm in a

water solution as a dip significantly reduces typical aerobic spoilage types on chicken compared to chilling poultry with no chlorine added in the water. Lactobacilli, enterococci and coliforms are more resistant to chlorine treatment than the general mesophilic or psychrotrophic flora.

7. In general, dry packing of poultry provides better microbiological quality and shelf life than a dip treatment with a chill solution containing either chlorine or no chlorine.

8. Cutting of poultry into parts for retail marketing increases the incidence of Salmonella and coagulase positive staphylococci compared with whole birds.

9. When Clostridium perfringens is considered as an index of the effect of vacuum packaging on growth of anaerobes on poultry, little or no hazard may be anticipated.

10. Supplementing KF Streptococcus agar with potassium tellurite has a selective effect in the isolation of Streptococcus faecalis and thus enhances the usefulness of bacteriological tests for sanitary quality of poultry.

11. Rapid chemical tests, particularly determination of tyrosine value as a measure of proteolytic activity, or 2-thiobarbituric acid number as a measure of lipid oxidation, are useful tests tools to assess the quality of poultry meat under refrigerated storage.

12. Freezing of turkey by means of cryogens or by conventional air blast at -29C results in an increase of the Myofibrillar Fragmentation Index (MFI). If MFI is taken as a measure of tenderization, then freezing produces changes associated with tenderization of turkey muscle.

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