INFLUENCE OF LACTIC CULTURES ON MICROBIAL DETERIORATION IN GROUND BEEF

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Dedicated to my mother whose love, affection and encouragement made this study possible

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CHAPTER I

INTRODUCTION

Ground beef keeps well for only a short period at refrigerated temperatures. This is attributed mainly to the deterioration caused by the rapid growth of spoilage micro organisms which cause decomposition of meat. The availability of favorable amounts of water (50-70%), protein (15-20%), fat (18-30%), carbohydrate (1%), and other metabolites in meat, present a good nutrient medium for rapid growth of the spoilage organisms. The involved chopping and grinding processes in making ground beef increases the exposed tissue surface area enabling greater contamination and more growth of the micro organisms than in the intact meat. Hence, any technique which can reduce the microbial deterioration in ground beef such as inhibiting the growth of spoilage organisms, to delay meat decomposition, will be of practical use. Such methods must render the meat safe for human consumption.

At present very little research is being conducted on the use of micro organisms as a tool for retention of meat quality. On the other hand, lactic cultures are commonly used to improve the quality and shelf-life of dairy products. Since milk products and meat are similar in regard to nutritive properties and types of micro organisms causing spoilage, it was presumed that lactic cultures could be used to retard bacterial deterioration by inhibiting undesired growth, and thus improving the shelf-life.

Most of the rapid spoilage of milk and meat products is due to the growth of gram-negative non-spore forming, rod shaped bacteria. Pseudomonas and Achromobacter are the species most predominant in meat. They constitute about 85% of the bacterial population. Hence, this study was undertaken to determine what effect the inoculation of ground beef with lactic cultures had on the rate and extent of gram-negative bacterial growth, meat deterioration and shelf-life improvement.

Since no reports were available in this area of work, several preliminary trials using different culture concentrations, combinations, and forms were conducted. These studies were designed to learn their effect on inhibiting the gram-negative bacterial growth thus reducing microbial deterioration in ground beef. Results of the preliminary trials were used to formulate this study.

CHAPTER II

REVIEW OF LITERATURE

Bacteriology of Ground Beef

Very little progress has been made in the meat industry to reduce bacterial numbers in fresh meat. Today, bacterial counts in fresh meat are practically the same as that of 50 years ago, indicating no evident improvement in sanitary practices of meat processing. These bacterial loads in fresh meat are mainly attributable to contamination during the steps involved in processing the whole carcass to the finished product.

Kirsch et al. 1952 reported that the bacterial load in prepackaged ground beef was comparable to the data reported in 1919 and 1924 (one to ten million/gm). Halleck et al. 1958; Saffle et al. 1961; Jay et al. 1964; Terrell 1967; Pearson 1968; and Stringer et al. 1969 are among many current reports which indicate that the bacterial load in refrigerated ground beef varies from 5 to 7 log numbers per gram of tissue at the initial day of storage.

Ample evidence has been established that the majority of the psychrophilic meat-spoilage bacteria (about 90% of the total population) are of the gram-negative type. A study was made by Brown et al. 1958 on selected properties of 189 psychrophilic bacteria isolated from chilled beef and associated sources. Of these, 182 were gram-negative and 7 were gram-positive. In the same study, they also

reported that there were 170 pseudomonads out of 182 gram-negative bacteria isolated. Bacterial flora in refrigerated ground meat was quantitatively and qualitatively studied by Krisch et al. 1952. Their study indicated that gram-negative, non-spore forming rods predominated throughout the storage period. They also concluded that the Pseudomonas and/or Achromobacter species were a major portion of the total load in the refrigerated ground beef. Halleck et al. 1958 found that non-pigmented Achromobacter-Pseudomonas type organisms constituted about 85% of the total bacterial population in prepacked ground beef during the first two weeks of storage at 34-38°F, and during the first week at 40-44°F. Also, bacteria of the Pseudomonas fluorescens type constituted approximately 80% of the total counts in the latter part of the storage period. Mossell et al. 1955; Ayres 1960; Gardner et al. 1966; and Stringer et al. 1969; are among several other researchers who also concluded the Pseudomonas-Achromobacter group as the most predominant microflora in fresh beef under refrigerated storage. During refrigerated storage of meat, coliformbacteria, yeasts, molds, and species of Micrococcus and Streptococcus increased. At all periods these organisms formed only a minor proportion of the total flora (Gardner et al. 1966).

Lactic Cultures

Lactic Cultures most commonly used in the dairy industry consist of a mixture of Streptococcus lactis or Streptococcus cremoris, which produce lactic acid from lactose, and Leuconostoc species or Streptococcus diacetilactis, which produce biacetyl and other compounds (acetyl methyl carbinol, acetic acid, propionic acid, CO_2, and 2, 3-butylene glycol) from citrates in milk (Hammer et al. 1957).

Zuraw et al. 1952; Whitehead et al. 1958; Sandine et al. 1961; Niven, 1944; Prouty, 1961; Mizuno et al. 1959; Kizer et al. 1955, Anderson et al. 1953; Marth, 1962; Speckman et al. 1968; Friedman, 1939; Keenan et al. 1968; Vakil et al. 1969; and several other workers studied the metabolism of lactic cultures and reported it to be complex.

Proteolytic activity and enzyme production by the lactic streptococci were studied by Vanderzant et al. 1953, 1953a, 1954, and 1954a; Williamson et al. 1962; and Speck, 1962. They observed the production of some proteinase and proteolytic activity by Streptococcus lactis. This activity was reported to be needed by the organism in order to obtain certain nitrogenous constituents from the medium for metabolic activity. However, this organism is considered as non-proteolytic because of its very slight proteolytic activity.

Production of the inhibitory substances and inhibitory action of lactic cultures towards spoilage organisms in dairy products have been studied by Baribo et al. 1951; Collins, 1961; Marth et al. 1962; Marth 1962; and Mather et al. 1959. They reported that the lactic cultures produce antibiotic-like inhibitory substances which have a profound effect on varieties of gram-negative spoilage type bacteria in dairy products. Mather et al. 1959 also showed that inoculation of cream dressing with Leuconostoc citrovorum prevented spoilage in cottage cheese by certain gram-negative organisms.

Chemical Methods for Beef Quality Assessment

Autolytic and bacterial proteolysis and changes in fat tissue are the main processes responsible for meat spoilage during storage at refrigeration temperatures.

In proteolytic deterioration the changes in the free amino acid levels is brought about by the protein break-down in the muscle. Pearson, 1968, noted that protein deamination was likely to be the predominant action under aerobic conditions. However, amine production by decarboxylation was considered important under anaerobic conditions. Gardner, 1965; Ayres, 1960 and 1960a; also reported that the psychrophilic bacteria growing on and causing spoilage in beef were principally Pseudomonas, which were likely to cause the production of ammonia by deamination of amino acids under aerobic conditions.

Pearson, 1968, explained that the fat spoilage was mainly due to the oxidation of unsaturated bonds, microbial hydrolysis, and production of free acidity due to tissue enzymes.

Numerous chemical techniques have been developed and proposed for the estimation or determination of proteolytic spoilage brought about by microorganisms in meat. Determination of hydrogen sulfide, ninhydrin and biuret positive substances, indole, free amino nitrogen, volatile nitrogen including ammonia and amines, tyrosine value, resazurin dye reduction time, picric acid turbidity, and the changes in the free amino and other nitrogen compounds are among several other chemical tests employed to study meat spoilage due to proteolysis. These techniques have been evaluated and reviewed by Kirch et al. 1952. Bowlby et al. 1953; Saffle et al. 1961; Broumand et al. 1958; Burks et al. 1959; Folin et al. 1917; Jay et al. 1964; Jay 1964; Bradely et al. 1940; Gardner et al. 1966; and Pearson 1968a. Most of these chemical methods are either complicated or not reliable enough to give reproducible results. Some of these methods were also reported to have non-significant correlations for proteolytic breakdown of meat.

However, in recent studies by Pearson 1968a, it was concluded that the determination of volatile nitrogen in meat by macro-distillation procedures was the more reliable method to measure protein breakdown in meat. Volatile nitrogen produced in meat consisted almost entirely of ammonia (Burks et al. 1959). Production of the ammonia due to deamination of protein by bacterial enzymes in meat increases during spoilage. Hence, determination of ammonia produced in meat was explained by Pearson, 1968c to represent a simple method of following the course of deterioration of lean meat. Macro or semimicro distillation, micro-diffusion, aeration or colorimetry are some of the methods used for volatile base determination in fish and meat. The first three methods have been evaluated and slightly modified by Pearson, 1968a. He concluded that the macro and semi-micro distillation methods were superior to other methods in view of time of performance. However, the macro-distillation method was preferred to other techniques in view of the good agreement between replicates and general simplicity in performance.

Measures of the free fatty acids due to lipase action on the triglycerides, oxidative rancidity due to the action of the air, and ketonic rancidity due to microorganisms were employed to assess fat spoilage in meat. Various techniques such as the determination of free fatty acids (Broumand et al. 1958; Mahlenbacher 1960; Hills et al. 1946; Pearson, 1968b, etc.); iodine values (Broumand et al. 1958 and Pearson 1968b); Kreis test (Mahlenbacher, 1960 and Pearson, 1968b); Thiobarbituric acid values (Keskinel et al. 1964; and Pearson 1968b) are among others employed for determination of fat spoilage in meat. Most of these tests have proved to be impractical due to irregular and inconsistent results. Titrimetric determination of free fatty acids (FFA) was proved to be the more reliable method for fat spoilage

determination in meat (Pearson, 1968b).

Besides proteolysis and lipolysis, some physicochemical changes also take place during meat storage. Values for pH, total acidity, volatile acidity, oxidation-reduction potential, water holding capacity, extract release volume (ERV), and volatile compounds are among some changes taking place during meat spoilage at refrigeration temperatures. Pearson, 1968c and 1968d, reviewed and evaluated some of these changes. He concluded, that the extract-release volume and pH are useful criteria for meat spoilage assessment.

Bodwell, et al. 1965; Jay, 1964; and Pearson, 1968d; reported a pH decline followed by a rise in pH in meat examined from the slaughter stage to several days of storage.

The extract release volume (ERV) phenomenon in relation to meat spoilage was studied by Jay et al. 1964; Jay 1964, Jay 1964a; Kontou et al. 1966; and Pearson, 1968d. They concluded that the ERV values decreased as spoilage in meat progressed. Jay, 1964a, also concluded that the pH of the meat exerted a significant influence on the ERV volume.

Sensory Evaluation for Meat Freshness

Sensory evaluation criteria such as flavor, color, aroma, juiciness and tactility are used for subjective evaluation of meat freshness. Kramer et al. 1962, explained that, because of the subjective nature of these evaluating criteria, taste panel results are influenced by human psychological factors. In an attempt to eliminate these human imperfections, statistical guides are used.

Several taste panel procedures are described by different authors for sensory

evaluation of food products. Each procedure has been described suitable for a particular set of data. Single stimulus (Kramer et al. 1962 and Hunter 1959), paired comparison, duo-trio test, triangle test, multiple comparison, (Peryam 1958 and Kramer et al. 1962) and hedonic scale method (Peryam et al. 1957) are among other procedures described for sensory evaluation of food products.

Saffle et al. 1961 correlated odor scores with total bacterial counts, ninhydrin, resazurin reduction, and picric acid turbidity tests for meat spoilage evaluation. They reported significant correlations between odor scores and the chemical tests used for evaluating meat spoilage. Jay et al. 1964 studied the correlations between sensory (tactile, odor, and color) and other spoilage detecting tests (bacterial numbers, extract release volume phenomenon and ninhydrin positive substance) in beef. Significant correlations were reported between sensory scores and other tests used to evaluate beef spoilage. However, they also reported an increase in panel sensory scores for the meat after 3 days of storage at refrigeration temperature. Similar results were reported by Kontou et al. 1966. Overall sensory scores for raw meat dropped until 4 days of storage and then a slight increase was noticed until 7 days.

CVT Medium for Gram-Negative Bacterial Determinations

Crystal violet and its impure form, gentian violet, have long been used in culture media because of their selective inhibitory action toward the gram-positive bacteria. Bacto-crystal Violet (Difco, 1966) has a wide range over which it is not significantly toxic to the gram-negative bacteria and is still definitely bacteriostatic toward the gram-positive organisms. Crystal Violet agar is described in Difco,

1966 for detecting gram-negative bacteria.

An improved test (CVT test) for detecting gram-negative bacteria was developed by Olson, 1967. This test was used for detecting contamination of milk and other dairy products subsequent to pasteurization. The organisms which survive proper pasteurization are mainly gram-positive rods and cocci, while those that cause rapid spoilage in dairy products at refrigerated storage are the gram-negative non-spore forming rods. To detect these spoilage type of organisms a medium was developed which inhibited the growth of gram-positive and permitted the growth of gram- negative bacteria. The plating medium consisted of Standard Plate Count agar with 1 to 2 ppm of crystal violet added to inhibit the gram-positive bacteria and with 50 ppm 2, 3, 5 triphenyl tetrazolium chloride (TTC) to impart a distinctive purple-red color to the colonies of the gram-negative bacteria. This test is designated as the "crystal violet-tetrazolium test" or "CVT test". A concentration of 2 ppm of crystal violet was needed when 1 ml quantities of milk were plated and 1 ppm was needed when less than 1 ml was used. The TTC was added as a 1% solution in 50% alcohol just prior to pouring the plates. The CVT test has proven to be very useful in detecting gram-negative bacterial contamination in milk and other dairy products.

CHAPTER III

MATERIALS AND METHODS

Several preliminary and principal trials were conducted to determine the effect of added lactic cultures on inherent gram-negative bacterial growth in ground beef stored at refrigeration temperature (7°C). To facilitate this study different concentrations, combinations, and culture forms along with different substances were tried. The information gained from the preliminary tests is also reported. Materials and and methods, and the results for each of the preliminary trials (I to VI) are independently reported. Experimental data for the principle study (trials VII to XI) were pooled and reported.

Ground Beef

Coarse ground beef with 15 to 20% fat was obtained from the Oklahoma State University Meat Laboratory and from a retail store. No specification was made with regard to the breed, age, sex, or grade of the carcass meat. No specific muscle or muscles were chosen in obtaining the meat samples to be used. No history was known of the aging period or sanitary conditions of the carcass. However, in each case the material obtained was considered fresh coarse ground beef. Treated and untreated meat samples were stored at 7°C for different intervals before studying the treatment effects.

Propagation of the Cultures

The lactic cultures used were propagated as described by Hammer et al. 1957a. Pasteurized skim milk was heated under flowing steam in the autoclave for 20 min., cooled to room temperature, and inoculated with 1% of the lactic culture. In order to have higher concentrations of the organisms, concentrated cultures were prepared with 20% milk solids (20 gm milk solids in 80 ml of distilled water). This concentrated milk was heated and inoculated with the cultures as previously stated. Each culture was ripened by incubating at 21°C for 16 hours before use. Cultures grown in regular skim milk and used in Trial I only, consisted of about 1 billion/ml Streptococcus lactis, and 100 million/ml Leuconostoc citrovorum organisms. Concentrated cultures grown in reconstituted milk with 20% milk solids consisted of about twice the viable organisms as cultures grown in regular skim milk. Streptococus lactis, Leuconostoc citrovorum, or both Streptococcus lactis and Leuconostoc citrovorum together were inoculated in both milk types to make the desired culture. In the work herein reported the term "lactic culture" refers to the culture containing both S. lactis and L. citrovorum.

Preparation of Crystal Violet-Tetrazolum (CVT) Agar

Standard Plate Count agar (Difco) was prepared according to the manufacturer's recommended directions. Bacto-crystal violet 1 ppm (Difco) was added to the standard Plate Count agar at the time of heating the medium. The medium was sterilized in an autoclave for 15 minutes at 15 pounds pressure, then promptly cooled and held at 45°C in a water bath until used. Just before pouring the plates, 50 ppm of 2, 3,

5 triphenyl tetrazolium chloride (TTC) was added to the medium as a 1% solution in 50% alcohol.

Bacteriological Determination

Gram-negative bacterial counts in the meat were determined by standard bacteriological pour plate methodology (A.P.H.A., 1967) using CVT agar for plating. For the determinations in trials I, II and III the required meat tissue homogenates were directly pipetted from the samples in test for making serial dilutions. For the determinations in the remaining trials, 11 gms representing the meat samples were blended with 99 ml of phosphate-buffered saline diluent (Sulzbacher, 1953; Lewis and Angelotti, 1964) in a sterile Omni-mix can fixed to a Servall Omnimixer. Samples were blended for a period of two and one-half minutes at the low speed setting (approximately 10,000 rpm). Required sample aliquots were pipetted directly from the homogenates into the blanks to make appropriate dilutions. The procedure for shaking the dilution blanks and pipetting the sample was as described by A.P.H.A., 1967. Duplicate platings at two appropriate decimal dilutions using CVT agar were made for each sample. Plates were incubated at 32°C for 2 days before the counts were made. Colonies in appropriate plates were counted and the number of gram-negative bacteria per gram of original meat sample was computed.

pH Determination

Approximately 10 gms of the appropriate meat sample was placed in a glass beaker and mixed with 5 ml of distilled water. Each sample was allowed to equilibrate with room temperature (22°C). The hydrogen ion concentration for the samples

from trials I to VI was measured with a standarized (pH 5.0 ± 0.01) pH meter (Beckman) by placing the electrodes directly into the sample. The pH for samples from trial VII to XI was obtained by using a standardized (pH 5.0 ± 0.01) single probe electrode Corning model 10 pH meter.

Total Volatile Nitrogen Determination

Total soluble nitrogen (extraction method - Saffle et al. 1964, determination method, A.O.A.C., 1965); total protein (macro-Kjeldahl nitrogen determination method A.O.A.C., 1965); extract release volume (Jay, 1964a); and total volatile nitrogen (Pearson, 1968a) criteria were used to assess the protein breakdown in the samples from initial trials. Except for total volatile nitrogen, all other criteria gave erratic and inconsistent results. Extract release volume was greatly influenced due to the large variation in the pH of the treated and untreated samples. Adjustment of the treated and untreated samples to pH 5.6 also failed to give any consistent results by the extract release volume procedure. Further study is needed before comment can be made on the usefulness of this method.

Total volatile nitrogen determinations gave very consistent results throughout the study. The method used for the determination of total volatile nitrogen was essentially the same as described by Pearson, 1968a.

A 10 gm sample of ground beef, 2 gms of magnesium oxide, and 300 ml of distilled water were added to a one liter distilling flask. The flask was connected to a macro-Kjeldahl distillation apparatus. Twenty-five ml of a 2% boric acid solution and 10 drops of methyl red indicator were added to a 600 ml receiving beaker. The sample was placed in the receiving beaker and about 300 ml of distillate was

collected. Condenser washings were added to the distillate and sulphuric acid (0.1N) was used to titrate the distillate. Total volatile nitrogen (TVN) was calculated as milligrams of nitrogen per 100 gms of sample. Duplicate determinations were made from each sample in the test.

$$\text{mg TVN/100 gms of sample} = \frac{(\text{ml of } 0.1\text{ N } H_2SO_4 \text{ required to titrate the distillate}) \times (1.4)^* \times (100)}{\text{sample in grams used for distillation}}$$

*1 ml of 0.1 N H_2SO_4 = 0.0014 gm of nitrogen = 1.4 mg N.

Organoleptic Evaluation

The general method adopted for organoleptic evaluation was as described by Peryam et al. 1957. A 150 gm sample was prepared for flavor evaluation. The sample was filled into a glass petri dish cover, placed about 3 inches from the flame and broiled in a gas oven. Both meat surfaces were exposed to the flame. All the samples were broiled for approximately the same time and at the same temperature. The cooked sample was divided into six approximately equal portions for panel evaluation. About 500 gms of the uncooked sample was displayed against a white background for color and aroma evaluations. Light intensities and other environmental conditions were the same throughout the study. The panel of six members consited of staff, graduate students, and secretaries. The panel was not trained primarily for this study, however, the members were considered competent to evaluate organoleptic criteria of meat. Panel members were asked to score the cooked samples for flavor and the uncooked samples for color and aroma. A nine point hedonic scale with a neutral point was used for scoring the samples. A score of nine was the highest rating and one the lowest.

Trial I

Regular ground meat was obtained from Oklahoma State University Meat Laboratory. To facilitate sampling for plating and uniform mixing of the test materials, a 200 gm meat sample was blended in a sterile glass Waring blender jar containing 800 ml of sterile distilled water to make a 1 to 5 dilution. Each meat sample was blended for two and one-half minutes using the low speed setting (approximately 10,000 rpm) on a Waring Blender. 100 ml of the homogenate containing 20 gms of meat was dispensed into sterile dilution bottles with screw caps. Test materials were added directly to the homogenate and stored at 7°C. The effect of the test materials on the CVT count and pH change was tested against the control (homogenate without test material) at different intervals.

The test materials used in this trial were 5 and 10% sterile skim milk and 5 and 10% lactic culture propagated in regular skim milk. The concentration was obtained by adding sterile skim milk or culture respectively to 100 ml of meat homogenate containing 20 gms of meat.

Trial II

Preparation of the meat homogenate and the experimental design was the same as indicated in trial I. However, the test materials used were 2.5, 5 and 10% reconstituted milk; 2.5, 5 and 10% culture. These concentrations were obtained by adding 0.5, 1 or 2 ml of reconstiuted milk or culture, respectively, to 100 ml of meat homogenate with 20 gms of meat.

The culture used consisted of Streptococcus lactis and Leuconostoc citrovorum

grown in sterile reconstituted milk with 20% milk solids. The CVT count and pH determination were both made on the meat samples treated with the different test materials at various time intervals.

Trial III

This trial was conducted to learn the effect of the addition of 10% pure culture of S. lactis; 10% pure culture of L. citrovorum plus lactic acid to bring the pH of the homogenate to 5.0; 10% L. citrovorum plus lactic acid to bring the pH of the homogenate to 4.5; lactic acid to bring the pH of the homogenate to 5.0; lactic acid to bring the pH of the homogenate to 4.5; and 10% lactic culture. (2ml of the culture added to 100ml homogenate provided the 10% culture). The meat homogenate preparation and experimental design were the same as indicated in trial I. The CVT count and pH determination were made on the samples at 0, 2 and 5 days.

Trial IV

Eighteen pounds of beef lean trim was obtained from the OSU meat supply. The meat was coarse ground using a sanitized grinder with 1/2 inch bore plate. The meat was divided into three six-pound aliquots for further treatment. One of these three aliquots was considered as a control sample and received no treatment. Each of the other two aliquots was treated with cultures, one consisted of a 20% pure culture of S. lactis and the other 20% culture containing S. lactis and L. citrovorum (20ml of culture to 100gm of ground beef). The cultures, grown in reconstituted milk with 20% milk solids, were thoroughly mixed into the meat sample by hands which were previously sanitized. The control sample aliquote was also mixed

by hand to the same extent as the treated sample aliquots and then reground through the 3/16 inch bore plate to provide a sample of regular ground beef. After thorough mixing of the cultures, the treated sample aliquots were also run through the grinder with a 3/16 inch bore plate to obtain regular size ground beef with added cultures.

Each of these three sample aliquots was divided into five approximately equal parts. Each part was placed into a polyethylene bag. The meat in the bag was squeezed and pressed to eliminate excess air and to provide a uniform rectangular shape. The open end of the bag was folded and sealed by an adhesive tape. Meat in one of the five bags from each sample aliquot was used for the 0 day analysis. The remaining four samples were stored in a 7°C cooler with fluorescent lights for 3, 5, 7, and 10 day analysis. CVT count, pH, total volatile nitrogen content and organoleptic evaluation for flavor, color and aroma were determined.

Trial V

Preparation of the meat sample and the experimental procedure was the same as indicated in trial IV. The deviations from trial IV are as follows: Coarse ground meat (1.2 inch diameter bore plate) was divided into four 5 pound sample aliquots for the control; 5, 10, and 20% cultured meat. Cultured meat samples were prepared by adding lactic cultures grown in reconstituted milk with 20% milk solids. The culture was added to the coarse ground beef to obtain meat containing 5, 10, and 20% culture. After mixing and regrinding the samples through a 3/16 inch bore plate, each of these four sample aliquots was divided into 5 portions and packed in polyethylene bags as previously indicated. Samples were analyzed at 0, 2, 5,

7, and 9 day intervals after being stored at 7°C as indicated in the previous trial.

Trial VI

Twenty pounds of ground beef from the meat laboratory was divided into 5 four pound aliquot samples. One of these samples was used as the control and received no treatment. The remaining four aliquot samples were treated with various concentrations and/or combinations of frozen concentrated lactic cultures. These cultures were received fresh from Angevine-Funke Co., St. Louis, Mo. and contained about 14 billion organisms of S. lactis plus L. citrovorum per ml.

For uniform distribution, the cultures were first mixed with sterile distilled water to make 100 ml and then added to the meat to prepare the cultured meat. Sample aliquots receiving the treatment were inoculated with 1% frozen concentrated culture (1 ml of the culture/100 gms of sample); 2% frozen concentrated culture (2 ml of the culture/100 gms of sample); 1% frozen concentrated culture plus 1% lactose (1 ml of the culture plus 1 gm of lactose/100 gms of the sample); and 2% frozen concentrated culture plus 1% lactose (2 ml of the culture plus 1 gm of lactose/100 gms of sample). All the sample aliquots were ground through 3/16 inch bore plate and divided into four approximately equal parts for 0, 3, 5, and 8 day analysis. Procedures adopted for mixing the cultures, grinding, storing the samples, and analysis were the same as indicated in trials IV and V. Samples were analyzed for CVT counts, pH, and volatile nitrogen content.

Trials VII to XI

Experimental procedure and sample preparation for mixing the cultures were

essentially the same as indicated in trials IV, V and VI. However, the meat used in trials VII, VIII, and X were obtained from the OSU meat supply and the samples used for trials IX and XI were from a retail grocery store. The effects of adding 10% lactic culture and 10% culture plus 450 ppm ascorbic acid were studied in these five trials. The experimental procedure and sample analysis were the same throughout this study. Figure 1 illustrates the experimental procedure used in these trials. Fifteen pounds of coarse ground beef were used in each trial. The coarse ground meat was divided into three 5 pound aliquot samples of which one was used as the control and other two were inoculated with cultures. One of the treated sample aliquots received 10% lactic culture grown in reconstituted milk with 20% solids and the other received a 10% similar culture plus 450 ppm ascorbic acid. Propagation and amount of the cultures added to make 10% were similar to that used in trial V. Ascorbic acid was added directly to the desired amount of culture before the culture was mixed with the aliquot meat sample.

After grinding through a 3/16 inch bore plate, each of the treated sample aliquots was divided into 4 equal parts, dispensed in polyethylene bags and stored at 7°C for 0, 3, 5 and 7 day analysis. CVT counts, pH, volatile nitrogen contents, and organoleptic evaluations for flavor, color and aroma were determined.

Statistical Analysis

Mean values for trials I to VI are presented in the tables and were used to make the graphs. Analysis of variance, F-test and least significant difference (LSD) were used to determine the effects of the treatments in trials VII to XI. A split plot design was used for this analysis. Two error terms were used in the analysis. One

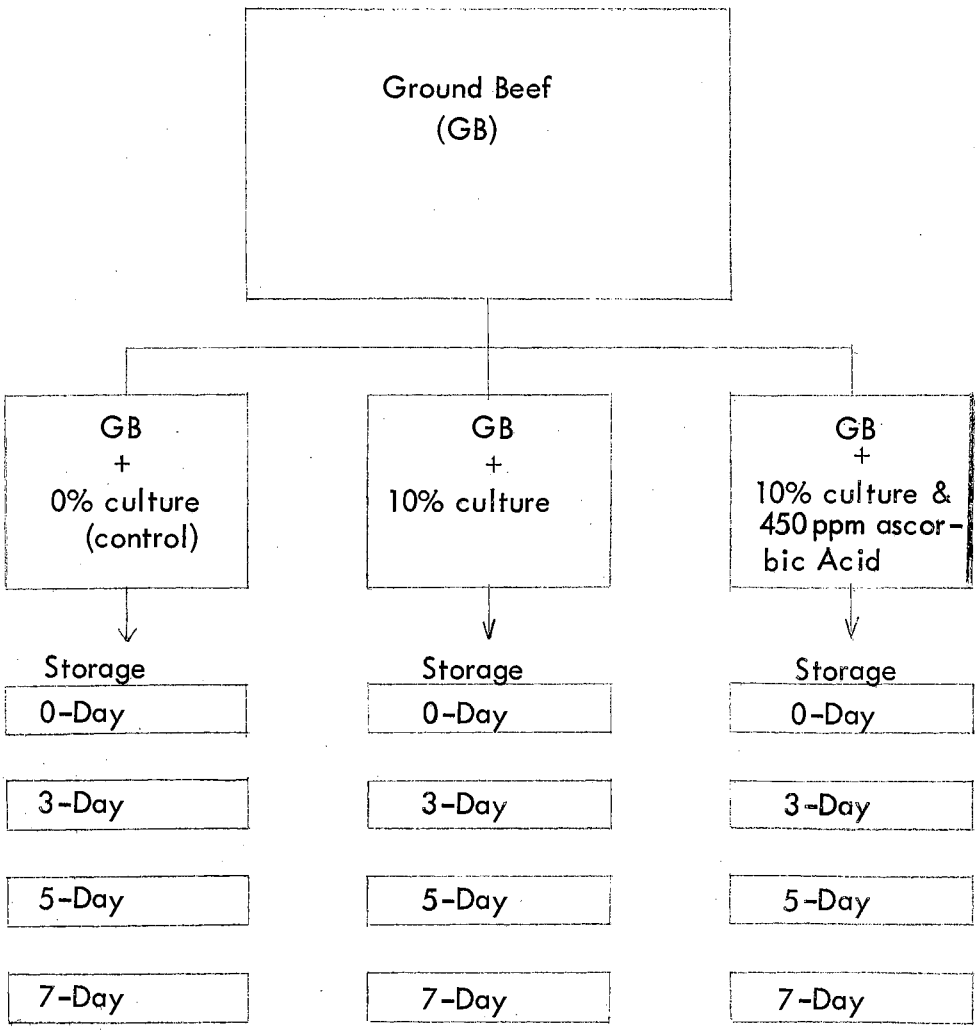

Figure 1. Experimental Plan for Trials VII -XI

was trial x treatment interaction to test treatment and trial effect. The other was trial x day and trial x treatment x day interactions to test day and trial x day effect. Neither of these two error terms were true estimates of the experimental error.

CHAPTER IV

RESULTS AND DISCUSSION

Trial I

CVT Count

The addition of milk and lactic cultures to a ground meat homogenate exerted some inhibitory effect on the inherent gram-negative bacterial growth. The effects on CVT count and pH are summarized in Table I and shown graphically in Figure 2. Meat samples without treatment had a higher CVT count than the treated samples at 3 and 6 days of storage. Gram-negative bacterial counts (CVT counts) for the meat with cultures were generally lower than in the meat samples with milk. Meat inoculated with a 10% culture reflected lower gram-negative counts than the samples with 5% culture.

pH

The pH values for the samples at the various storage periods are reported in Table I. Graphical illustrations of these values are shown in Figure 3. Higher pH values were noted for the samples receiving no treatment than the treated samples. A pH decline was more pronounced in the cultured samples than in those with milk. An increase in the amount of added culture relatively decreased the pH

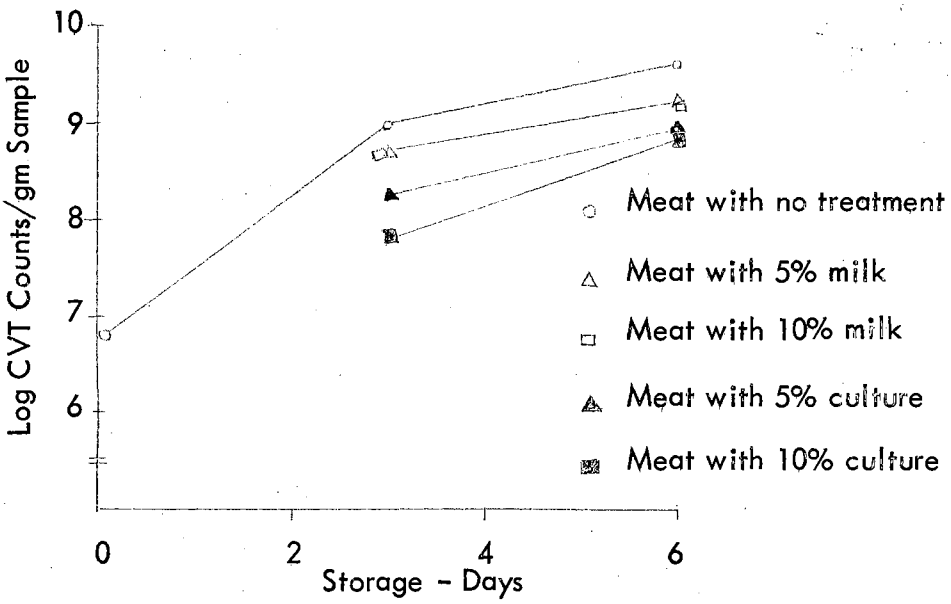

Figure 2. Log CVT Counts as Influenced by the Treatments (Trial I)

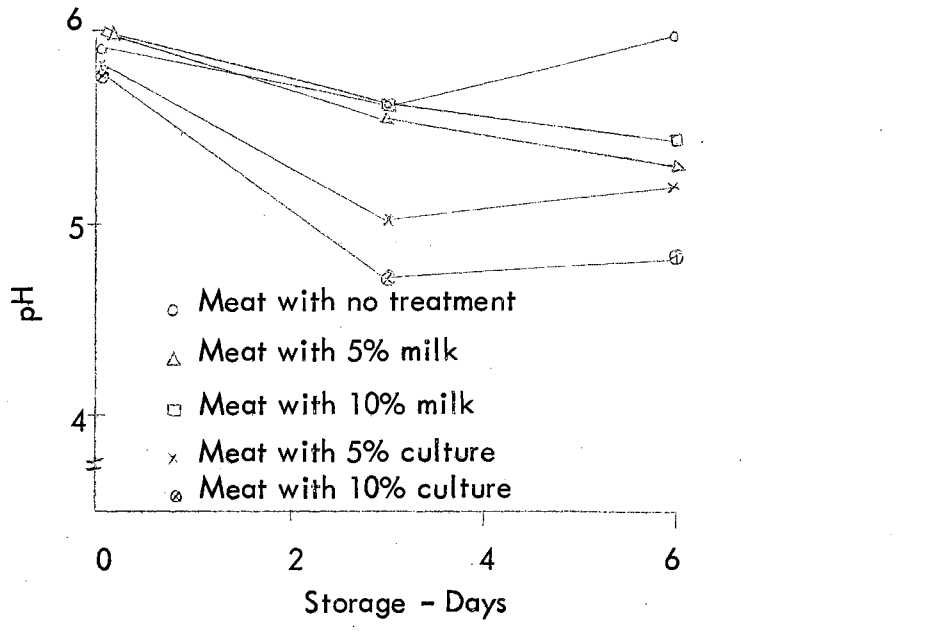

Figure 3. pH as Influenced by the Treatments (Trial I)

TABLE I

LOG CVT COUNTS AND pH AS INFLUENCED BY VARIOUS TREATMENTS IN GROUND BEEF (TRIAL I)

Treatment	Log CVT count/gm sample			pH		
Storage (Day)	0	3	6	0	3	6
Meat (control)	6.81	9.00	9.59	5.9	5.62	5.98
Meat + 5% milk	-	8.72	9.20	6.0	5.55	5.30
Meat + 10% milk	-	8.72	9.18	6.0	5.63	5.44
Meat + 5% culture	-	8.28	8.95	5.83	5.02	5.20
Meat + 10% culture	-	7.81	8.89	5.80	4.72	4.82

- counts not determined

TABLE II

LOG CVT COUNTS AND pH AS INFLUENCED BY VARIOUS TREATMENTS IN GROUND BEEF (TRIAL II)

Treatment	Log CVT count/gm sample			pH		
Storage (Day)	0	3	6	0	3	6
Meat (control)	3.40	6.38	8.36	5.48	5.50	5.40
Meat + 2.5% reconstituted milk	-	-	8.75	5.48	5.50	5.40
Meat + 5% reconstituted milk	-	-	8.81	5.55	5.55	5.40
Meat + 10% reconstituted milk	-	-	8.34	5.62	5.60	5.40
Meat + 2.5% culture	-	-	7.75	5.19	4.80	5.00
Meat + 5% culture	-	-	6.98	5.10	4.60	4.90
Meat + 10% culture	3.38	4.26	4.38	5.00	4.60	4.75

- counts not determined

values.

The inhibitory effect due to the addition of milk was probably due to the pH decline in these samples incident to some growth of the inherent acid producing organisms resulting in the production of some inhibitory action toward the gram-negative bacteria. The results obtained in this trial were not sufficient to conclude whether the lower CVT count in the samples with milk were due to experimental error or due to the inhibitory effect of acid producing organisms.

Trial II

A decrease in CVT count in the meat homogenate was found to increase with the amount of added culture in the previous trial. The addition of 10% lactic culture to meat homogenate resulted in a considerably lower CVT count than in the control sample. However, the count in the sample containing 10% culture increased by 2 log numbers in the 6 day stored sample. Hence the influence of adding higher concentrations of lactic cultures grown in reconstituted milk and of adding like amounts of reconstituted milk to the meat homogenate was determined in trial II.

CVT Counts

The results indicated (Figure 4 and Table II) that the lactic cultures had a definite inhibitory effect on the gram-negative bacteria in ground meat. The effect became greater as the concentration of culture used increased. After 6 days of storage the log of CVT count in the untreated sample was 8.36, while those for the inoculated samples were 7.75, 6.98 and 4.38 respectively for 2.5, 5 and 10%

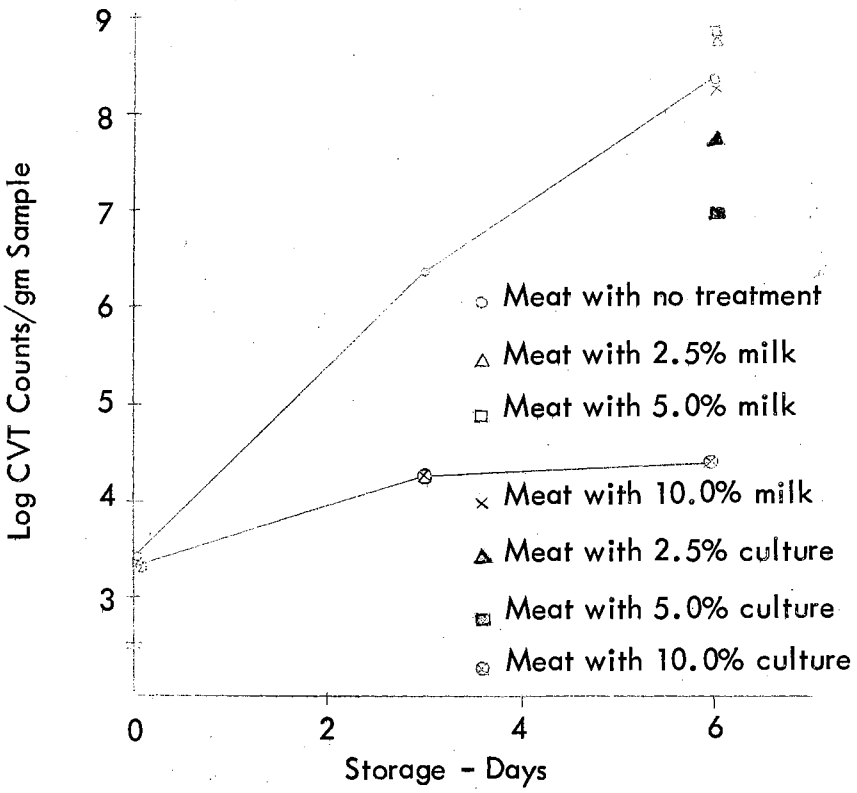

Figure 4. Log CVT Counts as Influenced by the Treatments (Trial II)

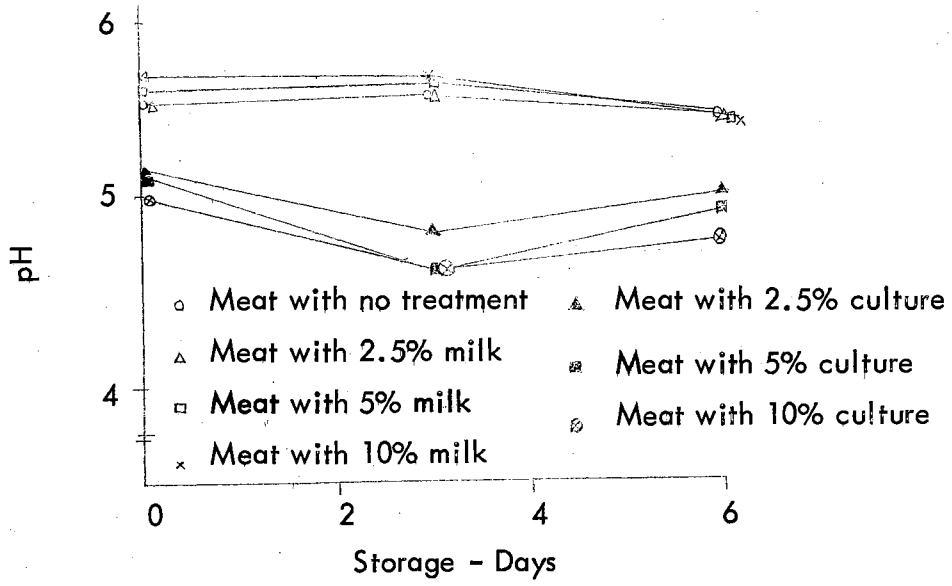

Figure 5. pH as Influenced by the Treatments (Trial II)

cultured samples. The addition of uninoculated reconstituted milk appeared to enhance the growth of gram negative organisms with 2.5 and 5% concentrations, while the 10% concentration appeared to be slightly inhibitory.

pH

Very little change in pH was observed for the untreated and treated samples without cultures (Table II and Figure 5), whereas in the cultured samples pH was considerably decreased during 6 day storage time. Increased culture concentrations accordingly resulted in decreased pH values. The pH decline in the cultured samples was attributed to the lactic acid production by S. lactis.

Trial III

This trial was conducted to determine if the inhibitory effect of added cultures was due to the production of lactic acid, causing a reduction in pH or to some other effects of the culture organisms in the samples. Log of CVT counts and pH values are reported in Table III and Figure 6 and 7.

CVT Counts

The terminal log CVT counts after 5 days storage ranged from 4.69 for the sample inoculated with L. citrovorum at a pH of 4.5 to 8.73 for the untreated control. It appeared that pH was a major factor in inhibiting the gram negative bacteria. The log CVT count in the sample inoculated with L. citrovorum at pH 5.0 was much higher (6.18) than that on the similar sample with a pH of 4.5. Also, reduction of the pH to 4.5 with lactic acid definitely reduced development in gram-

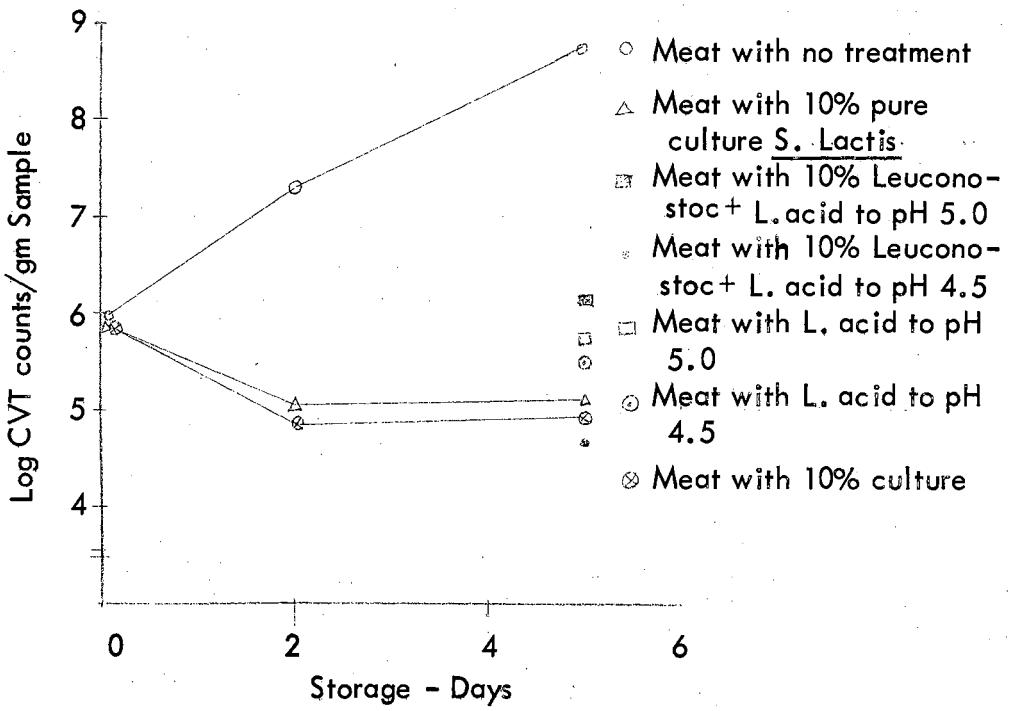

Figure 6. Log CVT Counts as Influenced by the Treatments (Trial III)

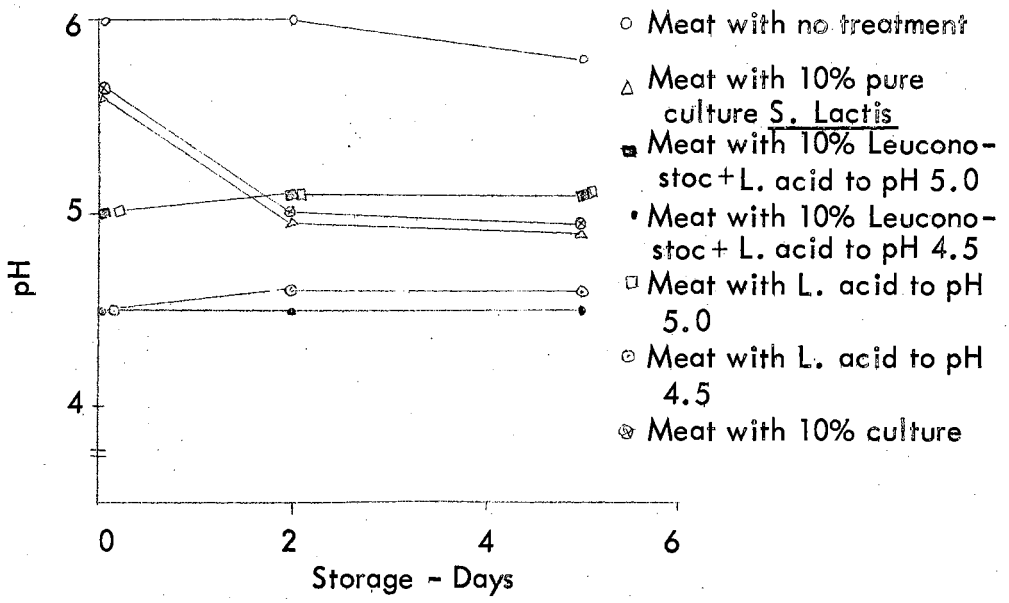

Figure 7. pH as Influenced by the Treatments (Trial III)

TABLE III

LOG CVT COUNTS AND pH AS INFLUENCED BY VARIOUS TREATMENTS (TRIAL III)

Treatment	Log CVT count/gm sample			pH		
Storage (Day)	0	2	5	0	2	5
Meat (control)	5.98	7.30	8.73	6.00	6.00	5.81
Meat with 10% pure culture S. Lactis	5.95	5.04	5.15	5.60	4.95	4.90
Meat with 10% Leuconostoc + L. acid to pH 5.0	-	-	6.18	5.00	5.10	5.10
Meat with 10% Leuconostoc + L. acid to pH 4.5	-	-	4.69	4.50	4.50	4.50
Meat with L. acid to pH 5.0	-	-	5.77	5.00	5.10	5.10
Meat with L. acid to pH 4.5	-	-	5.53	4.50	4.60	4.60
Meat with 10% culture	5.93	4.85	4.91	5.65	5.00	4.95

- counts not determined

negative bacteria; however, at the same pH level, the sample with added L. citrovorum had a considerably lower CVT count. The sample inoculated with a pure culture of S. lactis had a slightly higher log CVT count (5.15) than the one with the lactic culture (4.91), indicating some inhibitory action of L. citrovorum. These results indicated that the inhibitory action of the lactic cultures is due not only to the reduction in pH by the lactic acid produced by the cultures, but also to some other factor exerted by the culture organisms. These results are in general agreement with those reported by Baribo et al. 1951; Collins 1961; Mather et al. 1959, who concluded that lactic cultures had a definite inhibitory action toward gram-negative organisms in dairy products. Addition of lactic acid to bring the sample pH to 5.0 or 4.5 severely affected the color and aroma of the samples. However, when the pH of the cultured samples was brought to 5.0 or 4.5 by the culture organisms, the color and aroma were not affected to the extent observed in the samples with lactic acid. This suggested the impracticality of the addition of lactic acid to meat to inhibit the growth of inherent gram-negative bacteria.

pH

Changes in pH were very small for all the samples except meat with the pure culture of S. lactis and the lactic culture, where the pH sharply declined for 2 days then held constant for the rest of the storage period. The pH increased slightly in meat with added lactic acid whereas a slight decrease was noted in the control.

Trial IV

This trial was conducted to study the effects of adding pure culture of S. lactis

and lactic culture to ground beef. The effects were evaluated using microbial, chemical, and organoleptic analytical criteria.

CVT Counts

The results indicated (Table IV and Figure 8) that the log CVT counts on the untreated control sample increased steadily to a terminal count of 8.56, while the counts on the cultured samples decreased slightly during the first 3 days of storage and then remained constant during the next 7 days. The sample inoculated with pure S. lactis maintained almost the same level of gram-negative bacteria from the third to the tenth day (4.14) while the sample with the lactic culture had a lower terminal count (3.72). These results demonstrated the pronounced inhibitory action of added culture and indicated that the lactic culture was more effective than a pure culture of S. lactis.

pH

Addition of cultures to meat resulted in decreasing the pH of the samples from 5.7 to 5.35 on the initial day (Table IV and Figure 9). The pH of both stored cultured samples declined further at the 5 and 7 days storage periods (4.9). Meat samples without treatment increased in pH from 5.7 to 5.95 during 7 day storage period.

Volatile Nitrogen

Pronounced change was noted in the volatile nitrogen content (VNC) of the untreated control sample and steadily increased throughout the storage period

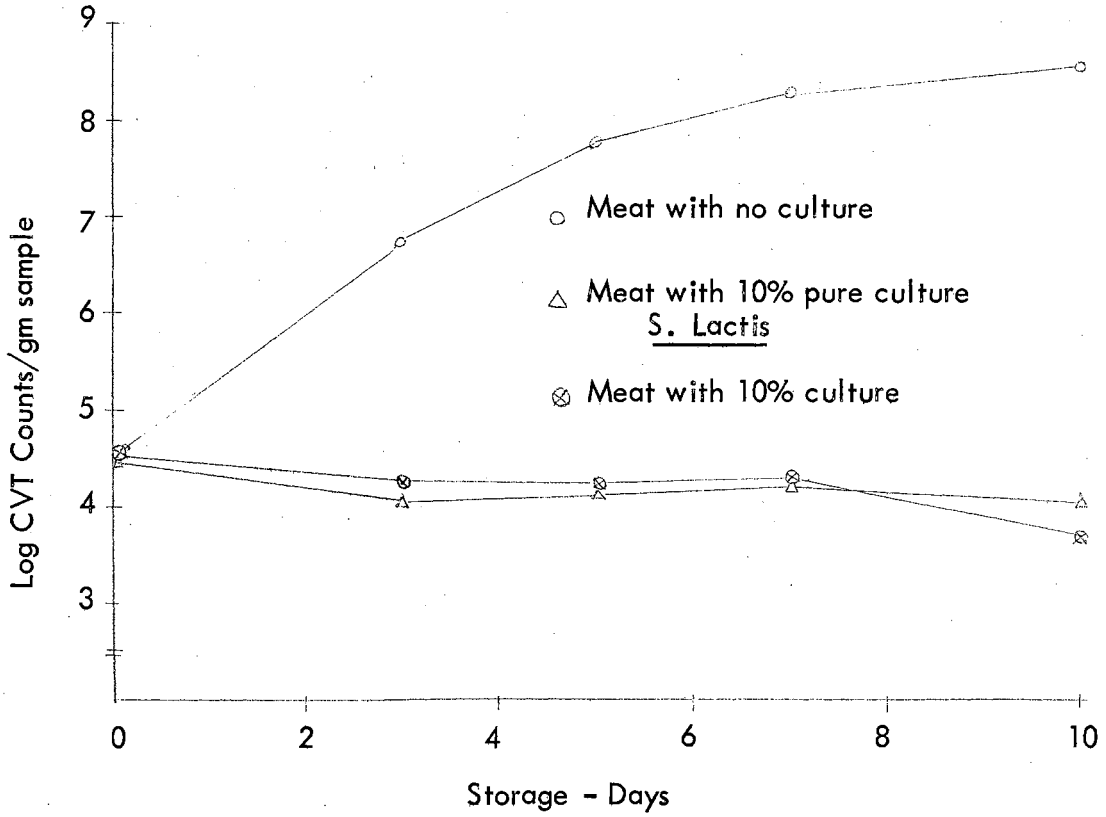

Figure 8. Log CVT Counts as Influenced by Lactic Cultures in Ground Beef (Trial IV)

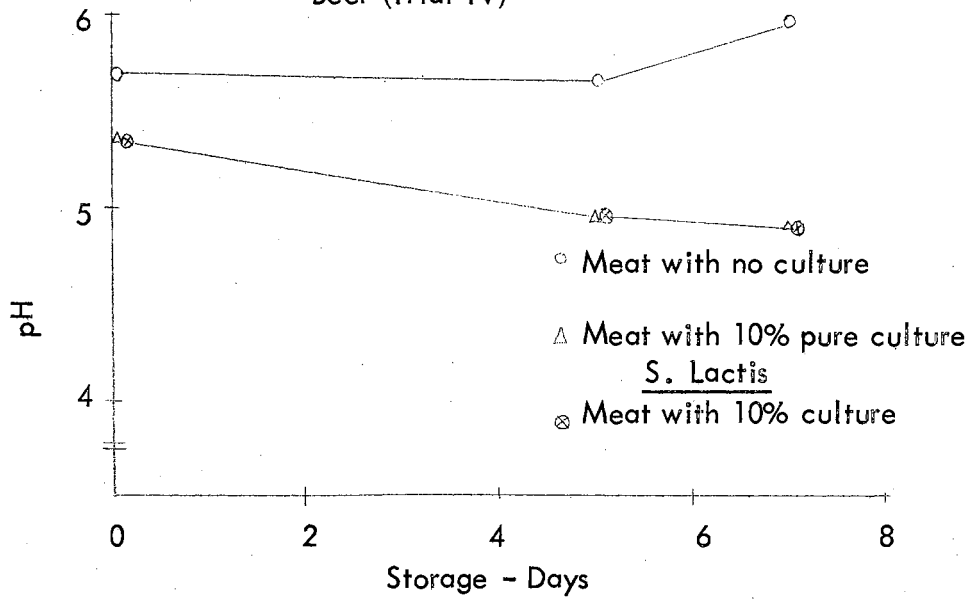

Figure 9. pH as Influenced by Lactic Cultures in Ground Beef (Trial IV)

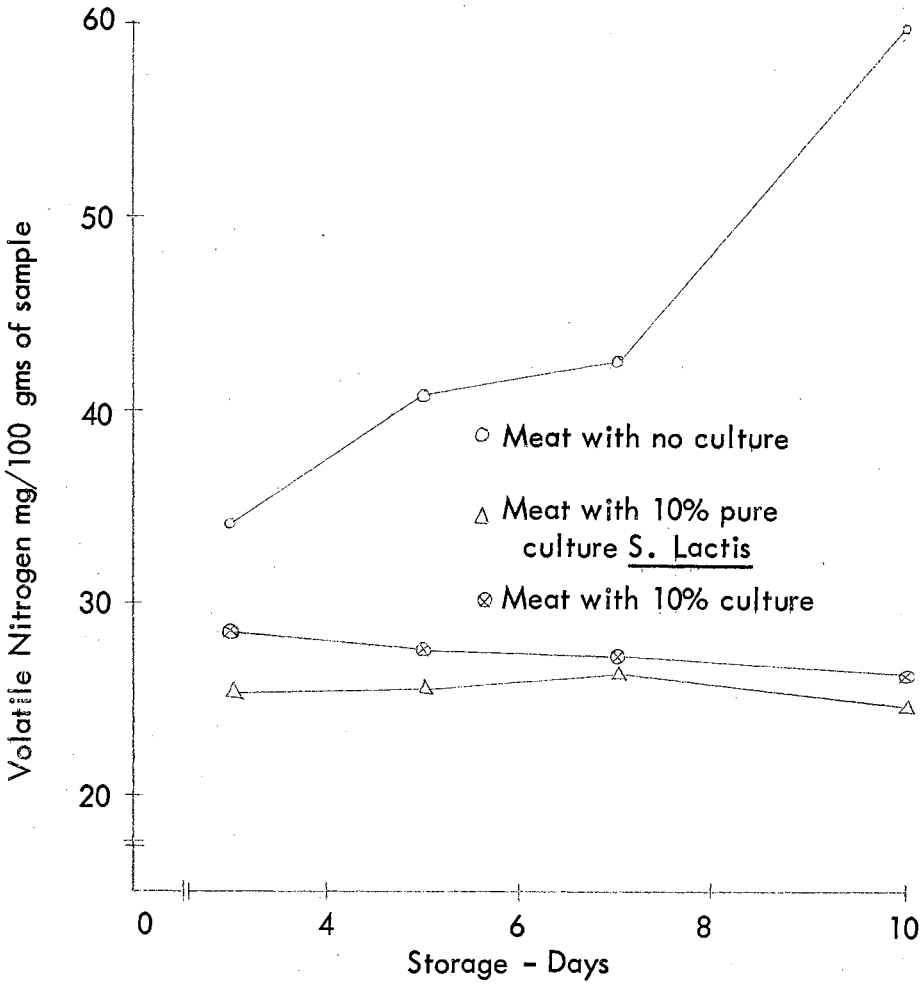

Figure 10. Volatile Nitrogen as Influenced by Lactic Cultures in Ground Beef (Trial IV)

TABLE IV

LOG CVT COUNTS, pH AND VOLATILE NITROGEN AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIAL IV)

Treatment	Log CVT count/gm sample					Volatile Nitrogen* mg/100 gms of sample				pH		
Storage (Day)	0	3	5	7	10	3	5	7	10	0	5	7
Meat (control)	4.57	6.79	7.77	8.28	8.56	34.28	40.73	42.69	59.85	5.70	5.68	5.95
Meat with 10% pure culture S. Lactis	4.54	4.08	4.18	4.20	4.08	25.55	25.63	26.26	24.51	5.35	4.95	4.90
Meat with 10% Lactic culture	4.56	4.28	4.28	4.23	3.72	28.68	27.67	27.00	26.04	5.35	4.95	4.90

*Mean values for 2 observations

(Table IV and Figure 10). In meat samples containing cultures, the VNC held steadily constant throughout the storage period. On the tenth day VNC in the control sample was almost twice as much as it was on the initial day (increase from 34.28 mg to 59.85 mg/100 gms sample). Consistently less VNC was detected in the samples inoculated with pure culture of S. lactis when compared to the samples with the lactic cultures (slightly lower VNC in the cultured samples at the 0-day analysis might be due to the weight of the added cultures which was not substracted from the sample weight to get actual weight of the meat sample). These data suggested that the bacterial deamination in the cultured meat was markedly reduced due to the prounced inhibitory action of the cultures on the growth of the inherent gram-negative spoilage types of bacteria in ground beef.

Organoleptic Evaluations

Flavor scores for the control sample were generally higher when compared to the treated samples (Table V and Figure 11). Sample with lactic culture was scored slightly higher than the sample with pure culture S. lactis.

Aroma scores for both cultured samples were generally higher than the control sample after 3 days of stroage. However, the score for the control sample was high at the initial day (Table V and Figure 12).

Color score for the control sample was consistently higher than that for the cultured samples throughout the study (Table V and Figure 13).

Since the variation in panel scores was high (ranged 9 to 5 for any given sample at any given time), the effects of culture on the organoleptic criteria were not correctly known. Statistical analysis using more data was felt necessary to resolve this

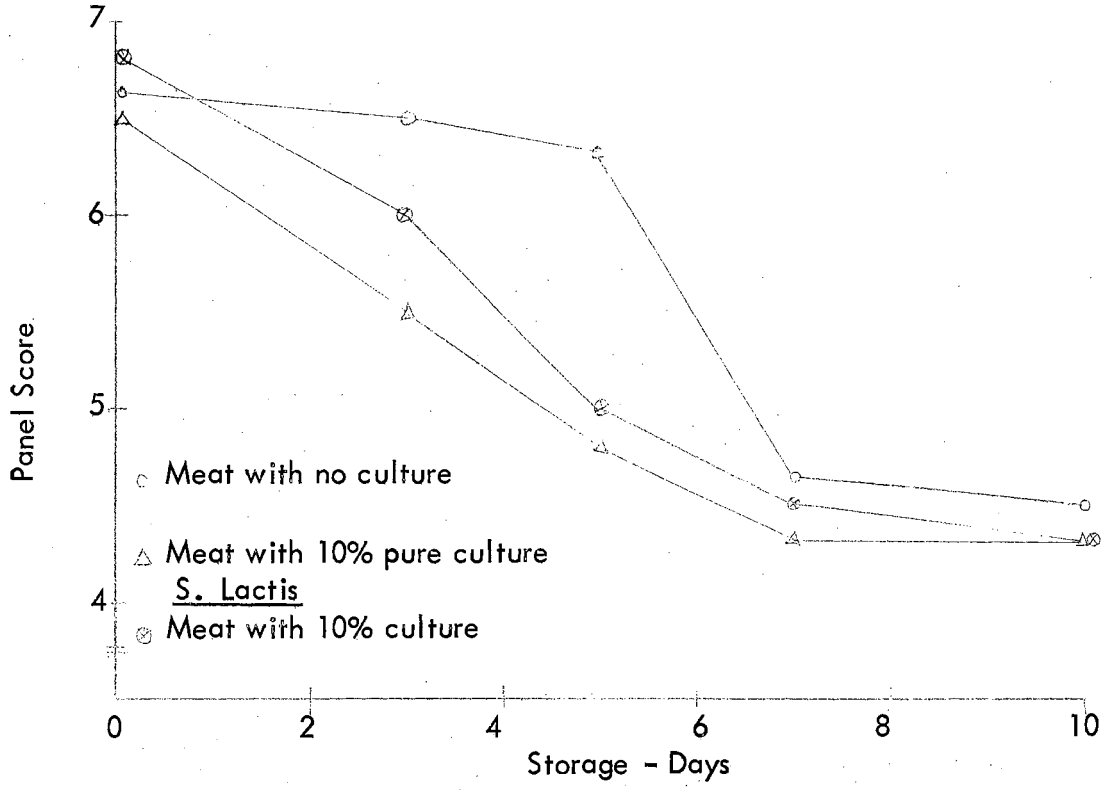

Figure 11. Flavor as Influenced by Lactic Cultures in Ground Beef (Trial IV)

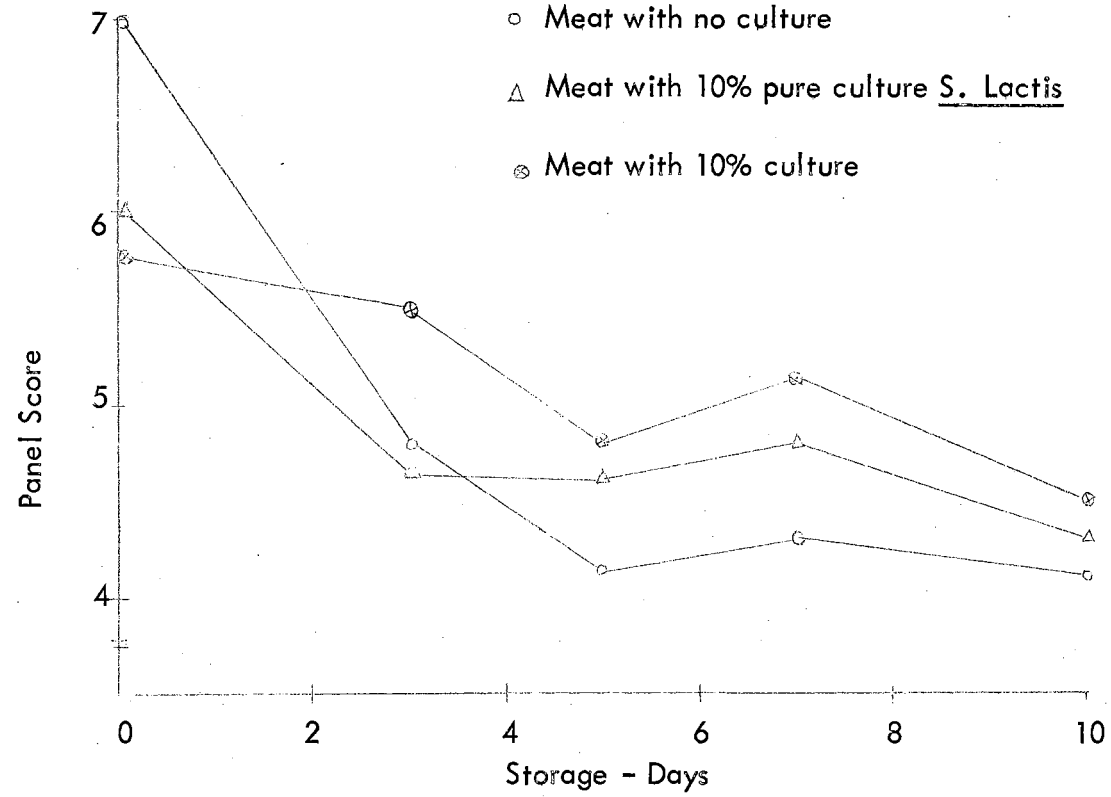

Figure 12. Aroma as Influenced by Lactis Cultures in Ground Beef (Trial IV)

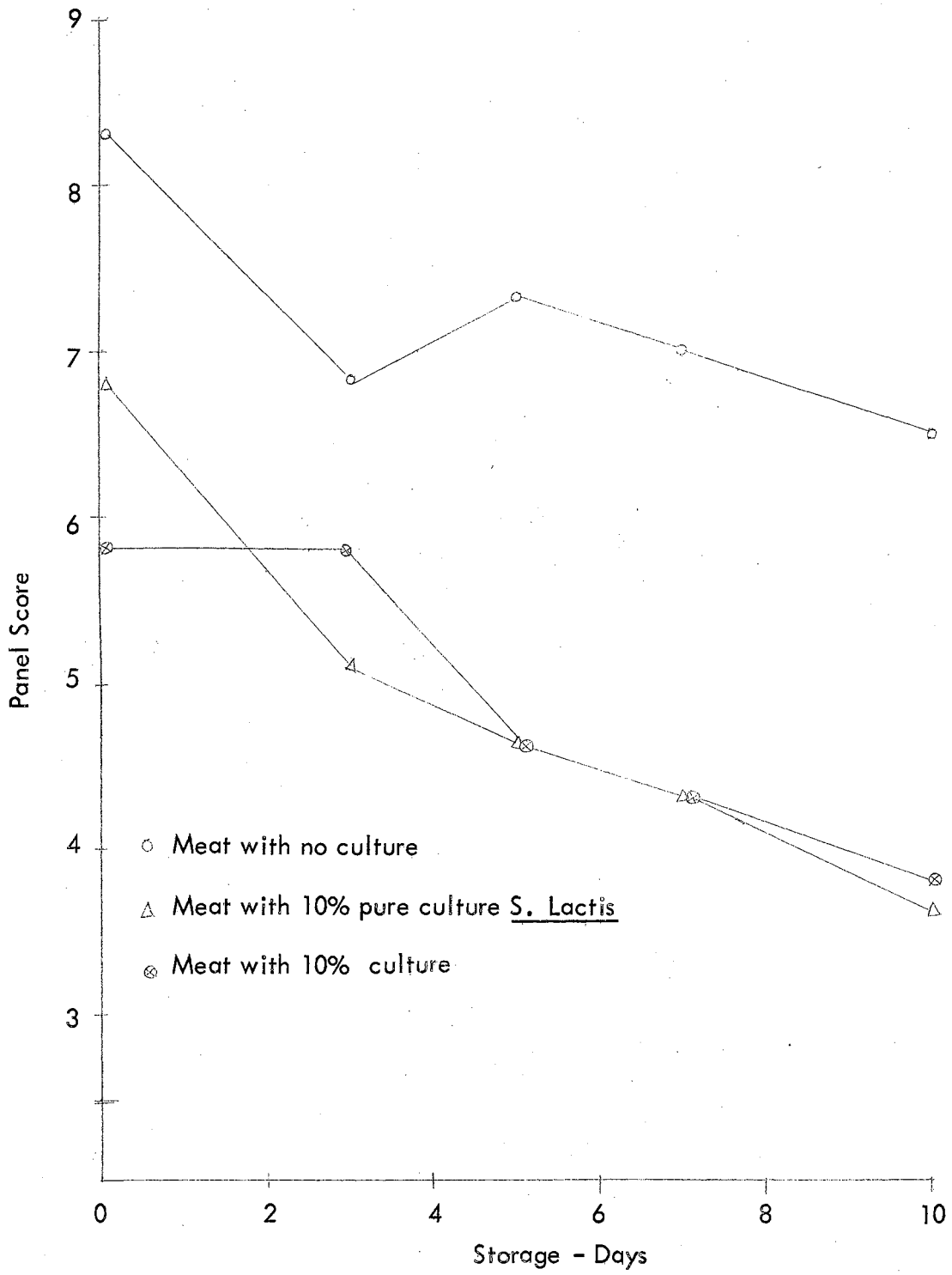

Figure 13. Color as Influenced by Lactic Cultures in Ground Beef (Trial IV)

TABLE V

FLAVOR, AROMA AND COLOR AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIAL IV)*

Treatment	Flavor Score					Aroma Score					Color Score				
Storage (Day)	0	3	5	7	10	0	3	5	7	10	0	3	5	7	10
Meat (control)	6.66	6.50	6.33	4.66	4.50	7.00	4.83	4.16	4.33	4.16	8.33	6.83	7.33	7.00	6.50
Meat with 10% pure culture S. Lactis	6.50	5.50	4.83	4.33	4.33	6.00	4.66	4.66	4.83	4.33	6.83	5.16	5.16	4.66	3.66
Meat with 10% culture	6.83	6.00	5.00	4.50	4.33	5.83	5.50	4.83	5.16	4.50	5.83	5.83	4.66	4.66	3.83

*Mean values for 6 observations

point.

Trial V

Data in the previous trial indicated that the lactic cultures have a slight edge over pure cultures of S. lactis in prolonging shelf-life of ground beef. Hence, only the lactic culture having both S. lactis and L. citrovorum was used in the later trials.

Since the results of previous trials (trial I and II) indicated that inhibitory action increased as the concentration of the culture used increased, this trial was conducted to establish a minimum level of culture concentration to be used for providing the required effect.

CVT Counts

The addition of 5% culture was found to be effective in reducing the rate and extent of gram-negative bacterial growth in the ground beef, but 10 and 20% concentrations were completely effective in preventing gram-negative bacterial growth (Figure 14). The terminal log CVT counts were 9.73, 8.80, 6.32 and 6.11 respectively for the samples with 0 (control), 5, 10 and 20% culture added (Table VI).

pH

The control sample with no treatment exhibited a constant increase in its pH during the storage time, while the cultured samples declined in pH during the first 2 days, then held fairly constant during the remainder of the storage (Table VI and Figure 15).

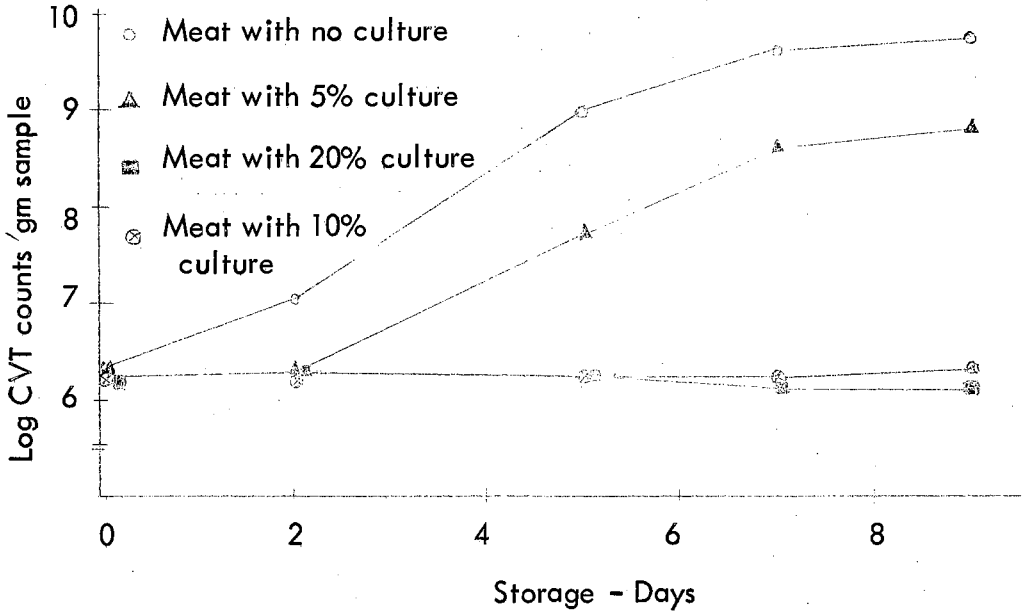

Figure 14. Log CVT Counts as Influenced by Lactic Cultures in Ground Beef (Trial V)

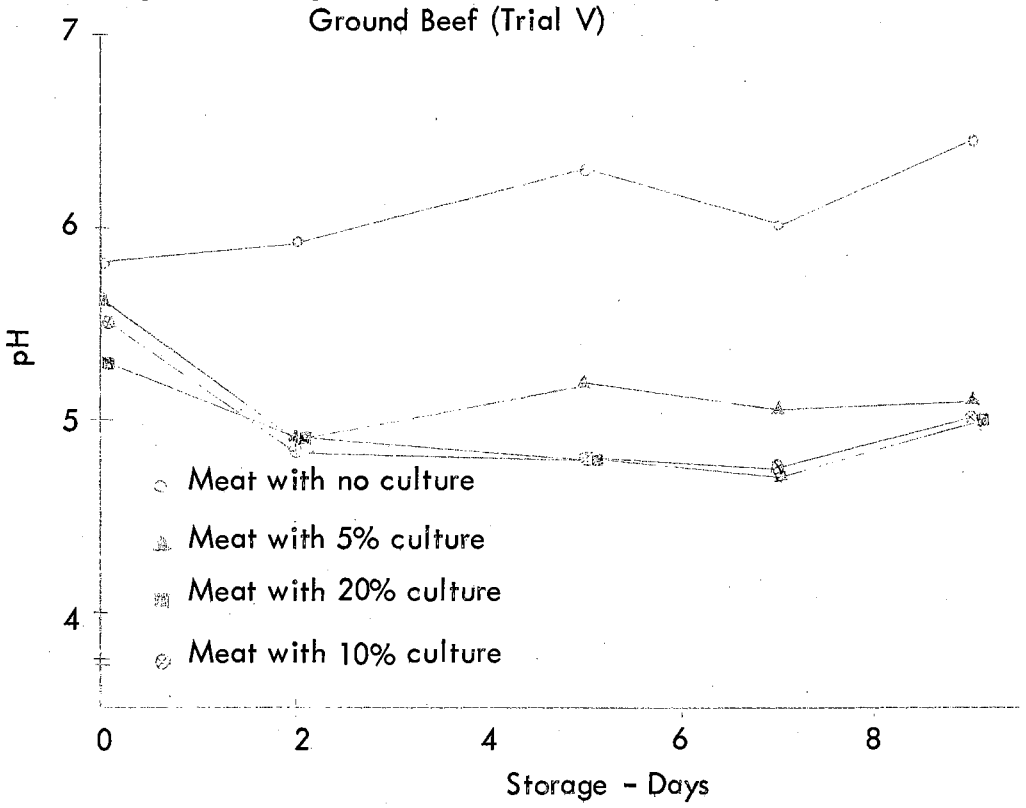

Figure 15. pH as Influenced by Lactic Cultures in Ground Beef (Trial V)

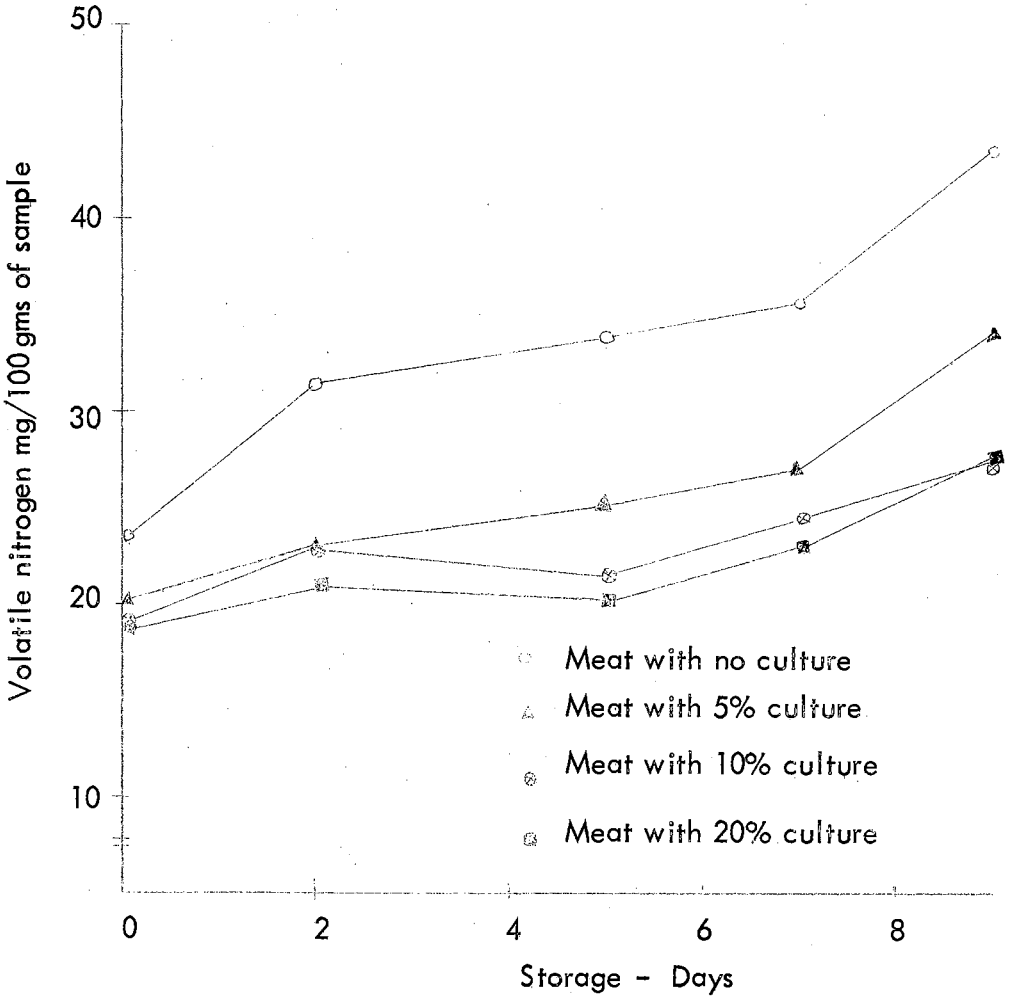

Figure 16. Volatile Nitrogen as Influenced by Lactic Cultures in Ground Beef (Trial V)

TABLE VI

LOG CVT COUNTS, pH AND VOLATILE NITROGEN AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIAL V)

Treatment	Log CVT count/gm sample					Volatile Nitrogen* mg/100 gms of sample					pH				
Storage (Day)	0	2	5	7	9	0	2	5	7	9	0	2	5	7	9
Meat (control)	6.34	7.04	9.00	9.61	9.73	23.76	31.84	33.97	35.68	43.76	5.82	5.90	6.30	6.00	6.45
Meat with 5% Culture	6.34	6.30	7.73	8.61	8.80	20.27	23.43	25.20	26.93	34.29	5.62	4.90	5.20	5.05	5.10
Meat with 10% Cutlure	6.32	6.28	6.26	6.23	6.32	19.24	23.08	21.70	24.52	27.26	5.50	4.85	4.80	4.75	5.00
Meat with 20% Culture	6.32	6.32	6.26	6.18	6.11	18.90	21.33	20.44	23.10	27.99	5.30	4.90	4.80	4.70	5.00

*Mean values for 2 observations

Volatile Nitrogen

Consistently less VNC was detected in the cultured meat samples (Table VI and Figure 16). These results confirm the data obtained in Trial IV. It was also seen that the increased concentrations of added cultures decreased the volatile nitrogen production in the samples. At the end of 9 days, 43.76, 34.29, 27.26 and 27.99 mg of VNC/100 gm samples was detected respectively for 0 (control), 5, 10 and 20% cultured samples.

Organoleptic Evaluations

Flavor scores indicated that the 10% cultured sample was preferred over the 20% cultured or the control on the initial day (Figure 17 and Table VII). However, at the end of 9 days both the 10 and 20% cultured samples were scored much higher than the samples with 0 (control) and 5% culture.

The control and 5% cultured samples were scored slightly higher for aroma on the initial day, but were generally scored lower than the 10 and 20% cultured samples throughout the later part of the study (Table VII and Figure 18).

Sample with 5% added culture was scored slightly higher for color than the control on the initial day. However, color scores were generally much lower for all the cultured samples throughout the study (Table VII and Figure 19).

Again the data in this trial was considered insufficient to conclude the effect of cultures on organoleptic criteria of ground beef due to large variations in the scores among the panel members. However, an improvement in the color of cultured meat was seriously thought to be necessary and attempts were made to improve

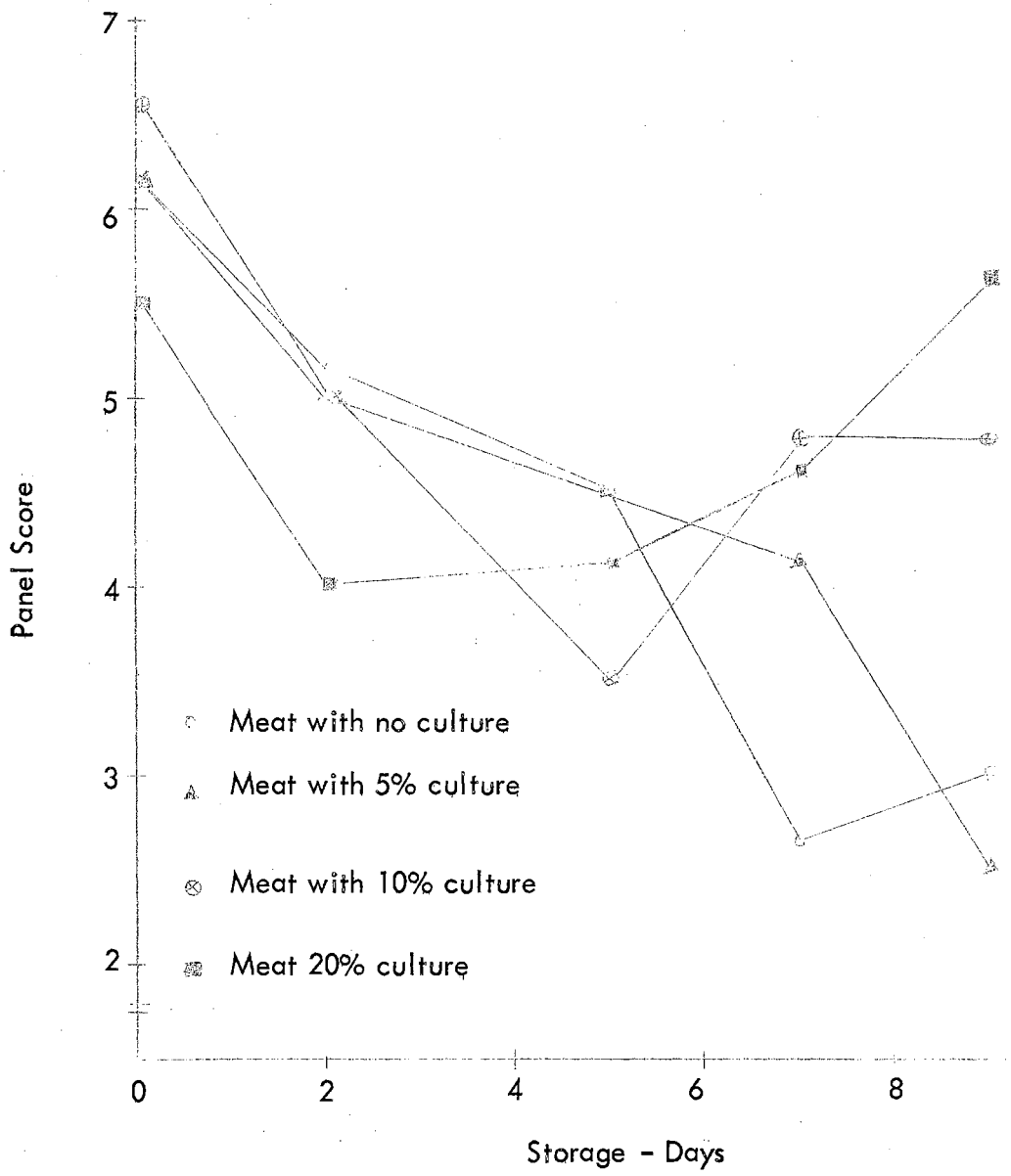

Figure 17. Flavor as Influenced by Lactic Cultures in Ground Beef (Trial V)

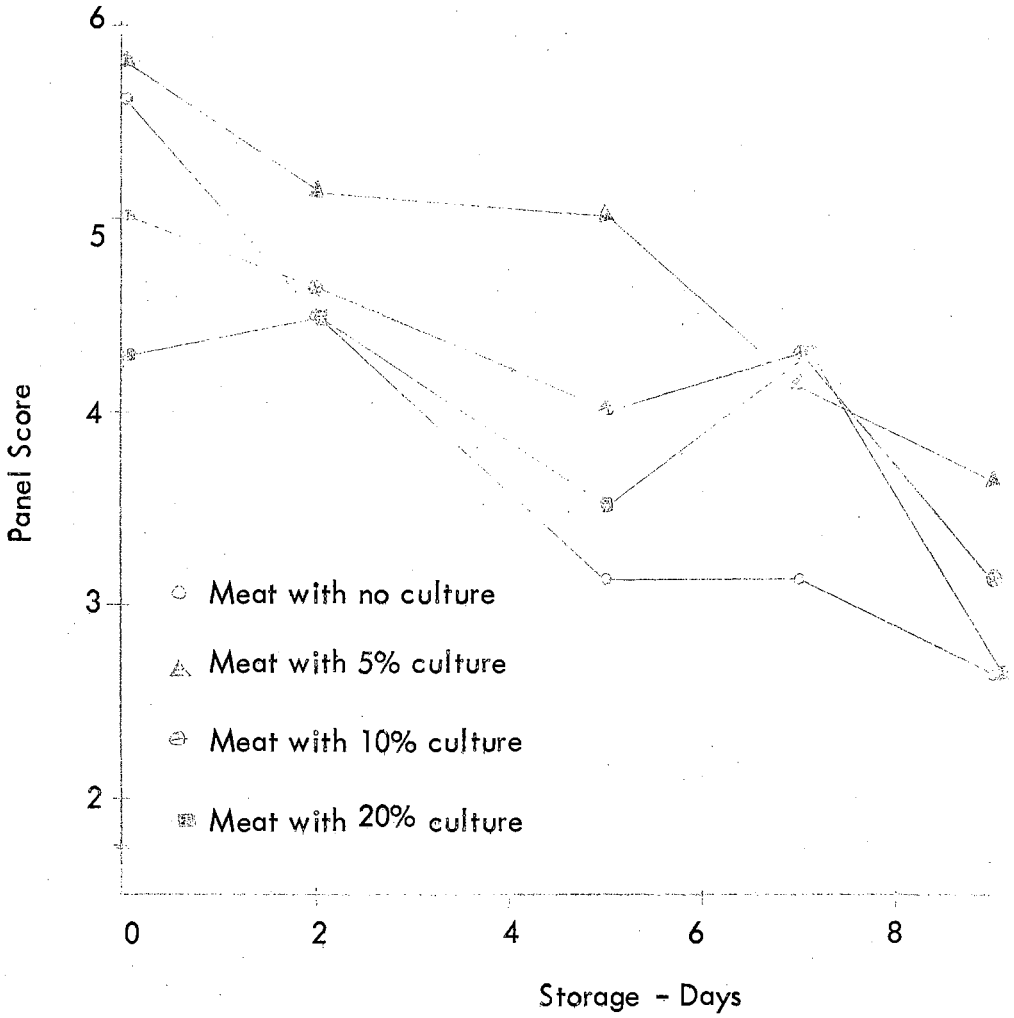

Figure 18. Aroma as Influenced by Lactic Cultures in Ground Beef (Trial V)

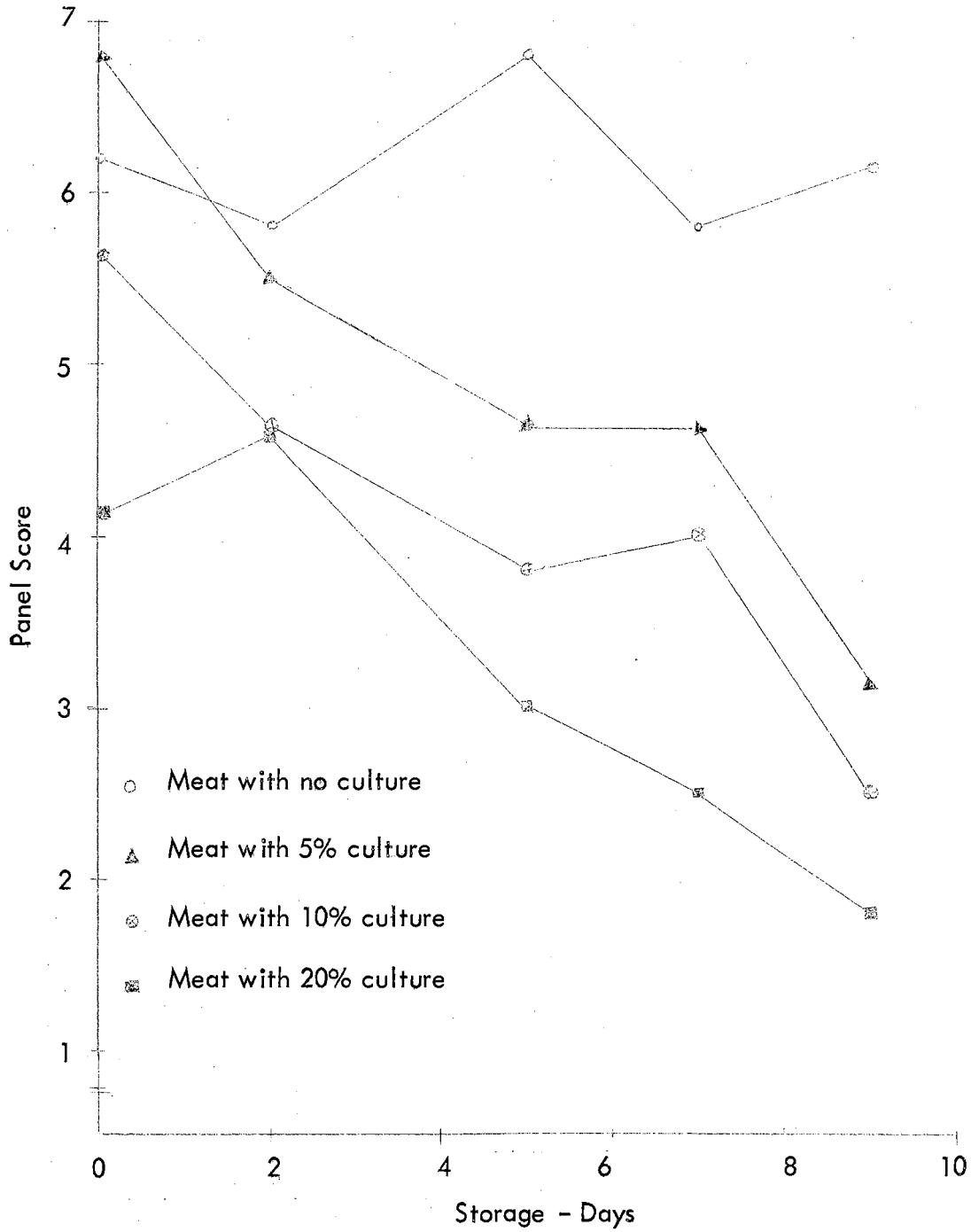

Figure 19. Color as Influenced by Lactic Cultures in Ground Beef (Trial V)

TABLE VII

FLAVOR, AROMA AND COLOR AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIAL V)*

Treatment	Flavor Score					Aroma Score					Color Score				
Storage (Day)	0	2	5	7	9	0	2	5	7	9	0	2	5	7	9
Meat (control)	6.16	5.16	4.50	2.66	3.00	5.66	4.50	3.16	3.16	2.66	6.20	5.83	6.83	5.83	6.16
Meat with 5% Culture	6.16	5.00	4.50	4.16	2.51	5.83	5.16	5.00	4.16	3.66	6.83	5.50	4.66	4.66	3.16
Meat with 10% Culture	6.83	5.00	3.50	4.83	4.83	5.00	4.66	4.00	4.33	3.16	5.66	4.66	3.83	4.00	2.50
Meat with 20% Culture	5.50	4.00	4.16	4.66	5.66	4.33	4.51	3.50	4.33	2.66	4.16	4.51	3.00	2.50	1.83

*Mean values for observations of 6 panel members.

it in the later trials.

Trial VI

Decreases in mean panel color scores of cultured samples were believed due to the addition of the reconstituted milk used for propagating the cultures. Hence, in this trial frozen concentrated cultures were added to the ground beef samples and their effects determined.

CVT Counts

Cultured samples with 1% lactose were generally lower in log CVT counts than the control and the cultured samples without lactose (Figure 20 and Table VIII). However, the inhibitory effect was not as pronounced as was seen with the cultures grown in the reconstituted milk. Cultures used in this trial did not indicate any inhibitory effect on CVT counts when lactose was not added. At the terminal day of analysis the log CVT counts were 8.92, 9.81, 9.08, 7.99 and 8.34 respectively for control, 1 and 2% culture, and 1 and 2% culture with lactose.

pH

Consistenly lower pH was noted in the cultured samples when lactose was added than in both the sample without culture and the cultured samples without lactose (Figure 21 and Table VIII).

Volatile Nitrogen

Volatile nitrogen content (VNC) was comparatively lower in the cultured

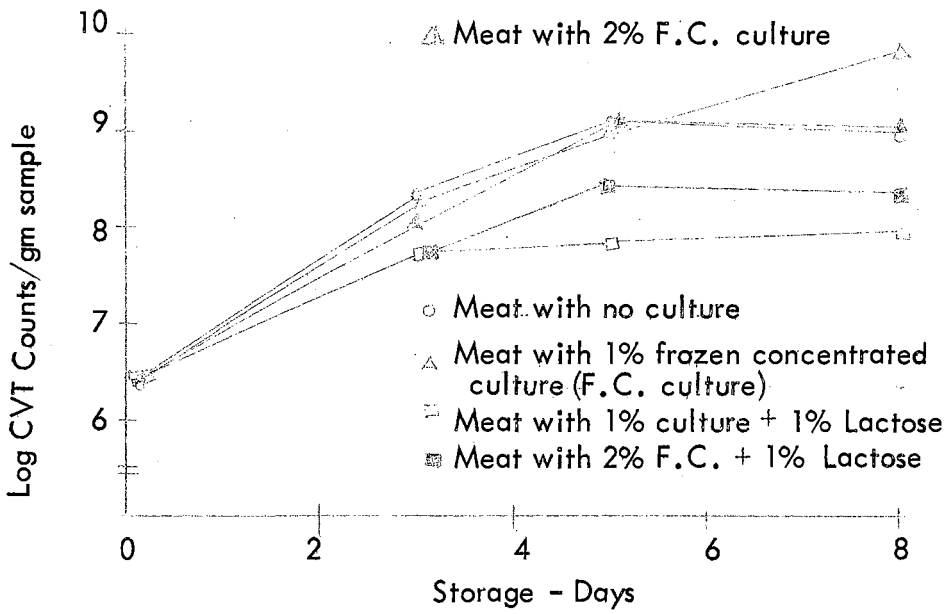

Figure 20. Log CVT Counts as Influenced by Lactic Cultures in Ground Beef (Trial VI)

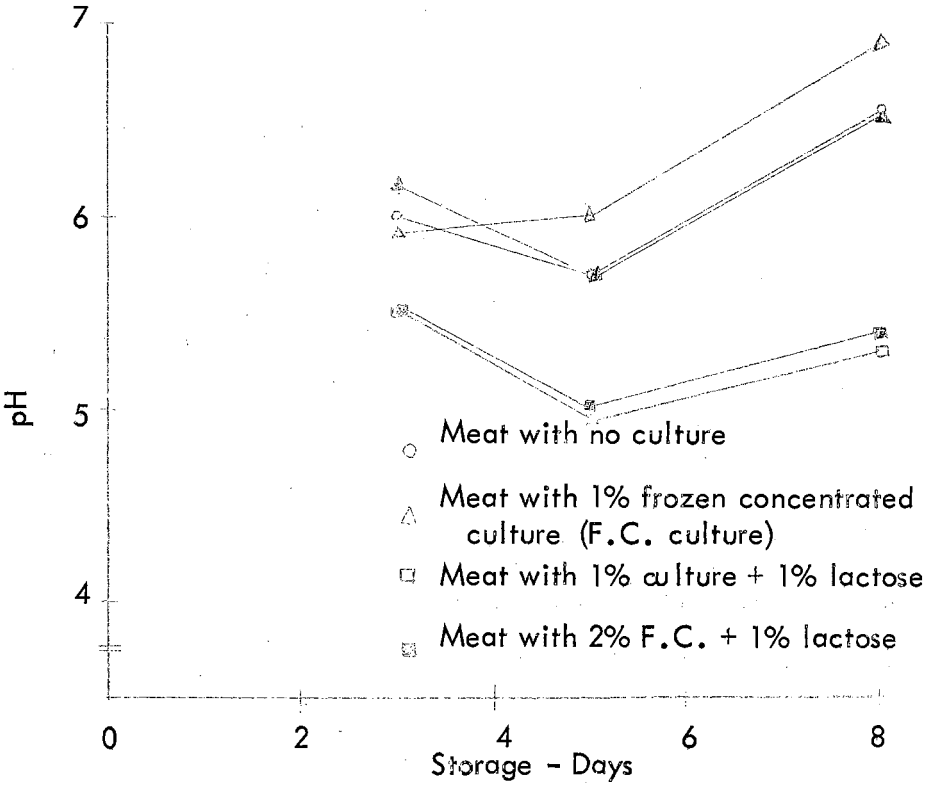

Figure 21. pH as Influenced by Lactic Cultures in Ground Beef (Trial VI)

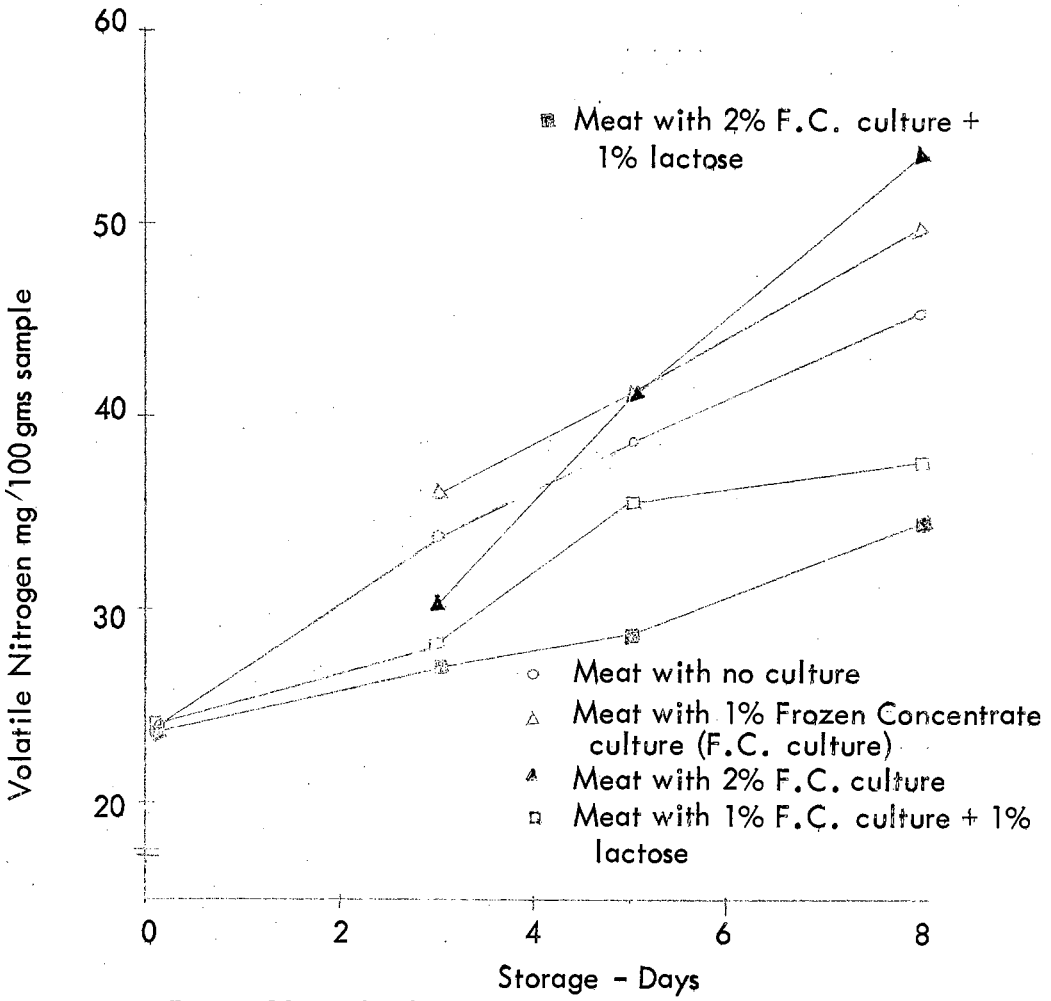

Figure 22. Volatile Nitrogen as Influenced by Lactic Cultures in Ground Beef (Trial VI)

TABLE VIII

LOG CVT COUNTS, pH AND VOLATILE NITROGEN AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIAL VI)

Treatment	Log CVT count/gm sample				pH				Volatile Nitrogen* mg/100 gms of sample			
Storage (Day)	0	3	5	8	0	3	5	8	0	3	5	8
Meat (control)	6.43	8.36	9.08	8.92	-	6.00	5.70	6.55	23.80	33.61	38.85	45.15
Meat with 1% frozen concentrated culture	6.43	8.34	8.98	9.81	-	5.90	6.00	6.90	-	36.05	41.30	49.71
Meat with 2% frozen concentrated culture	6.42	8.04	9.08	9.08	-	6.15	5.70	6.50	-	30.10	41.30	53.55
Meat with 1% frozen concentrated culture +1% Lactose	6.43	7.75	7.85	7.99	-	5.50	4.95	5.30	24.15	28.35	35.35	37.80
Meat with 2% frozen concentrated culture +1% Lactose	6.41	7.72	8.42	8.34	-	5.55	5.00	5.40	23.80	27.30	28.70	34.65

- Not determined

* Mean values for 2 observations

samples with lactose. However, the cultures without lactose resulted in higher VNC than the control sample at the later part of the storage (Figure 22 and Table VIII). These data suggested that the culture organisms did not grow due to insufficient lactose in the samples where 1% lactose was not added. There appeared to be little or no growth of the culture organisms in the samples without lactose. Consequently no inhibitory action was observed.

Visual appraisal of color in all the cultured samples suggested no improvement over the previous trials. Therefore, it was decided not to use the frozen concentrated cultures in future trials.

Trials VII - XI

In an attempt to retain and/or improve the cultured meat color, different levels of food color and ascorbic acid were added to the cultured meat. No improvements were observed when the food color was added to the cultured meat samples. However, adding 450 ppm of ascorbic acid (460 ppm of ascorbic acid is legally permitted in the meat products to be processed) was considered adequate to retain and/or improve the color in the cultured meat samples. Hence, in trials VII - XI the effect of adding culture and culture plus 450 ppm of ascorbic acid was tested. Pooled data for these 5 trials are used for graphical illustrations, tables, and statistical analysis.

CVT Counts

Statistical analysis (Analysis of Variance - AOV) indicating F-tests is shown in Table X. A significant treatment effect ($P < .01$) indicated the profound effect

of the cultures in altering the log CVT counts in the samples. Graphical illustration of the log CVT count in the samples with and without lactic culture at different storage intervals is reported in Figure 23. Steady increase in CVT count was noted in the uncultured meat sample throughout the storage time. The count in the cultured sample and in the cultured sample with ascorbic acid were practically identical throughout the storage period and were much lower (at least 2 log count difference) than those of the control sample. These data indicated a definite inhibitory effect of lactic cultures on the growth of inherent gram-negative bacteria in ground beef. Counts in both the cultured samples did not show any significant increase until the 5th day, after which an apparent increase was noticed. Further analysis, using the least significant difference (LSD) test, indicated no significant differences in log CVT counts for the initial day samples (Table IX). This was expected because all the samples were taken from one main sample and the cultures had no time to grow and exert their inhibitory effect. Non-significant difference in log CVT counts in the treated and untreated samples on the initial day also assured a uniform bacterial load and thus homogenity in the starting samples. However, at later part of the storage the counts in the cultured samples were significantly lower than the contro sample ($P < .05$, $P < .01$, and $P < .01$ respectively) at 3, 5 and 7 days storage, substantiating the inhibitory effect of the cultures.

These results are in general agreement with Baribo et al. 1951; Collins, 1961; Marth et al. 1962; Mather et al. 1959; who reported the inhibitory effect of the lactic cultures toward gram-negative spoilage type organisms in dairy products.

A non-significant difference in log CVT counts was found (LSD) between meat with only the culture and culture with ascorbic acid. This suggested that 450 ppm

of ascorbic acid had no effect on the CVT counts in ground meat.

A significant day effect ($P < .01$), suggested that the log CVT count at different storage intervals were different in the same sample. These results are supported by other research (Jay 1964; Gardner et al. 1966. etc.) who reported a significant increase in the counts as the meat storage period is prolonged. Analysis by LSD test indicated a significant increase ($P < .01$) in the CVT count in 3 and 7 days stored control samples when compared with the same samples at 0 and 3 days storage. On the other hand, cultured samples did not exhibit a significant increase in the CVT count until 5 days of storage. Only after 7 days of storage there was a significant difference ($P < .05$) between the 0 and 7 day samples.

A significant day x treatment interaction ($P < .01$) was also found using F-test. This is due to the typical growth pattern of the bacteria in the uncultured samples (logarithmic and stationary phases of growth) and also might be due to the microbes in the cultured meat which developed a resistance to the inhibitory effect of the culture and started growing in later part of the storage periods or to the growth of inherent resistant types which developed into countable numbers as the storage period lengthened. Log CVT counts were significantly different due to trial effect ($P < .01$), indicating that a heterogenous meat sample was used in the different trials.

pH

A significant difference in pH ($P < .01$) between cultured and uncultured meat samples existed due to the treatment effect (Table XI). The pH in both cultured meat samples was essentially the same but lower ($P < .01$) than the uncultured samples.

The pH declined in the cultured samples during the first 3 days of storage after which the pH change was very insignificant (Figure 24). A slight increase in pH of the uncultured meat samples was noticed after 3 days storage. The LSD test indicated a significant decrease ($P < .01$) in pH of the cultured meat samples throughout the study (Table IX). A significant decline in pH for the initial day cultured samples was due to the acidity (pH 4.5-4.6) of the cultured medium. The decline in pH for the cultured samples indicated a steady growth of the culture in the sample.

A significant day effect ($P < .01$) on pH change was substantiated by the F-test (Table XI). The uncultured control sample at 7 days storage had a greatly different ($P < .01$) pH from the sample at 3 days stroage (Table IX). These results are in agreement with Bodwell, et al. 1965, Jay, 1964, and Pearson, 1968d, who reported a pH decline followed by a rise in meat from slaughter stage to several days of storage. During the first 3 days of storage, cultured samples had a highly significant decline ($P < .01$) in pH which was held constant until 7 days storage.

Day x treatment interaction was also found by F-test (Table XI). This could be due to the fact that the culture organisms would only grow and produce lactic acid until the pH of the medium reaches a certain level. A slight increase in the pH of the control sample was assumed to be due to the increased proteolysis as a result of higher CVT counts at the later part of the storage period. These factors were considered to be the cause for this interaction.

The pH of the treated or control meat in the different trials was not significantly different.

Volatile Nitrogen

Treatment effect was significant ($P < .01$) and superseded day, trial and day x treatment effects (Table XII). Volatile nitrogen content continuously increased ($P < .01$) in the control sample throughout the study (Figure 25) and was always, greater ($P < .01$) than that in the cultured samples. These findings are in agreement with Pearson, 1968a who reported increase in VNC of meat as the days of storage increased. Samples with culture and culture plus ascorbic acid did not significantly differ from each other at any tested storage time.

Volatile nitrogen content of the meat was not significantly different (LSD test, Table IX) in the control and treated samples at 0-day analysis. However, a significant increase ($P < .01$) was seen in the 3, 5 and 7-day control sample. Non-significant difference in 0-day analyzed samples was expected because the treatment effect was not anticipated at this time. Significantly lower VNC in the cultured samples assured a definite inhibitory action of the cultures during storage.

The F-test (Table XII) also showed a highly significant day effect ($P < .01$) which was supported by further analysis (LSD test, Table IX). The latter test indicated a highly significant increase in VNC ($P < .01$) of control sample at 3, 5 and 7-day storage. Significantly increased VNC ($P < .01$ and $P < .05$) was observed in both the cultured samples as the storage time increased. However, this increase was significantly lower than the control (Figure 24 and Table IX).

Day x treatment interaction proved to be significant ($P < .01$). This could be explained due to the different growth phases of microbes affecting the volatile nitrogen production in control sample.

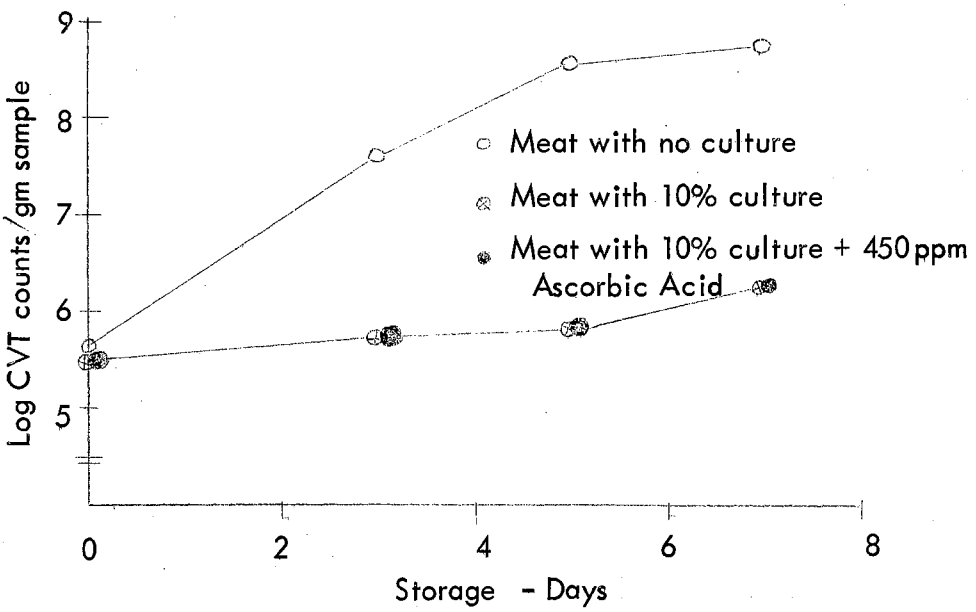

Figure 23. Log CVT Counts as Influenced by Lactic Cultures in Ground Beef (Trials VII-XI)

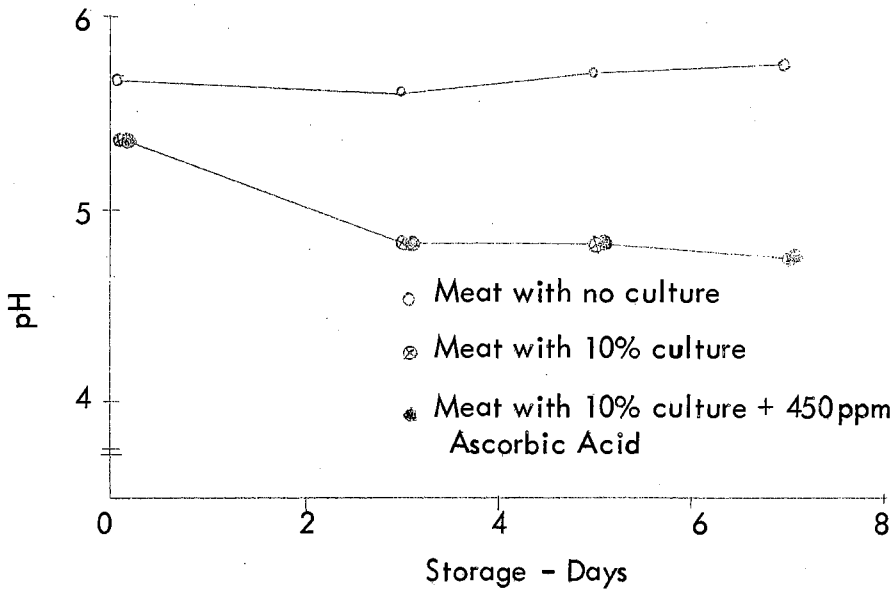

Figure 24. pH as Influenced by Lactic Cultures in Ground Beef (Trials VII - XI)

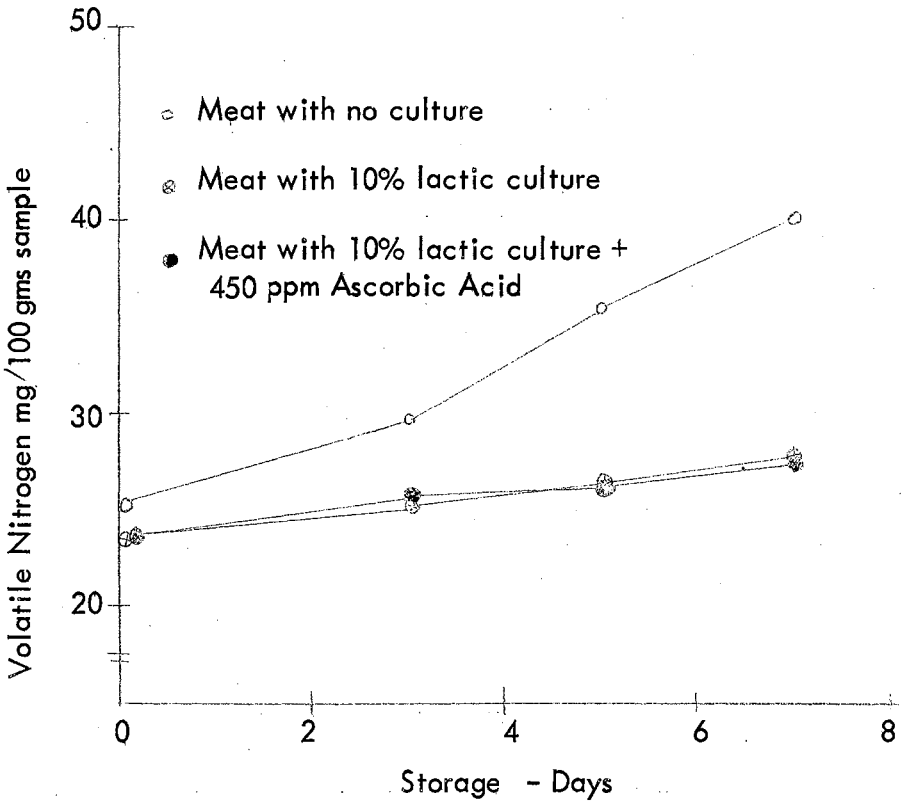

Figure 25. Volatile Nitrogen as Influenced by Lactic Cultures in Ground Beef (Trials VII-XI)

TABLE IX

LOG CVT COUNTS, pH AND VOLATILE NITROGEN AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIALS VII-XI)

Treatment / Storage (Day)	Log CVT Count* per gram sample				pH*				Volatile Nitrogen* mg/100 gms sample			
	0	3	5	7	0	3	5	7	0	3	5	7
Meat with no culture (control)	5.65	7.60	8.56	8.70	5.65	5.61	5.70	5.75	25.15	29.64	35.49	40.18
Meat with 10% culture	5.50	5.73	5.78	6.24	5.37	4.84	4.82	4.75	23.80	25.27	26.60	27.93
Meat with 10% culture + Asc. acid 450ppm	5.54	5.73	5.84	6.26	5.37	4.84	4.83	4.78	23.91	25.97	26.25	27.65

*Mean values for five trials

Least Significant Differences (LSD)

		Day	Log CVT Count	pH	Volatile Nitrogen mg/100 gms sample
Treatment Effect		0	Not Significant	5.65>5.37 (P<.01)	Not Significant
		3	7.60 > 5.73 (P<.05)	5.61>4.84 (P<.01)	29.64 > 25.97 (P<.01)
		5	8.56 > 5.84 (P<.01)	5.70>4.83 (P<.01)	35.49 > 26.60 (P<.01)
		7	8.70 > 6.26 (P<.01)	5.75>4.78 (P<.01)	40.18 > 27.93 (P<.01)
Day Effect	Treatment				
	Control		8.70 > 7.60 > 5.65 (P < .01)	5.75 > 5.61 (P < .01)	40.18 > 35.49 > 29.64 > 25.15 (P < .01)
	10% Cul.		6.24 > 5.5 (P < .05)	5.37 > 4.84 (P < .01)	27.93 > 25.27 26.60 > 23.80 (P < .01)
	10% Cul. + Asc. Acid		6.26 > 5.54 (P < .05)	5.37 > 4.84 (P < .01)	27.65 > 25.97 > 23.91 (P < .05) 27.65 > 23.91 } (P < .01) 26.25 > 23.91 }

TABLE X

ANALYSIS OF VARIANCE FOR LOG CVT COUNT AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF

Source	df	SS	MS	F
Total	59	141.44	-	-
Trial	4	51.74	12.93	14.69**
Treatment	2	43.10	21.55	24.49**
Error A	8	7.04	0.88	-
Trial x treatment	8	7.04	0.88	-
Day	3	18.72	6.24	31.20**
Day x treatment	6	13.68	2.28	11.40**
Error B	36	7.16	0.20	-
Trial x Day	12	2.99	0.25	-
Trial x treatment x Day	24	4.17	0.17	-

**$P < 0.01$

TABLE XI

ANALYSIS OF VARIANCE FOR pH AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF

Source	df	SS	MS	F
Total	59	10.017	-	-
Trial	4	0.118	0.030	2.308
Treatment	2	7.072	3.535	271.923**
Error A	8	0.107	0.013	-
Trial x treatment	8	0.107	0.013	-
Day	3	1.473	0.491	61.375**
Day x treatment	6	0.961	0.160	20.00**
Error B	36	0.286	0.008	-
Trial x Day	12	0.125	0.010	-
Trial x treatment x Day	24	0.161	0.007	-

**$P < 0.01$

TABLE XII

ANALYSIS OF VARIANCE FOR VOLATILE NITROGEN AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF

Source	df	SS	MS	F
Total	119	3558.90	-	-
Trial	4	680.34	170.08	73.95**
Treatment	2	1194.12	597.06	259.59**
Error A	8	18.42	2.30	-
Trial x treatment	8	18.42	2.30	-
Day	3	967.56	322.52	98.63**
Day x treatment	6	499.19	83.20	25.44**
Error B	36	117.73	3.27	-
Trial x Day	12	59.97	5.00	-
Trial x treatment x day	24	57.75	2.41	-
With in sample	60	81.55	1.36	-
Sample	1	0.09	0.09	-
Trial x sample	4	1.65	0.41	-
Treatment x sample	2	4.06	2.03	-
Day x sample	3	6.41	2.14	-
Trial x treatment x sample	8	12.67	1.58	-
Trial x day x sample	12	18.76	1.56	-
Treatment x day x sample	6	6.21	1.03	-
Trial x treatment x day x sample	24	31.70	1.32	-

**$P < 0.01$

Samples obtained in different trials were significantly different ($P < .01$) from each other. Considerably small mean squares for within sample (Table XII) suggested precision in the experimental procedure (less variation between the duplicate samples).

Flavor

The analysis of variance (Table XIV) revealed a non-significant treatment effect for flavor scores. However, the "F" value of 2.69 for treatment effect lead to the conclusion that, although a difference was present, the number of samples and/or the techniques were not precise enough to detect. Considerably large within sample mean square value (2.74) as shown in Table XIV also indicated a large variation in the panel score for any given sample due to the varied prference of the panel members for the cultured meat samples. Graphical illustration in Figure 26 and mean values in Table XIII clearly showed panel preference for cultured meat samples over the control meat sample throughout the storage time. Among the two cultured samples, the one with ascorbic acid was scored higher at any given storage time.

The F-test (Table XIV) suggested a highly significant day effect ($P < .01$). Further analysis by LSD test indicated (Table XIII LSD) significant decreases in flavor scores ($P < .01$ and $P < .05$) in both the treated and untreated samples due to the storage day effect. The decline in the flavor score during storage was greatest in the control sample and was least in the cultured sample with ascorbic acid. The reason for a non-significant increase in the flavor scores of cultured samples after 5 days storage was not clearly known, but can be postulated due to the effect of

comparing a very low quality product (control sample) with low quality products (cultured samples).

The effect of trials and day x treatment interaction were not found to be significant. These results explained non-significant changes in the general behavior of stored meat due to the addition of cultures, except a comparatively slow decline in the flavor quality of cultured meat. The results also lead to the conclusion that the significant differences in CVT count and VNC due to the trial effect were not sufficient to affect the flavor of the meat samples.

Aroma

Treatment influenced the aroma score ($P < .1$) and the cultured samples were generally rated higher than the control sample except on the initial day (Tables XIII, XV and Figure 27). Cultured sample with ascorbic acid was preferred over the sample with culture alone. Within sample variation as indicated by the mean square in Table XIV (2.30) was considerably larger and indicated a selective preference of the panel members to a specific treatment.

Analysis of variance (Table XIV) showed a significant day effect ($P < .01$). Scores were significantly decreased ($P < .01$) in the control and treated samples after 3 days and subsequent storage time (Table XIII LDS). As the storage time increased, a decline in the score was most in the control sample and least in the cul- cultured sample with ascorbic acid.

Trial and day x treatment interaction was not significant by F-test (Table XIV). However, the non-significant trial effect suggested that the differences in trials as shown by CVT counts and VNC were not sufficient to be picked up by the

aroma criterion.

Color

Panel scores for the color criterion were affected due to treatment ($P < .1$). However, the significance of the treatment effect was not high (Table XVI). This was due to the considerable variation in the panel scores for any sample at any day. Panel score variation was indicated by within sample mean square (2.16) in Table XVI. Graphical illustration of the scores in Figure 28 showed the higher rating for color in the cultured meat sample with ascorbic acid than the control and the sample with culture alone. The control sample was rated higher than the sample with culture alone except for the initial day. A drop in the panel scores for color was greatest for the sample with culture alone and least for the cultured sample with ascorbic acid (Figure 28). These data indicated the influence of ascorbic acid in maintaining the color in the cultured meat sample.

The analysis of variance indicated a highly significant influence ($P < .01$) of storage time (Table XVI), which was substantiated by LSD test (Table XIII).

The color scores for the control and the sample with culture alone significantly decreased ($P < .01$) on 3 days of storage. However, no significant drop in color score in the cultured meat with ascorbic acid was observed until 5 days of storage. Color score for the control sample was improved after 5 days of storage. These findings are in agreement with those of Kontou et al. 1966 and Jay et al. 1964 who reported a rise in the ground beef color scores after 3-4 days of storage at refrigerated temperatures.

Day x treatment interaction was significant ($P < .05$) which might be mainly due

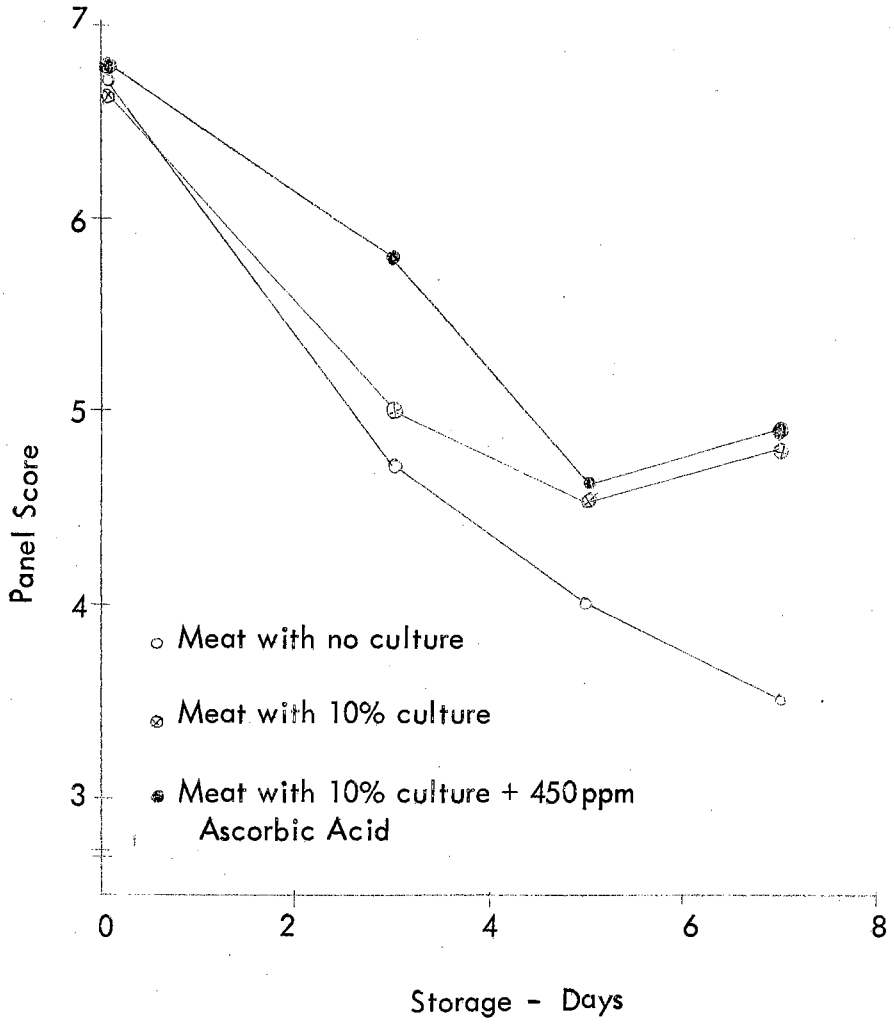

Figure 26. Flavor as Influenced by Lactic Cultures in Ground Beef (Trials VII-XI)

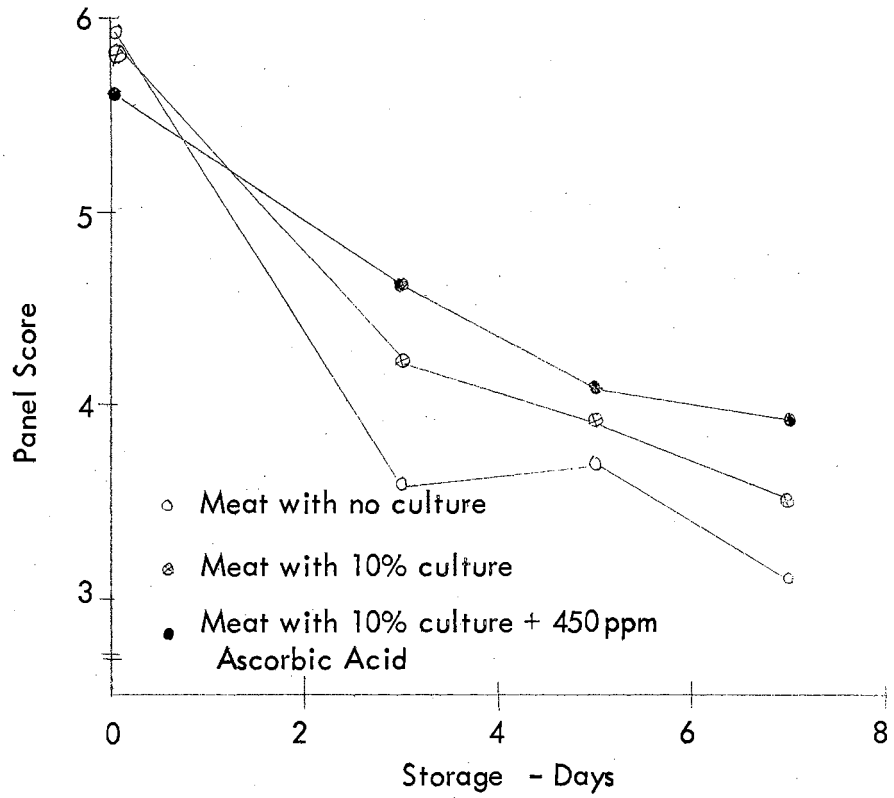

Figure 27. Aroma as Influenced by Lactic Cultures in Ground Beef (Trial VII-XI)

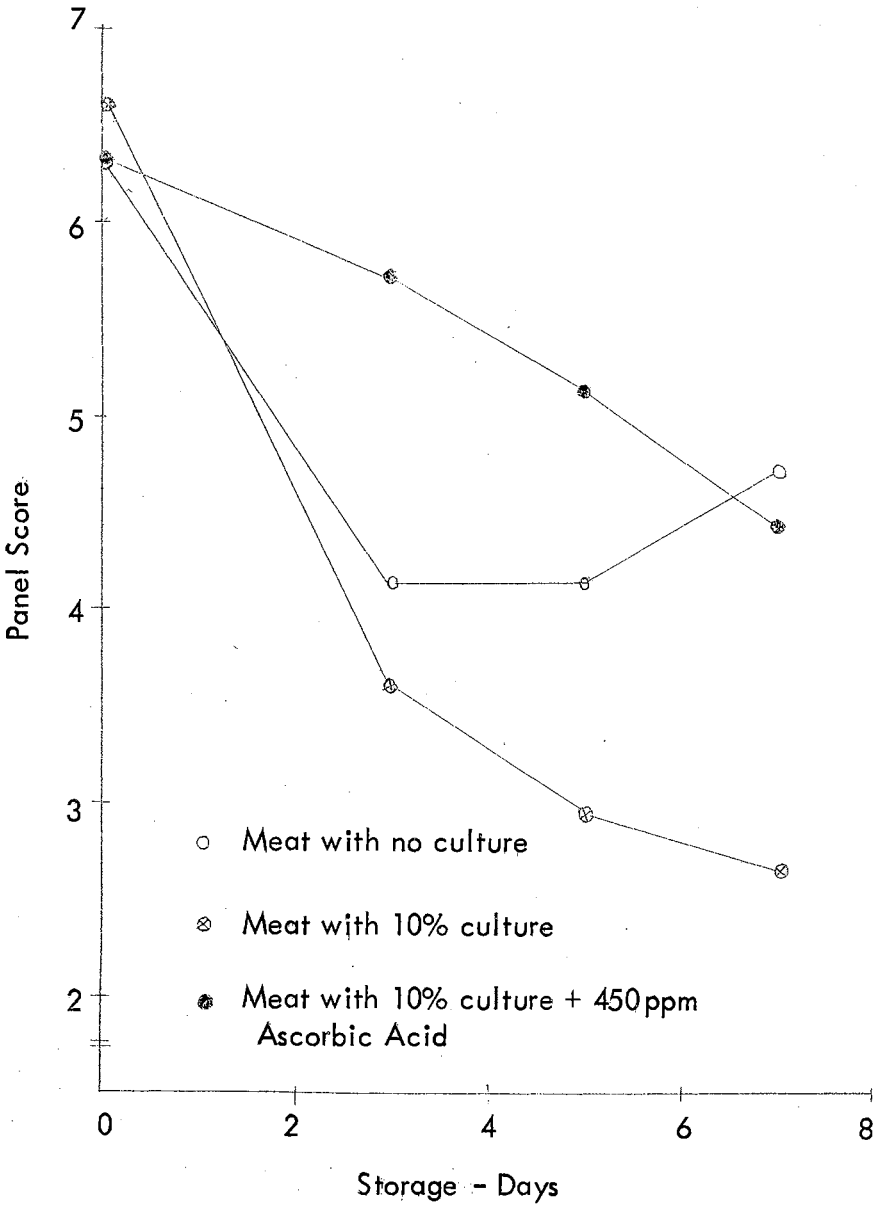

Figure 28. Color as Influenced by Lactic Cultures in Ground Beef (Trials VII-XI)

TABLE XIII

FLAVOR, AROMA, AND COLOR AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF (TRIALS VII-XI)

Treatment / Storage (Day)	Flavor Score* 0	3	5	7	Aroma Score* 0	3	5	7	Color Score* 0	3	5	7
Meat with no culture (control)	6.77	4.77	4.03	3.50	5.97	3.63	3.70	3.13	6.30	4.17	4.17	4.73
Meat with 10% culture	6.67	5.00	4.53	4.83	5.83	4.20	3.93	3.50	6.63	3.63	2.97	2.67
Meat with 10% culture + Asc. acid 450ppm	6.80	5.80	4.63	4.90	5.60	4.63	4.10	3.93	6.37	5.77	5.10	4.43

*Mean values for 5 trials

Least Significant Differences (LSD)

		Day	Flavor Score	Aroma Score	Color Score
Treatment Effect		0 3 5 7	Not Tested (Treatment effect was not significant by AVO)	Not Tested (Treatment effect was not highly significant by AVO)	Not Tested (Treatment effect was not highly significant by AVO)
Day Effect	Treatment		6.77>4.77>3.50 (P<.01)	5.97>3.70>3.13 (P<.01) 3.63>3.13 (P<.01)	6.30>4.73 (P<.05) 6.30>4.17 (P<.01)
	Control				
	10% Cul.		6.67>5.00 (P<.01)	5.83>4.20>3.13 (P<.01) 3.63>3.13 (P<.05) 3.93>3.50 (P<.01)	6.63>3.63 (P<.01)
	10% Cul. + Asc. Acid		6.80>5.80 (P<.05) 6.80>4.90 (P<.01) 5.80>4.63 (P<.05)	5.60>4.63>4.10 (P<.01) 4.63>3.93 (P<.01)	6.37>5.10 (P<.05) 6.37>4.43 (P<.01) 5.77>4.43 (P<.05)

TABLE XIV

ANALYSIS OF VARIANCE FOR FLAVOR SCORE AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF

Source	df	SS	MS	F
Total	359	1406.53	-	-
Trial	4	26.41	6.60	0.98
Treatment	2	36.21	18.10	2.69
Error A	8	53.88	6.73	-
Trial x treatment	8	53.88	6.73	-
Day	3	328.23	109.41	34.41**
Day x treatment	6	25.33	4.22	1.33
Error B	36	114.32	3.18	-
Trial x day	12	60.73	5.06	-
Trial x treatment x day	24	53.59	2.23	-
Within sample	300	822.18	2.74	-
Sample	5	136.91	27.38	-
Trial x sample	20	75.96	3.8	-
Treatment x sample	10	57.16	5.72	-
Day x sample	15	83.39	5.56	-
Trial x treatment x sample	40	58.76	1.47	-
Trial x day x sample	60	174.91	2.92	-
Treatment x day x sample	30	44.91	1.50	-
Trial x treatment x day x sample	120	190.18	1.58	-

**$P < .01$

TABLE XV

ANALYSIS OF VARIANCE FOR AROMA SCORE AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF

Source	df	SS	MS	F
Total	359	1095.60	-	-
Trial	4	10.14	2.53	1.40
Treatment	2	12.67	6.34	3.50(*)
Error A	8	14.49	1.81	-
Trial x treatment	8	14.49	1.81	-
Day	3	271.63	90.54	40.97**
Day x treatment	6	16.53	2.75	1.24
Error B	36	79.64	2.21	-
Trial x day	12	54.22	4.52	-
Trial x treatment x day	24	25.42	1.06	-
Within sample	300	690.49	2.30	-
Sample	5	256.21	51.24	-
Trial x sample	20	41.16	2.06	-
Treatment x sample	10	38.16	3.82	-
Day x sample	15	57.75	3.85	-
Trial x treatment x sample	40	35.01	0.88	-
Trial x day x sample	60	100.48	1.67	-
Treatment x day x sample	30	34.24	1.14	-
Trial x treatment x day x sample	120	127.48	1.06	-

**$P < .01$
(*)$P < .1$

TABLE XVI

ANALYSIS OF VARIANCE FOR COLOR SCORE AS INFLUENCED BY LACTIC CULTURES IN GROUND BEEF

Source	df	SS	MS	F
Total	359	1572.49	-	-
Trial	4	37.13	9.28	0.62
Treatment	2	126.41	63.20	4.25(*)
Error A	8	118.96	14.87	-
Trial x treatment	8	118.96	14.87	-
Day	3	358.76	119.59	22.52**
Day x treatment	6	92.86	15.48	2.92*
Error B	36	191.05	5.31	-
Trial x day	12	96.49	8.04	-
Trial x treatment x day	24	94.56	3.94	-
Within sample	300	647.33	2.16	-
Sample	5	179.69	35.94	-
Trial x sample	20	68.34	3.42	-
Treatment x sample	10	45.49	4.55	-
Day x sample	15	38.04	2.54	-
Trial x treatment x sample	40	54.48	1.36	-
Trial x day x sample	60	130.04	2.17	-
Treatment x day x sample	30	32.04	1.07	-
Trial x treatment x day x sample	120	99.21	0.83	-

*$P < .05$
**$P < .01$
(*)$P < .1$

to the increased color scores in the control sample after 5 days of storage.

Analysis of variance did not indicate significant trial effect. This again assured that the differences in CVT counts and VNC found due to the trial were not sufficient to be picked up by this criterion.

CHAPTER V

SUMMARY AND CONCLUSION

Six preliminary (I to VI) and 5 principal trials (VII to XI) were conducted to determine the effect of adding lactic cultures to ground beef stored at 7°C. The results obtained in the preliminary trials were used to formulate the prinicpal experiments.

To facilitate the preliminary study, different concentrations, combinations and culture forms containing either Streptococcus lactis, Leuconostoc citrovorum, or both along with different substances were tried. The effects of 5 and 10% lactic culture grown in skim milk; 2.5, 5, 10 and 20% lactic culture grown in reconstituted milk; and 1 and 2% frozen concentrated culture were studied. Other substances used in the preliminary study were different concentrations of skim milk, reconstituted milk, lactic acid, ascorbic acid and lactose. The effects of these substances were tested either alone or with combinations of some of the above cultures.

Regular fresh ground beef obtained from the Oklahoma State University meat laboratory was used as a test material in the preliminary trials. However, the principal study was conducted using fresh ground beef obtained from the meat laboratory and

one of the local supermarkets. The effects of adding 10% lactic culture grown in the reconstituted milk and like amount of culture with 450 ppm of ascorbic acid were determined in the principal study. Log CVT counts, pH, volatile nitrogen content and organoleptic evaluation for flavor, aroma and color were used to test the effect of the treatments. In the preliminary trials either all or some of these criteria were studied; however, the principal study included all criteria.

Data obtained in the preliminary study indicated a profound inhibitory action of lactic cultures on the growth of the inherent gram-negative bacteria in the ground beef. The addition of 10% culture grown in the reconstituted milk was completely effective in preventing growth of these organisms.

Lactic culture (S. lactis plus L. citrovorum) and lactic acid plus L. citrovorum exhibited greater inhibitory effect than the pure culture of S. lactis and lactic acid alone, respectively. This indicated that the inhibitory effect was due to both S. lactis and L. citrovorum organisms in the culture. However, the inhibition by S. lactis was considerably greater than by L. citrovorum. The inhibition observed with S. lactis appeared to be due not only to the pH reduction by the lactic acid produced but also to some other factor. Addition of lactic acid affected the sample color and aroma to a great extent. Addition of a frozen concentrated cultures did not exert any inhibition on the CVT counts; however, when 1% lactose was added to these cultures some inhibition was observed. Increase in pH readings suggested no growth of the frozen cultures when lactose was not added. This was concluded to be the reason for no inhibition by frozen cultures when added without lactose. The addition of 5 and 10% milk and 450 ppm of ascorbic acid did not show any considerable effect on CVT counts.

The pH of the cultured meat dropped below 5.0 during 2-3 days of storage except in sample with frozen concentrated cultures without lactose where a general increase was noted. The control and other treated samples without cultures indicated a general increase in the pH during the 7 days of storage. However, no general pattern was observed.

Volatile nitrogen content constantly increased during the storage period and was considerably greater in the control sample at any given storage period than in the cultured sample, except when the frozen concentrated culture without lactose was used. These results indicated that the added culture was responsible for lowering the rate of deamination by inhibiting the growth of inherent gram-negative type of bacteria in the ground beef.

Information obtained in the preliminary trials was insufficient to clearly explain the influence of cultures on the organoleptic criteria of the ground beef, however, a general trend indicated slight preference for the aroma of the cultured samples. Color scores for culture samples in the preliminary study were considerably lower and the results were consistent.

The results obtained in the preliminary study were generally confirmed in the principal study. A significant difference ($P < .01$) was noted in the log CVT counts between the cultured and uncultured samples. Counts were remarkably lower in the cultured samples indicating a profound inhibitory action of lactic cultures on the growth of inherent gram-negative bacteria in the ground beef. A significant increase ($P < .01$) in log CVT counts was observed due to day effect in the uncultured sample. However, significant increases ($P < .05$) in the counts were not noticed in the cultured samples unitl 7 days storage time.

The pH of the cultured meat was significantly lower ($P<.01$) than in the uncultured meat. A significant decline in pH ($P<.01$) of the cultured samples occurred during the first 3 days of storage, whereas an increase ($P<.01$) in pH of the uncultured sample was noted after the 5 days of storage.

A significant increase ($P<.01$) in volatile nitrogen content was observed in the uncultured samples when compared with the cultured samples. Storage time significantly ($P<.01$ and $P<.05$) influenced the volatile nitrogen content in both cultured and uncultured samples which increased as the storage time progressed.

Differences in the organoleptic criteria due to treatment were either significant at a low level ($P<.1$) or approaching significance.

Cultured meat samples with 450ppm ascorbic acid were consistently preferred over the samples without culture and the samples with culture alone for flavor, aroma and color. However, the samples with culture alone were preferred over uncultured samples for flavor and aroma. No specific preference was observed for any sample on the initial day.

A significant difference ($P<.01$) in log CVT counts and volatile nitrogen contents due to trial was not enough to be picked up by the organoleptic criteria used in this study.

With the information obtained in this study it was concluded that the addition If lactic culture along with ascorbic acid would help in retaining the quality of ground beef during storage at refrigeration temperatures. Further studies on the organoleptic criteria of the cultured meat are suggested.

LITERATURE CITED

American Public Health Association Inc. 1967. Standard Methods for the Examination of Dairy Products. 12th Edition. Chapter 4. A.P.H.A., Inc. New York City.

Anderson, A. W. and Elliker, P. R. 1953. "The Nutritional Requirements of Lactic Streptococci Isolated from Starter Culture." J. Dairy Sci. 36:161.

Association of Official Agricultural Chemists. 1965. Official Methods of Analysis. 10th Ed. Assoc. Official Agric. Chemists. Washington, D. C. pp. 346.

Ayres, J. C. 1960. "Temperature Relationships and Some Other Characteristics of the Microbial Flora Developing on Refrigerated Beef." Food Res. 25:1.

Ayres, J. C. 1960a. "The Relationship of Organisms of Genus Pseudomonas to the Spoilage of Meat, Poultry and Eggs." J. Appl. Bact. 23:471.

Baribo, L. E. and Foster, E. M. 1951. "Some Characteristics of the Growth Inhibitor Produced by a Lactic Streptococcus." J. Dairy Sci. 34:1128.

Bodwell, C. E., Pearson, A. M. and Sponner, M. E. 1965. "Post-Mortem Changes in Muscle. I. Chemical Changes in Beef." J. Food Sci. 30:766.

Bowlby, C., Tucker, H., Miller, B. S. and Johnson, J. A. 1953. "A Comparison of Methods for Determining Proteolytic Acitivity." Cereal Chem. 30:480.

Bradley, H. C. and Bailey, B. E. 1940. "Estimation of Decomposition of Fish Muscle." Food Res. 5:487.

Broumand, H., Ball, C. O. and Stier, E. F. 1958. "Factors Affecting Quality of Prepackaged Meat." Food Tech. 12:159.

Brown, A. D. and Weidemann. J. F. 1958. "The Taxonomy of the Psychrophilic Meat-Spoilage Bacteria. A Reassessment." J. Appl. Bact. 21:11.

Burks, R. E., Baker, E. B., Clark, P., Esslinger, J. and Lacey, J. C. 1959. "Detection of Amines Produced on Irradiation of Beef." J. Agric. Food Chem. 1:778.

Collins, E. B. 1961. "Domination Among Strains of Lactic Streptococci with Attention to Antibiotic Production." Appl. Microbiol. 9:200.

Difco Manual. 1966. Difco Laboratories Incorporated. Detroit, Michigan. pp. 194 and 294.

LITERATURE CITED (Continued)

Folin, R. and Bell, R. D. 1917. "Applications of a New Reagent for the Separation of Ammonia." J. Biol. Chem. 29:329.

Friedman, T. E. 1939. "The Carbohydrate Metabolism of Streptococci." J. Biol. Chem. 130:757.

Gardner, G. A. 1965. "The Aerobic Flora of Stored Meat with Particular Reference to the Use of Selective Media." J. Appl. Bact. 28:252.

Gardner, G. A. and Stewart, D. J. 1966. "Changes in the Free Amino and Other Nitrogen Compounds in Stored Beef Muscle." J. Sci. Food Agric. 17:491.

Halleck, F. E., Ball, C. O. and Stier, E. F. 1958. "Factors Affecting Quality of Prepackaged Meat." Food Technol. 12:198.

Hammer, B. W. and Babel, F. J. 1957. Diary Bacteriology. 4th Edition. Chapter 16, p. 373. John Wiley & Sons, Inc. New York

Hammer, B. W. and Babel, F. J. 1957a. Dairy Bacteriology. 4th Edition. Chapter 16, p. 387. John Wiley & Sons, Inc. New York.

Hills, G. and Thiel, C. C. 1946. "The Ferric Thiocyanate Method of Estimating Peroxide in the Fat of Butter, milk and Dried Milk." J. Diary Res. 14:340.

Hunter, J. J. 1959. "Consumer Preference for a 6 - to - 1 Apple Juice Concentrate." Marketing Research Report. 343, USDA.

Jay, J. M. 1964. "Beef Microbial Quality Determined by Extract Release Volume (ERV)." Food Tech. 18:133.

Jay, J. M. 1964a. "Release of Aqueous Extracts by Beef Homogenates, and Factors Affecting Release Volume." Food Tech. 18:129.

Jay, J. M. and Kontou, K. S. 1964. "Evaluation of the Extract-Release Volume Phenomenon as a Rapid Test for Detecting Spoilage in Beef." Appl. Microb. 12:378.

Keenan, T. W. and Bills, D. D. 1968. "Metabolism of Volatile Compounds by Lactic Strater Culture Microorganisms. A Review." J. Dairy Sci. 51:1561.

Keskinel, A., Ayres, J. C. and Snyder, H. E. 1964. "Determination of Oxidative Changes in Raw Meats by the 2-Thiobarbituric Acid Method." Food Tech. 18:101.

LITERATURE CITED (Continued)

Kirsch, R. H., Berry, F. E., Boldivin, C. L. and Foster, E. M. 1952. "The Bacteriology of Refrigerated Ground Beef." Food Res. 17:495.

Kizer, D. E. and Speck, M. L. 1955. "Observation on the Acetate and Citrate Metabolism of Streptococcus lactis and Streptococcus cremoris." J. Dairy Sci. 38:96.

Kontou, K. S., Huyck, M. C. and Jay, J. M. 1966. "Relationship Between Sensory Test Scores, Bacterial Numbers, and ERV on Paired Raw and - Cooked Ground Beef from Freshness to Spoilage." Food Tech. 20:128.

Kramer, A. and Twigg, B. A. 1962. Fundamentals of Quality Control for the Food Industry. AVI Publishing Co., Westport, Conn. p. 105.

Lewis, K. H. and Angelotti, R. 1964. "Examination of Foods for Enteropathogenic and Indicator Bacteria." Publich Health Serv. Pub. No. 1142. p. 1.

Mahlenbacher, V. C. 1960. The Analysis of Fats and Oils. Garrard Press, Illinois.

Marth, E. H. 1962. "Certain Aspects of Starter Culture Metabolism." J. Dairy Sci. 45:1271.

Marth, E. H. and Hussong, R. V. 1962. "Effect of Skim Milk Cultured with Different Strains of Leuconostoc citrovorum on Growth of some Micro-Organisms Associated with Cottage Cheese Spoilage." J. Dairy Sci. 45:652.

Mather, D. W. and Babel, F. J. 1959. "Inhibition of Certain Types of Bacterial Spoilage in Creamed Cottage Cheese by the Use of Creaming Mixture Prepared with Streptococcus citrovorum." J. Dairy Sci. 42:1817.

Mizuno, W. G. and Jezeski, J. J. 1959. "Studies on Starter Metabolism." J. Dairy Sci. 42:251.

Mossell, D. A. A. and Ingram M. 1955. "The Physiology of the Microbial Spoilage of Foods." J. Appl. Bacteriol. 18:232.

Niven, C. F., Jr. 1944. "Nutrition of Streptococcus lactis." J. Bacteriol. 47:343.

Olson, H. C. 1967. "The CVT Test for Controlling the Sanitary Quality of Dairy Products." Proc. Assoc. Southern Agr. Workers. Inc. 1:180.

Pearson, D. 1968. "Application of Chemical Methods for the Assessment of Beef Quality." I. J. Sci. Food Agric. 19:364.

LITERATURE CITED (Continued)

Pearson, D. 1968a. "Application of Chemical Methods for the Assessment of Beef Quality." II. J. Sci. Food Agric. 19:366.

Pearson, D. 1968b. "Application of Chemical Methods for the Assessment of Beef Quality." III. J. Sci. Food Agric. 19:553.

Pearson, D. 1968c. "Assessment of Meat Freshness in Quality Control Employing Chemical Techniques. A Review." J. Sci. Food Agric. 19:357.

Pearson, D. 1968d. "Application of Chemical Methods for the Assessment of Beef Quality." IV. J. Sci. Food Agric. 19:556.

Peryam., D. R. and Pilgram, F. J. 1957. "Hedonic Scale Method of Measuring Food Preferences." Food Tech. 11. insert 9.

Peryam, D. R. 1958. "Sensory Difference Test." Food Tech. 12:231.

Prouty, C. C. 1961. "Amino Acid Requirements of Leuconostoc citrovorum and Leuconostoc dextranicum Isolated from Dairy Starters and Cultured Milk Products." J. Dairy Sci. 44:2100.

Saffle, R. L., May, K. N., Hamid, H. A. and Irby, J. D. 1961. "Comparing Three Rapid Methods of Detecting Spoilage in Meat." Food Tech. 15 :465.

Saffle, R. L. and Galbreath, J. W. 1964. "Quantitative Determination of Salt Soluble Protein in Various Types of Meat." Food Tech. 18 9:119.

Sandine, W. E., Seitz, E. W. and Elliker, P. R. 1961. "Studies on the Mechanisms of Diacetyl Synthesis by Streptococcum diacetilactis." J. Dairy Sci. 44:1158.

Speck, M. L. 1962. "Starter Culture Growth and Action in Milk." J. Dairy Sci. 45:1281.

Speckman, R. A. and E. B. Collins. 1968. "Diacetyl Biosynthesis in Streptococcus diacetilactis and leuconostoc citrovorum." J. Bact. 96:174.

Stringer, W. C., Bilskie, M. E. and Naumann, H. D. 1969. "Microbial Profiles of Fresh Beef." Food Tech. 23 1:97.

Sulzbacher, W. L. 1953. "Report of Subcommittee on Microbiological Methods of Meat Research." Proc. 5th Recip. Meat Conf. p. 170.

LITERATURE CITED (Continued)

Terrell, R. N. 1967. "Bacterial Contamination. Its Relationship to Product Shelf Life." Report presented at the Annual Meeting of the Alabama Meat Packers Association.

Vakil, J. R. and Shahani, K. M. 1969. "Carbohydrate Metabolism of Streptococcus lactis and Their Sensitivity to Antibiotics." J. Dairy Sci. 52:162.

Vanderzant, W. C. and Nelson, F. E. 1953. Proteolysis by Streptococcus lactis Grown in milk with and without Controlled pH." J. Dairy Sci., 36:1104.

Vanderzant, W. C. and Nelson, F. E. 1953a. "Characteristics of an Endocellular Proteolytic Enzyme System of Streptococcus lactis." J. Dairy Sci. 36:1212.

Vanderzant, W. C., and Nelson, F. E. 1954. "Amino Acids and Peptides in Protein Free Fraction of Milk before and after incubation with Streptococcus lactis." J. Dairy Sci. 37:790.

Vanderzant, W. C. and Nelson, F. E. 1954a. "Characteristics of Some Endocellular Peptidases of Streptococcus lactic." J. Dairy Sci. 37:795.

Whitehead, H. R., Jones, P. A. and Robertson, P. S. 1958. "The Influence of Carbondioxide on the Growth of Lactic Streptococci." J. Dairy Res. 25:24.

Williamson, W. T., Tove, S. B., and Speck, M. L. 1962. "Purification and Properties of Extracellular Protein Produced by Streptococcus Lactis." Bacteriol. Proc. 26:1962.

Zuraw, E. and Morgan, M. R. 1952. "Acetaldehyde Production by Streptococcus lactis and Streptococcus lactis var. maltigenes." J. Dairy Sci. 35:483.

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