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# QUANTITATIVE DETERMINATIONS OF SELECTED BACTERIA IN GROUND BEEF EXTENDED WITH VARYING LEVELS OF TEXTURED VEGETABLE PROTEIN

A Dissertation

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Doctor of Philosophy

Degree

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## ABSTRACT

The objective of this study was to determine the influence of various levels of textured vegetable protein (TVP) on selected groups of bacteria present in ground beef. This influence was assessed by five selected bacteriological assays: aerobic count, psychrophilic-mesophilic count, coliform count, KF (fecal streptococci) count and the counts of Staphylococcus aureus. Samples were prepared by extending ground beef to contain by weight 0, 5, 10, 20 and 40 percent of hydrated TVP. The samples were stored at 6°C for 4 days, at 0°C for 10 days and at -16°C for 90 days.

The bacteriological determinations of the TVP revealed that this material contained very small numbers of bacteria as determined by the selected assays.

Variation among replications during storage at the three selected temperatures was highly significant ($P < 0.01$).

Highly significant ($P < 0.01$) or significant ($P < 0.05$) increases in logarithmic counts were observed with increased time of storage at 6°C and 0°C, but not at -16°C, in all assays except those for Staphylococcus aureus. Highly significant ($P < 0.01$) decreases were observed with time in logarithmic counts of Staphylococcus aureus at all storage temperatures.

With the exception of percent protein, highly significant ($P < 0.01$) changes were observed in proximate analyses with increasing levels of TVP. Percent moisture and the unanalyzed fraction increased while the percent ether extract decreased. The protein analyses varied little.

Highly significant ($P < 0.01$) increases in logarithmic aerobic and psychrophilic-mesophilic counts accompanied increased level of TVP during storage at 6°C and 0°C, but not at -16°C. The increases in the logarithmic counts of these bacteria at higher levels of TVP commenced early and proceeded rapidly during storage. The interaction between levels of TVP and storage time was observed to be significant ($P < 0.05$) or highly significant ($P < 0.01$) at storage temperatures of 6°C and 0°C respectively.

No significant differences in logarithmic coliform counts or logarithmic counts of Staphylococcus aureus attributable to varying levels of TVP were observed during storage at any of the selected temperatures.

Although highly significant ($P < 0.01$) increases in logarithmic KF counts due to varying levels of TVP were observed during storage at 0°C, the magnitudes of change as well as observed numbers were small.

Coagulase-positive staphylococci were observed in all of the initial samples.

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# CHAPTER I

## INTRODUCTION

According to Carlin and Neilson (1974), the increase in production of soybeans in the United States during the past 43 years has been phenomenal. Approximately 5,000,000 bushels were produced in 1924, compared to 1,600,000,000 in 1973.

Described as efficient sources of high quality protein and edible oil, soybeans have been used largely as feed for animals or as an oil seed, but not as a direct human food. Because of their low cost and high nutritive value, soybeans have been suggested as a potential "goldmine" for solving world food problems, especially the shortage of protein. However, many people do not select foods on a nutritive basis only; therefore, the motives of the nutritionist and the factors that affect acceptance of a new food are rarely the same.

Several things have happened recently to change the resistance of Americans to foods containing soy products. Spaeth (1974a) stated that as food prices increased, American homemakers closely examined the growing array of fabricated and "substitute" foods on local grocery shelves, since the usage of these products would reduce food expenditures.

In addition to rising food prices, Spaeth (1974a) indicated that other diverse influences, such as the United States school lunch program, the shift of the Humbolt Current (resulting in fish meal shortage) and the world-wide protein shortage contributed to the growing acceptance of meat analogs and meat extenders made from processed soybeans. These seemingly unrelated factors played important roles.

In an examination of the factors, Carlin and Neilson (1974) observed the sale of ground beef products containing soy additives. These products were called by various names, such as "Patty Mix" or "Juicy Burger" and some contained as much as 25 percent TVP. In addition, large restaurant chains began adding TVP to hamburger and selling it at a lower price than all-meat hamburger and sandwiches. Carlin and Neilson (1974) speculated that the use of TVP as a meat extender would increase, since foods served in restaurants were not subject to the same labeling and identification requirements as were foods sold at retail.

In February of 1971, a regulation was put into effect by the United States Department of Agriculture's Food and Nutrition Service, which permitted the use of as much as 30 percent textured protein products in meat dishes served in school lunch programs to some 25 million children. Spaeth (1974a) quoted Greenberg (publisher of Science and Government Report and author of The Politics of Pure Science) as calling

the USDA enabling document, FNS Notice 219, the "Magna Carta" of textured vegetable protein.

Nearly 23 million pounds of TVP were used during the first year after the passage of the FNS regulation. This amount doubled the next year, and indications are clear that it will continue to increase. Spaeth (1974a) stated that when mixed with ground beef, textured vegetable protein reduced the price of the finished product by 10 to 20 cents per pound.

Another of the important factors that stimulated TVP acceptance and use was the shift of the Humbolt Current. About two years ago, the Humbolt Current, off the coast of Peru in South America, shifted from its normal course. This sudden change in the icy current that carries chilled water from the fringes of Antarctica displaced schools of anchovies, and these fish disappeared from the usual fishing grounds. Therefore, the production of fish meal, the world's largest single livestock feed supplement, was reduced, obviously resulting in higher feed costs and higher priced meat products.

The utilization of soy products as ground meat extenders has become widespread (Judge et al., 1974). Carlin and Neilson (1974) concluded that if the trend continues, and if 8 percent of potential sales could be replaced by soy protein by 1980 as predicted, the importance of obtaining technological information about the use of TVP as meat extenders will become evident.

Tiwari and Maxey (1971) reported that logarithmic bacteria counts observed in samples of retail ground beef were so high that such products were on the threshold of organoleptic spoilage. These workers stated that the common use of ground red meats provides a major potential vector for microorganisms of public health significance.

The present study was initiated because of the increased practice of extending natural meat systems with TVP, coupled with the fact that there is little information in the literature which reported the effect this extension has on the major groups of bacteria present in ground beef. Further impetus was given by the industrial interest in the effect of TVP extension on the shelf-life of ground beef. The objective of this study was to determine the influence of various levels of TVP on selected bacterial groups occurring in raw refrigerated ground beef stored at three selected temperatures. This influence was assessed by five selected bacteriological assays: aerobic count, psychrophilic-mesophilic count, coliform count, KF (fecal streptococci) count and counts of Staphylococcus aureus.

# CHAPTER II

# REVIEW OF LITERATURE

## Microbiological Aspects

According to Jay (1970), meats are the most perishable of all important foods. The indicated reason is that meats contain an abundance of all nutrients required for the growth of bacteria, yeasts and molds. Table 1 (Lawrie, 1966) presents the chemical composition of a typical adult mammalian muscle after rigor mortis, but before degradative changes postmortem.

Jay (1970) listed the following genera of bacteria which have been reported to be present in the flora of fresh and cured meats, fresh poultry and fresh seafoods: Achromobacter, Aeromonas, Aerobacter, Alcaligenes, Bacterium, Bacteroides, Brevibacterium, Clostridium, Corynebacterium, Escherichia, Flavobacterium, Halobacterium, Lactobacillus, Lenconostoc, Micrococcus, Pediococcus, Pseudomonas, Salmonella, Serratia, Sarcina, Staphylococcus, Streptococcus, Spirillum, Photobacterium and Vibrio. However, when spoiled meat products are examined, only a few of these many genera are found. In almost all cases, one or more genera is found to be characteristic in the spoilage of a given type of meat product.

Table 1—Chemical composition of typical adult mammalian muscle after rigor mortis, but before degradative changes postmortem (percent wet weight)[a]

| | | |
|---|---|---|
| Water | | 75.5% |
| Protein: | | 18.0% |
| Myofibrillar | 10.0 | |
| Sarcoplasmic | 6.0 | |
| Mitochondrial | 0.002 | |
| Sarocoplasmic reticulum | 2.0 | |
| Fat: | | 3.0% |
| Soluble nonprotein substances: | | 3.5% |
| Nitrogenous | 1.57 | |
| Carbohydrate | 1.18 | |
| Inorganic | 0.632 | |
| Traces of glycolytic intermediates, metals, vitamins | 0.10 | |

[a]Lawrie (1966).

Unlike the spoilage of whole fresh beef, which is sometimes spoiled by molds, ground beef is spoiled exclusively by bacteria, with the following seven genera reported to be most important: Pseudomonas, Achromobacter, Bacillus, Flavobacterium, Microbacterium, Micrococcus and Aeromonas. Pseudomonas and Achromobacter species are generally agreed to be the primary cause of spoilage (Brown and Weidemann, 1958; Kirsch et al., 1952; Ayres, 1955; Ayres et al., 1950; and Jay, 1967).

Duitschaever et al. (1973) explained that the process of manufacturing ground beef involves grinding of cellular tissue. Bacteria normally present on the surface of meat are distributed by this process throughout the product, creating ideal conditions for multiplication. Further, ground beef is not heated or otherwise processed to ensure the absence of pathogenic and spoilage organisms. Thus, the microbiological quality depends on the materials used, sanitary conditions imposed, practices followed during preparation, storage time and temperature of storage.

Ingram and Dainty (1971) indicated that fresh meat develops off-odors when superficial bacterial numbers exceed 10,000,000 per square centimeter. Other researchers (Kirsch et al., 1952, and Barnes, 1957) suggested that off-odors develop at about 100,000,000 organisms per gram. Figure 1 (Ayres, 1960) illustrates this phenomenon.

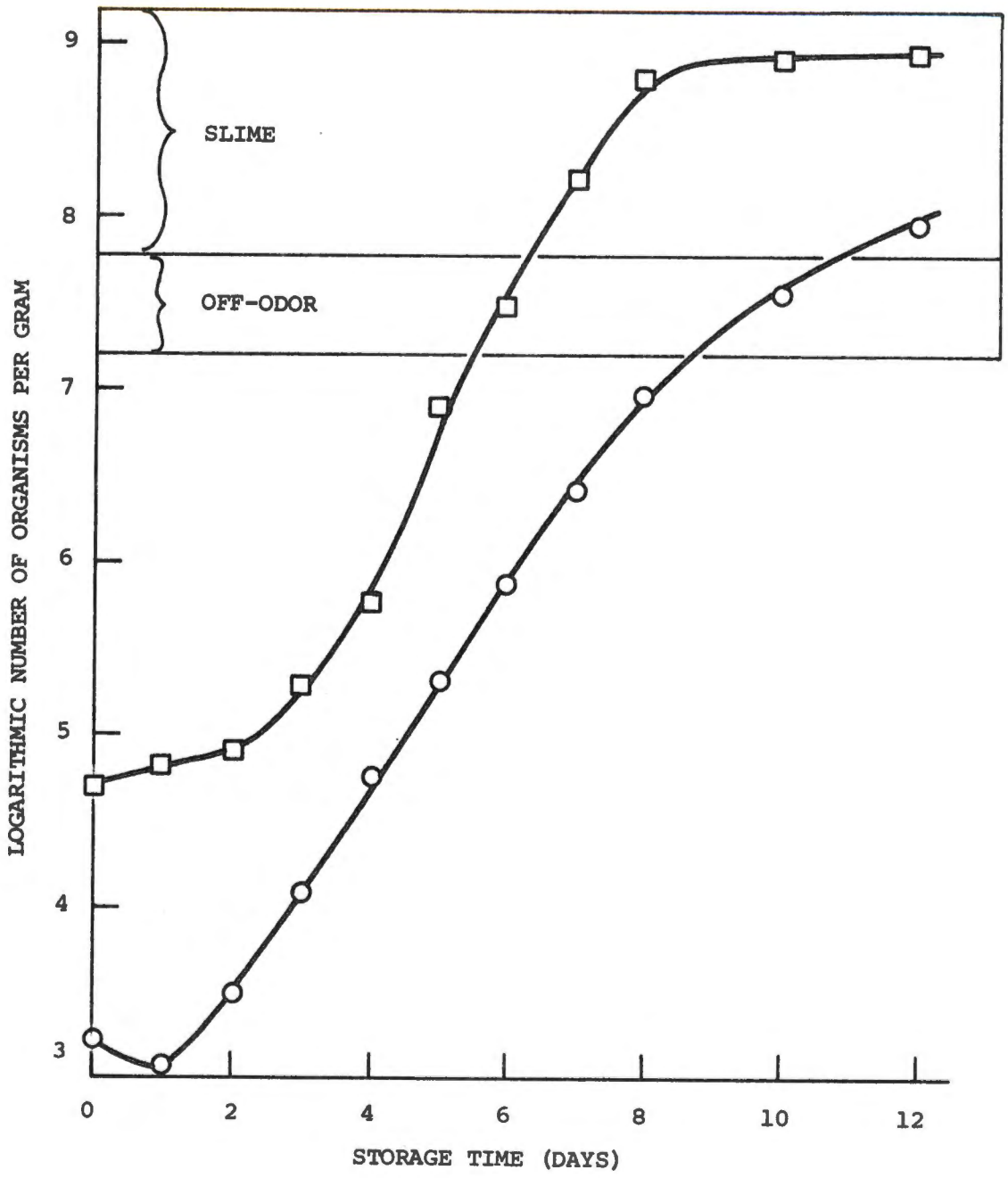


Figure 1. The development of off-odor and slime on dressed chicken (squares) and packaged beef (circles) during storage at 5°C. (Ayres, 1960)

In a survey of ground beef samples from six local super supermarkets, Tiwari and Maxey (1971) observed an average logarithmic count of 7.54 per gram for total aerobic organisms and 5.11 for coliform organisms. After storage for six days at 5°C, the average logarithmic count per gram was 9.6 for total aerobic organisms and 7.9 for coliform organisms. These workers indicated that the samples were organoleptically unacceptable after the six day storage at 5°C. Rey et al. (1970) observed a logarithmic aerobic bacterial count of 6.3 per gram of ground beef at the beginning, and 8.6 at the end of seven days of storage at 5°C. These workers found that the counts of enterococcus organisms did not increase substantially during refrigerated storage, while coliforms did.

Empey and Scott (1939) observed that the numbers of bacteria on contaminated meat surfaces showed a very wide nonnormal variation, while the distribution of the logarithms of the bacterial counts was approximately normal. Following confirmation of these findings in a study applied to food processing plants, Hansen (1962) explained that the bacterial population on a contaminated surface generally shows a nonnormal distribution. The numbers of bacteria in individual samples differ greatly, and as a result, the averages for small groups of samples taken from a common source do not agree well. This suggests that the arithmetic mean is not suitable for the evaluation of surface contamination. Since

the distribution of logarithmic bacterial numbers is approximately normal, it would be better to express surface contamination by the logarithms of the counts rather than the actual counts. Hansen (1962) further suggested that the principal of this method could find wider application.

Pierson et al. (1970) found that the total bacteria count of aerobically packaged beef increased until about the tenth day of storage at 3.3°C but only slightly thereafter, with a final count of about 10,000,000,000 organisms per square centimeter (surface area count) after the 15 days of storage. These researchers determined numbers of enterococcus organisms also and found them to increase slowly or remain constant during anaerobic storage at 3.3°C.

Rey et al. (1971) performed quantitative determinations of various bacteria present in beef steaks stored at 5°C. These steaks, in the form of an entire loin, had been subjected previously to an aging treatment of three days at 22°C. Dramatic increases were observed in total aerobic and coliform counts per gram while levels of enterococci varied little. The average logarithmic count per gram of the total aerobic organisms increased from 5.5 at day zero to about 8.0 during the five-day storage at 5°C, while that of the coliform organisms increased from 4.5 to 6.75. Logarithmic counts of enterococci per gram were approximately 3.5 throughout the entire storage period. A 60 percent incidence

of coagulase-positive staphylococci was observed in samples examined by these researchers.

Duitschaever et al. (1973) assayed 213 samples of various types of raw refrigerated ground beef from 51 different retail stores. Mesophilic and psychrotrophic counts in 64 percent of the samples were in excess of ten million per gram. Psychrotrophic and mesophilic counts were comparable for different types of meat with the exception of bulk hamburger in which psychrotrophic counts were almost twice as high as mesophilic counts. Average coliform counts ranged from 1,400 to 19,000 per gram while counts of enterococcus organisms ranged from less than 10 to 10,000 per gram. Thirty-seven percent of the samples contained coagulase-positive staphylococci ranging from 5 to 100 percent of the total staphylococci count. The presence of these organisms indicated the potential dangers present in such products.

According to Lechowich (American Meat Institute Foundation, 1971), the fate of the microorganisms which have been added to the cut surfaces of meat during the various stages of processing depends upon several important environmental factors such as the ability of the organisms to utilize the high protein-low carbohydrate meat substrate at low temperatures. The highly oxygenated conditions and high moisture content of meat are also important environmental conditions. All of these conditions favor the proliferation

of the members of the microbial genera Pseudomonas and Achromobacter, the most commonly found organisms in fresh meat stored at refrigerated temperatures.

According to Jay (1970), the moisture requirements of microorganisms should be defined in terms of water activity in the environment. This parameter is defined as the ratio of the water vapor pressure of a food substrate to the vapor pressure of pure water at identical temperatures. The minimum water activity values for the growth of Pseudomonas and Achromobacter (the predominant spoilage bacteria in fresh meat) are 0.97 and 0.96 respectively. These values are relatively high when compared to those required by other genera of bacteria. Lechowich (American Meat Institute Foundation, 1971) stated that the water activity of fresh meat is generally 0.99, or above, which is near the optimum for many varieties of bacteria. However, a majority of meat products including fresh, cured, pasteurized, dried and vinegar packed are refrigerated and develop therefore, a psychrophilic bacterial flora. Psychrophiles are described as bacteria that grow at 0°C or lower, but their optimal growth temperature is between 20°C and 30°C. Therefore, growth of Pseudomonas and Achromobacter are favored by the high water activity and low temperature in which meat is stored.

The presence of nonproteolytic strains of bacteria in spoiled beef was interpreted by Jay (1967) to indicate that

nutrients, other than the primary proteins, were utilized by spoilage organisms. Lechowich (American Meat Institute Foundation, 1971) stated that many microorganisms preferentially use carbohydrates as a source of energy for growth. Those organisms that grow aerobically on the surface of meat (such as pseudomonads, molds, yeasts and micrococci) generally oxidize sugars almost completely to carbon dioxide and water.

The physical properties of meat are markedly affected by pH [Lechowich (American Meat Institute Foundation, 1971)]. The pH values of fresh meat are generally between 5.3 and 6.5. Most types of microorganisms can initiate growth in this range, but meat has been shown to spoil bacteriologically much more rapidly at a pH of 6.5 than 5.3.

## Textured Vegetable Protein Aspects

Boyer, Atkinson and Calvert, originally "fellow" employees of the Ford Motor Company, were cited by Spaeth (1974b) as important early industrial soy researchers.

Calvert was described as a "pioneer of soybean protein" by Stone (1974). Stone explained that Calvert began soy research upon instruction from Henry Ford, who had observed that soybeans had been used by Orientals for four thousand years. Calvert, along with colleague Boyer, made plastic car parts from the soybean and even made the parts for the famous Ford "soybean car."

While conducting soybean research (Spaeth, 1974c), Boyer first extracted the oil and then solubilized the remaining protein, producing a viscous substance which could be spun into resilient threads. Using this fibrous texture as a base, analogs that look and taste like pieces of ham, chicken and turkey were produced.

In 1970, Atkinson (Spaeth, 1974c) patented a process for extruding soy protein under high temperature and pressure. This process, which was both relatively inexpensive and uncomplicated, actually realigned molecules and the extruded product had a meat-like texture.

According to Wolf (1972), the approximate composition of soybeans, on a dry basis, is 40 percent protein, 20 percent oil, 30 percent carbohydrate and 10 percent ash and minor constituents. Soybeans are processed in a variety of forms. An increase in protein to 70 percent is achieved by removal of about half of the carbohydrate in "defatted flakes" and flours by extraction with such solvents as aqueous alcohols or dilute acid. Such extraction eliminates the sugars and some minor constituents and produces soy protein concentrates. Altschul (1974) stated that a method used to introduce texture involves thermoplastic extrusion of soy flour or mixtures of soy flour and other materials to form granules which can be mixed in various proportions with meat in hamburger-style dishes.

Bontrae crumbles, produced by General Mills, Minneapolis, Minnesota, are manufactured by an "expanding" technique that has evolved from the conventional "extruded" process. The proximate analyses per 100 grams edible portion on a hydrated basis are as follows: 19.6 grams protein, 3.2 grams fat, 65.0 grams water, 2.6 grams ash and 9.6 grams carbohydrate (General Mills, 1973).

According to Wolf (1972) the majority of soy proteins are classified as globulins. Such proteins are insoluble in water, but dissolve in water or dilute salt solutions at pH values above or below their isoelectric point. The high water extractibility of the proteins from defatted meal indicates that the phospholipid membrane surrounding the protein bodies is easily broken. When defatted meal is dispersed in distilled water at a pH of 6.5, nearly maximum protein solubility occurs. Wolf indicated that soy protein solubility was reduced rapidly as the pH values fall below 6.5.

## Development and Use of Soy Extended Ground Beef

Textured soy proteins (Spaeth, 1974b) are prepared and used for two general purposes: as a partial or complete substitute in processed items, or as a meat analog that resembles specific meats. Spaeth (1974c) indicated that textured soy protein can be combined with many meats, poultry

or fish, but its present use is predominantly in ground beef. Wolford of General Mills, Minneapolis, Minnesota, was quoted by Spaeth (1974c) as saying that the annual beef consumption in the United States is 116 pounds per capita and of this total, consumption of ground beef makes up just over 22 percent, or approximately 26 pounds per person. Spaeth (1974b) stated that during the spring of 1973, when meat prices increased dramatically, General Mills tested what was initially called "Burger II" (meat containing 25 percent of soy product). This product, which was priced 20 cents below the all-meat product, was marketed in the Minneapolis-St. Paul area. The name of the product was changed to "Juicy Blend II" six months after introduction, in response to action by the Minnesota State Department of Agriculture. The soy extended product, however, outsold regular ground beef by a substantial margin, and ran at 17 to 18 percent of the total meat sales.

Read and Baer (1974) indicated that since microbial content in most foods is controllable through good manufacturing practices, it is a criterion of quality. Judge et al. (1974) reported one of the few studies involving the bacteriology of soy extended ground beef and indicated that a low bacterial count is a criterion of quality in ground beef.

The research reported by Judge et al. (1974) evaluated the effects of two soy products on the various indicators of

quality in ground beef patties, including bacterial count. The two commercially prepared soy products used in this study were soy flour, with a protein content of 40 to 60 percent, and soy protein concentrate containing not less than 70 percent protein. These ingredients were added to ground beef samples which contained fat contents of 20 and 30 percent. The protein content of the two types of meat was roughly standardized by adding a relatively high level of the soy additives to the beef containing 30 percent fat and a relatively low level to that containing 20 percent fat. Bacterial numbers were estimated by plating rinses of one gram portions of each ground beef patty. The one gram portion was placed directly into a dilution blank and shaken prior to plating. The patties were sampled immediately after preparation and at the end of seven days of storage at 4°C. The observed effect of storage time was highly significant ($P < 0.01$). The interaction between storage time and additives also was highly significant ($P < 0.01$) in samples containing soy flour. According to these workers, this resulted from higher initial counts in the meat samples containing soy flour, as compared to those containing soy protein concentrate. After storage for seven days at 4°C, there were no significant differences in bacterial counts among the samples.

Busta and Schroder (1971) found that natural meat systems were highly stimulatory to growth of Clostridium

perfringens. However, the addition of protein additives did not affect the potential for fast growth of this organism in meat.

## Present Status of Microbiological Implications of Ground Beef Extended with TVP

The literature reviewed revealed that ground beef is highly susceptible to microbial spoilage. While spoilage of ground beef is mainly due to seven genera of bacteria, at least 25 have been reported to be present in fresh meats, including several which are pathogenic. Bacteria normally present on the surface of meat are distributed throughout the product by grinding, which creates ideal conditions for multiplication. Therefore, since ground beef is not subjected to any kind of sterilization or pasteurization treatment, microbiological quality depends upon the materials used, sanitary conditions imposed, practices followed during preparation, storage time and temperature of storage.

The development of off-odors and slime in meat occurs at a logarithmic bacterial count per gram of between seven and nine. Logarithmic bacterial counts, which were observed in retail ground beef samples, approached these actual numbers and therefore, indicated that such products were on the threshold of organoleptic spoilage.

The predominant use of TVP is in the extension of ground beef, and such products are gaining an ever increasing share

of the "Hamburger" market. The few studies which have been reported indicated that the addition of TVP to ground beef products did not appear to affect shelf-life, and neither did it affect the potential for fast growth of Clostridium perfringens in natural meat systems. However, Judge (1974) reported that the bacterial counts observed in meat samples containing soy flour showed a highly significant ($P < 0.01$) interaction between storage time and additives.

# CHAPTER III

# MATERIALS AND METHODS

## Manufacture and Sample Preparation

Samples were prepared by extending raw ground beef to contain by weight 0, 5, 10, 20 and 40 percent of hydrated TVP. The hydrated TVP consisted of water, previously autoclaved at 121°C for 20 minutes, and textured vegetable protein in a two to one ratio. The actual TVP used, called "Bontrae," was manufactured by General Mills.

The ground beef and the extended ground beef materials were prepared by mixing the ingredients in a meat chopper for approximately two minutes. This mixture was then ground through a 1/8 inch plate. Sufficient quantity was prepared to determine the selected bacteriological assays at the three storage temperatures. Bulk samples of about 500 grams of each of the various levels of TVP were packed into aseptic polyethylene bags and stored at 6°C and 0°C. Seven samples of about 250 grams of each of the various levels of TVP also were packed in aseptic polyethylene bags to be stored at -16°C. Five samples were needed; however, extra samples were prepared for unexpected eventualities. The bacteriological assays were determined at varying times during the storage periods as shown in Table 2.

Table 2—Distribution of sample days (storage time) at the various selected temperatures[a]

| Temperature | Storage time—days |
|---|---|
| -16°C | 7 - 15 - 30 - 60 - 90 |
| 0°C | 2 - 4 - 6 - 8 - 10 |
| 6°C | 0 - 1 - 2 - 3 - 4 |

[a]The selected temperatures of storage were -16°C (frozen storage), 0°C (retail showcase storage) and 6°C (home refrigeration).

Three determinations were made from each sample drawn, and the entire experiment was replicated three times.

## Preparation of Food Homogenate

The food homogenate was prepared according to the method described in the manual edited by Olson (1973) with one modification. A three- instead of two-minute blending time was used because of the coarseness of the sample material. To aid uniformity of results, each sample was mixed quickly and aseptically prior to removal from the polyethylene bag for weighing. Frozen samples were allowed to thaw at room temperature for one to two hours prior to weighing.

## Bacteriological Assays

The aerobic count assay was determined according to the method described in the manual edited by Olson (1973).

The procedure for the determination of the psychrophilic-mesophilic count assay was identical to that used for the aerobic count assay with the exception that the temperature of incubation was 20 ± 1°C rather than 35 ± 1°C.

The procedure for the determination of the coliform count assay was to prepare the food homogenate as in the aerobic count procedure. Appropriate dilutions (inoculum) were transferred to triplicate petri plates which were then poured with Violet Red Bile agar. This medium and the

inoculum were mixed thoroughly and allowed to solidify. An additional four to five milliliters of medium was distributed over the surface of the agar to inhibit surface and spreader-type growth. As soon as the agar was solidified, the plates were inverted and incubated for 18 to 24 hours at 35°C. The dark red colonies (one to two millimeters in diameter and surrounded with a reddish zone of precipitated bile) were counted and reported as coliform colonies per gram. This procedure was similar to that described by Sharf (1966).

The KF (fecal streptococci) count assay was determined according to the method described in the manual edited by Olson (1973). A special medium (KF Streptococcus agar), developed and studied by Kenner et al. (1960), was used in this research to determine approximate numbers of fecal streptococci. This medium, although restrictive, is not 100 percent selective for members of this group of organisms. Therefore, the bacterial enumerations determined, utilizing this medium, were reported as KF counts per gram since the suspect colonies observed might not have actually represented fecal streptococci.

The assay of the counts of Staphylococcus aureus was determined according to the method described in the manual edited by Olson (1973) with the following modification. Two shiny black colonies (with or without grey edges and exhibiting a clear zone extending into the surrounding agar)

were picked from each plate and cultured on Standard Methods agar slants. Subsequently, these cultures were tested for coagulase reaction.

## Proximate Analyses

The proximate analyses of moisture, protein (Nitrogen times 6.25) and ether extract were conducted according to "Methods of Analysis" (Association of Official Analytical Chemists, 1970). Proximate analyses of ash and carbohydrate were not determined. The fraction, represented by these constituents, was obtained by difference (one hundred minus the sum of the proximate analyses), and was reported as unanalyzed material.

## Statistical Analysis

The influence of various levels of TVP on selected groups of bacteria present in ground beef was assessed by five selected bacteriological assays: aerobic count, psychrophilic-mesophilic count, coliform count, KF (fecal streptococci) count and counts of Staphylococcus aureus.

Table 3 shows the experimental design employed. Fixed effects included five levels of TVP and five storage times, while random effects included three sample determinations and three replications of the entire experiment.

Since the quantitative determinations in this study showed a wide nonnormal variation, and since other

Table 3—Experimental design of each bacteriological assay[a] and selected storage temperature[b]

| Level of TVP | Storage time[c] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 1 (0%) | 3 | 3 | 3 | 3 | 3 |
| 2 (5%) | 3 | 3 | 3 | 3 | 3 |
| 3 (10%) | 3 | 3 | 3 | 3 | 3 |
| 4 (20%) | 3 | 3 | 3 | 3 | 3 |
| 5 (40%) | 3 | 3 | 3 | 3 | 3 |

[a]The five selected bacteriological assays were as follows: aerobic count, psychrophilic-mesophilic count, coliform count, KF (fecal streptococci) count, and counts of Staphylococcus aureus.

[b]The selected temperatures of storage were 6°C, 0°C and -16°C.

[c]The five storage times varied with temperature. At 6°C the samples were taken at 0, 1, 2, 3 and 4 days of storage. At 0°C the samples were taken at 2, 4, 6, 8 and 10 days of storage. At -16°C the samples were taken at 7, 15, 30, 60 and 90 days of storage.

researchers have found that the distribution of the logarithms of bacterial counts was approximately normal, these data were transformed to logarithms prior to statistical analysis. The following mathematical model and analysis of variance table (Table 4) is representative of each assay at each of the storage temperatures.

$$Y_{ijkl} = Y \ldots + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + E_{ijkl}$$

where

$Y$ = overall mean;

$A_i$ = effect of the $i^{th}$ replication; $i = 1, 2, 3$;

$B_j$ = effect of the $j^{th}$ level of TVP; $j = 1, 2, \ldots, 5$;

$C_k$ = effect of the $k^{th}$ storage time; $k = 1, 2, \ldots, 5$;

$E_{ijkl}$ = random error associated with the $ijkl^{th}$ determination; $l = 1, 2, 3$.

Table 4—Sample analysis of variance table

| Source of variance | | Degrees of freedom | F/ratio |
|---|---|---|---|
| Replication | (A) | 2 | A/E |
| Level of TVP | (B) | 4 | B/AB |
| Storage time | (C) | 4 | C/CA |
| Replication × TVP | (AB) | 8 | AB/E |
| Replication × storage time | (AC) | 8 | AC/E |
| TVP × storage time | (BC) | 16 | BC/ABC |
| Replication × TVP × storage time | (ABC) | 32 | ABC/E |
| Random error | (E) | 150 | — |
| Total | | 224 | |

# CHAPTER IV

# RESULTS AND DISCUSSION

## Bacteriological Examination of TVP

A sample of the TVP used in the manufacture of the variously extended ground beef materials was examined by the five selected bacteriological assays just prior to the preparation of each replication. The data shown in Table 5 reveal little growth per gram for any of the assays. Therefore, the population levels of bacteria in the ground beef should have been reduced by dilution with increasing levels of TVP. Although this logic was supported by the initial quantitative determinations, it was not as pronounced as had been anticipated. Additional handling and preparation was necessary, however, in the case of the samples extended with TVP which subjected these materials to additional chance of bacterial contamination.

## Proximate Analyses of Sample Materials

Proximate analyses of the three replications of samples containing the five levels of TVP were determined. The summary of the analysis of variance is shown in Table 6. Variation due to the level of TVP was highly significant ($P < 0.01$) in the case of percent ether extract and percent

Table 5—Bacteriological analyses of TVP

| Assay | Replication | Count/gram |
|---|---|---|
| Aerobic count | 1 | < 100 |
| | 2 | < 100 |
| | 3 | < 100 |
| Psychrophilic-mesophilic count | 1 | < 100 |
| | 2 | < 100 |
| | 3 | < 100 |
| Coliform count | 1 | < 10 |
| | 2 | < 10 |
| | 3 | < 10 |
| KF count (fecal streptococci) | 1 | < 10 |
| | 2 | < 10 |
| | 3 | < 10 |
| Counts of *Staphylococcus aureus* | 1 | < 10 |
| | 2 | < 10 |
| | 3 | < 10 |

Table 6—Summary of analysis of variance of proximate analyses

| Source of variance | Degrees of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | % Ether extract | % Moisture | % Protein | % Unanlyzed material |
| Replication | 2 | 1.71 ** | 2.13 * | 0.78 | 0.18 |
| Level of TVP | 4 | 48.85 ** | 6.13 ** | 0.31 | 21.14 ** |
| Error | 8 | 0.10 | 0.32 | 0.21 | 0.59 |

** P < 0.01

* P < 0.05

moisture, but was not significant in the case of percent protein. The effect of varying levels of TVP on the percent unanalyzed material also was highly significant ($P < 0.01$).

The data presented in Table 7 show that the moisture content rose with increasing levels of TVP although the increase was not large in proportion to the total amount present. The percent ether extract decreased considerably with increasing levels of TVP. The product prepared with 40 percent TVP contained almost one-half as much percent ether extract as the product prepared without any TVP. The unanalyzed material, which increased from 0.26 percent to almost 6.5 percent, is primarily carbohydrate and ash.

No attempt was made in this study to adjust the percentage of protein in the preparation of the samples as was done by Judge et al. (1974). Furthermore, protein was the only chemical constituent analyzed which varied little. This was as anticipated because the hydrated TVP contained approximately 19 percent protein, which was approximately the same as that present in the ground beef. If chemical constituents had been adjusted using differing raw materials, additional sources of bacterial contamination would have been introduced and the possibility of confounding might have developed. However, since highly significant ($P < 0.01$) changes were observed in the percentages of all the other chemical constituents with varying levels of TVP, it is

Table 7—Means of proximate analyses

| % TVP | % Ether extract | % Moisture | % Protein | % Unanalyzed material |
|---|---|---|---|---|
| 0 | 18.8 ± 0.76 | 62.3 ± 0.51 | 18.6 ± -.82 | 0.26 ± 0.33 |
| 5 | 18.5 ± 0.93 | 63.2 ± 0.81 | 17.8 ± 0.53 | 0.50 ± 0.30 |
| 10 | 17.6 ± 0.58 | 63.7 ± 0.64 | 18.1 ± 0.40 | 0.53 ± 0.42 |
| 20 | 14.0 ± 0.50 | 64.6 ± 1.25 | 18.1 ± 0.65 | 2.97 ± 0.87 |
| 40 | 9.3 ± 0.41 | 66.0 ± 0.86 | 17.8 ± 0.31 | 6.47 ± 1.04 |

possible that these differences might have contributed to the observed variations in logarithmic aerobic and psychrophilic-mesophilic counts.

According to Jay (1970), the moisture requirements of microorganisms should be defined in terms of water activity in the environment. An increase in water activity would result in a more favorable environment for spoilage bacteria to proliferate. The water activity of fresh meat is generally 0.99 or above [Lechowich (American Meat Institute Foundation, 1971)] which is near the optimum for many varieties of bacteria. However, virtually all such bacteria are restricted to the surface of the meat, and drying of the surface will progressively restrict the growth of most spoilage organisms. The minimum water activity values for the growth of Pseudomonas and Achromobacter (the predominant spoilage bacteria in meat) are 0.97 and 0.96 respectively. These values are relatively high when compared to those required by other genera of bacteria. Therefore, the highly significant ($P < 0.01$) increase in moisture content with increased level of TVP is suggested to be responsible, in part, for the observed differences in logarithmic aerobic and psychrophilic-mesophilic counts.

The presence of nonproteolytic strains of bacteria in spoiled beef was interpreted by Jay (1967) to indicate that nutrients, other than the primary proteins, were utilized by

spoilage organisms. Lechowich (American Meat Institute Foundation, 1971) stated that microorganisms such as pseudomonads preferentially utilize carbohydrates as a source of energy for growth. Therefore, the highly significant ($P < 0.01$) increase in the unanalyzed fraction (carbohydrate and ash) with increased level of TVP is also suggested to be partly responsible for the observed differences in logarithmic aerobic and psychrophilic-mesophilic counts.

Pseudomonas and Achromobacter species of bacteria are strongly proteolytic. Since proteolysis occurs only at population levels of about 100,000,000 organisms per gram (Ingram and Dainty, 1971), the soluble proteins would be utilized first. The pH of fresh meat is generally between 5.4 and 6.5 [Lechowich (American Meat Institute Foundation, 1971)]. The possibility is strong that addition of the hydrated TVP may have increased the pH above 6.5. Since this is a value which would result in nearly maximum protein solubility (Wolf, 1972), extension with TVP would tend to favor spoilage. Therefore the possible increase in pH is also suggested to be responsible, in part, for the observed differences in logarithmic aerobic and psychrophilic-mesophilic counts.

## Effect of Replication

The summaries of the analysis of variance of the five selected assays are shown in Tables 8, 9, 10, 11 and 12.

Table 8—Summary of analysis of variance of logarithmic aerobic counts per gram

| Source of variance | Degrees of freedom | Mean squares at 6°C | Mean squares at 0°C | Mean squares at -1.6°C |
|---|---|---|---|---|
| Replication | 2 | 59.4074 ** | 67.1827 ** | 63.0870 ** |
| Level of TVP | 4 | 0.8875 ** | 2.7045 ** | 0.0336 |
| Storage time | 4 | 69.2613 ** | 22.1413 ** | 0.0381 |
| TVP × storage time | 16 | 4.7776 ** | 0.3420 ** | 0.0071 * |
| Replication × TVP | 8 | 0.1414 | 0.1427 ** | 0.0093 ** |
| Replication × storage time | 8 | 1.1930 ** | 0.6211 ** | 0.0678 ** |
| Replication × TVP × storage time | 32 | 0.1414 | 0.1257 ** | 0.0033 ** |
| Error | 150 | 0.1073 | 0.0009 | 0.0017 |

** $P < 0.01$

* $P < 0.05$

Table 9—Summary of analysis of variance of logarithmic psychrophilic-mesophilic counts per gram

| Source of variance | Degrees of freedom | Mean squares at 6°C | Mean squares at 0°C | Mean squares at -16°C |
|---|---|---|---|---|
| Replication | 2 | 18.7272 ** | 15.8665 ** | 84.9926 ** |
| Level of TVP | 4 | 2.6632 ** | 7.6730 ** | 0.0058 |
| Storage time | 4 | 71.4769 ** | 50.1091 ** | 0.0200 |
| TVP × storage time | 16 | 0.3687 * | 0.3840 ** | 0.0017 |
| Replication × TVP | 8 | 0.0931 ** | 0.1172 ** | 0.0031 ** |
| Replication × storage time | 8 | 2.8182 ** | 3.7955 ** | 0.0334 ** |
| Replication × TVP × storage time | 32 | 0.1519 ** | 0.1004 ** | 0.0023 ** |
| Error | 150 | 0.0004 | 0.0011 | 0.0006 |

** $P < 0.01$

* $P < 0.05$

Table 10—Summary of analysis of variance of logarithmic coliform counts per gram

| Source of variance | Degrees of freedom | Mean squares at 6°C | at 0°C | at -16°C |
|---|---|---|---|---|
| Replication | 2 | 3.8575 ** | 2.1727 ** | 5.2890 ** |
| Level of TVP | 4 | 0.1349 | 0.0218 | 0.0718 |
| Storage time | 4 | 38.1234 ** | 1.9889 ** | 0.1374 |
| TVP × storage time | 16 | 0.0518 | 0.0087 | 0.0160 |
| Replication × TVP | 8 | 0.6431 ** | 0.0135 ** | 0.1426 ** |
| Replication × storage time | 8 | 0.5734 ** | 0.0719 ** | 0.0909 ** |
| Replication × TVP × storage time | 32 | 0.1691 | 0.0064 ** | 0.0209 ** |
| Error | 150 | 0.0011 | 0.0014 | 0.0058 |

** $P < 0.01$

Table 11—Summary of analysis of variance of logarithmic KF (fecal streptococci) counts per gram

| Source of variance | Degrees of freedom | Mean squares | | |
|---|---|---|---|---|
| | | at 6°C | at 0°C | [illegible]°C |
| Replication | 2 | 1.4040 ** | 0.5746 ** | 4.9434 ** |
| Level of TVP | 4 | 0.0277 | 0.0787 ** | 0.1696 ** |
| Storage time | 4 | 19.1392 ** | 0.3254 ** | 0.2687 |
| TVP × storage time | 16 | 0.0227 ** | 0.0070 * | 0.0064 |
| Replication × TVP | 8 | 0.0102 ** | 0.0046 ** | 0.0071 ** |
| Replication × storage time | 8 | 0.2622 ** | 0.0532 ** | 0.2208 ** |
| Replication × TVP × storage time | 32 | 0.0053 ** | 0.0030 ** | 0.0043 ** |
| Error | 150 | 0.0012 | 0.0012 | 0.0015 |

** $P < 0.01$

* $P < 0.05$

Table 12—Summary of analysis of variance of logarithmic counts of *Staphylococcus aureus* per gram

| Source of variation | Degrees of freedom | Mean squares at 6°C | Mean squares at 0°C | Mean squares at -16°C |
|---|---|---|---|---|
| Replication | 2 | 23.5111 ** | 29.5283 ** | 15.0601 ** |
| Level of TVP | 4 | 0.1706 | 1.6682 | 0.3977 |
| Storage time | 4 | 11.4815 ** | 8.7670 ** | 35.7156 ** |
| TVP × storage time | 16 | 0.1418 | 0.2504 | 0.1677 |
| Replication × TVP | 8 | 0.0663 * | 0.6847 ** | 0.2290 ** |
| Replication × storage time | 8 | 1.0770 ** | 0.3260 ** | 2.0744 ** |
| Replication × TVP × storage time | 32 | 0.1023 ** | 0.2923 ** | 0.2833 ** |
| Error | 150 | 0.0277 | 0.0368 | 0.0146 |

** $P < 0.01$

* $P < 0.05$

Observed variation among replications during storage at the three selected temperatures was highly significant ($P < 0.01$) in all the selected assays. This was not surprising since the three replications were manufactured at different times using raw material from different sources. These results indicated that there was great variability in bacterial counts in different lots of raw ground beef materials. Further, it should be noted that sanitation and manufacturing practices vary widely from one source of raw beef material to another. Future investigations of this kind should consider eliminating this source of variability by using identically produced lots, or by assessing treatment effects using a common lot of raw material.

## Effect of Storage Time

The summaries of the analysis of variance of the logarithmic aerobic, psychrophilic-mesophilic, coliform, and KF (fecal streptococci) counts are shown in Tables 8, 9, 10 and 11. Highly significant ($P < 0.01$) or significant ($P < 0.05$) increases in logarithmic counts with time were observed during storage at 6°C and 0°C, but not at -16°C. These results indicate that ground beef extended with TVP is a medium in which bacterial numbers can increase during storage at 6°C and 0°C for four and ten days respectively. Although no data were taken, most samples stored at 6°C and 0°C

developed offensive odors by the end of the storage time. Particularly offensive were those samples containing the high levels of TVP.

The 90-day storage at -16°C did not result in significant changes in logarithmic aerobic, psychrophilic-mesophilic, coliform or KF (fecal streptococci) counts with time. Also, as was noted by visual observation, little change occurred in the appearance of any of the samples during the 90-day storage period at -16°C. These results indicated that bacteriological quality, as measured by quantitative determination, did not change curing storage at this temperature. Since most products of this type are stored at temperatures approaching -16°C, there is little likelihood of bacteriological deterioration of products extended with TVP during frozen storage.

The summary of the analysis of variance of the logarithmic counts of Staphylococcus aureus is shown in Table 12. Highly significant ($P < 0.01$) reductions in logarithmic counts with time occurred during storage at all three temperatures. The reductions in mean logarithmic counts at the three temperatures are shown in Table 13.

Coagulase-positive staphlococci were observed in all of the initial samples. This does not agree with the findings reported by Duitschaever et al. (1973), who found these organisms present in only 37 percent of the retail ground

Table 13—Mean logarithmic counts per gram of *Staphylococcus aureus* during storage

| Storage time (days) | Logarithmic counts per gram | | |
|---|---|---|---|
| | at 6°C | at 0°C | at -16°C |
| 1 | 2.31 | 1.95 | 2.01 |
| 2 | 1.97 | 1.57 | 1.81 |
| 3 | 1.79 | 1.30 | 1.39 |
| 4 | 1.15 | 1.24 | 0.37 |
| 5 | 1.18 | 0.75 | 0.00 |

beef samples examined. However, no indication of times or temperatures of storage were given. Since the present study indicated a rapid reduction in coagulase-positive staphylococci with time, the results of the two studies are not comparable.

## Effect of Level of TVP

### Aerobic Count

The summary of the analysis of variance of the logarithmic counts of aerobic bacteria is shown in Table 8 (page 35). Increased levels of TVP resulted in highly significant ($P < 0.01$) increases in logarithmic counts during storage at 6°C and 0°C, but not at -16°C. The interaction between the levels of TVP and storage time also was highly significant ($P < 0.01$) at 6°C and 0°C. These results are in general agreement with those reported by Judge et al. (1974). However, the differences observed by these workers were attributed to raw material differences rather than the addition of soy additives.

The increases in logarithmic aerobic counts with time during storage at 6°C are shown in Figure 2. The initial logarithmic counts were nearly the same or lower with higher levels of TVP. This was due to lower bacteriological counts of the TVP. By day two, however, this trend had completely reversed, with higher levels of TVP resulting in higher

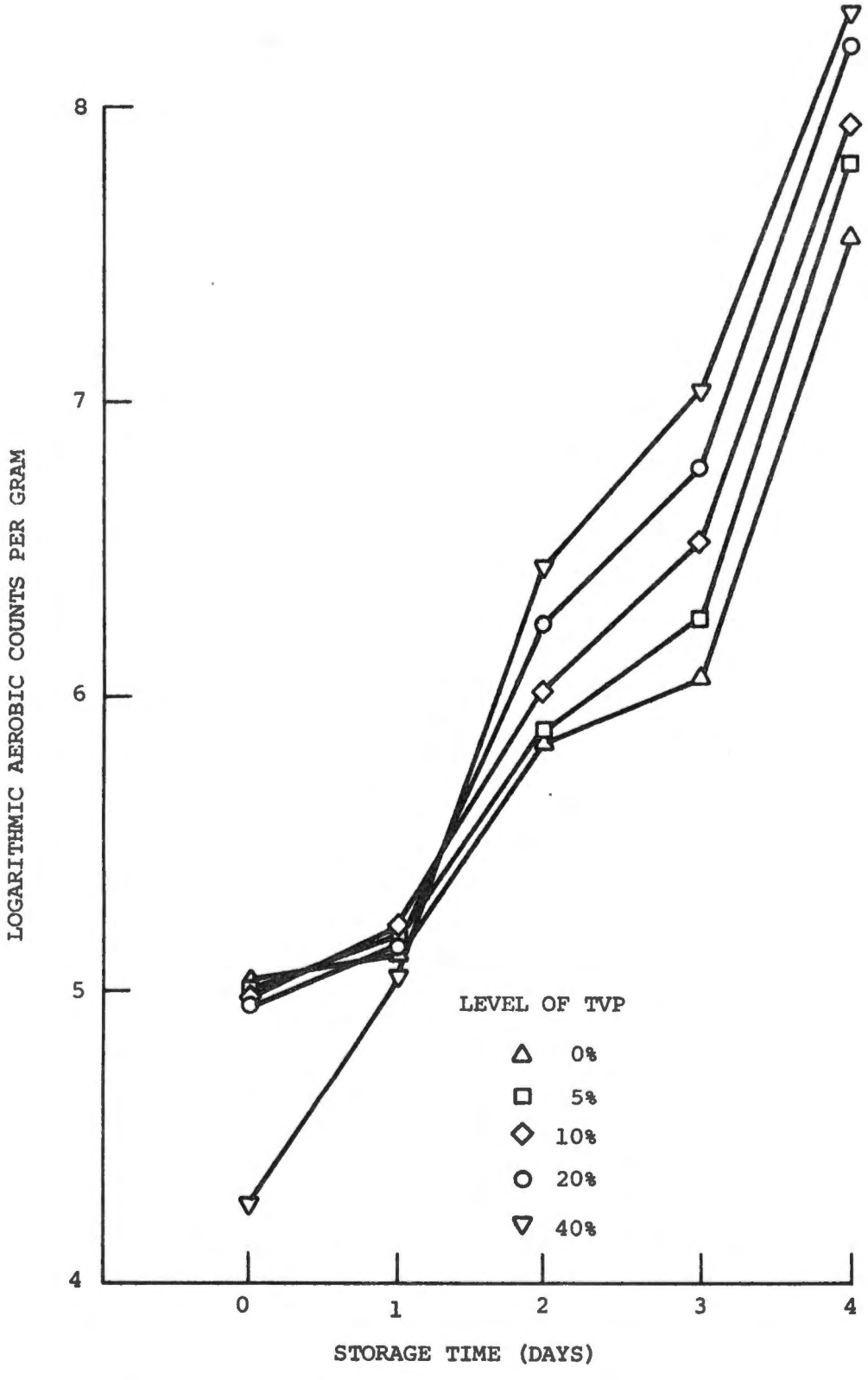


Figure 2. Logarithmic aerobic counts per gram at 6°C and the interaction between levels of TVP and storage time.

logarithmic counts. The increases in the logarithmic counts of these bacteria at higher levels of TVP commenced early and proceeded rapidly during the storage period.

The trends shown in Figure 3 are somewhat similar to those shown in Figure 2. However, limitations of the design of this study resulted in commencement of quantitative determinations on day two of storage at 0°C rather than day zero as in the case of the storage at 6°C. Nevertheless, by day four, the trend of increased logarithmic counts with increased level of TVP had developed, and continued through the 10-day storage at 0°C.

The aerobic count was one of the assays used to estimate the number of spoilage organisms present. Multiplication of these bacteria would result in a reduction of the shelf-life of meat. Therefore, these findings indicated that the shelf-life was adversely affected by extension with TVP at 6°C and 0°C, but not at -16°C.

Psychrophilic-Mesophilic Count

The summary of the analysis of variance of the logarithmic psychrophilic-mesophilic counts is shown in Table 9 (page 36). Increasing levels of TVP resulted in highly significant ($P < 0.01$) increases in logarithmic counts during storage at 6°C and 0°C, but not at -16°C. The interaction between levels of TVP and storage time also was

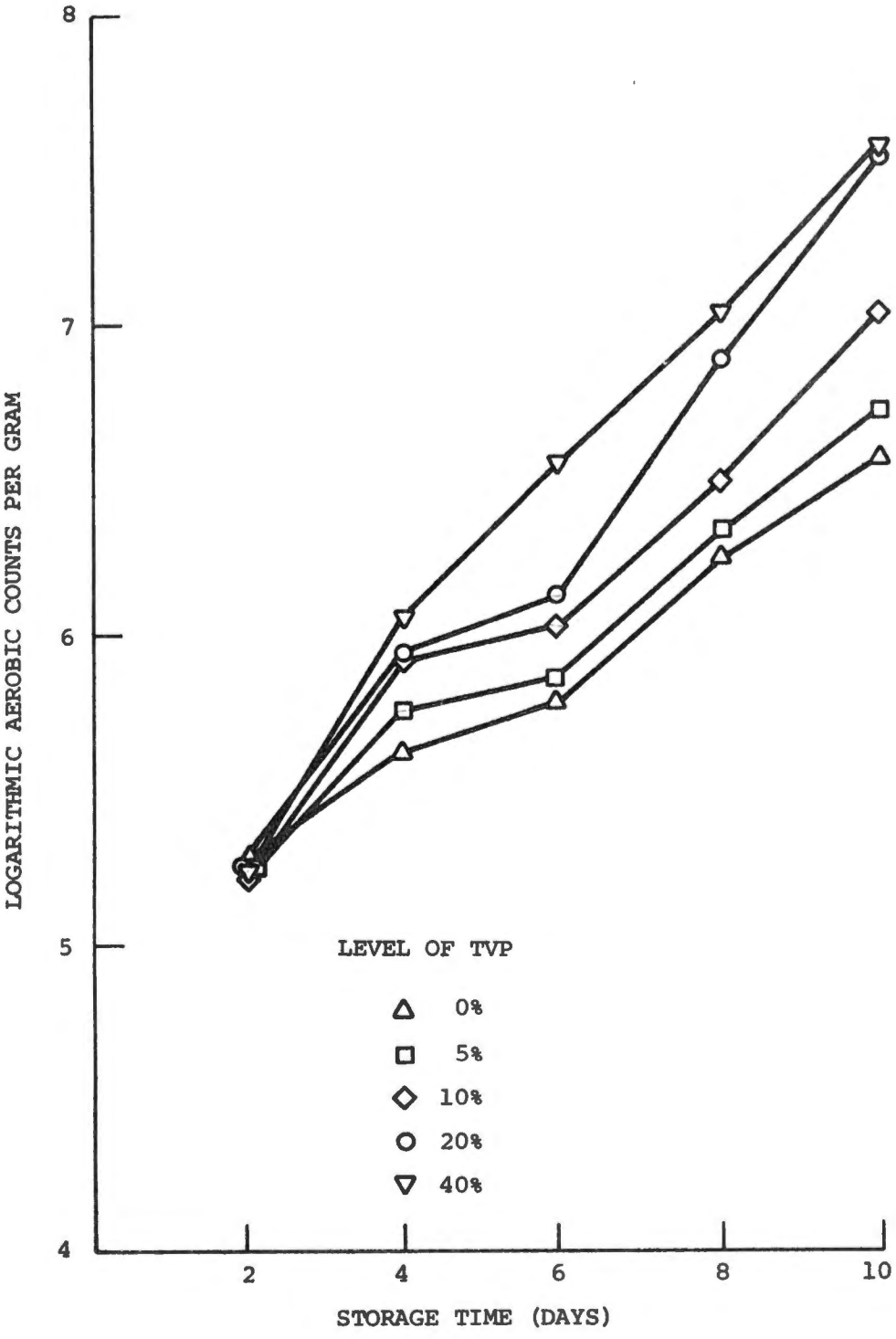


Figure 3. Logarithmic aerobic counts per gram at 0°C and the interaction between levels of TVP and storage time.

highly significant ($P < 0.01$) in the case of the 0°C storage temperature.

Figure 4 shows the initial counts to be nearly the same or decreasing with higher levels of TVP at day zero. Also shown is the increase in logarithmic counts during storage at 6°C. These trends were much like those depicted in Figure 2 (page 44), with higher levels of TVP resulting in higher logarithmic counts by day two of storage at 6°C.

The trends shown in Figure 5 reveal that the increases in logarithmic counts during the 10-day storage at 0°C were similar to those shown in Figure 4 from the second day of storage through day four. However, as in the case of the a aerobic count assay, limitations of the design of this study resulted in commencement of quantitative determinations on day two of storage at 0°C rather than day zero, as in the case of the storage at 6°C.

The psychrophilic-mesophilic count assay differed from the aerobic count assay only in the temperature of incubation. The psychrophilic-mesophilic count assay incubation temperature was 20 ± 1°C, compared to 35 ± 1°C for the aerobic count assay. Since these results show that much higher counts were obtained using the lower incubation temperature, the psychrophilic-mesophilic count assay estimated more accurately the actual number of spoilage organisms present.

Increased numbers of spoilage organisms, as measured by the aerobic and psychrophilic-mesophilic count assays, would

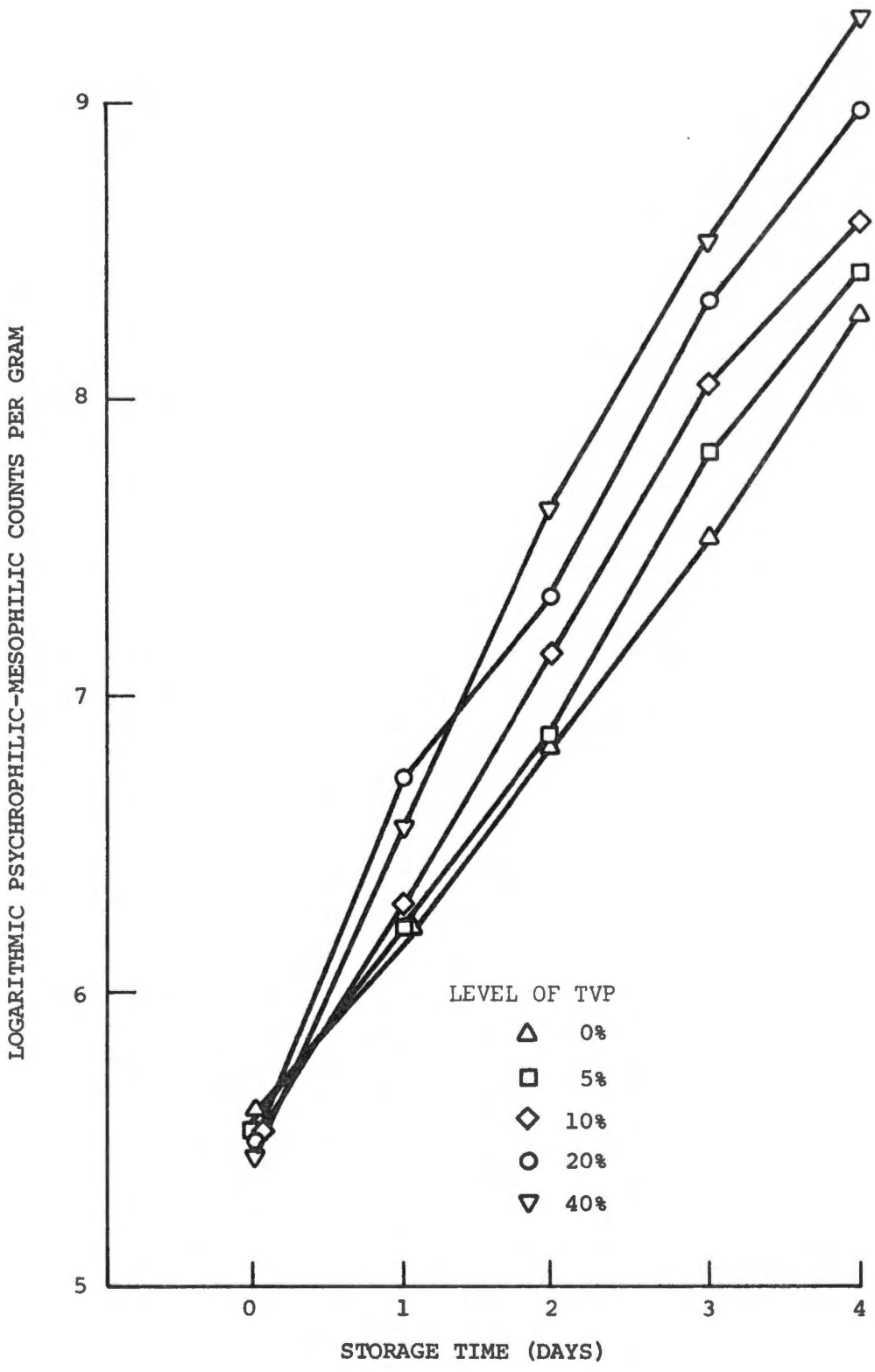


Figure 4. Logarithmic psychrophilic-mesophilic counts per gram at 6°C and the interaction between levels of TVP and storage time.

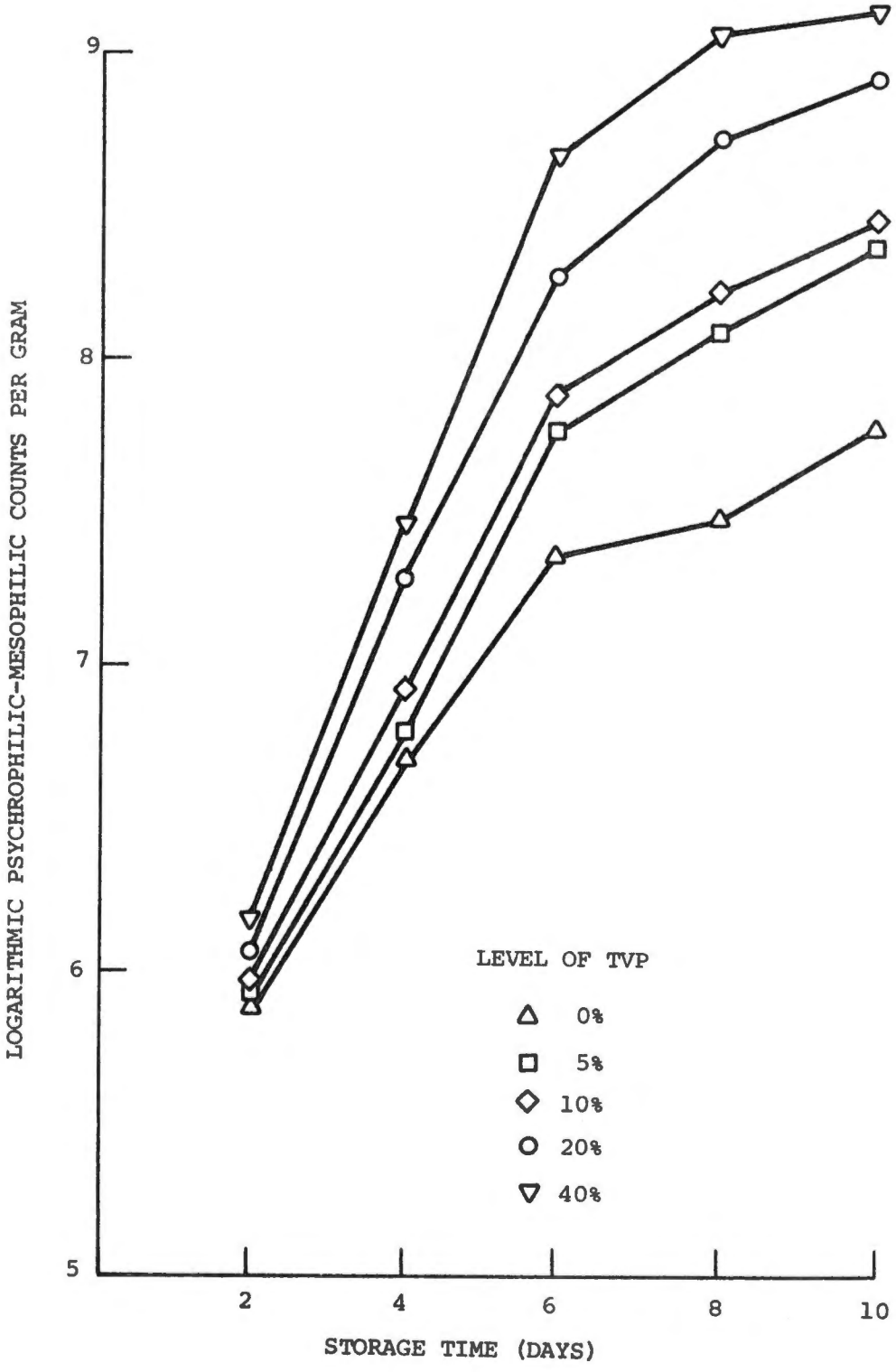


Figure 5. Logarithmic psychrophilic-mesophilic counts per gram at 0°C and the interaction between levels of TVP and storage time.

result in a reduction of the shelf-life of meat. Therefore, these findings indicated that the shelf-life was adversely affected by extension with TVP at 6°C and 0°C, but not at -16°C.

Coliform Count

The summary of the analysis of variance of the logarithmic coliform counts is shown in Table 10 (page 37). There were no significant variations in logarithmic coliform counts attributable to varying levels of TVP during storage at any of the three selected temperatures.

KF (Fecal Streptococci) Count

The summary of the analysis of variance of the logarithmic KF counts is shown in Table 11 (page 38). There were no significant variations in logarithmic KF counts attributable to varying levels of TVP during storage at 6°C. However, highly significant ($P < 0.01$) increases in logarithmic KF counts were observed with increasing levels of TVP during storage at 0°C. These increases are shown in Figure 6. Also shown is the significant ($P < 0.05$) interaction between levels of TVP and storage time. The total logarithmic counts were not large, however, when compared to those determined by the aerobic and psychrophilic-mesophilic count assays and the magnitudes of change also were small.

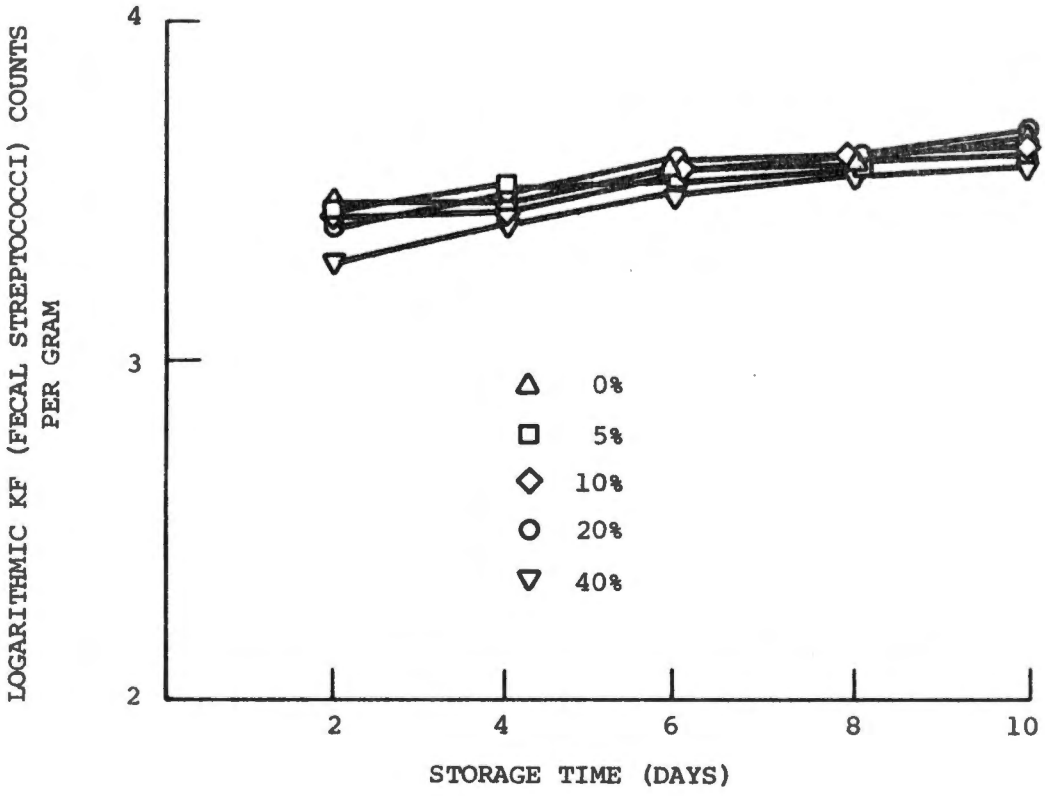


Figure 6. Logarithmic KF (fecal streptococci) counts per gram at 0°C and the interaction between levels of TVP and storage time.

The highly significant ($P < 0.01$) decreases in logarithmic KF counts at -16°C, with increasing levels of TVP, are shown in Figure 7. This reflects the relative uniformity in logarithmic KF counts observed during the 90-day storage at -16°C.

Counts of Staphylococcus aureus

The summary of the analysis of variance of the logarithmic counts of Staphylococcus aureus is shown in Table 12 (page 39). There were no significant differences in logarithmic counts of Staphylococcus aureus due to varying levels of TVP at any of the three selected temperatures.

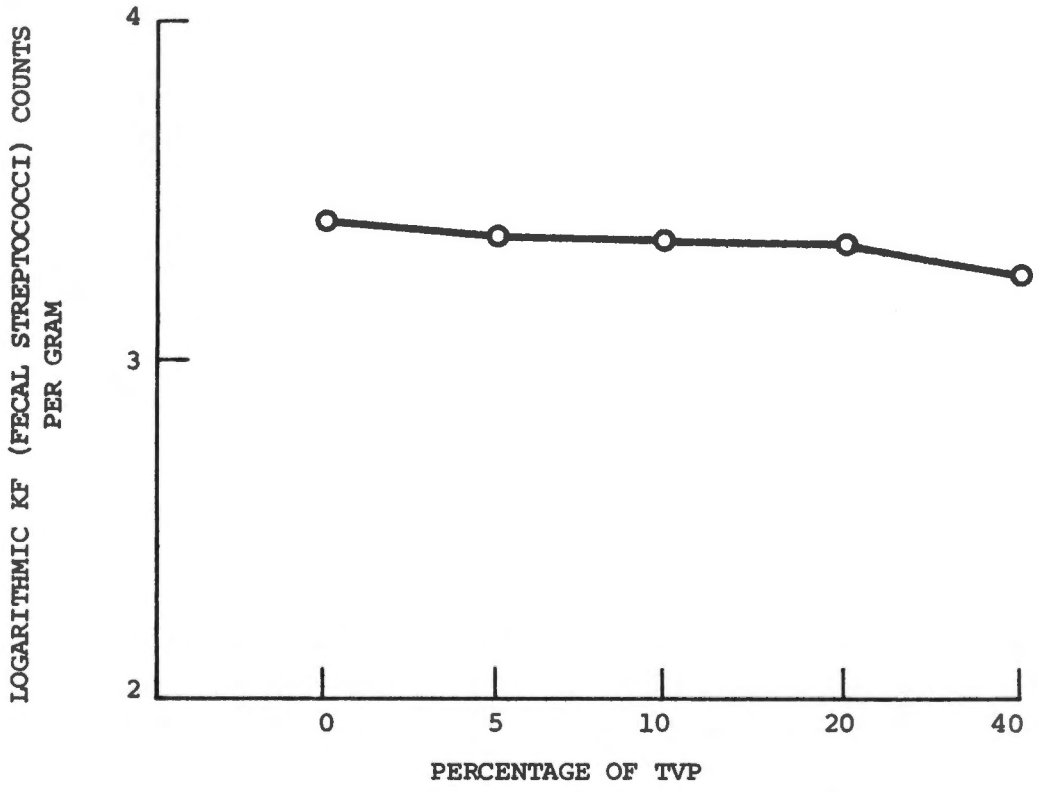


Figure 7. Logarithmic KF (fecal streptococci) counts per gram at -16°C showing the effect of varying levels of TVP.

# CHAPTER V

## SUMMARY

The objective of this study was to determine the influence of various levels of textured vegetable protein (TVP) on selected groups of bacteria present in ground beef. This influence was assessed by five selected bacteriological assays: aerobic count, psychrophilic-mesophilic count, coliform count, KF (fecal streptococci) count and counts of Staphylococcus aureus. Samples were prepared by extending ground beef to contain by weight 0, 5, 10, 20 and 40 percent of hydrated TVP. The samples were stored at 6°C for 4 days, at 0°C for 10 days and at -16°C for 90 days.

The bacteriological determinations of the TVP revealed that this material contained very small numbers of bacteria as determined by the selected assays. Therefore, the population levels of bacteria in the ground beef materials should have been reduced by dilution with increasing levels of TVP. Although this logic was supported by the initial quantitative determinations, it was not as pronounced as had been anticipated.

Observed variation among replications during storage at the three selected temperatures was highly significant ($P < 0.01$). Therefore, future investigations of this kind should

use identically produced lots or assess treatment effects using a common lot of raw material.

With the exception of percent protein, highly significant ($P < 0.01$) changes were observed in proximate analyses with varying levels of TVP. The percentages of unanalyzed material (carbohydrate and ash) and moisture were increased with increased level of TVP while that of the fat (ether extract) decreased.

Highly significant ($P < 0.01$) increases in logarithmic aerobic and psychrophilic-mesophilic counts accompanied increasing levels of TVP during storage at 6°C and 0°C, but not at -16°C. The interaction between levels of TVP and storage time was significant ($P < 0.05$) or highly significant ($P < 0.01$) at storage temperatures of 6°C and 0°C respectively. These results were in general agreement with those reported by Judge et al. (1974). However, the differences observed by these workers were attributed to raw material differences rather than the addition of soy additives. The increases in logarithmic counts of these bacteria with increased level of TVP commenced early and proceeded rapidly during storage at 6°C and 0°C.

The aerobic and psychrophilic-mesophilic count assays were used to estimate the number of spoilage organisms present. Multiplication of these bacteria would result in a reduction of the shelf-life of meat. Therefore, these findings indicated that the shelf-life of ground beef was

adversely affected by extension with TVP at 6°C and 0°C, but not at -16°C.

No significant differences in logarithmic coliform counts or logarithmic counts of Staphylococcus aureus attributable to varying levels of TVP were observed during storage at any of the selected temperatures.

The highly significant ($P < 0.01$) changes in the proximate analyses with increasing levels of TVP were suggested to be responsible for the significant ($P < 0.05$) and highly significant ($P < 0.01$) increases in logarithmic counts in those instances where such increases were observed. Also suggested as being responsible for the observed increases in logarithmic aerobic and psychrophilic-mesophilic counts with increasing levels of TVP was the probable rise in pH and subsequent increase in protein solubility.

The 90-day storage at -16°C did not result in significant increases in logarithmic counts determined by any of the selected assays. Therefore, since most products extended with TVP are stored at temperatures approaching -16°C, there is little likelihood of bacteriological deterioration during frozen storage. Caution, however, is advised when storing such products at temperatures of 0°C, and above, because of reduced shelf-life with increasing levels of TVP.

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## LITERATURE CITED


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# VITA

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