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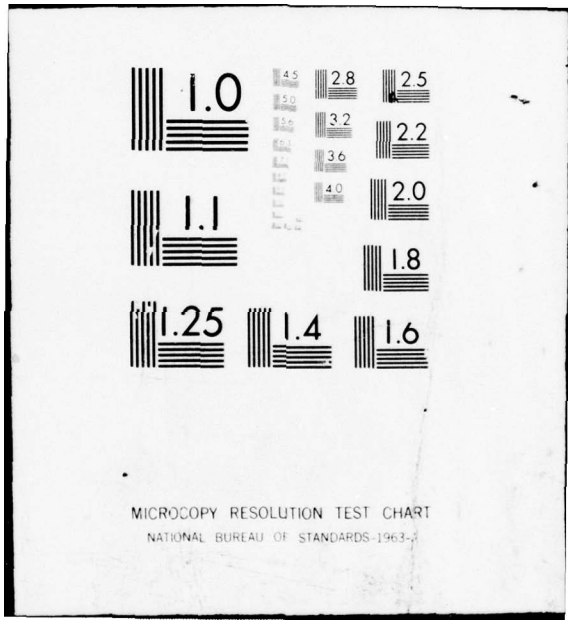
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REPORT NO. 43

A SURVEY OF THE MICROBIAL FLORA OF GROUND BEEF, TEXTURED SOY PROTEIN AND TEXTURED SOY PROTEIN EXTENDED GROUND BEEF AFTER 3 DAYS' AND 10 DAYS' STORAGE AT 4 C

FOOD HYGIENE DIVISION
DEPARTMENT OF NUTRITION

PATHOLOGY AND COMPARATIVE STUDIES DIVISION
DEPARTMENT OF COMPARATIVE MEDICINE

SAN FRANCISCO STATE UNIVERSITY
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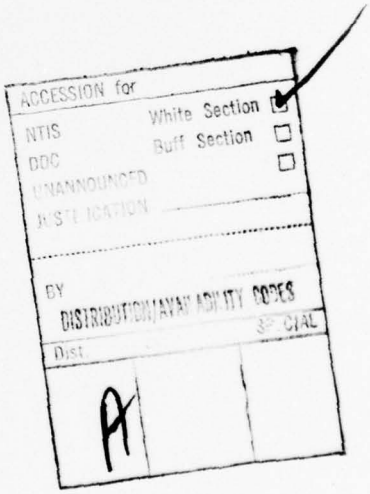
ABSTRACT

A survey of the microbial populations of 31 samples of ground beef (GB), textured soy protein (TSP) and TSP extended ground beef (SGB) after 3 days' and 10 days' storage at 4 C was performed. Analyses included aerobic plate count (APC), psychrotrophic plate count (PPC), coliform most probable number (MPN) and plate determinations (CMPN and CPC), Escherichia coli MPN and plate determinations (EMPN and EPC), Staphylococcus aureus MPN (SMPN), fecal streptococci count (FSC), Clostridium perfringens determinations, isolation and identification of gram-positive and gram-negative organisms and screening for enteric virus. Statistical analyses of the enumeration procedures showed significant increases in the total microbial flora after 10 days' storage. PPCs were significantly higher than APCs. CMPNs were significantly higher than CPCs for GB and SGB. The EMPNs were significantly higher than EPC in SGB only. E. coli was the predominant gram-negative isolate from GB and SGB. Few gram-negative organisms were found in TSP. C. perfringens was the predominant gram-positive isolate in GB and SGB while Bacillus sp. predominated in TSP. Salmonella enteriditis ser. worthington was isolated from GB and TSP. These products contained a wide variety of microorganisms, many in large numbers. If properly handled and cooked before consumption, these products should present no public health problems.



PREFACE

The authors wish to thank Mr. John Dacey and SP5 Fred Tillman for their technical assistance during this study. We also wish to express our thanks to Mr. Alan Hopkins for his assistance with the statistical analyses and Mrs. Karen Trefz for her perseverance in typing the manuscript and tables. COL James L. Fowler's present address is 929 Pineview Circle, Live Oak, FL 32060.

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INTRODUCTION

The United States Armed Forces is one of the world's largest single consumers of ground beef items. It is estimated that 50 million pounds of ground beef are consumed annually by the Armed Forces (Department of Defense information, TELECON, 1976). This includes purchases by both the Defense Personnel Support Center and the Army and Air Force Exchange. This figure not only represents the bulk purchases of ground beef, but also includes ground beef produced by military facilities from carcass trim, bull meat, rounds, and suet. With the current diet preferences of the young soldier being short-order type foods, the per capita consumption of ground beef items could easily increase in the future. In addition the cost of red meat items has been steadily increasing. In order to meet this challenge, dietitians have had to look for methods by which they can stretch their food dollar and at the same time provide a nutritious food item. The use of soy protein extended ground beef (beef/soy) has been proposed as a partial solution to this problem.

The first use of soybeans by man has been placed in the 24th to 29th century BC by Morse (1). According to Hymowitz (2) the use of soybeans for food originated around the 11th century BC during the latter part of the Shang dynasty in China. Soybean products have been a primary protein source in the Orient historically and still continue to be a major part of the diet.

In the U.S., soybeans were initially grown and utilized for the soybean oil and soybean meal around the turn of the century. Soybeans have been grown in quantity in the U.S. only since the late 1920s. However, soybeans have developed into a major cash crop, second only to corn. Additionally, there have been many technological advances in the processing techniques of soybeans which have resulted in a variety of soy products in the food industry. These products include soy flour and grits, soy protein concentrates, soy protein isolates, textured soy protein, spun soy protein, and textured soy protein isolates (3).

Soy flour has been used in food products in the U.S. for about 50 years, and in some meat products on a limited basis for about 40 years. In 1962, soy protein concentrate was authorized as an extender in meat products from federally inspected meat plants (4,5). Isolated soy protein and textured vegetable proteins were authorized for use in federally inspected meat plants in 1964 (4,5). However, in 1971, a significant breakthrough was realized in the use of soy protein for meat extension. The United States Department of Agriculture (USDA) approved the use of soy protein at the maximum level of 30% in the Class A government

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subsidized school lunch program (5). In early 1973, ground beef extended with textured soy protein began to appear in supermarkets (6). The product has many advantages to the consumer over regular ground beef. Data presented by Wolford (6) indicate that nutritionally the beef/soy combination was equivalent to ground beef. In addition, Wolford's data showed that the consumer can realize a 21% cost savings on a raw basis and a 30% savings on a cooked basis. Although dollar savings are substantial, any major changes in the price of beef and soy protein could significantly alter the savings previously reported. Studies have been conducted which indicate that ground beef patties containing 20% soy protein concentrate were about equal in flavor, appearance, aroma, juiciness, and overall acceptability when compared will all beef patties (7). Other researchers have shown that soy protein extended ground beef is superior to regular ground beef in total shrinkage and cooking loss measurements (8-10).

Although the physical and nutritional characteristics of beef/soy have been well investigated, the question of wholesomeness from a bacterial standpoint remains unanswered. Microbiological standards for ground beef and vegetable protein extended ground beef are pending legislative action in Canada (11). Similar although more stringent standards for these same products are expected to be rescinded in the State of Oregon (12). The proposal and initiation of standards for ground meat products have resulted in considerable discussion between regulatory agencies and industry. Agreement on standards for ground meat items will be slow in coming. However, it would seem to be only a matter of time before public awareness and consumer group pressure will force government and industry to come to some kind of agreement on the issue of microbiological standards.

Before equitable standards can be formulated, extensive knowledge of the product must be obtained. Many investigators (13-21) have published research pertaining to the microbiology of regular ground beef

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and an extensive data base exists for this item. Microbiological data on ground beef with added soy protein are almost nonexistent. In reviewing the literature, only three studies (9,11,22) reporting aerobic plate counts, coliform counts, or specific organism characterizations for fresh soy extended ground beef could be found. Researchers (23) using this product experimentally inoculated with C. perfringens indicated that the soy protein had no noticeable effect on the growth of this pathogen. The authors stated that further testing with additional strains was warranted. In another study, the same researchers (24) found that four of the 16 ingredients comprising synthetic soybeef significantly stimulated the growth of C. perfringens.

During processing, the bacterial contamination present on the meat surface is distributed throughout the product. Therefore, the bacterial flora present in ground beef is dependent upon the bacterial levels present on the meat and trimmings, sanitary conditions during processing, temperature and storage time before sale. Rogers and McClesky (17) found that the numbers of bacteria in market samples of ground meat are clearly indicative of the history of the product.

Additionally, human enterovirus isolation has been reported from ground beef and other foods (25,26). Food products have been implicated as a vehicle of transmission for several viral agents. In a current review, Bryan (27) listed seven groups of viruses (Adenovirus, Coxsakivirus, Echovirus, Poliovirus, Reovirus, Hepatitis and Norwalk agent) which may be transmitted by food. Many of the reports are based on epidemiologic evidence since enteric viruses can be conveyed by more than one vehicle. In an earlier review, Cliver (28) described several instances of food-associated poliomyelitis and infectious hepatitis. For both diseases, "the clinical pictures were so distinctive as to permit these to be diagnosed on that basis by the attending physician" (28). Since "clinically distinct" viral agents have been demonstrated in food-associated illnesses, other human enteroviruses with less discrete clinical syndromes could be transmitted via food. However, relatively few reports of laboratory isolation of viruses from foods exist in the literature. Sullivan et al. (25) isolated poliovirus types 1 and 2 and echovirus type 6 from 3 of 12 commercial ground beef samples. Metcalf and Stiles (26) isolated several enteric viruses from oysters.

Due to inherent technical and/or economic difficulties, methods for the detection of foodborne viruses have been met with varying degrees of success. Clarification of the sample suspension, elimination of

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cytotoxic agents and concentration of sample suspension are crucial to virus detection in foods. Several investigators have treated sample suspensions with ethyl ether (26,29), fluorocarbon (30), acid precipitation (31), low speed centrifugation (32), or glasswool filtration (33) to optimize virus detection. In order to increase the probability of virus detection, sample suspensions have been concentrated by a variety of laboratory procedures including ultracentrifugation (26,34), dialysis against hydrophilic solutions (35), the application of aqueous two phase system (36,37), and ultrafiltration (32,38).

If regular ground beef is extended with textured soy protein the bacteria present will be diluted accordingly. With the addition of the soy protein, a new environment has been created for the microorganisms present. Therefore, regular ground beef, textured soy protein, and textured soy protein extended ground beef were analyzed to determine the microbial flora present. Additionally, since regular ground beef is known to have a limited shelf life (19,39), analyses were performed in order to determine the changes in microbial flora following storage at 4 C for 7 days. The regular ground beef and textured soy protein were screened for human enterovirus by using celite filtration to clarify the food suspension and molecular filtration to concentrate the sample. Known quantities of poliovirus type 1 were added to samples of regular ground beef to determine the sensitivity of this virus recovery method.

MATERIALS AND METHODS

Samples: Duplicate units from 31 production lots of ground beef (GB), textured soy protein (TSP), and the corresponding lots manufactured into TSP extended ground beef (SGB) were obtained from a production facility in the San Francisco Bay Area. Units were held at 4 ± 1 C and analyzed after 3 and 10 days' storage from the date of production.

Sample Preparation: A 25 g portion of each unit was weighed into a sterile one liter blender. Following addition of 225 ml of sterile

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phosphate buffered water, the sample was blended at high speed for 3 min. Serial dilutions from 10^{-2} through 10^{-7} were prepared.

Aerobic plate count (APC): Duplicate plates for dilutions 10^{-1} through 10^{-7} were prepared and poured in accordance with Bacteriological Analytical Manual for Foods (BAM) (40). Plates were incubated at 32 C for 72 ± 2 h.

Psychrotrophic plate count (PPC): Duplicate plates for dilutions 10^{-1} through 10^{-7} were prepared as in the APC procedure. Plates were incubated at 7 C for 10 days.

Total coliform and Escherichia coli plate count: Total coliform and E. coli plate counts were made in accordance with the procedures described in Reference Methods for the Microbiological Examination of Foods (41).

Total coliform and Escherichia coli MPN count: Total coliform and E. coli MPN determinations were made using the techniques described in the BAM (40).

Staphylococcus aureus analyses: S. aureus MPN determinations were performed in accordance with the AOAC method (42) except that tellurite polymyxin egg yolk (TPEY) agar was substituted for Baird-Parker agar. The tube coagulase test (42) was performed as needed.

Clostridium perfringens analyses: Approximately 1 g of sample was inoculated into each of four 25 x 150 mm tubes containing 20 ml fluid thioglycollate medium (FTM). Additionally, 10 ml of the original food homogenate were inoculated into each of four 25 x 150 mm tubes containing 20 ml of FTM. One pair of tubes (one tube with blended and the other with unblended sample) was incubated for 24 ± 2 h at 37 C. Another pair was heat shocked at 75 C for 20 min and incubated for 24 ± 2 h at 37 C. The third pair was heat shocked at 95 C for 5 min and incubated for 24 ± 2 h at 37 C. The remaining pair was incubated at 46 C for 8 ± 2 h followed by incubation at 37 C for 16 ± 2 h. All tubes were incubated aerobically. Gas formation was recorded for all at the end of the incubation period. Approximately 0.01 ml of material from each FTM tube was then transferred to cooked meat medium (CMM) in 16 x 125 mm tubes and incubated at 37 C for 24 ± 2 h with gas formation again being recorded. Material from all CMM tubes was used to streak sulfite polymyxin sulfadiazine (SPS) agar plates, which were overlayed and incubated anaerobically at 37 C for 24 ± 2 h. Isolated black colonies were transferred to FTM and incubated at 37 C for

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24 ± 2 h. FTM cultures were Gram stained and transferred to duplicate liver veal egg yolk (LVEY) agar plates which were incubated aerobically and anaerobically at 37 C for 24 ± 2 h. Isolated lecithinase-producing colonies from anaerobic LVEY agar plates were transferred to motility, indole-nitrite, iron milk, and gelatin media and incubated at the appropriate temperature (40). Cultures showing only typical nonmotile gram-positive rods, no growth on aerobic LVEY agar plates, lecithinase production on LVEY agar plates incubated anaerobically, reduction of nitrate to nitrite, stormy fermentation in iron milk, and gelatin hydrolysis were reported as confirmed C. perfringens.

Direct enumeration of C. perfringens was determined by the use of sulfite polymyxin sulfadiazine (SPS) agar and the nitrite-motility reactions. Duplicate pour plates, inoculated with 1 ml each of the original food homogenate were prepared and incubated anaerobically at 35 C for 24 ± 2 h. Black colonies on SPS agar were counted as presumptive C. perfringens. Representative colonies were transferred into indole-nitrite medium and incubated at 35 C. After 24 ± 2 h incubation, tubes showing nonmotile and nitrate positive reactions were reported as C. perfringens.

Fecal streptococci analyses: The fecal streptococci analyses were performed in accordance with the procedures outlined in BAM (40). In addition, representative colonies from the KF streptococcal agar plates were inoculated into ethyl violet azide broth. After incubation at 35 C for 48 ± 2 h, tubes exhibiting a yellow color and sediment were reported as confirmed fecal streptococci.

Salmonellae analyses: The procedure in BAM (40) for raw and highly contaminated products utilizing selenite and tetrathionate broths was used to determine the presence of Salmonellae. Colonies exhibiting positive reactions from this procedure were verified biochemically using the API 20E Enterobacteriaceae System and serologically following procedures outlined in Identification of Enterobacteriaceae (43).

Isolation and identification of aerobic bacteria: Gram-negative and gram-positive organisms were isolated and identified by use of the methods described by Guthertz et al. (44).

Virological Analyses

Tissue culture: The following cell lines were used: African green monkey kidney (Vero) (American Tissue Culture Association, Rockville, MD) and bovine turbinate (BT-8) (courtesy of Dr. B. Casto, Biolabs, Inc., Northbrook, IL). The cells were seeded in 25 cm^2 plastic flasks at concentrations adequate for the formation of confluent monolayers

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in time for the test. The cells were incubated at 37 C in a humidified atmosphere of 5% CO_2 in air.

Virus: Poliovirus type 1 (POL-1) (courtesy of Dr. G. French, Fort Detrick, MD) was used as the test virus. The stock was prepared by passing the virus in vero cultures. The cultures with advanced cytopathic effect (CPE) were freeze-thawed 2 times and cleared by low speed centrifugation. The supernatant was stored in 1 ml aliquots at -70 C until used. Infectious bovine rhinotracheitis (IBR) (courtesy of Dr. B. Casto, Biolabs Inc., Northbrook, IL) was also used. Using BT-8 cells, IBR stock was prepared as described above for POL-1.

Growth Medium: Eagle's minimal essential medium with Earle's salts (MEM) was supplemented with 10% bovine fetal serum (BFS) for Vero, or 10% horse serum (HS) for BT-8 cells. The sera had been screened for virus and mycoplasma and were heat inactivated (HI) for 30 min at 56 C. The medium was also supplemented with 1% non-essential amino acids (100 x), 1% L-glutamine (200 mM), penicillin (100 U/ml), Streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml). A 7.5% solution of $NaHCO_3$ was used to adjust the pH to 7.2.

Sample Processing Medium (MEM-Tris): MEM with the supplements for growth medium and 2% BFS HI was used for sample processing except that 0.05 M Tris-buffer was substituted for $NaHCO_3$ to adjust the pH at 8.0.

Agar Overlay Medium: The agar overlay consisted of 1% purified agar in modified Eagle's medium supplemented with 4% BFS HI, 1% L-glutamine, gentamicin (50 µg/ml) and 0.01% $NaHCO_3$. This medium was prepared by mixing equal volumes of 2 x Eagle's medium with the above mentioned supplements and a sterile suspension of 2% purified agar in deionized water. Each component was tempered at 43 C before mixing.

Virus Detection, Plaque Forming Unit (PFU) Assay: The principles of the double overlay technique (45) were applied by using 25 cm^2 flasks of confluent monolayer of Vero and BT-8 cells. After removal of tissue culture fluid, 0.1 ml of sample suspension was inoculated per flask. Samples of food suspensions before and after concentration were inoculated in duplicate flasks. Controls included: (a) Vero cells inoculated with stock POL-1 and BT-8 cells with IBR virus (0.1 ml of 10-fold dilutions); and (b) cells inoculated with MEM-Tris. After one hour for adsorption at 37 C in a 5% CO_2 humidified incubator, each flask received 4 ml of agar overlay medium. The agar was allowed to solidify and the flasks were incubated in the inverted position. After 3 days incubation, 4 ml of agar overlay medium with 1.8% of 1:300 neutral red was added. Plaques were counted with the aid of an X-ray film viewer 6, 24, and 72 hours after overlaying.

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Procedures for Sample Processing: Thirty-one samples of GB and TSP were tested. A 25 g sample was placed in a sterile plastic bag, kneaded and suspended in 75 ml of MEM-Tris. Individually, the bags were vigorously shaken by hand then placed on a low speed shaker for 15 min. If necessary, the pH was readjusted to 8.0 with Tris buffer, and the bags were returned to the shaker for an additional 10 min. Each suspension was transferred to two 50 ml conical tubes and centrifuged for 10 min (200 x G at 25 C). Each sample supernatant was passed through a glasswool column (0.3 g glasswool loosely packed in a 60 ml syringe barrel) onto a diatomaceous earth filter (Celite, Johns-Manville Products Corp, Lompoc, CA). The Celite filter was prepared by pouring 250 ml of 2.6% suspension of washed Celite in deionized water onto an 11 cm Whatman No. 1 filter in a Buchner funnel. The water was removed with partial vacuum. The Buchner funnel was transferred to a sterile filtration flask to receive the food sample filtrate. Vacuum was used to promote this filtration and to keep the Celite packed. After filtration, a 1.0 ml aliquot of food suspension was saved for virus isolation and remainder transferred to an assembled 47 mm molecular filtration cell (MFC) (Millipore Corp, Bedford, MA) with a Pellicon ultrafiltration membrane filter (PTHK, 1 x 10^5 molecular wt retention) (Millipore Corp, Bedford, MA). Five such MFCs were attached to a Pellicon Carrousel Manifold (Millipore Corp, Bedford, MA). With the application of nitrogen pressure (40 psi) to the MFC, the five samples were each concentrated to 1.5 ml or less within 2 hours. A 0.1 ml aliquot of concentrate was used for virus isolation and the remainder stored in sterile vials at -70 C for further testing if needed.

Sensitivity of POL-1 virus recovery: To determine the sensitivity of virus recovery, POL-1 virus in 5 ml of MEM-Tris was added to 25 g of sample. After kneading for 2 minutes to disperse the virus throughout, the sample was processed as described above. The POL-1 virus stock used to contaminate the sample, as well as aliquots of the food sample suspensions that were collected after concentration, was tested for virus by the plaque assay. The sensitivity of virus recovery was calculated as the number of plaque forming units (PFU) detectable per gram of the original food sample using the following equation:

$$\text{Sensitivity (PFU/gm)} = \frac{\text{Total Virus Added (PFU)}}{\text{Virus Detected (PFU)*}} \div \frac{\text{wt of food}}{\text{sample (gm)}}$$

* Number of PFU in 0.1 ml of the food sample suspension after concentration (average of duplicate Vero flasks).

The percent of virus recovery was determined as follows:

$$\text{Percent of virus recovery} = \frac{\text{Total virus detected (PFU)}}{\text{Total virus added (PFU)}} \times 100$$

Statistical Analyses: Friedman's two-way analysis of variance with multiple comparisons based upon rank sums was applied to data

obtained by the enumeration procedures (46). The Wilcoxon matched-pairs signed-ranks test was applied to determine if significant differences existed between APC and PPC, CPC and CMPN, and EPC and EMPN (46). All statistical analyses were done with $\alpha = .05$ level of significance.

RESULTS

The aerobic plate count (APC) distributions for ground beef (GB), textured soy protein (TSP), and TSP extended ground beef (SGB) for the 3- and 10-day storage times are presented in Table 1. Analyses of the data show that 96.8% of the GB samples at 3 days had APCs of less than 5×10^6 organisms per gram while 90.3% of the TSP and SGB samples evaluated had APCs of less than this value. After 10 days' storage at 4 C this pattern was significantly altered with 26.7, 24.1, and 10.0% of the GB, TSP, and SGB samples having APCs of less than 5×10^6 organisms per gram, respectively.

Statistical analyses of these data showed a significant increase in APCs after 10 days' storage for all products (Table 2). It is interesting to note that significant differences existed between products at 3 days' storage; however, after 10 days' storage no significant differences in APCs existed for all products analyzed.

Psychrotrophic plate count (PPC) (Table 3) distributions were similar to the APC determinations. After the 3-day storage, 93.6% of the GB, 90.3% of the TSP and 87.1% of the SGB units resulted in PPCs of less than 5×10^6 organisms per gram. The 10-day determinations resulted in 10.0, 24.1 and 0.0% of the GB, TSP and SGB with counts of less than 5×10^6/g. As found in the APC determinations, significantly higher PPCs were demonstrated after storage for 10 days at 4 C. The same pattern of differences among 3-day and 10-day determinations for APC was also found in the PPCs (Table 2).

The APC and PPC procedures were compared using the Wilcoxon matched-pairs, signed-ranks test (Wilcoxon Test) (46). With the exception of the 3-day SGB and the 10-day TSP, the PPC determinations were significantly higher than the corresponding APC determinations for the products at both 3- and 10-day storage times.

Total coliform determinations were performed by both the plate and most probable number method. Coliform plate count (CPC) distributions are presented in Table 4. Following the 3-day storage period, 90.3, 100.0 and 80.7% of the GB, TSP and SGB samples contained fewer than 1×10^3 coliform organisms per gram. These percentages decreased somewhat with an additional 7 days' refrigerated storage resulting in 53.3, 96.7and 63.3% of the GB, TSP and SGB samples, respectively, having less than 1×10^3 coliforms per gram. With the exception of

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46. Hollander, M. and Wolfe, D.A., Nonparametric Statistical Methods (First edition), 1973, pp. 27-33, 139-146, 151-158

the 10-day GB all of the products had total coliform counts of less than 1×10^4/g. Statistical analyses of these data showed that no significant difference existed between the counts obtained from the 3- or 10-day storage periods. Additionally, no significant difference was indicated in the coliform counts from GB and SGB (Table 2).

Coliform most probable number (CMPN) determinations (Table 5) were similar to the CPC results, however, some interesting differences were noted. Statistical analyses of these data indicated that no significant difference existed between 3- and 10-day determinations for TSP and SGB, however, the 10-day GB coliform determinations were significantly higher than the 3-day results (Table 2).

The CPC and CMPN procedures were compared with the Wilcoxon test (46). Except for the 3-day TSP determinations, the CMPN procedure resulted in significantly higher counts than the CPC procedure. Although the CMPN counts for the 3-day TSP were higher than the CPC results there was no significant difference in the counts at the 95% confidence level.

E. coli determinations were performed utilizing both the plate and MPN procedures. Results of the E. coli plate count (EPC) analyses are presented in Table 6. Among the 3-day analyses only 64.5, 93.6 and 48.4% of the GB, TSP and SGB samples resulted in counts of less than 50/g. The 10-day analyses were similar with 63.3, 96.7 and 46.7% of the GB, TSP and SGB samples containing less than 50 E. coli per gram.

Statistical analyses (Table 2) revealed that no significant differences were present when comparing the EPCs after 3 and 10 days' storage. No significant difference was indicated between counts from GB and SGB, and GB and TSP. However, a significant difference in counts from TSP and SGB was noted.

The E. coli MPN (EMPN) determinations produced count distributions similar to those found in the EPC procedure (Table 7). The 3-day data show that 74.2, 100.0 and 35.5% of the counts for GB, TSP and SGB had less than 50 E. coli per gram. The 10-day determinations resulted in 66.7, 100.0 and 46.7% of the GB, TSP and SGB with E. coli counts of less than 50/g. Only 8.2% of all TSP samples tested contained E. coli, while 78.7 and 96.7% of the GB and SGB samples were E. coli positive.

Statistical analyses of the EMPN data showed that there was no significant difference in the counts obtained after 3 and 10 days' storage; however, the 10-day determinations were lower than the 3-day determinations in all cases (Table 2). E. coli counts from TSP were significantly lower than counts from GB and SGB.

Comparison of the EPC and EMPN procedures by the Wilcoxon test (46) indicated: (1) no significant differences were shown for GB and

TSP at the 3- and 10-day storage intervals; (2) the EMPN counts for the 3- and 10-day SGBs were significantly higher than the EPC counts for the same intervals; (3) with the exception of the 3-day TSP, all of the EMPN determinations were higher than the corresponding EPC determinations.

S. aureus MPN (SMPN) distributions are presented in Table 8. Less than 7% of the samples for all product types and both storage times had SMPN counts in excess of 150/g. Statistical analyses (Table 2) indicated that no significant differences were found between GB and SGB for the 3- or 10-day storage time and the 3- and 10-day TSP determinations were significantly different from all other determinations.

Fecal streptococci plate count (FSC) distributions are outlined in Table 9. Only 6.4, 0.0 and 9.7% of the GB, TSP and SGB 3-day analyses produced FSCs in excess of 1000/g. For the 10-day analyses, 0.0, 3.3 and 10% of the GB, TSP and SGB samples exceeded 1000 fecal streptococci per gram. There were no significant differences indicated between counts obtained after 3 and 10 days' storage for all product types (Table 2). However, 3- and 10-day TSP determinations differed significantly from all others.

The aerobic gram-negative organisms isolated from each product after 3 and 10 days' storage are presented in Table 10. The most frequent isolates from the 3-day GB, in order of occurrence, were Escherichia coli, Enterobacter cloacae, Citrobacter freundii, Acinetobacter calcoaceticus var. anitratum, Klebsiella pneumoniae, Aeromonas hydrophila, Proteus vulgaris, and Enterobacter hafniae. Arizona hinshawii (Salmonella arizonae) was isolated from 1 sample. A number of changes in percent of samples positive were noted after 10 days' storage at 4 C. E. coli predominated as the most frequently isolated organism with E. hafniae, C. freundii, E. cloacae, A. calcoaceticus var. anitratum, Serratia liquefaciens, K. pneumoniae and A. hydrophila following. Salmonella enteriditis ser. worthington was isolated from 1 sample of the 10-day GB.

The 3-day TSP contained considerably fewer gram-negative organisms than the ground beef. Isolates occurring most frequently included E. coli, C. freundii, and A. calcoaceticus var. anitratum all of which occurred in less than 10% of the samples. S. enteriditis ser. worthington was isolated from 1 sample of 3-day TSP. Isolates from the 10-day TSP presented a somewhat different picture than the 3-day analyses. E. hafniae and S. liquefaciens were isolated most often followed by Pseudomonas fluorescens grp., E. coli and C. freundii. In one sample, S. enteriditis ser. worthington was isolated (same lot as the 3-day isolate). One isolate of Yersinia enterocolitica was found in the 10-day TSP.

Fewer types of organisms were isolated from SGB than GB. After 3 days' storage E. coli was the predominant organism isolated followed

by K. pneumoniae, E. cloacae, A. calcoaceticus var. anitratum, C. freundii, Pseudomonas sp., and E. hafniae; A. hinshawii (S. arizonae) was isolated from 1 sample. After 10 days' refrigeration there were even fewer species of organisms found, however, substantial increases in the occurrence of some species were observed. E. coli remained the predominant isolate with E. hafniae, A. calcoaceticus var. anitratum, K. pneumoniae, C. freundii, E. cloacae, S. liquefaciens, Pseudomonas sp., A. hydrophila and P. vulgaris following in order of occurrence.

Gram-positive organisms isolated from GB, TSP and SGB are shown in Table 11. The most frequent isolates from GB at 3 days' storage were Clostridium perfringens, Streptococcus faecalis var. liquefaciens, Staphylococcus epidermidis, Staphylococcus aureus, and Bacillus cereus. After 10 days' storage at 4 C there was an overall reduction in the number of gram-positive isolates. C. perfringens remained the predominant organism with 73% of the samples positive. The incidence of S. aureus and S. faecalis increased while the incidence of the majority of other isolates decreased.

The 3-day TSP produced few gram-positive isolates with Bacillus sp., B. cereus and C. perfringens being isolated most frequently. Following the 10-day storage period the percent of samples positive for C. perfringens, B. cereus, and S. faecalis var. liquefaciens was reduced. The percent recovery of all other gram-positive organisms was increased.

The 3-day SGB contained the largest variety of isolates and in most cases the highest number of positive samples for all products tested. C. perfringens remained as the most frequently isolated organism (96.8% positive). Micrococcus sp., S. epidermidis, B. cereus, diphtheroids, S. faecalis var. liquefaciens, and S. aureus were all present in at least 50% of the samples.

In the 10-day SGB the previous pattern of isolates was found with few exceptions. However, B. cereus, diphtheroids and the S. aureus isolations were notably reduced. Several Streptococcus sp. and Bacillus sp. showed a marked increase in occurrence.

The percent recovery of C. perfringens after using various isolation procedures is presented in Table 12. C. perfringens was recovered by at least one of the nine isolation procedures from 68% of all units analyzed. It was found in only 40% of the units with the use of the SPS agar pour plate procedure. C. perfringens counts obtained directly on SPS agar pour plates ranged from $<30/g$ to $>10^3/g$. Only one unit was found to be positive by all eight enrichment isolation procedures and the SPS agar pour plate method. C. perfringens was isolated from 60% of all units when samples were blended and from 59% of all units when samples were not blended. However, it was isolated concurrently by both blended and unblended methods from 51% of all units analyzed.

The isolation percentages by method for all food categories from unblended samples incubated and/or heat shocked at 37, 46, 75 and 95 C

were 47, 48, 9 and 5%, respectively. Similarly isolations from blended samples treated at the temperatures stated above were obtained from 44, 46, 8 and 1% of the units, respectively. When the data from blended and unblended isolation methods were grouped, C. perfringens was isolated from samples incubated and/or heat shocked at 37, 46, 75 and 95 C from 58, 56, 14 and 6% of the units, respectively.

Friedman's two-way analysis of variance with multiple comparisons based upon rank sums was applied to the sample means (46). This test showed there was no difference in isolation efficiency at the 5% level between the blended and unblended samples incubated at 46 C and unblended at 37 C. These methods were significantly superior to all other methods. The blended samples incubated at 37 C were the second most efficient method followed by the SPS agar pour plate method. Both of these methods were significantly different from all other recovery methods. Recoveries from blended and unblended samples heat shocked at 75 C were not statistically different at the 5% level. In addition, isolation from samples blended and heat shocked at 75 C was not significantly different from unblended samples heat shocked at 95 C. Isolation from blended samples heat shocked at 95 C was significantly lower than all other isolation methods. From samples heat shocked at 75 C, only 2 units yielded isolates not also isolated at 37 or 46 C. Heat shocking at 95 C yielded no additional isolates.

C. perfringens was isolated from 97 and 73% of GB, 26 and 20% of TSP, and 97 and 90% of SGB units after storage for 3 and 10 days, respectively. Overall, C. perfringens was isolated from 85, 23 and 94% of the GB, TSP and SGB units, respectively.

There were no enteroviruses detected in the GB and TSP samples tested in this study. Evaluation of the enterovirus detection method showed that viral concentrations >2.4 PFU/gm could be detected in ground beef.

DISCUSSION

The microbial quality of raw ground beef has been well documented. In a recent report by Foster et al. (21), studies of the microbial quality of raw ground beef for the past 63 years were tabulated. Although numerous reports pertaining to the microbial quality of raw ground beef are available, few studies have investigated the microbial quality of soy protein extended ground beef (SGB) (9,11,22-24).

The addition of textured soy protein (TSP) appears to have no effect on the total microbial load of regular ground beef (GB). Statistical analysis of the data in Table 1 shows that there was no significant difference in the APCs from GB and SGB at either 3 or 10 days' storage. However, a significant difference existed between 3-day APCs and 10-day APCs for both products. This indicates that the addition of TSP has no effect on the APC even after a 10-day storage period. This same result was found in the analyses of the PPC (Tables 2 and 3). Comparison

of the APC and PPC data by the Wilcoxon test (46) indicated that the PPCs were significantly higher than the APCs in all but two cases. This indicates that the predominant microbial flora in raw beef products is psychrotrophic in nature and that current incubation temperatures (i.e., 30, 32 and 35 C) for testing raw meat products are questionable. This point is supported by Goepfert (47), Westhoff and Feldstein (20) and Foster et al. (48).

The APCs for products stored at 4 C in this study were in agreement with the findings of Judge et al. (9) and Craven and Mercuri (22). Analysis of the 3- and 10-day APC data in this study agrees with the findings of Judge et al. (9) who found significant increases in plate counts in soy protein extended ground beef after 7 days' storage at 4 C. Additionally, they reported significant differences in plate counts initially and no significant difference in plate counts after 7 days' storage when comparing soy extended and regular ground beef. Craven and Mercuri (22) showed that the APC increased faster in beef patties extended with textured soy protein than in regular ground beef patties. Also they found that the counts increased over storage time for all samples. Craven and Mercuri (22) found 2.5 to 3 $\log_{10}$ increases in the APC for hydrated textured soy protein over an 11-day storage period at 4 C.

As previously cited, the State of Oregon has revoked its APC standard of 5×10^6/g for ground beef. Although this standard is no longer in effect it will be retained as a guideline for use by state regulatory agencies involved in the sanitary inspection of retail meat stores. This guideline includes all raw meat products including soy protein extended ground beef. Comparing the APC data to the Oregon guideline, we found that 96.8% of the GB and 90.3% of the SGB samples were in compliance after the 3-day storage time (Table 1). Comparison of the PPC data showed that 93.6% of the GB and 87.1% of the SGB samples would comply after the 3-day storage time (Table 3). After 10 days' storage the percent of samples which comply with the Oregon guideline was dramatically reduced (Tables 1 and 3). This shows that ground meat products can be produced in compliance with what some consider an extremely rigid guideline, however, as expected these percentages are reduced with increased storage time. At this time the use of microbiological standards, with all their legal and enforcement complications, to ensure the quality of various food items seems questionable. Alternatively, the use of microbiological guidelines coupled with increased sanitary inspection and laboratory testing, and cooperation with the industry could result in a product of improved microbial quality and longer shelf-life.

The coliform analyses presented in Tables 4 and 5 show distributions similar to those previously reported for ground beef (20,21,47). Statistical analyses showed that no significant differences in counts existed when comparing ground beef with or without soy protein. These

47. Goepfert, J.M., J Milk Food Technol. 39:175, 1976
48. Foster, J.F. et al, J Food Protect. 40:300, 1977

results are in conflict with the findings of Craven and Mercuri (22) who reported that coliform counts increased with the addition of soy protein in beef patties. Only in the case of raw ground beef without soy protein did the coliform count increase significantly during refrigerated storage.

Statistical comparison of the coliform plate and MPN procedures showed that the most probable number procedure yielded significantly higher counts for all products except the 3-day TSP. Since the manufacturing steps necessary to produce TSP include high pressure heat extrusion, the result should be a product with a low bacterial load. Therefore, the fact that no significant difference existed between the MPN and plate determinations for the 3-day TSP was not unexpected.

Currently, eight states have microbial guidelines based upon coliform counts (49). These guidelines range from 1×10^2 to 1×10^4 organisms per gram. Fowler et al. (50) recently reported that a coliform plate count limit of 1×10^4/g for ground beef appears to be feasible. This recommendation was based upon the analyses of 1856 samples of ground beef. Comparing the data from this study to the limit of 1×10^4/g, 100% of the GB and SGB samples after the 3-day storage time were in compliance, using the coliform plate method (Table 4). However, when utilizing the most probable number procedure it was found that only 90.3% of the GB and 96.8% of the SGB samples would comply with the limit of 1×10^4 coliforms per gram. The percent of samples in compliance was reduced after the 10-day storage time. This finding re-emphasizes the point that ground beef is a product of limited shelf-life and that improved sanitary conditions during processing which favor lower microbial populations would be beneficial to both the consumer and producer.

The E. coli counts were determined by both the plate and MPN procedures. The results of the E. coli analyses, as determined by both procedures, indicated that the addition of TSP had no effect on the E. coli count. Comparisons of the different procedures indicated that the MPN method gave higher counts but these counts were not significantly higher.

There are eleven states that have microbiological guidelines for E. coli in ground beef. These guidelines range from 0 to 1×10^3 organisms per gram. The majority of the states use the value of 50 E. coli per gram as their guideline. Comparing the E. coli plate count data to this guideline, 64.5% of the GB and 48.4% of the SGB samples would be acceptable after the 3-day storage time. These percentages changed very little after an additional 7 days' storage. Comparison of the E. coli MPN data was quite different, with 74.2% of the GB and 35.5% of the SGB samples in compliance after the 3-day storage time. The 10-day storage data showed 66.7% of the GB and 46.7% of the SGB samples

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49. Wehr, H.M., 37th Annual Meeting, Institute of Food Technologists, 1977
50. Fowler, J.L. et al, J Food Protect. 40(3):166, 1977

having E. coli counts of 50 or less. The wide difference in the 3-day values of GB and SGB was probably due to the dilution of the GB with TSP. Only 8.2% of all TSP samples tested contained E. coli and 100% of the samples tested by the MPN method had values less than 50/g at both 3 and 10 days' storage (Table 7).

S. aureus as determined by the MPN method was found in limited numbers. Less than 7% of all the samples tested had SMPN counts in excess of 150/g. The recovery of S. aureus in low numbers from ground beef is in concurrence with the findings of a number of other investigators (11,19,21,51). Currently, eight states have guidelines pertaining to the numbers of S. aureus allowable in ground beef. These limits range from 0 to 2.5×10^2 S. aureus organisms per gram. The samples tested in this study compared favorably to these guidelines. Although S. aureus is recognized as a potential food poisoning organism, to date there have been no reported cases of S. aureus food poisoning from ground beef. This could be attributed to the fact that this organism's ability to compete with the microbial flora of ground beef is questionable (19,52). However, S. aureus is a potential hazard and should be handled in a manner which will minimize the possibility of the growth of this organism.

The fecal streptococci determinations indicated that the addition of TSP to GB and/or refrigerated storage did not effect an increase in this group of organisms.

A review of the literature revealed only one report where specific organisms from ground beef and soy extended ground beef were isolated (22). In both ground beef patties and soy extended ground beef patties, Serratia and Enterobacter were the predominant genera reported. Gram-positive organisms were not identified. In this study a more complete investigation of specific organisms present in GB, TSP and SGB was performed. E. coli was the predominant isolate found in GB and SGB at the 3-day and 10-day sampling periods. Specific organisms of public health significance which were isolated include S. enteriditis ser. worthington, E. coli, K. pneumoniae and A. hinshawii. E. hafniae and S. liquefaciens showed the largest increase in the percent of samples positive after 10 days' storage at 4 C. E. cloacae and K. pneumoniae showed the largest decrease in percent of samples positive after 10 days' storage at 4 C. There was no indication that the addition of soy protein to the ground beef had any stimulatory effect upon any one organism or group of organisms. However, it must be noted that this cannot be directly shown from these data since all organisms present in each sample were not identified, only morphologically different organisms were examined. Overall the SGB contained fewer species of organisms than the GB. This might be due to the extension of the product with TSP. The TSP contained few species of gram-negative organisms with

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51. Emswiler, B.S. et al, Appl Environ Microbiol. 31:826, 1976
52. Goepfert, J.M. and Kim, H.U., J Milk Food Technol. 38:449, 1975

many samples yielding no isolates. Since the SGB in this study contained 20% TSP by weight, the result would be a product with a reduced microbial load. The fact that fewer species of gram-negative organisms were present in the SGB after 10 days' storage at 4 C suggests that either the product was not a favorable growth medium or that they were overgrown by other microorganisms adaptable to cold storage and able to utilize available nutrients.

In contrast, the gram-negative isolation procedures which showed that fewer organisms were present in the SGB than the GB, the gram-positive isolations showed the opposite (Table 11). Overall, SGB yielded a larger variety of gram-positive organisms than either of its two components, which further indicates that soy protein when combined with ground beef offers some form of protection to organisms normally susceptible to refrigerated storage. C. perfringens was the predominant isolate found in the beef products, while Bacillus sp. predominated in the TSP. The presence of C. perfringens in ground meat products in low numbers has been well documented (51,53,54). Therefore, the high recovery rates of this organism were unexpected. Studies by Schroder and Busta (23,24) and Kokoczka and Stevenson (55) have indicated that soy protein extension of ground beef products has variable effects on the growth of C. perfringens. Further studies of the characteristics of C. perfringens have shown that this organism has limited ability to survive refrigerated storage (56,57,58). The results of this study also indicate that the survival of C. perfringens is reduced with refrigerated storage. However, a reduction of 23.5% in the frequency of positive samples was found in GB while only a 6.8% reduction was shown in SGB after 10 days' refrigerated storage. Additionally, a reduction of 5.8% was noted in the TSP after the same storage period. This finding could indicate that the addition of soy protein provides some protection for C. perfringens during refrigerated storage. If this is the case, food handlers should be aware of the extended potential of these food products to cause food poisoning.

Generally a low level of C. perfringens was found in all products as would be expected from refrigerated meat products. The need for heat shocking during isolation appears to be unnecessary. The use of enrichment incubation temperatures of 46 C for either blended or unblended samples and 37 C for unblended samples were most effective in the recovery of C. perfringens from minimally contaminated foods.

53. Ladiges, W.C. et al, J Milk Food Technol. 37:622, 1974
54. U.S. Dept of Health, Education, and Welfare, Public Health Service. Morbidity and Mortality. 24:229, 1975
55. Kokoczka, P.J. and Stevenson, K.E. J Food Sci. 41:1360, 1976
56. Fruin, J.T. Ph.D. Thesis, Purdue Univ., West Lafayette, IN, 1974
57. Woodburn, M. and Kim, C.H. Appl Microbiol. 14:914, 1966
58. Strong, D.H. and Ripp, N.M. Appl Microbiol. 15:1172, 1967

Few isolations of enteroviruses from foods have been reported. However, Sullivan et al. (28) reported isolation enteroviruses from ground beef. The fact that no enteroviruses were isolated in our study could be attributed to the small sample size utilized or ideally to the absence of enteroviruses in the product. Evaluation of the enterovirus screening method used in this study showed that viruses could be detected in concentrations of >2.4 PFU/gm (Table 13).

RECOMMENDATIONS AND CONCLUSIONS

1. The addition of textured soy protein appears to have no effect on the total microbial load of regular ground beef.
2. The coliform most probable number method produced significantly higher counts than the plate method.
3. The microflora of the products tested appears to be psychrotrophic in nature because the PPCs were significantly higher than the APCs.
4. The predominant gram-negative isolate of GB and SGB was Escherichia coli yet few gram-negative organisms were found in TSP.
5. The predominant gram-positive isolate from GB and SGB was Clostridium perfringens while the genus Bacillus was most frequently found in the TSP.
6. The use of TSP as an extender in GB appeared to have a cryoprotective effect upon C. perfringens, however, additional investigations are necessary to show a direct effect.
7. Although potentially pathogenic organisms were isolated from SGB, this product is no more or less hazardous than GB, if properly handled.
8. Additional studies to evaluate the effect of TSP on specific foodborne pathogenic organisms are warranted.
9. Evaluation of currently accepted incubation times and temperatures for meat analyses is needed.

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TABLE 1: Aerobic plate counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

Log_{10} Plate Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤2.7					19	61.3	1	3.5				
2.8 to 3.0					5	77.4	1	6.9				
3.1 to 3.7					1	80.7	1	10.3				
3.8 to 4.0	1	3.2			1	83.9	1	13.8				
4.1 to 4.7	7	25.8			1	87.1	1	17.2				
4.8 to 5.0	1	29.0			1	90.3						
5.1 to 5.7	13	70.9							12	38.7		
5.8 to 6.0	4	83.9					2	24.1	8	64.5		
6.1 to 6.7	4	96.8	8	26.7					8	90.3	3	10.0
6.8 to 7.0			2	33.3	1	93.5	3	34.5	1	93.6	3	20.0
7.1 to 7.7			7	56.7	1	96.8	7	58.6			9	50.0
7.8 to 8.0			4	70.0			2	65.5			6	70.0
8.1 to 8.7			5	86.7			7	89.7			7	93.3
8.8 to 9.0			2	93.3			1	93.1				
>9.0	1	100.0	2	100.0	1	100.0	2	100.0	2	100.0	2	100.0
TOTAL UNITS	31		30		31		29		31		30	
RANGE[c]	3.9 to 9.5		6.2 to 9.5		1.5 to 9.5		1.8 to 9.5		5.3 to 9.5		6.5 to 9.5	
MEAN[c]	5.4		7.5		3.0		7.0		6.1		7.7	

[a] U - Number of samples within each count range

[b] CP - Cumulative Percentage of samples

[c] Log_{10} of counts

TABLE 2: Friedman two-way analysis of variance with multiple comparisons for ground beef (GB), textured soy protein (TSP) and TSP extended ground beef (SGB) after 3 days' and 10 days' storage*

Aerobic Plate Count -

TSP-3**	GB-3	SGB-3	TSP-10	GB-10	SGB-10

Psychrotrophic Plate Count -

TSP-3	GB-3	SGB-3	TSP-10	GB-10	SGB-10

Coliform Plate Count -

TSP-3	TSP-10	GB-3	SGB-3	SGB-10	GB-10

Coliform MPN Count -

TSP-3	TSP-10	GB-3	SGB-3	SGB-10	GB-10

E. coli Plate Count -

TSP-10	TSP-3	GB-10	GB-3	SGB-10	SGB-3

E. coli MPN Count -

TSP-10	TSP-3	GB-10	GB-3	SGB-10	SGB-3

S. aureus MPN Count -

TSP-3	TSP-10	GB-3	GB-10	SGB-10	SGB-3

Fecal streptococci Count -

TSP-10	TSP-3	GB-10	GB-3	SGB-10	SGB-3

* Mean counts are ranked from lowest to highest (left to right).

** Products underscored by the same line are not significantly different at the .05 level.

TABLE 3: Psychrotrophic plate counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

Log_{10} Plate Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤2.7					23	74.2	1	3.5				
2.8 to 3.0					1	77.4						
3.1 to 3.7	1	3.2			2	83.9	2	10.3				
3.8 to 4.0					1	87.1	1	13.8				
4.1 to 4.7	7	25.8					1	17.2				
4.8 to 5.0	2	32.3			1	90.3						
5.1 to 5.7	12	71.0							10	32.3		
5.8 to 6.0	1	74.2							9	61.3		
6.1 to 6.7	6	93.6	3	10.0			2	24.1	8	87.1		
6.8 to 7.0	1	96.8	1	13.3	1	93.5	2	31.0			2	6.7
7.1 to 7.7			8	40.0	1	96.8	6	51.7	3	96.8	2	13.3
7.8 to 8.0			7	63.3			3	62.0			7	36.7
8.1 to 8.7	1	100.0	8	90.0	1	100.0	6	82.8			13	80.0
8.8 to 9.0			2	96.7			3	93.1			5	96.7
>9.0			1	100.0			2	100.0	1	100.0	1	100.0
TOTAL UNITS	31		30		31		29		31		30	
RANGE[c]	3.3 to 8.4		6.3 to 9.1		<0 to 8.7		2.9 to 9.1		5.1 to 9.3		6.8 to 9.2	
MEAN[c]	5.4		7.7		2.7		7.1		6.1		8.2	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 4: Coliform plate counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

Log_{10} Plate Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
<0	5	16.1	3	10.0	28	90.3	25	83.3	1	3.2	2	6.7
0 to 0.7												
0.8 to 1.0												
1.1 to 1.7	6	35.5	1	13.3			1	86.7	2	9.7	1	10.0
1.8 to 2.0	5	51.6	5	30.0	1	93.6			6	29.0	3	20.0
2.1 to 2.7	11	87.1	6	50.0	2	100.0	3	96.7	13	70.9	10	53.3
2.8 to 3.0	1	90.3	1	53.3					3	80.7	3	63.3
3.1 to 3.7	3	100.0	7	76.7			1	100.0	5	96.8	7	86.7
3.8 to 4.0									1	100.0	4	100.0
4.1 to 4.7			5	93.3								
>4.7			2	100.0								
TOTAL UNITS	31		30		31		30		31		30	
RANGE[c]	<0 to 3.5		<0 to 5.5		<0 to 2.3		<0 to 3.2		<0 to 3.9		<0 to 4.0	
MEAN[c]	1.8		2.8		0.1		0.4		2.4		2.6	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 5: Coliform Most Probable Number (MPN) counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

MPN Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤3	1	3.2			27	87.1	20	66.7				
3.6 to 10					1	90.3	2	73.3				
20 to 42	2	9.7					2	80.0				
43 to 64	4	22.6	1	3.3					1	3.2	1	3.3
72 to 150	3	32.3	1	6.7	1	93.5			3	12.9		
160 to 460	11	67.7	4	20.0			2	86.7	14	58.1	3	13.3
530 to 1100	2	74.2	3	30.0	1	96.7	1	90.0	7	80.7	4	26.7
1200 to 9500	5	90.3	9	60.0	1	100.0			5	96.8	7	50.0
>11000	3	100.0	12	100.0			3	100.0	1	100.0	15	100.0
TOTAL UNITS	31		30		31		30		31		30	
RANGE[c]	<0 to 4.7		<0 to 6.0		<0 to 3.4		<0 to 4.4		1.6 to 4.2		1.6 to 6.0	
MEAN[c]	2.5		3.7		0.3		0.9		2.8		3.7	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 6: Escherichia coli plate counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

Log_{10} Plate Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
<0	13	41.9	18	60.0	29	93.6	28	93.3	9	29.0	14	46.7
0 to 0.7												
0.8 to 1.0												
1.1 to 1.7	7	64.5	1	63.3			1	96.7	6	48.4		
1.8 to 2.0	4	77.4	2	70.0			1	100.0	2	54.8	3	56.7
2.1 to 2.7	5	93.6	4	83.3	2	100.0			7	77.4	8	83.3
2.8 to 3.0			1	86.7					2	83.9	1	86.7
3.1 to 3.7	2	100.0	4	100.0					4	96.8	3	96.7
3.8 to 4.0									1	100.0	1	100.0
TOTAL UNITS	31		30		31		30		31		30	
RANGE[c]	<0 to 3.2		<0 to 3.7		<0 to 2.3		<0 to 1.7		<0 to 3.8		<0 to 3.7	
MEAN[c]	1.1		0.9		0.1		0.1		1.7		1.2	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 7: *Escherichia coli* Most Probable Number (MPN) counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

MPN Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤3	4	12.9	9	30.0	28	90.3	28	93.3	1	3.2	2	6.7
3.6 to 19	12	51.6	6	50.0	2	96.8			4	16.1	1	10.0
20 to 42	6	70.9	3	60.0	1	100.0	1	96.7	2	22.6	5	26.7
43 to 64	1	74.2	2	66.7			1	100.0	4	35.5	6	46.7
72 to 150	3	83.9	5	83.3					7	58.1	7	70.0
160 to 460	5	100.0	1	86.7					6	77.4	3	80.0
530 to 1100			2	93.3					4	90.3	4	93.3
1200 to 9500			2	100.0					3	100.0	2	100.0
TOTAL UNITS	31		30		31		30		31		30	
RANGE[c]	<0 to 2.7		<0 to 3.2		<0 to 1.4		<0 to 1.6		<0 to 3.7		<0 to 3.4	
MEAN[c]	1.3		1.3		0.1		0.1		2.1		1.9	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 8: Staphylococcus aureus Most Probable Number (MPN) counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

MPN Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤3	11	35.5	6	20.0	30	96.8	28	93.3	2	6.5	3	10.0
3.6 to 19	8	61.3	13	63.3	1	100.0	1	96.7	7	29.0	7	33.3
20 to 42	3	70.9	5	80.0			1	100.0	7	51.6	10	66.7
43 to 64	3	80.7	3	90.0					8	77.4	4	80.0
72 to 150	4	93.6	1	93.3					6	96.8	5	96.7
160 to 460	2	100.0	1	96.7					1	100.0	1	100.0
1200 to 9500			1	100.0								
TOTAL UNITS	31		30		31		30		31		30	
RANGE[c]	<0 to 2.7		<0 to 3.4		<0 to 0.6		<0 to 1.4		<0 to 2.7		<0 to 2.4	
MEAN[c]	0.9		1.0		.02		0.1		1.4		1.3	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 9: Fecal streptococci counts for ground beef, textured soy protein (TSP) and TSP extended ground beef

Log_{10} Plate Count Range	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
<0	1	3.2	3	10.0	28	90.3	27	90.0	1	3.2	3	10.0
0 to 1.7	12	41.9	3	20.0	2	96.8	2	96.7			5	26.7
1.8 to 2.0	3	51.6	14	66.7			4	16.1	3	36.7		
2.1 to 2.7	10	83.9	8	93.3	1	100.0			15	64.5	13	80.0
2.8 to 3.0	3	93.6	2	100.0					8	90.3	3	90.0
3.1 to 3.7	2	100.0					1	100.0	3	100.0	3	100.0
TOTAL UNITS	31		30		31		30		31		30	
RANGE[c]	<0 to 3.3		<0 to 3.0		<0 to 2.2		<0 to 3.0		<0 to 3.3		<0 to 3.4	
MEAN[c]	2.0		1.8		0.2		0.2		2.4		2.1	

[a] U - Number of samples within each count range

[b] CP - Cumulative percentage of samples

[c] Log_{10} of counts

TABLE 10: Gram-negative organisms[a] isolated from ground beef, textured soy protein (TSP) and TSP extended ground beef

ORGANISMS	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[b]	P[c]	U	P	U	P	U	P	U	P	U	P
Acinetobacter calcoaceticus var. anitratum	11	35.5	10	33.3	3	9.7	1	3.3	13	41.9	14	46.7
Achromobacter xylosoxidans	0	-	1	3.3	0	-	0	-	0	-	0	-
Aeromonas hydrophila	6	19.4	3	10.0	2	6.5	2	6.7	2	6.5	3	10.0
Alcaligenes sp.	1	3.2	1	3.3	0	-	0	-	3	9.7	0	-
Arizona hinshawii	1	3.2	0	-	0	-	0	-	1	3.2	0	-
Chromobacterium typhiflavum	0	-	1	3.3	0	-	1	3.3	0	-	0	-
Citrobacter diversus	0	-	1	3.3	0	-	0	-	0	-	0	-
Citrobacter freundii	18	58.1	13	43.3	3	9.7	3	10.0	10	32.3	11	36.7
Citrobacter sp.	1	3.2	0	-	0	-	0	-	0	-	0	-
Enterobacter aerogenes	1	3.2	0	-	0	-	0	-	0	-	0	-
Enterobacter agglomerans	0	-	0	-	1	3.2	1	3.3	1	3.2	0	-
Enterobacter cloacae	20	64.5	12	40.0	2	6.5	1	3.3	15	48.4	9	30.0
Enterobacter hafniae	4	12.9	20	66.7	2	6.5	5	16.7	4	12.9	19	63.3
Escherichia coli	26	83.9	26	86.7	3	9.7	3	10.0	31	100.0	28	93.3
Klebsiella pneumoniae	7	22.6	4	13.3	0	-	1	3.3	16	51.6	11	36.7
Pasteurella multocida	0	-	0	-	0	-	0	-	1	3.2	0	-
Proteus mirabilis	2	6.5	1	3.3	0	-	1	3.3	0	-	0	-
Proteus morganii	1	3.2	1	3.3	0	-	0	-	0	-	0	-
Proteus vulgaris	5	16.1	1	3.3	1	3.2	0	-	1	3.2	3	10.0
Pseudomonas aeruginosa	2	6.5	0	-	0	-	1	3.3	0	-	0	-
Pseudomonas fluorescens	3	9.7	1	3.3	2	6.5	2	6.7	2	6.5	1	3.3
Pseudomonas fluorescens grp.	1	3.2	2	6.7	0	-	4	13.3	2	6.5	2	6.7
Pseudomonas maltophilia	0	-	0	-	1	3.2	0	-	0	-	0	-
Pseudomonas putida	1	3.2	1	3.3	0	-	0	-	1	3.2	0	-
Pseudomonas sp.	3	9.7	1	3.3	1	3.2	1	3.3	9	29.0	5	16.7
Salmonella enteriditis ser. worthington	0	-	1	3.3	1	3.2	1	3.3	0	-	0	-
Serratia liquefaciens	2	6.5	10	33.3	2	6.5	5	16.7	2	6.5	6	20.0
Serratia marcescens	0	-	0	-	0	-	1	3.3	0	-	0	-
Yersinia enterocolitica	0	-	0	-	0	-	1	3.3	0	-	0	-

[a] Genus and species names are from Analytical Profile Index, Analytab Products, Inc.

[b] U - Number of samples positive for each organism

[c] P - Percent of samples positive

TABLE 11: Gram-positive organisms isolated from ground beef, textured soy protein (TSP) and TSP extended ground beef

ORGANISMS	GROUND BEEF				TEXTURED SOY PROTEIN				TSP EXTENDED GROUND BEEF			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	P[b]	U	P	U	P	U	P	U	P	U	P
Bacillus cereus	14	45.2	0	-	17	54.8	3	10.0	17	54.8	1	3.3
Bacillus sp.	14	45.2	13	43.3	21	67.7	22	73.3	14	45.2	16	53.3
Clostridium perfringens	30	96.8	22	73.3	8	25.8	6	20.0	30	96.8	27	90.0
Corynebacterium sp.	1	3.2	1	3.3	0	-	1	3.3	0	-	0	-
Diphtheroids	11	35.5	11	36.7	5	16.1	6	20.0	16	54.8	12	40.0
Erysipelothrix sp.	0	-	2	6.7	0	-	0	-	0	-	1	3.3
Micrococcus sp.	13	41.9	15	50.0	4	12.9	9	30.0	19	61.3	15	50.0
Staphylococcus aureus	15	48.4	9	30.0	3	9.7	3	10.0	16	51.6	12	40.0
Staphylococcus epidermidis	17	58.8	17	56.7	4	12.9	8	26.7	18	58.1	16	53.3
Streptococcus anginosus	0	-	0	-	0	-	1	3.3	1	3.2	2	6.7
Streptococcus avium	0	-	0	-	0	-	0	-	1	3.2	0	-
Streptococcus casseliflavus	0	-	0	-	0	-	0	-	0	-	1	3.3
Streptococcus cremoris	0	-	0	-	0	-	1	3.3	0	-	1	3.3
Streptococcus durans	3	9.7	1	3.3	0	-	0	-	1	3.2	0	-
Streptococcus faecalis	11	35.5	11	36.7	2	6.5	1	3.3	12	38.7	7	23.3
Streptococcus faecalis var. liquefaciens	20	64.5	13	43.3	6	19.4	4	13.3	16	54.8	17	56.7
Streptococcus faecium	2	6.5	0	-	2	6.5	1	3.3	1	3.2	6	20.0
Streptococcus lactis	0	-	0	-	0	-	1	3.3	1	3.2	1	3.3
Streptococcus sanguis	3	9.7	1	3.3	1	3.2	3	10.0	5	16.1	2	6.7
Streptococcus sp.	11	35.5	7	23.3	0	-	3	10.0	9	29.0	11	36.7

[a] U - Number of samples positive for each organism

[b] P - Percent of samples positive

TABLE 12: The percent recovery of Clostridium perfringens from ground beef, textured soy protein (TSP) and TSP extended ground beef using different isolation procedures

	Unblended Sample				Blended Sample				
	Incubation/Heat Shock Temperature in Fluid Thioglycollate Media				Incubation/Heat Shock Temperature in Fluid Thioglycollate Media				SPS Agar Pour Plates
FOOD ITEM	37C	46C	75C	95C	37C	46C	75C	95C	37C
Ground Beef 3-Day	65	71	13	3	61	65	16	3	45
Ground Beef 10-Day	53	53	13	3	47	43	13	0	33
TSP 3-Day	13	10	3	6	13	6	0	0	0
TSP 10-Day	3	10	0	0	10	10	3	0	0
Ground Beef + TSP 3-Day	77	81	10	10	68	84	10	3	84
Ground Beef + TSP 10-Day	70	60	13	10	63	70	6	0	77
Mean	47^{a}	48^{a}	9^{c}	5^{d}	44^{a}	46^{a}	8^{cd}	1^{e}	40^{b}

Mean values for each treatment followed by the same letter are not significantly different at the 5% level of significance (46).

TABLE 13: Poliovirus type 1 recovery from laboratory contaminated ground beef

Sample No.	Total Virus Input (PFU)	Virus Detected (PFU/0.1 ml)	Volume of Concentrate (ml)	Virus Recovery (%)	Sensitivity (PFU/gm)
1	2.7×10^7	5.7×10^5	1.3	27	1.9
2	3.4×10^5	4.3×10^3	3.4	42	3.2
3	3.4×10^2	5.0×10^0	3.2	47	2.7
4	6.8×10^1	2.0×10^0	2.6	76	1.4
5	3.4×10^1	0.5×10^0	2.2	32	2.7
			Mean	45	2.4

GLOSSARY OF ABBREVIATIONS

AOAC - Association of Official Analytical Chemists
APC - Aerobic plate count
BAM - Bacteriological Analytical Manual
BFS - Bovine Fetal Serum
BT-8 - Bovine Turbinate cell line
CMM - Cooked Meat Media
CMPN - Coliform Most Probable Number
CPC - Coliform Plate Count
CPE - Cytopathic Effect
EMPN - *Escherichia coli* Most Probable Number
EPC - *E. coli* Plate Count
FTM - Fluid Thioglycollate Medium
FSC - Fecal streptococci count
GB - Ground Beef tested in this study
HI - Heat Inactivated
HS - Horse Serum
LVEY - Liver Veal Egg Yolk
MEM - Eagle's minimum essential medium with Earle's salts
MPN - Most Probable Number
PFU - Plaque forming unit
POL-1 - Poliovirus type 1
PPC - Psychrotrophic Plate Count
SGB - Textured soy protein extended ground beef tested in this study
SMPN - *Staphylococcus aureus* Most Probable Number
SPS - Sulfite polymyxin sulfadiazine
TPEY - Tellurite polymyxin egg yolk
TSP - Textured soy protein tested in this study
Vero - African green monkey kidney cell line

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