SURVIVAL OF SELECTED PATHOGENIC ORGANISMS DURING PROCESSING OF A FERMENTED TURKEY SAUSAGE PRODUCT

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
WILLIAM LEE BARAN
1973

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This is to certify that the

thesis entitled

SURVIVAL OF SELECTED PATHOGENIC ORGANISMS DURING PROCESSING OF A FERMENTED TURKEY SAUSAGE PRODUCT

presented by

William Lee Baran

has been accepted towards fulfillment of the requirements for

~~Ph. D.~~ degree in ~~Food Science~~

R. E. Stevenson

Major professor

Date May 14, 1975

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ABSTRACT

SURVIVAL OF SELECTED PATHOGENIC ORGANISMS DURING PROCESSING OF A FERMENTED TURKEY SAUSAGE PRODUCT

By

William Lee Baran

The potential growth or survival of selected pathogens in a fermented turkey sausage process was determined. A fermented sausage was developed using 45% dark turkey meat, 45% light turkey meat, and 10% turkey fat. Sorbitol was used as a humectant and improved the binding properties of the sausage mixture. The sausage mixture was stuffed into 14 mm casing and heated for 3 hrs. at 27 C, 4 hrs. at 32 C, and 5 hrs. at 46 C. The product could be developed further for commercial use. The complete time for processing was reduced to 8 days. Pathogens such as Salmonella spp., Staphylococcus aureus, Clostridium perfringens, and enteropathogenic strains of Escherichia coli were inoculated into the sausage at various concentrations from 14 organisms/g to 2.2 X 10^6 organisms/g. Most of these selected pathogens have been responsible for food-borne disease outbreaks associated with turkey products.

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Standard methods were used to enumerate the pathogens. Aerobic plate counts, lactic acid bacteria counts, and counts of coliforms and yeasts and molds were determined along with the counts of the selected pathogens. Aerobic plate counts were reported for the various spices, freshly processed turkey meat, and meat formulation before and after processing.

The results of this investigation indicated that most pathogens were not completely destroyed by the process at the inocula used. Salmonellae populations decreased $\geq$ 5.1 to 1.3 logs depending on strain of Salmonellae and initial inoculum used. Clostridium perfringens populations were reduced 0.55 to 3.4 logs. There was very little difference in destruction of C. perfringens between heat-sensitive and heat-resistant strains. A reduction of 0.83 to 3.0 logs occurred with enteropathogenic Escherichia coli. E. coli strain 0128 was relatively resistant to the fermented turkey sausage process. Staphylococcus aureus strain 243 multiplied during the processing of the sausage even with as low an inoculum as 5500 organisms/g meat. However, no detectable enterotoxin was found in any of the sausages sampled. The highest S. aureus cell cound in the finished product was 2.3×10^7 cells/g meat. These results indicate that most pathogens, if present in high numbers, survive the process used for fermented turkey sausage.

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SURVIVAL OF SELECTED PATHOGENIC ORGANISMS DURING PROCESSING OF A FERMENTED TURKEY SAUSAGE PRODUCT

By

William Lee Baran

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1973

DEDICATION

To my wife, Sara

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This author wishes to express appreciation and gratitude for the guidance and interest given by his major professor, Dr. Kenneth E. Stevenson, during the course of the investigation and preparation of this thesis.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Walker and Ayres (1959) investigated the microorganisms present on carcasses of commercially processed turkeys, but few investigations have been reported on the microbiology of new further-processed turkey products such as turkey frankfurters, turkey bologna, or turkey-fermented sausage. Turkey carcasses and turkey rolls have been examined for the presence of salmonellae, Clostridium perfringens, and Staphylococcus aureus. However, the quantitation of salmonellae in further-processed turkey products has been omitted in most of the research to date.

Turkey meat has been implicated as a vehicle of foodborne disease. An averaging of data from yearly foodborne disease reports indicates that turkey products were the vehicles in 8.4% of the outbreaks [United States Department of Health, Education, and Welfare (USDHEW), 1967-1971]. In the same five-year period, C. perfringens was the most prevalent pathogen present in turkey-related outbreaks of foodborne disease, accounting for 37%, followed by Salmonella spp. 27%, Staphylococcus aureus 23%, and unknown sources 12%.

Foodborne disease has been grossly under-reported (USDHEW, 1971). In addition, the problem of determining the source of the pathogenic organisms has been neglected in most cases which have been reported.

The parasite, Toxoplasma gondii, may be of concern to food processors if new turkey products are developed which do not rely on heating to assure a safe product. In order to destroy T. gondii, turkey meat used in fermented sausage formulations should be processed to a temperature of at least 137 F or should be frozen for 15 to 20 days prior to use.

In this study a small diameter fermented turkey sausage was developed and microbiological tests were made to determine if such a product presents any potential public health hazard. The sausage was inoculated with various initial numbers of C. perfringens, Salmonella spp., enteropathogenic Escherichia coli, and Staphylococcus aureus. The effect of processing on the survival and possible growth of the inocula was determined. The implications of the results with respect to health were evaluated.

LITERATURE REVIEW

Poultry Carcass Quality

Initial contamination. Ayres (1951) stated that the type of flora encountered as well as the initial number of microorganisms has a definite influence on the ultimate storage life of meat. Studies have determined the generic distribution of microorganisms isolated from the surfaces of beef sausage and chickens. Jensen (1945) found that the surface of beef sausage normally contained eight genera of bacteria. Ayres et al. (1950) reported that surfaces of chicken carcasses contained 14 genera of bacteria and Gunderson et al. (1947) reported an additional 7 genera as being present on chicken carcasses.

Although there have not been definite studies concerning the generic distribution of microorganisms isolated from the surfaces of cured meats or turkey carcasses, Pseudomonas spp. and Achromobacter spp. have been suggested as the organisms primarily responsible for the spoilage of turkey meat (Mast and Mountney, 1970). Lactobacillus was the predominant bacterial

genus found in cecal feces of turkeys (Harrison and Hansen, 1950), and Corynebacterium, Pseudomonas, Sarcina, Micrococcus, and Brevibacterium species were prevalent in turkey giblets (Salzer et al., 1967).

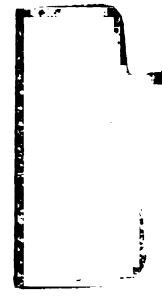
The numbers and types of microorganisms encountered in the evisceration and dressing of poultry were established by Gunderson et al. (1954) and Walker and Ayres (1959). Walker and Ayres (1959) investigated the microorganisms associated with commercially processed turkeys and found the bacterial population on the turkey skin increased ten-fold during processing. The final product contained a median count of 44,000 bacteria/cm^2 of skin surface.

Salmonellae. Since Salmonella spp. were reported as the prevalent species in poultry products, emphasis has been placed on research investigations of salmonellae in turkey products. Salmonella spp. were isolated more often from turkeys than from chickens, probably due to lower numbers or more fastidious types of salmonellae present on chickens (Walker and Ayres, 1959).

A survey of market poultry by Sadler and Corstvet (1965) showed that 4.16% of the turkeys, 1.42% of the chicken fryers, and 0.65% of the chicken hens being commercially slaughtered carried salmonellae in their intestinal tracts. Their results also indicated that

birds contaminated with salmonellae were slaughtered on 56% of the days for turkey, 32% for chicken fryers, and 14% for chicken hens.

Bryan et al. (1968) evaluated "further-processed" turkey products and found that swab samples from chilled, eviscerated turkey carcasses, and finished products, most frequently contained serotypes of Salmonella san-diego and Salmonella anatum. S. anatum was the most common of 7 serotypes of Salmonella spp. isolated from turkey giblets by Salzer et al. (1967).

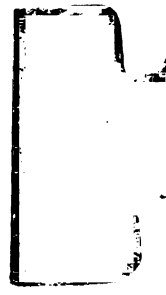


Baran et al. (1973) failed to recover Salmonella spp. from turkey carcass meat samples which were chilled in three rocker chiller tanks containing chlorinated water. Thus, the number of Salmonella spp. found on turkey products may be related to the sanitation procedures practiced in the various plants.

Surveys conducted at turkey processing plants indicated that both equipment and plant workers were associated with the transfer of salmonellae to finished products (Brobst et al., 1958; Bryan et al., 1968; Dixon and Pooley, 1961, 1962; Galton et al., 1955). In one survey of a turkey processing plant, the defeathering equipment was contaminated with salmonellae 76% of the time; also, 63% of the carcasses leaving the defeathering machine were positive for salmonellae (Bryan, 1965). However, airborne contamination by

salmonellae in turkey processing plants was found only in the live-bird and picking room areas, whereas the processing areas were free from airborne salmonellae contamination (Zottola et al., 1970).

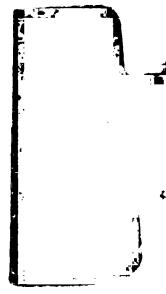
Surveys of dressed poultry collected from poultry processing plants or from retail markets have shown varying, but frequently high, levels of salmonellae (Schneider and Gunderson, 1949; Thatcher and Loit, 1961; Felsenfeld et al., 1950; Wilson et al., 1961; Woodburn, 1964; Bryan et al., 1968; and Wilder and MacCready, 1966). No studies were found in which Salmonella spp. were quantitated for turkey products.



Clostridium perfringens. In studies by Yamamoto et al. (1961), 110 turkeys were sampled using Ellners' medium and 28 samples contained C. perfringens. However, Baran et al. (1973) using direct plating methods found low numbers of C. perfringens (< 10 organisms/g to 435 organisms/g) from turkeys chilled in rocker chillers containing chlorinated water. Commercial turkey products contained 110 to 500 viable cells of C. perfringens/g (Shahidi and Ferguson, 1971). Frozen further-processed turkey products contained C. perfringens in 20% of the 35 samples examined.

Lillard (1971) recovered C. perfringens from chicken carcasses before processing in the range of < 10 to 10^5 cells/g, but found < 10 cells/g after the

chill tank treatment. However, she indicated that using enrichment techniques, 10% of the turkey breasts surfaces were contaminated with C. perfringens after chilling in a chill tank which did not contain chlorinated water. This result may show a spreading of C. perfringens from contaminated birds to relatively clean birds during the chilling process. In another study, Hall and Angelotti (1965) reported that 58% of 26 commercially processed chickens contained C. perfringens.



Although large populations of C. perfringens were not found using direct counting methods, the use of enrichment techniques did show frequent contamination of frozen turkey products by C. perfringens (Zottola and Busta, 1971). Roast turkey involved in a food poisoning incident contained 3.3×10^5 C. perfringens/g (Shahidi and Ferguson, 1971). Thus, the numbers of C. perfringens present on turkey products initially is low, but improper handling and storage may cause sufficient growth of the organism to produce a food-poisoning outbreak.

Enteropathogenic Escherichia coli. For years, enteropathogenic strains of Escherichia coli (EEC) have been associated with gastroenteritis occurring mostly in infants. Recently, food has been implicated as a source in a few cases of EEC infections in adults.

Adults can be asymptomatic carriers of EEC and transfer the organisms to children.

The identification and survival of EEC in dairy products have been the subjects of several investigations (Charter, 1965; Jones et al., 1967; Read et al., 1961; Wilson and Weiser, 1949; and Yang and Jones, 1969). Yang and Jones (1969) studied the physiological characteristics of EEC and nonenteropathogenic E. coli (NEEC) isolated from pasteurized dairy products produced in Canada. They were interested in finding a simple biochemical test to differentiate between the pathogenic and nonpathogenic E. coli. They determined that some EEC were capable of surviving a laboratory batch pasteurization of 145 F for 30 minutes. Strain PE 616 (serotype 0128:B12) and strain PE 815 (serotype 0119:B14) had D_{147} values of 20 and 10 minutes, respectively. Storage tests indicated some EEC strains could survive and multiply at 7 C whereas the NEEC strains did not survive. One strain, PE 712 (serotype 026:B6), was able to increase 1,000-fold in 6 days at 7 C.

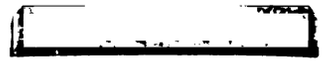
Recently, imported French cheese was shown to contain EEC ("serogroup" 0124). The organism was isolated from the cheese and from stools of several patients who acquired EEC infections as a result of ingesting the cheese. This was the first well-documented

food-poisoning outbreak caused by enteropathogenic E. coli in the United States [Center for Disease Control (CDC), 1971].

Research to determine if EEC is present in poultry products has been limited. However, Tamura et al. (1971) found 9.3% of 188 frozen broilers contaminated with EEC.

Staphylococcus aureus. Contamination of poultry products by Staphylococcus aureus is usually a result of human negligence. The organism does not grow when high numbers of Streptococcus spp. are present (Gilliland and Speck, 1972). Contamination of poultry products by Staphylococcus aureus is potentially significant and usually occurs after processing.

Walker and Ayres (1959) found Staphylococcus spp. present on turkey skin surfaces in the range of < 10 to 3,100 cells/cm^2, whereas counts from the scald and chill water tanks did not exceed 500 cells/ml. However, the skin surface counts reported were high since only 38% of 260 colonies tested were coagulase-positive staphylococci. The quantities of Staphylococcus spp. isolated from turkey products were no higher than those reported for chickens (Walker and Ayres, 1959). Ostovar et al. (1971) did not find Staphylococcus spp. present on



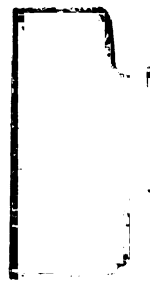
deboned turkey meat. However, Zottola and Busta (1971) found 71% of the frozen raw turkey products they sampled contained staphylococci.

Clostridium botulinum. The presence of Clostridium botulinum spores in raw meats in the United States and Canada is rare (Ingram and Roberts, 1966). Botulism resulting from the ingestion of poultry is also rare. From 1899-1968 only 2 of 647 reported botulism outbreaks were associated with poultry products (CDC, 1968). Abrahamsson and Riemann (1971) sampled 372 meat products and found 5 samples contained type A and 1 sample contained type B C. botulinum. Of 41 samples of smoked turkey examined, only 1 contained type B toxin (Abrahamsson and Riemann, 1971).

Toxoplasma gondii. A sausage mixture which contains pork is required by law to be heated to an internal temperature of 137 F or held frozen for 20 days prior to use in order to destroy the organisms which cause trichinosis. Turkey sausage which contains no pork would be exempt from this law. However, Toxoplasma gondii, an intracellular protozoan which is widely distributed geographically, could possibly be a problem in undercooked poultry.

Jacobs et al. (1962) studied the amount of T. gondii infections which occurred naturally in poultry

originating along the eastern seaboard of the United States and processed in a Baltimore poultry plant. They tested 124 pooled samples each consisting of 10 oviducts or ovaries from hens that appeared normal on macroscopic inspection of their viscera. They found 9 ovary pools and 10 oviduct pools contained T. gondii. Forty-six individual birds were also examined using the ovary, shelled eggs, leg muscle, and brain from each bird. Four ovaries and 1 leg muscle sampled contained T. gondii while the other tissues of these birds were negative for T. gondii.



Jacobs and Melton (1966) using the digestion-inoculation technique examined 108 individual hens and discovered 4 hens with chronic toxoplasmosis; none of the 108 shelled eggs taken from these birds contained T. gondii. One egg was found to contain T. gondii when 327 eggs were examined from 16 birds with experimentally induced chronic infections. When sacrificed 3 to 10 months after inoculation with T. gondii, all inoculated birds had T. gondii cysts in 1 or more of the following organs or tissues: brain, ovary, oviduct, kidney, gizzard, and intestines.

Bickford and Saunders (1966) examined chickens that received intramuscular inoculations of T. gondii. They found the parasite present in the heart, testicles, liver, pancreas, and brain. Zarde et al. (1968) also

detected toxoplasmic infections in 176 chickens. Simitch et al. (1965) determined that turkeys are susceptible to infections by T. gondii, especially the cystic form. The chance of acquiring toxoplasmosis from infected meat can be minimized either by thorough cooking or by freezing and thawing of the meat as recommended for destruction of trichinosis.

Further-Processed Products

New products and legislation. For many years whole carcasses have been the primary way of merchandising poultry meat. Recently, new products have been merchandised using poultry meat. Dawson (1970) emphasized that products containing combinations of poultry and beef could be developed if federal and state regulations did not prohibit this practice. If products containing poultry and beef were permitted, the processors could take advantage of the "generally lower" price of poultry and provide the consumer with lower priced meats which have good nutritional value.

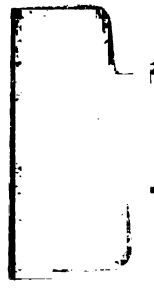
The development of poultry sausage products has been reported by some researchers. Baker et al. (1966) reported the use of 100% fowl meat to produce "chicken franks" and "chicken bologna." Other products have been developed such as: "chicken sausage" (Mori and Zambonini, 1968; Fujita, 1968), cooked poultry "hamburgers"

(Gorizontova et al., 1962), and fresh turkey sausage (Dawson, 1970). Kebede (1969) produced fermented poultry sausage from 100, 75, and 50% poultry meat mixed with 0, 25, and 50% beef, respectively, using 7 different binders. Sodium chloride was the best meat binder and the optimum concentration was 1-3%.

Microbiology of new products. Recently, microbial studies have been conducted on some of the new poultry products such as turkey rolls, deboned poultry meat, frankfurters containing 15% deboned turkey meat, and further-processed frozen turkey products. Wilkinson et al. (1965) determined the relationship between the internal temperature at the end of cooking and the destruction of specific species of Salmonella, Staphylococcus, and Streptococcus which had been inoculated into uncooked turkey rolls. They suggested that non-frozen turkey rolls should be cooked to an internal temperature of 71 C to assure a safe product for the consumer.

da Silva et al. (1967) reported that Staphylococcus aureus was present on 60% of the uncooked turkey roll surfaces, but not on the cooked turkey roll surfaces. Fewer than 300 aerobes/cm^2 were found on the cooked turkey roll surfaces, whereas raw rolls had counts of approximately 100,000/cm^2.

Eastern and western types of cooked turkey rolls, differing by the methods of cooking, packaging, and subsequent storage, are produced in the United States. Kinner et al. (1968) microbiologically examined the ingredients of eastern-type turkey rolls. They also examined the skin, "meat jelly," and meat of the eastern-type, "ready-to-eat," turkey rolls for total aerobes, coliforms, and enterococci. The results indicated that the hot flowing meat jelly contaminated the skin and internal parts of the turkey rolls.



Mercuri et al. (1970) studied the microbial flora of eastern-type turkey rolls immediately after processing and after various periods of refrigerated storage. After 2 weeks at 5 C the concentrations of aerobes on the surface of sliced and whole rolls was 10^6 to $10^7/cm^2$. In stored whole rolls, counts of coliforms and enterococci, respectively, ranged from 10^4 to $> 10^6/g$ and from $< 10^2$ to $10^6/g$. Post-cooking operations did not significantly affect the total count of turkey rolls. Out of 28 finished rolls sampled, 8 contained coagulase-positive staphylococci. No salmonellae or C. perfringens were detected in any of the rolls sampled. The initial bacteria counts for eastern-type turkey rolls and counts from rolls stored in a laboratory refrigerator for 4 days were relatively low.

[redacted]

Recently poultry meat from deboning machines has been evaluated microbiologically. Studies of processed deboned meat, when stored at 3 and -15 C, have shown the presence of salmonellae in 8.3 and 16.6% of the samples examined from 2 different poultry plants. C. perfringens was detected in 2.7 and 16.6% of the poultry samples examined (Ostovar et al., 1971). The majority of the psychro-tolerant organisms isolated from deboned turkey meat were Pseudomonas, Flavobacterium and Achromobacter species (Ostovar et al., 1971).

Investigations by Froning et al. (1971) compared frankfurters containing 15% fresh, mechanically deboned turkey meat against red meat frankfurters and found no difference in flavor or stability of the products. Both types of frankfurters showed some increase in total counts during refrigerated storage. The use of mechanically deboned turkey meat which was frozen for 90 days, resulted in inferior products with low flavor acceptance.

Zottola and Busta (1971) investigated the microbiological quality of further-processed turkey products and suggested microbial counts for raw and cooked products which would indicate unsanitary preparation of these products. They analyzed 35 samples of raw turkey products and found all contained coliforms, 19 contained E. coli, 25 contained S. aureus, 7 contained C. perfringens, and 3 contained salmonellae. In the 38 cooked

products which were analyzed, the incidence of these food-poisoning organisms was lower than the incidences in raw turkey products. Neither salmonellae nor E. coli was found, whereas, 16 contained coliform types, 6 contained C. perfringens, and only 1 contained S. aureus.

Fermented Sausages

Use of starter cultures. The use of pure cultures for fermented meat products was introduced in the United States in 1921 (Kurk, 1921). New concepts were introduced in 1955 that led to the development of Pediococcus cerevisiae as a strain suitable for use in the fermentation of meat products (Niven et al., 1955). Other organisms have been suggested for use as starter cultures, such as Micrococcus strain M53 (Niinivaara, 1955), Aspergillus oryzae, Bacillus mesentericus, and Brevibacterium linens (Redel et al., 1969). Everson et al. (1970) developed a frozen Pediococcus cerevisiae starter culture which is recommended for use in semi-dry fish and poultry sausages.

Microbiology of fermented sausage. The microbiology of sausage products is complicated because of variations in types of meats used, processing methods used, and types of products produced. Steinke and Foster (1951) studied the microbial flora present in liver sausage and bologna and the change in organisms during

storage at various temperatures when packaged in "Saran." The predominant organisms were micrococci in both products when they were stored at 30 C for 20 days. The only organism isolated from bologna was M. candidus, but both M. candidus and M. epidermidis were present in liver sausage. Bacillus spp. were also present in liver sausage at the two higher storage temperatures (16 C and 30 C) and these organisms eventually became predominant in the sausage after prolonged storage at 30 C.

The bacterial genera present in fresh pork sausage were determined by Sulzbacher and McLean (1951). The authors believed that Microbacterium spp. may be responsible for the development of the acid taste in stored sausage. They reported that the prevalent genera were Bacterium, 20.6% of the total isolates, Achromobacter, 12.7%, and Pseudomonas, 10.8%. The microbial flora of raw sausages were determined by Maleszewski et al. (1969). They examined 370 samples of "Metka" and 88 samples of "Kielbasa polska surowa," two types of Polish sausage, and reported that 96% of the samples contained coliforms, 73% contained enterococci, and 18% contained coagulase-positive staphylococci. No salmonellae were detected in the sausages.

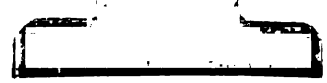
Sidorenko et al. (1969) examined 1,000 samples of raw meat and found 30-50% contained C. perfringens. Before stuffing, 275 samples of sausage mixtures were

examined and 45-100% contained C. perfringens. They examined ingredients added to the sausage mixture and found 79.6% of the flour samples and 66.6% of the starch samples contained C. perfringens. The microbial content of the spices was not reported.

Aroma-producing bacterial strains were isolated from raw sausage during the ripening process by Zanguchi and Debindate (1956). The bacteria found belonged to the genera Alcaligenes, Achromobacter, Escherichia, Aerobacter, and Pseudomonas.

Deibel et al. (1961) determined that total viable counts in processed sausage mix were relatively low and the predominant flora were lactobacilli. The authors mentioned that ruptured casings were observed, probably due to the formation of gas, and that large numbers of heterofermentative lactobacilli were detected in the sausage. The rupturing of sausage casings during fermentation was attributed by Kebede (1968) to over-stuffing of the sausage.

The microbial content of summer sausage, thuringer, and some types of fermented sausages such as salami, cervelat, genoa, goteborg, and lebanon was described by Deibel et al. (1961). The predominant flora of the fermented sausages consisted of lactobacilli. Although sausage mixes contained small numbers of pseudomonads and other gram-negative rods, coagulase-positive



staphylococci were not detected in any of the samples. The predominant flora of the finished thuringer sausage were Pediococcus spp., even though these bacteria were not detected in the initial sausage mixture. No explanation was given for this finding by the authors.

Goepfert and Chung (1970) studied the behavior of salmonellae during the production and storage of a fermented semi-dry sausage product. The authors found that salmonellae were able to grow at pH 5.2 while the sausage was being heated at 46 C. Although the finished thuringer sausage contained lower numbers of salmonellae than the sausage mixture, the process did not insure complete destruction of salmonellae when the sausage was inoculated with 10^3 to 5 X 10^5 cells/g.

Processing of fermented sausage. The manufacturing of fermented sausage is sometimes considered an art more than a science. Most industrial sausage formulations are trade secrets and new methods of production are jealously guarded.

Niinivaana et al. (1964) reported that the processing time for European dry sausages varied from 10 to 100 days or more. For example, Hungarian salami can be 6 months old when marketed. Some sausages are cooked or smoked while others are not. Sometimes mold growth is allowed to develop on the sausage exterior, imparting a characteristic flavor to the product. Meat formulations

and processing conditions for the various type of semi-dry and dry sausages were described by Adajian and Gartner (1946).

The classification of fermented sausages was explained by Price and Schweigert (1971) as being based on meat formulations, particle size, spicing, degree of tang, intensity of smoked flavor, finished temperature, and type of casing. They mentioned the pH of fermented sausages ranged from 4.8 to 5.4 and semi-dry sausages differ from dry sausages by having a more tangy flavor, and softer less chewy texture. In addition, semi-dry sausages contain approximately 50% moisture whereas dry sausages contain about 35%. The majority of the dry sausages in the United States have diameters of 3 to 4 inches and are dried for 50 to 70 days. Two common types are genoa and "b.c." salamis.

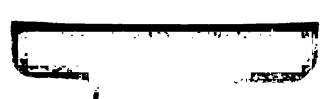
The only recent major change in the production of semi-dry and dry sausages has been the use of starter cultures of Pediococcus cerevisiae and Micrococcus. Deibel et al. (1961) demonstrated that holding the curing mixture for 48 to 72 hours before stuffing was not required for an acceptable product. The production time can also be reduced from the traditional 150 hours to 12-15 hours with the use of a frozen starter culture (Everson et al., 1970).

MATERIALS AND METHODS

Sausage Ingredients and Spices

A flock of broad-breasted white-breeder tom turkeys, approximately 1 1/2 years old, was obtained from the Department of Poultry Science, Michigan State University, slaughtered, and processed. The carcasses were wrapped in "cryovac" bags and placed in a freezer (-29 C) until used. The carcasses were defrosted in a walk-in refrigerator (3 C) and cut into strips. The dark meat, white meat, and fat were divided into separate portions which were frozen and then ground through a plate containing 1/4-inch holes attached to a model 5010 meat grinder (Toledo Scales Co., Toledo, Ohio). The ground meat and fat were then distributed into 15-lb. batches consisting of 45% dark meat, 45% white meat, and 10% fat including skin which were mixed by hand, placed into "cryovac" bags, frozen, and stored at -29 C until used.

The ground mixture was defrosted at 3 C for 2 days and placed through a meat grinder containing a plate with 3/16-inch holes. The 15 lbs of formulated mixture were divided into 3 5-lb batches and placed into



shallow aluminum pans. Spices and other ingredients (Table I) were added and mixed into the formulation by hand. For inoculation of the mixture with selected pathogens, the appropriate pathogen was sprayed on the meat using a Sprayon sprayer (Sprayon Products Inc., Cleveland, Ohio) and mixed thoroughly by hand. The pans of formulated mixture were incubated for 24 hours at 10 C and 72% relative humidity. The incubation period provided time for the salt to extract out the myosin which is necessary for proper binding of the meat. The 24-hour period also allows the inoculated pathogen to acclimate to the new environmental conditions. The third reason for this incubation period was to simulate the greening room procedure which is used in some processing operations. After the incubation period, 3 1.41-g portions of Accel (Merck & Co., Rahway, N.J., 07065) were each placed into 45 ml of water and mixed by hand into 5-lb batches of the meat mixture.

Sausage Process

A 4-liter hand stuffer (F. Dick) was used to pack 14 mm artificial "collagen" casing (Brechteen Co., Mount Clemens, Mi., 48043). The stuffed sausage was placed into a smoking chamber and heated in air with a relative humidity of 80-90% according to the following schedule: 27 C for 3 hours, 32 C for 4 hours, and 46 C for 5 hours. During the heating process, no smoke was

TABLE I. Spices and ingredients used in the turkey sausage formulation.

Ingredient	Grams/5 lbs Sausage	Source
Allspice	1.41	Archibald & Kendall, Inc. Chicago, Ill.
Black pepper	8.49	The Frank Tea & Spice Co. Cincinnati, Ohio
Red pepper	5.66	The Frank Tea & Spice Co. Cincinnati, Ohio
Paprika	5.66	B. Heller Co. Chicago, Ill.
Garlic powder	0.80	Meisel Co. Detroit, Mich.
D-Sorbitol	15.0	Pfanstiehl Lab. Inc. Waukegan, Ill.
Salt	45.4	Hardy Salt Co. St. Louis, Mo.
Glucose	16.95	Fisher Scientific Co. Fairlawn, N.J.
Sodium Nitrate	0.177	J. T. Baker Chem. Co. Phillipsburg, N.J.
Sodium Nitrite	0.177	Mallinckrodt Chem. Works St. Louis, Mo.

used. The heated sausage was cooled by spraying with cold water until an internal temperature of 16-18 C was obtained. The sausage was dried in a chamber at 10 C and 72% relative humidity for 8 days.

Sample Preparation for Microbial Evaluation

The first sample for enumeration of the selected pathogens was taken just prior to stuffing the sausage into the casing. The second sample was examined for the selected pathogens after 8 days of storage. For each sample, 5 separate 100-g portions of meat were each placed into 900 ml 0.1% peptone solution and blended for 2 minutes in Waring blendors. Duplicate samples from each of 5 blenders were then placed into the appropriate broth or agar medium for the enumeration of the selected pathogens.

Preparation of Culture Inocula

All pathogenic cultures were treated similarly during preparation of the inocula. Each culture was transferred into duplicate nutrient agar (Difco Laboratories, Detroit, Michigan) slants before each new experiment. One tube was used for culture maintenance while the other was used for preparing the inoculum.

Strains of Salmonella pullorum and Salmonella Senftenberg 775 W were obtained from the culture collection of the Department of Food Science and Human

Nutrition, Michigan State University. The cultures were thawed at 37 C and transferred into lactose broth (Difco). The cells were streaked onto brilliant green sulfadiazine (BGS) agar (Difco) and incubated for 24 hours at 37 C. A typical salmonellae colony from each culture was transferred to both nutrient agar slants and triple sugar iron (TSI) agar (Difco) slants which were incubated for 24 hours at 37 C. TSI agar slants showing an alkaline slant, acid butt, and H_2S production were considered positive for salmonellae. Inocula were obtained from nutrient agar slants which contained colonies that had given typical reactions for salmonellae on TSI agar slants. A 5-ml aliquot of lactose broth was added to each nutrient agar slant. The tube was shaken and transferred to a 100-ml flask of preheated lactose broth (37 C). The culture broth was incubated at 37 C on a Gyrotory Shaker (New Brunswick Scientific Co., New Brunswick, N.J.) and used to inoculate the sausage product.

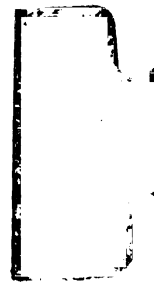
A heat-sensitive Clostridium perfringens strain ATCC 3624 and a heat-resistant strain NCTC 8238 were obtained from the Department of Food Science and Human Nutrition, Michigan State University. The C. perfringens cultures were maintained in cooked meat medium (Difco) at 5 C. Thioglycollate medium (Difco) with resazurin as an oxidation-reduction indicator was used as the

growth medium prior to inoculation. The medium was incubated at 37 C without agitation.

Three strains of enteropathogenic Escherichia coli cultures were used in these studies. E. coli strains 0128:B12 and 0125:B12-HIC were obtained from the University of Wisconsin and strain 026:B6 was obtained from the Center for Disease Control, Atlanta, Ga. Hereafter these strains will be referred to as 0128, 0125, and 026, respectively. The cultures were received on agar slants and transferred to lactose broth. The broth was incubated at 45.5 C for 24 hours. A loopful of the lactose broth was streaked on eosin methylene blue (EMB) agar (Difco) and incubated at 35 C for 24 hours. A colony with a metallic sheen was transferred to a nutrient agar slant, a tube of EC medium (Difco), and a slant of veal infusion agar (Difco). The EC medium was incubated at 45.5 C. Both strains 026 and 0125 did not grow well in EC medium when incubated at this temperature. The growth on the veal infusion agar (Difco) slants was used for the slide agglutination method to determine the serological identity of the culture.

Staphylococcus aureus strain 243 ATCC 14458 was obtained from the Department of Food Science and Human Nutrition, Michigan State University. A typical S. aureus colony was fished from a mannitol salt agar

(Difco) plate and inoculated into brain heart infusion (BHI) broth (Difco). After 24 hours at 37 C the BHI culture was streaked onto a Vogel-Johnson agar (Difco) plate and incubated at 37 C for 24 hours. A typical S. aureus (black) colony from the Vogel-Johnson agar plate was transferred to a tryptose agar (Difco) slant and maintained at 5 C. Prior to use, the culture was inoculated into BHI broth and incubated at 37 C for 24 hours.



Enumeration of Selected Pathogens

Salmonellae. The sausage samples were prepared as described previously. The procedure for the detection of Salmonella spp. is described by Galton et al. (1968). Dilution of 10^{-4}, 10^{-5}, and 10^{-6} were made from each of the duplicate samples from the 5 blenders and 1-ml aliquots of each dilution were placed into three tubes of selenite-cysteine broth (Difco) and incubated for 48 hours at 37 C. The most probable number (MPN) technique was used to quantitate the salmonellae present in the sausage. BGS agar plates were streaked with inocula from the selenite-cysteine broth tubes and incubated for 24 hours at 37 C. Colonies from the BGS agar plates were transferred to TSI agar slants for presumptive identification. Further identification of the colonies from the BGS agar plates was accomplished

with the use of the microcolony indirect fluorescent antibody technique (Thomason, 1971).

For samples taken after processing a pre-enrichment lactose broth step was used prior to the selenite-cysteine broth. The pre-enrichment tubes were incubated at 37 C for 36 hours. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were used for the processed sausage samples.

The microcolony indirect fluorescent antibody technique employed a 1:8 dilution of specific salmonellae antisera (Thomason, 1971). The specific serotype antiserum used for Salmonella pullorum was Salmonella O group D, factor 9 (Difco), and for Salmonella senftenberg 775 W the specific antiserum was Salmonella O group E^4, factor 19 (Difco). Anti-rabbit globulin labeled with fluorescein isothiocyanate (FI) [Baltimore Biological Laboratory (BBL), Div. Becton, Dickinson & Company, Cockeysville, Maryland, 21030] was used as the indicator of the antigen-antibody reaction. The Leitz-Ortholux fluorescence microscope system used consisted of a HBO-200 mercury lamp for a light source with an optics system consisting of a 1:20 dark field condensor, BG-38 and BG-12 exciter filters, and a K 430 barrier filter.

Three loopfuls of a 48-hour selenite-cysteine broth culture were placed on a BGS agar plate at three different points and incubated for three hours at 37 C.

A slide cleaned with 95% ethanol was placed on the micro-colonies and removed with forceps. The slide was air-dried and fixed in a solution of 6 parts 95% ethanol:4 parts chloroform:1 part formaldehyde. The slides were then washed in 95% ethanol and dried (Thomason, 1971). A 1:8 conjugate, diluted with phosphate buffered saline (PBS), was placed on each smear, and the slide was held at 37 C for 30 minutes. The slides were washed in PBS for three minutes, rinsed with distilled water, and dried. A drop of anti-rabbit globulin labeled with FI was placed on each smear and the slides were incubated at 37 C for 20 minutes. After washing the slides in PBS, a drop of 9:1 glycerol-PBS solution was placed on the smear and covered with a cover glass (18 RD # 1, E. H. Sargent & Co., Chicago, Ill.). The slide was viewed under the microscope and the presence of fluorescent cells was considered a positive reaction and indicated that the specific serotype of salmonellae was present.

Clostridium perfringens. Sulfite-polymyxin-sulfadiazine (SPS) basal medium (Angelotti et al., 1962) with 0.4 μg/g D-cycloserine added was used for the recovery of C. perfringens from inoculated turkey sausages. The basal medium contained 1.5% tryptone (Difco), 3.0% agar (Difco), 1.0% yeast extract (Difco), 0.05% ferric citrate (K & K Laboratory Inc., Plainview,

N.Y.), and 0.1% sodium metabisulfite. The basal medium was autoclaved at 121 C for 15 minutes.

Seromycin (Eli Lilly & Co., Indianapolis, Ind.), a brand of D-cycloserine, was used as the antibiotic in place of polymyxin and sulfadiazine (Harmon et al., 1971). The seromycin powder was removed from the capsules which contained a filler (1.678 g seromycin powder = 1.00 g of D-cycloserine). The D-cycloserine was mixed with distilled water (1.00 g/12.5 ml) and filtered using a Millipore 3 µm pore-size filter (Millipore Corp., Bedford, Mass.). Five ml of filtrate were placed into tubes, steamed for 20 minutes for sterilization, and frozen at -73 C until used. D-cycloserine was used at a final concentration of 400 µg/ml.

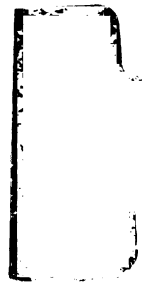
The pouch method developed by Bladel and Greenberg (1965) was used for the isolation and enumeration of C. perfringens. A polyester film was used for the pouch material (Kapak Pouches B-1215-2; Scotchpack-3M Co., Kapak Ind. Inc., Minneapolis, Minn.). The pouches did not need to be sterilized when properly handled.

The pouches were placed into a holder with walls spaced 0.5 cm apart and 23 ml of agar were added to each pouch. It was not necessary to seal the pouches since the agar, which formed in the neck of the pouch, produced an oxygen barrier.

The plastic pouches with agar were held in a 55 C incubator until inoculated with a sausage sample. After solidification of the agar the pouches were then removed from the holder and placed in an incubator at 37 C for 24 hours. During growth, C. perfringens reduces sulfites to sulfides producing black colonies when the ferric citrate is converted to ferrous sulfide. All black colonies from one of the middle dilution pouches suspected of being C. perfringens were tested in trypticase nitrate-motility agar and iron milk tubes; both media were prepared in the laboratory. Colonies were considered to be C. perfringens if they contained nonmotile, nitrate-reducing bacteria which produced a stormy fermentation in iron milk.

Enteropathogenic Escherichia coli. A three-tube MPN technique was employed for the enumeration of EEC. The turkey sausage dilutions were placed into lauryl tryptose broth (Difco) tubes which contained Durham vials. The tubes were incubated at 35 C for 24-48 hours. A loopful from each tube exhibiting gas was streaked onto EMB agar and incubated at 35 C for 24 hours. Typical E. coli colonies, showing a metallic sheen and dark centers, were transferred to EC medium and incubated in a water bath at 45.5 C for 24-48 hours. The samples showing gas production were considered to

contain EEC organisms. The organisms were identified using the biochemical tests contained in the API system for the identification of Enterobacteriaceae (Analytab Products Inc., N.Y.). The slide agglutination test was used for serological confirmation which employed the use of antisera OB poly A and B, and OK poly C (Difco).

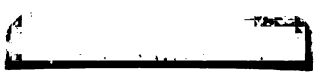


Coagulase-Positive Staphylococcus aureus.

Dilutions were made using phosphate buffered dilution blanks (APHA, 1966). Samples were spread in 0.1 ml volumes over the surfaces of Vogel-Johnson agar plates using a bent glass rod. The inoculum was allowed to absorb into the agar and the plates were incubated at 37 C for 48 hours. Black colonies larger than 1 mm in diameter were picked, transferred into BHI broth, and incubated for 24 hours at 37 C. One drop of BHI culture was added to 0.5 ml of citrated rabbit plasma (Difco) in a 13 X 100 mm test tube. The tubes were incubated at 37 C and coagulation of the plasma within 6 hours indicated the presence of coagulase-positive staphylococci.

Detection of Staphylococcus Aureus Enterotoxin

The micro-slide gel double diffusion method developed by Casman and Bennett (1965) was used, with slight modifications, for the extraction, purification, and detection of enterotoxin in fermented turkey sausage. After centrifugation at 32,800 X g for 20 minutes,



the supernatant fluid was heated in a 56 C water bath followed by chilling in ice (Barber and Deibel, 1972) in order to eliminate substances which might interfere with serological test for enterotoxin. The fluid was again centrifuged at 32,800 X g for 15 minutes. The supernatant fluid was adjusted to a pH of 4.5 and centrifuged at 32,800 X g for 15 minutes. The supernatant was saved and the pH was adjusted to 5.7 before being applied to a carboxymethyl cellulose (CMC) column. The enterotoxin was absorbed onto the CMC column and eluted from the column with 0.2 M sodium phosphate buffer containing 0.2 M NaCl, pH 7.4.

Enumeration of Nonpathogenic Microorganisms

Aerobic plate counts. For aerobic plate counts, 50-g portions of sausage mixture were added to 450 ml of 0.1% peptone and blended for 2 minutes. Serial dilutions of the homogenate were prepared and 1-ml portions were used as inocula for pour plates of plate count agar (Difco). The plates were incubated at 30 C for 72 hours (APHA, 1966).

Lactic acid bacteria. LBS agar (BBL) was used for pour plates for the enumeration of lactic acid bacteria (Rogosa et al., 1951). These plates were incubated at 30 C for 72 hours. Three percent hydrogen peroxide

was poured over the surface of the agar plates to determine the presence of the enzyme catalase. Most organisms growing on aerobically incubated plates possess the enzyme catalase which will release oxygen from the hydrogen peroxide causing an effervescence. However, the lactic acid bacteria, including the Lactobacillus and Pediococcus genera, do not normally produce a detectable catalase (Harrigan and McCance, 1969). Therefore, a negative catalase test indicated the presence of lactic acid bacteria.

Coliform. Coliforms were determined using violet red bile agar (Difco) as described in Recommended Methods for the Microbiological Examination of Foods (APHA, 1966). The plates were incubated for 48 hours at 35 C. Dark red colonies, at least 0.5 mm in diameter, were considered to be coliforms.

Yeast and molds. Yeast and molds were enumerated using potato dextrose agar (Difco) acidified as described in Microbiological Examination of Foods (APHA, 1966). The plates were incubated at 25 C for 48 hours.

pH and Total Acidity Determinations

The pH and total acidity of the fermented sausage products were determined with the aid of an automatic titrimeter model No. 36 (Fisher Scientific, Pittsburgh, Pa.). The standard AOAC method for

determining total acidity in cheese was used to determine total acidity in sausage products (AOAC, 1970). Ten g of meat were placed in a blender containing 100 ml of distilled water at 4.4 C. After blending for two minutes, the homogenate was filtered through a Seitz filter (Hercules Filter Corp., Hawthorne, N.J.). Portions of filtrate corresponding to 2.5 g of sample were titrated against 0.1N sodium hydroxide. An end-point of pH 8.7 was used for the sausage sample titration by plotting pH vs ml of titrant which was determined using the automatic titrimeter. The point on the titration curve at which the pH changes most rapidly with addition of titrant was chosen as the endpoint. This endpoint was used throughout the experiments. Percent total acidity, expressed as lactic acid, was determined by the formula:

$$\frac{(\text{ml N/10 NaOH})\ (.009)}{2.5\text{ g sample}} \times 100 = \begin{matrix}\text{\% total acidity of} \\ \text{the sausage product}\end{matrix}$$

RESULTS AND DISCUSSION

Spice Contamination

Table II indicates the aerobic plate counts of each spice and ingredient used in the formulation of the turkey sausage. The results show sorbitol and glucose supplied some microorganisms to the product. However, the spices contributed more organisms than the other ingredients. An average of 9×10^3 organisms/g meat was contributed to the sausage mixture by the spices. No lactic acid bacteria or coliforms were recovered from the spices and ingredients. Some molds and yeasts were found in allspice (5.5×10^2 /g meat) and red pepper (2.5×10^4 /g meat). The microorganisms introduced to the product by the spices could be reduced by using gas-sterilized spices.

Krishnaswamy et al. (1971) enumerated the microorganisms present in spices and spice mixtures. Their results for numbers of total organisms were similar to those in Table II.

Processing Parameters

Table III shows the pH, total acidity, and percent loss of moisture obtained in the sausage when

TABLE II. Microbial counts of spices and ingredients added to the turkey meat mixture.

Ingredient	g/5 lbs Meat	Aerobic Plate Count/ Gram of Spice
Allspice	1.41	3.7×10^6
Black pepper	8.49	3.5×10^5
Red pepper	5.66	8.5×10^5
Paprika	5.66	1.3×10^6
Garlic powder	0.80	1.4×10^4
Sorbitol	15.0	1.5×10^1
Glucose	16.95	1.0×10^1
Salt	45.40	nc
Sodium nitrate	0.177	nc
Sodium nitrite	0.177	nc

nc represents $< 1.0 \times 10^1$

TABLE III. Processing results for a fermented turkey sausage product inoculated with four different pathogenic organisms.

Organism	% Loss of Moisture	pH	% Total Acidity With Respect to Lactic Acid
Control	35.2	5.3	.31
Salmonella spp.	35.4		
After heating		5.8	.10
After processing		5.9	.14
Clostridium spp.	37.5		
After heating		5.6	.12
After processing		5.7	.17
Enteropathogenic *E. coli*	46.6		
After heating		5.5	.09
After processing		5.2	.21
Staphylococcus aureus	38.2		
After heating		5.4	.15
After processing		5.6	.23

various pathogenic inocula were used. The indicated parameters were examined before processing (after inoculation of pathogens, addition of spices, and incubation at 50 F for 24 hours) and after processing (addition of P. cerevisiae, heat processing, and storage at 50 C and 72% relative humidity for 8 days).

There was a difference in percent loss of moisture between the control sausages and the inoculated sausages. This was the result of fluctuation in the control chamber due to mechanical problems. A pH of 5.3 provided an acceptable organoleptic product. The sausages inoculated with Clostridium spp., Salmonella spp., and Staphylococcus spp. had a slight pH increase during processing. The pH did not drop as in the control sausage which may be the result of competition of the inoculated organisms with the P. cerevisiae. The sausages inoculated with E. coli showed a decrease in pH after processing. This may be due to acid products produced by the E. coli strains or the inability of E. coli to compete with P. cerevisiae.

The percent total acidity, expressed as lactic acid, increased during the processing indicating that lactic acid was being produced. The sausages inoculated with the Salmonella spp. and Clostridium spp. had a lower total acidity than the control sausage.

Microbial Contamination During Processing

Data in Table IV represent the microbial development at various processing stages. Initially the fresh processed turkey meat had a low count because the turkeys were processed in our own facilities under sanitary conditions which may not be obtained with continuous processing. The total initial aerobic count of 2.7×10^4 organisms/g meat was an acceptable figure and no off-odor or discoloration was noted in the product.

The final aerobic plate count was measured after the heating and drying process. In general a 10-fold increase in aerobic plate counts occurred during processing. One experiment with a sausage product which did not contain the starter culture, P. cerevisiae, showed a similar increase in aerobic plate counts. This indicates that other organisms besides the starter culture are able to grow during the processing. Yeast and molds were completely destroyed by processing in all runs.

For each sausage batch inoculated with a selected pathogen, lactic acid bacteria, coliform, and yeast and mold counts were obtained. The lactic acid bacteria counts of the sausage mixture prior to inoculation with the starter culture were high. Even without the addition of the lactic starter culture, P. cerevisiae, a 10-fold increase in numbers of lactic acid bacteria was noted.

TABLE IV. Aerobic plate counts of turkey sausage at various processing steps.

Sample	Aerobic Plate Count Org/ Gram
Fresh processed turkey meat	1.3×10^4
Added spices	9.0×10^3
Pathogenic inoculation	6.0×10^3
Initial sausage mixture	2.7×10^4
Sausage mixture after inoculation of pathogens, addition of spices, and incubation at 50 F for 24 hours	1.3×10^7
Sausage in casings, after the addition of Pediococcus cerevisiae and processing	5.0×10^8
Sausage in casings, after processing without addition of Pediococcus cerevisiae	4.1×10^8

Coliforms were found at low levels in the initial product, and they were completely destroyed by the process when C. perfringens were used as the pathogenic inoculum. However, there were some coliform type colonies found after processing when Salmonella spp. and E. coli were used as the pathogenic inocula. These coliforms could have been part of the salmonellae inoculum or E. coli inoculum that had survived the processing.

Studies of Selected Pathogens

Salmonellae. As shown in Figure 1, during processing, Salmonella pullorum was reduced in numbers by $\geq$ 4.1 to $\geq$ 5.1 log cycles when the initial inoculum was 99,000 to 380,000 organisms/g meat. S. pullorum would be expected on poultry products although not in as high a number as used in the inoculation studies.

Figure 2 indicates that an initial inoculum of > 2,200 to 61,000 organisms/g meat of Salmonella senftenberg 775 W was reduced by 1.3 to 1.7 log cycles during processing. These results indicate S. senftenberg has a higher tolerance than S. pullorum to the processing conditions used. The higher processing tolerance may be due to factors other than heat since the heating process was very mild. Possibly tolerance to pH, drying, and greater ability to compete with other organisms, or a combination of these factors were responsible for the greater survival of S. senftenberg.

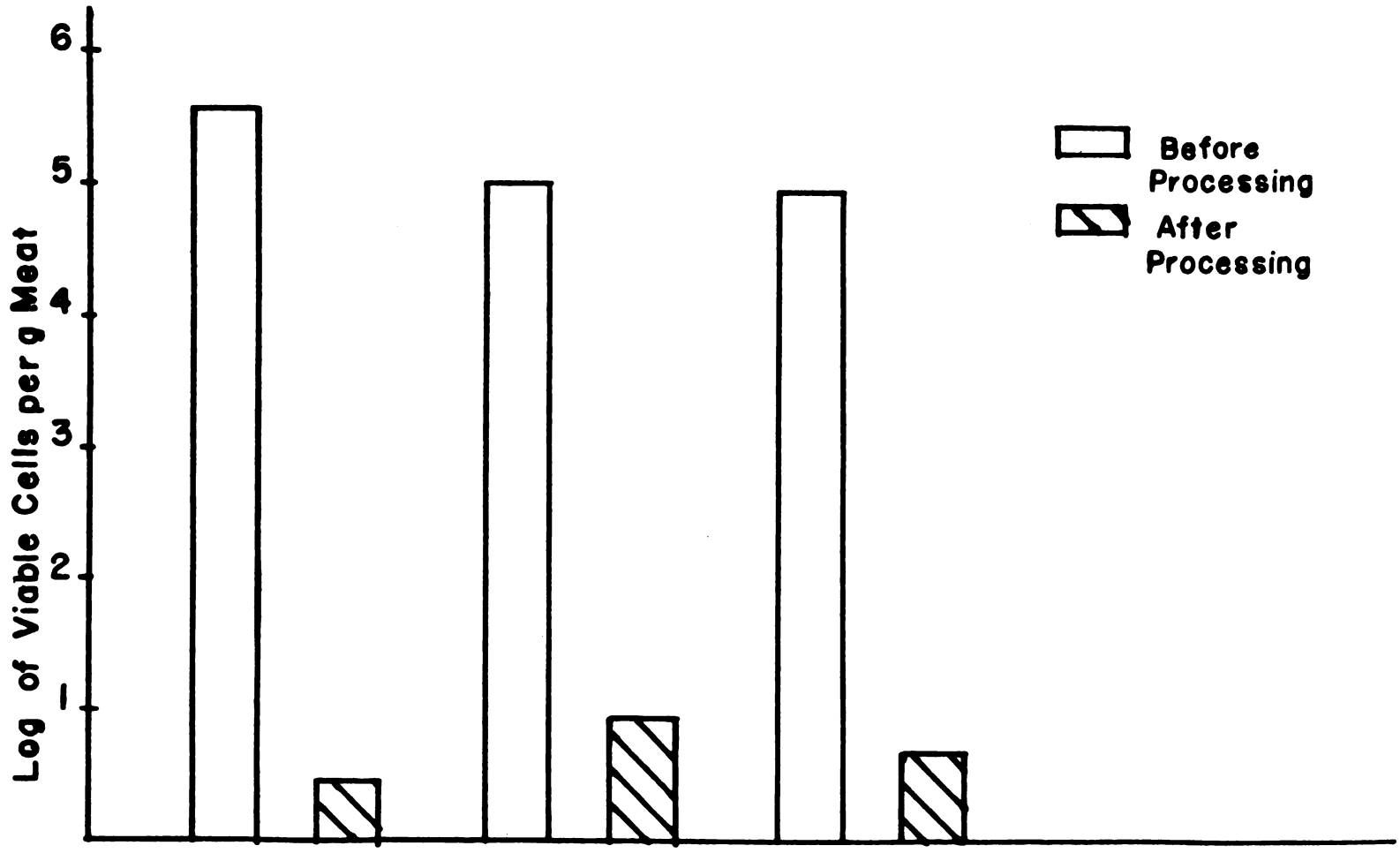


Figure 1. Effect of fermented turkey sausage processing on the survival of Salmonella pullorum inoculated at three different concentrations and enumerated by the three tube MPN technique.

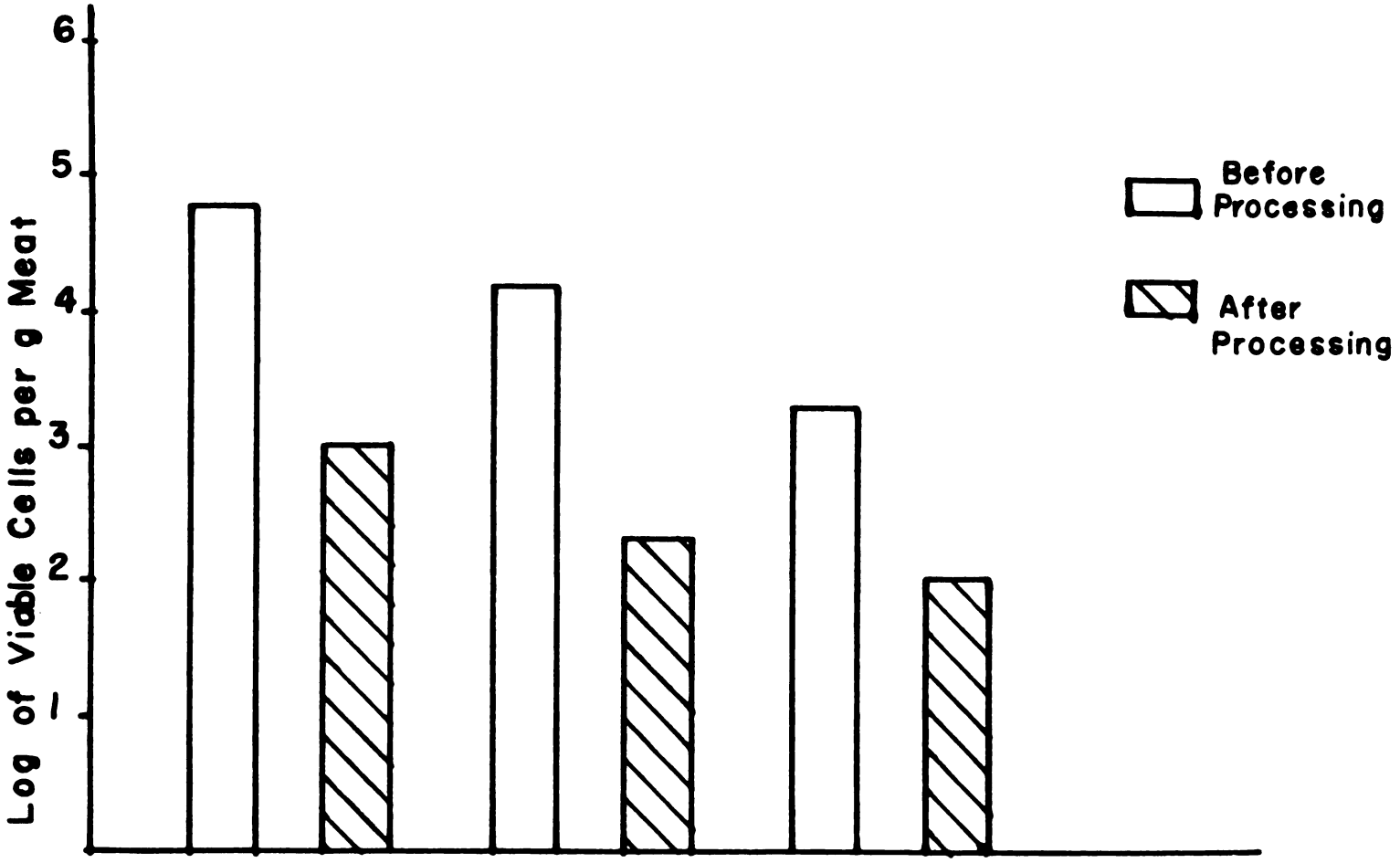


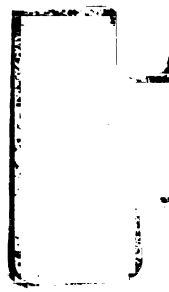
Figure 2. Effect of fermented turkey sausage processing on the survival of Salmonella senftenberg 775 W inoculated at three different concentrations and enumerated by the three tube MPN technique.

A comparison of the results obtained using S. pullorum and S. senftenberg 775 W as inocula indicates that a difference in tolerance to processing exists between salmonellae species. This result emphasizes the importance of determining the species, and presumably the strains, in solving salmonellae problems.

Matches and Liston (1972a) indicated that three serotypes of salmonellae were capable of growth when incubated at 12 C in a medium containing 0 to 4% sodium chloride. This indicates that the salt concentration used in the sausage mixture may not be sufficient to inhibit the growth of Salmonella spp. In another study Matches and Liston (1972b) suggested that some salmonellae grew at low temperatures in a narrow pH range. Growth was reported at pH 6.0 with a temperature of 5 C. Therefore, with the temperature used in these studies, 10 C or higher, the possibility of salmonellae growth exists.

Takacs and Simonffy (1970) studied the fate of Salmonella spp. during maturation and storage of inoculated dry sausages. Declines in the concentration of Salmonella spp. were apparent after 7-9 days of storage and were related to pH, salt concentration, and water content. However, when initial counts were > 20,000 organisms/g meat, the sausages contained viable salmonellae up to the time of consumption.

Goepfert and Chung (1972) reported growth of salmonellae occurred in a low-acid sausage product heated to 46 C during processing. Reduction of the number of viable salmonellae occurred during refrigerated storage. The results of the present study indicated that even when salmonellae were inoculated in high numbers into turkey sausage, the salmonellae were greatly reduced in numbers, but complete destruction of the organisms did not occur.



Clostridium perfringens. Figure 3 demonstrates the survival of the heat-sensitive strain of C. perfringens ATCC 3624. The numbers inoculated varied from 14 to 250,000 organisms/g meat. The number of log cycle reductions obtained after processing declined as the inoculum declined. The range of reduction was 0.55 to 3.4 log cycles, depending on the inoculum. This result may be due to a protection phenomenon at the lower concentrations. However, it should be noted that the plate count technique has a relatively high degree of error at the lower concentrations measured in this investigation.

Figure 4 shows the survival of the heat-resistant C. perfringens strain NCTC 8238. The range of inoculation was 21.5 to 27,300 organisms/g meat. The numbers of C. perfringens were reduced by 1.0 to 2.1 log cycles during processing. The samples were not heat shocked to determine if spores were present. The results indicate that

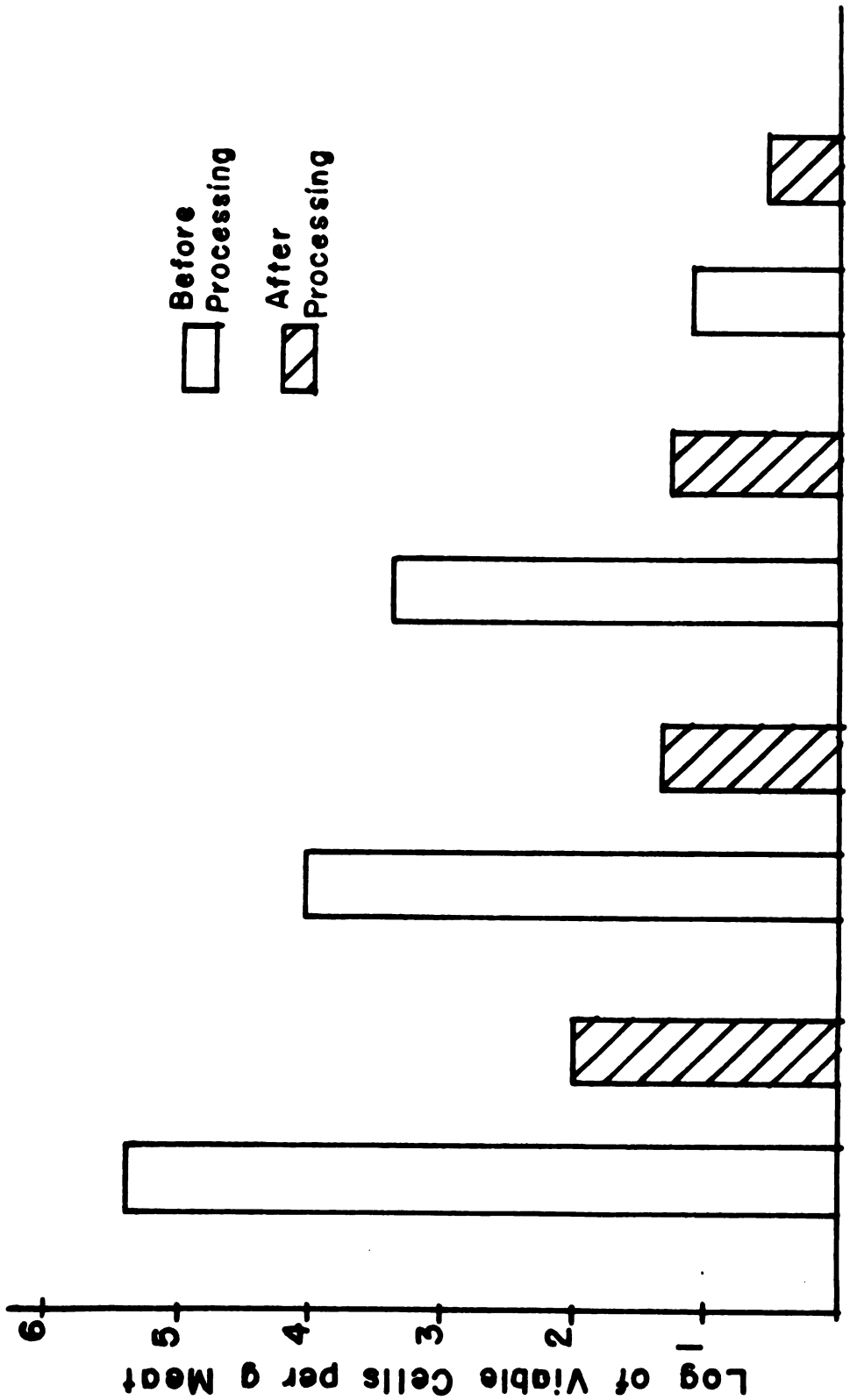


Figure 3. Effect of fermented turkey sausage processing on the survival of Clostridium perfringens ATCC 3624 inoculated at four different concentrations and enumerated by the SPS pouch method.

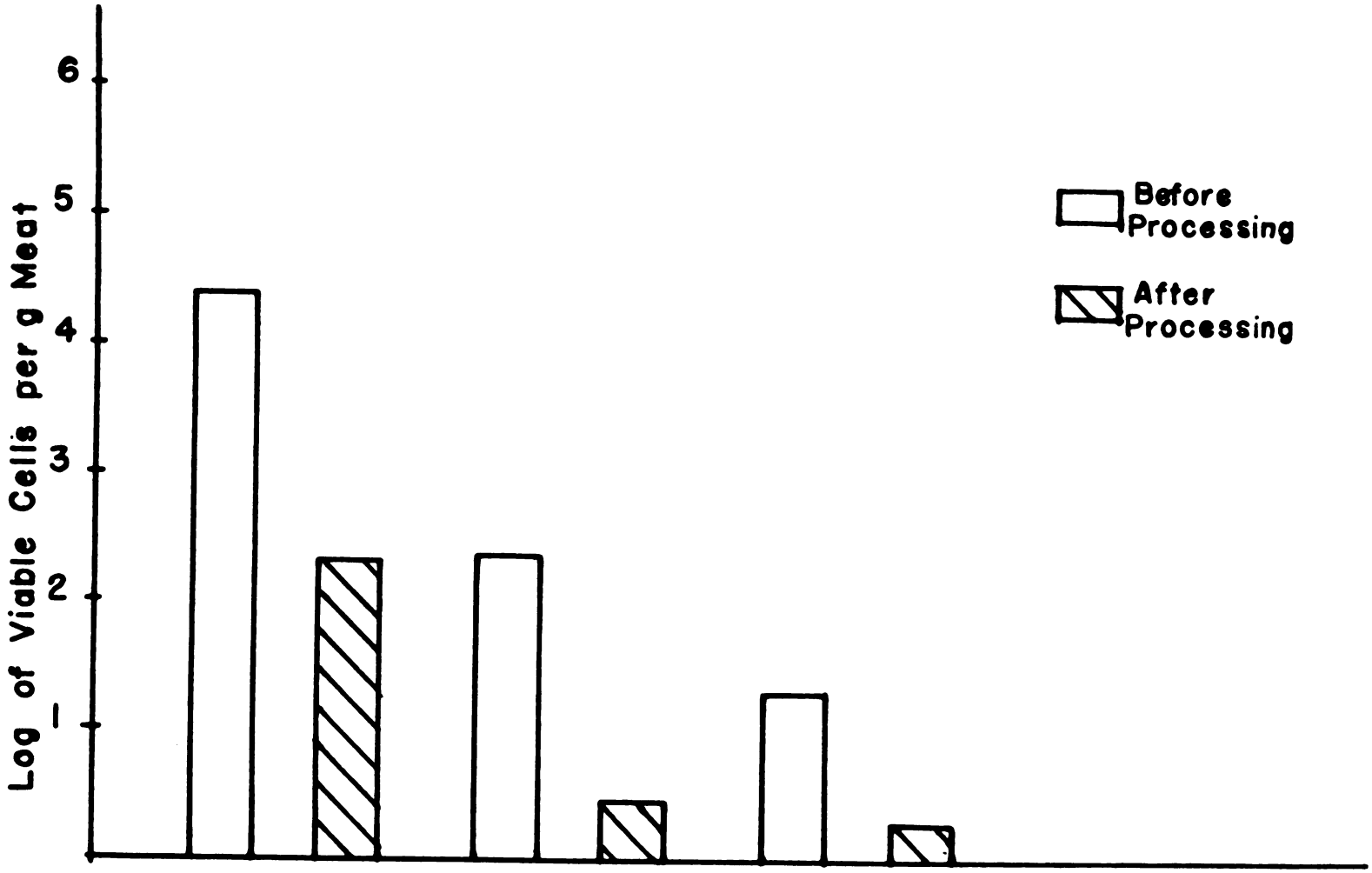


Figure 4. Effect of fermented turkey sausage processing on the survival of Clostridium perfringens NCTC 8238 inoculated at three different concentrations and enumerated by the SPS pouch method.

C. perfringens can survive the process, even when present at low concentrations, and, if mishandling of the fermented product occurred, the surviving organisms could multiply and cause a foodborne disease outbreak.

Solberg and Elkind (1970) conducted a study with "frankfurters" inoculated with C. perfringens strain 1362 and S-80. After heating the "frankfurters" to an internal temperature of 68-69 C in 30-48 min., they reported growth of these strains of C. perfringens at 12 C during storage, but not at 10 C. C. perfringens strain 1362, inoculated at 4.5 X 10^3 to 7.0 X 10^3 organisms/g meat, was reduced in numbers by .16 to .62 log cycles.

The results presented here vary from those reported by Solberg and Elkind (1970). This may be due to the addition of a starter culture which may compete with C. perfringens, the 8-day drying process, the higher levels of organisms used for inoculation, the use of different strains of C. perfringens, or the addition of both nitrate and nitrite to the fermented sausage mixture.

Enteropathogenic Escherichia coli. Three enteropathogenic strains of Escherichia coli were used in these studies. Figure 5 shows the results using EEC strain 026. The initial inoculum of < 1,000 to 10,000 organisms/g meat was reduced by 2.5 to 3.0 log cycles during processing.

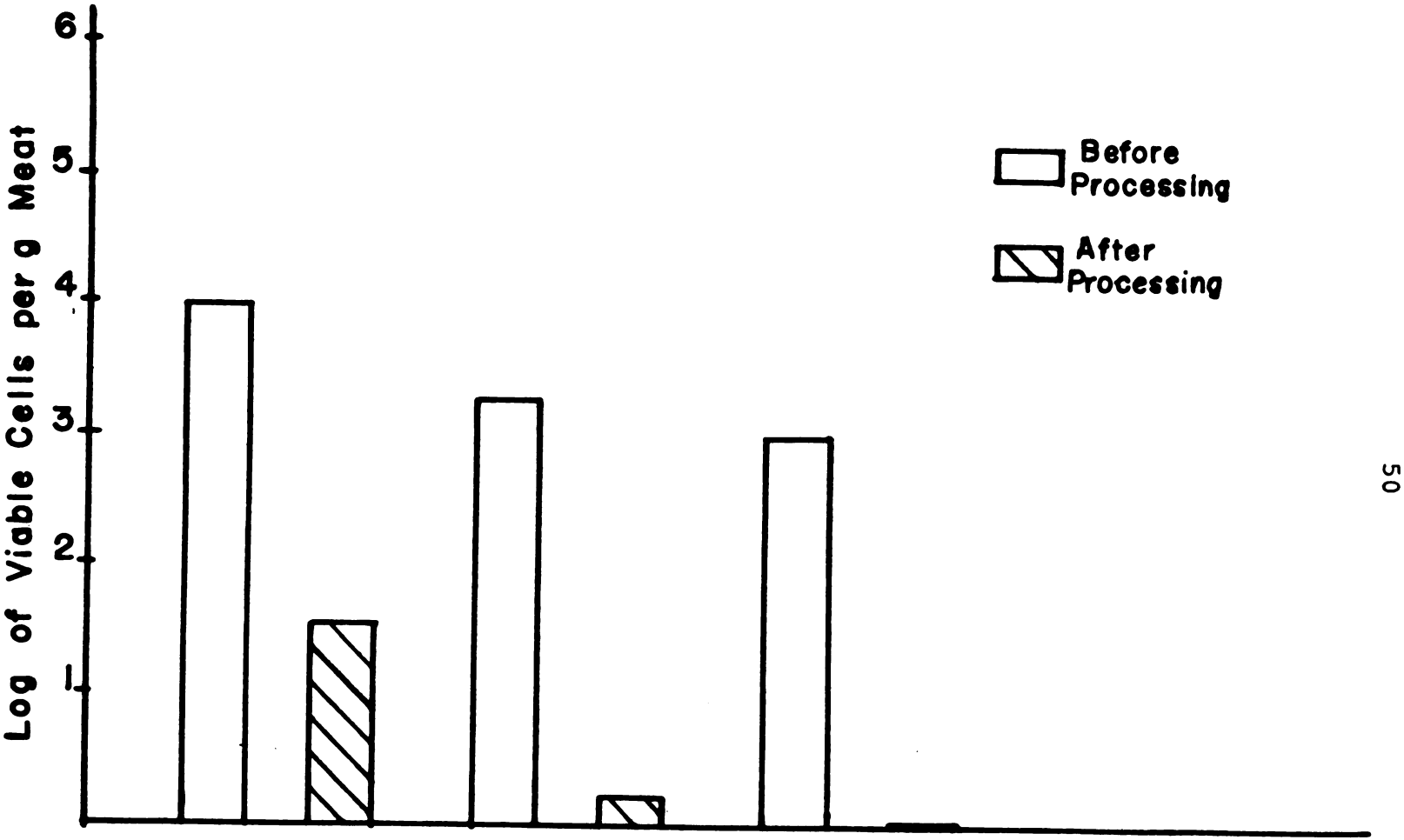
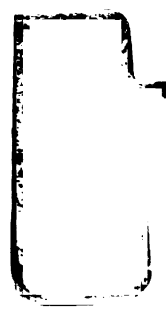


Figure 5. Effect of fermented turkey sausage processing on the survival of Escherichia coli strain 026 inoculated at three different concentrations and enumerated by the three tube MPN technique.

In contrast to the results reported above, EEC strain 0128 was relatively resistant to the fermented turkey sausage process (Figure 6). The initial numbers of organisms, 10,000 to 130,000 organisms/g meat, were reduced by only .83 to 1.8 log cycles.

As shown in Figure 7, EEC strain 0125 was inoculated at concentrations of 5,500 to 55,000 organisms/g meat which were reduced by 2.4 to 2.7 log cycles. The resistance of EEC strain 0125 was comparable to strain 026, and less than that of strain 0128. A comparison of Figures 5, 6, and 7 shows a variation between EEC strains. The EEC strain 0128 was very resistant to the process considering it does not produce spores.



Staphylococcus aureus. Staphylococcus aureus strain 243 was used to inoculate sausages with 25,000 to 1,700,000 organisms/g meat. With an inoculum of 1.0×10^6 organisms/g meat, growth occurred during the process, i.e., the final count was larger than the inoculum (Figure 8). In sausages inoculated with lower cell concentrations, no growth occurred. In contrast to that result, in a second experiment using washed cells as the inocula, an increase in the number of cells occurred even when the inoculum was only 5,500 organisms/g meat (Figure 9).

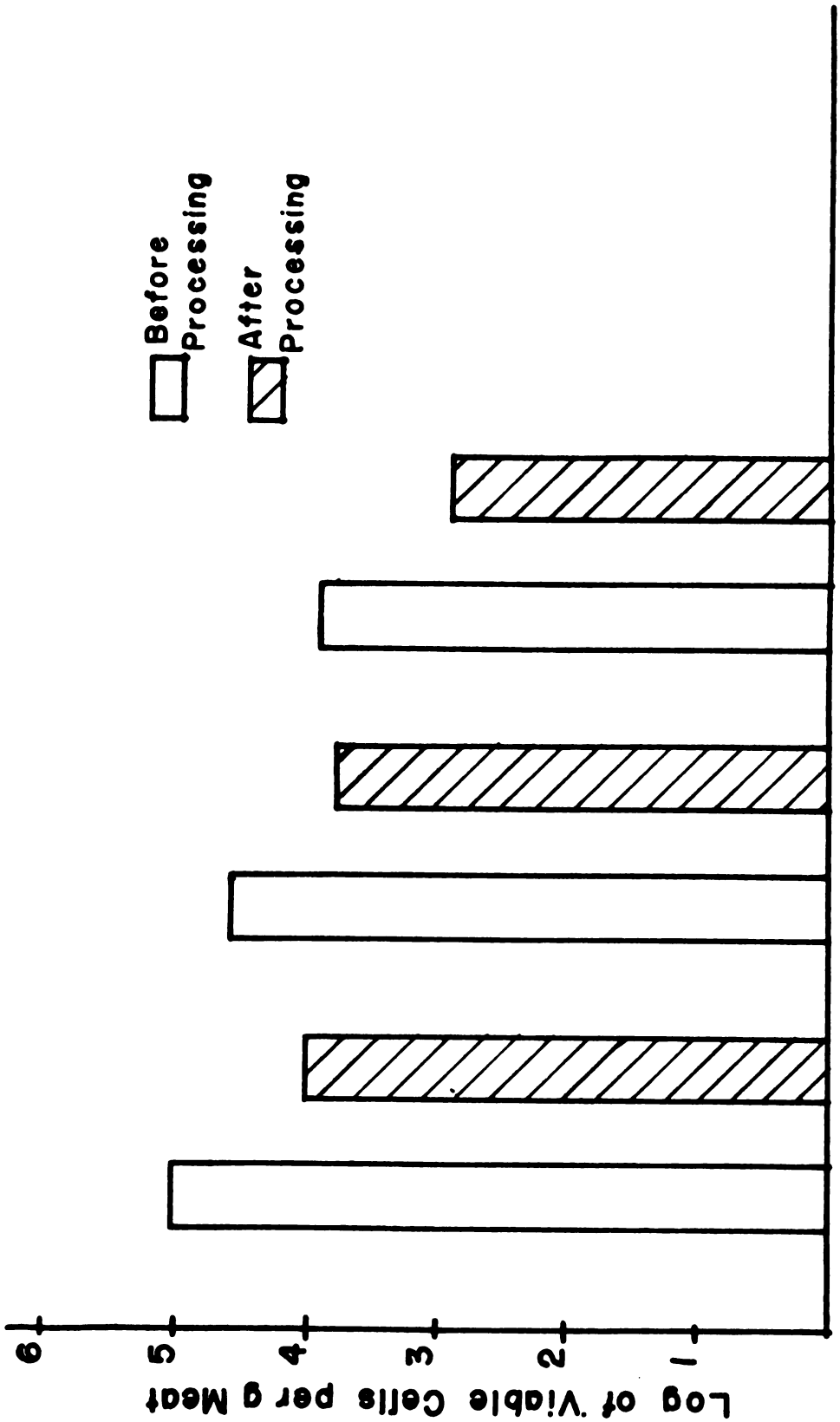


Figure 6. Effect of fermented turkey sausage processing on the survival of Escherichia coli strain 0128 inoculated at three different concentrations and enumerated by the three tube MPN technique.

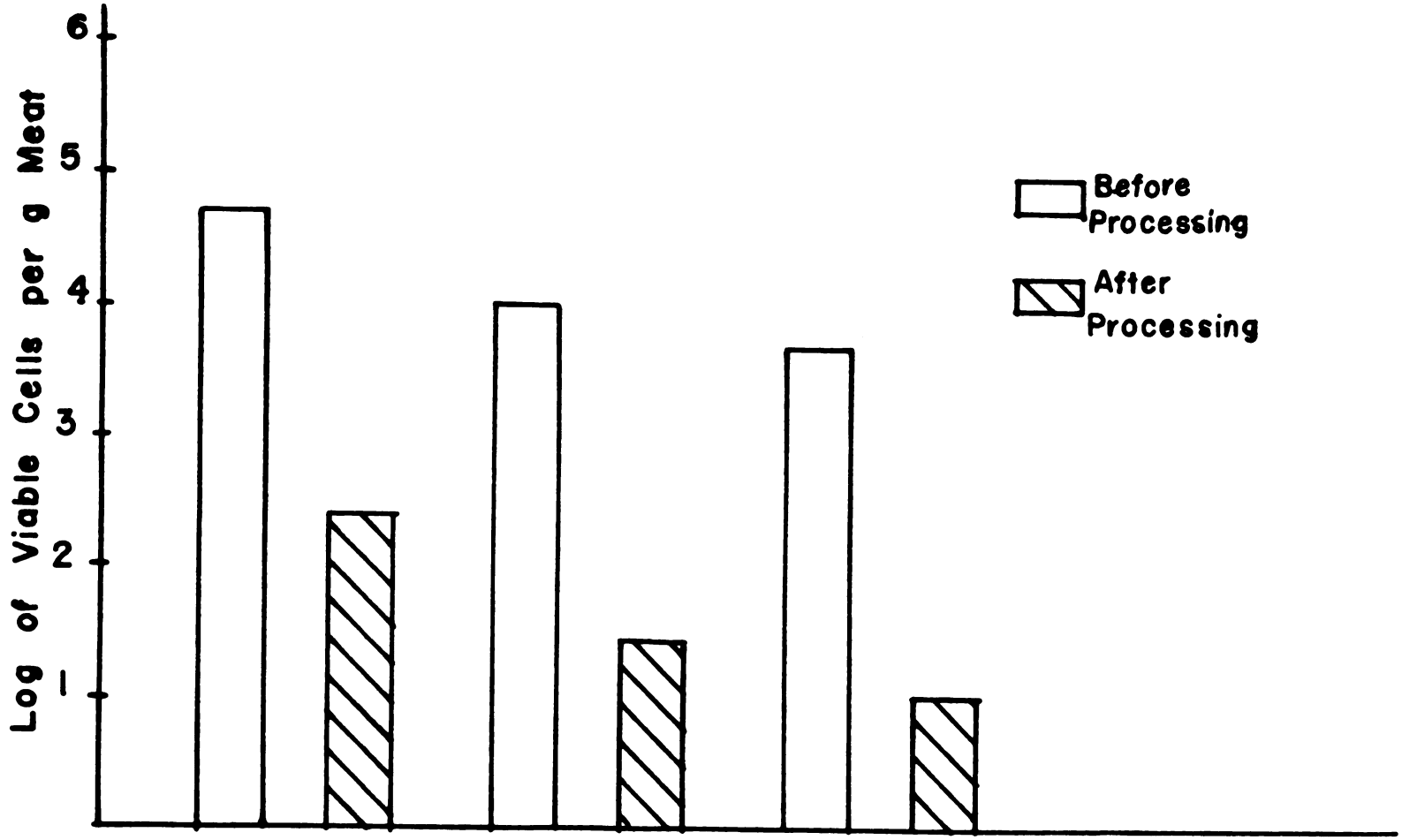


Figure 7. Effect of fermented turkey sausage processing on the survival of Escherichia coli strain 0125 inoculated at three different concentrations and enumerated by the three tube MPN technique.

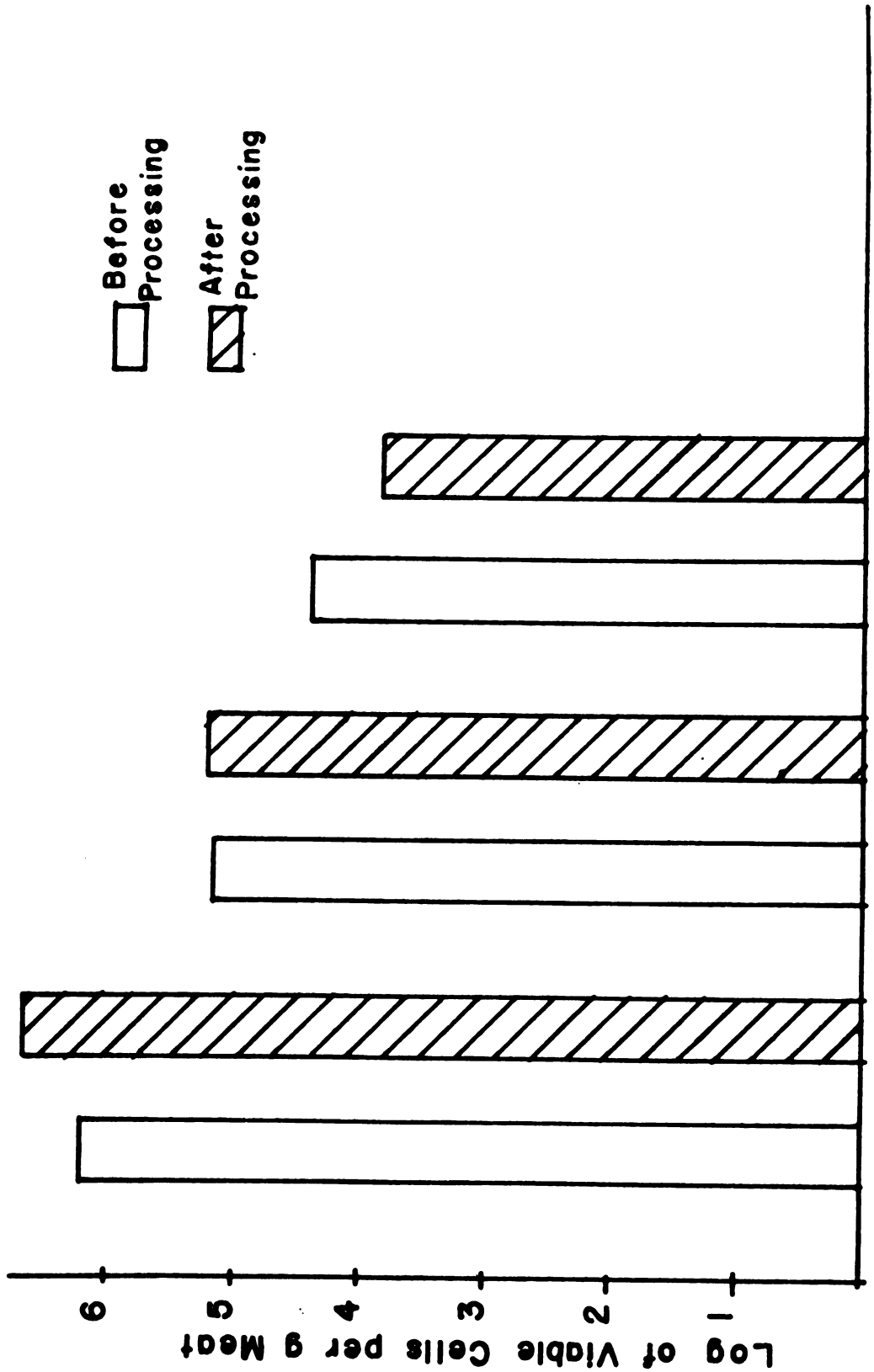


Figure 8. Effect of fermented turkey sausage processing on the survival of unwashed cells of Staphylococcus aureus strain 243 inoculated at three different concentrations and enumerated by the Vogel-Johnson plate count method.

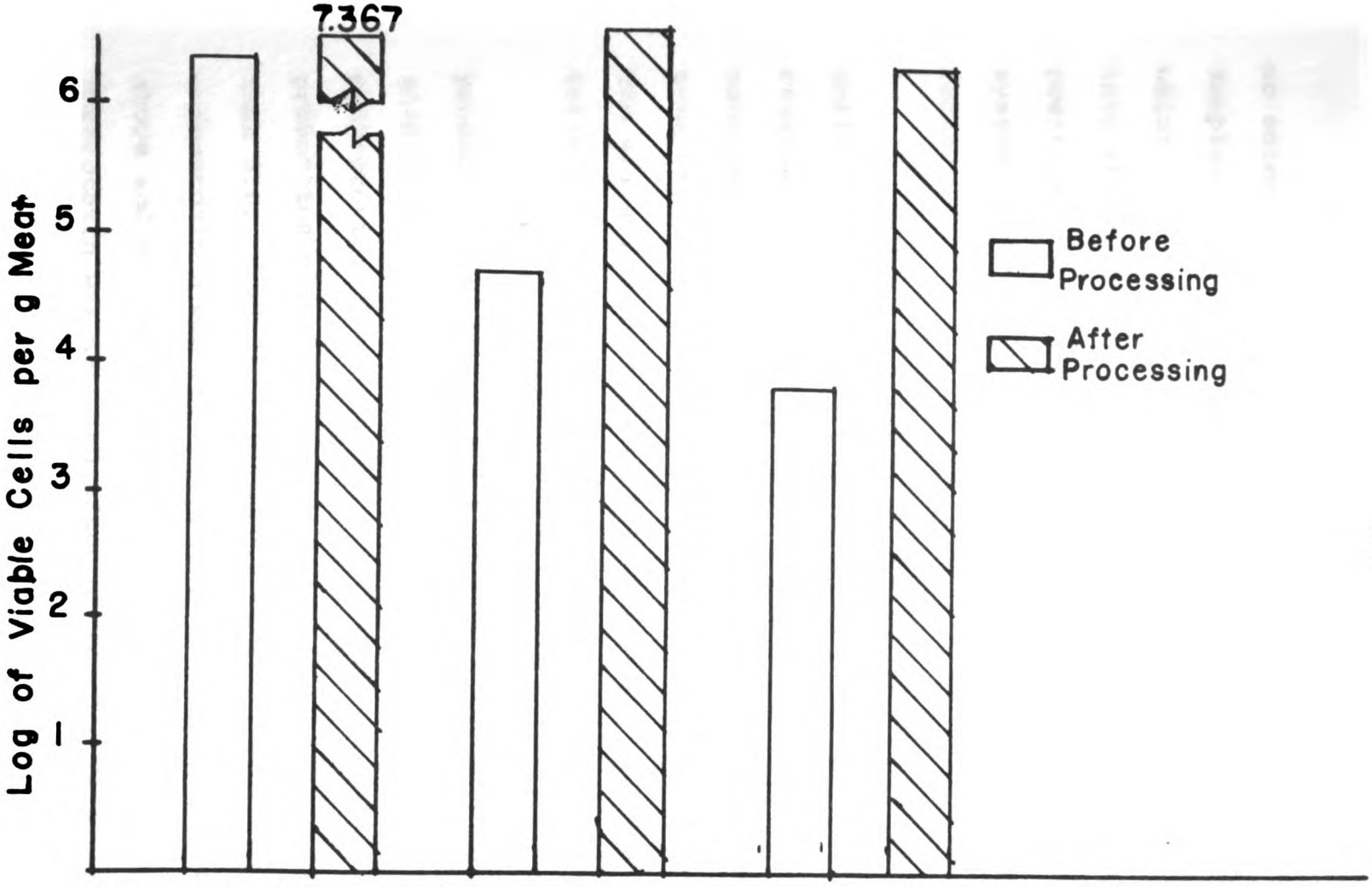
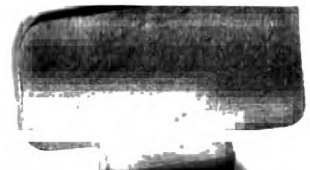


Figure 9. Effect of fermented turkey sausage processing on the survival of washed cells of Staphylococcus aureus strain 243 inoculated at three different concentrations and enumerated by the Vogel-Johnson plate count method.



Using the micro-slide double gel diffusion method no enterotoxin was detected in any of the three sausage samples or in the spent medium. A sausage mixture in which 1 μg enterotoxin per g of meat was incorporated into the blender before mixing of the sample, gave a positive result for enterotoxin indicating that the system used for detection of enterotoxin was satisfactory.

Aerobic plate counts and counts of lactic acid bacteria indicated the starter culture grew during processing and a 20-fold increase in lactic acid bacteria occurred. However, Barber and Deibel (1972) showed that even with a 6:1 ratio of P. cerevisiae to staphylococci, the staphylococci grew to populations of 10^3 to 10^4 cells/g of surface sausage.

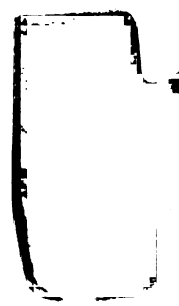
The final pH (5.64) of the finished sausage product inoculated with S. aureus was not sufficiently acid to eliminate growth and subsequent production of enterotoxin. Morse et al. (1969) reported little or no production of enterotoxin occurred at pH values less than 5.0. However, Genigeorgis et al. (1969) reported enterotoxin B was formed in cured hams at pH 5.3 or above and at sodium chloride concentrations up to 9.2%. Enterotoxin was detected after at least 2 weeks of incubation at 10 C and most samples contained enterotoxin after 8 weeks when the pH was > 5.6. Barber and Deibel

(1972) reported the majority of strains tested initiated growth and produced detectable amounts of enterotoxin aerobically in buffered BHI broth at pH 5.1. However, under anaerobic conditions most strains failed to produce detectable amounts of enterotoxin in media with pH values below 5.7. Kao and Frazer (1966) reported that type B toxin production could occur at pH values as low as 5.0 to 5.1.

The number of cells required for production of detectable amounts of enterotoxin varies from strain to strain. Barber and Deibel (1972) reported that strain S-6 required 9 X 10^8 cells/g before enterotoxin B was formed, and that strains 272 and 334 did not produce enterotoxin. Genigeorgis et al. (1969) also reported that toxic hams contained 4 X 10^6 cells/g. The highest cell concentration obtained in this study was 2.3 X 10^7 organisms/g meat.

Scott (1953) was the first to report that growth of S. aureus was related to water activity (a_w). He observed growth at water activities between .999 and 0.86, with a reduction in growth when the a_w was less than 0.94. Troller (1971) reported that toxin production in broth cultures was greatly reduced if there was a slight decrease in a_w. He indicated that the rapid growth and presence of high numbers of staphylococci did not necessarily indicate the presence of enterotoxin. Therefore, although the conditions were

present for growth of the staphylococci in the turkey sausage, a reduction in a_w during processing could have prevented the production of enterotoxin B. The starter culture did grow, and acid was produced, but not in sufficient quantities to lower the pH and thereby reduce the growth of staphylococci.



CONCLUSIONS

1. A small (14 mm) diameter fermented turkey sausage was prepared using a processing time of 8 days. This sausage was produced using only turkey meat and turkey fat, i.e., no beef or pork. Binding was improved with the use of sorbitol as a humectant.

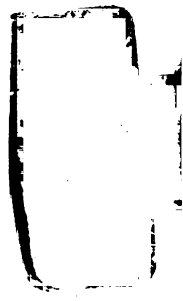
2. The aerobic plate count and lactic acid bacteria count increased 10- to 20-fold during the processing.

3. Salmonella pullorum and Salmonella senftenberg 775 W survived the process at the concentrations used in this investigation. However, the concentrations used would seldom be found in poultry meat.

4. The concentrations of Clostridium perfringens cells in the sausage were significantly reduced during processing. However, even when inoculated with low concentrations of cells, some cells survived the process. There was a surprisingly small difference in the results obtained with the heat-sensitive and heat-resistant strains.

5. Enteropathogenic strains of Escherichia coli varied in rate of survival although none of the strains grew during processing. E. coli strain 0128 was relatively resistant to the processing.

6. Washed cells of Staphylococcus aureus strain 243 grew during the processing even when inoculated at low concentrations (5,500 organisms/g meat). However, even though growth occurred, no enterotoxin was produced.



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APPENDIX

APPENDIX A

TABLES

APPENDIX A

TABLE IA. Determination of total acidity, pH, salmonellae counts, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Salmonella pullorum.

Batch Number	1	2	3
salmonellae			
(org/g.)			
before processing	$>1.1 \times 10^5$	$>1.1 \times 10^5$	3.8×10^5
after processing	≤ 4.6	8.6	< 3
pH			
after heating	5.6	6.3	5.8
after processing	5.7	6.7	5.8
% total acidity			
after heating	.08	.08	.10
after processing	.13	.10	.13
% weight loss (moisture)	38%	70%	34%

TABLE IIA. Determination of total acidity, pH, salmonellae counts, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Salmonella senftenberg 775 W.

Batch Number	1	2	3
salmonellae			
(org/g)			
before processing	6.1×10^4	1.6×10^4	$>2.2 \times 10^3$
after processing	$>1.1 \times 10^3$	2.4×10^2	1.2×10^2
pH			
after heating	6.1	5.9	5.9
after processing	5.9	6.2	6.2
% total acidity			
after heating	.11	.12	.12
after processing	.16	.12	.15
% weight loss (moisture)	40%	30.2%	28%

TABLE IIIA. Determination of total acidity, pH, counts of *Clostridium perfringens*, and percent weight loss in four experimental batches of fermented turkey sausage inoculated with *Clostridium perfringens* ATCC 3624.

Batch Number	1	2	3	4
Clostridium perfringens (org/g)				
before processing	2.5×10^5	1.1×10^4	2.6×10^3	1.4×10^1
after processing	1.0×10^2	2.4×10^1	1.8×10^1	4
pH				
after heating	5.4	5.5	5.4	6.0
after processing	5.0	5.6	5.2	6.2
% total acidity				
after heating	.12	.15	.12	.12
after processing	.19	.21	.18	.13
% weight loss (moisture)	47.4%	40.8%	40.1%	26.8%

TABLE IVA. Determination of total acidity, pH, counts of *Clostridium perfringens*, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with *Clostridium perfringens* NCTC 8238.

Batch Number	1	2	3
Clostridium perfringens			
(org/g)			
before processing	2.7×10^4	2.2×10^2	2.2×10^1
after processing	2.1×10^2	3	2
pH			
after heating	5.3	5.9	5.8
after processing	5.0	6.4	6.3
% total acidity			
after heating	.10	.10	.11
after processing	.22	.15	.14
% weight loss (moisture)	49.6%	30.1%	28.8%

TABLE VA. Determination of total acidity, pH, counts of Escherichia coli, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Escherichia coli 026.

Batch Number	1	2	3
E. coli			
(org/g)			
before processing	1.0×10^4	1.8×10^3	$< 1.0 \times 10^3$
after processing	3.3×10^1	1.6	< 3
pH			
after heating	5.8	5.6	5.3
after processing	5.1	5.2	4.9
% total acidity			
after heating	.08	.08	.08
after processing	.21	.21	.25
% weight loss (moisture)	51.3%	50.9%	53.9%

TABLE VIA. Determination of total acidity, pH, counts of Escherichia coli, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Escherichia coli 0128.

Batch Number	1	2	3
E. coli			
(org/g)			
before processing	1.3×10^5	4.5×10^4	9.8×10^3
after processing	$\geq 1.1 \times 10^4$	6.7×10^3	$\geq 7.9 \times 10^2$
pH			
after heating	5.3	5.1	5.8
after processing	5.1	5.3	5.5
% total acidity			
after heating	.10	.10	.09
after processing	.21	.19	.21
% weight loss (moisture)	51.3%	50.4%	52.4%

TABLE VIIA. Determination of total acidity, pH, counts of Escherichia coli, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Escherichia coli 0125.

Batch Number	1	2	3
E. coli			
(org/g)			
before processing	$\geq 1.1 \times 10^3$*	1.1×10^4	$\geq 1.1 \times 10^4$**
after processing	1.2×10^1	$< 3.0 \times 10^1$	2.2×10^2
pH			
after heating	5.7	5.8	5.5
after processing	5.7	5.5	5.0
% total acidity			
after heating	.12	.11	.10
after processing	.19	.18	.21
% weight loss (moisture)	35.2%	35.9%	37.5%

*1.1×10^3 approximately 5500 org/g

**1.1×10^4 approximately 55,000 org/g

TABLE VIIIA. Determination of total acidity, pH, counts of Staphylococcus aureus, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Staphylococcus aureus strain 243 (unwashed cells).

Batch Number	1	2	3
S. aureus			
(org/g)			
before processing	2.5×10^4	1.4×10^5	1.7×10^6
after processing	6.9×10^3	1.5×10^5	4.7×10^6
pH			
after heating*	5.5	5.2	5.5
after processing	5.8	5.6	5.6
% total acidity			
after heating*	.16	.14	.15
after processing	.27	.20	.22
% weight loss (moisture)	39.8%	36.3%	38.5%

*Samples taken after 12 hours drying.

TABLE IXA. Determination of total acidity, pH, counts of Staphylococcus aureus, and percent weight loss in three experimental batches of fermented turkey sausage inoculated with Staphylococcus aureus strain 243 (washed cells).

Batch Number	1	2	3
S. aureus			
(org/g)			
before processing	5.6×10^3	5.3×10^4	2.2×10^6
after processing	1.8×10^6	4.2×10^6	2.3×10^7
pH			
after heating*	5.5	5.2	5.2
after processing	5.4	5.3	5.3
% total acidity			
after heating*	.11	.12	.12
after processing	.21	.22	.22
% weight loss (moisture)	45.9%	45.7%	46.9%

*Samples taken after 12 hours drying

TABLE XA. Various nonpathogenic microorganisms enumerated in processed sausages inoculated with *Salmonella pullorum* and *Salmonella senftenberg* 775 W.

Salmonella pullorum (batch number 3)	After Processing
	org/g
aerobic plate count	> 1000
lactic bacteria	> 1000
coliform	76
yeast and mold	nc

Salmonella senftenberg 775 W (batch number 3)	After Processing
	org/g
aerobic plate count	4.3×10^6
lactic bacteria	1.4×10^6
coliform	6.0×10^2
yeast and mold	nc

nc = < 10 organisms detected

TABLE XIA. Various nonpathogenic microorganisms enumerated in processed sausages inoculated with Clostridium perfringens ATCC 3624.

Clostridium perfringens ATCC 3624 (Batch number 3)	Before Processing	After Processing
	org/g	org/g
aerobic plate count	$1.5 \times 10^{7}_{6}$	$4.6 \times 10^{8}_{8}$
lactic bacteria	$5.0 \times 10^{3}_{3}$	1.7×10^{8}
coliform	3.2×10^{3}	nc
yeast and mold	1.0×10^{3}	nc

nc = < 10 organisms detected

TABLE XIIA. Various nonpathogenic microorganisms enumerated in processed sausages inoculated with Escherichia coli strain 026.

Batch Number	Before Processing		
	1	2	3
	org/g	org/g	org/g
aerobic plate count	3.6×10^6	4.7×10^6	1.7×10^7
lactic bacteria	3.3×10^6	3.9×10^6	1.5×10^7
coliform	1.0×10^2	1.0×10^2	$< 1.0 \times 10^3$
yeast and mold	3.4×10^3	5.0×10^4	1.1×10^3

Batch Number	After Processing		
	1	2	3
	org/g	org/g	org/g
aerobic plate count	2.0×10^8	2.5×10^8	1.1×10^9
lactic bacteria	1.6×10^8	1.5×10^8	-
coliform	1.2×10^4	4.6×10^3	-
yeast and mold	nc	nc	nc

nc = < 10 organisms detected

TABLE XIIIA. Various nonpathogenic microorganisms enumerated in processed sausages inoculated with Staphylococcus aureus strain 243 (washed cells).

Batch Number	1	2	3
	org/g	org/g	org/g
Before processing			
aerobic plate count	8.6×10^6	7.5×10^6	1.2×10^7
lactic bacteria	5.6×10^5	1.5×10^6	1.5×10^6
After processing			
aerobic plate count	3.3×10^8	7.3×10^8	5.7×10^8
lactic bacteria	2.8×10^8	2.6×10^8	4.0×10^7