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Microbiological examination of beef prepared by bacterial fermentation in brine containing papaya

Mario Armando Castro

To the Graduate Council:

I am submitting herewith a thesis written by Mario Armando Castro entitled "Microbiological examination of beef prepared by bacterial fermentation in brine containing papaya." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

F. A. Draughon, Major Professor

We have read this thesis and recommend its acceptance:

C. C. Melton, H. O. Jaynes

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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To the Graduate Council:

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We have read this thesis
and recommend its acceptance:

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Date March 1986

MICROBIOLOGICAL EXAMINATION OF BEEF PREPARED BY BACTERIAL FERMENTATION IN BRINE CONTAINING PAPAYA

A Thesis

Presented for the

Master of Science

Degree

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Mario Armando Castro

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ABSTRACT

Salt and papaya were used to brine-ferment fresh round beef pieces which were later smoked and dried (Ubbaonu, 1982). This study was undertaken to conduct microbial examinations which were performed to aid in the selection of an appropriate salt-papaya brine concentration in Ubbaonu's study (1982) and to produce a product fit for human consumption. Identification of the microflora of the beef pieces was performed before fermentation, after fermentation, and after drying and storing the meat for 30 days.

During the fermentation, beef pieces were separated and treated with 36 different brine solutions made of varying levels of NaCl (0% to 10% in 2% increments) in combination with varying levels of papaya (0% to 25% in 5% increments).

At the end of the brine-fermentation, presumptive coliforms and anaerobes were reduced when 8% salt or more was used in the brine ($P<0.0001$). Presumptive C. perfringens were not detected at salt concentrations higher than 6%. Salmonella was found at 2% and 8% salt. The increase of papaya in the brine decreased the meat's final pH ($P<0.0001$).

A 10% salt-10% papaya fermentation was selected as the best combination based on the results of this study and Ubbaonu's (1982) observations. A new batch of fresh beef round pieces was subjected to this treatment to collect bacterial isolates for identification.

Fresh beef pieces had a microflora composed mainly of Micrococcus (95% on SMA and 87% on SMA plus 3% NaCl). Gram-positive heterofermentative

lactics predominated in the APT agar microflora (33.3%). S. faecium (40.9%) and S. faecalis (36.3%) were the predominant microorganisms found on SPS anaerobic agar. Only Rhodotorula was found in malt extract agar.

At the end of the fermentation, the contaminant P. fluorescens predominated among the bacteria isolated from SMA (78%), SMA plus 3% NaCl (68%) and APT agar (63.3%); Streptococcus formed 87% microaerophilic/anaerobic isolates. Malt extract agar showed no yeasts or molds.

After drying, the homofermentative bacteria (Streptococcus and Pediococcus) formed the predominant fermented-dried meat microflora. Malt extract agar showed no yeasts or molds.

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CHAPTER I

INTRODUCTION

Prehistoric men developed the first food preservation methods. By reducing the water content of fruit, meat, and fish, they were able to prevent spoilage. This process was accomplished by exposing the foods to the sun for several days or over fires. Later, salt was added to the process, thus improving the preservation method. Today, drying still constitutes an important role in the developing countries where the lack of refrigeration in rural areas does not allow cold storage of meat. Animals used for food must be consumed shortly after slaughter. The only option is to store meat by drying it. Such an alternative also presents its problems when meat is mishandled. Villagers often use the wrong proportions of salt and drying time. To avoid the unpleasant flavor caused by high salt concentrations and toughness of the dry meat, villagers frequently let the meat dry below safe levels of salt and water activity.

In the underdeveloped countries, the lack of proper sanitation that starts in the slaughter house continues to the butcher's booth where the meat chunks are placed on tables without refrigeration, or exposed to dust and insects. All these factors, combined with the lack of bacterial and other quality standards, aggravates the already unsafe drying methods. This results in an unreliable product.

Dr. Collins N. Ubbaonu and Dr. Curtis C. Melton have developed a dehydrated, fermented beef product which can be processed and

stored without refrigeration (Ubbaonu, 1982). The product was developed using common natural ingredients (salt, papaya and fresh beef).

The primary objective of this study was to conduct a microbiological survey of the dehydrated fermented beef product and to determine an acceptable curing treatment using sodium chloride and papaya. A secondary objective was to identify the microflora of the fermented beef during and after the curing treatment.

CHAPTER II

REVIEW OF LITERATURE

1. MICROBIOLOGY OF BEEF

Sources of Contamination

The bacteriological quality of beef is affected by factors prior to, during, and after slaughter. Prior to slaughter, the quality of the beef is affected by the microbiological population of the feeding areas; unclean feeding areas may increase bacteria on the surfaces of the cattle. Bacteria are carried on the skin, hair, intestines and hooves of the cattle to the slaughter house (Price and Schweigert, 1978). From these areas bacteria are then passed to the surfaces of the carcass during the skinning operation. Contamination may also occur during the handling and sticking operation (Ayres et al., 1980; Price and Schweigert, 1978). The rump, brisket, and forelegs are the areas which are likely to show greater contamination during slaughter (Murray, 1969). Stringer et al. (1969) found that the neck, the lean surface area above the aitch muscle, and the cod area, were the most highly contaminated. Aerobic plate counts were log 5.34/in^2, 5.68/in^2, and 5.42/in^2, respectively. Kotula et al. (1975) and Stringer et al. (1969) reported that seasonal variation had little or no effect on the degree of initial contamination of the carcasses.

After slaughter, bacterial contamination continues throughout the various processes related to preparing the meat for consumption. The air and walls of the chilling room are subjected to contamination.

From here, organisms could be later transferred to the carcass by the circulating air and by the workers manipulating the carcasses; the areas closer to the floor are especially receptive (Ayres, 1955). Carcasses leave the chilling room and are then quartered, cut, trimmed, and transferred to the retail stores. Bacteria which have been multiplying since evisceration are then redistributed by cutting and handling the beef. Knives, saws, tables, air, and workmen are some of the sources that may increase and disperse bacteria (Ayres, 1955; Rey et at., 1970; Stringer et al., 1969).

Generally, standard plate counts of beef carcasses immediately after slaughter range from log $3.3/in^2$ to log $5.3/in^2$. By the time meat reaches the retail stores, counts range from log $4.9/in^2$ to log $6.3/in^2$ (Stringer et al., 1969). Kotula et al. (1975) reported that mold and yeast counts remained less than $10/cm^2$ on the carcasses in all instances. Organisms on cut, packaged, raw beef range from 10^3 to 10^5/g for aerobic bacteria, and 10^4 to 10^5/g for anaerobic bacteria (Ayres et al., 1980).

Effect of Temperature on the Microflora of Beef

Temperature has an important effect on growth of bacteria. The final bacterial population of beef is influenced by the temperature at which the beef was held before it arrived at the retail store. The initial flora of beef after slaughter consists mainly of *Micrococcus*, *Pseudomonas* and *Microbacterium* spp. (Ayres, 1955, 1960; Ayres et al., 1980). *Corynebacterium*, *Moraxella-Acinetobacter*, *Flavobacterium*, *Enterobacter*, and *Penicillum* spp. are also commonly

found in meat at the beginning of the storage period. During storage, at temperatures around 4°C, the number of Micrococcus spp. is kept constant or decreases leaving Pseudomonas, Enterobacter, Lactobacillus, and Microbacterium spp. as the principal genera of refrigerated meat (Ayres, 1969; Ayres et al., 1980; Gill et al., 1978).

It is generally recognized that gross neglect has occurred if the aerobic count reaches 10^6/g on fresh, refrigerated beef (Murray, 1969). By practicing good hygiene and lowering the temperature of the carcass rapidly after butchering to about 4°C, it is possible to keep bacteria from reaching these high numbers during storage (Ayres, 1955; Price and Schweigert, 1978). Another good reason for lowering the temperature is to prevent hazardous bacteria from reaching high enough concentrations to constitute a health hazard. Refrigerated beef contains a predominately psychrotrophic flora (Ayres, 1960).

When beef carcasses are stored at temperatures higher than normally used in refrigeration (4°C), the population of bacteria starts to change as higher temperatures favor mesophilic organisms. At temperatures of 15°C to 20°C, psychrotrophic and mesophilic organisms (Pseudomonas and Micrococcus) are approximately equal in numbers (Ayres, 1960; Ayres et al., 1980).

Ayres (1960) reported the type of microorganisms surviving in beef stored at different temperatures. Despite the low bacterial counts of the beef at the beginning of his experiments, meat held at 25°C developed off-odor by the second day with counts above

$10^7/cm^2$. Similar counts were observed by the third day when held at 20°c, by the fifth day when stored at 10°C, by the tenth day when held at 5°C, and by the twentieth day when stored at 0°C. When meat was held at 5°C and 0°C, the microbial population progressively decreased during the first and second day, respectively. The population increased after that. Gram negative rods were the predominant flora on meat stored at 0°C and 5°C; gram positive cocci were the next most numerous. At 15°C, gram negative rods and gram positive cocci were in approximately equal numbers; at this temperature and higher, there was about an equal incidence of Pseudomonas and Micrococci.

Gill et al. (1980) found that Acinetobacter (44%) and Enterobacteriaceae (36%) dominated the major aerobic flora of beef steaks held at 30°C for three days; whereas, Pseudomonas (60%) dominated at 20°C. The anaerobic flora, on the other hand, was dominated by Clostridium (62%) and Lactobacillus (33%) when the steaks were held at 30°C; whereas, Enterobacteriaceae (80%) and Lactobacillus (20%) dominated at 20°C.

Yeasts and molds, although they do not constitute a large part of the natural flora of meats, have been reported growing in refrigerated beef. Yeasts seldom cause spoilage of fresh, red meats, and when they do so, it is because bacterial numbers have been restricted (Ayres et al., 1980). Torulopsis, Candida and Rhodotorula were reported by Ayres (1960) as some of the genera of yeasts occasionally recovered from refrigerated meat. Molds, much

more like yeasts, will spoil meats whenever bacterial growth is inhibited (Gill et al., 1981). This happens when chilled meat is held at a humidity that will prevent bacterial growth. Koburger (1973) reported that fungi recovered from meats grow better at temperatures between 16°C to 22°C.

Effects of pH on Microorganisms

The living muscle has a pH of 7.0 to 7.2 (Gill and Newton, 1978). After death, glycolysis results in the conversion of glycogen to lactic acid and pH falls to a level of 5.5 to 5.7. Most bacteria have an optimum pH for growth near neutrality, with maximum and minimum values around 8.0 and 5.0, respectively (Price and Schweigert, 1978).

An ultimate high pH (above 6.0), resulting from a muscle deficient in glycogen because of exercise or stress prior to slaughter, produces dark, firm, dry (DFD) meat (Newton and Gill, 1980). Such meat spoils more rapidly than normal. Organisms responsible for such spoilage include species of Acinetobacter, Alteromonaas putrefaciens, Enterobacter liquefaciens, and Pseudomonas spp. (Gill and Newton, 1978; Newton and Gill, 1978; Newton and Gill, 1981). Such spoilage is due to two factors according to Newton and Gill (1981). One factor is the depletion of glycogen reserves in the muscle which leads bacteria to use amino acids as a source of energy. This results in the production

of ammonia and off-odors when the population exceeded $10^6/cm^2$; whereas, in normal pH meat, a cell density in excess of $10^8/cm^2$ must be attained before release of ammonia is observed (Gill 1976; Newton and Gill, 1978). The second factor is the effect of pH on bacteria that produce off-odors. Acinetobacter and Alteromonas putrefaciens are inhibited by the pH of normal meat. Production of hydrogen sulfide by bacteria is inhibited at pH values below 6.0 (Nicol et al., 1970). Normal pH meat does not have much effect on Pseudomonas spp., but favors Lactobacilli spp. which are known to inhibit growth of other bacteria. Lactobacillus has been reported to inhibit the growth of Pseudomonas, Staphylococcus aureus, and Salmonella (Daly et al., 1973; Price and Lee, 1970; Raccach and Baker, 1978).

A pH of 4.7 is the minimum for staphylococcal growth; the maximum being 9.5 to 9.8 (Raccach and Baker, 1978; Troller, 1976). On the other hand, Peterson et al. (1964) reported that staphylococcal growth was inhibited at pH values of 5.2 when grown in competition with saprophytic bacteria. This was observed at different temperatures ranging from 0°C to 37°C with the exception of 10°C where staphylococci were the predominant population for 6 days before they decreased. The pH of the medium decreased at all temperatures. Troller (1976) reported that at pH levels of 5.0 and below, little or no Staphylococcus enterotoxin is produced. Lee et al. (1977) reported that when Genoa salami was inoculated with S. aureus (about 10^7 to $10^8/g$), enterotoxin A was detected; whereas, enterotoxin B was not. This, perhaps, is due to the low pH of salami and the

ability of S. aureus to produce more enterotoxin A than enterotoxin B at lower pH values (Troller, 1976).

Roberts and Ingram (1973) reported growth of C. botulinum types A, B, and F at pH values of 5.4 to 6.2 (at 35°C over a period of 3 months). Type E failed to grow at pH 5.4 but grew at 5.6 to 6.2. C. botulinum type A did not grow at pH 5.4 when NaCl was added to a concentration of 5%; whereas, type B was inhibited at the same pH when NaCl was 6%. Vibrio spp. prefer a neutral or alkaline medium; acidic conditions of pH 5.0 to 5.5 will inhibit Vibrio spp. according to Beuchat (1975) and Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Bacillus cereus toxin production occurs within a pH range of 6.0 to 8.5; with an optimum of 7.0 to 7.5. Bacillus cereus growth ranges from a pH of 4.3 to 9.3 (Johnson, 1984).

Effects of Water Activity

Availability of free water in foods is important for bacterial growth. It influences their rate of growth. By removing water from food we reduce or stop bacterial spoilage of food. Water in food serves as a solvent for many constituents, and any removal of water will concentrate these solutions. Thus, alternatives such as salting and syruping do preserve foods by increasing solute concentrations (Ayres et al., 1980; Troller and Christian, 1978).

Labuza (1975) defines water activity (Aw) as

$$Aw = P/Po = \%ERH/100$$

Where

Aw = water activity,

P = vapor pressure of water in a food system,

Po = vapor pressure of pure water, and

%ERH = relative humidity at which food neither gains nor loses moisture to the atmosphere.

Most bacteria found in meat are favored by its high Aw (the value for fresh meats is Aw = 0.99). The growth of psychrotrophic bacteria, predominantly Pseudomonas spp. which causes spoilage of fresh meats, is reduced during the initial chilling period, by the reduction in Aw that results from surface drying. The minimum Aw reported for Pseudomonas spp. is 0.95 (Juven and Gertshouki, 1976), although generally it is believed to be 0.97 (Ayres et al., 1980). Clostridium botulinum types A and B along with Escherichia coli are reported to have a minimum Aw of 0.95; that of Staphylococcus aureus is 0.88 (Ayres et al., 1980; Troller, 1976). Although yeasts and molds are not able to compete with bacteria at the higher Aw because of their rate of growth, they are able to grow at much lower Aw values than bacteria. Molds like Aspergillus spp. and Penicillium spp. are reported to have a minimum Aw between 0.70 to 0.85. Yeasts are commonly found at levels near 0.90 Aw; although there are some like Saccharomyces rouxii which had been reported grown as low as 0.62 (Ayres et al., 1980; Troller and Christian, 1978).

Effects of Sodium Chloride on Microorganisms

Sodium chloride (NaCl) has been used since prehistoric times to prevent food spoilage. Most bacteria and yeast are relatively

sensitive to a low concentration of NaCl. They are inhibited at concentrations as low as 3% to 5% in fluids. Staphylococcus aureus has been reported to grow at NaCl concentrations of 15-20% (Minor and Marth, 1971). However, when pH is lowered to 5.1 at 16% NaCl, no cells survived in Brain Heart Infusion broth (37°C) after 10 days. Raccach and Baker (1979) reported that 3% NaCl decreased the basal thermal resistance of S. aureus by 37% when the bacterium was heated at 60°C for 60 minutes in mechanically deboned poultry meat at a pH of 4.7. Enterotoxin B production is totally suppressed when the Aw is lowered to 0.96 (with a NaCl concentration of approximately 6.5%) (Troller, 1976). Stern et al. (1979) noticed that S. aureus, grown in Trypticase Soy Broth at 37°C and pH 5.0, reduced its rate of growth when NaCl concentration increased from 3% to 7%. At 3% NaCl, the organism took 6 hours before any increase in the number of cells was noticeable; whereas, at 7%, it needed 24 hours to start increasing in numbers (initial population was 10^5 cells/ml).

Yersinia enterolitica has been implicated in causing water and food borne enteritis. Stern et al. (1980) reported that concentrations of 7% NaCl inhibit growth of this organism for 48 hours in Brain Heart Infusion Broth at 25°C. Vibrio parahaemolyticus, a known halophilic organism which has been responsible for food borne disease outbreaks, requires a minimum of 0.5% NaCl but it is unable to grow in a suitable medium containing 10% (Beuchat, 1975). Salmonella is another organism that has been implicated with food borne diseases in meat products. It has been reported that the

addition of 3% NaCl accelerates the rate of death of Salmonella in fermented sausage during the first 12 hours of storage (Goepfert and Chug, 1970). Bryan (1968) stated that Salmonella will not grow at 8% NaCl. Ayres et al. (1978) reported that Salmonella could initiate growth in broth containing 7-8% salt at 37°C.

2. PAPAYA AND ITS ENZYMES

Papaya (Carica papaya) is a native fruit of tropical America that is now widespread in tropical regions around the world (Samson, 1980). Its composition is shown in Appendix 1, page 85 (Watt and Merrill, 1963). Natives of Central America had been utilizing papaya juice and papaya leaves to tenderize meat for many centuries (Reed, 1975). From the early 1900's to the late 1930's, papaya latex was collected by cutting or scratching the surface of the green fruit while still on the papaya tree. This latex was dried, pulverized and used as a tenderizing agent. Papaya latex contains three major enzymes: papain, chymopapain, and lysozyme. Their relative amounts in latex, molecular weights, and isoelectric points are shown in Appendix 2, page 86 (Reed, 1975). Balls and Hoover succeeded in separating and crystallizing papain in 1937 (Balls and Hoover, 1937).

Gottschall and Kies (1942) found that the optimum temperature for the digestion of meat and pure proteins by papain was higher than the optimum temperature for the activity of most other enzymes. Gottschall and Kies (1942) also reported that the results of papain digestion of meat at room temperature and at the cooking temperatures

ordinarily employed were inferior to those obtained at 55°C and 75°C. Tappel et al. (1956) suggested that this increase of papain hydrolyzation may be partially due to denaturation of the heat-labile muscle proteins. Tappel et al. (1956) stated that since both elastin and collagen contribute to the toughness of meat it is very significant that papain catalyzes their hydrolysis with maximum effects at 60°C. Hinrichs and Whitaker (1962) also found that collagen in meat is degrated more rapidly by ficin, bromelain, papain, and trypsin at elevated temperatures (60-70°C), and that sodium chloride (at concentrations of 0.8M) produce a small but significant increase in solubilization of collagen. Kang and Rice (1970) found that papain has a stronger activity on the salt soluble fraction of meat than on the insoluble.

Papain depends on the amino acid number 25 (cysteine) for its activity. The oxidation of this amino acid inhibits the activity of the enzyme. Cyanide, cysteine, and sulfides are commonly used as activating agents. In solution, papain has a good stability at pH 5 and it drops off sharply at pH values below 3 and above 11. Papain hydrolyzes small peptides as well as proteins; it is very active in the hydrolysis of amides and esters. On the other hand, it is strongly inhibited by oligopeptides that possess phenylalanine as the second amino acid from the carboxyl terminus. Papain has a synthetic activity that produces protein like substances (plasteins) from the proteolitic hydrolyzates of proteins (Reed, 1975).

Chymopapain was first crystallized by Jansen and Balls (1941). Reed (1975) refers to it as a protease very similar to papain in its action on synthetic substrates, the effect of activating chemicals and its synthetic activity. It is different from papain in its activity in the hydrolysis of hemoglobin and casein (only 50% of that of papain). It hydrolyzes some of the esters more slowly than papain. Also, it has greater stability at 75°C than papain.

Lysozyme is the third major portion of the papaya latex enzymes. It constitutes 20% of the soluble protein of the latex (Reed, 1975). Meyer et al. (1946) observed that crude preparations of plant proteinases such as ficin and papain were very rich in lysozyme activity. Smith et al. (1955) isolated lysozyme from papaya latex while attempting to isolate papain. The reports of Smith et al. (1955) and Howard and Glazer (1967, 1969) revealed several major differences between this plant enzyme and lysozymes of animal origin. Papaya lysozyme is a single polypeptide chain of molecular weight of about 25,000; whereas, animal lysozymes have a molecular weight of about 14,000 (Bender and Brubacher, 1973; Blackburn, 1976; Howard and Glazer, 1967). Lytic activity of papaya lysozyme toward bacterial cell walls is reported to be one-third of that of egg white lysozyme; also, papaya lysozyme appeared to have a great chitinase activity toward tetra-N-acetyl-glucosamine (about 400 times more than egg white lysozyme). However, on chitin it was only 10 times more active than egg white lysozyme. These lysozymes also differ in their mode of attack upon the bacterial cell wall, their

relative activity toward various substrates and the nature of the residues in the binding sites. They are, however, similar in their hydrolysis of β, 1-4 glycosidic bonds of poly-N-acetylhexosamine and the exclusive hydrolysis at the β 1-4-N-acetylmuramic acid bonds in Micrococcus lysodeikticus cell walls. Both lysozymes are inhibited by imidazole derivatives. Papaya lysozyme has an optimum activity at pH values of 4.5-4.7 for lysis of M. lysodeikticus and an optimum pH value of 4.2-6.4 for hydrolysis of chitin (egg white lysozyme optimum values are 5.0-7.0 and 4.5-6.0, respectively). Papaya lysozyme is stable from pH 1.8 to 10 at room temperature and remains active for 5 minutes up to pH 4.6 (Howard and Glazer, 1967, 1969). In general, lysozymes decompose the cell walls of certain gram positive cocci and bacilli (Ayres et al., 1980).

Purified lysozyme is presently being used in the food industry because of its bactericidal properties. Egg white lysozymes are the most important commercially available lysozyme. Akashi (1961) found that cured ground beef was preserved more effectively with a combination of 3% NaCl, 12.5 ppm of $NaNO_2$ and 50 or 200 ppm of lysozyme than either by the salts alone or by lysozyme alone. Later, Akashi (1971) found that dipping Vienna sausage in a solution of 0.05% of lysozyme gave better results than using other chemical preservatives. Lysozyme has been found to inhibit growth of Clostridium spp. (at 0.002% w/w) in pasteurized cheese milk while it had no effect on the lactic acid and coliform bacteria.

Chan et al. (1973) discussed the release of other enzymes in papaya that had been pureed. In a later report, Chan et al. (1975) discussed the presence of invertase and its effects on the concentration of naturally occurring sugars in papaya. Sucrose constitutes 48.3% of the total sugars of papaya, followed by glucose 29.8%, and fructose 21.9%. Chan et al. (1975) reported that if invertase is not inactivated prior to the blending of papaya flesh, 50% of the sucrose will be lost within 2.66 minutes following the blending.

Today, enzymatic tenderization of meat can be achieved by direct applications of enzymes to surfaces of meat cuts or by introduction of enzyme solutions into the live animal. Papain, ficin (from figs), bromelain (from pineapple), and fungal proteases are widely used in the developed countries as meat tenderizers. Generally, ficin is considered more potent than papain and bromelain (see Appendix 3, page 87) (Reed, 1975).

3. SMOKE AND ITS EFFECTS ON BACTERIA

The practice of smoking foods has been used by practically all the peoples of the earth since prehistoric times. It was observed that when food was exposed to smoke, its keeping qualities were greatly increased (Jensen, 1954).

Today smoke is mainly used for its color and flavor properties since refrigeration and new ways of food preservation are taking away the importance of smoke as a preservative (Hruza et al., 1974; Issenberg et al., 1971). Yet, some parts of the world such as

the Pacific Northwest, Eastern Canada, countries surrounding the North Sea, and many undeveloped countries still rely on the action of smoking and salting to preserve their fish and meat (Ayres et al., 1980; Jensen, 1954).

Wood is formed of three major constituents: cellulose, hemicellulose, and lignin (Gilbert and Knowles, 1975). The pyrolysis of cellulose forms acetic acid and its homologs, water, and occasionally small amounts of furans and phenols. Hemicellulose, being the least heat-stable of the wood components is rapidly decomposed yielding furan and its derivatives with a range of aliphatic carboxylic acids; hardwoods contain larger amounts of pentosans and yield higher amounts of acids than softwoods. Pyrolysis of lignin produces the compounds of most importance in smoke flavoring and smoke preservation (Draudt, 1963; Gilbert and Knowles, 1975; Jensen, 1954). These compounds are pehnols and phenolic ethers (guaiacol and syringol), and their homologs and derivatives (Gilbert and Knowles, 1975).

Wood composition and its chemistry has been discussed by Fiddler et al. (1966, 1967, 1970), Gilbert and Knowles (1975), Hruza et al. (1974), Issenberg et al. (1971), Lustre and Issenber (1969, 1970), and Porter et al. (1965).

Smoke not only provides flavor and color to smoked products, but it also substantially contributes to preservation by acting as an antioxidant and a bacteriostatic and/or bactericidal agent. It provides also a protective film on the surface of the smoked products

(Draudt, 1963). Hess (1928) reported that an increase of the density of smoke increases the bactericidal power of smoke. The rate of reduction of nonsporeformers was greater after 30 minutes to 2 hours of exposure to fairly weak smoke. Hess (1928) found sporeforming bacteria to have a high resistance to smoke (older cultures particularly); whereas, nonsporeforming organisms were destroyed in one or two hours by the same smoke.

White et al. (1942) reported that the smoking of Wilshire bacon reduced the surface bacteria and was effective in retarding growth during storage. Rancidity was also reduced by smoking. The shelf life of the smoked bacon was twice as long compared to the unsmoked bacon under the same conditions.

Janky et al. (1975) studied the microbiological behavior of smoked Cornish hens. They reported a reduction of the total aerobes (from $10^5/cm^2$ to less than 10), and coliforms (from $10^2/cm^2$ and $10^3/cm^2$ to undetectable numbers) after the hen carcasses were smoked. The counts remained the same after a storage period of 3 weeks.

Hooshyar and Oblinger (1982) studied the survival of Salmonella infantis and Staphylococcus aureus on smoked broilers stored at 5°C and -18°C with a low and a high inoculum. In this experiment, S. aureus was significantly reduced ($P<0.05$) at 5°C compared to that at -18°C; the low inoculum produced higher mortality rates than did the higher inoculum. The same phenomena was observed on S. infantis but to a greater degree. Bacterial isolates from aerobic plate counts of uninoculated smoked broiler halves indicated that the

majority of the flora were S. epidermitis, followed by Micrococcus and Bacillus spp. No gram negative bacteria were detected.

Hayes et al. (1970) reported the presence of Clostridium botulinum type E in smoked fish products with a low content of salt (<4.7%). Inhibition of aflatoxin production in smoked coconut and potato dextose broth was reported by Arseculeratne et al. (1976). Mycelial growth, on the other hand, was inhibited to a lesser degree.

4. MICROBIOLOGY OF BACTERIA IN CURED AND FERMENTED MEAT

Although it is not known when man first learned to use fermentation to preserve and to enhance the sensory properties of his foods, it is known that fermentation has been used for many centuries by different civilizations. Examples of this fermentation are alcoholic beverages, sauerkraut, bread, cheeses, fermented fish and dried and semidry sausages.

Fermented sausages (Lebanon bologna, Thuringer salami, summer sausage, and pepperoni) vary in their composition. Moisture content ranges from 58.9% to 26.4%, salt content ranges from 2.8% to 4.7% and pH values vary from 4.4 to 5.6. Their total bacterial counts per gram ranges from 10^3 to 10^8 and their lactic bacteria counts per gram are reported from less than 10^2 to 10^6 (Acton and Dick, 1976). Their fermentation process is however similar. Meat is aged at relatively low temperatures (usually 5°C) for several days. Salt is added to the meat (3 to 4%) to suppress the development of

Pseudomonas while lactic acid bacteria increase. After aging, the ingredients are blended and the mixture cased. The sausages are then placed in the smokehouse where fermentation takes place. The length of time and temperatures of fermentation and smoking vary according to the manufacturer. The product is then allowed to cool at room temperature and stored to mellow (Palumbo et al., 1976; Pederson, 1979; Smith and Palumbo, 1973).

During fermentation lactic acid is usually produced by lactobacilli, thus lowering the pH while micrococci takes care of the nitrate reduction (Smith and Palumbo, 1973). Starter cultures are available consisting of Lactobacillus, Pediococcus, Micrococcus, and Streptococcus (Deike, 1978; Scharner and Hofmann, 1978).

While the fermentation process is carried out by the natural flora of the meat, the concentration of salt plays an important role in the selection of the fermenting bacteria. Smith and Palumbo (1973) reported that low concentrations of salt (2 to 4%) prevent the growth of catalase positive, gram negative bacteria in the manufacture of Lebanon bologna. Concentrations of 3 and 4% salt were more effective against the former bacteria while 2% salt allowed equal numbers of catalase positive, gram negative bacteria and catalase positive, gram positive bacteria. Smith and Palumbo (1973) also reported in their work an increase of pH as salt concentration increased. On the other hand, APT agar counts and rogosa SL agar counts decreased as salt increased.

Goepfert and Chung (1970) reported that when sucrose was reduced during the manufacture of a fermented sausage, a final pH of 5.2 to 5.4 resulted. Salmonella were able to multiply during emulsion and fermentation. When sucrose was increased, the number of salmonellae decreased during fermentation. During heat treatment, it was found that 46°C (for 5 hr) was not effective in killing Salmonella when the product had a low acid content (49°C and especially 52°C were more effective in reducing Salmonella). This finding is similar to that of Smith et al. (1975). They report that temperatures of 51.7°C or higher were more effective against Salmonella.

There has been some concern about Staphyilococcus aureus contamination in fermented meats (Pullen and Genigeorgis, 1977; Tatini et al., 1976). Production of staphilococcal enterotoxin requires one or more factors; high initial inoculum of Staphylococcus aureus, low inoculum of starter culture (lactic acid producer), high pH of sausages, high water activity and low salt concentration (Pullen and Genigeorgis, 1977). Daly et al. (1973) reported that when starter bacteria were not used in simulated sausage mixes, a relatively low inoculum (3.0×10^4/gram) of Staphylococcus aureus proliferated to numbers (more than 10^6/gram in 25 hours at 37°C) high enough to become a health problem before the natural lactic acid bacteria were able to reduce the pH of the sausage mixes. On the other hand, when a starter culture (Lactobacillus plantarum and Pediococcus cerevisiae) was used, the pH of the mixes was reduced substantially, inhibiting S. aureus. However when a higher inoculum of S. aureus (1.8×10^5/gram)

was used, the starter culture was not able to keep it from reaching levels hazardous to health (more than 10^6/g). Lee et al. (1977) reported that when they inoculated S. aureus (10^5 to 10^7/g) in Genoa salami, enterotoxin A production was restricted to the surface of the salami. Smoking has been reported to reduce substantially the growth of S. aureus on the surfaces of pepperoni (Tatini et al., 1976). Lactic acid bacteria have been reported to inhibit Pseudomonas, Salmonella, Staphylococcus and other type bacteria by producing bactericidal substances other than lactic acid (Raccach and Baker, 1979; Stamer, 1979).

Cured and fermented meats have several similarities. They are both designed to preserve meats by the action of salt, sodium nitrate, sodium nitrite, and low water activity. However, cured meats do not depend on bacteria to lower the pH of the product, just to reduce nitrate.

Curing procedures take place at temperatures in the range of 0.5°C to 4.5°C. Salt is the chief bacteriostatic substance (Jensen, 1954). Nitrate and nitrite contribute to development of the color and flavor of cured meats in addition to inhibiting putrefactive anaerobic bacteria (Ayres et al., 1980). There are four general methods of curing; dry curing, brine immersion, artery pumping, and stich pumping.

Ingram and Dainty (1971) pointed out that sodium chloride at a concentration of 5% is partially, and of 7% totally, inhibitory to putrefying Pseudomonas in fresh meat. Micrococci spp. replace them

as the predominating flora at these levels of salt. These investigators also suggested that sodium chloride might inhibit the proteolytic metabolism of amino acids by pseudomonads. The same effect has been reported previously on gram-positive cocci growing in bacon by Tonge et al. (1964).

Tonge et al. (1964) reported that cured, sliced, vacuum-packed bacon with a salt concentration of 8% to 12% had a microflora composed mainly of catalase positive cocci when stored at 20°C and 30°C. The same was observed during the first 9 days of storage of bacon containing 5 to 7% salt. The flora then switched to group D streptococci and lactic acid bacteria.

CHAPTER III

MATERIALS AND METHODS

1. PREPARATION AND FERMENTATION OF MEAT

The meat product was prepared and developed by C. N. Ubbaonu and C. C. Melton. The methods used are described in Ubbaonu's work (1982). The preparation of the product is summarized as follows: Top and bottom round were cut in pieces of about 3 x 4 x 5 cm. Fresh, partially ripe papaya was ground after the seeds were removed. The curing solutions were made utilizing non-iodized salt, ground papaya flesh, and tap water. There were 36 different treatments, 6 aqueous concentrations of salt (0, 2, 4, 6, 8 and 10% w/w) and 6 aqueous concentrations of papaya (0, 5, 10, 15, 20 and 25% w/w). The pieces of meat were placed into the different brine solutions in covered plastic containers and left at room temperature for 48 hours. The meat pieces were then rinsed and placed in a food processing oven for drying. The temperature was set at 48.9°C and wood smoke was applied for 1 hour after 30 minutes of drying. The process was stopped after 45 hours. The meat was allowed to cool and was then packed in plastic bags. The product was stored at room temperature for 30 days.

2. MICROBIOLOGICAL ANALYSIS OF TREATMENTS

Collection of Samples

Samples of fresh meat, brine-fermented (after 48 hours of brining), and dried fermented meat (stored for 30 days) were taken from each treatment at random to be analyzed. The scheme of analysis is outlined in Table 1.

Preparation of Meat Samples

The meat samples, each weighing 25 g, were added to 225 ml of the appropriate diluent (see Table 1) and blended for 1 minute at medium speed using a Waring blender.

Preparation of Diluents

The diluent used for the analysis of mesophiles, psychrophiles, yeast and molds, presumptive coliforms, presumptive Staphylococcus aureus, lactic acid bacteria, and streptococcal bacteria was peptone water. It was prepared by dissolving peptone (BBL) in distilled water to a concentration of 0.1%. The solution was distributed into 360 ml blender jars and 100 ml dilution bottles. The amounts added to the blender jars and dilution bottles were 225 ml and 99 ml of the diluent, respectively. Bottles and jars containing diluent were sterilized by autoclaving at 121°C for 15 minutes.

The diluent used for the analysis of mesophilic and psychrophilic halophiles was 0.1% peptone water, to which sodium chloride was added at a concentration of 3% (w/v) before sterilization. The distribution and sterilization were performed in the same manner as for the peptone water without sodium chloride.

TABLE 1. Plating Media and Incubation Conditions Used for Each of the Organisms Sought During the Microbiological Analysis of the Beef Pieces Subjected to the 36 Different Treatments.

Type of Organism Sought	Plating Media	Source	Plating Technique	Incubation Time and Temperature (°C)	Special Conditions	Diluent
1. Mesophiles	Standard methods agar	BBL	Spread	48 hrs at 32°		Peptone water
2. Psychrophiles	Standard methods agar	BBL	Spread	7-10 days at 4°		Peptone water
3. Mesophilic halophiles	Standard methods agar plus 3% NaCl	BBL	Spread	48 hrs at 32°		Peptone water plus 3% NaCl
4. Psychrophilic halophiles	Standard methods agar plus 3% NaCl	BBL	Spread	7-10 days at 4°		Peptone water plus 3% NaCl
5. Yeast and molds	Malt extract agar	BBL	Spread	5-7 days at 25°	pH 3.5	Peptone water
6. Presumptive coliforms	Violet red bile	BBL	Spread	24 hrs at 37°	Overlaid	Peptone water
7. Presumptive *Clostridium perfringens*	Sulfite polymyxin sulfadiazine agar	BBL	Pour	24 hrs at 37°	Anaerobic jar	Reinforced clostridial medium
8. SPS agar anaerobic bacteria	Sulfite polymyxin sulfadiazine agar	BBL	Pour	24 hrs at 37°	Anaerobic jar	Reinforced clostridial medium
9. Presumptive *Staphylococcus aureus*	Baird-Parker agar	BBL	Spread	24 hrs at 37°	With EY tellurite enrichment	Peptone water
10. Lactic acid bacteria	APT agar	BBL	Pour	48 hrs at 32°		Peptone Water
11. Streptococcal bacteria	KF agar plus 0.001% triphenyl tetrazolium chloride	BBL	Pour	48 hrs at 37°		Peptone water

Reinforced clostridial medium (Speck, 1976) was used as a diluent for the analysis of Cl. perfringens and anaerobes. It was prepared with the following ingredients: Yeast extract, 3.0 g; Peptone, 10.0 g; Lab-Lemco meat extract (Oxoid), 10.0 g; D-Glucose, 5.0g; Sodium acetate, hydrated, 5.0 g; Cysteine, 0.5 g; Soluble starch, 1.0 g; Agar, 0.5 g; and distilled water 1 liter. The mixture was heated in a steamer until all ingredients dissolved in the distilled water. The reinforced clostridial medium was then filtered through a hot paper pulp filter.

The hot paper pulp filter consisted of 38 g of Whatman No. 1 filter paper soaked in water and mashed to a pulp. The pulp was boiled and poured into a Buchner-type filter funnel while applying suction. When the hot paper pulp stopped dripping, the receiving flask was replaced by a clean flask and the medium filtered immediately afterwards. The medium was allowed to cool to about 25°C and the pH adjusted to 7.4. The diluent was then distributed between 360 ml blender jars and 100 ml screw-capped dilution bottles. The amount of the diluent added to the blender jars and to the dilution bottles was 225 ml and 99 ml, respectively. Sterilization was performed by autoclaving at 121°C for 15 minutes.

Dilutions

Ten-fold serial dilutions were prepared in the following manner. After blending, the jars were labeled 10^{-1} dilution. From each jar, 11 ml was transferred aseptically by sterile pipette to

the corresponding dilution bottles (either peptone water, peptone water plus NaCl, or reinforced clostridial medium). These dilution bottles were labeled 10^{-2} dilutions. Successive serial dilutions were performed until the desired dilution was achieved.

Plating

Spread plate technique. Approximately 15 ml of the appropriate medium (see Table 1) were poured into each sterile plate and allowed to cool and dry at room temperature for 24 hours. The plates were labeled with the appropriate dilution factor, medium name, sample code, and date of plating. From each dilution 0.1 ml aliquots were spread with a sterile glass rod as quickly and carefully as possible on the surface of the agar media. The plates were allowed to dry for 15 to 20 minutes at room temperature, then inverted and incubated.

Poured plate technique. Sterile empty plates were labeled with the appropriate dilution factor, medium name, sample code, and date of plating.

Approximately 15 ml of each melted medium was poured (at 45°C) as quickly and carefully as possible. The agar was mixed with the diluted sample (1.0 ml) by rotating the dish first in one direction and then in the opposite direction at least five times successively. The mixture was allowed to cool until it solidified at room temperature.

Incubation

All the plates were incubated upside down with the exception of the 10^{-1} dilution spread plate in which 1 ml of dilution was used. Such plates were left in an upright position due to the larger amount of dilution plated. The time, temperature and other incubation conditions used for each medium are listed in Table 1.

Recording the Number of Microorganisms

Selected plates with 30 to 300 colonies were used to compute the number of organisms. The colonies were counted with the aid of a Quebec colony counter.

Analysis for Salmonella

The presence or absence of Salmonella was determined in the following manner. Tetrathionate broth was used as a selective enrichment medium, it was prepared according to Harrigan and McCance (1976). Calcium carbonate (25 g) was added to 780 ml of sterile nutrient broth. The solution was distributed by pouring 206 ml of it into 500 ml screw-capped jars. Sterilization was done by steaming for 30 minutes on each of 3 successive days. When cool, 15 ml of sodium thiosulphate solution (24.8 g of sodium thiosulphate, hydrated in 100 ml of distilled water and sterilized by steaming for 30 minutes on each of 3 successive days) was added to each jar. In addition, 4 ml of iodine solution (12.7 g of iodine, 20 g of potassium iodine and 100 ml of distilled water) was poured into each jar. The meat samples, 25 g each, were placed in the tetrathionate broth jars and

incubated for 24 hours at 37°C. A 3 mm loopful of the incubated broth was streaked on 3 plates of brilliant green agar and MacConkey's agar. The plates were incubated for 24 hours at 37°C. If the selective media showed indication of presence of Salmonella (colorless to pink or red colonies surrounded by brilliant green agar, and transparent-colorless colonies on MacConkey's agar), colonies were picked with a needle into triple sugar iron (TSI) agar and lysine iron agar (LIA) slants. If the selective media showed no indication of containing Salmonella, they were incubated an additional 24 hours.

The TSI and LIA slants were inoculated with a portion of the suspected colony by streaking the slant and stabbing the bottom of each agar. The slants were incubated 24 hours at 37°C. If the media showed a change of color typical of Salmonella (red slants with yellow butts and H_2S production on TSI slants, and purple with H_2S production on LIA), then Gram stains, motility tests, urease tests, MR-VP tests, and serological tests were performed to confirm the isolate as a Salmonella culture.

Statistical Analysis

An analysis of variance was performed to determine the effect (if any) of salt and papaya on the bacterial counts and pH of the meat product. The experiment was repeated once. Duncan's Multiple Range Test was employed using the "Proc GLM" program prepared by the Statistical Analysis System (SAS) (Duncan, 1955; Barr et al., 1979).

3. IDENTIFICATION OF BACTERIAL ISOLATES

Treatment Preparation

A combination of salt and papaya was selected from the 36 original treatments. This selection was based on Ubbaonu's (1982) results and on the preliminary microbial examinations. The preparation of such selected treatment was done in the same manner as described on page 24.

Preparation of Isolates

Samples of fresh beef, brine-fermented meat after 48 hours of brining at ambient temperature, and dried-fermented meat stored for 30 days were taken from each treatment at random. The meat samples, weighing 25 g each, were added to 225 ml of the appropriate diluent (see Table 2) and blended for 1 minute at medium speed using a Waring blender. The diluents were prepared in the same manner as described on page 25.

The preparation of plates, preparation of dilutions, and plating for this treatment were done in the same manner as previously mentioned during the microbial analyses of the 36 treatments. The media used were standard methods agar, standard methods agar plus 3% NaCl, malt extract agar, APT agar, and SPS agar. Their respective incubation time, temperatures, and diluent used are listed in Table 2.

A plate containing 30 to 300 colonies was selected from each medium to collect the isolates. A maximum of 60 colonies were picked from each plate using a Harrison template (Harrigan and McCance, 1976).

TABLE 2. Plating Media, Incubation Condition and Type of Diluent Used to Obtain Microbial Cultures for Identification From the 10% Salt-10% Papaya Treatment.

Media Employed	Source	Plating Technique	Incubation Time and Temperature (°C)	Special Conditions	Diluent
Standard methods agar	BBL	Spread	48 hrs at 32°		Peptone water
Standard methods agar plus 3% NaCl	BBL	Spread	48 hrs at 32°		Peptone water plus 3% NaCl
Malt extract agar	BBL	Spread	5-7 days at 25°	pH 3.5	Peptone water
APT agar	BBL	Poured	48 hrs at 32°		Peptone water
SPS agar	BBL	Poured	24 hrs at 37°	Anaerobic jar	Reinforced clostridial medium

To assure that the isolates were pure, the following procedures were followed. Each isolate was picked into a broth and incubated. Table 3 gives the type of broth, incubation times and incubation temperatures used for the various isolates. A 3 mm loopful of the incubated broth was streaked on a solid medium for isolation and incubated. Table 3 gives the type of solid medium, incubation times and incubation temperatures used for each isolate type. This procedure was repeated at least three times or until the colonies on the streak plates had a similar appearance. The isolates were picked onto their respective agar slants and incubated. Table 4 shows the type of media, incubation time and incubation temperature used. Finally, a gram stain of each culture was made to determine their gram reaction, purity and cell shape. Biochemical tests for their identification followed.

Identification of Bacteria

According to Ayres et al. (1980), bacteria most commonly found in foods can be placed under eight different groups: (1) gram-negative aerobic rods and spheres; (2) gram-negative facultatively anaerobic rods; (3) gram-negative anaerobic bacteria; (4) gram-negative rods or coccobacilli; (5) gram-positive spheres; (6) gram-positive asporulating catalase-negative rods; (7) gram-positive endospore-forming rods; and (8) bacteria with branching cells. These groups are taken from Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

The following tests and observations were performed on all isolates: (1) gram-stain and determination of cell shape and

TABLE 3. Media and Incubation Conditions for Purification of Bacterial Isolates.

Original Plating Medium	Transfer Medium	Incubation Time and Temperature (°C)	Isolation Medium	Incubation Time and Temperature (°C)
Standard methods agar	Nutrient broth	24 hrs at 32°	Trypticase soy agar	24 hrs at 32°
Standard methods agar plus 3% NaCl	Nutrient broth plus 3% NaCl	24 hrs at 32°	Trypticase soy agar plus 3% NaCl	24 hrs at 32°
Malt extract agar	Mycophil broth	3-4 days at 25°	Malt extract agar	5-7 days at 25°
APT agar	MRS broth	24 hrs at 32°	APT agar	48 hrs at 32°
Sulfite polymyxin sulfadiazine agar	Thioglycollate semi-solid medium	24-48 hrs at 37°	Thioglycollate agar	48 hrs at 37°

TABLE 4. Storage and Isolation Media for Bacterial Isolates

Isolation Medium	Storage Medium	Incubation Time and Temperature (°C)
Trypticase soy agar	Trypticase soy agar slants	24 hrs at 32°
Trypticase soy agar plus 3% NaCl	Trypticase soy agar plus 3% NaCl slants	24 hrs at 32°
Malt extract agar	APT agar slants	5-7 days at 25°
Thioglycollate agar	Thioglycollate semi-solid medium	48 hrs at 37°
APT agar	APT agar slants	48 hrs at 32°

arrangement; (2) examination of a wet mount to determine motility and shape; (3) flagella stain of motile organisms; (4) catalase test; (5) ability to grow under anaerobic or aerobic conditions; (6) oxidase test of aerobic organisms; (7) determination of the oxidative or fermentative metabolism; (8) shape, appearance and color of agar colonies; and (9) spore stain on catalase-positive rods.

The bacterial isolates were then placed into one of the groups shown above according to Ayres et al. (1980). Identification schemes were prepared to aid in the identification of bacteria to the genus level. The identification schemes used are presented as follows: gram-negative aerobic rods, spheres and coccobacili (Figures 1 and 2), gram-negative facultatively anaerobic rods (Figure 3), gram-positive spheres (Figure 4), and gram-positive rods (Figure 5).

Identification of species, when possible are based on additional biochemical test as provided by the individual genera sections in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

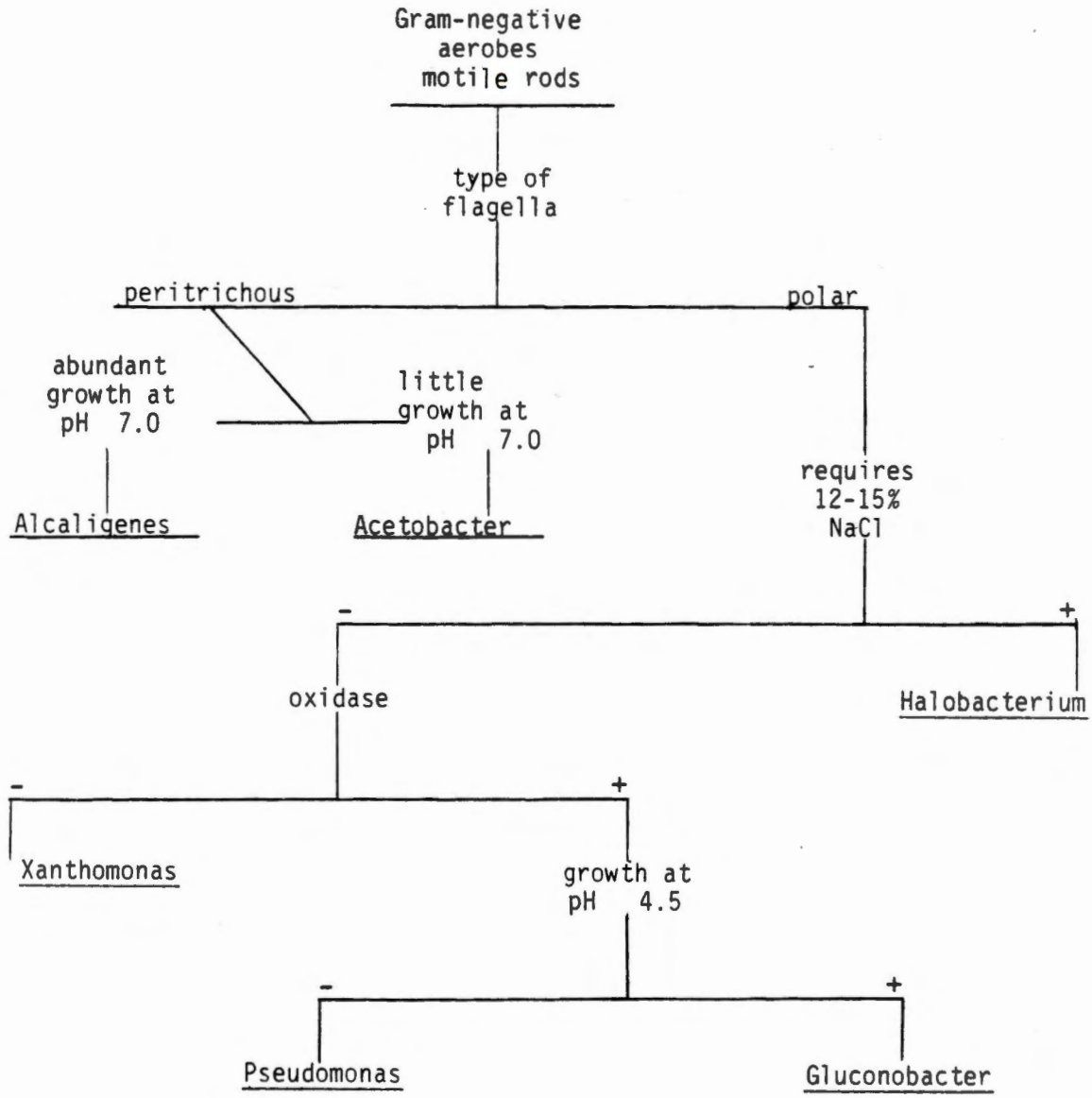

FIGURE 1. Scheme used in the identification of gram-negative aerobic motile rods isolated from fresh pieces of beef, from brine-fermented meat with 10% salt and 10% papaya, and from fermented-dried meat.

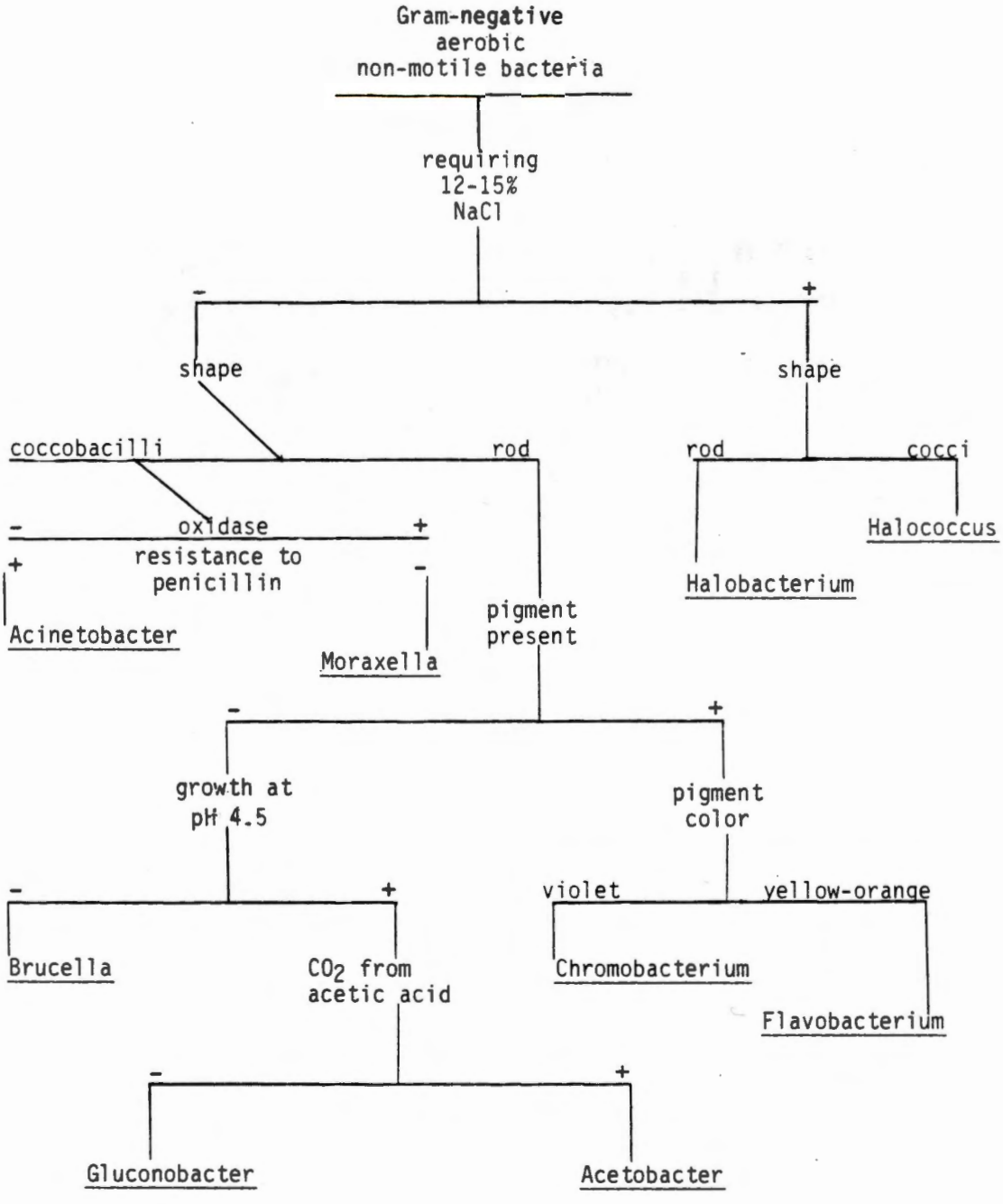

FIGURE 2. Scheme used in the identification of gram-negative aerobic non-motile bacteria isolated from fresh pieces of beef, from brine-fermented meat with 10% salt and 10% papaya, and from fermented-dried meat.

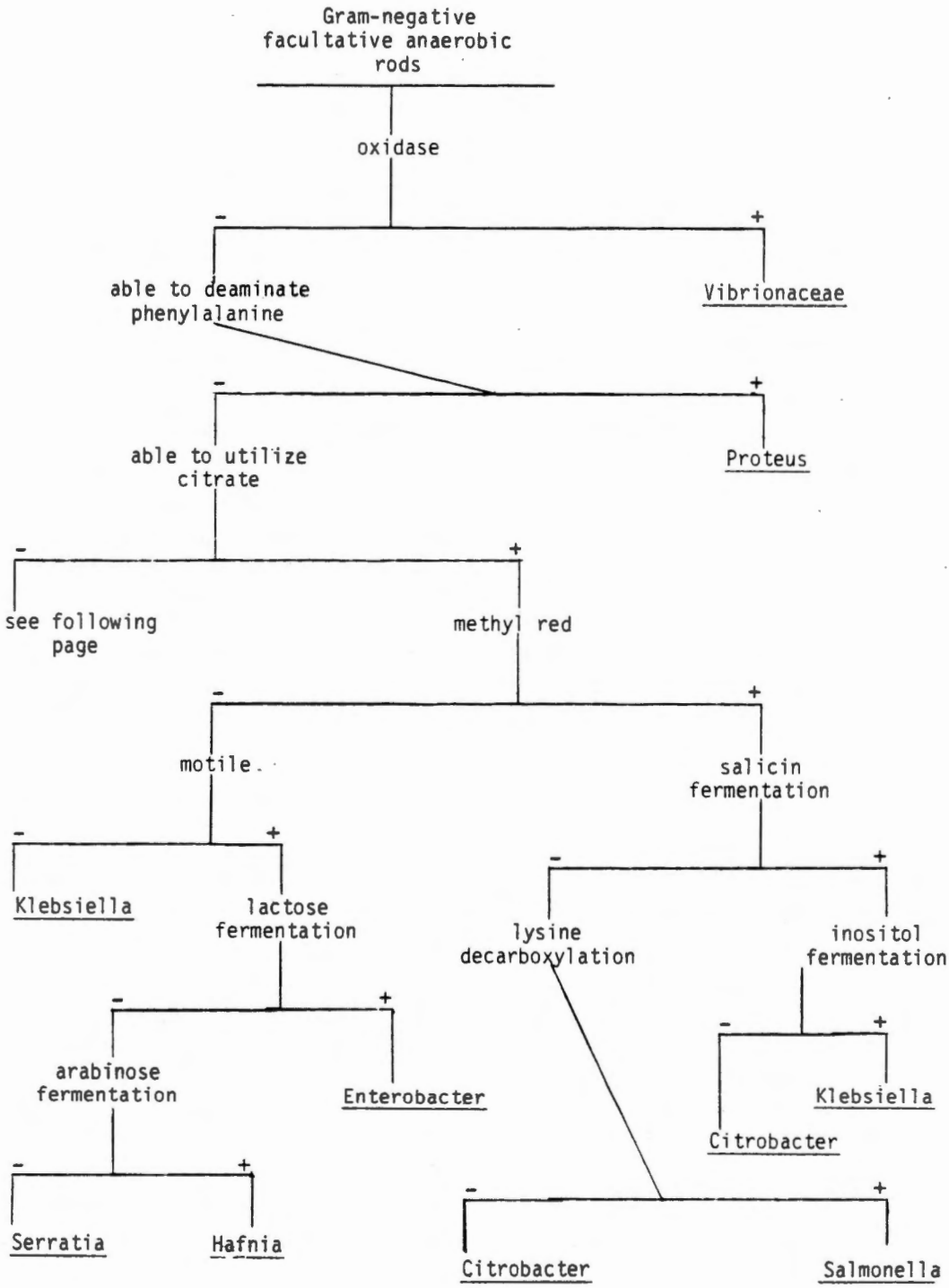

FIGURE 3. Scheme used in the identification of gram-negative facultatively anaerobic rods isolated from fresh pieces of beef, from brine-fermented meat with 10% salt and 10% papaya, and from fermented-dried meat.

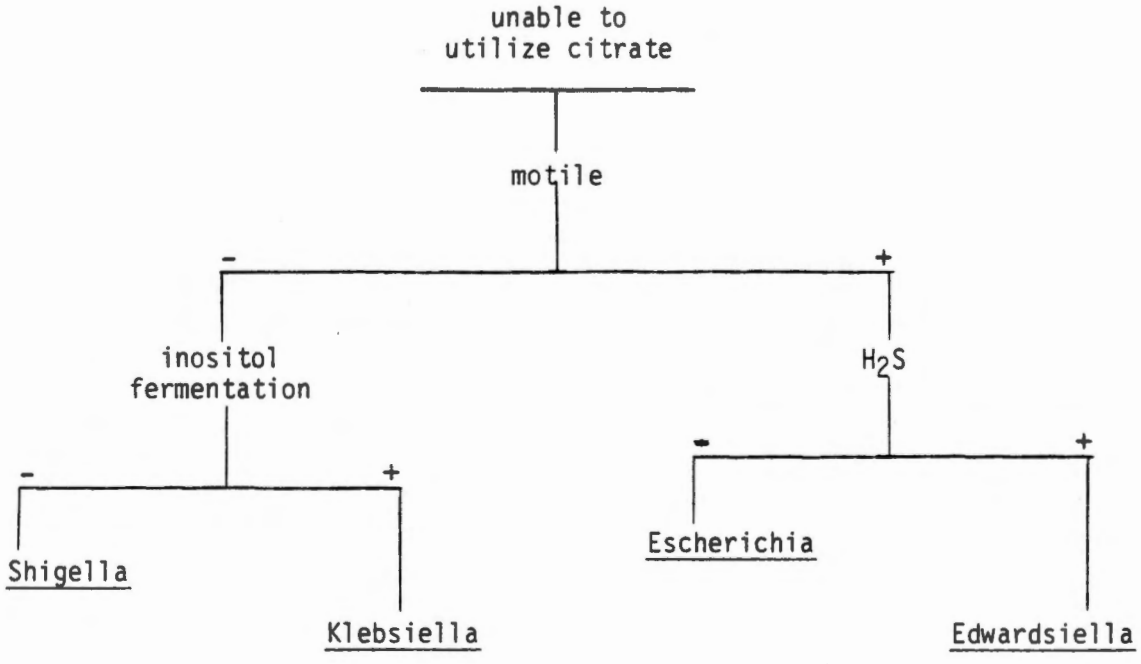

FIGURE 3. (Continued)

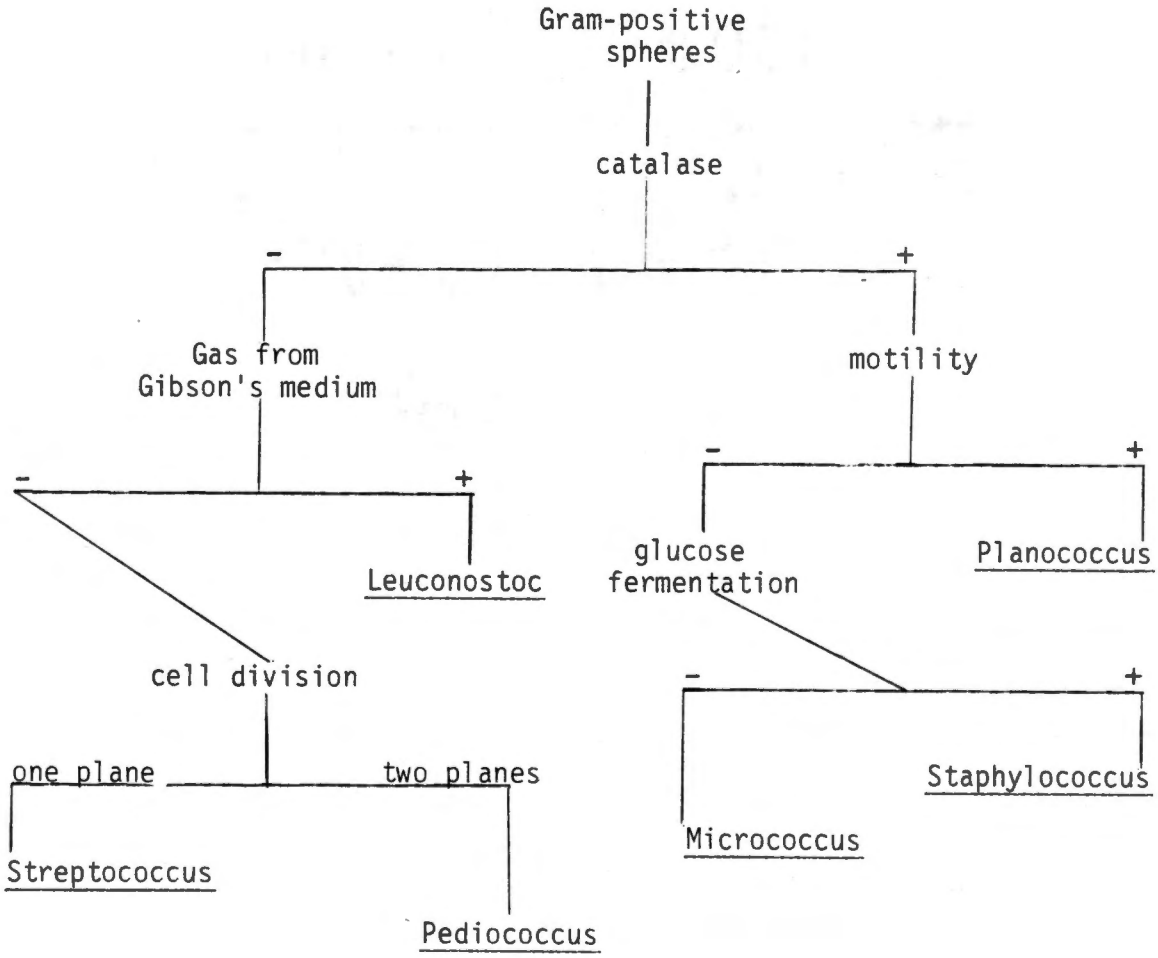

FIGURE 4. Scheme used in the identification of gram-positive cocci isolated from fresh pieces of beef, from brine-fermented meat with 10% salt and 10% papaya, and from fermented-dried meat.

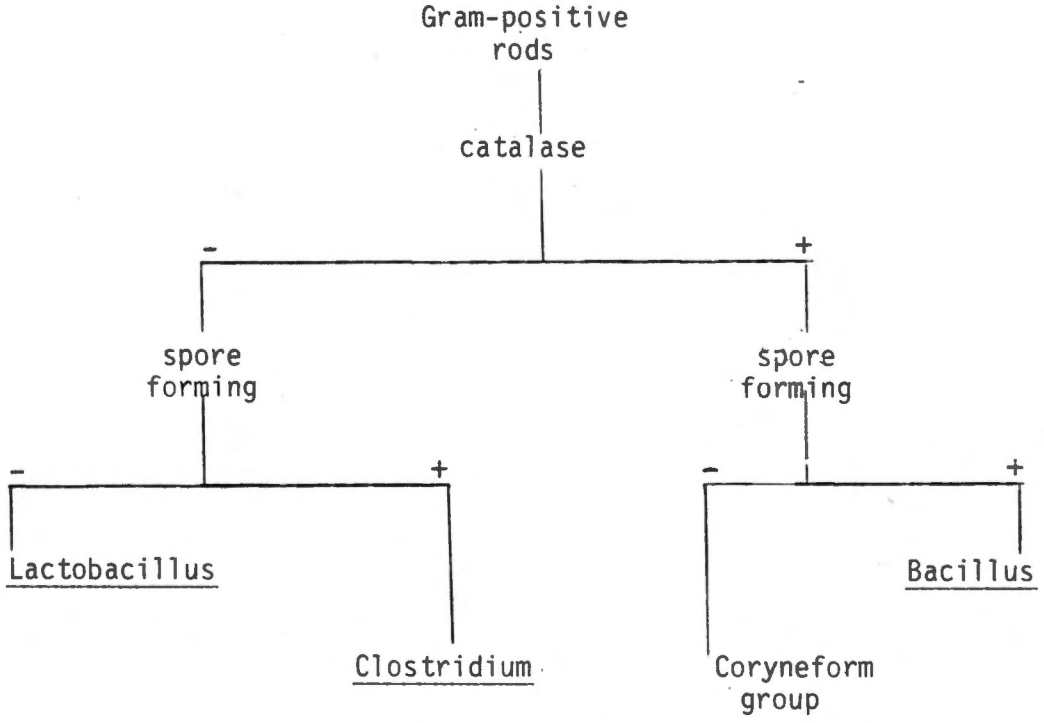

FIGURE 5. Scheme used in the identification of gram-positive rods isolated from fresh pieces of beef, from brine-fermented meat with 10% salt and 10% papaya, and from fermented-dried meat.

CHAPTER IV

RESULTS AND DISCUSSION

1. MICROBIOLOGICAL QUALITY OF FRESH BEEF ROUND

The fresh beef round pieces were analyzed to establish the microbiological quality of the meat before the fermentation process.

All microbial counts performed on the fresh beef pieces indicated that the meat had a high bacteriological quality (Ayres et al., 1980). The colony counts are shown in Table 5. Presumptive coliforms, presumptive Staphylococcus aureus and presumptive Clostridium perfringens were in acceptable numbers (Ayres et al., 1980; Wilson et al., 1981). No Salmonella were detected. The pH of the meat (5.6) was found within the normal beef pH range (Price and Schweigert, 1978).

2. BRINE FERMENTED BEEF

Effect of Salt Concentration

The effect of salt on bacterial counts and pH of the brine-fermented beef was observed at the end of the fermentation process. Mesophiles, psychrophiles, mesophilic halophiles, psychrophilic halophiles, presumptive coliforms, presumptive C. perfringens, SPS agar anaerobes, lactic acid bacteria, presumptive S. aureus, streptococcal bacteria, and yeast and mold counts are shown in Table 6. The presence or absence of Salmonella and the pH values of the brine-fermented beef are shown in the same table as well.

TABLE 5. Bacterial Load and pH of Fresh Beef Pieces Before the Fermentation Process.

Microorganism	Microbial Count (log CFU/g)
Mesophiles	4.7
Psychrophiles	4.2
Mesophilic halophiles	4.8
Psychrophilic halophiles	4.2
Presumptive coliforms	2.4
Presumptive *C. perfringens*	3.0
SPS agar anaerobic count	3.9
Lactic acid bacteria	4.5
Presumptive *S. aureus*	3.2
Streptococcal bacteria	3.2
Yeast and molds	2.9
Salmonella	not detected
pH	5.6

TABLE 6. Bacterial Counts (Log_{10} CFU/g) and pH Values of Brine-Fermented Meat at Different Salt Concentrations. Each salt concentration includes all papaya levels (0, 5, 10, 15, 20, 25%)

Salt (%)	Final pH	Mesophiles	Psychrophiles	Mesophilic Halophiles	Psychrophilic Halophiles	Coliforms	SPS Agar Anaerobes	Lactic Acid Bacteria	Presumptive *S. aureus*	Streptococcal Bacteria	Yeast and Molds
0	4.6 a	8.5 a[4]	8.1 a[1]	8.4 a	7.1 a[4]	6.5 b[1]	6.8 a[1]	8.0 a	3.7 a	6.0 c[2]	5.2 ab[3]
2	4.7 a	8.1 ab	8.0 a	8.0 a	6.8 ab	7.9 a	6.8 a	7.9 a	3.9 a	6.2 c	5.8 a
4	4.8 a	8.2 ab	8.1 a	8.1 a	6.3 b	7.8 a	6.9 a	8.0 a	3.3 a	6.9 ab	6.0 a
6	4.7 a	8.1 ab	6.7 b	8.3 a	6.5 ab	7.8 a	6.8 a	8.2 a	3.2 a	6.5 bc	5.5 ab
8	4.8 a	8.2 ab	6.5 bc	8.4 a	6.7 ab	7.1 b	6.4 b	8.1 a	3.2 a	7.1 a	5.8 a
10	4.8 a	7.9 b	6.2 c	8.2 a	6.3 b	5.9 c	5.9 c	7.8 a	3.9 a	6.4 bc	4.7 b

[1]Means followed by different letters in columns are significantly different ($P<0.0001$).

[2]Means followed by different letters in columns are significantly different ($P<0.001$).

[3]Means followed by different letters in columns are significantly different ($P<0.05$).

[4]Means followed by different letters in columns are significantly different ($P<0.1$).

Statistical analyses indicated that the brine-fermented beef final pH was not significantly affected by salt. According to Ubbaonu's (1982) analysis, salt affected the final pH of the brine solutions. This effect, however, was not seen on the meat final pH probably because of the meat's buffer capacity whigh might have blocked any significant change in the meat pH.

Mesophilic halophiles, lactic acid bacteria, and presumptive S. aureus colony counts were not significantly affected by salt at the end of the fermentation process.

Mesophiles and psycrophilic halophiles were affected slightly ($P<0.1$) by salt at the end of the fermentation process. The number of mesophiles decreased significantly ($P<0.1$) only when 10% salt was used in the brine solutions. The mesophilic count before the brine-fermentation was log 4.7 CFU/g. Psycrophilic halophiles behaved differently; the highest count was found at 0% salt (log 7.1 CFU/g), whereas the lowest was at 4% and 10% salt in the brine solution (log 6.3 CFU/g each).

Psychrophiles were affected significantly ($P<0.0001$) by salt at the end of the brine fermentation process. The number of colonies recorded were unchanged by salt concentrations of 0% 2%, and 4%; they were between log 8.0 CFU/g and log 8.1 CFU/g. When the salt concentration in the brine reached 6%, the number of psychrophilic bacteria decreased considerably to log 6.7 CFU/g.

These microorganisms continued to decrease to their lowest number (log 6.2 CFU/g) at 10% salt. Thus, salt concentrations of 6% and higher had an inhibitory effect on psychrophiles.

Presumptive coliforms were affected significantly by salt ($P<0.0001$). Salt concentrations of 2%, 4%, and 6% appeared to favor these microorganisms; the bacterial counts were between log 7.8 CFU/g and log 7.9 CFU/g. A salt concentration of 10% was most effective against presumptive coliforms; at this salt concentration they had a colony count of log 5.9 CFU/g.

Salt also had a significant effect on the SPS agar anaerobes ($P<0.0001$). These microorganisms showed similar counts in the brine solutions with 0%, 2%, 4%, and 6% salt; they were between log 6.8 CFU/g and log 6.9 CFU/g. At 8% salt, the SPS agar anaerobes in the brine-fermented beef were reduced to log 6.4 CFU/g. These microorganisms reached their lowest number when 10% salt was used in the brine; it was log 5.9 CFU/g.

Streptococcal bacteria were affected significantly by salt ($P<0.001$). These microorganisms appeared to be favored by salt concentrations of 4%, 6%, and 8% in which they varied from log 6.5 CFU/g to log 7.1 CFU/g. This was probably due to the halo-tolerance of the streptococcal bacteria and to the selective elimination of other competitive bacteria. The highest streptococcal count was log 7.1 CFU/g at 8% salt, the lowest was log 6.0 CFU/g at 0% salt.

Yeast and molds vary greatly in their salt tolerance (Banwart, 1979). A salt concentration of 10% showed the lowest yeast and mold count.

Presumptive C. perfringens was detected in the beef pieces fermented with 0% and 6% salt but only reached log 1.1 CFU/g. Thus, it appears that 8% and 10% salt would be more effective in inhibiting these microorganisms. Roberts and Derrick (1978) tested the inhibitory effect of NaCl on different strains of C. perfringens; they found that only 1 out of the 22 strains of C. perfringens was able to grow in a suitable broth containing 7% NaCl at pH 6.0.

Salmonella was detected in two fermented meat samples that had a brine salt concentration of 2% and 8%, respectively. Salmonella has been reported to grow on pork meat with 8% salt at 20°C and pH 6.5 (Banwart, 1979). At the moment, there is no evidence indicating the ability of this microorganism to grow at 10% salt.

Effect of Papaya Concentration

The effect of papaya on bacterial counts and final pH of the brine-fermented meat was observed. These values are shown in Table 7.

There was a decrease ($P<0.0001$) in the final pH of brine-fermented meat as papaya concentration increased in the brine solutions. Beef fermented without papaya had the highest pH (5.2). Papaya concentrations of 20% and 25% produced the lowest final pH (4.5 each) in the brine-fermented beef.

TABLE 7. Bacterial Counts (Log_{10} CFU/g) and pH Values of Brine-Fermented Meat at Different Papaya Concentrations. Each papaya concentration includes all salt levels (0, 2, 4, 6, 8, 10%).

Papaya (%)	Final pH	Mesophiles	Psychrophiles	Mesophilic Halophiles	Psychrophilic Halophiles	Coliforms	AOS Agar Anaerobes	Lactic Acid Bacteria	Presumptive *S. aureus*	Streptococcal Bacteria	Yeast and Molds
0	5.2 a[1]	8.3 a*	7.1 a*	8.5 a*	6.7 a*	7.6 a*	6.6 a*	8.0 a*	3.7 a*	6.3 a*	5.9 a*
5	5.0 b	8.2 a	7.4 a	8.1 a	6.7 a	7.5 a	6.6 a	7.9 a	4.0 a	6.4 a	5.8 a
10	4.7 c	8.0 a	7.3 a	8.1 a	6.5 a	7.1 a	6.6 a	7.9 a	3.1 a	6.4 a	5.0 a
15	4.6 cd	8.1 a	7.2 a	8.2 a	6.6 a	7.0 a	6.6 a	8.1 a	3.8 a	6.6 a	5.1 a
20	4.5 d	8.2 a	7.1 a	8.2 a	6.7 a	7.0 a	6.7 a	8.0 a	3.5 a	6.7 a	5.8 a
25	4.5 d	8.2 a	7.4 a	8.2 a	6.5 a	6.9 a	6.7 a	8.2 a	3.1 a	6.7 a	5.6 a

[1]Means followed by different letters in vertical columns are significantly different ($P<0.0001$).

*Means followed by the same letter were not significantly different ($P<0.1$).

The decrease of the meat's final pH with the increase of papaya in the brine was probably due to several factors. First, the papaya fruit had a lower pH (5.2) than the beef used (5.6). Second, according to Carreno and Chan (1982) and Chan et al. (1973), pureed papaya held at room temperature for 24 hours shows a decrease in pH to about 4.3 due to enzymatic and microbial (gram-positive diplococci and rods) activity. Third, the addition of papaya increases the concentration of fermentable sugars. This fruit has a total sugar concentration of 10% to 15% and it is represented by fructose (45.4%) and glucose (54.2%) in pureed papaya (without inactivation of the papaya enzymes).

The bacterial composition of the brine-fermented beef was not affected significantly by the increase of papaya in the fermentation brines.

3. FERMENTED-DRIED BEEF

Effect of Salt Concentration

Fermented-dried beef showed no evidence of psychrophiles, psychrophilic halophiles, and presumptive *S. aureus* at the lowest dilution used (10^{-2}). There was neither evidence of presumptive coliforms nor presumptive *C. perfringens* at the lowest dilution used (10^{-1}). *Salmonella* was not detected in any fermented-dried meat sample.

Salt had a significant effect on the fermented-dried meat pH ($p<0.05$) only at 10% salt (see Table 8). Apparently the residual

TABLE 8. Bacterial Counts (Log_{10} CFU/g) and pH Values of Fermented-Dried Meat at Different Salt Concentrations. Each salt concentration includes all papaya levels (0, 5, 10, 15, 20, 25%).

Salt (%)	Final pH	Mesophiles	Mesophilic Halophiles	SPS Agar Anaerobes	Lactic Acid Bacteria	Streptococcal Bacteria
0	5.0 b[1]	5.0 a*	4.8 b[1]	5.8 a*	5.3 a*	4.7 b[1]
2	5.1 b	5.7 a	5.8 a	5.7 a	5.9 a	5.6 a
4	5.2 ab	5.8 a	6.3 a	6.2 a	6.2 a	5.9 a
6	5.1 ab	5.6 a	5.9 a	5.8 a	5.4 a	5.7 a
8	5.3 ab	5.0 a	5.6 ab	6.0 a	5.8 a	5.6 a
10	5.4 a	5.3 a	5.6 ab	6.1 a	5.6 a	5.7 a

[1]Means followed by different letters in vertical columns are significantly different ($P<0.05$).

*Means followed by the same letter were not significantly different ($P<0.1$).

salt left in the meat after drying affected the production of acidic substances by bacteria during storage. Ubbaonu (1982) found a reduction in the titrable acidity of fermented-dried beef with the increase of salt in the brine solution.

Mesophiles, SPS agar anaerobic count, and lactic acid bacteria of the fermented-dried beef were not significantly affected by salt.

Mesophilic halophiles were significantly higher ($P<0.05$) only when no salt was used.

Streptococcal bacteria was significantly lower ($P<0.05$) at 0% salt (log 4.7 CFU/g). Streptococcal microorganisms grew better in dried meat with salt at all concentrations added during fermentation than without it ($P<0.05$).

Mesophiles, SPS agar anaerobic count, and lactic acid bacteria, were not significantly affected by salt concentration.

Yeast and molds were not found in fermented-dried beef when fermented with 2% salt or more at the lowest dilution used (10^{-2}). When no salt was used in the fermentation brine, yeast and molds were detected at a concentration of log 2.6 CFU/g.

Effect of Papaya Concentration

Psychrophiles, psychrophilic halophiles, and presumptive *S. aureus* were not detected at the lowest dilution used (10^{-2}). Presumptive coliforms and presumptive *C. perfringens* were not detected at their lowest dilution (10^{-1}).

Salmonella, as was mentioned before, was not found in any of the fermented dried samples. Thus, although Salmonella was detected in two beef samples at the end of the fermentation, it did not survive the smoking-drying process.

Papaya concentration affected the pH of the fermented-dried beef measured 30 days after drying ($P<0.001$) (see Table 9). As papaya concentration increased in the fermentation brine, the pH of the dried meat decreased. The lowest pH values were reached when 15% papaya or more were used in the fermentation brine; these values were 5.1 to 5.0. The lowest, when no papaya was used in the fermentation brine, was 5.4.

The numbers of mesophiles, halophiles, SPS agar anaerobic count, lactic acid bacteria, and streptococcal bacteria in fermented-dried beef were not significantly affected by the addition of papaya during fermentation.

Yeast and mold counts were below log 2.0 CFU/g at all papaya concentrations.

4. SELECTION OF A CURING TREATMENT

The selection of an appropriate salt-papaya combination was based on the microbiological findings and Ubbaonu's (1982) observations concerning the appearance, odor, and chemical analysis. A salt concentration of 10% was chosen because of its inhibitory effects on presumptive coliform counts, presumptive C. perfringens counts and Salmonella.

TABLE 9. Bacterial Counts (Log_{10} CFU/g) and pH Values of Fermented-Dried Meat at Different Papaya Concentrations. Each papaya concentration includes all salt levels (0, 5, 10, 15, 20, 25%).

Papaya (%)	Final pH	Mesophiles	Mesophilic Halophiles	SPS Agar Anaerobes	Lactic Acid Bacteria	Streptococcal Bacteria
0	5.4 a[1]	5.8 a*	6.1 a*	6.2 a*	5.5 a*	5.6 a*
5	5.3 ab	5.2 a	5.4 a	6.1 a	5.8 a	5.5 a
10	5.2 bc	5.6 a	5.7 a	6.0 a	5.8 a	5.6 a
15	5.0 c	5.2 a	5.7 a	5.9 a	5.8 a	5.6 a
20	5.1 bc	5.6 a	5.8 a	5.7 a	5.9 a	5.5 a
25	5.0 c	5.1 a	5.3 a	5.7 a	5.5 a	5.4 a

[1]Means followed by different letters in verticle columns are significantly different ($P<0.001$).

*Means followed by the same letter were not significantly different ($P<0.1$).

Presumptive S. aureus counts were found well below the number at which enterotoxin is produced. It was considered that a combined effect of a low pH during fermentation (obtained by the addition of 10% papaya), and 10% salt would slow the rate of growth of this organism enough to keep it in safe numbers. S. aureus at pH 5.0 reduces its rate of growth when 3% NaCl or more is used in TSB at 37°C (Stern et al., 1979).

Thus, considering Ubbaonu's (1982) observations and the above, a 10% salt and 10% papaya treatment was determined to be most acceptable for human consumption.

5. IDENTIFICATION OF BACTERIA FROM FRESH BEEF

Mesophilic Bacteria

The SMA plate from which isolates for identification were selected showed a mesophilic count of log 4.1 CFU/g (colony forming unit per gram).

The bacterial isolates picked from the mesophilic plate had a high incidence of gram-positive aerobic cocci belonging to the genus Micrococcus (see Table 10). This bacterium formed 85% of the total colonies isolated. According to the literature, Micrococcus, being a mesophile, is most commonly found as part of the initial flora of beef carcasses after slaughter and also on sliced beef held at 15°C to 20°C (Ayres, 1955, 1960; Ayres et al., 1980). On the other hand, Stringer (1969) found a predominance of Micrococcus on beef carcasses after the chilling process.

TABLE 10. Percentages of Mesophilic Bacteria Isolated From Samples of Fresh Meat, Meat That Had Been in a Brine Fermentation for 48 Hours, and Meat That Had Been Stored for 30 Days After a Smoking-Drying Process.

Organism[a]	Fresh Meat	Brine Fermented Meat	Fermented Dried Meat
S. faecium	1.6%	0.0%	60.0%
S. faecalis	0.0%	0.0%	18.0%
P. pentosaceus	0.0%	0.0%	8.3%
L. fermentum	1.6%	0.0%	0.0%
L. brevis	1.6%	0.0%	0.0%
L. viridescens	3.3%	0.0%	0.0%
Leuc. paramesenteroides	0.0%	0.0%	10.0%
M. varians	85.0%	21.6%	0.0%
Flavobacterium spp.	3.3%	0.0%	0.0%
P. alcaligens	1.6%	0.0%	0.0%
P. fluorescens	0.0%	78.0%	0.0%
E. coli	1.6%	0.0%	0.0%
Lost	0.0%	0.0%	3.3%
Total colony count[b]	4.1	8.7	5.7
Total number isolates	60	60	60

[a]The organisms were isolated from SMA incubated at 32°C for 48 hours.

[b]Microbial plate counts at the time of bacterial identification. Numbers are expressed as log CFU/g.

Gram-negative bacteria were found in unexpectedly low numbers. Pseudomonas is usually found as one of the predominant organisms of refrigerated beef (Ayres, 1959; Ayres et al., 1980; Stringer et al., 1969). In this experiment, Pseudomonas formed only 1.6% of the total mesophilic flora of the fresh meat pieces. Flavobacterium (3.3%) and Escherichia coli (1.6%) were also found among the gram-negatives.

Homo and heterofermentatives gram-positive rods and cocci were found as 8.1% of the total number of isolates. The gram-positive heterofermentative flora, represented by Lactobacillus brevis, L. viridescens, and L. fermentum formed 1.6%, 3.3%, and 1.6% of the total mesophilic isolates, respectively. Streptococcus faecium, a homofermentative organism, formed 1.6% of the total mesophilic isolates.

Halophilic Bacteria

The halophilic plate from which isolates for identification were selected showed a total halophilic count of log 4.2 CFU/g. Speck (1976) recommends the addition of 3% NaCl to a non-selective agar to enumerate halophilic organisms. SMA plus 3% NaCl was used in this experiment. The type of bacteria isolated from this medium were found not to be truly halophilic organisms but rather halotolerant (see Table 11).

Gram-positives formed nearly 97% of the bacteria isolated on SMA plus 3% NaCl. Micrococcus, as with the mesophilic isolates was the predominant genus representing 86.6% of the total number of

TABLE 11. Percentages of Halophilic Bacteria Isolated From Samples of Fresh Meat, Meat That Had Been in a Brine Fermentation for 48 Hours, and Meat That Had Been Stored for 30 Days After a Smoking-Drying Process.

Organism[a]	Fresh Meat	Brine Fermented Meat	Fermented Dried Meat
S. faecium	0.0%	0.0%	64.0%
S. faecalis	0.0%	0.0%	22.2%
P. pentosaceus	0.0%	0.0%	4.4%
L. brevis	5.0%	0.0%	0.0%
L. viridecens	5.0%	0.0%	0.0%
Leuc. paramesenteroides	0.0%	0.0%	2.2%
M. varians	86.6%	31.6%	0.0%
Flavobacterium spp.	1.6%	0.0%	0.0%
P. fluorescens	1.6%	68.0%	0.0%
Lost	0.0%	0.0%	6.6%
Total colony count[b]	4.2	8.8	5.6
Total number isolates	60	60	45

[a]The organisms were isolated from SMA plus 3% NaCl and incubated at 32°C for 48 hours. Organisms were found to be halotolerant rather than halophilic.

[b]Microbial plate counts at the time of bacterial identification. Numbers are expressed as log CFU/g.

isolates. The second most predominant organisms were heterofermentative lactobacilli (10%). These were represented by L. brevis (5%) and L. viridecens (5%). There were no gram-positive homofermentatives isolated from this medium.

Gram-negatives, as with the mesophilic isolates, remained as a small fraction of the total number of isolates. Flavobacterium and a fluorescent type of Pseudomonas were present forming 1.6% of the total number of isolates each.

Lactic Acid Bacteria

The APT agar plate from which isolates for identification were selected showed an APT colony count of log 4.2/g. APT agar is recommended for the enumeration of lactics in meat (Speck, 1976).

The bacteria isolated from this medium showed that not only lactic acid producers are isolated but others as well (see Table 12).

Gram-positive heterofermentative lactics were the dominant flora. They represented 33.3% of the total number of isolates. L. viridescens, which was the most common species found (25%), and Leuconostoc paramesenteroides (7.4%) were the only two heterofermentatives found. L. casei and Pediococcus pentosaceus represented the homofermentative organisms; they formed 3.7% of the total number of isolates each. Lactobacilli were the most common type of microorganisms found in APT agar.

TABLE 12. Percentages of Bacteria Isolated From APT Agar for Samples of Fresh Meat, Meat That Had Been in a Brine Fermentation for 48 Hours, and Meat That Had Been Stored for 30 Days After a Smoking-Drying Process.

Organism[a]	Fresh Meat	Brine Fermented Meat	Fermented Dried Meat
S. faecium	0.0%	6.6%	56.6%
S. faecalis	0.0%	5.0%	11.6%
L. casei	3.7%	0.0%	0.0%
P. pentosaceus	3.7%	0.0%	18.3%
L. viridescens	25.9%	0.0%	0.0%
Lactobacillus spp.	0.0%	6.6%	0.0%
Leuc. paramesenteroides	7.4%	0.0%	13.3%
M. varians	14.8%	18.3%	0.0%
M luteus	3.7%	0.0%	0.0%
Micrococcus spp.	7.4%	0.0%	0.0%
Flavobacterium spp.	3.7%	0.0%	0.0%
P. fluorescens	0.0%	63.3%	0.0%
Lost	18.5%	0.0%	0.0%
Total colony count[b]	3.4	7.1	5.4
Total number isolates	27	60	60

[a]The organisms were isolated from APT agar incubated at 32°C for 48 hours.

[b]Microbial plate counts at the time of bacterial identification. Numbers are expressed as log CFU/g.

Micrococcus was found to represent 25.9% of the bacterial isolates from APT agar. There were two identified species, M. varians (14.8%) and M. luteus (3.7%), and a group of gram-positive cocci (7.4%) that share the same generic characteristics of the two previous Micrococcus spp. These microorganisms did not fit staisfactorily in any of the species mentioned in the 1974 Edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Gram-negative bacteria isolated from APT agar remained as a small fraction of the total isolates. Flavobacterium was the only gram-negative found, the bacterium represented 3.7% of the total number of isolates.

Of the colonies isolated from APT agar, 18.5% were lost before they were identified. These organisms grew very slowly in MRS broth and produced very scanty growth or none at all on APT agar. They apparently lost their ability to grow on artificial media after successive streaking for isolation.

Anaerobic Bacteria

SPS agar was used to obtain anaerobic isolates for identification, the reasons for this are explained on page 67. The total number of colonies found on the SPS agar was log 2.6/g.

The identified isolates were exclusively homofermentative gram-positive cocci (see Table 13). They were identified as S. faecium and S. faecalis which represented 40.9% and 36.3% of the total number of SPS isolates respectively.

TABLE 13. Percentages of Bacteria Isolated From SPS Agar for Samples of Fresh Meat, Meat That Had Been in a Brine Fermentation for 48 Hours, and Meat That Had Been Stored for 30 Days After a Smoking-Drying Process.

Organism[a]	Fresh Meat	Brine Fermented Meat	Fermented Dried Meat
S. faecium	40.9%	57.9%	51.3%
S. faecalis	36.3%	28.9%	27.0%
Lost	22.7%	13.1%	21.6%
Total colony count[b]	2.6	5.5	3.9
Total number isolates	44	38	37

[a]The organisms were isolated from SPS agar incubated anaerobicly at 37°C for 48 hours.

[b]Microbial plate counts at the time of bacterial identification. Numbers are expressed as log CFU/g.

There were some bacterial isolates that were lost before they were identified. These represented 22.7% of the total number of isolates.

Yeast and Mold

The PDA plate from which isolates for identification were selected showed an estimated total count of log 1.9 CFU/g.

Mold was not found among the PDA isolates from fresh beef pieces. Rhodotorula were the only type of yeasts found and represented 100% of the total number of isolates from PDA.

6. IDENTIFICATION OF BACTERIA FROM BRINE FERMENTED BEEF

Mesophilic Bacteria

High numbers of bacteria were recorded at the end of the brine-fermentation. The SMA showed a total colony count for the brine fermented meat of log 8.7/g. Isolates for identification were selected from the countable plate.

There was a dramatic increase in the number of gram-negatives at the end of the brine-fermentation (see Table 10). These micro-organisms constituted 78% of the total number of mesophilic isolates. They were represented solely by P. fluorescens. This organism was the dominant mesophilic isolate from brine-fermented meat even though there was no evidence of this organism in the mesophilic isolates from the fresh meat pieces.

The increase of P. fluorescens during fermentation was probably due to several reasons. First, the motility of this organism would

improve its ability to compete with other microorganisms for nutrients. Second, the high rate of growth of Pseudomonas would place this organism in a more competitive place over many meat bacteria (Gill and Newton, 1977). Third, the organism's adaptability to its environment and its versatile nutritional requirements could have also played an important factor (Draughon, 1984). Finally, they could have entered the fermentation through handling by people.

A peculiarity of this particular strain of P. fluorescens was that when it was cultivated on TSB containing NaCl, its size decreased as NaCl concentration increased. The rod shaped bacterium became shorter and somewhat thinner giving a rather oval shape at a NaCl concentration of 7.5%; P. fluorescens is known to behave in such a manner in old cultures (Buchanan and Gibbons, 1974), although this has not been reported previously during exposure to salt.

The second and only other microorganism found among the mesophilic isolates on the brine-fermented meat was M. varians. This organism represented 21.6% of the total mesophilic count.

Halophilic Bacteria

Brine-fermented meat also showed high numbers of bacteria when SMA plus 3% NaCl was used to collect bacterial isolates for identification. The total halophilic count of the meat was log 8.8 CFU/g.

P. fluorescens was also found to be the predominant microorganism among the halophilic (halotolerant) bacteria (see Table 11). It represented 68% of the total number of isolates; slightly lower (10%)

than its representation of the mesophilic bacteria. The bacteria isolated in SMA plus 3% NaCl were not truly halophilic organisms but rather halotolerants.

M. varians occupied the second place among the halotolerant isolates of brine-fermented meat as it did in the mesophilic isolates of the same product.

Lactic Acid Bacteria

The APT lactic colony count of the brine-fermented meat was log 7.1 CFU/g. P. fluorescens, as with the mesophilic and halophilic isolates, was the predominant organism isolated (see Table 12). It was found to represent 63.3% of the total microflora isolated from APT agar. M. varians, as in the mesophilic and halophilic isolate study, was found to be the second most common organism isolated.

Despite the predominance of the previously mentioned micro-organisms which overwhelmed any other organisms and prevented them from being detected from SMA and SMA plus 3% NaCl, lactic acid producers were able to show up in APT agar. Thus, Streptococcus spp. were the third most common genera found; it represented 11.6% of the total APT agar isolates. S. faecium and S. faecalis were the two species identified; they represented 6.6% and 5.0% of the total number of isolates respectively.

Heterofermentative lactobacilli, representing 6.6% of the total number of isolates, were classified as Lactobacillus spp. because they did not fit under any of the species listed in the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

It can be noticed that the APT agar colony count (log 7.1 CFU/g) was lower than that of SMA (log 8.7 CFU/g) and SMA plus 3% NaCl (log 8.8 CFU/g). This phenomenon could be due to two main reasons. First, although APT agar is not a selective medium, the poured plate technique used when plating with this medium put at a disadvantage the predominantly aerobic flora found in the brine-fermented meat (P. fuorescens and M. varians). Second, APT agar satisfies better the nutritional needs of lactic acid producers which by growing could have partially inhibited some of the aerobic microflora. Inhibition of bacteria by lactic acid producers has been discussed by Bacus and Brown (1981), Raccach and Baker (1979), and Stamer (1979). Kafel and Ayres (1969) studied the inhibition of several organisms by enterococci when grown in APT agar. The same reasons that explain the lower APT colony could also explain why lactic acid producers were able to show up in APT agar despite the very high numbers of the predominantly aerobic microflora.

Anaerobic Bacteria

The SPS agar count was log 5.5 CFU/g. The isolates identified from it belonged to the enterococci group (see Table 13). No C. perfringens were isolated. S. faecium represented almost 58% of the total number of isolates. S. faecalis represented almost 29% of the bacterial isolates. There were about 13% bacterial isolates lost which did not survive the streaking procedure used to assure the purity of the isolates.

There were no true anaerobes isolated from this medium perhaps because they were among the lost isolates, or they were inhibited by the antibiotics in the medium, or they were overwhelmed by the enterococci or a combination of these. In addition, enterococci are able to inhibit Clostridium spp. among others (Kafel and Ayres, 1969).

The isolation of anaerobic organisms from a non-selective medium (Thioglycollate agar) was not done because of the enormous amount of gas produced by some microorganisms. This gas disrupted the agar breaking it in several places, mixing the microorganisms and preventing enumeration.

Yeast and Mold

No yeast or mold were isolated from the brine-fermented meat.

7. IDENTIFICATION OF BACTERIA FROM FERMENTED DRIED BEEF

Mesophilic Bacteria

At the end of the storage period (30 days), the fermented-dried meat had a mesophilic count of log 5.7 CFU/g. Isolates from the countable plate were collected for identification.

Homofermentative organisms were the predominant flora (see Table 10, page 56). These organisms made 86.3% of the mesophilic isolates of fermented-dried meat. They were identified as S. faecium, S. faecalis, and P. pentosaceus; each represented 60.0%, 18.0%, and 8.3% of the total number of isolates, respectively.

One heterofermentative lactic, Leuc. paramesenteroides, was found representing 10% of the total number of isolates. There were 3.3% bacterial isolates lost before their complete identification.

The radical change occurring in the bacterial composition of the meat product at this stage may be explained by the high thermostability of streptococci, Leuconostoc and Pediococcus (Buchanan and Gibbons, 1974; Kafel and Ayres, 1969; Skinner and Quesnel, 1978). These organisms are capable of surviving the temperature that the fermented meat was exposed to during drying and smoking (48.9°C). Chyr et al. (1981) reported that S. faecalis was able to survive in Braunschweiger when heated at 60°C for 30 minutes if the cell concentration was 10^6/g or higher. Streptococcus, Leuconostoc, and Pediococcus are also known to be halotolerant, a feature that would help them survive the increase in salt concentration in the meat during drying. Ayres et al. (1980) reported that lactic acid bacteria are one of the most common bacterial forms associated with dried food products.

Smoking could also be playing an important factor. There is evidence that gram-negative bacteria are much more sensitive to smoking than gram-positives (Kitchell and Shaw, 1975; Hooshyar and Oblinger, 1982). Thus, the elimination of Pseudomonas during drying and the survival of gram-positive cocci appears to have occurred.

Halophilic Bacteria

SMA plus 3% NaCl showed a halophilic colony count of log 5.7 CFU/g. The microorganisms were identified and their percentages were very similar to those found in the mesophilic isolates of dried meat.

Gram-positive homo- and heterofermentative lactic acid producers were the only type of bacteria recovered (see Table 11, page 58). Homofermentatives were the dominant bacterial flora; they represented 90.6% of the total number of isolates. Leuc. paramesenteroides was the only heterofermentative lactic found; it constituted 2.2% of the bacterial isolates. There were 6.6% of the bacterial isolated lost before they were identified.

Lactic Acid Bacteria

The APT agar plate count of the dried meat was log 5.4 CFU/g. The bacteria found were composed of gram-positive homo- and heterofermentative lactic acid producers (see Table 12). Homofermentatives were the dominant flora; they made up 85% of the total number of bacterial isolates. Homofermentatives were represented by S. faecium (56.6%), S. faecalis (11.6%), and P. pentosaceus (18.3%).

Heterofermentatives were represented only by Leuc. paramesenteroides. This organism constituted 13.3% of the total number of isolates.

Anaerobic Bacteria

The colony count on the SPS agar was log 3.9 CFU/g. Homofermentative streptococci were the only types of bacteria identified; they formed 78.3% of the total number of isolates (see Table 13). S. faecium (51.3%) and S. faecalis (27.0%) were the species found.

Isolates that were lost before they were identified represented 21.6% of the total number of bacterial isolates.

Yeast and Mold

No yeast or mold were isolated from the fermented-dried meat.

CHAPTER V

SUMMARY AND CONCLUSIONS

A procedure to produce a brine-fermented meat product which could be manufactured and stored without refrigeration was developed by C. N. Ubbaonu and C. C. Melton (Ubbaonu, 1982). The product was to be manufactured with salt, papaya, and beef round pieces. In this study, microbial examinations were performed to aid in the selection of an appropriate salt-papaya brine concentration that could be used during the fermentation to produce an acceptable and safe product for human consumption. Additionally, the bacteria growing on the meat product treated with the chosen brine treatment were identified.

There were 36 different brine solutions (prepared by Ubbaonu, 1982), containing 0% to 10% salt (in increments of 2%) and 0% to 25% papaya (in increments of 5%). Fresh beef round pieces were brine-fermented for 48 hours at room temperature. The meat was then placed in a food processing oven and dried at 48.9°C for 45 hours. Wood smoke was applied for one hour and after one-half hour of drying. The dried beef pieces were placed in plastic bags and stored at room temperature.

The microbial examinations were performed on the fresh beef, on the fermented beef at the end of the brine-fermentation, and on the dried meat after 30 days of storage. Such examinations consisted of the following colony counts: mesophiles, psychrophiles, mesophilic halophiles, psychrophilic halophiles, presumptive coliforms, presumptive

C. perfringens, SPS agar anaerobes, lactic acid bacteria, presumptive S. aureus, streptococcal bacteria, and yeast and molds. In addition, the presence or absence of Salmonella and the meat Ph at the time of the examinations were determined.

The microbial counts and pH readings of the fresh beef indicated that the fresh meat used had a good bacteriological quality.

The brine-fermented beef bacterial counts appeared to be affected only by the salt concentration. Colony counts of psychrophiles were reduced considerably when 6% salt or more was used ($P<0.0001$). Presumptive coliforms counts and SPS agar anaerobic counts were reduced when 8% salt or more was used ($P<0.0001$). Streptococcal bacteria were favored by salt concentrations of 4% to 8% ($P<0.001$). Yeast and mold counts were more effectively reduced when 10% salt was used ($P<0.05$). Presumptive C. perfringens were detected at 0% and 6% salt. Salmonella was detected at 2% and 8% salt. The meat final pH, on the other hand, appeared to be affected only by the papaya concentration ($P<0.0001$); it decreased as papaya concentration increased.

The bacterial examinations of the fermented-dried meat after 30 days of storage showed no presence of psychrophiles, psychrophilic halophiles, presumptive coliforms, Salmonella, presumptive C. perfringens, and presumptive S. aureus. The increase of salt appeared to increase the meat pH ($P<0.05$). Streptococcal bacteria were favored when salt (2% or greater) was used ($P<0.05$). Mesophiles, SPS agar anaerobes, and lactic acid bacteria were not affected by salt. Yeast and molds were not detected when 2% salt or more was used. The papaya

concentration appeared to affect only the same meat pH ($P<0.001$); it decreased as papaya concentration increased.

Thus, a salt-papaya concentration of 10% each (in the brine solution) was considered bacteriologically acceptable and safest for human consumption. This decision was based on the effect that the salt concentration had on the bacterial counts of pathogens. The effect of papaya on the meat pH was also considered.

The identification of bacteria growing on the meat treated with 10% salt-10% papaya solution is summarized as follows. Bacterial colonies were picked for identification from SMA (mesophiles), SMA plus 3% NaCl (mesophilic halophiles), APT agar (lactic acid bacteria), SPS agar (SPS agar anaerobes), and malt extract agar (yeast and molds). The stages at which the meat was sampled were: before fermentation; after fermentation, and 30 days after the drying process.

The identification of fresh beef isolates yielded the following results. Micrococcus predominated among the mesophiles (85%) and the mesophilic halophiles (86.6%). Gram-positive heterofermentatives (L. viridescens and Leuc. paramesenteroides) were the predominant flora of the APT agar isolates (33.3%); they were followed by Micrococcus (25.9%). SPS agar anaerobic isolates were dominated by S. faecium (40.9%) and S. faecalis (36.3%). Yeasts and molds isolates had a total dominance of Rhodotorula.

The identification of brine-fermented beef isolates yielded the following results. P. fluorecens predominated among the mesophiles (78%) from SMA, mesophilic halophiles (68%) from SMA plus 3% NaCl,

and APT agar isolates (63.3%); it was followed in predominance by M. varians in all three media (21.6%, 31.6%, and 18.3%, respectively). Although there was dominance by P. fluorecens, there were no off-odors or off-flavors detected. Salt apparently had an effect on the proteolytic enzymes of the bacterium, also the presence of fermentable carbohydrates in papaya could have delayed these enzymes. S. faecium (58%) and S. faecalis (29%) represented the majority of the SPS agar anaerobes. There were no yeasts or molds isolated.

The identification of bacteria from fermented-dried beef produced the following results. Homofermentatives (S. faecium, S. faecalis, and P. pentosaceus) were the predominant microflora of the isolates from SMA (60%, 18%, and 8.3%, respectively), from SMA plus 3% NaCl (64%, 22.2%, and 4.4%, respectively), and from APT agar (56.6%, 11.6%, and 18.3%, respectively). SPS agar anaerobic isolates were represented by S. faecium (51.3%) and S. faecalis (27%). Yeast and mold counts were not detected.

The identification of bacteria confirmed the fact that a 10% salt-10% papaya brine would produce a satisfactory meat product without the aid of refrigeration. As could be seen by the identification of bacteria, pathogenic bacteria were effectively inhibited.

LIST OF REFERENCES

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Acton, J. C. and Dick, R. L. 1976. Composition of some commercial dry sausage. J. Food Sci. 41:971.

Akashi, A. 1971. Preventive effect of egg white lysozyme on Vienna sausage. Jap. J. Zootechnical Sci. 42:289-295. (Cited in Food Sci. Technol. Abst. 4:1 S-66).

Akashi, A. 1961. Preservative effect of egg white lysozyme added to cooked sausage. Jap. J. Zootechnical Sci. 40:243. (Cited in Food Sci. Technol. Abst. 1:12 S-868).

Arseculeratne, S. N., Samarajeewa, U. and Welianga, L. V. 1976. Inhibition of aflatoxin accumulation in smoked substances. J. Appl. Bact. 41:223.

Ayres, J. C. 1955. Microbiological implications in the handling, slaughtering and dressing of meat animals. Adv. Food Res. 6:109.

Ayres, J. C. 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res. 25:1.

Ayres, J. C., Mundt, J. O. and Sandine, W. E. 1980. "Microbiology of Foods." W. H. Freeman and Company, San Francisco, CA.

Bacus, J. N. and Brown, W. L. 1981. Use of microbial cultures: meat products. Food Technol. 35(1):74.

Balls, A. K. and Hoover, S. R. 1937. The milk clotting time of papain. J. Biol. Chem. 121:737.

Banwart, G. J. 1979. "Basic Food Microbiology." AVI Publishing Co., Inc., Westport, CT.

Barr, A. J., Goodnight, J. H., Sall, J. P., Blair, W. H. and Chilko, D. M. 1979. "Statistical Analysis System" SAS Institute, Inc., Raleigh, NC.

Bender, M. L. and Brubacher, L. J. 1973. In: "Catalisis and Enzyme Action." McGraw-Hill Book Co., NY.

Beuchat, L. R. 1974. Combined effects of water activity, solute, and temperature on the growth of Vibrio parahaemolyticus. Appl. Microbiol. 27:1075.

Beuchat, L. R. 1975. Environmental factors affecting survival and growth of Vibrio parahaemolyticus; a review. J. Milk Food Technol. 38:476.

Blackburn, S. 1976. In: "Enzyme Structure and Function." Marcel Dekfer Inc., NY

Bryan, F. L. 1968. What the sanitarian should know about staphilococci and Salmonella in non-dairy products. II. Salmonellae. J. Milk Food Technol. 31:131.

Buchanan, R. E. and Gibbons, N. E. 1974. "Bergey's Manual of Determinative Bacteriology. 8th Ed. Williams and Wilkins Co., Baltimore, MD.

Carreno, R. and Chan, H. T., Jr. 1982. Partial purification and characterization of acid phosphates from papays. J. Food Sci. 42:1498.

Chan, H. T., Jr., Flat, R. A., Forrey, R. R., Cavaletto, C. G., Nakayams, T. O. M. and Brekke, J. E. 1973. Development of off-odors and off-flavors in papays puree. J. Ag. Food. Chem. 21:566.

Chan, H. T., Jr. and Kwok, S. C. M. 1975. Importance of enzyme inactivation prior to extraction of sugars from papaya. J. Food Sci. 40:770.

Chyr, C. Y., Walker, H. W. and Sebranek, J. G. 1981. Bacteria associated with spoilage of braunschweiger. J. Food Sci. 46:468.

Daly, C., La Chance, M., Sandine, W. E., and Elliker, P. R. 1973. Control of Staphilococcus aureus in sausage by starter cultures and chemical acidulation. J. Food Sci. 38:426.

Deike, K. H. 1978. Starter culture for meat substrates, their production and use. German Fed. Rep. Pat. Appl. 2 718 791.

Draudt, H. N. 1963. The meat smoking process: a review. Food Technol. 17(12):85.

Draughon, F. A. 1984. Personal communication.

Duncan, D. 1955. Multiple Range and Multiple F Tests. Biometrics 11:1.

Fiddler, W., Doerr, R. C., Wasserman, A. E. and Salay, J. M. 1966. Composition of hickory sawdust smoke, furans and phenols. J. Ag. Food Chem. 14:659.

Fiddler, W., Parker, W. E., Wasserman, A. E. and Doerr, R. C. 1967. Thermal decomposition of ferulic acid. J. Ag. Food Chem. 15:757.

Fiddler, W., Wasserman, A. E. and Doerr, R. C. 1970. A smoke flavor fraction of a liquid smoke solution. J. Ag. Food Chem. 18:934.

Gilbert, J. and Knowles, M. E. 1975. The chemistry of smoked foods: a review. J. Food Technol. 10:245.

Gill, C. O. 1976. Subtrate limitation of bacterial growth at meat surfaces. J. Appl. Bact. 41:401.

Gill, C. O. and Newton, K. G. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bact. 43:189.

Gill, C. O. and Newton, K. G. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. Meat Sci. 2:207.

Gill, C. O. and Newton, K. G. 1980. Growth of bacteria on meat at room temperatures. J. Appl. Bact. 49:315.

Gill, C. O., Lowry, P. D. and Di Menna, M. E. 1981. A note on the identities of organisms causing black spot spoilage of meat. J. Appl. Bact. 5:183.

Goepfert, J. M. and Chung, K. C. 1970. Behavior of Salmonella during the manufacture and storage of a fermented sausage product. J. Milk and Food Technol. 33(5):185.

Gottschall, G. Y. and Kies, M. W. 1942. Digestion of beef by papain. Food Research 7:373.

Hayes, S., Craig, J. M. and Pilcher, K. S. 1970. The detection of Clostridium botulinum type E in smoked fish products in the Pacific Northwest. Can. J. Microbiol. 16:207.

Hess, E. 1928. The bactericidal action of smoke. J. Bact. 15:33.

Hinrichs, J. R. and Whitaker, J. R. 1962. Enzymatic degradation of collagen. J. Food Sci. 27:250.

Hooshyar, P., Oblinger, J. L., and Janky, D. M. 1982. Survial of Salmonella infantis and Staphylococcus aureus on smoked broiler halves. Poultry Sci. 61:79.

Howard, J. B. and Glazer, A. N. 1969. Papaya Lysozyme. J. Biol. Chem. 244:1399.

Howard, J. B. and Glazer, A. N. 1967. Studies of the physiochemical and enzymatic properties of papaya lysozyme. J. Biol. Chem. 242:5715.

Hruza, D. E., Van Praag, M. and Heinsohn, H. Jr. 1974. Isolation and identification of the tar of hickory wood smoke. J. Ag. Food Chem. 22:123.

Ingram, M. and Dainty, R. H. 1971. Changes caused by microbes in spoilage of meats. J. Appl. Bact. 34:24.

Issenberg, P., Kornreich, M. R. and Lustre, A. O. 1971. Recovery of phenolic wood smoke components from smoked food and model systems. J. Food Sci. 36:107.

Janky, D. M., Oblinger, J. L. and Koburger, J. A. 1975. Sensory and microbiology evaluation of smoked cornish game hens. Poultry Sci. 54:1942.

Jansen, E. F. and Balls, A. K. 1941. Chymopapain; a new crystalline proteinase from papaya latex. J. Biol. Chem. 137:459.

Jensen, L. B. 1954. "Microbiology of Meats." 3rd Ed. The Garrad Press Publishers, Champain, IL.

Johnson, K. M. 1984. Bacillus cereus foodborne illness; an update. J. Food Prot. 47:145.

Juven, B. J. and Gertshouki, R. 1976. The effect of salting on the microbiology of poultry meat. J. Milk Food Technol. 39:13.

Kafel, S. and Ayres, J. C. 1969. The antagonism of enterococci on other bacteria in canned hams. J. Appl. Bact. 32:217.

Kang, C. K. and Rice, E. E. 1970. Degradation of various meat fraction by tenderizing enzymes. J. Food Sci. 35:563.

Kitchell, A. G. and Shaw, B. G. 1975. "Lactic Acid Bacteria in Beverages and Foods." Academic Press, London.

Koburger, J. A. 1973. Fungi in foods. J. Milk Food Technol. 36:434.

Kotula, A. W., Lusby, W. R. and Crouse, J. D. 1975. Variability in microbiology counts on beef carcasses. J. Anim. Sci. 40:834.

Labuza, T. P. 1975. Interpretation of sorrption data in relation to the state of constituent water. In: "Water Relation of Foods." (R. B. Duckworth, Ed.). Academic Press, NY.

Lee, I. C., Harmon, L. G. and Price, J. F. 1977. Growth and enterotoxin production by Staphylococci in Genoe salami. J. Food Protection 40:325.

Lustre, A. O. and Issenberg, P. 1969. Volatile components of hardwood sawdust smoke. J. Ag. Food Chem. 17:1387.

Lustre, A. O. and Issenberg, P. 1970. Phenolic components of smoked meat products. J. Ag. Food Chem. 18:1056.

Meyer, K., Hahnel, E. and Steinberg, A. 1946. Lysozyme of plant origin. J. Biol. Chem. 163:733.

Minor, T. E. and Marth, E. H. 1971. Staphylococcus aureus and staphylococcal food intoxications. J. Milk Food Technol. 34:557.

Murray, J. G. 1969. An approach to bacteriological standars. J. Appl. Bact. 32:123.

Newton, K. G. and Gill, C. O. 1978. Storage quality of dark, firm, dry meat. Appl. Environ. Microbiol. 36:375.

Newton, K. G. and Gill, C. O. 1980. Control of spoilage in vacuum packaged dark, firm, dry (DFD) meat. J. Food Technol. 15:227.

Newton, K. G. and Gill, C. O. 1981. The microbiology of DFD fresh meats. Meat Sci. 5:223.

Nicol, D. J., Shaw, M. K. and Ledward, D. A. 1970. Hydrogen sulfide production by bacteria and sulfmyoglobin formation in prepacked chilled beef. Appl. Microbiol. 19:937.

Palumbo, S. A., Zaika, L. L., Kissinger, J. C. and Smith, J. L. 1976. Microbiology and technology of the pepperoni process. J. Food Sci. 41:12.

Pederson, C. S. 1979. "Microbiology of Food Fermentations." 2nd Ed. AVI Publishing Co., Inc., Westport, CT.

Peterson, A. C., Black, J. J. and Gunderson, M. F. 1964. Staphylococci in competition. III. Influence of pH and salt on staphylococcal growth in mixed population. Appl. Microbiol. 12:70.

Price, J. F. and Schweigert, B. S. 1978. "The Science of Meat and Meat Products." 2nd Ed. Food and Nutrition Press, Inc., Westport, CT.

Price, R. J. and Lee, J. S. 1970. Inhibition of Pseudomonas species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol. 33:13.

Porter, R. W., Bratzler, L. J. and Pearson, A. M. 1965. Fractionation and study of compounds in wood smoke. J. Food Sci. 30:615.

Pullen, M. M. and Genigeorgis, C. A. 1977. A study of coagulase positive staphylococci in salami before fermentation. J. Food Protection 40:704.

Raccach, M. and Baker, R. C. 1978. Lactic acid bacteria as an antispoilage and safety factor in cooked, mechanically deboned poultry meat. J. Food Protection 41:703.

Raccach, M. and Baker, R. C. 1979. Fermented mechanically deboned poultry meat and survival of Staphilococcus aureus. J. Food Protection 42:214.

Reed, G. 1975. "Enzymes in Food Processing." Academic Press, NY.

Rey, C. R., Kraft, A. A., Walker, H. W. and Parrish, F. C. 1970. Microbial changes in meat during aging at elevated temperatures and later refrigerated storage. Food Technol. 24(1):67.

Roberts, T. A. and Derrick, C. M. 1978. The effect of curing salts on the growth of Clostridium perfringens (welchii) in a laboratory medium. J. Food Technol. 13:349.

Roberts, T. A. and Ingram, M. 1973. Inhibition of growth of Clostridium botulinum at a different pH values by sodium chloride and sodium nitrite. J. Food Technol. 8:467.

Samson, J. A. 1980. "Tropical Fruits." Longman Group Lt. London.

Scharner, E. and Hofmann, H. P. 1978. Use of starter cultures in the manufacture of raw sausages and cured products. Fleisch 32:133.

Skinner, F. A. and Quesnel, L. B. 1978. Streptococci. Academic Press, NY.

Smith, E. L., Kimmel, J. R., Brown, D. M. and Thompson, E. O. P. 1955. Isolation and properties of a crystalline mercury derivative of a lysozyme from papaya latex. J. Biol. Chem. 215:67.

Smith, J. L., Palumbo, S. A., Kissinger, J. C., and Huhtanen, C. N. 1975. Survival of Salmonella dublin and Salmonella typhimurium in Lebanon bologna. J. Milk Food Technol. 38:150.

Smith, J. L. and Palumbo, S. A. 1973. Microbiology of Lebanon bologna. Appl. Microbiol. 21:489.

Speck, M. L. (Ed.). 1976. Compendium or Methods for the Microbiological Examination of Food. Prepared by the APHA Intersociety Agency Committee on Microbiological Methods for Foods. American Public Health Association, Washington, D.C.

Stamer, J. R. 1979. The lactic acid bacteria; microbes of diversity. Food Technol. 33(1):60.

Stern, N. J., Pierson, M. D. and Kotula, A. W. 1980. Effect of pH and sodium chloride on Yersinia enterolitica growth at room and refrigeration temperatures. J. Food Sci. 45:64.

Stern, N. J., Smoot, L. A. and Pierson, M. D. 1979. Inhibition of Staphylococcus aureus growth by combinations of butylated hydroxy anisole, sodium chloride and pH. J. Food Sci. 44:710.

Stringer, W. C., Bilskie, M. E. and Naumann, H. D. 1969. Microbial profiles of fresh beef. Food Technol. 23( ):97.

Tappel, A. L., Miyada, D. S., sterling, C. and Maier, V. P. 1956. Meat tenderization. II. Factors affecting the tenderization of beef by papain. Food Research 21:375.

Tatini, S. R., Lee, R. Y., McCall, W. A. and Hill, W. M. 1976. Growth of Staphylococcus aureus and production of enterotoxins in pepperoni. J. Food Sci. 41:223.

Tonge, R. J., Baird-Parker, A. C. and Cavett, J. J. 1964. Chemical and microbiological changes during storage of vacuum packed sliced bacon. J. Appl. Bact. 27:252.

Troller, J. A. 1976. Staphylococcal growth and enterotoxin production factors for control. J. Milk Food Technol. 39:499.

Troller, J. A. and Christian, J. H. B. 1978. "Water Activity and Foods." Academic Press, NY.

Ubbaonu, C. N. 1982. Development and acceptability of cured and dried beef prepared at ambient temperatures in NaCl and papaya brine. Ph.D. Dissertation, The University of Tennessee, Knoxville.

Watt, B. K. and Merrill, A. L. 1963. "Composition of Foods." Agriculture Handbook 8. U.S. Department of Agriculture, Washington, D.C.

White, W. H., Gibbons, N. E., Woodcock, A. H. and Cook, W. H. 1942. Smoked meats. Canad, J. Res. 20:263.

Wilson, N. R. P., Dyett, E. J., Hughes, R. B. and Jones, C. R. V. 1981. "Meat and Meat Products." Applied Sci. Publishers, London.

APPENDICES

APPENDIX 1

COMPOSITION OF PAPAYA[a]

TABLE A1. Composition of Papaya[a].

Constituent	Papaya (Carica papaya) Per 100-g
Water (percent)	88.7
Calories	39
Protein (grams	0.6
Fat (grams)	0.1
Carbohydrates (grams):	
Total	10.0
Fiber	0.9
Ash (Grams)	0.6
Calcium (milligrams)	20
Phosphorus (milligrams)	16
Iron (milligrams)	0.3
Sodium (milligrams)	3
Potassium (milligrams)	234
Vitamin A (milligrams)	1750
Thiamine (milligrams)	0.04
Niacin (milligrams)	0.3
Vitamin C (milligrams)	56
Riboflavin (milligrams)	0.04

[a]From Watt and Merrill (1975).

APPENDIX 2

CHARACTERISTICS OF PAPAYA LATEX ENZYMES[a]

TABLE A2. Characteristics of Papaya Latex Enzymes[a].

Enzyme	Molecular Weight	Isoelectric Point	Concentration in the Latex[b] (%)
Papain	21,000	8.75	10
Chymopapain	36,000	10.1	45
Lysozyme	25,000	10.5	20

[a]From Reed (1975).

[b]Expressed as percent of the soluble protein latex.

APPENDIX 3

RELATIVE POTENCIES OF PLANT PROTEOLITIC ENZYME PREPARATIONS ON THE MUSCLE TISSUE COMPONENTS[a]

TABLE A3. Relative Potencies of Plant Proteolitic Enzyme Preparations on the Muscle Tissue Components[a].

Enzyme	Muscle fiber	Connective tissue	
		Collagen	Elastin
Ficin	+++	+++	+++
Papain	++	+	++
Bromelain	Trace	+++	+

[a]From Reed (1975).

VITA

Mario Armando Castro was born in Navojoa, Sonora, Mexico on August 27, 1956. He attended elementary and high school in Los Mochis, Sinaloa, Mexico. In August 1973 he entered the Institute de Tecnologia y de Estudios Superiores de Monterrey, and in December 1977 he received a Bachelor of Science degree in Food Technology and Science. During 1978 and 1979, he worked for El Satelite Snack Food Company in Los Mochis, Sinaloa, Mexico as Production and Quality Control Manager. In 1980, he entered the Graduate School of The University of Tennessee, Knoxville.