

EFFECTS OF GLUCOSE PHOSPHATE, SALT, AND COOKING  
TEMPERATURES ON THE MICROBIOLOGY OF PRE- AND  
POST-RIGOR RESTRUCTURED BEEF ROASTS

by

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## INTRODUCTION

The restructuring of meat products introduces a variety of microbiological problems; these include alteration of the redox potential and distribution of microorganisms throughout the product. Hot-processing produces a meat product with a higher pH. This pH is closer to neutral and the optimum for microbial growth. The addition of non-meat ingredients (salt, phosphates or nitrites) alter the natural ecological environment of the product. Therefore, it is imperative that a microbiological study be included when reformulating or establishing new procedures. The consumer must be assured of a "safe" meat product.

The demand for low salt products is increasing, and the meat industry continues to look for ways to reduce the warmed over flavor (WOF) in meats caused by lipid oxidation. New ingredients, combinations of ingredients, or the elimination of commonly added ingredients may serve both of these requirements, but at the same time must be determined to be microbiologically "safe".

The experiment described in this thesis was initiated by Steve Goll (1987), Kansas State University, to investigate if antioxidants to retard lipid oxidation would be produced through the Maillard reaction by the addition of glucose, a reducing sugar. Tests measuring TBA values and

consumer acceptability tests were used to assess antioxidant activity. Along with Goll's testing it was determined that microbiological testing would be in order. At least three reasons made this necessary: 1) salt, a preservative, was eliminated from one treatment, 2) glucose, a primary metabolite, was added to three treatments, and 3) there was some evidence that Maillard reaction products also affect the growth of microorganisms (Davidson et al., 1983). This study, therefore, was conducted to establish the safety of the restructuring, and reformulating done in Goll's project.

## LITERATURE REVIEW

This literature review has several purposes. First, the microbiology of meats will be reviewed in general terms. The discussion will then focus on the types of microorganisms that may be present at slaughter, processing or storage, their requirements for growth, and methods of control of these microorganisms. The discussion will also include three specific areas of concern: 1) the effect of hot boning on the microbiology of meats, 2) the microbiology of restructured meats, and 3) the effect of the addition of glucose, phosphate, salt and sugar in meat systems.

### History

Before these issues can be adequately addressed, the reader should be familiar with the historical evolution of food science and in particular, meat microbiology.

Man is a meat eater. Through our evolution we have developed the interest and skills that extend our meat supply beyond the kill. The technological and scientific advances of the last three centuries have enabled us to understand why and how some preservation techniques have worked and others have not. We have also learned to coordinate the sciences of chemistry, biology, and physics to develop new methods of preservation and to ascertain their impact on human health.

There is a microscopic world around us. The soil, the air, and our skin harbor many tiny creatures. Antony Van Leewenhoek was the first to enjoy seeing "animalcules" present in this tiny world when he invented the microscope. Only later was the importance of this tiny world recognized when Louis Pasteur and other scientists saw the connection between these single celled creatures and disease and the preservation of foods.

Before technology, successful methods of food preservation were reestablished through trial and error and chance in combination with the human ability to learn. Our ancestors found that salting or drying meat or fish would preserve it. Fermentation (with back inoculation) could be used to preserve milk, grains or fruits. Some unsuccessful methods met with illness or death. Packing meats in oil probably fostered the growth of what we now know as Staphylococcus aureus (Jensen, 1954). Why methods succeeded or failed was not known until microbiology became an established science.

#### Types of Microorganisms

Three categories of microorganisms have emerged through the scientific examination of our food supply. They are those that are "good", those that are a nuisance in that they cause spoilage, and those that are "bad" or pathogenic.



The "good" microorganisms inhibit the growth of pathogenic or spoilage microorganisms by successful competition and produce by-products that give foods their characteristic flavor and aroma.

The "nuisance" (spoilage) microorganisms bring about undesirable changes in the food product to render it unacceptable to the consumer. Consumption of large enough amounts of some spoilage microorganisms may also cause illness.

Illness is almost a certainty when consumed food is contaminated with "bad" pathogenic microorganisms or their toxins. Much present day microbiology is concerned with the detection and control of pathogens.

Pathogenic, spoilage, or "good" microorganisms may also be classified by their tolerance of or requirements for a certain range of temperature. Microorganisms exist which will tolerate very low to very high temperatures. In our foods three classes are the most important. Psychrotrophs will grow at 0-10C, but may grow best at 15-21C. Mesophiles are limited to the range of 15-45C, this includes 37C--body temperature for most animals. It is where most pathogenic species of microorganisms will be classified. Thermophilic microorganisms grow above 45C and may form spores which survive heat treatments.

### Extrinsic and Intrinsic Parameters

Modern-day microbiologists have learned to control microbial growth by manipulating the "intrinsic" and "extrinsic" parameters by which they grow. Intrinsic parameters for microbial growth are the chemical components of the substrate (foodstuff). At the simplest level the intrinsic parameters are oxygen, water, and a carbon source, but are usually seen at the substrate level as:

1. Nutrient content i.e. carbohydrate, protein, fat, etc.
- 2 pH
3. Oxidation-Reduction Potential (aerobic, anaerobic, or facultative anaerobic)
4. Water activity ( $A_w$ )
5. Natural inhibitors
6. Physical structure of the food

We are able to manipulate the intrinsic parameters somewhat, but most likely the food scientist will manipulate the extrinsic parameters to foster or inhibit growth.

Extrinsic parameters are:

1. Temperature
2. Relative humidity
3. Atmosphere of storage
4. Time in storage

Each species of microorganism, pathogenic, spoilage, or "good" has a unique optimum combination of intrinsic and

extrinsic parameters; conversely, there will be a combination which is least likely to allow growth.

In fermentation, the food microbiologist matches the organisms he desires to grow with an optimum set of parameters to produce a desired set of by-products. Microorganisms are responsible in fermentation for the characteristic aroma, color, texture and flavor of the product. For example, blue cheese is produced by the growth of Streptococcus lactis and Penicillium roqueforti. The characteristic flavor and aroma of Italian hard salami is due to Micrococcus and Lactobacillus or Pediococcus (Smith and Palumbo, 1981). Through fermentation there is an alteration of the intrinsic parameters of the substrate; usually a drop in the pH or a production of inhibitors to other competing microorganisms. Proper fermentation is used to prevent spoilage or pathogenicity.

#### Microbial Growth on Meat

Any food without chemical or microbial inhibitors can spoil. Meats especially have a combination of intrinsic parameters most conducive to growth of microorganisms with the particular balance of air, water, temperature, pH, and nutrients.

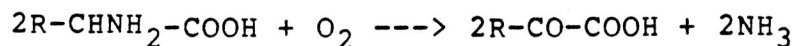
Meat is comprised of about 75% water, 19% protein, 2.5% fat, 1.2% carbohydrate (.1% glycogen, .2% glycoposphates,

.9% lactic acid), .65% ash, and 1.65% soluble nitrogenous substances. The soluble nitrogenous compounds consist of .35% amino acids, .55% creatine and .75% other minor components (ICMSF, 1980). The high percentage of water present in uncured meats enables microorganisms to flourish, as the  $A_w$  is above 0.97. Since water availability does not limit meat spoilage, the pattern of chemical degradation by microorganisms is determined by the ease at which the individual components can be metabolized.

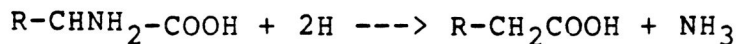
Most microbial meat spoilage begins with the fermentation of carbohydrates. Monosaccharides are metabolized first with glucose as the sugar of choice. Disaccharides and polysaccharides must be transformed by enzymatic action to glucose and then metabolized. Glucose is converted to pyruvate which will then proceed either through oxidation or fermentation. The products of these reactions depend upon the unique enzyme systems of each microorganism.

Of the soluble nitrogenous substances, the amino acids are most readily metabolized by microorganisms. Products that are formed contribute to changes in the character of the meat, both the intrinsic parameters for microbial growth and organoleptic properties. The type of microorganism and its enzyme system, the types of amino acids present, the temperature, and the redox potential will determine the reactions. As outlined by Banwart (1981) these are:

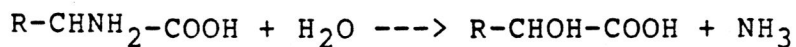
## 1. Oxidative deamination



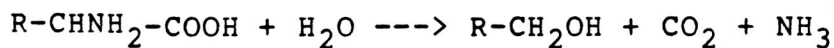
## 2. Reductive deamination



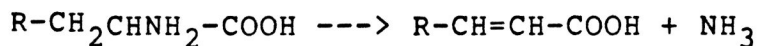
## 3. Hydrolytic deamination



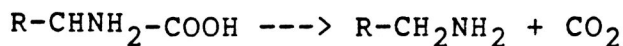
## 4. Hydrolytic deamination and decarboxylation



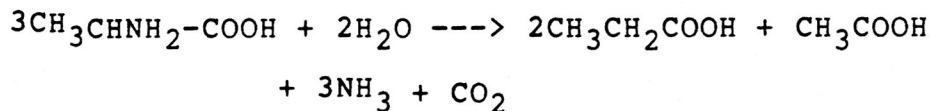
## 5. Deamination and Desaturation



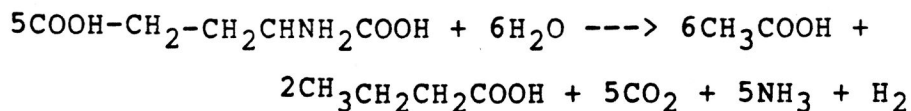
## 6. Decarboxylation



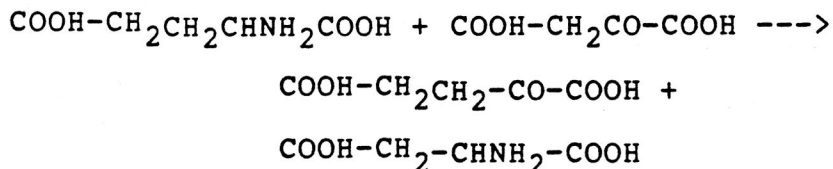
## 7. Oxidation-reduction



## 8. Anaerobic degradation--&gt;release of hydrogen



## 9. Transamination--&gt;alteration of substrate



10. Mutual oxidation and reduction: This occurs when some amino acids serve as hydrogen donors, and other amino acids serve as hydrogen acceptors.

Common to most of these reactions is the release of ammonia

or amines which create the odor associated with putrified meat and also cause an increase in the pH.

Spoilage may also be a function of lipid oxidation. While some oxidative rancidity (the warmed over flavor) will occur in meats, it is the result primarily of autooxidation or the action of tissue lipases. Microbial enzymes play a minor role in lipid oxidation. To breakdown lipids, some water must be available, therefore degradation will occur at the tissue-lipid interface where some of the soluble components of the tissue are provided by damaged blood vessels (Lowry and Gill, 1985). Ground or mixed meats will be more susceptible to the action of microbial enzymes which break down lipids to their component parts. As a result free fatty acids will react with available oxygen to produce hydroperoxides and further to produce volatile carbonyl compounds. Whether by microbial action, tissue enzymes or autooxidation the production of volatile compounds is an objectional symptom of spoilage.

The pH of meat ranges from 5.5 to 7.0 depending on the amount of glycogen at slaughter. The production of lactic acid from glycogen will drop the pH. An animal with little or no glycogen will have an ultimate pH of 7.0. One percent lactic acid will cause a drop in pH to 5.5 (ICMSF, 1980). A pH of 7.0 is neutral and optimum for most bacteria. A pH 5.5 inhibits some species of bacteria but is tolerated by many

spoilage species (e.g. Pseudomonas and Enterobacter). Foods at pH greater than 4.6 are considered to be "low-acid" and at prime risk for spoilage and pathogenicity. Many strict regulations have been designed by the Food and Drug Administration (FDA) and much money is invested each year to educate food processing personnel in proper preservation of these foods.

The growth of aerobic, facultative anaerobic or strict anaerobic bacteria depends upon the oxidation-reduction potential of the substrate. Yeasts may grow and multiply with or without air; molds will require air for growth. Surfaces of meat have more available oxygen and will support the growth of aerobic bacteria. Towards the interior of meat muscle, less and less  $O_2$  is available to the organisms and anaerobic bacteria would be favored. Fortunately for food scientists, meat processors, and consumers, meat muscle from healthy animals is intrinsically sterile. Grinding and mixing of meat alters the redox potential and at the same time distributes microorganisms from the surface to throughout the product. Spoilage of the raw product may then occur rapidly. Cooked meat products may also be affected by the distribution of oxygen and microorganisms.

In the U.S., our meat supply comes from warm-blooded animals. Bacteria from animal intestine, hide, or hooves will be mesophilic, meaning that these microorganisms grow

best at 15-45C, with the most prolific growth at 37C, or body temperature. This group includes most pathogens. Lee et al., (1982a) isolated and identified mesophilic microorganisms from both hot-boned and conventionally processed meat at the time of fabrication. A level of 1.95 CFU/cm<sup>2</sup>, and 1.2 CFU/cm<sup>2</sup> mesophiles was noted for hot-boned and conventionally processed meats, respectively. A mixture of strains were found, including Lactobacillus, Bacillus, Staphylococcus, and Micrococcus among others (Lee et al., 1982a). In the same study, psychrotrophs were enumerated and identified. Hot-boned beef contained an average of .84 CFU/cm<sup>2</sup> and conventionally processed beef supported .72 CFU/cm<sup>2</sup>. These included Micrococcus, Brevibacterium, Moraxella, and Streptococcus (in cold-boned only) (Lee et al., 1982a).

Growth of microorganisms after fabrication depends to a great deal upon temperature, treatment and packaging. At refrigeration temperature where meat is usually stored, psychrotrophs will be favored including Pseudomonas, Streptococcus or Lactobacillus. These organisms are in turn favored or inhibited by the availability of oxygen. Lee et al. (1982a) found that after fourteen days of storage in vacuum packaging Pseudomonas was not detected and that the lactic acid bacteria, Lactobacillus and Streptococcus were favored.



Cooked meats have similar microbiological responses to available air which can be deduced from studies done on packaging. McDaniel et al. (1984) studied the different effects of CO<sub>2</sub>, vacuum packaging, or packaging in a mixture of 15% CO<sub>2</sub>:30% O<sub>2</sub>:55% N<sub>2</sub> on microbial growth of a cooked boneless roast (internal temperature 60C) stored at 4C. Roasts were sampled at 1, 7, 14, and 21 days and enumerated for psychrotrophs and mesophiles. There was no statistical difference at 0 and 7 days, but after 14 days storage the differences between treatments began to surface. Vacuum packaging had significantly higher mesophile and psychrotroph counts. CO<sub>2</sub> storage had the least with mixed air storage bearing intermediate counts. The microbiological significance of the study was two-fold: 1) As the CO<sub>2</sub> content was increased in the packaging treatment, microbial counts were lowered. This relationship indicates that CO<sub>2</sub>, a metabolic by-product of aerobic metabolism, inhibits further growth of aerobic microorganisms and 2) Mesophilic and psychrotrophic counts were statistically the same--possibly indicating the bacteria present could grow at both psychrotrophic and mesophilic temperatures. The identification of microorganisms present was not attempted (McDaniel et al., 1984).

Moisture availability is a primary requirement for bacterial growth. It is more accurately called water

activity ( $A_w$ ) in terms of its effects. It is one of the intrinsic parameters of a food system.  $A_w$  interacts with and is greatly influenced by relative humidity and solute concentration. Water activity can be defined by relative humidity as follows:

$$A_w = \frac{\% \text{ Relative Humidity}}{100} = \frac{P}{P_o}$$

where P = water vapor pressure exerted by the food material

$P_o$  = vapor pressure of pure water at temperature  $T_o$

$T_o$  = equilibrium temperature of system

(Labuza, 1968)

It can also be defined by solute concentration as in Raoult's law:

$$A_w = \frac{\text{Moles of Water}}{\text{Moles of water} + \text{Moles of Solute}}$$

Bone (1973) has noted that not all solutes behave ideally by Raoult's law. Some solutes act as if they are more concentrated and some less. This is measured and reported as "activity coefficient". Sucrose, for example, at a 40% concentration in a 30% moisture food behaves as if it were a 63.6% concentration, or an activity coefficient of less than 1.0. Combinations of solutes may have a synergistic effect, that is "salting in" or they have a subtractive effect, i.e. "salting out".

Salting or drying have long been the methods employed to preserve meats. By lowering the water activity the growth

of pathogens or spoilage microorganisms can be effectively reduced or inhibited. Staphylococcus aureus is perhaps our most salt-tolerant pathogen. It will grow at an  $A_w$  of .84-.92. Salmonella and most other pathogens grow only at greater than .93  $A_w$  (Banwart, 1981). In addition to lowering the  $A_w$  of meats through salting, drying is another method used to lower the  $A_w$  of meats. The cold storage of conventionally processed meat in a low humidity environment dries the surface which is the most common site of contamination. Concern with the microbiological safety of hot processed meats is in part due to lack of this drying time.

Less significant in comparison to water, but also important to the growth of microorganisms are the trace elements present in the substrate. Most abundant in meats are iron, calcium, phosphorus, and magnesium. Of lesser importance are copper, manganese, and zinc.

Microorganisms produce their own chelators which allow easy transport of the necessary minerals through cell walls and membranes. Iron, although bound to hemoglobin and myoglobin in meats, is readily transportable because the microbial chelators are stronger than the porphyrin groups (Brock, 1979).

The fluorescent pigment produced by Pseudomonas syringae in iron deficient media was studied by Torres et

al. (1968) and was found to be a stable Fe (III) complex consisting of an amino acid moiety. This is an example of the mechanism by which microorganisms can act to maximize their nutritional availability of minerals.

Of the mineral elements, magnesium is perhaps most important to microbial growth. It is involved in stabilization of ribosomes, cell membranes, nucleic acids, and enzymatic phosphate transfer. Gram positive organisms require as much as ten times the amount of magnesium for growth as Gram negative organisms (Brock, 1979). Jen and Shelef (1986) used magnesium measurements to evaluate the antimicrobial effects of phosphates in BHI media. Growth inhibition was reversed by the addition of  $Mg^{++}$ , and only partially by  $Fe^{++}$  and  $Ca^{++}$ .

#### Pathogens of Concern in the Meat Supply

The nutritive content of meat is perfect for microbial growth. The organisms most likely to be found in the meat supply will be those intrinsic to the animal or transferred in handling. These include mesophilic and psychrotrophic organisms. Psychrotrophs usually cause spoilage. They grow at 1-10C, but may grow best at 15-21C thus may also be called mesophilic by plate count enumeration.

Fortunately, pathogens do not grow well in refrigerated storage. An exception is the organism Yersinia

enterocolitica. This is a gram negative, non-spore forming motile facultative anaerobic rod. It can be found in animal intestines and lymph nodes, and may be found in meat, seafood, poultry and dairy products. Ingestion of Yersinia enterocolitica will cause gastroenteritis, mesenteric lymphadenitis and terminal ileitis. Yersinia produces a heat stable enterotoxin and a virulence plasmid. Serotypes (0:3, 0:9 and to some degree 0:8) are associated with human outbreaks (Wauters, 1981).

Of most historical concern to the meat microbiologist are the mesophilic pathogens; Salmonella, Staphylococcus aureus, and Clostridium species. Abuse of foods (i.e. holding at temperatures conducive to bacterial growth) may result in their growth and possibly toxin formation. Of new concern to the meat microbiologist are the "emerging" pathogens: Campylobacter jejuni, Listeria monocytogenes, and as mentioned, Yersinia enterocolitica.

Campylobacter is a greater problem to the farmer than the food scientist. This organism causes abortion to the fetus and economic loss. It is the cause of venereal disease in cattle, is a non-sporeforming spirally curved motile rod, gram negative, microaerophilic or anaerobic bacteria. Campylobacter causes gastroenteritis perhaps as often as Salmonella. Pasteurization will destroy this organism (Roberts, 1982).

Listeria monocytogenes has received much attention recently due to outbreaks from dairy products. Listeria is a short gram positive non-sporeforming rod. Infection causes meningoencephalitis and abortion; it is therefore of special concern to pregnant women. Proper pasteurization destroys Listeria (Oosterom et al. 1981).

The non-sporeforming Yersinia enterocolitica, Listeria monocytogenes and Campylobacter jejuni are assumed to occur in small numbers. The small numbers combined with the effectiveness of heat treatment in destroying the bacteria reduces the chance of outbreak.

The heat treatments required to render Clostridium species innocuous are more rigorous. The survival of spores during pasteurization and subsequent mishandling create conditions for germination and outgrowth of these gram-positive spore-forming rods. The two species of concern to the health care professionals are Clostridium botulinum and Clostridium perfringens.

The commercial canning industry is most concerned with Clostridium botulinum because of its heat resistant spores and the production of its virulent toxin. Proper thermal processing techniques have been instrumental in preventing incidences of botulinal intoxication. Unfortunately, C. botulinum may be more of a problem in refrigerated cooked meats than was previously thought. In a recent review by

Simunovic et al. (1985) it was pointed out that Type E and non-proteolytic strains B and F may survive pasteurization, grow and produce toxin at refrigeration temperatures (3-4C) depending upon other conditions. These psychrotrophic strains of Clostridium botulinum are associated with aquatic animals, but even so the relevance to warm-blooded meat sources should not be discounted. Lucke et al. (1981) investigated the behavior of C. botulinum inoculated into meat products. They were looking for the intrinsic and extrinsic parameters involved in control of growth and toxin production. Fermented sausages did not support growth, and toxin formation was prevented through a lowering of  $A_w$  and pH. Perishable liver sausage and bologna supported growth and toxin production at greater than 5C. At 15C growth was rapid (15 days for liver sausage and 5 days for bologna). At 5C, growth and toxin production did not occur until more than 100 days. Under commercial conditions of storage (2-4 weeks) followed by several days in a household refrigerator, Lucke et al. (1981) concluded that no toxin production would occur. They also found that cured hams may indeed be a vehicle for nonproteolytic C. botulinum. In their study, neither curing salts, competition by Enterobacteriaceae, or a wet or dry cure made any difference in toxin formation. Of most concern to the consumer, very little organoleptic changes occurred to signal spoilage--so that consumption and

intoxication would be possible (Lucke et al., 1981).

While Clostridium perfringens is not as important in terms of the consequences of ingestion, it appears frequently enough to be of concern to the food scientist. It is a gram positive spore-forming rod. Spores survive common heating temperatures. Improper holding temperatures following cooking may facilitate sporulation and then growth. Illness is due to ingestion of large numbers of bacteria which form toxins in the intestine. 10% of all the confirmed outbreaks of meat or poultry borne illness from 1968-1977 were due to Clostridium perfringens (Bryan, 1980).

Another organism common to meat-borne intoxication is Staphylococcus aureus. This facultative anaerobe clusters on food and on human skin. S. aureus produces heat stable enterotoxins which when ingested cause vomiting, diarrhea, nausea and abdominal cramps. A food which has been formulated to lower the water activity may, through the inhibition of other organisms, actually select for S. aureus which can tolerate  $A_w$  down to 0.84. As the enterotoxin produced by this organism is heat stable, the only way to prevent intoxication is by prevention of cross contamination. Most intrinsic flora would inhibit growth, but when these bacteria have been reduced by heat treatment S. aureus may easily grow and produce toxin.

Recent serious outbreaks of Salmonella have made this



gram-negative rod a major concern to food microbiologists and food processors. Often mistaken for a viral infection, ingestion of Salmonella causes vomiting, headache, chills, diarrhea and fever. It resides in the animal intestine and may assume a carrier state in some individuals. It may be transferred by cross contamination from these individuals or through the slaughtering procedure. Poultry is noted for the incidence of Salmonella; some estimates place it at 40% of all flocks.

Other organisms implicated in food borne illness are Bacillus cereus, Shigella, Streptococcus, Trichinella, Vibrio parahemolyticus (in seafoods), possibly Aeromonas and the virus, Hepatitis A (Bryan, 1980).

Meat products implicated in food borne illness range from whale meat, pork sausage, smoked ham, genoa salami, and precooked roast beef (Bryan, 1980). Contributing to these reported illnesses were improper cooling of foods, inadequate heating, reinoculation of cooked foods, inadequate reheating, improper sanitation, improper fermentation, slaughtering of unhealthy animals, or feeding animals with tainted grain. Often a combination of these factors will encourage growth of microorganisms. One recent concern is that animals treated with antibiotics may select for antibiotic resistant organisms (Bryan, 1980).

### Meat Spoilage

While pathogenicity is of critical concern to food microbiologists with their dedication to consumer health, spoilage is of great economic concern. It affects the grocer, the distributor, the farmer and ultimately the consumer. By monitoring microbial loads and organoleptic changes in meats on the grocers shelves, microbiologists assess the shelf life of those foods. Much work is done in the meat processing industry to find means of extending the shelf life of meat products. The microbiologist plays an important role in the design of methods, not the least of which is monitoring the effects that any new procedure such as hot-boning or restructuring, will have upon the microbiology of the product.

Indications of spoilage are found not only through culturing and counting microorganisms, but also by changes in color, odor and texture. Lipases formed by microorganisms will result in liberation of free fatty acids and rancidity. Putrefaction of protein to ammonia by microbial proteases result in adverse changes in flavor and odor. Some microorganisms will contribute to color changes in meats; for example, Pseudomonas at refrigeration temperatures produces a fluorescent pigment. Some microorganisms produce polysaccharides and slime, and the breakdown of sugars results in lactic acid accumulation and a drop in pH.

Spoilage at refrigeration temperatures is by psychrotrophs. By far the most important of these are the Pseudomonas. They are ubiquitous in our soil, air, water, and will be found in meat processing facilities. To control growth of psychrotrophs, environmental conditions must be altered to ensure sanitation of slaughter room and crews, temperature kept below or above rapid growth temperatures (<10C or >50C), and proper processing and packaging to prevent recontamination.

In a mixed meat system (ground or restructured meats), it is possible for the food scientist to reformulate the environment to control microorganisms. The intrinsic and extrinsic parameters affect which organisms grow. Environmental changes, such as decrease in pH, change in atmosphere, and production of metabolites, may result from their growth. When the intrinsic parameters become unacceptable for growth of the microorganisms which created the condition, another genus may take over and grow. This is called microbial succession.

Intrinsic parameters of processed meat microbiology are in part determined by the method of slaughter and processing. In a conventionally processed carcass, a side of beef is initially chilled for 48 hours. The surface of the meat is allowed to dry somewhat to protect it from microbial proliferation. The microbial profile depends upon the

quality of equipment sanitation and the presence of contamination from the skin and intestinal tract (Nottingham, 1982; Lechowich, 1971; Jensen, 1954).

### Hot-Processing

Hot-boning of meat presents a new set of challenges to the processor. The pH is higher and the warm temperature during processing results in a redistribution of the mesophilic flora from the intestine, the hide, and the hooves. The temperature and pH also facilitate growth of microorganisms from the environment, the equipment, and crew.

Conventionally processed meat is usually chilled in a -1C cooler for 48 hours. The carcass is then transferred to a holding cooler of 0C for 48 hours. After which it is transferred to a boning line where it is boned, trimmed, packaged, and boxed, or otherwise processed. Following fabrication, raw meat is held in a shipping cooler (-2C) for 48 hours (Erickson et al., 1980).

Fung et al. (1980) determined that through conventional processing the microbial loads of psychrotrophs and mesophiles are essentially equal. In one experiment, after storage of conventionally processed meats at refrigeration temperature and vacuum packaging, they found that mesophiles and psychrotrophs measured Log 4.64 CFU/cm<sup>2</sup> and Log 4.43

CFU/cm<sup>2</sup>, respectively. At zero time Fung found very low levels of both mesophiles and psychrotrophs (log 0-2).

Similar results occurred with hot-boned meat, although Fung et al. (1980) found significantly higher counts of both types after storage. There were Log 5.26 CFU/cm<sup>2</sup> and Log 5.5 CFU/cm<sup>2</sup> for mesophiles and psychrotrophs, respectively, in one experiment and Log 6.62 CFU/cm<sup>2</sup> and Log 6.61 CFU/cm<sup>2</sup> in another experiment.

It can be concluded from these studies that 1) conditions are slightly more favorable for microbial proliferation in hot-processing and 2) there is an overlap between these two classes of organisms (similar mesophilic and psychrotrophic counts), and 3) conditions are slightly more favorable for psychrotrophs at refrigerator storage (Fung et al., 1980).

Hot-processing, as its name implies, describes a process where the cooler storage stage of fabrication is eliminated. From slaughter, the carcass goes either to a conditioning room for 8 hours (15C) or through electrical stimulation directly to the boning line, then to processing and packaging. The economic savings can be substantial, not only by reducing refrigeration requirements, but also by speeding the rate of product turnover (Kastner, 1982).

Primary to the safety of hot-processed meats is a rapid cooling time. Fung et al. (1981) compared internal and

external portions of hot-boned beef to the same portions of conventionally processed beef. The hot-processed cuts were microbiologically safe when cooled to 21C within 9 hours, while those chilled to 21C after 12 hours had high counts of microorganisms. Critical to the safety of the product and the proliferation of bacteria was the "C-h" (degrees above 21C and time to reach 21C). Differences in curves (temperature versus time) of the variations were shown to be related to the initial temperature. This study also demonstrated a higher initial contamination of mesophiles. Lee et al. (1985) studied time versus temperature with the same results and expanded the study to include the microbial profile. This study revealed that slower chilling resulted in greater numbers of lactic acid and saccharolytic bacteria.

The establishment of criteria for hot-processing (i.e. sanitation, chilling, packaging, and storage) will result in a safe meat product. The concentration of studies has been on whole muscle tissues: steaks and roasts. Little work has been done with restructured meats.

### Restructured Meats

Restructuring has been pursued recently as a method for economic expansion in the food industry. Restructured foods, e.g. chicken nuggets, restructured steaks, etc., have

generally been limited to industrial or fastfood settings (Field, 1982). Little microbiological research has been published. In fact, when outlining long term research needs, John L. Secrist of the U.S. Army Natick Research and Development Laboratories only mentions the microbial aspects once in regards to reducing sodium content (Secrist, 1982).

Unlike whole steaks or roasts where the interior of the product is essentially sterile, restructured meats contain microorganisms throughout as a result of handling, contaminated equipment, and contaminated ingredients. Some ingredients, e.g. nitrite and/or salt, will serve to inhibit growth of microorganisms. Other such as sugar or the incorporation of air into the product ingredients may increase the possibilities for growth. A recent concern has been the use of hot-boned carcasses in restructured meats because they may have a slightly higher microbial load than conventionally processed meats. If the restructured product is to be precooked, then the higher mesophilic (or thermoduric) counts of hot-boned meats may make a significant difference in the shelf life of the product.

Newsome et al. (1986) compared raw restructured steaks from hot-boned and cold-boned steers. These were studied over a period of 1 week for non-vacuum packaged steaks and 1,3, and 6 weeks for vacuum packaged steaks. Salt and an antioxidant were the only ingredients added to the

restructured steaks. Microbial counts were higher in restructured steaks for both hot and cold-boned steers.

Enteric, aerobic, psychrotrophic, anaerobic, lactobacilli, and staphylococci were enumerated. Except for enteric and staphylococci, all counts were higher for hot-boned meats, whether restructured or not. With vacuum packaging, only anaerobic and lactobacilli were found after one week and throughout the storage period.

The development of any new food product requires extensive testing for shelf-life limits and potential pathogens. A restructured, precooked product may have the most promising future, since heating eliminates most organisms. Shelf life would be dependent upon the temperature of cooking, subsequent handling, the inhibition of organoleptic changes in the product, and the choice of additives.

While nitrite is commonly added for the prevention of microbial growth, the organoleptic changes are undesirable for a roast beef type restructured product. It will not be discussed here except to say that it has been quite successful at the legal levels in eradicating the incidence of Clostridium botulinum toxin formation in cured meats.

### Sodium

Salt has long been used as a preservative which is



perhaps why the taste for it is so widely established throughout the world. The elemental composition was fully established by the early nineteenth century. For a good part of that century it was generally believed that sodium was needed to balance potassium intake (Meenely, 1973). Sodium occurs naturally in drinking water and in all foods. In addition, the food processor may add salt, or the consumer may add it in cooking or at the table. The trend in recent years is to reduce salt consumption. This is based on public interest in health and the awareness that sodium intake is associated with hypertension. This awareness is not new. In 1904, work by Ambard and Beaujard, as cited by Meenely (1973) associated the sodium factor with high blood pressure. By the 1940's therapeutic use of lowered salt was established as a remedy for congestive heart failure. Today, this association is firmly established (Meenely, 1973).

Food processors have responded to a health conscious public by developing foods lower in salt, and the FDA has responded by targeting a date for salt declarations on labels. Processing meats with less salt is not an easy matter. A long history of salt dependence must be overcome. Not only is salt in processed meats necessary for binding, texture, and flavor, but it has a tremendous impact on the microbial safety of the product.

Salt concentration in the water component of meat

causes a reduction in the water activity which slows or totally inhibits the growth of many bacteria. General inferences about salt in foods have been outlined by Sofos (1983), including the types of organisms affected, the effects of varying levels of salt (high and low) and the effects of food composition. Most pathogens will not grow at lowered  $A_w$ . Staphylococcus is the most tolerant of the pathogenic bacteria to a low  $A_w$  (.84-.92). Salt is not the only determinate of  $A_w$  but interacts with any water binding substance present including carbohydrate and protein (Sofos, 1983).

Besides lowering the water activity, salt acts to reduce pH in muscle tissue. The chloride ions bind tightly to positively charged groups on the protein molecule, while sodium ions only weakly bond to negatively charged groups. The net result is a shift downward of the isoelectric point of the molecule (Pedersen, 1960). This "anion effect" changes the water holding capacity of the meat, but may also have significant antimicrobial implications.

NaCl is not the only form of sodium in the diet. Also included are sodium nitrate, sodium nitrite, sodium ascorbate, and isoascorbate, sodium phosphates, sodium bicarbonate, sodium citrate, monosodium glutamate, sodium benzoate, and sodium propionate. Factors related to salt content in regards to shelf-safety are as follows:

NaCl concentration  
Type of microorganisms  
Number of microorganisms  
Type of food or medium  
Substrate pH  
Other salts  
Sodium Nitrite  
Other preservatives  
Heat treatment  
Storage temperature  
Length of storage  
Storage atmosphere

(Sofos, 1983)

When searching for substitutions or reduction of salt, all of these factors must be considered.

Barbut et al. (1986) formulated mechanically deboned turkey meat with varying levels of salt and three types of phosphate (at a constant nitrite level) and inoculated it with C. botulinum. He found that increasing salt levels reduced toxin production. The addition of hexametaphosphate had no effect on toxin production, sodium tripolyphosphate allowed toxin formation, and sodium acid pyrophosphate delayed it. The effects might be entirely due to the combinations of pH (different for the three phosphate

components) and salt levels. Madril and Sofos (1986) saw similar results when they studied the effect of pH and reduced salt along with the addition of sodium acid pyrophosphate. They found an increase in the safety (lowered microbiological counts) at lowered pH with all salt levels - showing the synergistic effect between these two aspects. And while sodium acid pyrophosphate holds the pH steady and low, Madril and Sofos (1986) hypothesized that it may in other ways have antimicrobial properties--so that salt reduction and sodium acid pyrophosphate addition may be a viable combination in comminuted meat systems.

Natural means have been suggested when looking for alternatives to salt addition. Marcy et al. (1985) reduced salt levels in a fermented sausage from 3.3% (minimum required by the USDA) to 2.48% and to 1.65%. Growth of Staphylococcus aureus is inhibited by lactic acid produced by starter cultures, with the greatest inhibition at the lowest level of salt. Increasing salt up to 10% allows the growth of S. aureus. Low levels of salt (1.65%) in a product fermented by lactic acid bacteria produced a safer product as compared to one of a higher salt content.

In a cured meat product, Whiting et al. (1984; 1985) found that salt levels under 2.5% had no effect on the formation of Clostridium botulinum toxin or the enterotoxin of Staphylococcus aureus. The growth and toxin formation of

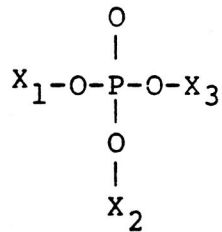
these two species was measurably sensitive to nitrite, ascorbate, and temperature abuse.

Refrigeration is commonly thought to prevent growth of pathogenic bacteria. Stern et al. (1980) conducted tests at refrigeration and room temperature to see if Yersinia enterocolitica could be controlled by pH and salt addition. Yersinia could grow at up to 7% salt, and between pH 4.6 and pH 9.0, and will survive in more acid foods (Stern and Pierson, 1979). It is seldom targeted as a problem in meat-borne illness as compared to Salmonella or Staphylococcus aureus.

Campylobacter jejuni is sensitive to salt concentration, but the effects are temperature dependent. Doyle and Roman (1981) conducted tests with C. jejuni in brucella media at 4, 25, and 42C and at varying sodium chloride (NaCl) levels. Salt concentration of at least .5% at all temperatures was necessary for growth. At 42C greatest tolerance of increasing NaCl concentration was noted. At 25C and 4C, NaCl concentration above 1% was detrimental to growth. However the death rate was greatly increased at 25C compared to 4C.

Phosphate

Phosphates have recently been investigated as a substitute for part of the salt in meat. The maximum legal limit is .5%. Phosphates come in many forms, the simplest the ortho-phosphate:



Adding the orthophosphate groups together gives either pyrophosphate (two groups), tripolyphosphate (three groups) or metaphosphate (many groups with an average of thirteen).  $\text{X}_1$ ,  $\text{X}_2$ , and  $\text{X}_3$  may be filled by Na, K, or H to give the various phosphates approved in meats:

Orthophosphates

monosodium phosphate  
monopotassium phosphate  
disodium phosphate  
dipotassium phosphate

Pyrophosphates

sodium acid pyrophosphate  
tetra sodium pyrophosphate  
tetra potassium pyrophosphate

Tripolyphosphates

sodium tripolyphosphate

potassium tripolyphosphate

Metaphosphates

sodium hexametaphosphate

(Sofos, 1986)

Phosphates are involved in water binding, color, inhibition of rancidity, texture, leavening, coagulation, emulsification, curing, handling, buffering, and sequestering. In fresh meats the long chain phosphates will be hydrolyzed to the orthophosphate form by phosphatase enzymes (Sofos, 1986). The greater number of ionic groups relative to the size of the molecule in orthophosphates makes it the best buffer of all phosphate types.

Antimicrobial effects of phosphates may be related to the sequestering ability which may also affect the extent of oxidative rancidity. Free  $Mg^{++}$  concentration in the substrate is reduced by phosphate (as are Ca, Fe, and Cu ions) which tends to make  $Mg^{++}$  the limiting factor in microbial growth. On the contrary, the chelation of some mineral elements may accentuate the availability of these elements to the microorganism. In fact some microorganisms produce their own chelators to serve as a mechanism to pass minerals through cell membranes (Sofos, 1986). The basis for antimicrobial activity of phosphates is still largely uncertain. Research on each meat product is still necessary

and may show a synergistic or additive effect between product components.

The effect on toxin production by C. botulinum produced by various phosphates in turkey meat emulsions was studied by Barbut et al. (1986). Results show sodium acid pyrophosphate delayed production of toxin, while sodium hexametaphosphate had no effect and sodium tripolyphosphate accelerated production of toxin. The action of sodium acid pyrophosphate is due in part to the reduced pH, but also due to other reactions. Other tests show that sodium acid pyrophosphate had antimicrobial effects independent of pH, although there is an interaction between salt levels and pH (Madril and Sofos, 1986). Mechanism for the antimicrobial action of sodium acid pyrophosphate may be attributed to the large number of anionic groups, and to its hydrolysis in thermal processing to orthophosphate.

Chelating agents (phosphates included) have a mixed response on the growth of microorganisms in general and may vary from species to species, and between chelators. The effects are due to both the strength of the chelator-mineral bond, the strength of microbial chelators, the ability of the microorganism to transport chelated minerals, and the toxicity of the ionic form of the mineral.

In meats, iron is bound to heme groups which in theory could make it unavailable to microorganisms. In reality some



microorganisms, specifically Pseudomonas syringae, produce siderophores which have a greater affinity for iron than hemoglobin or myoglobin (Torres et al. 1986).

The toxicity of filtered sea water was reversed by the addition of chelating agents (EDTA, hydroxyquinilone, etc.) and metal complexing agents ( $\text{Na}_2$  NaCN, etc.). When Escherichia coli was grown in media prepared with seawater, the addition of  $\text{Na}_2$  EDTA significantly reduced the lag phase attributed to toxic amounts of copper and other ions (Jones, 1964).

Where Jones found that chelating agents in seawater reduce the toxicity of heavy metals to the microorganism, Lankford et al. (1957) studied many compounds that had a stimulatory effect on the growth of Bacillus globigii and found the common denominator to be their metal chelating potential. Some of these were sugar-phosphate products formed by autoclaving the monosaccharide and two potassium phosphates. Neither compound alone had this response. Bacillus subtilis var. niger needs a balance between chelators and manganese for the initiation of growth. The addition of chelators increased lag time at pH above or below but not at pH 7.0 (Mayer and Traxler, 1962).

That chelating activity is pH dependent was also demonstrated by Jen and Shelef (1986). Chelators become increasingly dissociated at alkaline pH and their highly

anionic nature serves to attract more cations. As these cations ( $Mg^{++}$ ,  $Ca^{++}$ , and  $Fe^{++}$ ) become unavailable to the microorganism, initiation and growth are hindered. Using Staphylococcus aureus as the test organism, they found that the longer chain phosphates are more inhibitory to growth. This contradicts Barbut's findings on C. botulinum (Barbut et al., 1986). Differences may be due to differing requirements between organisms, the inability to produce microbial chelators, or the ease/difficulty in transporting chelated ions across cell membranes.

One of Jen and Shelef's (1986) findings could be important to the food processor concerned with the development of toxins by S. aureus. When initial counts of microorganisms were low (Log 4 CFU/ml), after 24 hours incubation no growth was detected in broth containing sodium tripolyphosphate, or sodium hexametaphosphate. When initial populations were high (above Log 4 CFU/ml) a 100 to 1,000 decline in numbers occurred after 24 hours at 35C. If these findings are transferred to a food system, phosphates are definitely indicated to provide additional safety from food borne intoxication.

Phosphates have little effect upon Salmonella in poultry products at the .5% legal limit (Foster and Mead, 1976). Scientists are investigating the use of phosphates in the processing steps of poultry. Use of 8% commercial

phosphate blend in the cooling water resulted in lowered bacterial counts, due to reduced lipolytic type organisms. The spoilage indicators, slime and odor, were significantly less noticeable in carcasses dipped in phosphate treated cooling water (Steinhauer and Banwart, 1964). Contradictory results were found by Thomson et al. (1979) in their study. Addition of 6% phosphate in cooling water had no effect on survival of Salmonella, but did reduce the rancidity of the carcasses. Temperatures above 70C caused destruction of microorganisms. It may be that agitation of polyphosphates (and hydrolysis) in warm water could be the cause of reduced antibacterial effectiveness, or that the added phosphates had a positive or neutral effect on growth as in Barbut et al. (1986) studies. Analysis of the water following processing might have offered an explanation.

Some microorganisms may only be stressed by the same temperatures that destroy Salmonella. Firstenberg-Eden et al. (1981) studied stressed and unstressed Moraxella-Acinetobacter cells and the effects of salt and phosphates. Unstressed cells were sensitive to the following phosphates in descending order: sodium tripolyphosphate, sodium pyrophosphate and then sodium orthophosphate. Filter sterilized phosphates were more inhibitory than heat sterilized phosphates. NaCl and sodium tripolyphosphate combinations were additive. NaCl plus sodium pyrophosphate

combinations were synergistic in their inhibition. Cells inoculated into meat and heated to an internal temperature of 70C were inhibited to a slight degree by the maximum concentration of the two phosphates, sodium tripolyphosphate, and sodium acid pyrophosphate. When salt alone (.80%) or salt plus sodium tripolyphosphate (0.03%) was added, there was a dramatic decrease in cell recovery. (Firstenberg-Eden, 1981)

Molins et al. (1984) monitored the inhibitory effects of phosphates in media inoculated with Salmonella typhimurium, Pseudomonas aeruginosa, and Staphylococcus aureus. Sodium acid pyrophosphate had no effect or enhanced recovery whether autoclaved, heated with the media, or added aseptically after sterilization. Of all other phosphates tested, greatest inhibition was found with unheated tetra sodium polyphosphate and slightly less inhibition with heated tetra sodium polyphosphate. Sodium phosphate glassy and sodium tripolyphosphate showed some inhibition which lessened upon heating.

High temperature has a hydrolytic effect upon polyphosphates which may change their antimicrobial activity in food systems. What about uncooked refrigerated meats formulated with phosphates? Will phosphates act in the same manner as the filter sterilized unhydrolyzed polyphosphates? This question was approached by Molins et al. (1986). Their

results show that survivor curves were not affected significantly by any phosphate, although sodium acid pyrophosphate and sodium tripolyphosphate increased the lag phase of mesophilic microorganisms and sodium acid pyrophosphate slightly reduced numbers of psychrotrophs at the end of a seven day storage period. The reason for the lack of difference can be explained by the presence of naturally occurring phosphatases in meat. These phosphatases produce an effect similar to hydrolysis by heat sterilization.

The effects of phosphates in meat are interrelated to other additives in the system, the most important of which is salt. Recent attempts to lower salt content has focused on the synergistic properties of salt plus phosphate in the hope of creating a product with all the organoleptic properties and shelf-stability of a highly salted product.

Although, sodium tripolyphosphate appeared to be the phosphate with the most promising outlook. Sofos (1985) found evidence to the contrary. Sofos (1985) used various levels of salt with .38% sodium tripolyphosphate in meat batters inoculated with Clostridium sporogenes either prior to or after processing to 70C. Sodium tripolyphosphate did not extend the shelf life of the meat batters at any level of salt.

### Sugar

The search for a combination of ingredients which could approximate the protein solubilization effect of salt has led to investigation of sugar. It has no legal limit and it is generally acceptable to the consumer. The simple monosaccharide glucose is the easiest sugar to metabolize, and in microbiology it is the standard by which other sugars are compared.

Since glucose is preferentially metabolized, the presence of this chemical will delay the use of other nutrients. In meats, the addition of glucose and the action of microorganisms will bring about the production of lactic acid and reduced pH. The safety of fermented sausages relies on this phenomenon as it inhibits spoilage and growth of pathogenic bacteria (Smith and Palumbo, 1981).

Some recent studies have used glucose as an addition to non-fermented meats. Shelef (1977) found that the addition of 2-10% glucose dropped the pH of meat from 5.8 to between 5.0-5.2. When the glucose was depleted, metabolism of amino acids occurred which eventually brought the pH back up to 6.0. The succession of metabolism from glucose to the nitrogen containing nutrients was not due to a bacterial shift, other than a slight suppression of non-pigmented pseudomonads. The metabolic succession resulted in a prolonged shelf-life at refrigeration temperatures. Other

studies showed similar results with less glucose (2%) (Barua and Shelef, 1980).

Cured meat products must contain nitrite to control botulinal toxin formation. Sugar and starter cultures added to bacon increase the effectiveness of nitrite. Tanaka et al. (1985) formulated bacon with 40 or 80 ppm nitrite, lactic acid bacteria, and sucrose. These were also inoculated with C. botulinum and monitored for toxins. They found that conversion of sugar to lactic acid reduces pH thereby rendering nitrite more effective in controlling botulinal toxin formation.

The microbiological impact of sugar in meat products is variable. Vanderzant et al. (1983) adjusted the pH of normal steaks and dark firm and dry (DFD- high pH) steaks with citric acid and citric-lactic acid. No extension was noted on the shelf-life of any of these steaks. Possibly results are dependent upon microflora present. An inoculation of lactic acid bacteria may have been necessary to extend the shelf-life of the product (Vanderzant et al., 1983).

These studies on meat formulations would not be complete without a discussion of phosphate and glucose when combined in a meat product. Nielsen and Zeuthen (1983) used a bologna type sausage formulated with and without a low pH phosphate blend and sodium tripolyphosphate. Each treatment was formulated with or without glucose. Glucose alone stimulated growth of Brochothrix thermospacta, Serratia

liquefaciens, and lactic acid bacteria. Of the phosphates, only the low pH blend was an effective inhibitor of the two aforementioned bacteria. Lactic acid bacteria were not affected.

The interactions between sugar and other additives have a variable effect; the general trend is that glucose acts as a primary metabolite except when the metabolic functions of the microorganisms are disrupted. This may be due to injury in processing, chelation of crucial minerals, or a lowering of pH through chemical addition. Effects vary by microorganisms; some are more sensitive to environmental changes, and some microorganisms may be selected for by the relative ease of glucose metabolism.

Sugar in combination with processing variations has also been assessed. Irradiation is now under scrutiny as an antimicrobial process for foods. It is not as successful as hoped in controlling toxin formation when combined with sugar. Huhtanen et al. (1986) found that when fermenting microorganisms are injured by irradiation, they are unable to convert sugar to lactic acid as in the experiments of Tanaka et al. (1985). When not irradiated, the fermenting microorganisms were able to drop the pH for successful inhibition of botulinal toxin formation.

Processing affects the microbial system due to cell injury. Thermal processing may create another variable, i.e.



the development of heat induced anti-microbials.

### Non-Enzymatic Browning Products

Non-enzymatic browning (Maillard reaction) products are formed when an amino derivative and a reducing sugar are allowed to react. The reaction is influenced by pH, temperature, time, humidity, and the concentration of the reacting compounds.

The ease at which a sugar reacts is related to its ability to go to the reducible form from the ring form. Of the hexoses, D-galactose, D-mannose, and D-glucose exhibit decreasing ring opening and hence, browning. Sucrose, if hydrolyzed, may also participate in the reaction. Of the amino groups, lysine is the preferred amino acid for reaction (Saltmarch and Labuza, 1982).

Maillard reaction products are formed with greater ease as the pH is increased. As the pH rises above the isoelectric point of the protein more amino groups of lysine are exposed and available. Lysine is the primary target of the reaction and the rate of browning is often measured as loss of lysine. As the epsilon amino group of lysine is bound to sugar, the pH will drop (Saltmarch and Labuza, 1982).

Water plays a significant part in the ease of the reaction. High amounts of water dilute the reacting species

and inhibit the rate of browning. The most likely reason for the ease of browning at the intermediate range ( $A_w$  .60-.80) of moisture is due to a balance in the amount of water-- enough to dissolve the reacting species, but not enough to dilute it. Similarly, humectants may dissolve the reacting species at a lower water activity thereby facilitating the reaction, or sulfites may tie up the sugar thereby preventing the reaction (Saltmarch and Labuza, 1982).

Temperature plays an important part in the development of browning products. At storage temperatures, secondary products formed are L-dicarbonyls, reductions, furfural, hydroxy-methyl furfural, and dehydroreductones. Through the Strecker degradation, increases in processing temperatures will produce sugar fragmentation products and pyrazines (typical sources of aroma changes). All of these products will further react to produce nitrogen containing or nitrogen free brown polymers. These may be water soluble (premelanoidins), or insoluble (melanoidins) (Finot, 1982).

Oxygen availability also influences the production of non-enzymatic browning products. It influences degradation of lipids and the production of carbonyl reducing compounds which may serve as intermediary reactants (Saltmarch and Labuza, 1982).

Loss of lysine may result in reduced protein efficiency ratio (PER) and low level toxicity for mammalian systems

(Lee et al., 1982b). Effects upon microorganisms may depend upon the loss of amino acids or sugars, the intermediate products formed, or the end-products formed.

Some research has been conducted on the effect of Maillard reaction products on the lipid oxidation. The L-dicarbonyls, reductones and dehydroreductone intermediate products act as antioxidants and are also assumed to act as antimicrobials due to the sequestering of metal ions.

The difficulty in studying the effects of Maillard reaction products in microbiology is due to the great variety of products, the volatile nature of the low-molecular weight compounds (Walter and Fagerson, 1968) and the complexity of the melanoidins. Because of the nature of the products, the route to studying their effects has been limited to measuring the initial reactants, heating and then measuring the effect on microorganisms. These studies must be done in media.

Jemmali (1969) is noted for the original research of Maillard reaction products on the growth of intestinal bacteria. This research was begun not to assess shelf-stability of foods, but rather to enlighten animal scientists on the metabolic effects of browning products on the microflora in the gut of the animal. The initial reacting compounds were dextrose (glucose) and glycine. Solutions were heated for varying times and then added to

filter-sterilized media which were inoculated with three strains of Lactobacillus and Escherichia coli. After eight hours of heating, the Maillard reaction products increased the rate of growth in all bacteria, shortened the lag phase in Lactobacillus and increased the lag phase of E. coli.

Other studies have shown inhibition of microflora by browning products. Einarsson et al., (1983) studied bacterial growth in the presence of the reaction products of arginine plus xylose and histidine plus glucose. The resultant products were separated into low and high molecular weight fractions and added to media for growth of E. coli, Salmonella seftenberg, Staphylococcus aureus, Pseudomonas fragi, Pseudomonas fluorescens, Lactobacillus plantarum, and Bacillus subtilis. High molecular weight products had a greater inhibitory effect measured in a longer lag phase. The greatest inhibition was seen in B. subtilis. The two Pseudomonas species died off eventually at four parts per million of Maillard reaction products. The other species exhibited eventual growth. The authors attempted to assess the minimum inhibitory concentration (MIC) of the Maillard reaction products. MIC values varied by bacterial species and by initial reacting amino acid plus sugar (Einarsson et al., 1983).

Other work was done by Field and Lichstein in (1958). They studied the effect of filter sterilized glucose,

autoclaved glucose and glucose plus a variety of amino acids on the CO<sub>2</sub> requirement of Propionibacterium. The filter-sterilized glucose control had no growth, and the autoclaved glucose control exhibited growth with an optical density value at 650 nanometers of 39. Other combinations except casein hydrolysate plus glucose with no phosphate had optical densities of greater than 39 nanometers. The casein hydrolysate + glucose exhibited no growth even after 54 hours. The effect of autoclaved glucose could be approximated by adding CO<sub>2</sub>, sodium bicarbonate, several dicarboxylic acids, asparagine or glutamine. The degree of browning was deemed irrelevant (Field and Lichstein, 1958).

Early work on sterilized media was done by Lewis (1930). Sterilized media containing reducing sugars and sodium ammonium phosphate inhibited ammonia utilization by Phytomonas malvaceara. Aseptically added glucose was not inhibitory, nor were potassium salts when added. Extent of inhibition was proportional to the amount of glucose in the media prior to sterilization. 0.2% ammonium phosphate was the minimum amount required for any inhibitory effect. Alkaline pH and high temperature were both factors. Lewis (1930) concluded that alkaline conditions maintained by the phosphate influence the dissociation of sugar in order that it may produce unassimilable forms of nitrogen (Maillard reaction products).

Maltol has been isolated as a browning reaction product that inhibits growth of Lactobacillus casei. It may interfere with the metabolic uptake of amino acids or peptides. This seems likely as the addition of excess glutamic acid (a metabolic intermediary) overcomes inhibition (Banerjee et al., 1980).

The relationship between metabolism in the organism and the Maillard reaction products is further emphasized by Horn et al. (1968). The model compound, 1-deoxy-1-methionino-D-fructose was exchanged for methionine in media used for the growth of Leuconostoc. It was also fed to rats. Both were assayed for growth potential. Rats were totally unable to use this form of methionine, but L. mesenteroides demonstrated 74 percent of the growth previously demonstrated on an equimolar amount of methionine. When the compound was hydrolyzed to yield methionine, growth resumed to 94% of the control. These studies were performed to find a rapid diagnostic system using L. mesenteroides for amino acid availability. They were unsuccessful in this endeavor, but demonstrate that L. mesenteroides (and possibly other microorganisms) have the necessary enzyme systems to split some Maillard reaction compounds.

Another form of inhibition is demonstrated by Malkki (1964). Heated solutions especially glucosamine and kojic acid inhibited growth and germination of C. sporogenes. This

effect was increased when copper salts were added. Reversal of the effect could be accomplished by addition of zinc, nickel, chromium, and cobalt salts. Purine and pyrimidine bases, 2-deoxy-2-ribose, and peptones could reverse toxicity. Apparently glucosamine and kojic acid interfere in metabolism or act as sequestering agents to make minerals unavailable for use as co-factors.

Microbial inhibition has been attributed to the following compounds of the Maillard reaction which have been isolated: catechol, 3-methylcatechol, 4-methylcatechol, and methyl-hydroquinone and all phenolics. The soluble fraction of Maillard reaction products (individual components not identified) was determined to be inhibitory to S. aureus, B. subtilis, E. coli, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida utilis, and to a lesser degree Aspergillus niger (Kato and Shibasaki, 1974).

### Summary

The subject of meat microbiology is complicated due to the nature of the product. Intrinsic and extrinsic parameters must be assessed before trying to control the growth of microorganisms. The microbiology will vary by the health and treatment of the animal prior to slaughter; which processing method is used, hot boning or cold boning; which microorganisms are initially present; how the meat product

is fabricated; and whether the product is raw, cooked or cured.

Control of growth will be determined by the temperature of storage, the atmosphere of storage, the length of time in storage, and if thermally processed, by the final internal temperature. Meat scientists and microbiologists must work together to find an optimum set of conditions to achieve a consumer acceptable, shelf-stable product.

The reduction of salt in the American diet has created new challenges. When investigating possible substitutions for NaCl, meat scientists may include phosphates and sugars into meat products. By chelating metal ions, phosphates do two things: 1) reduce lipid oxidation, and 2) inhibit microbial growth. Phosphates increase water holding capacity and buffer the system at a pH particular to the phosphate, thereby affecting microbial growth.

Sugars in a meat system serve two functions. First, in the pure form they are preferentially metabolized by microorganisms which convert them to lactic acid and drop the pH to extend the shelf-life. Second, reducing sugars react with the amino groups of free amino acids or with the epsilon amino group of lysine to produce the many reaction products of non-enzymatic browning. The intermediate products of this reaction will act as sequestering agents and antioxidants. Depending upon the microbial systems of



each species, these compounds will inhibit growth to a lesser or greater degree.

There are two ways that Maillard reaction compounds can limit growth of microorganisms. Either they reduce essential amino acids during the reaction or they sequester mineral ions which are necessary to cell function. The degree of inhibition depends in part upon the presence of enzymes which split reaction products. Most if not all of the research to date has been in media due to the difficulties in isolating reaction products. Research needs to be done to assess the antimicrobial value of Maillard reaction products in food systems such as meat products where the limiting reactant will be the sugar and not the amino component of the reaction.

MATERIALS AND METHODS

## 1. Meat Processing

Seven Holstein steers were conventionally slaughtered at the Department of Animal Sciences and Industry facility. After slaughter, 1/2 of each carcass was hot-boned within 2 hrs postmortem. The other half of the carcass was chilled conventionally at approximately 3C and cold-boned at 48 hrs postmortem. Of these steers, only meat from 13 sides was used in the experiment; 6 hot-boned and 7 cold-boned sides.

Following the boning operation, the fat and connective tissue was removed from the major muscles of the round, loin, rib and chuck. The meat was blade tenderized and then ground: 90% through a 1" plate and 10% through a 1/8" plate.

The 2 sizes of meat chunks were then mixed together in the 90% : 10% ratio to give 4 10 kg batches. Each batch was blended with a different set of ingredients, resulting in 4 different treatments. The ingredient treatments were:

Salt, Phosphate and Glucose (SPG)

4% H<sub>2</sub>O  
 2% NaCl  
 .5% Lem-O-Fos® (a commercial blend of sodium tripolyphosphate and lemon juice)  
 2% Glucose

Salt and Phosphate (SP)

4% H<sub>2</sub>O  
 2% NaCl  
 .5% Lem-O-Fos®

Salt and Glucose (SG)

4% H<sub>2</sub>O  
 2% NaCl  
 2% Glucose

Phosphate and Glucose (PG)

4% H<sub>2</sub>O  
 .5% Lem-O-Fos®  
 2% Glucose

The water used in the formulation was city water from the city of Manhattan, Kansas. The NaCl was obtained from Fisher Scientific, catalog #S-671. The Lem-O-Fos® blend of sodium tripolyphosphate and lemon juice was obtained from Stauffer Chemicals, Westport, Connecticut. Glucose (dextrose) was obtained from ADM Corn Sweeteners, Clinton, Iowa.

Water was common to all treatments. The SPG treatment contained all three ingredients and is the "control" in the experiment. Each of the other 3 treatments lacked one ingredient. It is helpful to see and evaluate the treatments as follows:

<u>Treatment</u>	<u>Additive Missing</u>
SPG	None
SP	Glucose
SG	Phosphate
PG	NaCl

Each treatment batch mixed in a Hobart mixer for 15 min, and then stuffed into moisture proof casings. Each log

contained approximately 5 kg and there were two per treatment. One was cooked in the smokehouse to 63C and the other to 94C. The beef logs were then chilled at 4C for 24 hrs, then frozen and stored at - 15C before being thawed at 4C for bacteriological analyses.

## II. Bacteriological Procedures:

### A. Raw materials and beef from hot and cold-boned.

1. Sampling: Samples were obtained aseptically and placed into sterile whirl-pak bags for transfer. Samples and sampling protocols are described below.

#### a. Raw ground untreated meat mixture

#### b. Raw ground treated batches

1. SPG
2. SP
3. SG
4. PG

#### c. Cooked treated batches

1. SPG cooked to 63C
2. SPG cooked to 94C
3. SP cooked to 63C
4. SP cooked to 94C
5. SG cooked to 63C
6. SG cooked to 94C
7. PG cooked to 63C

8. PG cooked to 94C
  - d. All treatment logs listed in "c" above were sliced and evaluated at "0" day following thawing to 4C.
  - e. All treatments listed in "c" were evaluated after 2, 4, and 10 days of display. Slices were covered with Resinite Packaging Film. The film was RMF-61-Oxygen permeable, gauge H-Y, obtained from Borden, Inc., Chemical Division. Packaged samples displayed at 4C were continuously illuminated by 40 watt natural fluorescent light at 200 ft candles.
  - f. Sample slices from each of the treatments listed in "c" above were vacuum packaged and stored at 4C in the dark for 21 days.
2. Blending procedure
  - a. A 5 g portion of the sample was removed aseptically from the whirl-pak bag and
  - b. 45 ml of sterile phosphate buffer was added for a 1:10 dilution.
  - c. Sample was blended 1 min in a Stomacher blender.
3. Pour plate procedure
  - a. Appropriate dilution aliquots were drawn

with sterile pipets and placed into the center of disposable petri dishes.

- b. Media stored at 48C was poured into petri dishes and "swirled" for complete distribution of microorganisms. Test dishes for coliforms were overlaid with media after the initial media had gelled.

#### 4. Counting of Colony Forming Units

- a. Number of Colony Forming Units (CFU's) were counted with the aid of a Quebec Colony Counter and a 10X Dissecting Scope.
- b. Number of Colony Forming Units (CFU's) were multiplied by the dilution factor to determine CFU's/g.

### B. Media Preparation

#### 1. Plate Count Agar

- a. Suspended 23.5 gm in 1 liter distilled water. Heat to boiling to dissolve completely.
- b. Sterilized in autoclave at 15 lbs pressure (121C) for 15 min.
- c. Store in water bath to stabilize at 48C before using (Difco, 1984).

#### 2. Bacto KF Streptococcus Agar

- a. Suspended 76.4 in 1 liter cold

distilled water and heat to boiling to dissolve completely. Heat for 5 min.

b. Cooled to 50C, add 1 ml 1% triphenyltetrazolium chloride (TTC).

c. Cooled and hold at 48C for making pour plates (Difco, 1984).

3. Violet Red Bile Agar

a. Suspended 41.5 g in 1 liter distilled water. Heated to boiling to dissolve but did not boil more than 2 min.

b. Cooled to 48C and held in water bath for immediate use (Difco, 1984).

C. Analysis: The raw ground untreated meat mixture, the raw ground treated batches, and the cooked batches were monitored as discussed below.

1. To obtain fecal coliform counts, dilutions were pour plated into petri dishes using Violet Red Bile agar allowed to gel and overlaid with the same agar. The plates were inverted and incubated for 24 hrs at 45C. A positive Colony Forming Unit (CFU) is a colony 0.55mm or larger with a halo, or 1-2 mm in diameter (Klein and Fung, 1976).

2. To obtain streptococci counts dilutions were pour plated with Bacto KF Streptococcus agar. They were inverted and incubated at 37C for 48 hrs (APHA, 1976).
  3. To obtain mesophile counts, dilutions were pour plated with Standard Plate Count agar, inverted and incubated at 32C for 48 hrs.
  4. To obtain psychrotroph counts, dilutions were pour plated with Standard Plate Count agar, inverted and incubated at 7C for 10 days. A positive CFU was determined with the use of 10X dissecting scope.
- D. Analysis: The cooked treated batches sliced at "0" day, cooked treated batches stored aerobically at 4C for 2, 4 and 10 days, and cooked treated batches vacuum packaged and stored at 4C for 21 days were sampled, blended, and monitored as discussed below.
1. To obtain mesophile counts, dilutions were pour plated with Standard Plate Count agar, inverted and incubated at 32C for 48 hrs.
  2. To obtain psychrotroph counts, dilutions were pour plated with Standard Plate Count agar, inverted and incubated at 7C for 10 days. A positive CFU was determined with



the use of a 10X dissecting scope.

- E. pH values were monitored throughout processing and display with the use of an Orion model 211 digital hand-held pH meter. Readings were recorded for each treatment after grinding, prior to stuffing, and over the display period of days 1, 2, 4, and 10.

### III Statistical Methods

Statistical analyses were performed using the paired T test, the General Linear Models Procedure, Fisher's Least Significant Difference Test, and Duncans Multiple Range Test with consultation of the Kansas State University Department of Statistics. Data were analyzed by SAS Program (SAS, 1985).

#### Data Treatments

Data was transformed from the numeric to the logarithmic value in order to overcome large variation in the experimental data. This statistical procedure is commonly used to make inferences regarding the microbiological impact of the results (Ott, 1984).

## RESULTS AND DISCUSSION

### Initial Microbial Profile

Prior to treatment no differences were noted (Table 1) between mesophile populations of hot-boned and cold-boned beef with Log 3.84 CFU/g and Log 3.54 CFU/g, respectively. This agrees with studies by Fung et al., (1980). Psychrotrophs were undetected at the lowest dilution (Log 3 CFU/g).

No coliforms were found on the untreated hot-boned or cold-boned raw beef (Table 2). Cold-boned sides contained a slightly higher incidence of coliforms after treatment; and three animals showed somewhat more contamination. Two of the hot-boned steers also exhibited coliforms. Any coliforms detected were in the range of Log 1-2 CFU/g, which is an acceptable level of contamination and "unavoidable" (Johnston and Elliott, 1976). The raw samples had no detectable coliforms indicating that the meats were processed and handled in a sanitary manner.

Streptococci were found in one out of six samples of untreated hot-boned beef and two out of seven samples of cold-boned beef. Relatively low levels (Log 1-3 CFU/g) are common in foods. In no case in this experiment were levels of Fecal Streptococci higher than Log 2 CFU/g. (Table 2).

### The Effects of Heat Treatment

No Fecal Coliforms or Fecal Streptococci were detected

TABLE 1. Mesophile populations of hot-boned and cold-boned beef before and after treatment.

Pre-Treatment		Post-Treatment		
Boning	Log CFU/g	Log CFU/g	Treatment <sup>a</sup>	Log CFU/g
Hot-boned	3.84 <sup>b</sup>	4.11 <sup>c</sup>	SPG	4.06 <sup>d</sup>
			SP	3.86 <sup>d</sup>
			SG	4.39 <sup>d</sup>
			PG	4.21 <sup>d</sup>
Cold-boned	3.54 <sup>b</sup>	4.03 <sup>c</sup>	SPG	3.63 <sup>d</sup>
			SP	3.64 <sup>d</sup>
			SG	4.12 <sup>d</sup>
			PG	4.76 <sup>d</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl, and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>bcd</sup> Means in the same column bearing common superscripts are not significantly different ( $P > 0.05$ ).

TABLE 2. Fecal Streptococci and Fecal Coliforms in beef before and after treatment.

Treatment <sup>c</sup>	Hot-boned <sup>a</sup>		Cold-boned <sup>b</sup>	
	Coliform	Streptococci	Coliform	Streptococci
No treatment	0/6 <sup>d</sup>	1/6	0/7	2/7
SPG	0/6	2/6	1/7	2/7
SP	1/6	1/6	2/7	4/7
SG	2/6	1/6	2/7	4/7
PG	0/6	2/6	2/7	2/7
Animal <sup>e</sup>				
#2			0/5	1/5
#3	0/5	1/5	0/5	4/5
#4	0/5	5/5	0/5	1/5
#5	0/5	0/5	0/5	0/5
#6	0/5	1/5	1/5	2/5
#7	2/5	0/5	3/5	0/5
#8	1/5	0/5	3/5	3/5

<sup>a</sup>n=24

<sup>b</sup>n=28

<sup>c</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl, and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>d</sup>Indicates the occurrence of microorganisms out of total samples. "0" indicates 10 CFU/g. In no case were microorganisms detected above Log 2 CFU/g.

<sup>e</sup>Animal #1 was entirely eliminated from the experiment, and hot-boned side of Animal #2 was eliminated from the experiment.

following the cooking procedure. Coliforms are heat sensitive and easily destroyed by the temperatures reached in this experiment.

Fecal Streptococci have been suggested as a replacement for Coliform tests because they are somewhat more heat resistant (Banwart, 1981). In this case, however, both 63C and 94C internal temperatures were sufficient to destroy the bacteria.

As with Coliforms and Streptococci, mesophiles and psychrotrophs were undetected following heat treatment. Heating denatures microbial proteins and destroys all but the most heat resistant microorganisms.

Prior to treatment the differences between hot-boned and conventionally-boned (cold-boned) beef are shown in the pH (Table 3) where pH values are 6.50 and 5.75, respectively. This agrees with what is known about these two fabrication techniques. One would expect the pH of hot-boned, pre-rigor muscle to be greater than cold-boned, post-rigor muscle because pH decline is not complete in pre-rigor muscle.

Following heat treatment, there was a significant difference ( $P < 0.05$ ) between hot-boned and cold-boned samples at pH means of 6.28 and 6.02, respectively (Table 3).

When the roasts were made from hot-boned beef, SPG and SG were not significantly different at pH 6.33 and 6.34,

TABLE 3. pH values of beef before and after cooking.

Treatment <sup>a</sup>	pH--Raw		pH--Cooked	
	Hot-boned	Cold-boned	Hot-boned	Cold-boned
Untreated	6.50 <sup>b</sup>	5.75 <sup>f</sup>	-----	-----
SPG	6.18 <sup>c</sup>	5.77 <sup>f</sup>	6.33 <sup>i</sup>	6.07 <sup>l</sup>
SP	6.25 <sup>d</sup>	5.79 <sup>f</sup>	6.17 <sup>j</sup>	6.06 <sup>l</sup>
SG	6.18 <sup>c</sup>	5.65 <sup>g</sup>	6.34 <sup>i</sup>	6.03 <sup>m</sup>
PG	6.61 <sup>e</sup>	5.90 <sup>h</sup>	6.29 <sup>k</sup>	5.92 <sup>n</sup>
Composite	-----	-----	6.28 <sup>k</sup>	6.02 <sup>m</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl, and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>b-n</sup>Means bearing common superscripts in the same column are not significantly different ( $P > 0.05$ ).

respectively. SP at pH mean 6.17 and PG at pH mean 6.29 were each significantly different ( $P < 0.05$ ) than the other treatments (Table 3).

Between treatments of cold-boned cooked roasts, SPG and SP did not have significantly different pH means of 6.07 and 6.06 respectively, but they were different from both SG at pH 6.03 and PG at pH 5.92. SG and PG were each significantly different from the other three treatments ( $P < 0.05$ , Table 3).

When hot-boned is compared to cold-boned within each treatment, the hot-boned samples were in each case significantly higher ( $P < 0.05$ ) than their cold-boned counterparts (Table 3).

Higher pH values are noted when roasts are processed to 94C as compared to when processed to 63C in each treatment. This is consistent with the findings of Wierbicki et al. (1957). They suggested that this is due to the destruction of free sulfhydryl and carboxyl groups.

When beef logs were cooked to an internal temperature of 63C, the mean pH values are from low to high, 6.05, 6.09, 6.15 and 6.18 for PG, SP, SG and SPG, respectively. All were significantly different from the rest ( $P < 0.05$ ).

When beef logs were cooked to an internal temperature of 94C, the mean pH values of 6.14 and 6.13 for SP and PG, respectively, were not significantly different ( $P > 0.05$ ). Neither were SPG and SG significantly different ( $P > 0.05$ ) at

mean pH values of 6.21 and 6.19, respectively.

#### Changes During Storage at 4C

Microbiologically, the most important pH is the change noted over the 10-day storage time. The pH of SPG, SP and SG did not change significantly over time. At the 10-day storage time a significant difference ( $P < 0.05$ ) was noted between PG and the rest of the treatments. The mean pH of PG dropped .05 pH units indicating acid production by microorganisms (Table 4).

Figure 1 shows the change in pH of hot-boned roasts cooked to 63C and 94C internal temperatures. The only real dramatic change (a decrease) was with PG when cooked to 63C. Figure 2 shows the pH of cold-boned roasts over 10 days for both temperatures. Very little change was noted for any of the 4 treatments at either temperature.

Collectively, the pH data for hot-boned and cold-boned beef during the storage periods shows this: Between treatments sampled at 0, 2, and 4 days of storage, treatments SPG and SG were not significantly different, nor were treatments SPG and PG. However, at 10-days of storage each treatment was significantly different ( $P < 0.05$ ) from the rest of the treatments. Low to high, the mean pH values were 6.05, 6.13, 6.18, and 6.21 for PG, SP, SG, and SPG, respectively. This indicates that sometime at or before the



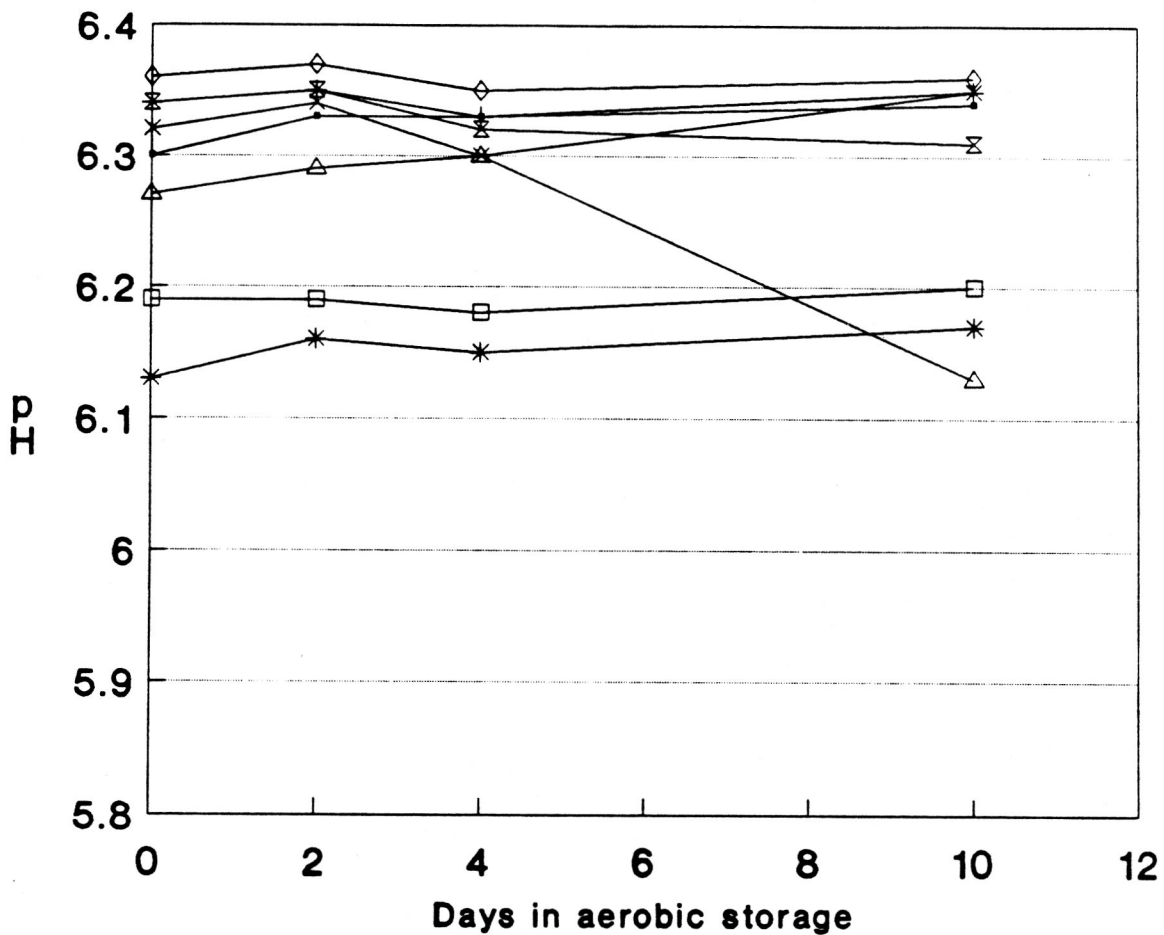
TABLE 4. pH values of restructured roasts during aerobic storage at 4C--treatment vs time.

Treatment <sup>a</sup>	0 Day	2 Day	4 Day	10 Day
SPG	6.18 <sup>b</sup>	6.19 <sup>b</sup>	6.20 <sup>b</sup>	6.21 <sup>b</sup>
SP	6.10 <sup>c</sup>	6.11 <sup>c</sup>	6.11 <sup>c</sup>	6.13 <sup>c</sup>
SG	6.17 <sup>b</sup>	6.17 <sup>b</sup>	6.17 <sup>b</sup>	6.18 <sup>b</sup>
PG	6.10 <sup>c</sup>	6.10 <sup>c</sup>	6.10 <sup>c</sup>	6.05 <sup>d</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>bcd</sup>All means in the same column and in the same row bearing common superscripts are not significantly different ( $P>0.05$ ).

Figure 1. pH of hot-boned roasts cooked to internal temperatures of 63 and 94C



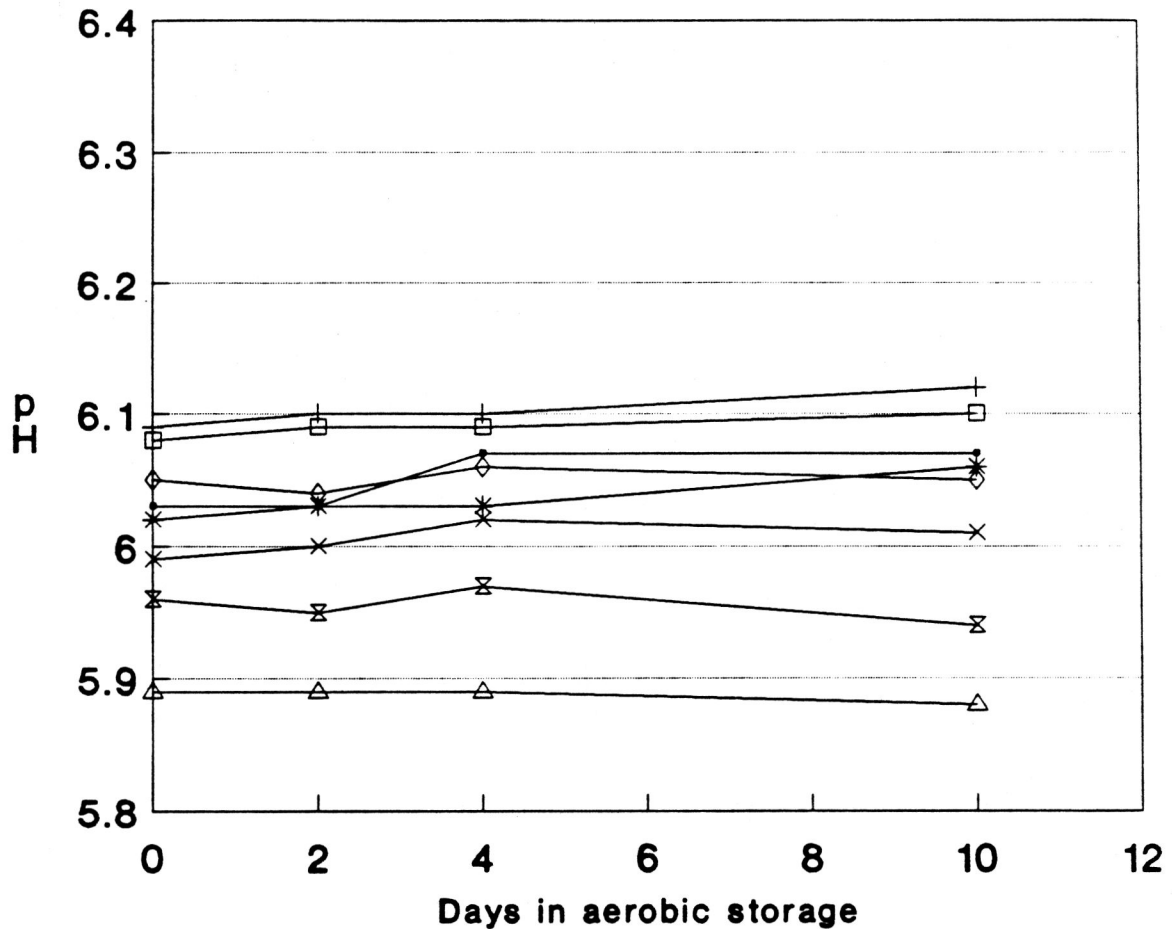
Treatment x Temp

—●— SPG 63C —+— SPG 94C —\*— SP 63C —□— SP 94C

—x— SG 63C —◇— SG 94C —△— PG 63C —x— PG 94C

Roasts displayed at 4C under 200 ft  
candle natural fluorescent light

**Figure 2. pH of cold-boned roasts cooked to internal temperatures of 63 and 94C**



**Treatment x Temp**

—●— SPG 63C —+— SPG 94C —\*— SP 63C —□— SP 94C  
 —x— SG 63C —◇— SG 94C —△— PG 63C —x— PG 94C

**Roasts displayed at 4C under 200 ft  
candle natural fluorescent light**

10-day sampling time, but after the 4-day sampling time, environmental changes are occurring most likely due to microbial action.

Mesophile counts were analyzed for differences over the storage period at 4C and separately analyzed at 10-days only. No statistical differences ( $P > 0.05$ ) were found between hot-boned and cold-boned samples (Tables 5 and 6). Over the storage period mean log counts for hot-boned and cold-boned restructured beef roasts were Log 2.01 CFU/g and Log 1.91 CFU/g, respectively. At 10-days only, mesophile counts for hot-boned and cold-boned restructured beef roasts were Log 4.03 CFU/g and Log 3.71 CFU/g, respectively.

Differences between treatments were significant when analyzed over time, but not when the 10-day mesophilic counts were isolated and analyzed separately. Over time, treatment PG exhibited significantly higher ( $P < 0.05$ ) mean counts at Log 2.25 CFU/g. (Table 5). Treatments SPG, SP, and SG had mean counts of Log 1.91 CFU/g, Log, 1.81 CFU/g and Log 1.90 CFU/g, respectively. When the mesophilic microbial counts taken after 10-days of storage were analyzed, no differences between treatments were noted with mean counts for SPG, SG and SPG at Log 3.30 CFU/g, Log 3.60 CFU/g, Log 3.91 CFU/g and Log 4.63 CFU/g, respectively (Table 6). While there appears to be a difference, the widely varying microbial counts after 10-days resulted in insignificance.

TABLE 5. Mesophile populations during aerobic storage at 4C-- differences by boning, treatment, time, and cooking temperature.

Boning		Treatment <sup>a</sup>	Time	63C	94C
	Log CFU/g			Log CFU/g	Log CFU/g
Hot-boned	2.01 <sup>b</sup>	SPG	0 Day	1.40 <sup>ef</sup>	1.14 <sup>e</sup>
Cold-boned	1.91 <sup>b</sup>	SP	2 Day	1.88 <sup>f</sup>	1.10 <sup>e</sup>
		SG	4 Day	1.30 <sup>e</sup>	0.96 <sup>e</sup>
		PG	10 Day	4.57 <sup>g</sup>	3.15 <sup>h</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>b-h</sup>Mean values bearing common superscripts in the same column are not significantly different ( $P > 0.05$ ).

TABLE 6. Mesophile populations at 10 day aerobic storage at 4C--differences by boning, cooking temperature, and treatment.

Boning	Temperature		Treatment <sup>a</sup>	
	Log CFU/g		Log CFU/g	Log CFU/g
Hot-boned	4.03 <sup>b</sup>	63C	4.57 <sup>c</sup>	SPG 3.30 <sup>e</sup>
Cold-boned	3.71 <sup>b</sup>	94C	3.15 <sup>d</sup>	SP 3.60 <sup>e</sup>
				SG 3.91 <sup>e</sup>
				PG 4.63 <sup>e</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>b</sup>Means not significantly different ( $P > 0.05$ ).

<sup>c,d</sup>Means are significantly different ( $P < 0.05$ ).

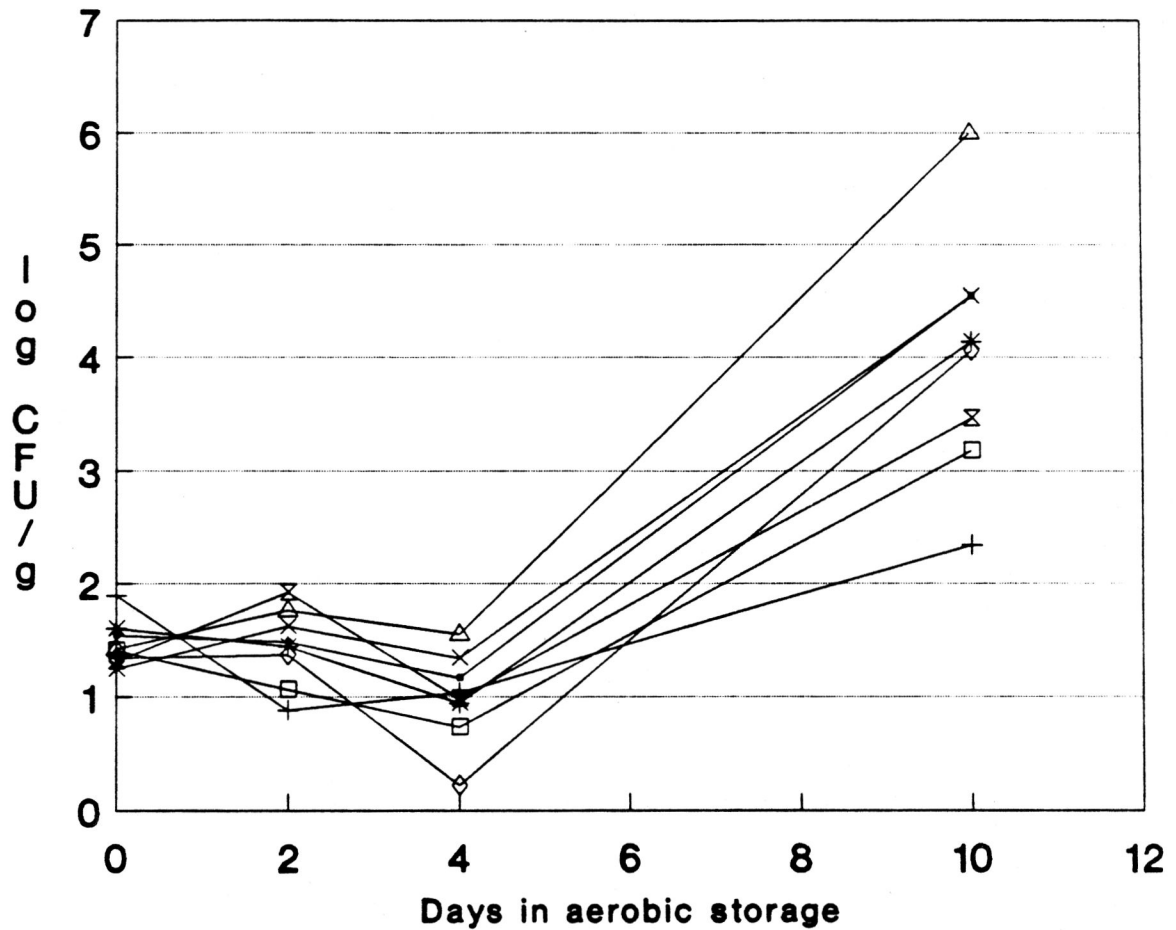
<sup>e</sup>Means are not significantly different ( $P = 0.06$ ).

Figures 3 and 4 show the growth of mesophilic microorganisms on hot-boned and cold-boned treatments at both temperatures. It seems that hot-boned treatment PG was the most susceptible to spoilage when cooked to 63C. The 94C internal cooking temperature reduced microbial numbers to less than Log 4 CFU/g as opposed to the Log 6 CFU/g noted for 63C. Bar charts of mesophile and psychrotroph populations of hot-boned and cold-boned samples aerobically displayed for 10-days are presented in Figures 5, 6, 7, and 8.

Analysis of mean counts of psychrotrophs after aerobic storage at 4C confirms the results of the analysis of mesophiles over time. Prior to the tenth day of storage, psychrotrophic microbial levels were either undetected or too infrequent to be statistically analyzable (Table 7). This gap may be explained by two things: 1) freezing the product before the storage test increased the likelihood of freeze-thaw injury, and 2) thermal destruction occurred during the cooking process. Freeze-thaw has been identified as one of the difficulties of enumerating microorganisms (Brown and Baird-Parker, 1982). Freeze injuries may include cellular leakage, increased nutritional needs and the need for a longer lag phase (Davies and Obafemi, 1985). Heating denatures microbial proteins to destroy most microorganisms.

Shown in Table 7, hot-boned and cold-boned samples are not significantly different ( $P>0.05$ ) with means of Log 4.13

**Figure 3. Mesophile populations of hot-boned roasts cooked to internal temperatures of 63 and 94C**



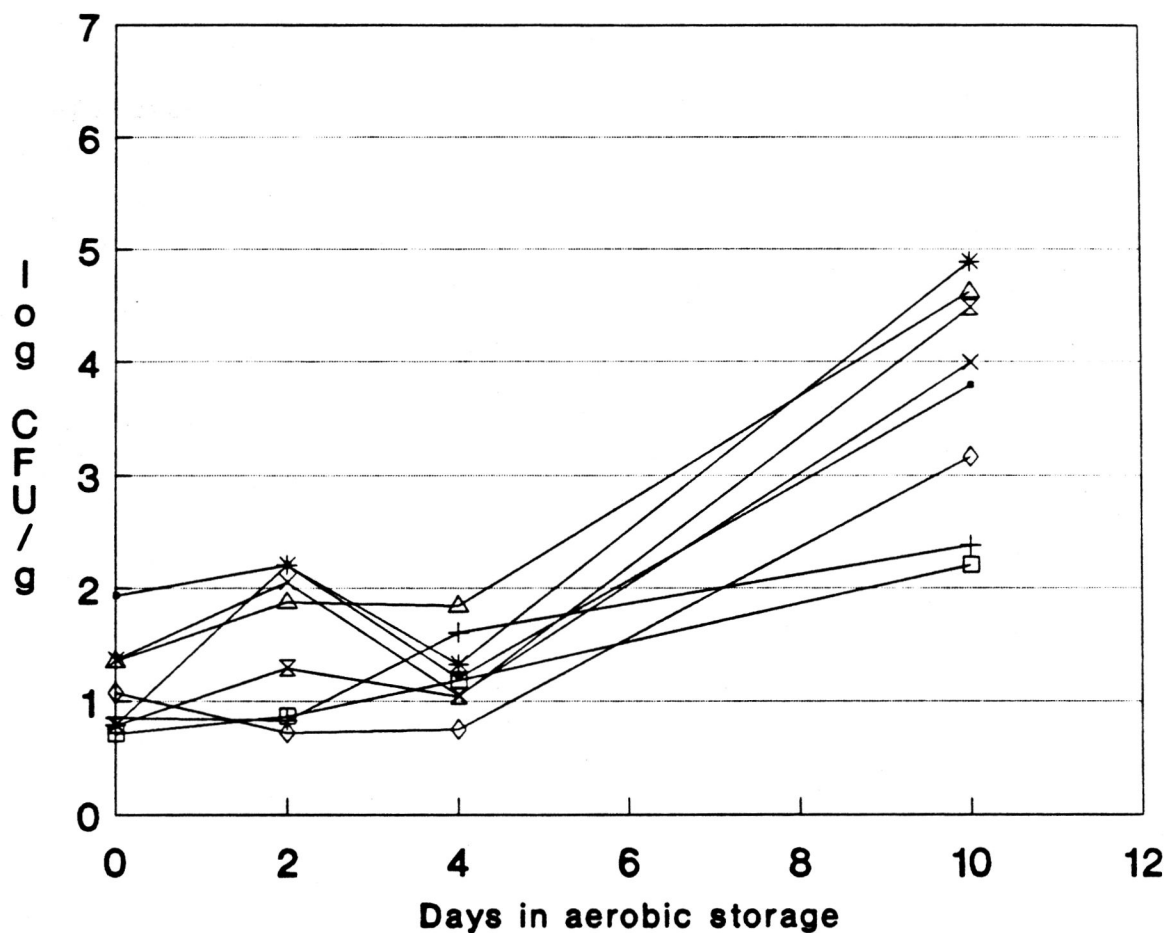
**Treatment x Temp**

—●— SPG 63C —+— SPG 94C —\*— SP 63C —□— SP 94C  
 —×— SG 63C —◇— SG 94C —△— PG 63C —⊗— PG 94C

**Roasts displayed at 4C under 200 ft  
 candle natural fluorescent light**



Figure 4. Mesophile populations of cold-boned roasts cooked to internal temperatures of 63 and 94C

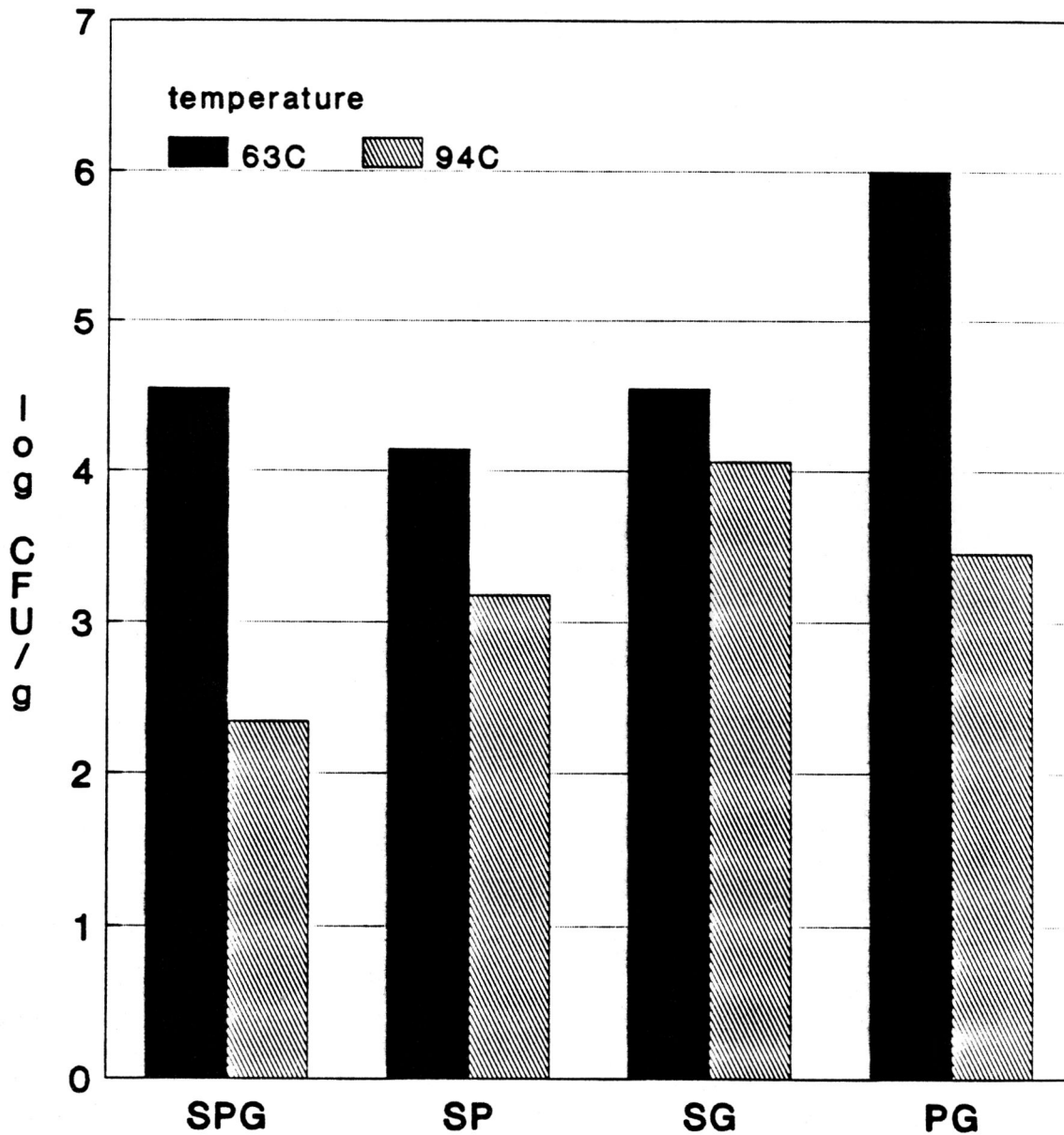


Treatment x Temp

—●— SPG 63C    —+— SPG 94C    —\*— SP 63C    —□— SP 94C  
 —x— SG 63C    —◇— SG 94C    —△— PG 63C    —⊗— PG 94C

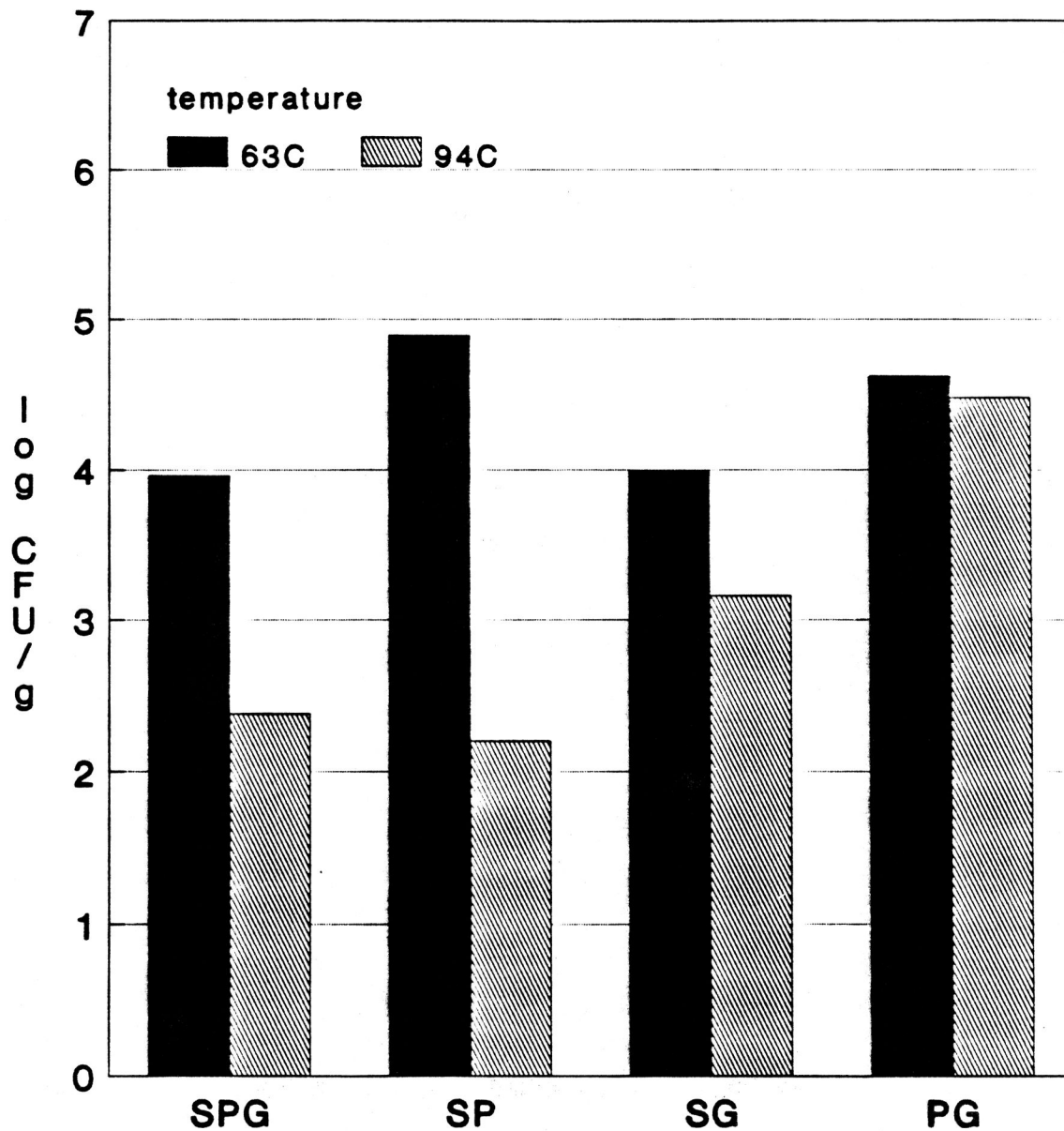
Roasts displayed at 4C under 200 ft  
candle natural fluorescent light

Figure 5. Mesophile populations of hot-boned roasts cooked to internal temperatures of 63 and 94C--10-day aerobic storage



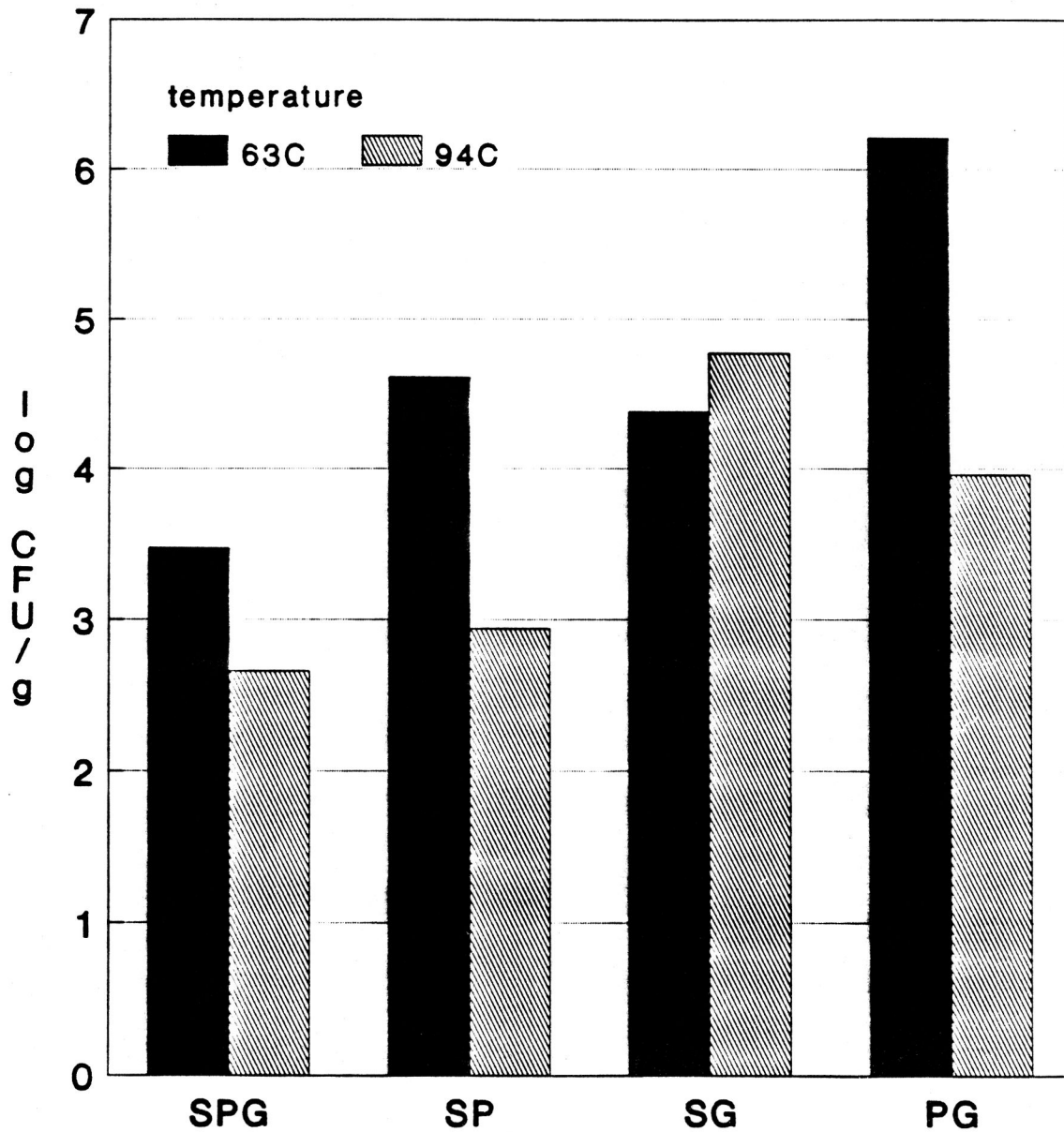
Roasts displayed at 4C under 200 ft  
candle natural fluorescent light

Figure 6. Mesophile populations of cold-boned roasts cooked to internal temperatures of 63 and 94C--10-day aerobic storage



Roasts displayed at 4 C under 200 ft  
candle natural fluorescent light

Figure 7. Psychrotroph populations of hot-boned roasts cooked to internal temperatures of 63 and 94C--10-day aerobic storage



Roasts displayed at 4C under 200 ft  
candle natural fluorescent light

Figure 8. Psychrotroph populations of cold-boned roasts cooked to internal temperatures of 63 and 94C--10-day aerobic storage

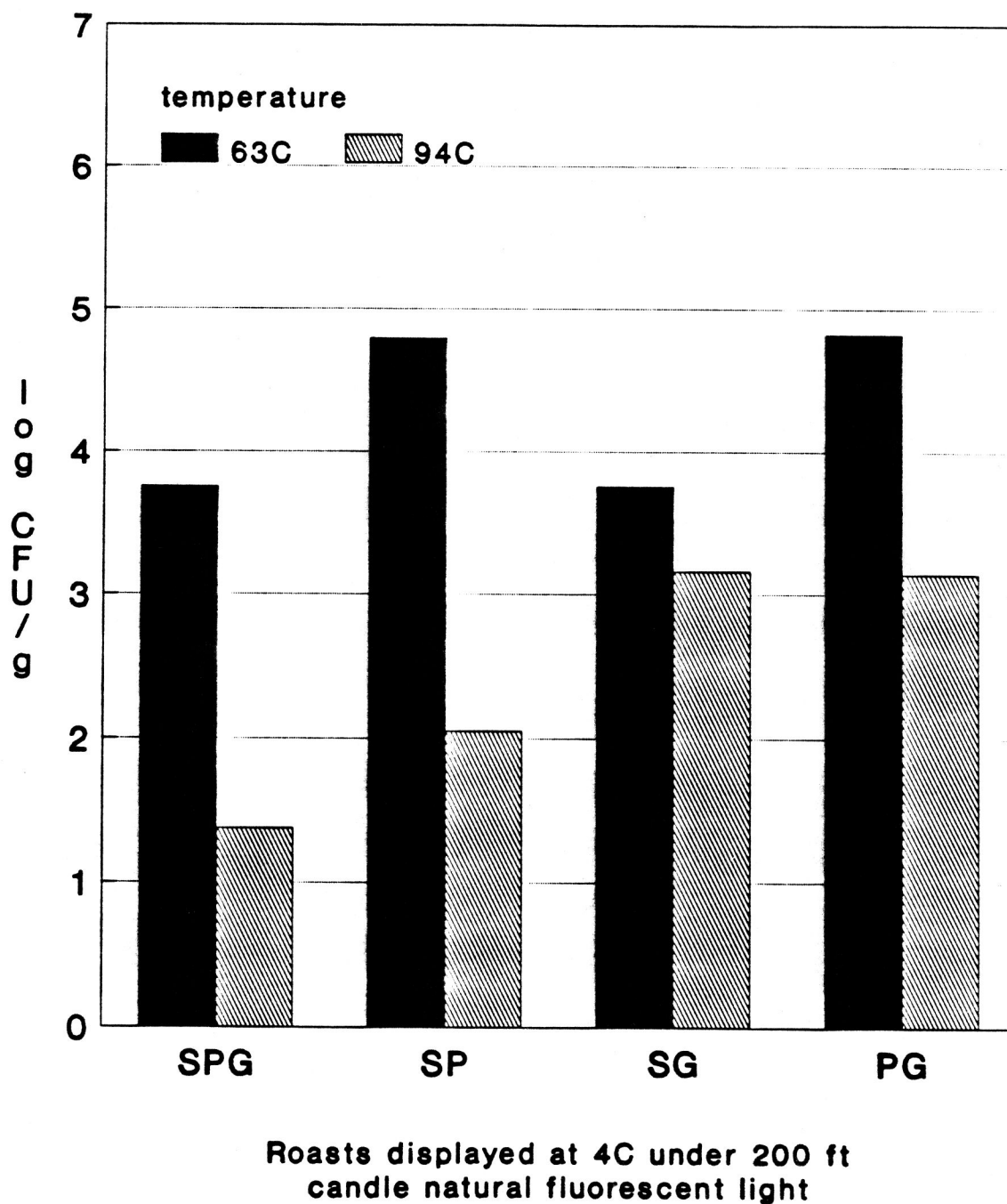


TABLE 7. Psychrotrophs at 10 day\* aerobic storage at 4C-- differences by boning, cooking temperature, and treatment.

Boning	Temperature		Treatment <sup>a</sup>		
	Log CFU/g		Log CFU/g		Log CFU/g
Hot-boned	4.13 <sup>b</sup>	63C	4.47 <sup>c</sup>	SPG	2.75 <sup>e</sup>
Cold-boned	3.30 <sup>b</sup>	94C	2.96 <sup>d</sup>	SP	3.58 <sup>ef</sup>
				SG	3.97 <sup>fg</sup>
				PG	4.49 <sup>g</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>b-g</sup>Means in the same column bearing common superscripts are not significantly different (P<0.05).

\*Prior to 10 day sampling time, psychrotrophs could not be statistically analyzed.

CFU/g and Log 3.30 CFU/g psychrotrophs, respectively. There were significant differences ( $P < 0.05$ ) between samples cooked to 63C and those cooked to 94C, with means of Log 4.47 CFU/g and Log 2.96 CFU/g, respectively. The highest microbial load was seen with treatment PG, but not significantly different from SG. SG was not statistically different from SP although it was slightly higher. SPG had the lowest load of psychrotrophs, but not statistically different from SP ( $P > 0.05$ ). This agrees with Nielsen and Zeuthen's (1983) studies on bologna which showed that the addition of glucose increased microbial growth. This finding disagrees with Shelef's (1977) study using uncooked hamburger plus glucose, where glucose increased the shelf-life of the product.

NaCl was the additive excluded from the treatment with highest microbial load (i.e. PG) confirming the efficacy of salt treatment in preservation. It was noted that SP at low temperature was carrying as high a mean psychrotrophic count as PG at low temperature on cold-boned samples. PG on hot-boned beef appears to be the most susceptible to psychrotrophic growth in cooler storage.

#### Vacuum Packaged Restructured Roasts

Samples were taken and plate counts performed on vacuum packaged treatments stored at 4C in the dark. The statistical analysis is summarized in Tables 8 and 9. Bar

TABLE 8. Mesophile populations after 21 day vacuum storage at 4C, in the dark--differences by boning, cooking temperature, and treatment.

Boning	Temperature		Treatment <sup>a</sup>	
	Log CFU/g		Log CFU/g	Log CFU/g
Hot-Boned	2.42 <sup>b</sup>	63C	3.71 <sup>c</sup>	SPG 2.63 <sup>e</sup>
Cold-Boned	3.61 <sup>b</sup>	94C	2.24 <sup>d</sup>	SP 2.35 <sup>e</sup>
				SG 3.52 <sup>e</sup>
				PG 3.38 <sup>e</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>b</sup>Means with common superscripts not significantly different (P>0.05).

<sup>c,d</sup>Means are significantly different (P<0.05).

<sup>e</sup>Means with common superscripts not significantly different (P>0.05).



TABLE 9. Psychrotroph populations after 21 day vacuum storage at 4C, in the dark--differences by boning, cooking temperature, and treatment.

Boning	Temperature		Treatment <sup>a</sup>		
	Log CFU/g		Log CFU/g		Log CFU/g
Hot-boned	3.90 <sup>b</sup>	63C	3.83 <sup>c</sup>	SPG	3.00 <sup>e</sup>
Cold-boned	2.75 <sup>b</sup>	94C	2.72 <sup>d</sup>	SP	3.09 <sup>e</sup>
				SG	3.97 <sup>e</sup>
				PG	3.05 <sup>e</sup>

<sup>a</sup>Treatment codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG.

<sup>b</sup>Means not significantly different ( $P > 0.05$ ).

<sup>c,d</sup>Means are significantly different ( $P < 0.05$ ).

<sup>e</sup>Means not significantly different ( $P > 0.05$ ).

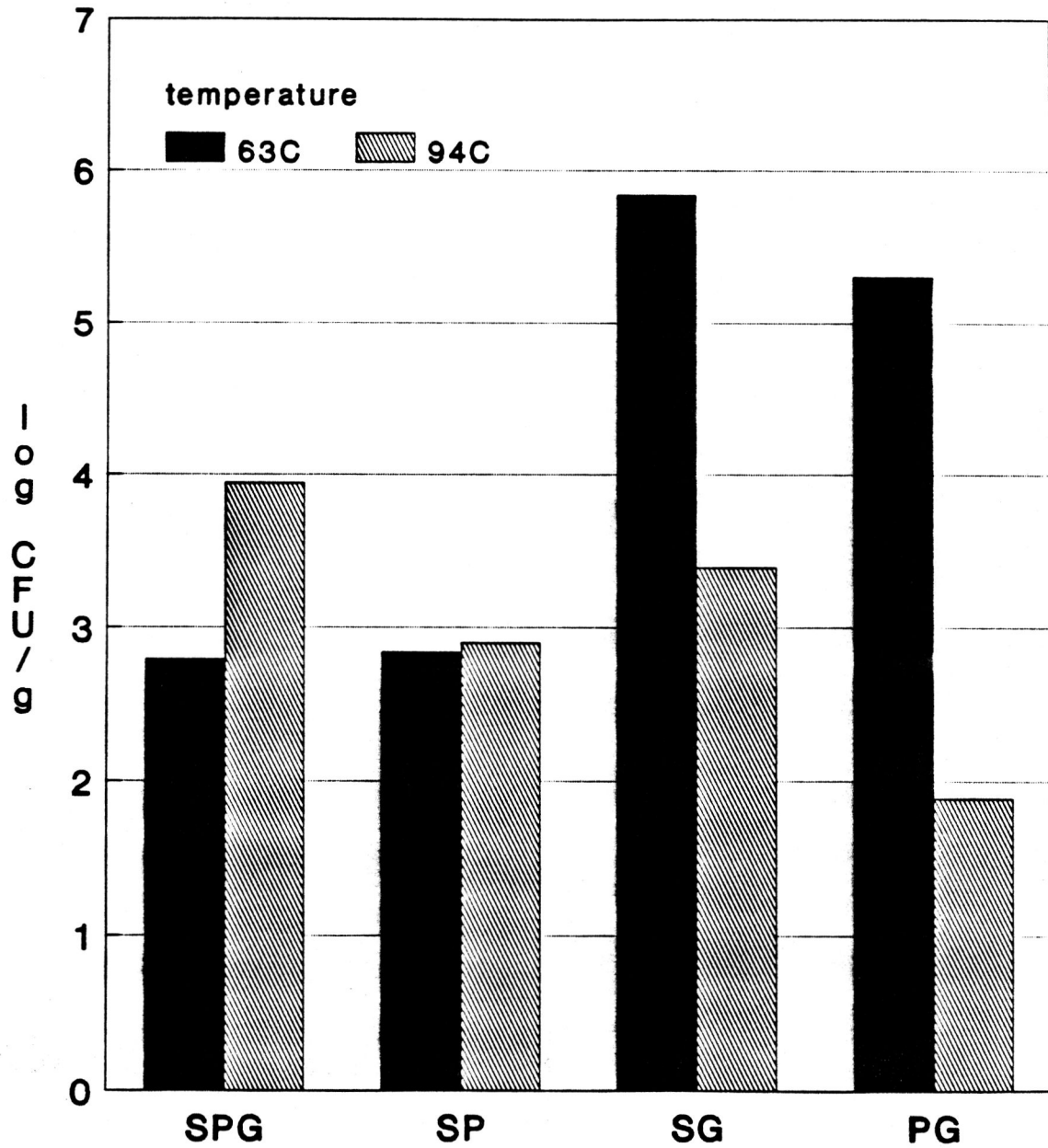
charts of mean log counts are shown in Figures 9, 10, 11 and 12. No significant differences were noted between boning techniques or treatments ( $P > 0.05$ ). Significant differences were noted between samples cooked to 63C vs 94C. The mean mesophilic counts for samples cooked to 63C and 94C were Log 3.71 CFU/g and Log 2.24 CFU/g, respectively. Mean psychrotrophic populations for samples cooked to 63C and 94C were Log 3.83 CFU/g and Log 2.72 CFU/g, respectively.

#### Analysis of Microbiological Data by Grouping and Ranking

The statistical analysis of microbiological data in the previous sections did not include an analysis of all treatment combinations due to the design of the experiment. This section is a discussion of the trends of microbial growth by grouping and ranking of data of all treatment combination.

The mesophilic populations of hot-boned and cold-boned roasts cooked to internal temperature of 63C and 94C during storage are presented in Figures 3 and 4, respectively. During the first 4 days of storage the numbers were low (about Log 2 CFU/g or less) indicating that the products were microbiologically safe. After 10 days of storage, variations of mesophilic counts and psychrotrophic counts were observed when comparing all treatments of samples. Using the microbiological scale developed by Fung et al.

Figure 9. Mesophile populations of vacuum packaged hot-boned roasts cooked to 63 and 94C--held for 21 days



Roasts stored at 4C in the dark

Figure 10. Mesophile populations of vacuum packaged cold-boned roasts cooked to 63 and 94C--held for 21 days

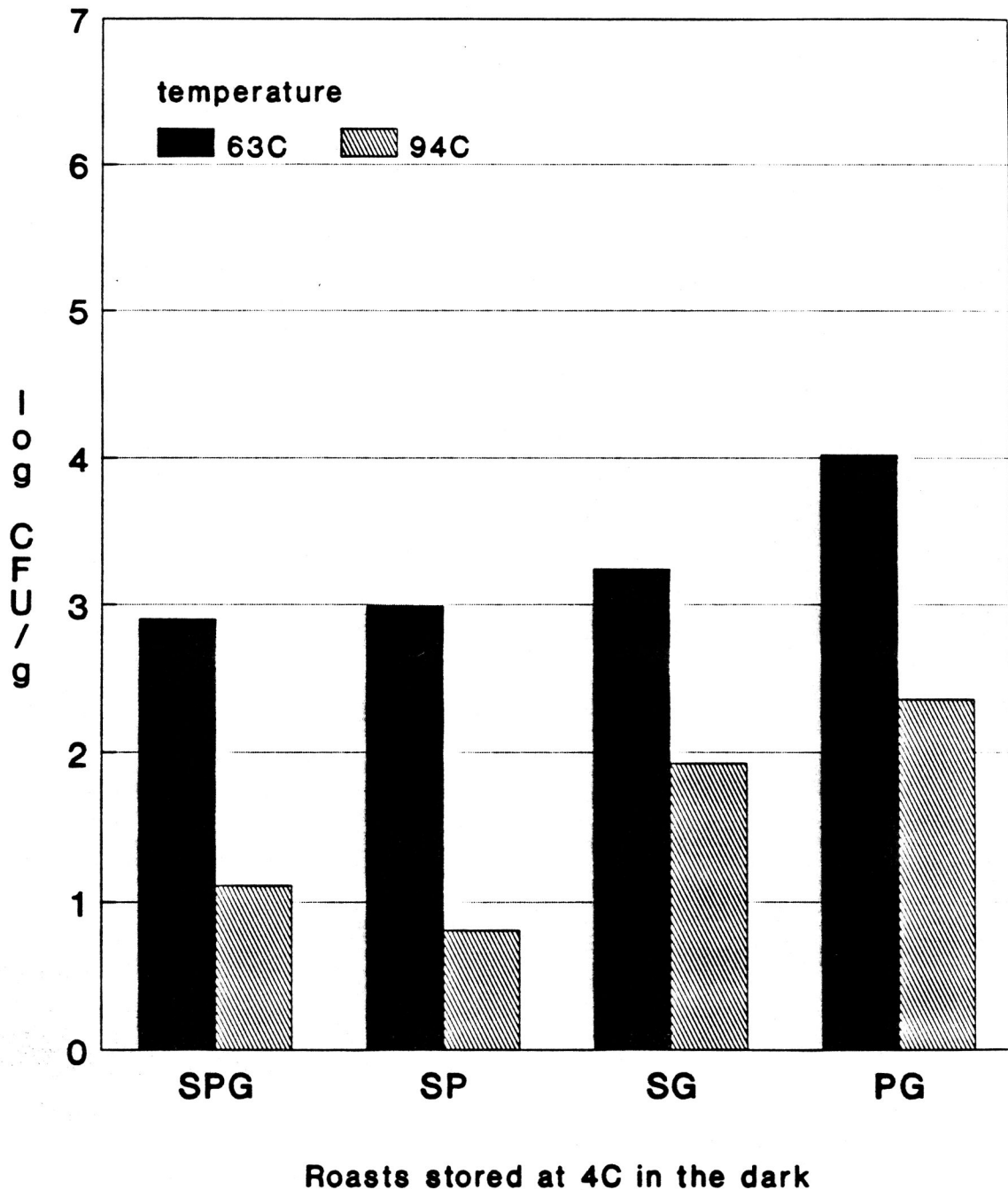


Figure 11. Psychrotroph populations of vacuum packaged hot-boned roasts cooked to 63 and 94C--held for 21 days

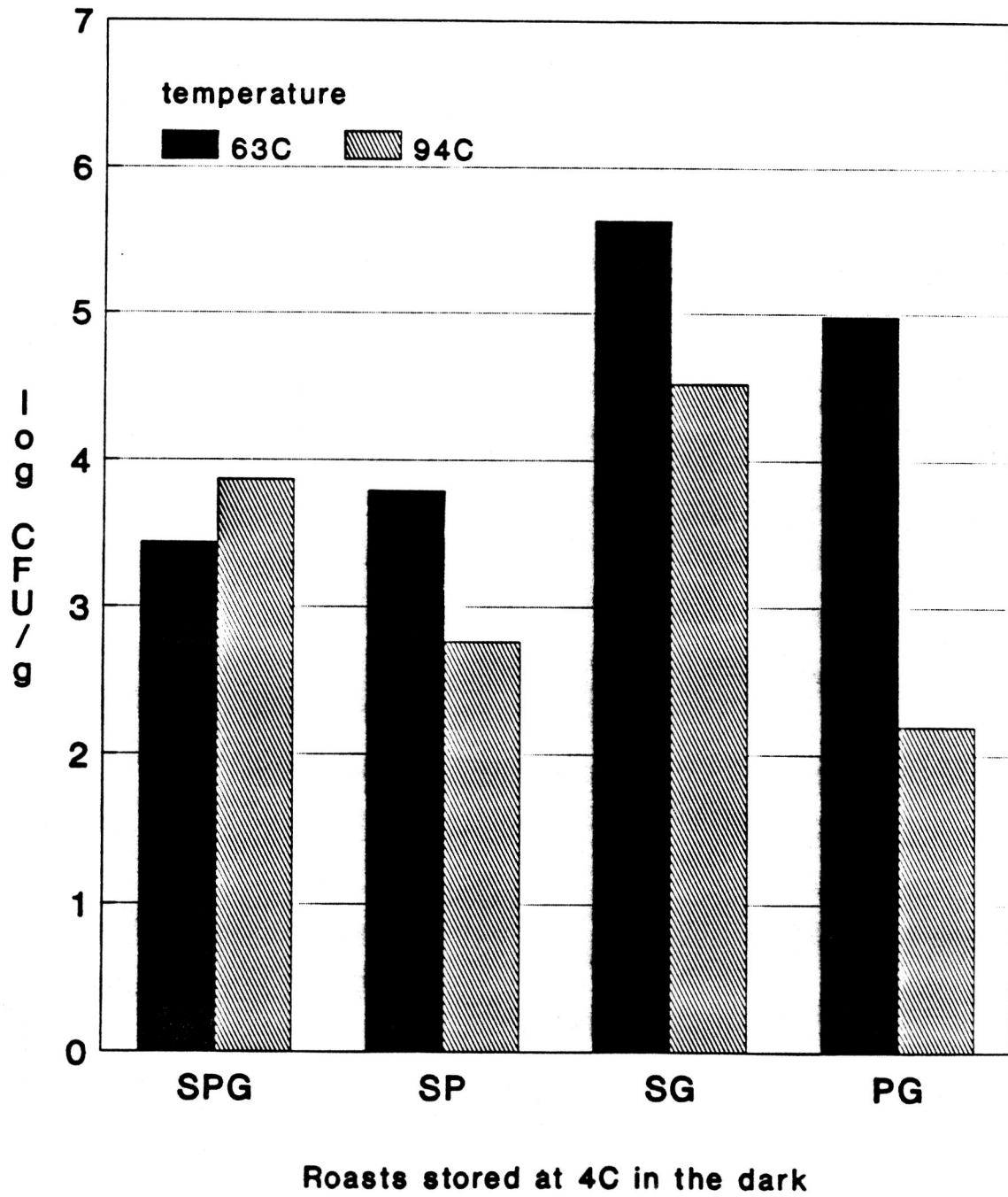
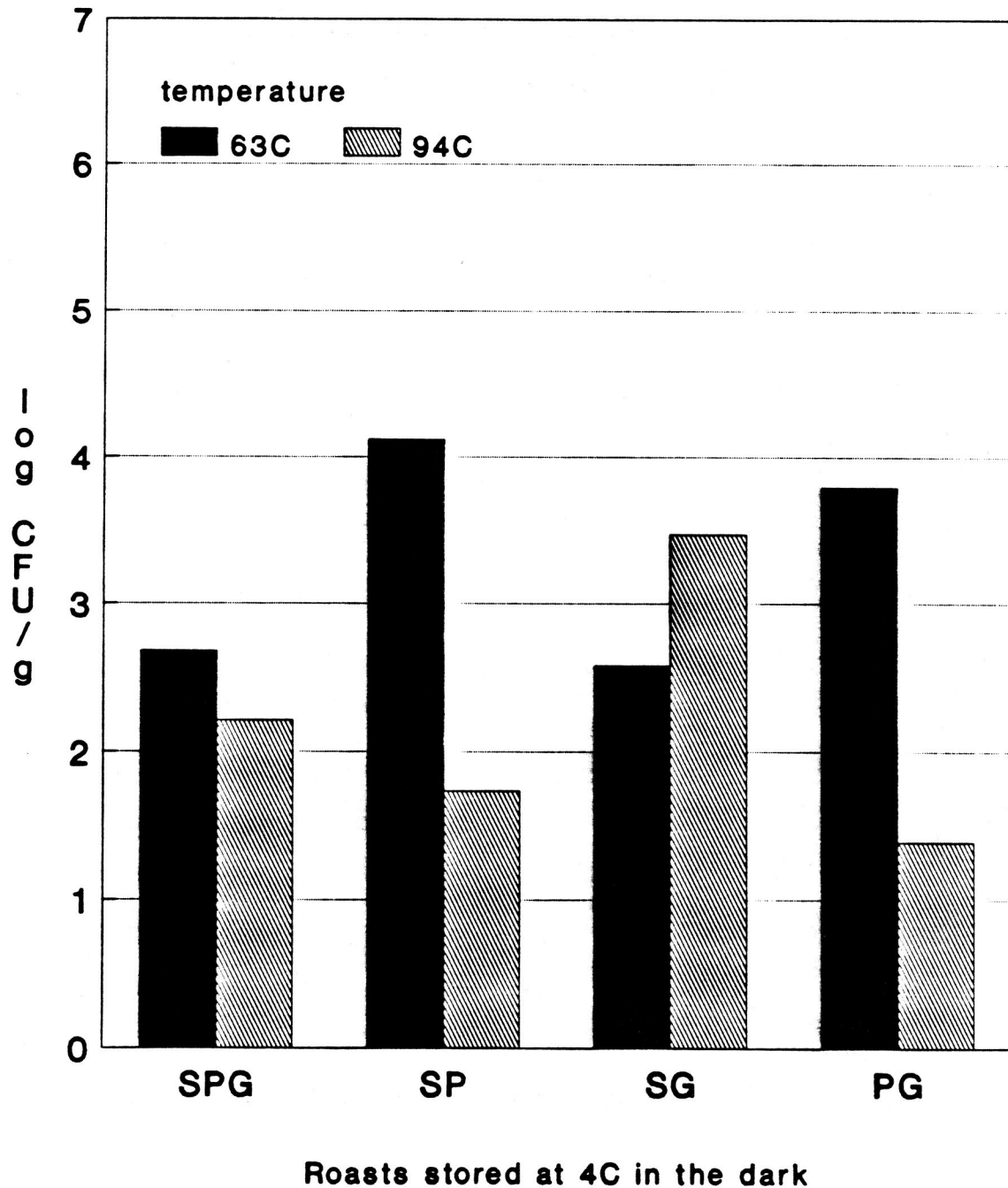


Figure 12. Psychrotroph populations of vacuum packaged cold-boned roasts cooked to 63 and 94C--held for 21 days



(1980) the data can be presented in ranking and grouping format from the lowest count to the highest. The scale of Fung et al. (1980) separates microbial counts of meat into low (Log 0-2 CFU/g), intermediate count (Log 3-4 CFU/g), high count (Log 5-6 CFU/g), and very high count (Log 7 CFU/g). For mesophilic counts of hot-boned samples, 1 treatment (SPG High) fell in the low count region, 6 (SP High, PG High, SG High, SP Low, SPG Low, and SG Low) fell in the intermediate count region and 1 (PG Low) was in the high count region (Table 10). For psychrotroph counts of hot-boned samples, 2 (SPG High and SP High) fell in the low count region, 5 fell in the intermediate count region and 1 (PG Low) fell in the high count region. For mesophilic counts of cold-boned samples 2 treatments (SP High and SPG High) fell in the low count region and the other six fell in the intermediate count region. For psychrotroph counts of cold-boned samples, 2 (SPG High and SP High) fell in the low count region and the other 6 fell in the intermediate region.

It is apparent by examining these data that high temperature treated samples fell in the lower count region while low temperature treated samples resulted in samples in the high count region. PG treated at low temperature had higher counts than samples treated with other combinations after 10 days of aerobic storage. These data indicate that

TABLE 10. 10-day aerobic storage--analysis by grouping and ranking.

	Hot-boned Roasts				Cold-boned Roasts			
	Mesophiles		Psychrotrophs		Mesophiles		Psychrotrophs	
	Rank	Log CFU/g	Rank	Log CFU/g	Rank	Log CFU/g	Rank	Log CFU/g
Low <sup>b</sup> Count	1. SPG-94C <sup>a</sup>	2.38	1. SPG-94C	2.66	1. SP-94C	2.20	1. SPG-94C	1.38
			2. SP-94C	2.94	2. SPG-94C	2.38	2. SP-94C	2.05
Intermediate Count	2. SP-94C	3.18	3. SPG-63C	3.47	3. SG-94C	3.16	3. PG-94C	3.14
	3. PG-94C	3.46	4. PG-94C	3.96	4. SPG-63C	3.97	4. SG-94C	3.16
	4. SG-94C	4.06	5. SG-63C	4.38	5. SG-63C	3.99	5. SG-63C	3.75
	5. SP-63C	4.14	6. SP-63C	4.61	6. PG-94C	4.48	6. SPG-63C	3.75
	6. SPG-63C	4.54	7. SG-94C	4.77	7. PG-63C	4.61	7. SP-63C	3.75
	7. SG-63C	4.54			8. SP-63C	4.89	8. PG-63C	4.82
High Count	8. PG-63C	6.00	8. PG-63C	6.21				

<sup>a</sup>Treatment-Temperature codes: NaCl, sodium triphosphate, and glucose, SPG; NaCl and sodium triphosphate, SP; NaCl and glucose, SG; sodium triphosphate and glucose, PG; 63C internal cooking temperature, 63C; 94C internal cooking temperature, 94C.

<sup>b</sup>Low count = Log 0-2 CFU/g; intermediate count = Log 3-4 CFU/g; high count = Log 5-6 CFU/g.



samples without salt (PG) and treated at low temperature (63C) showed consistently higher counts of both mesophilic and psychrotrophic organisms regardless of the boning type and treatment combination.

For vacuum packed samples only 21 day storage data were analyzed (Table 11). Again using the scale of Fung et al. (1980) the data are ranked and grouped similar to the 10 day aerobic storage data analysis.

For mesophilic count of hot-boned samples, 4 (PG High, SPG Low, SP Low, and SP High) fell in the low count region, 2 (PG Low and SG Low) were in the intermediate region, and 2 (PG Low and SG Low) were in the high count region. For psychrotroph count of hot-boned samples, 2 (PG High and SP High) were in the low count region, 5 (SPG Low, SP Low, SPG High, SG High and PG Low) were in the intermediate count region, and 1 (SG Low) was in the high count region. For mesophilic count of cold-boned samples 6 were in the low count region and 2 (SG Low and PG Low) were in the intermediate count region. For psychrotroph count of cold-boned samples 5 were in the low count region and 3 (SG High, PG Low and SP Low) were in the intermediate region. Similarly to the data of the 10 day aerobic storage data most of the vacuum packaged samples treated by high temperature had lower counts compared with those treated with low temperature. SG Low and PG Low showed higher counts

TABLE 11. 21-day vacuum storage--analysis by grouping and ranking.

	Hot-boned Roasts				Cold-boned Roasts			
	Mesophiles		Psychrotrophs		Mesophiles		Psychrotrophs	
	Rank	Log CFU/g	Rank	Log CFU/g	Rank	Log CFU/g	Rank	Log CFU/g
Low <sup>b</sup> Count	1. PG-94C <sup>a</sup>	1.89	1. PG-94C	2.19	1. SP-94C	0.81	1. PG-94C	1.39
	2. SPG-63C	2.79	2. SP-94C	2.76	2. SPG-94C	1.11	2. SP-94C	1.74
	3. SP-63C	2.84			3. SG-94C	1.93	3. SPG-94C	2.21
	4. SP-94C	2.90			4. PG-94C	2.36	4. SG-63C	2.58
					5. SPG-63C	2.90	5. SPG-63C	2.68
					6. SP-63C	2.99		
Intermediate Count	5. SG-94C	3.39	3. SPG-63C	3.44	7. SG-63C	3.42	6. SG-94C	3.47
	6. SPG-94C	3.94	4. SP-63C	3.79	8. PG-63C	4.02	7. PG-63C	3.79
			5. SPG-94C	3.87			8. SP-63C	4.12
			6. SG-94C	4.52				
			7. PG-63C	4.98				
High Count	7. PG-63C	5.30	8. SG-63C	5.63				
	8. SG-63C	5.84						

<sup>a</sup>Treatment-Temperature codes: NaCl, sodium tripolyphosphate, and glucose, SPG; NaCl and sodium tripolyphosphate, SP; NaCl and glucose, SG; sodium tripolyphosphate and glucose, PG; 63C internal cooking temperature, 63C; 94C internal cooking temperature, 94C.

<sup>b</sup>Low count = Log 0-2 CFU/g; intermediate count = Log 3-4 CFU/g; high count = Log 5-6 CFU/g.

compared with samples of other treatment combinations. Cold-boned vacuum packaged samples showed more treatments falling into the low count region than did the hot-boned vacuum packaged samples.

#### Relationship to Goll's (1987) Research

The major portion of research on these restructured roast beef logs was undertaken by Goll, 1987. Goll's research revealed that acceptable products are produced with all treatments. Parameters tested include proximate analysis, cohesiveness, yield, rancidity (warmed over flavor), protein extraction, and consumer acceptability.

Treatments cooked to the high temperature were perceived to have less warmed over flavor than those cooked to the low temperature; this result by taste panels is confirmed by TBA values. The least amount of rancidity was measured with treatment SPG (hot-boned and cold-boned) and SP (cold-boned) (Goll, 1987). The formation of antioxidants by the Maillard reaction with the addition of glucose resulted in less rancidity of meat lipids.

Bacteriological data in this study indicated that salt and high temperature treatment are most effective in preventing bacteriological proliferation during storage (aerobic and vacuum packaged).

### Conclusion

The most important result of this experiment is the significant part that proper cooking temperature (i.e. high enough to destroy most intrinsic microorganisms) plays in the process of preservation. Of secondary importance, the presence of NaCl inhibits growth of both psychrotrophic and mesophilic microorganisms. The evidence is seen in higher numbers of colony forming units per gram and a reduction in pH in the restructured products made without salt. The high microbial counts of cold-boned beef treated with NaCl and phosphate and cooked to 63C does cast some doubt on this conclusion.

NaCl changes the ecological environment of the beef logs by 1) lowering the water activity, and 2) dropping the pH by a slight margin in hot-boned beef. It is noteworthy that the hot-boned beef treated with NaCl and phosphate does not elicit the same growth response as with cold-boned beef. The addition of phosphate in the form of sodium tripolyphosphate does not affect the microbial loads to any significant degree. The combination of salt, phosphate and glucose provided consistent overall antimicrobial guarantees in a restructured roast beef product, both with hot-boned and conventionally-boned beef.

No proof of any antimicrobial activity of the part of Maillard reaction products is noted. There could be an

interaction in treatment SPG where NaCl served to lower the water activity to the optimum for the Maillard reaction, and the phosphate maintained a pH also optimum for the Maillard reaction. That may be why treatment SPG has consistently low microbial loads for both psychrotrophs and mesophiles at 10 days of display.

As in previous experiments (Lee et al., 1982; Fung et al., 1980), there was no significant difference in either mesophilic or psychrotrophic loads from hot-boned or cold-boned beef. The restructured products made in this experiment were made and cooked soon after slaughter in the case of the hot-boned beef and at 48 hrs post mortem in the case of cold-boned beef. No significant differences were noted ( $P > 0.05$ ) between the two boning techniques, but higher means were noted for the hot-boned beef for both mesophilic and psychrotrophic microorganisms especially when processed to a lower temperature.

The effect of temperature processing upon the shelf-life of this product type is underscored. Microbial loads increased by three log cycles between 4 and 10 days of display when cooked to 63C. When cooked to 94C, microbial loads increased by just two log cycles. This gives a fair idea when spoilage will begin to occur. Levels of Log 5-6 CFU/g obtained for psychrotrophic microorganisms was the highest achieved, with the highest noted in treatment PG.

This treatment also had the highest mean mesophilic count for hot-boned beef, while the cold-boned samples treated with salt and phosphate had the highest mesophilic levels. Most treatments did not achieve a growth of more than the intermediate count of Log 3-4 CFU/g.

In summary, of all treatment combinations the sample with the lowest bacteriological counts was cold-boned beef treated with NaCl, phosphate and glucose and thermally processed to an internal temperature of 94C. However, all treatments from both hot-boned and cold-boned beef and processed to 63C and 94C were microbiologically acceptable (i.e. not spoiled) during the display period of ten days at 4C, or vacuum packaged for 21 days at 4C.

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EFFECTS OF GLUCOSE PHOSPHATE, SALT, AND COOKING  
TEMPERATURES ON THE MICROBIOLOGY OF PRE- AND  
POST-RIGOR, RESTRUCTURED BEEF ROASTS

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## ABSTRACT

Restructured beef roasts were formulated from hot-processed and corresponding conventionally processed beef. The beef samples were split eight ways; two were treated with NaCl, sodium tripolyphosphate and glucose (SPG), two were treated with NaCl and sodium tripolyphosphate (SP), two were treated with NaCl and glucose (SG), two were treated with phosphate and glucose (PG). Paired samples were stuffed into logs and heat processed -- one to 63C and one to 94C. Samples for pH, and microbiological analysis were taken of the raw untreated meat, the raw treated meat, and after cooking. The logs were frozen, thawed and then displayed at 4C under oxygen permeable film or vacuum packaged. The displayed roasts were sampled at 0, 2, 4 and 10 days for pH, mesophilic, and psychrotrophic growth. The vacuum packaged roasts were sampled at 21 days for mesophilic and psychrotrophic growth. Hot-boned beef had a significantly ( $P < 0.05$ ) higher pH throughout the experiment. After 10 days aerobic storage treatment PG shows a significant ( $P < 0.05$ ) drop in pH while the other treatments remained the same. Over the 10-day display, PG showed a significantly ( $P < 0.05$ ) higher mesophilic count than other treatments. Analysis of the 10-day mesophilic counts alone did not show a treatment or



boning difference. Roasts treated to 63C vs 94C internal temperature showed significantly ( $P < 0.05$ ) higher mesophilic and psychrotroph counts.

No differences are noted in psychrotroph counts at 10-days between PG and SG, SG and SP, or SPG and SP, with the highest levels in PG. Vacuum packaged roasts heated to 63C showed significantly higher mesophilic and psychrotrophic counts than roasts heated to 94C, but showed no significant difference due to treatment or boning. By grouping and ranking the data, the aerobically stored and vacuum packaged samples both showed higher counts with the 63C treated samples. For the aerobically stored samples, PG treated to 63C consistently had higher counts than other treatment-temperature combinations. For the vacuum packaged samples, SG and PG treated to 63C had higher counts than other treatment-temperature combinations. Hot-boned samples had more treatment-temperature combinations falling into the intermediate and high count regions for both mesophiles and psychrotrophs when vacuum packaged. All treatments were acceptable (i.e. not spoiled) whether aerobically displayed or vacuum packaged for the storage period.