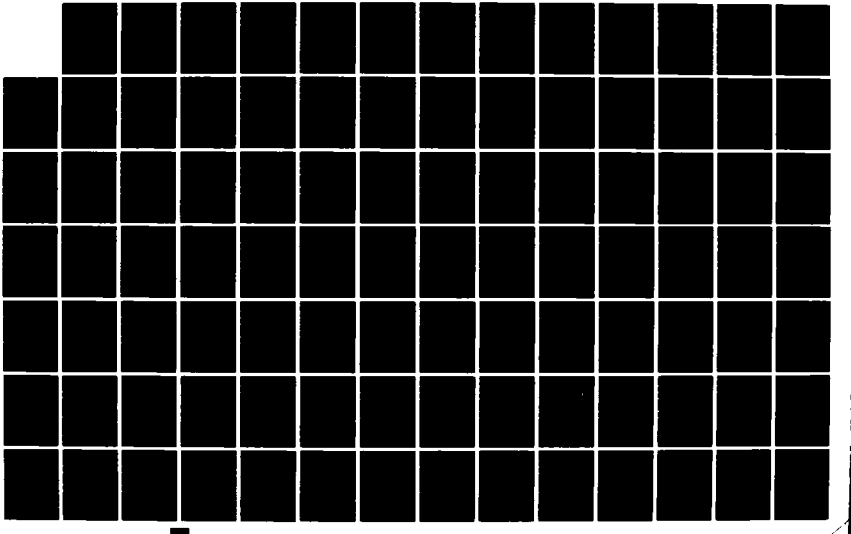
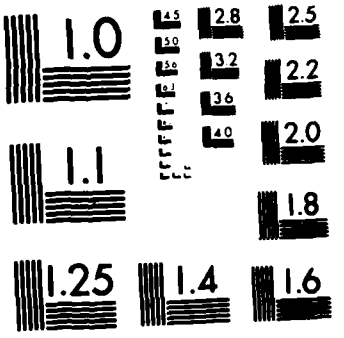
AD-A145 426 EFFECTS OF LACTIC ACID BACTERIA ON RESIDUAL NITRITE IN A SUMMER STYLE SAUSAGE(U) UTAH UNIV SALT LAKE CITY B L WOODBURY 1884 1/2

UNCLASSIFIED F/G 6/13 NL





EFFECTS OF LACTIC ACID BACTERIA ON RESIDUAL NITRITE IN A SUMMER STYLE SAUSAGE

by

Bruce L. Woodbury

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah
1984

EFFECTS OF LACTIC ACID BACTERIA ON RESIDUAL NITRITE IN A SUMMER STYLE SAUSAGE

by

Bruce L. Woodbury

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

Darrell T. Bartholomew
Major Professor

Fred J. Pat
Committee Member

Daren Comforth
Committee Member

John R. Simmons
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah
1984

ACKNOWLEDGEMENTS

Initially my gratitude is extended to the United States Army for this active duty assignment at Utah State University. I am honored to have been selected to participate in the Advanced Civil Schooling Program allowing me to study for this degree at the Army's expense, at my alma mater and in my hometown.

Appreciation goes to Dr. Darrell T. Bartholomew of my Graduate Committee for his enlightening supervision and guidance; Dr. Daren P. Cornforth for his willingness to answer questions and provide use of some needed materials; and to Dr. Frederick J. Post for his timely classroom instruction and review of this thesis.

My thanks goes to my children, Angela, Ben and Ryan for their interest in my work and desire to share some aspects of it with their friends and classes at school.

Finally, to my wife Pat, I extend eternal gratitude for her willingness to share the experience, her ability to listen, and her patience to endure.

Bruce L. Woodbury

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF FIGURES

Figure — Page

ABSTRACT

Effects of Lactic Acid Bacteria on Residual Nitrite in a Summer Style Sausage

by

Bruce L. Woodbury, Master of Science
Utah State University, 1984

Major Professor: Dr. Darrell T. Bartholomew
Department: Nutrition and Food Sciences

Lactic acid bacteria were evaluated for their ability to reduce nitrite and produce acid. Tests were conducted in broth, model meat systems, and a fermented sausage. The bacteria were screened for their ability to reduce nitrite irrespective of pH effects, in broth containing no added carbohydrate. Lactobacillus leichmannii, Streptococcus faecalis, and several atypical lactobacilli isolated from fresh beef and mutton reduced 200-1000 ppm nitrite in 48-72 hours. Reduction by L. leichmannii and S. faecalis was inhibited by carbon monoxide, suggesting a possible heme-containing nitrite reductase enzyme.

Studies conducted in broth and model meat systems showed significant differences in nitrite reduction and acid production with longer incubation times, higher temperatures, and increased carbohydrate levels. Differences were observed in degree of nitrite reduction due to meat source (beef versus mutton) and bacterial treatment.

Residual ritrite and pH were highly correlated in all media containing fermentable carbohydrate. Decreasing pH values enhanced significantly the rate of nitrite depletion in all bacterial media.

Three bacterial strains (L. leichmannii, S. faecalis and an atypical lactobacillus isolated from beef) showed abilities to reduce pH and residual nitrite to levels similar to L. plantarum and P. pentosaceus and deserve further evaluation for use in commercial starter cultures.

(107 pages)

INTRODUCTION

Historical

The addition of nitrate and nitrite to meats is an ancient practice. Although probably unintentional at first, nitrate was introduced to meats as an impurity of salt used as a preservative. The acceptability of the reddening effect of nitrate led to its deliberate addition to meat to provide color uniformity (Crosby and Sawyer, 1976). The original form of nitrate added to meat was calcium nitrate ($Ca(NO_3)_2$), produced by nitrifying bacteria on the walls of stables and caves (Jensen, 1954).

With time nitrate was used regularly as a curing agent. Toward the end of the ninteenth century chemists employed by the meat industry transformed meat curing from an art to a science (Sofos et al., 1979). A notable discovery of that period was that nitrite, reduced from nitrate by bacterial action, was the responsible agent for cured meat color development (Binkerd and Kolari, 1975). In 1901, Haldane reported the formation of nitrosohemoglobin by the interaction of hemoglobin and nitrite, and its subsequent transformation to the compound nitrosohemochrome, the cured meat color. Hoagland (1908) explained the microbial and enzymatic reduction of nitrate to nitrite (or nitrous acid in water) and further reduction to nitric oxide.

Regulation

The regulation of nitrate began in 1908 when the USDA permitted the addition of saltpeter to meat and meat food products (USDA, 1908). The Bureau of Animal Industry (USDA) gave permission in 1923 for research on the direct use of nitrite in meat products. The pioneer work of Kerr et al. (1926) dealt with the practicality of nitrite use as a curing agent and it's implication to human safety. Their extensive work led to the conclusions that: (a) sodium nitrite could replace nitrate in meat curing; (b) 1/4 to 1 oz of sodium nitrite (156 to 625 mg nitrite/kg meat) was sufficient for cured meat color production, based on the meat and curing method; (c) nitrate could be avoided since the levels of nitrite required for curing were not higher than those found in meat cured with nitrate; and (d) the quality and wholesomeness of meat cured with nitrite were not inferior to meat cured with nitrates (Sofos et al., 1979). As a result of these findings, the USDA released authorization in 1925 for the use of sodium nitrite in meat curing in Federally inspected plants, with the requirement that nitrite in the finished product be limited to not more than 200 mg nitrite/kg (USDA, 1925).

During the decade of the 1950's, the safety of nitrite in cured meats began to be questioned due to scientific findings. Magee and Barnes (1956) discovered the carcinogenic properties of dimethylnitrosamine on the

hepatic tissue of rats. N-nitrosodimethylamine was isolated as the toxic substance producing a rare liver disorder in ruminants and mink fed herring meal preserved with nitrite (Crosby and Sawyer, 1976). These reports raised some skepticism about the importance of nitrite as a food additive. As a result of this concern, a joint study was undertaken in 1972 by the USDA, the Food and Drug Administration (FDA) and the American Meat Institute (AMI) to ascertain the role of nitrite and nitrate in processed meats (Bard, 1977). Their thrust was in the areas of safety from Clostridium botulinum toxicity and nitrosamine formation. That extensive research concluded that: (a) nitrite reduced the risk of botulinal toxicity when used at regulated levels; (b) nitrosopyrrolidine (a nitrosamine) was found in parts per billion (ppb) in severely fried bacon; (c) lower nitrite levels combined with ascorbate or isoascorbate decreased nitrosamine levels in bacon; and (d) nitrate produced no apparent C. botulinum controlling effects.

Concern turned to controversy during the 1970's as science, industry and government became more aware of the implications of nitrite effects on human health. Upon recommendation by an Expert Panel on nitrites, nitrates and nitrosamines, the USDA published proposed regulatory changes to nitrite and nitrate levels to reduce their public health significance (USDA, 1975). The changes include: (a) a limit

of 2183 parts per million (ppm) (3.5 oz) sodium nitrate or 2597 ppm (4.2 oz) potassium nitrate to be added to 100 pounds of meat in dry cured products and 1716 ppm (2.75 oz) sodium nitrate or 2042 ppm (3.3 oz) potassium nitrate to be added to 100 pounds of meat in fermented sausages; (b) a limit of 624 ppm (1 oz) sodium nitrite or 768 ppm (1.23 oz) potassium nitrite to be added to 100 pounds of meat in dry cured products and 156 ppm (0.25 oz) sodium nitrite or 192 ppm (0.31 oz) potassium nitrite to be added to 100 pounds of meat in fermented sausages; (c) a residual limit of 200 ppm nitrite, calculated as sodium nitrite, in dry cured products and fermented sausages, whether cured with nitrate, nitrite or a combination; (d) a limit of 156 ppm sodium nitrite or 192 ppm potassium nitrite incorporated into canned cured products, whether shelf stable, sterile or perishable; in cooked sausages; in other cured perishable products (except bacon), with a residual nitrite limit of 50 ppm in canned cured sterile products, 125 ppm in all other canned cured products, and 100 ppm in cooked sausages; (d) the discontinued use of nitrates and nitrites in commercially prepared infant (strained) or junior (chopped) foods; and (e) a limit of 125 ppm added nitrite to bacon with an accompanying amount of ascorbate or isoascorbate (erythorbate) added at the maximum allowable limits.

Further study of the nitrite levels required in bacon resulted in the USDA regulation of 1978 limiting the ingoing

nitrite levels to 120 ppm with 550 ppm ascorbate or isoascorbate also added and the use of nitrate discontinued (USDA, 1978). Industry honors this regulation concerning bacon as well as the 1975 proposed changes to nitrite levels in all other cured meat products, even though the higher levels of the 1925 regulation are legally still in effect.

Role of Nitrite

The function of nitrite in processed meat products is fourfold: (a) it stabilizes the red color of the lean tissue (Fox, 1966); (b) it contributes to the characteristic flavor of cured meat (Dethmers et al., 1975); (c) it has antioxidant properties (Sofos et al., 1979); and (d) it acts generally as an antimicrobial agent (Widdus and Busta, 1982) and specifically provides protection against the growth of C. botulinum and subsequent toxin production (Marriot et al., 1981).

Color

The typical color of cured meat results from the reaction of nitrite with the heme pigments found in meat. The reduction of nitrite by both naturally occuring reducing compounds (e.g., NADH, cysteine, tocopherols, hydroquinones) and those added to the meat (e.g., ascorbate, erythorbate) provides sufficient nitric oxide to nitrosylate the ferrous (Fe^{+2}) heme iron of myoglobin and hemoglobin (Fox and Ackerman, 1968). Thermal denaturation of the protein

(globin) portion of the heme molecule, by temperatures of 65^{o} C or greater, results in the compound nitrosohemochrome, the cured meat color ranging from pink to red. Kerr et al. (1926) reported acceptable color and flavor developed in hams having as little as 20 mg residual nitrite/kg. Hustad et al. (1973) found that nitrite-free weiners had no cured meat color while weiners formulated with 50, 100 and 150 mg nitrite/kg showed no color difference.

Although the pink-red color of cured meat is a beneficial side condition of a nitrite-containing meat product, nitrite is not considered a color additive. Dr. Robert Terrell, Texas A&M University (Anon, 1980) submitted data to the Food and Drug Administration (FDA) showing that nitrite, a white crystalline powder (colorless in an aqueous solution) added to meat, produces no color change as a pigment or dye would. He explained that only when the naturally occuring meat pigment myoglobin is heated in the presence of nitrite is the typical pink cured meat color obtained. Dr. Terrell reasoned that the intensity of color in the product was more dependent on the concentration of myoglobin than that of nitrite, and that the fresh meat color is only fixed or stabilized rather an artificial color imparted.

Flavor

The majority of studies reporting the effect of nitrite on cured meat flavor are concerned with sensory evaluation

of meat products or chemical analysis of the reactions between nitrite and meat (Sofos et al., 1979). The scientific consensus is that less than 200 mg nitrite/kg is required to give meat the characteristic cured meat flavor (Mottram and Rhodes, 1974). Sensory evaluations conducted by Dethmers et al. (1975) found that at least 50 mg nitrite/kg was required to give thuringer sausage a typical flavor, and higher nitrite concentrations gave increased scores to flavor and appearance. Similar trends have been found with weiners (Wasserman and Talley, 1972), bacon (Herring, 1973), and fermented sausages (Kueper and Trelease, 1974).

Antioxidant

Nitrite added to cured meats provides some protection from the off-flavors and odors resulting from rancidity. This condition is caused by the breakdown of unsaturated fats, and subsequent release of free fatty acids, by the oxidative effects of free oxygen and to a lesser degree by the lipolytic and oxidative powers of bacterial enzymes (Kramlich et al., 1973). Cross and Ziegler (1965) concluded that nitrite interferes with the oxidation of unsaturated lipids, possibly by complexing with heme-containing catalysts found in the meat. Herring (1973) reported that off-flavors were higher and increased more rapidly in bacon cured with 0 and 15 mg nitrite/kg while off-flavors were

lower and acceptance scores higher in bacon formulated with 170 mg nitrite/kg.

Botulinal Protection

The most important role of nitrite in cured meat is the protection it provides against C. botulinum growth and toxin production. The overall success of commercially processed cured meat in remaining free of botulinal toxin has been attributed to nitrite; however, other influental factors include low spore incidence, heat processing, pH and refrigeration. Botulinal safety is at the expense of higher nitrite levels in the meat than those required for color and flavor development (Sofos and Busta, 1980).

The exact mechanism of the antibotulinal property of nitrite has received a considerable amount of research in the last 15 years. Two previously considered theories, which remain unsubstantiated, are: (a) that nitrite reacts initially with botulinal spores to cause inhibition; and (b) that nitrite reacts with some component of the meat to form an inhibitor or Perigo-like factor (Christiansen, 1980). It is accepted that botulism spores readily germinate in the presence of nitrite but are likewise prevented from outgrowth by nitrite (Christiansen, 1980). This has led researchers to examine the role of the naturally occuring, or added, vitamin and mineral content of meat in relation to the antibotulinal properties of nitrite. Tompkin et al. (1978c) examined the effects of calcium, iron, manganese,

zinc and riboflavin content on botulinal growth in perishable canned cured meat and found that only iron, as influenced by residual nitrite level and spore load, had an effect on botulinal inhibition. Current work by Reddy et al. (1983) suggests that nitrite may inactivate iron-containing or iron-activated enzymes in clostridia.

Nitrite also has been shown to have an increasing inhibitory effect toward C. botulinum growth at lower pH levels. Grindly (1929) noticed increased inhibition by nitrite under acid conditions and postulated that nitrite converts to nitrous acid under conditions of low pH. Tarr (1941a, 1941b, and 1942) confirmed this theory and showed that the inhibitory effect of nitrite upon several species of bacteria increased markedly at pH levels below 6.0. Roberts and Ingram (1966) reported a tenfold increase in the inhibitory effect of nitrite from pH 7.0 to 6.0. Shank et al. (1962) found that maximum protection from C. botulinum was achieved at lower pH levels due to the presence of undissociated nitrous acid as the active molecular species. They also showed that at approximately pH 5.5 the nitrite effect diminished as it reacted with constituents of the medium.

The role of nitrite initially introduced to a meat system versus the level remaining after processing and during storage on the inhibition of C. botulinum has caused some controversy. Christiansen et al. (1973 and 1974)

initially accomplished work which supported the idea that initial nitrite was responsible for curing the meat and providing botulinal inhibition. Tompkin et al. (1978b) indicated residual nitrite may only be indirectly related to botulinal inhibition by serving as a reservoir for a reactive intermediate such as nitrous acid. Christiansen (1980) readdressed this problem and reversed his position due to subsequent work. He showed that as residual levels of nitrite decreased in perishable canned cured meat, so did the germinated C. botulinum cell level (Christiansen et al. 1978). He found no difference in the rate of spore germination between the initial levels of 50 and 156 ppm nitrite; however, the number of germinated cells decreased concurrently with the depleting nitrite. Christiansen (1980) explained the inhibitory effect of nitrite as a race between residual nitrite depletion and the death of germinated botulinal spores. Tompkin et al. (1978a) gave further evidence for the importance of residual nitrite by showing the accelerated growth of germinated cells after extended refrigeration in a nitrite depleted system.

Nitrite and Nitrosamines

N-nitrosamines may be formed in the environment, particularly food and water, whenever secondary or even primary or tertiary amines are exposed to nitrite (Scanlan, 1975). Numerous studies have shown the presence of amines,

capable of forming nitrosamines, naturally occuring in meats (Gray and Randall, 1979). Approximately 21.2% of the human average daily intake of nitrite comes from cured meats. The majority (76.8%) comes from the action of normal bacteria, and other microorganisms of the mouth, which reduce nitrate introduced in the consumption of vegetables and fruits (White, 1975). N-nitroso compounds are known to be toxic, teratogenic, mutagenic and carcinogenic (Magee, 1971) but how these findings relate to cancer in man requires further study.

There are two opposite opinions on the deleterious effects of nitrosamines to humans. Jones and Grendon (1976) contend that continuous dietary exposure to bacon and other cured meats, having nitrite levels as currently regulated, would not produce cancers in the lifetime of humans. Conversely, Lijinsky (1976) concluded that the levels of N-nitrosopyrrolidine found in cooked bacon caused the development of tumors in rats, and because he considered these effects cumulative to other carcinogenic substances, it posed a definite health hazard to humans. Because nitrosamines have not been proven non-carcinogenic to humans, nitrite used as a curing agent has continued to be scrutinized and regulated while substitutes are sought and tested.

The existence of nitrosamines in cured meat varies from product to product. In response to a request by the USDA

for data, the Nitrite Safety Council (1980) sponsored a study to test a wide variety of cured meats for nitrosamines. Their conclusions were that: (a) cooked sausages, semi-dry and dry sausages are free of nitrosamines; (b) dry-cured hams and shoulders are generally free of nitrosamines, but the occurrence of N-nitrosopyrrolidine in 3 of 31 fried samples, occuring randomly across the several processing techniques, suggested further sampling was necessary; and (c) dry-cured bacon had an increased incidence of N-nitrosopyrrolidine over other dry-cured products and therefore would require further research. The fermented sausage results are enhanced by the findings of Dethmers et al. (1975) that no nitrosamines were detected in thuringer sausage, a semi-dry product fermented with lactic acid producing bacteria, with 0-150 ppm added nitrite and/or 0-1500 ppm added nitrate.

Nitrite Depletion

The role of residual nitrite in controlling *C. botulinum* and the formation of nitrosamines has caused researchers to examine both the fate of nitrite in cured meat products and the factors influencing the rate of nitrite depletion. Nitrite starts to deplete as soon as it is added to meat. This depletion is continuous and the rate depends upon product formulation (including natural and added reductants), pH, time and temperature relationships,

and subsequent storage (Fox and Nicholas, 1974; Nordin, 1969). Nordin (1969) found that the half-life of nitrite in canned ham at 30° C and pH 6.5 was 5 days. A simple relationship between nitrite level and time has not been found, probably due to uncontrolled variables such as pH (Sofos et al., 1979). While large amounts of nitrite are lost during processing, differences in quantities are observed between lots of meat, among laboratories, between products examined, and across storage temperatures (Sofos et al., 1979). Fiddler et al. (1972) suggested that 10-30% added nitrite is retained in commercially manufactured weiners while Hustad et al. (1973) found an average of 33% of added nitrite remained in weiners after manufacture. In canned comminuted pork, a large nitrite depletion was observed during formulation while cooking had little effect (Christiansen et al., 1973). Hustad et al. (1973) found however, that nitrite disappearance in weiners during cooking was higher than during processing. In observing a heated chopped beef product, nitrite depletion was shown to be directly proportional to the meat concentration (Olsman and Krol, 1972).

Temperature

The storage temperature of a cured meat product influences the rate at which nitrite depletion occurs. The higher the storage temperature, the faster the rate of nitrite disappearance . This nitrite depletion has been

shown in products such as weiners (Hustad et al., 1973), thuringer sausage (Dethmers et al., 1975), comminuted pork (Christiansen et al., 1973), and bacon (Christiansen et al., 1974).

Oxidation/Reduction

Nitrite (NO_2) may form a number of other nitrogen containing compounds, depending on the oxidation/reduction potential of the meat system. Ascorbate, the salt of ascorbic acid, and isoascorbate (erythorbate), the salt of an ascorbic acid isomer, are common reductants added to cured meats, and are required in bacon. Using a model system composed of myoglobin, nitrite, and ascorbate, Japanese researchers (Fujimaki et al., 1975) found all of the nitrogen in the added nitrite recovered as residual nitrite, nitrate, denatured nitric oxide myoglobin, and gaseous nitrogen compounds composed of nitric oxide, dinitric oxide and nitrogen gas. A study of nitrite in whole meat and meat fractions by Emi-Miwa et al. (1976) showed that 66 to approximately 90% was recovered as nitrite, nitrate, nitrosothiol, nitrosomyoglobin, and gaseous nitrogen compounds. The amount of nitrite unaccounted for varied depending on curing time and quantity of added ascorbate.

Several researchers have reported the presence of residual nitrate in products where no nitrate has been added. Herring (1973) reported 30% of nitrite added to

bacon was converted to nitrate within 1 week, and 40% by the 10th week of storage. Christiansen et al. (1974) noted that the amount of nitrate formed in bacon was in direct proportion to the amount of added nitrite.

The chemical state of myoglobin has been suggested as a factor in the oxidation of nitrite to nitrate. Lee et al. (1978) found that nitrate was formed from nitrite when both metmyoglobin and ascorbate were present. He theorized that ascorbate reduced metmyoglobin to myoglobin which in turn was oxidized back to metmyoglobin with a concurrent oxidation of nitrite to nitrate.

pH

The pH of cured meat has a definite effect on the rate of nitrite depletion. It can be generally said that the lower the pH of a meat system, the faster the rate of nitrite depletion. The pH values of cured meats generally fall within the range of pH 5.5 to 6.6 (Lechowich et al., 1978). Olsman and Krol (1972) considered the formation of nitrous acid (HNO_2) to be a product of nitrite depletion as the pH level decreases, and reported that at the pH of cured meats only a small amount of nitrite was present in the acid form. Nitrite loss followed first order kinetics at pH values of 6.0 or above while at levels below 6.0 the loss followed first to second order kinetics.

Goodfellow (1979) stated that an approach to the nitrosamine problem is to lower the residual nitrite level

of cured meat, such as bacon, by chemical or biological acidification. The pH of a system has an influence on the rate cured meat pigment is developed from the pool of available nitrite. A primary reason for lowering the pH of cured meat products is to accelerate pigment development by increasing the amount of nitrite converted to nitric oxide. The fraction of added nitrite (156 mg/kg product) used in color production varies with product formulation and nitrite distribution. Hustad et al. (1973) reported that only 25-50 mg nitrite/kg were necessary to provide weiners with good color production; however, Rubin (1977) suggested that 75 mg nitrite/kg may be needed under commercial processing conditions. He also stated that as little as 3 mg nitrite/kg is theoretically required for color production, assuming 50% of the myoglobin is converted to nitrosomyoglobin. In his review on the chemistry of meat pigments, Fox (1966) explained that reduced pH conditions enhance the reaction of nitrite with natural or added reductants to form nitric oxide, which binds with heme proteins in the formation of nitrosomyoglobin.

The reaction of nitrite with the sulfhydryl groups of meat proteins has been evaluated as a possible mechanism to explain the loss of some nitrite. Kubberod et al. (1974) reported the rate of reaction between sulfhydryl groups and nitrite was slow at the pH of meat and therefore could only account for a small amount of nitrite lost in curing;

however, Olsman and Krol (1972) found that blocking the sulfhydryl groups in chopped beef with a thiol alkylating agent slowed the rate of nitrite loss.

Woolford et al. (1976) suggested that a major pathway for nitrite depletion may be through its reaction with non-heme proteins. They found the reaction between nitrite and myosin or bovine serum albumin increased with decreasing pH, increased added nitrite levels, and increased incubation time. Olsman and Krol (1972) also found that as pH levels decreased, the protein-bound nitrite concentrations increased.

Bacterial Reduction

Lactic acid producing bacteria make up a significant portion of the natural microflora of both vacuum-packed fresh meats and cured meat and meat products (Kitchell and Shaw, 1975). Vacuum-packaging inhibits the growth of aerobic gram-negative rods, the chief spoilage organisms of unwrapped or gas-permeable film wrapped fresh meat (Gardner et al., 1967), but enhances the growth of facultative to anaerobic lactic acid producers. Likewise, the oxygen-impermeable wrap used to prevent oxidation of cured meat color insures a reduced oxygen, increased carbon dioxide environment conducive to these bacteria (Kitchell and Shaw, 1975). In addition, lactic acid bacteria have been used as starter cultures in fermented sausages (Deibel et al., 1961), added as single or double strains, to control

the fermentation rate and produce a consistent flavor (Reuter, 1975).

The contribution of both natural and added lactic acid producing bacteria to nitrite reduction in cured meats has received little attention in the literature. Ingram (1975) noted that certain lactobacilli have the ability to reduce nitrite in meat products, but made no reference to other published work. Bartholomew and Blumer (1980) also reported low residual nitrite levels in dry-cured ham inoculated with lactic acid producing bacteria. The depletion of nitrite by reduced pH from the lactic acid production of these bacteria is recognized by science, industry, and government. The USDA (1979) lists those lactic acid bacteria naturally present on meat as generally recognized as safe and allows their use to decrease the nitrosamine risk in bacon. The concept is that lactobacilli and pediococci added to bacon in the presence of a fermentable carbohydrate produce lactic acid which lowers the pH of the product. The reduced pH lowers residual nitrite levels by enhancing nitrite depletion, all without increasing the risk of C. botulinum toxin production. The low nitrite levels remaining in the bacon provide little substrate for the formation of nitrosamines.

The occurrence of nitrite reducing enzyme systems (nitrite reductase) has been observed in a number of bacteria. Youatt (1954) reported that a species of

Achromobacter reduced nitrite at a higher rate under anaerobic rather than aerobic conditions. These bacteria possess a dissimilatory pathway for reducing nitrite to gaseous nitrogen, or other oxides of nitrogen, by a series of respiratory processes (Payne, 1973). Thauer et al. (1977) stated that those bacteria possessing a dissimilatory pathway are facultative organisms which can grow anaerobically only when supplied with an inorganic substitute for oxygen. Payne et al. (1971) stated that the nitrite reductase enzymes are generally found in the soluble components of the cell, rather than being membrane-bound as in the nitrate reductase enzymes of denitrifying bacteria. Initially nitrite reductases were thought to show no metal involvement, where nitrate reductases are known to be complexed to molybdenum, but studies by Payne et al. (1971) also revealed a heme-nitric oxide complex during nitrite reductase action. A reduction in the iron concentration of a system used by Youatt (1954) also slowed the action of nitrite reductase.

Collins-Thompson and Rodriguez Lopez (1981) showed that various lactic acid bacteria, isolated from vacuum-packed bologna, reduced nitrite at different rates. Leuconostoc mesenteroides reduced nitrite at a faster rate than either Lactobacillus plantarum or Lactobacillus viridescens, while Lactobacillus brevis showed little reducing ability at all. They reported that pH levels for all samples remained very

nearly equal to each other at any one point in storage time, indicating that nitrite depletion was species dependent rather than pH dependent.

Evidence for a nitrite reductase enzyme system in lactobacilli was presented by Fournaud and Mocquot (1966). They found that under anaerobic conditions, and in the presence of sodium pyruvate, nitrite was reduced to either nitrogen dioxide, nitrous oxide or nitrogen by Lactobacillus lactis, Lactobacillus leichmannii and Lactobacillus buchneri. The optimum conditions were found to be pH 6.5 (6.2 for L. lactis) and an incubation temperature of 22° C. The reduction reaction was inhibited by potassium cyanide and para-chloromercuribenzoate, complimenting similar results found by Youatt (1954).

OBJECTIVE

The purpose of this research was to observe the effect of selected lactic acid bacteria on the residual nitrite of model meat systems and a fermented meat product. These bacteria were selected from commercial starter cultures, pure cultures, and from fresh beef and mutton isolates. Supporting objectives were to: (a) determine the acid production capabilities of these bacteria; and (b) determine their nitrite reduction rates versus reduction due to chemical acidification.

MATERIALS AND METHODS

Bacterial Treatments

Bacterial strains were obtained from several sources. Twelve species were purchased from the American Type Culture Collection, Rockville, Maryland (Table 1). Thirteen strains were obtained from commercial starter cultures donated by various manufacturers (Table 2). A strain of Streptococcus faecalis (Table 3) was isolated as a contaminant of fresh ground pork leg used in a study on Clostridium botulinum inhibition (Vahabzadeh et al., 1983). Six lactobacilli (Table 3) were obtained from a previous study, as yet unpublished, in which 320 bacteria were isolated from fresh beef and mutton using two different growth media both containing 120 parts per million (ppm) sodium nitrite, two incubation times, two temperatures, and two sodium chloride concentrations.

These 320 isolates were identified as strains of atypical lactobacilli using identification procedures (Table 4) at the VPI Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia and Bergey's Manual (Buchanan and Gibbons, 1974). They are gram positive, homofermentative lactic acid producers. These bacteria were grouped according to growth patterns, fermentation patterns and terminal pH, gel electrophoresis of the soluble proteins of the lysed cells, and gas

Table 1--Lactic acid producing bacterial strains from the American Type Culture Collection

Culture	ATCC Number
Lactobacillus acidophilus	4365
Lactobacillus casei	E393
Lactobacillus leichmannii	4797
Lactobacillus plantarum	4008
Lactobacillus bulgaricus	11842
Lactobacillus fermentum	9338
Lactobacillus casei subsp. *rhamnosus*	7469
Pediococcus acidilactici	8081
Lactobacillus viridescens	12706
Streptococcus faecalis	E19433
Streptococcus faecium	19432
Leuconostoc mesenteroides	8293

chromatography and optical rotation of the lactic acid end product. These six isolates were selected at random as representatives of the major groups.

All bacterial strains were stored on MRS agar slants (Appendix A) with .2% added dextrose (Difco).

Table 2--Lactic acid producing bacteria provided by various manufacturers

Culture	Product
Pediococcus cerevisiae	Lactacel 110[b]
Pediococcus cerevisiae	Lactacel 115[b]
Micrococcus varians	Saga 444[b]
Lactobacillus plantarum	([a])([c])
Pediococcus acidilactici	F100[c]
Pediococcus pentosaceus	LT II[c]
Micrococcus varians	([a])([c])
Pediococcus cerevisiae	PC 1[d]
Lactobacillus plantarum	LP 2[d]
Micrococcus sp	M[e]
Pediococcus acidilactici	PC[e]
Lactobacillus plantarum	18[e]
Lactobacillus plantarum	([a])([f])

[a]Product name not identified.

[b]Microlife Technics, Sarasota, FL

[c]Trumark Inc., Roselle, NJ

[d]Chr. Hansen's Laboratory, Inc., Milwaukee, WI

[e]ABC Research Corp., Gainsville, FL

[f]B. Heller & Co., Chicago, IL

Table 3--Lactic acid bacteria isolated from fresh meat using the conditions listed below including 120 ppm nitrite

Identification	Meat Source	Growth Media	Incubation Time (days)	Incubation Temp (^{o}C)	NaCl (%)
M-MRS-4-1	Mutton	MRS[a]	5	10	1.5
M-LBS-10-4	Mutton	LBS[b]	5	5	3.0
M-MRS-5-5	Mutton	MRS[a]	10	5	1.5
B-MRS-3-3	Beef	MRS[a]	5	10	1.5
B-LBS-1-3	Beef	LBS[b]	5	5	1.5
B-LBS-3-2	Beef	LBS[b]	5	10	1.5
Streptococcus faecalis	Pork	MRS[a]	2	35	2.5

[a]MRS (Difco)

[b]LBS (Difco)

Table 4--Methods of identification of bacteria

Bacteria	Identification procedures
All bacteria	Gram stain/morphology, catalase production Sugar fermentation pattern, gas production Gel electrophoresis of the soluble proteins of the lysed cells Gas chromatography of major end products Optical rotation of lactic acid produced Growth at 10^{o} C and 45^{o} C
Streptococci	Growth in 0.1% methylene blue milk, 6.5% NaCl, 40% bile, 0.02% azide Heat tolerance (60^{o} C for 30 min) Hydrolysis of hippurate, esculin, arginine Growth in and reduction of 0.04% tellurite

Qualitative Screening for Nitrite Reduction

The depletion of nitrite in a system is dependent on a number of relationships (e.g., time, temperature, media), a major contributor being pH (Sofos et al., 1979). Nitrite generally depletes more rapidly as pH levels decrease. Two methods were employed to study the ability of lactic acid bacteria to reduce nitrite concentration irrespective of pH. These methods utilized a basal media to preclude acid production resulting from carbohydrate metabolism by the bacteria.

MINITEK (BBL) Adaptation

This technique for examining nitrite reduction utilizes an adaptation of the hardware included in the MINITEK (BBL) system for identifying bacteria based on fermentation of selected sugars.

Bacterial strains were grown anaerobically on Brain Heart Infusion (BBL, Cockeysville, MD, 21030) agar plates for 48 hours at 35^{o} C. Cells were removed with a sterile cotton swab and immersed in 2 ml of basal MRS broth (Appendix A). The cells were transfered to the broth by rotating the swab quickly between the fingers. This eliminated oxygenating the broth which would have occured with the use of a vortex mixer.

Varying concentrations of potassium nitrite in distilled water (200, 400, 600, 800, 1000 as ppm sodium

nitrite) were filter sterilized and applied to separate sheets of MINITEK (BBL) stock paper (a thick porous card) so that each 10X20 cm sheet absorbed approximately 7-11 g of solution. Excess solution was removed with a rolling pin. The sheets were dried and 6 mm disks were punched with an ordinary paper punch. The disks were kept in a dessicant jar until used.

Duplicate MINITEK (BBL) plates (plastic plates having 20 wells and a loose-fitting cover) were prepared by placing the nitrite impregnated disks in the wells so that there was a row of disks containing all nitrite concentrations for each bacteria. Each row of disks was inoculated with .1 ml of the individual cell/broth suspension per well. Controls were made by inoculating a series of nitrite containing disks with sterile media. The plates were incubated anaerobically using Gaspaks (BBL) for 72 hours at 22^{o} C and 48 hours at 35^{o} C. A sponge containing distilled water was placed in each anaerobe jar to preclude evaporation of the cell/broth suspension.

Nitrite reduction was detected qualitatively by a modification of the AOAC test for nitrate reduction by Clostridium perfringens (AOAC, 1980). Here N-N-dimethyl-1-naphthylamine was substituted for α-naphthol. Each well received one drop, from a dropper or pasteur pipette, of a solution of sulfanilic acid (.8% in 5N acetic acid) followed by one drop of a solution of

N-N-dimethyl-1-naphthylamine (.6% in 5N acetic acid). The development of an orange color after two minutes was considered positive for nitrite, and thus negative for nitrite reduction. Absence of an orange color after two minutes was considered a positive indication of nitrite reduction by the organism.

Basal MRS Broth

Bacterial strains were grown anaerobically on MRS (Difco) agar plates for 48 hours at 35° C. Each cell culture was removed with a sterile cotton swab and immersed in a screw-cap tube containing 5 ml of basal MRS broth (Appendix A) with 120 ppm added nitrite. The nitrite was added to the autoclaved media as a filter sterilized solution to prohibit the formation of a possible Perigo-factor (Perigo et al., 1967). The swab was rotated between the fingers to transfer the cells to the media. Additional cells were added in a similar manner until the turbidity of all tubes was visually the same. Controls were made using tubes of uninoculated media. The tubes were capped loosely and incubated anaerobically using Gaspaks (BBL) at 22° C for 24 hours and a duplicate set at 35° C for 24 hours.

Nitrite reduction was determined colorimetrically using the same technique applied to the MINITEK (BBL) adaptation described earlier except that .2 ml of the sulfanilic acid

solution and .1 ml of the N-N-dimethyl-1-naphthylamine solution were used.

Inhibition

Bacterial strains were grown anaerobically on MRS (Difco) agar plates for 48 hours at 35° C. Each cell culture was removed with a sterile cotton swab and immersed in duplicate screw-cap tubes containing 10 ml sterile basal MRS broth (Appendix A) with 200 ppm nitrite added as a filter sterilized solution. One set was incubated anaerobically using Gaspaks (BBL) while the other set was placed in a Torbal anaerobe jar (Clifton, NJ), evacuated, and filled with carbon monoxide gas (CO) to 6 psi overpressure. Both sets were incubated at 22° C for 48 hours. Residual nitrite was determined spectrophotometrically by the AOAC (1980) method (Appendix B) using a Spectronic 21 spectrophotometer (Bausch and Lomb, Rochester, NY, 14625). Measurements of pH were taken using a Fisher ACCUMET 610 A pH meter (Pittsburg, PA, 15219).

Quantitative Nitrite Depletion and Acid Production

Inoculum Preparation

Cultures were grown in flasks containing 250 ml of MRS (Difco) broth for 24-36 hours at 30° C on an Eberbach (Ann Arbor, MI) shaker (100 rpm). Cells were harvested in 250 ml plastic bottles by centrifugation at 2800 X gravity for 10 minutes using a DAMON-IEC DPR-6000 centrifuge (300 Second

Ave., Needham Hts., MA, 02194). After decanting the supernatant broth the cells were washed by resuspending in 25 ml of sterile distilled water and recentrifuged. The cell pellet was taken up in 10 ml of sterile distilled water and an absorbance reading obtained using a Spectronic 21 (Bausch and Lomb) at 535 nanometers (nm). Each suspension was adjusted to a standard cell concentration (either 10^3, 10^5, or 10^7 cells per ml) by comparing it's absorbance reading to a table plotting absorbance versus actual plate count. These tables were produced by serially diluting a cell suspension, taking absorbance readings, plating the dilutions on MRS (Difco) agar plates, incubating, and counting the colonies. From this data a table of absorbance and corresponding cell numbers for each microorganism of differing cell size was established.

Analysis of Meats

Rapid analysis techniques were employed to determine fat and moisture of meat used as media. Fat was determined by a modified babcock method (Appendix C). Moisture was determined using the CENCO Moisture Balance (Griffith Laboratories, Chicago, IL; Appendix D). Ash was estimated to be 1.0% and protein was estimated by subtracting fat, moisture, and ash percentages from 100%.

Determinations in Laboratory Media

This method was employed to quantitatively examine the ability of lactic acid bacteria to reduce nitrite and produce acid in laboratory media.

Two sets of screw-cap tubes for each bacterial treatment, 6 tubes per set, were prepared with 10 ml basal MRS broth (Appendix A) in one set and 10 ml MRS (Difco) broth in the other set. The media in both sets contained 156 ppm added nitrite incorporated as a filter-sterilized solution of potassium nitrite. Each set was inoculated with a cell suspension providing 10^5 cells per ml of broth, prepared as previously described. Two additional treatments were prepared by chemically acidifying tubes of MRS (Difco) broth to pH 4.8-5.0 with gluconic acid and lactic acid. Controls were established using tube sets of uninoculated media. The tubes were capped loosely and incubated anaerobically using Gaspaks (BBL).

The treatments were incubated in a 20X2X3 factorial design using 20 bacteria, 2 incubation times (48 and 96 hours) and 3 incubation temperatures (5^o, 15^o, and 35^o C).

Model Meat System

This method was employed to quantitatively examine the ability of lactic acid bacteria to reduce nitrite and produce acid using meat as a media.

A 13X3X2 factorial design was used with 13 bacterial treatments, 3 meat/dextrose combinations (Table 5), and 2

Table 5--Formulations for three model meat systems

Ingredient	Beef[a]	Beef[a] without Dextrose	Mutton[b]
Ground Meat	95.5%	97.5%	95.5%
Salt (NaCl)	2.5%	2.5%	2.5%
Dextrose	2.0%	----	2.0%
Nitrite	156 ppm	156 ppm	156 ppm

[a]27.0% fat, 54.2% moisture, 17.8% protein, 1.0% ash.

[b]28.5% fat, 52.5% moisture, 18.0% protein, 1.0% ash.

repetitions. Two additional treatments were added for comparison; glucono delta lactone and encapsulated lactic acid (both 57° C melting point, 50% acid by weight) used at the 1% level. Controls consisted of uninoculated meat mixtures.

The meat was ground twice through a 3.2 mm plate. The ingredients were mixed in a Kitchenaid 5 quart mixer, Model K5-A (The Hobart Mfg. Co., Troy, OH), and divided into 100g portions. The portions were inoculated with a cell suspension providing 10^7 cells per g, prepared as previously described, and mixed in a Waring blender at low speed. The inoculated portions were placed in disposable plastic cups with lids and incubated at 35° C.

The treatments containing the two encapsulated acids were kept at 5° C for 36 hours, then brought to an internal temperature of 60° C in a water bath.

Residual nitrite determinations and pH measurements were taken on all treatments at 0, 12, 24, and 36 hours in a factorial split plot in time design.

Summer-style Sausage

This method was employed to quantitatively examine the ability of lactic acid bacteria to reduce nitrite and produce acid in a commercial-type product.

Seven bacterial treatments were applied to each of 2 beef sausage formulations (Table 6) in 2 repetitions for a 7X2X2 factorial design. Two additional treatments, added for comparison, consisted of chemical acidification with glucono delta lactone and encapsulated lactic acid (both 57° C melting point, 50% acid by weight) added at the 1% level. Controls were established with uninoculated sausages.

Meat trimmings were ground through a 6.4 mm plate and mixed with the appropriate amount of salt, spices, and nitrite in a Hollymatic 175 Mixer Grinder (Forest Park, IL, 60466). The meat was divided into two batches, one received dextrose, and both were ground through a 3.2 mm plate. One kg portions of the meat mixtures were inoculated with a cell suspension providing 10^7 cells per g, prepared as previously described, and mixed in a Kitchenaid (Model K5-A) 5 quart mixer. The sausages were stuffed into pre-soaked 5.1X76.2

Table 6--Formulations for summer-style sausage

Ingredient	With Dextrose	Without Dextrose
Ground beef[a]	94.985%	96.985%
Salt (NaCl)	2.500%	2.500%
Dextrose	2.000%	----
Spices[b]	.515%	.515%
Nitrite	156 ppm	156 ppm

[a] 28.0% fat, 53.0% moisture, 18.0% protein, 1.0% ash

[b] .015% garlic, .3% ground black pepper, .2% monosodium glutamate

cm Tee-Pak (2 N. Riverside Plaza, Chicago, IL, 60606) mahogany fibrous casings.

The sausages inoculated with bacterial cultures were hung in a smokehouse and processed at 38° C and 90% relative humidity (wet bulb-32° C, dry bulb-38° C). The sausages acidified with the encapsulated acids were held at 5° C and then cooked with other fermented sausage samples. All treatments were cooked to 60° C internal temperature (wet bulb-60° C, dry bulb-68° C).

Residual nitrite determinations and pH measurements were taken on all treatments at 0, 6, 12, 18, 24, 30, and 36 hours in a factorial split plot in time design.

Chemical Analysis

Residual nitrite was determined spectrophotometrically by the AOAC (1980) method (Appendix B) using a Spectronic 21 (Bausch and Lomb) spectrophotometer. Measurements of pH were taken using a Fisher ACCUMET 610 A pH meter.

Statistical Analysis

Residual nitrite determinations and pH measurements were analyzed by analysis of variance (Ostle and Mensing, 1975). Treatment means were analyzed by the multiple range test of Duncan (1955).

RESULTS AND DISCUSSION

Qualitative Screening for Nitrite Depletion

MINITEK (BBL) Adaptation

The reduction of varying amounts of nitrite by bacterial treatments in basal MRS broth is shown in Table 7. Time and temperature apparently had little effect on nitrite depletion in uninoculated control samples since abundant orange color was observed.

The effects of pH on the system could not be assessed since terminal values were not obtained with the extremely small inoculum used (.1 ml). The possible presence of minute amounts of fermentable carbohydrate in the tryptone and yeast extract used in the basal broth (Appendix A) probably had a negligible influence on pH since all treatments are acid producers and a majority had results comparable to the control. The pH of the autoclaved, uninoculated broth was 6.52.

Most strains were not able to reduce nitrite, which was either an expression of their inability to do so or perhaps evidence of susceptibility to its antimicrobial nature. The same species obtained from multiple sources (e.g., *Micrococcus varians*, *Lactobacillus plantarum*) exhibited different nitrite reducing abilities. *Lactobacillus leichmannii* (ATCC) and isolates Mutton LBS 10-4 and Mutton MRS 5-5 reduced the nitrite on the 1000 ppm disk under both

Table 7--Nitrite reduction by bacteria using an adaptation of the MINITEK (BBL) hardware

Bacterial Treatment	Incubation Conditions (anaerobic)										
	35° C 48 hrs					22° C 72 hrs					
	Nitrite Concentration on Disc (ppm)										
	200	400	600	800	1000	200	400	600	800	1000
Control	-[h]	-	-	-	-	-	-	-	-	-
Lactobacillus acidophilus[a]	-	-	-	-	-	-	-	-	-	-
Lactobacillus casei[a]	-	-	-	-	-	-	-	-	-	-
Lactobacillus leichmannii[a]	+	+	+	+	+	+	+	+	+	+
Lactobacillus plantarum[a]	-	-	-	-	-	-	-	-	-	-
Lactobacillus bulgaricus[a]	-	-	-	-	-	-	-	-	-	-
Lactobacillus fermentum[a]	-	-	-	-	-	-	-	-	-	-
Lactobacillus casei, subsp rhamnosus[a]	-	-	-	-	-	-	-	-	-	-
Pediococcus acidilactici[a]	-	-	-	-	-	-	-	-	-	-
Lactobacillus viridescens[a]	-	-	-	-	-	-	-	-	-	-
Streptococcus faecalis[a]	(+)	(-)	-	-	-	-	-	-	-	-
Streptococcus faecium[a]	-	-	-	-	-	-	-	-	-	-
Leuconostoc mesenteroides[a]	-	-	-	-	-	-	-	-	-	-

Table 7--cont.

Bacterial Treatment	Incubation Conditions (anaerobic)									
	35° C 48 hrs					22° C 72 hrs				
	Nitrite Concentration on Disc (ppm)									
	200	400	600	800	1000	200	400	600	800	1000
Pediococcus cerevisiae[b]	-	-	-	-	-	-	-	-	-	-
Pediococcus cerevisiae[b]	-	-	-	-	-	-	-	-	-	-
Micrococcus varians[b]	+	+	-	-	-	+	+	-	-	-
Lactobacillus plantarum[c]	-	-	-	-	-	-	-	-	-	-
Pediococcus acidilactici[c]	-	-	-	-	-	-	-	-	-	-
Pediococcus pentosaceus[c]	-	-	-	-	-	-	-	-	-	-
Micrococcus varians[c]	-	-	-	-	-	-	-	-	-	-
Pediococcus cerevisiae[d]	-	-	-	-	-	-	-	-	-	-
Lactobacillus plantarum[d]	-	(-)	-	-	-	-	-	-	-	-
Micrococcus sp.[e]	+	-	-	-	-	-	-	-	-	-
Pediococcus acidilactici[e]	-	-	-	-	-	-	-	-	-	-
Lactobacillus plantarum[e]	+	+	(-)	-	-	+	+	(-)	(-)	-
Lactobacillus plantarum[f]	-	-	-	-	-	-	-	-	-	-

Table 7--cont.

Bacterial Treatment	Incubation Conditions (anaerobic)									
	35° C 48 hrs					22° C 72 hrs				
	Nitrite Concentration on Disc (ppm)									
	200	400	600	800	1000	200	400	600	800	1000
Mutton MRS 4-1	-	-	-	-	-	-	-	-	-	-
Mutton LBS 10-4	+	+	+	+	+	+	+	+	+	+
Mutton MRS 5-5	+	+	+	+	+	+	+	+	+	+
Beef MRS 3-3	(+)	-	-	-	-	-	-	-	-	-
Beef LBS 1-3	-	-	-	-	-	-	-	-	-	-
Beef LBS 3-2	+	+	(+)	(-)	(-)	+	+	+	+	(+)
Streptococcus faecalis[g]	+	+	(+)	(+)	(+)	(+)	(-)	(-)	-	-

[a] ATCC

[b] Microlife Technics

[c] Trumark Inc.

[d] Chr. Hansen's Laboratory, Inc.

[e] ABC Research Corp.

[f] B. Heller & Co.

[g] pork isolate

[h] Symbols:
+ no nitrite remaining, completely reduced
(+) little nitrite remaining, mostly reduced
(-) most of the nitrite remaining, some reduction
- no change in nitrite level, no reduction

incubation conditions. Micrococcus varians (Microlife Technics), L. plantarum (ABC Research Corp.), and isolate Beef LBS 3-2 reduced the nitrite on the 400 ppm disk under both incubation conditions while Streptococcus faecalis (pork isolate) reduced the nitrite on the 400 ppm disk when incubated at 35° C for 48 hours.

Basal MRS Broth

The relative ability of bacteria to reduce 120 ppm nitrite in MRS broth (Appendix A) containing no added carbohydrate is shown in Table 8. Again nitrite depletion due to time and temperature was compensated for by comparison of treatments with identically incubated controls. The pH of the autoclaved, uninoculated broth was 6.64 and terminal pH values of the bacterial samples ranged from 6.48 to 6.71. Nitrite depletion due to the slight fluctuation in pH (+.07 to -.16) of the bacterial treatments compared to the control was probably minimal since the majority of the treatments had color values similar to the control. All bacteria showing ability to reduce nitrite in the MINITEK (BBL) adaptation showed similar ability in broth. P. pentosaceus and M. varians (both Trumark Inc.) showed an ability not expressed in the MINITEK system.

Inhibition

The values of residual nitrite and terminal pH for bacterial strains incubated in a carbon monoxide atmosphere

Table 8--Nitrite reduction by bacteria in basal MRS broth containing 120 parts per million nitrite

Bacterial Treatment	Incubation Conditions (anaerobic)			
	35° C 24 hrs		22° C 24 hrs	
	REP 1	REP 2	REP 1	REP 2
Control	-[h]	-	-	-
Lactobacillus acidophilus[a]	-	-	-	-
Lactobacillus casei[a]	-	-	-	-
Lactobacillus leichmannii[a]	(+)	+	+	+
Lactobacillus plantarum[a]	-	-	-	-
Lactobacillus bulgaricus[a]	-	-	-	-
Lactobacillus fermentum[a]	-	-	-	-
Lactobacillus casei, subsp *rhamnosus*[a]	-	-	-	-
Pediococcus acidilactici[a]	-	-	-	-
Lactobacillus viridescens[a]	-	-	-	-
Streptococcus faecalis[a]	(-)	(+)	(-)	(+)
Streptococcus faecium[a]	-	-	-	-
Leuconostoc mesenteroides[a]	-	-	-	-

Table 8--cont.

Bacterial Treatment	Incubation Conditions (anaerobic)			
	35° C 24 hrs		22° C 24 hrs	
	REP 1	REP 2	REP 1	REP 2
Pediococcus cerevisiae[b]	-	-	-	-
Pediococcus cerevisiae[b]	-	-	-	-
Micrococcus varians[b]	(-)	(-)	(-)	(-)
Lactobacillus plantarum[c]	-	-	-	-
Pediococcus acidilactici[c]	-	-	-	-
Pediococcus pentosaceus[c]	(-)	(-)	(+)	+
Micrococcus varians[c]	(-)	(+)	(+)	(+)
Pediococcus cerevisiae[d]	-	-	-	-
Lactobacillus plantarum[d]	-	-	-	-
Micrococcus sp.[e]	-	(-)	(-)	(+)
Pediococcus acidilactici[e]	-	-	-	-
Lactobacillus plantarum[e]	(+)	(+)	(+)	+
Lactobacillus plantarum[f]	-	-	-	-

Table 8--cont.

Bacterial Treatment	Incubation Conditions (anaerobic)			
	35° C 24 hrs		22° C 24 hrs	
	REP 1	REP 2	REP 1	REP 2
Mutton MRS 4-1	-	-	-	-
Mutton LBS 10-4	(+)	(+)	(+)	(+)
Mutton MRS 5-5	(+)	(+)	+	(+)
Beef MRS 3-3	-	(-)	-	-
Beef LBS 1-3	-	-	-	-
Beef LBS 3-2	(-)	(+)	(+)	(+)
Streptococcus faecalis[g]	(+)	(+)	+	+

[a]ATCC

[b]Microlife Technics

[c]Trumark Inc.

[d]Chr. Hansen's Laboratory, Inc.

[e]ABC Research Corp.

[f]B. Heller & Co.

[g]pork isolate

[h]Symbols: + no nitrite remaining, completely reduced
(+) little nitrite remaining, mostly reduced
(-) most of the nitrite remaining, some reduction
- no change in nitrite level, no reduction

or an anaerobic control atmosphere are shown in Table 9. The strains evaluated were selected for their ability to reduce nitrite in the previous screening studies. The difference between the ingoing nitrite level (200 ppm) and the residual nitrite values of the uninoculated controls is considered nitrite depletion due to incubation conditions. L. leichmannii (ATCC) and S. faecalis (pork isolate) had lower residual nitrite levels than the other bacteria in both gas environments but showed less ability to deplete nitrite when incubated in carbon monoxide. Since carbon monoxide is a strong inhibitor of heme-containing enzymes, a possible nitrite reductase enzyme system is suggested in these two species. Fournaud and Mocquot (1966) also reported an inhibition of the nitrite reducing ability in cell suspensions of L. lactis, L. leichmannii, L. buchneri, and two strains of lactobacilli isolated from ham curing brine when two enzyme inhibitors, potassium cyanide and para-chloromercuribenzoate were added.

The carbon monoxide may have been more inhibitory if the surface area of media exposed to the gas had been greater. As it was, the gas was required to diffuse through the 8 cm long column of media in the screw-cap tube. Thus bacteria at the bottom of the tube were provided with sufficient time to reduce a quantity of nitrite.

The pH of the media prior to inoculation was 6.60. Values of pH of the bacterial treatments were equal to or

Table 9--Residual nitrite and pH values in basal MRS broth, having 200 ppm added nitrite, by bacterial treatments grown in a carbon monoxide and anaerobic atmospheres at 22° C for 48 hours

Bacterial Treatments	Carbon Monoxide Atmosphere		Anaerobic Control	
	pH	ppm	pH	ppm
Control (uninoculated)	6.75	181	6.62	177
Lactobacillus leichmannii[a]	6.85	72	6.82	36
Streptococcus faecalis[a]	6.75	149	6.69	147
Pediococcus pentosaceus[b]	6.75	159	6.71	146
Lactobacillus plantarum[c]	6.89	167	6.78	162
Mutton LBS 10-4	6.77	173	6.70	170
Mutton MRS 5-5	6.80	171	6.73	169
Beef LBS 3-2	6.78	173	6.69	171
Streptococcus faecalis[d]	6.76	133	6.69	101

[a] ATCC

[b] Trumark Inc.

[c] ABC Research Corp.

[d] pork isolate

above those of the controls, thus eliminating any nitrite depletion due to pH/nitrite interaction.

Quantitative Nitrite Depletion and Acid Production

Determinations in Laboratory Media

Twenty bacterial strains and an uninoculated control were evaluated for their ability to reduce nitrite in basal broth with no fermentable carbohydrate added. A 20X3X2 factorial design was used with 3 incubation temperatures

(5^{o}, 15^{o}, and 35^{o} C) and 2 incubation times (48 and 96 hours)

Analysis of variance of residual nitrite in basal MRS media is shown in Appendix E, Table 21. Differences in mean residual nitrite levels between the two incubation times (48 and 96 hours) was significant ($p=.0001$) with the longest time (96 hours) showing the greatest depletion ($p=.05$). A significant difference ($p=.0001$) also occured between temperatures (5^{o}, 15^{o}, and 35^{o} C). However, mean residual nitrite levels were not different between 5^{o} and 15^{o} C while greater nitrite depletion occured at 35^{o} C ($p=.05$). Fournaud and Mocquot (1966) found that the optimum temperature for enzymatic nitrite reduction by L. leichmannii, L. lactis, L. buchneri, and two lactobacilli isolated from ham curing brine was 22^{o} C. Bacterial treatment means showed highly significant differences ($p=.0001$). Several bacteria were able to reduce nitrite to a greater degree than observed during screening. Two strains, S. faecium and P. acidilactici (ABC Research Corp.), which had not shown any nitrite reducing ability in screening, had lower mean nitrite levels than Mutton LBS 10-4, Mutton MRS 5-5, or Beef LBS 3-2, three strains showing good reduction in previous screening (Table 10). L. leichmannii and S. faecalis (pork isolate) reduced more nitrite ($p=.05$) than all other strains tested (Table 10; $p=.05$).

Table 10--Duncan's multiple range test comparing mean residual nitrite for bacterial treatments in basal MRS broth (Appendix A) containing no added carbohydrate, 156 ppm nitrite, incubated anaerobically at 5°, 15°, and 35° C for 48 and 96 hours

Duncan Grouping[a]			Mean Residual Nitrite (ppm)	Treatment
			135.17[h]	Control[i] (uninoculated)
	A		129.83	Beef LBS 1-3
B	A		127.92	Mutton MRS 4-1
B			127.17	Beef MRS 3-3
B	C		126.83	Lactobacillus viridescens[b]
D	C		125.08	Pediococcus cerevisiae[c]
D			124.67	Beef LBS 3-2
D	E		124.00	Pediococcus cerevisiae[e]
D	E	F	123.08	Mutton LBS 10-4
G	E	F	122.42	Lactobacillus plantarum[b]
G		F	121.83	Mutton MRS 5-5
G		F	121.83	Lactobacillus bulgaricus[b]
G		F	121.25	Leuconostoc mesenteroides[b]
G			120.83	Pediococcus acidilactici[f]
	H		110.33	Lactobacillus plantarum[f]
	I		105.75	Streptococcus faecalis[b]
	J		103.25	Lactobacillus plantarum[e]
	J		102.33	Pediococcus pentosaceus[d]
	K		93.33	Streptococcus faecium[b]
	L		89.83	Lactobacillus leichmannii[b]
	M		86.42	Streptococcus faecalis[g]

Table 10--cont.

[a]Means with the same letter are not significantly different at p = .05. Duncan value = 1.91.

[b]ATCC

[c]Microlife Technics

[d]Trumark Inc.

[e]Chr. Hansen's Laboratory, Inc.

[f]ABC Research Corp.

[g]pork isolate

[h]Analysis for nitrite is accurate to 1 ppm. Fractional values are due to mean computation (12 data points) and are necessary for Duncan procedure.

[i]Not given a Duncan grouping since not included in analysis of variance.

The same twenty strains used in the basal broth study above were evaluated for their ability to reduce 156 ppm nitrite in MRS broth containing 2% fermentable carbohydrate. A 20X3X2 factorial design was used with 3 incubation temperatures (5^{0}, 15^{0}, and 35^{0} C) and 2 times (48 and 96 hours).

Analysis of variance tables for pH and residual nitrite in MRS (Difco) broth containing 2% carbohydrate are contained in Appendix E, Tables 22 and 23. Means for bacterial treatments, time, temperature, and their interactions were all highly significant (p=.0001). Nitrite was reduced and acid produced to a greater degree (p=.05) at the longer time of 96 hours (Table 11) and higher temperature of 35^{0} C (Table 12). Nitrite reduction and acid production are highly correlated (r=.92, p=.0001). The

Table 11--Duncan's multiple range test comparing mean pH and residual nitrite (ppm) values for bacterial treatments incubated for various times in MRS (Difco) media containing 2% fermentable carbohydrate and 156 ppm nitrite

	Means/Duncan Grouping[a]	
Incubation Times	pH[b]	Residual Nitrite (ppm)[b]
48 hours	5.3819 A	94.542 A
96 hours	5.0621 B	75.708 B

[a]Means with the same letter are not significantly different at p = .05. Duncan value = .0626 (pH) and 3.238 (residual nitrite).

[b]Analysis for nitrite is accurate to 1 ppm and pH to .01. Fractional values are due to mean computation and are necessary for Duncan procedure.

Table 12--Duncan's multiple range test comparing mean pH and residual nitrite (ppm) values for bacterial treatments incubated at various temperatures in MRS (Difco) media containing 2% fermentable carbohydrate and 156 ppm nitrite

	Means/Duncan Grouping[a]	
Incubation Temperatures	pH[b]	Residual Nitrite (ppm)[b]
5 ^{o} C	5.8331 A	149.97 A
15 ^{o} C	5.3161 B	93.74 B
35 ^{o} C	4.5167 C	11.66 C

[a]Means with the same letter are not significantly different at p = .05. Duncan value = .0538 (pH) and 2.783 (residual nitrite).

[b]Analysis for nitrite is accurate to 1 ppm and pH to .01. Fractional values are due to mean computation and are necessary for Duncan procedure.

means of residual nitrite (85 ppm) and pH (5.22) for MRS broth containing fermentable carbohydrate were significantly lower (p=.05) than for the basal MRS broth (115 ppm, pH 6.55). This supports the findings of Olsman and Krol (1972) that nitrite depletion was enhanced by lower pH levels. In addition, broth acidified with gluconic and lactic acids had the lowest (p=.05) mean pH values of 4.71 and 4.26 respectively (Table 13) and the lowest mean residual nitrite of 49 and 38 ppm (Table 14).

Collins-Thompson and Rodriguez Lopez (1981) found L. mesenteroides reduced nitrite levels in APT broth at a greater rate than L. plantarum or L. viridescens. In this study results were found in MRS broth (Table 14) where three strains of L. plantarum had lower mean residual nitrite levels than L. mesenteroides (p=.05). All interactions of bacterial treatment, time, and temperature were significant (p=.05).

The atypical lactobacilli isolated from mutton and beef had lower mean pH values (p=.05) than all but three bacterial treatments (Table 13) and had the lowest mean residual nitrite levels (Table 14, p=.05). L. leichmannii and S. faecalis (pork isolate) were less efficient at acid production than most treatments (Table 13) and subsequently had higher corresponding mean nitrite levels (Table 14).

Table 13--Duncan's multiple range test comparing mean pH values for bacterial and chemically acidified treatments in MRS (Difco) media containing 2% carbohydrate, 156 ppm nitrite and incubated at 5°, 15°, and 35° C for 48 and 96 hours

Duncan Grouping[a]	Mean pH	Treatment
	6.0575[h]	Control[i] (uninoculated)
A	5.8242	*Lactobacillus viridescens*[b]
A	5.8142	*Streptococcus faecalis*[b]
B	5.5933	*Streptococcus faecalis*[g]
C	5.4908	*Streptococcus faecium*[b]
D	5.3792	*Leuconostoc mesenteroides*[b]
E	5.3525	*Lactobacillus bulgaricus*[b]
F	5.3308	*Lactobacillus leichmannii*[b]
G	5.1675	*Pediococcus acidilactici*[f]
G	5.1667	*Pediococcus cerevisiae*[c]
H	5.1200	*Lactobacillus plantarum*[b]
H	5.1183	*Lactobacillus plantarum*[e]
I	5.0742	Mutton MRS 4-1
I	5.0725	*Lactobacillus plantarum*[f]
J I	5.0692	Beef LBS 3-2
J	5.0508	Mutton LBS 10-4
K	5.0292	Beef LBS 1-3
L	5.0042	Mutton MRS 5-5
M	4.9625	*Pediococcus pentosaceus*[d]
N	4.9133	Beef MRS 3-3
N	4.9067	*Pediococcus cerevisiae*[e]
O	4.7075	gluconic acid
P	4.2608	lactic acid

Table 13--cont.

[a]Means with the same letter are not significantly different at p = .05. Duncan value = .0245

[b]ATCC

[c]Microlife Technics

[d]Trumark Inc.

[e]Chr. Hansen's Laboratory, Inc.

[f]ABC Research Corp.

[g]pork isolate

[h]Measurement of ph is accurate to .01. Fractional values are due to mean computation (12 data points) and are necessary for Duncan procedure.

[i]Not given a Duncan grouping since not included in analysis of variance.

Table 14--Duncan's multiple range test comparing mean residual nitrite for bacterial and chemically acidified treatments in MRS (Difco) broth containing 2% carbohydrate, 156 ppm nitrite and incubated at 5°, 15°, and 35° C for 48 and 96 hours

Duncan Grouping[a]	Mean Residual Nitrite (ppm)	Treatment
	125.29[h]	Control[i] (uninoculated)
A	118.58	Lactobacillus viridescens[b]
B	115.00	Streptococcus faecalis[b]
C	98.25	Lactobacillus leichmannii[b]
C	98.25	Leuconostoc mesenteroides[b]
C	97.75	Lactobacillus bulgaricus[b]
D	95.83	Pediococcus acidilactici[f]
E	92.17	Streptococcus faecium[b]
E	91.83	Pediococcus cerevisiae[c]
F	88.33	Lactobacillus plantarum[e]
F	87.92	Lactobacillus plantarum[b]
G	85.25	Streptococcus faecalis[g]
H	77.67	Pediococcus pentosaceus[d]
H	76.42	Lactobacillus plantarum[f]
I	72.67	Pediococcus cerevisiae[e]
J I	72.08	Mutton LBS 10-4
J I	71.75	Beef LBS 1-3
J	70.83	Mutton MRS 4-1
K	68.08	Mutton MRS 5-5
L	62.83	Beef LBS 3-2
M	61.00	Beef MRS 3-3
N	49.58	gluconic acid
O	38.50	lactic acid

Table 14--cont.

[a]Means with the same letter are not significantly different at p = .05. Duncan value = 1.2676.

[b]ATCC

[c]Microlife Technics

[d]Trumark Inc.

[e]Chr. Hansen's Laboratory, Inc.

[f]ABC Research Corp.

[g]pork isolate

[h]Analysis for nitrite is accurate to 1 ppm. Fractional values are due to mean computation (12 data points) and are necessary for Duncan procedure.

[i]Not given a Duncan grouping since not included in analysis of variance.

Model Meat System

Twelve bacterial treatments, selected from the previous broth studies for their nitrite reduction and acid production abilities, uninoculated controls, and two encapsulated acids were evaluated in a 3X2 factorial design using 3 model meat systems (beef and mutton with added carbohydrate and beef without) and 2 repetitions.

Analysis of variance tables for pH and residual nitrite are shown in Appendix E, Tables 24 and 25. The means of bacterial treatment, meat media, time, and all interactions for both pH and residual nitrite were significant at p=.0001, however; the mean residual nitrite level of mutton with added carbohydrate was not different from beef without carbohydrate.

Residual nitrite and pH were highly correlated in all three meats (r=.86 in both beef systems, r=.94 in mutton; p=.0001). Although the mean pH (5.54) of the basal beef formulation (Table 15) was significantly higher (p=.05) than pH values of beef with added carbohydrate (5.46) and mutton with added carbohydrate (5.43), its concommittant depletion of nitrite was not different than mutton with added carbohydrate. In fact, four of the twelve bacteria had equivalent or lower terminal nitrite levels in the basal beef mixture than in the two systems containing added carbohydrate (Table 16). Two strains, L. leichmannii and the atypical lactobacilli isolate Mutton MRS 5-5 had lower mean residual nitrite levels in the basal beef (Table 16). The reduction in pH level from the initial value of 5.81 to the terminal values listed for basal beef in Table 17 may be evidence of naturally occuring fermentable substrate in the meat which may mask any microbial depletion other than that due to pH.

The two encapsulated acids, glucono delta lactone and lactic, had pH values less than the uninoculated controls in all three meat systems (Table 18) yet the controls had significantly lower mean residual nitrite levels (Table 16; p=.05). Although the meat systems containing encapsulated acids were prepared with those inoculated with bacterial cultures, they were held at a lower temperature (5^{o} C) for 30 hours during incubation of the microbial treatments.

Table 15--Duncan's multiple range test comparing mean pH and residual nitrite (ppm) values of 3 model meat systems containing 156 ppm nitrite and incubated at 35° C with measurements taken at 0, 12, 24, and 36 hours

Model Meat System	Means/Duncan Grouping[a]	
	pH	Residual Nitrite (ppm)[b]
Beef without carbohydrate	5.54 A	74.25 A
Beef with carbohydrate	5.46 B	71.97 B
Mutton	5.43 C	75.61 A

[a]Means with the same letter are not significantly different at p = .05. Duncan value = .0117 (pH) and 1.605 (residual nitrite).

[b]Measurement of pH is accurate to .01. Fractional values due to mean computation and are necessary for Duncan procedure.

Table 16--Duncan's multiple range test comparing mean residual nitrite for bacterial and chemically acidified treatments in 3 model meat systems containing 156 ppm nitrite and incubated at 35° C with measurements taken at 0, 12, 24, and 36 hours

Treatment	Mean Residual Nitrite (ppm)[g]/Duncan Grouping[a]		
	Beef with Carbohydrate	Beef without Carbohydrate	Mutton
Control[h] (uninoc.)	72.88	68.38	80.75
glucono delta lactone	121.75 A	126.38 B	117.38 A
lactic acid (encap.)	117.00 B	128.38 A	117.75 A
Beef MRS 3-3	79.25 C	80.88 C	80.88 B
Mutton MRS 4-1	68.50 D	75.00 D	75.25 C
Mutton LBS 10-4	68.00 D E	75.12 D	75.13 C
Lactobacillus leichmannii[b]	67.00 F	44.50 K	68.88 E
Mutton MRS 5-5	65.00 G	61.88 I	71.50 D
Beef LBS 1-3	64.25 G	69.63 E	71.63 D
Streptococcus faecium[b]	62.63 H	67.00 F	62.00 H I
Pediococcus pentosaceus[c]	60.75 I	67.50 F	61.50 I
Streptococcus faecalis[f]	60.38 I J	65.38 G	63.88 G
Lactobacillus plantarum[e]	60.00 I J	64.00 H	67.00 F
Pediococcus cerevisiae[d]	58.75 K	66.00 G	62.63 H
Beef LBS 3-2	55.63 L	57.13 J	59.38 J

Table 16--cont.

[a]Means with the same letter are not significantly different at p = .05. Duncan value = .8437.

[b]ATCC

[c]Trumark Inc.

[d]Chr. Hansen's Laboratory, Inc.

[e]ABC Research Corp.

[f]pork isolate

[g]Analysis for nitrite is accurate to 1 ppm. Fractional values are due to mean computation (8 data points) and are necessary for Duncan procedure.

[h]Not given a Duncan grouping since not included in analysis of variance.

Table 17--Terminal pH and residual nitrite values for bacterial and chemically acidified treatments in model meat systems containing 156 ppm nitrite, taken after 36 hours incubation at 35 °C

Treatment	Model Meat System					
	Beef with Carbohydrate		Beef without Carbohydrate		Mutton	
	pH	Nitrite (ppm)	pH	Nitrite (ppm)	pH	Nitrite (ppm)
Initial values[a]	5.84	156	5.81	157	5.93	155
Control (uninoc.)	5.55	3	5.56	1	5.36	8
Beef MRS 3-3	5.39	19	5.51	9	5.39	8
glucono delta lactone	5.30	64	5.41	58	5.33	50
lactic acid (encap.)	5.21	56	5.40	69	5.20	50
Mutton MRS 4-1	5.21	9	5.43	20	5.08	11
Mutton LBS 10-4	5.15	9	5.30	20	5.09	11
Streptococcus faecalis[f]	5.01	4	5.40	9	5.03	6
Beef LBS 1-3	4.97	9	5.34	17	4.88	9
Mutton MRS 5-5	4.98	5	5.30	7	4.89	6
Lactobacillus leichmannii[b]	4.47	1	5.43	1	4.49	1
Lactobacillus plantarum[e]	4.77	6	5.19	6	4.94	4
Streptococcus faecium[b]	5.00	8	5.48	4	4.80	7
Pediococcus pentosaceus[c]	4.96	5	5.32	15	4.83	7
Beef LBS 3-2	4.84	4	5.29	7	4.79	3
Pediococcus cerevisiae[d]	4.77	6	5.30	10	4.76	6

[a]Taken at time of inoculation
[b]ATCC
[c]Trumark Inc.
[d]Chr. Hansen's Lab., Inc.
[e]ABC Research Corp.
[f]pork isolate

Table 18--Duncan's multiple range test comparing mean pH values for bacterial and chemically acidified treatments in 3 model meat systems containing 156 ppm nitrite and incubated at 35° C with measurements taken at 0, 12, 24, and 36 hours

Treatment	Mean pH[g]/Duncan Grouping[a]		
	Beef with Carbohydrate	Beef without Carbohydrate	Mutton
Control[h] (uninoc.)	5.7413	5.7275	5.6775
Beef MRS 3-3	5.6800 A	5.6800 A	5.6625 B
glucono delta lactone	5.6475 B	5.6575 C	5.6725 A
lactic acid (encap.)	5.6425 B	5.6650 B	5.6700 A
Mutton MRS 4-1	5.4700 C	5.5600 D	5.4688 C
Mutton LBS 10-4	5.4500 D	5.4913 H	5.4350 D
Streptococcus faecalis[f]	5.4375 E	5.5288 F	5.3675 F
Beef LBS 1-3	5.4163 F	5.5150 G	5.3450 G
Mutton MRS 5-5	5.3913 G	5.4725 I	5.3750 E
Lactobacillus leichmannii[b]	5.3700 H	5.5500 E	5.3263 H
Lactobacillus plantarum[e]	5.3638 I	5.4300 L	5.3625 F
Streptococcus faecium[b]	5.3388 J	5.5250 F	5.2550 K
Pediococcus pentosaceus[c]	5.3200 K	5.4700 I J	5.2688 I
Beef LBS 3-2	5.2900 L	5.4425 K	5.2650 I J
Pediococcus cerevisiae[d]	5.2550 M	5.4675 J	5.2675 I J

Table 18--cont.

[a]Means with the same letter are not significantly different at p = .05. Duncan value = .0061.

[b]ATCC

[c]Trumark Inc.

[d]Chr. Hansen's Laboratory, Inc.

[e]ABC Research Corp.

[f]pork isolate

[g]Measurement of pH is accurate to .01. Fractional values due to mean computation (8 data points) and are necessary for Duncan procedure.

[h]Not given a Duncan grouping since not included in analysis of variance.

It is projected from this data that sausages made with encapsulated acids may have higher nitrite levels just after processing than those produced with bacterial cultures. The lack of an incubation period at temperatures optimum for lactic acid bacteria growth may leave nitrite levels sufficiently high to offer increased botulinal protection early in the shelflife of the product.

Summer-style Sausage

Seven bacterial strains, selected from the model meat studies for their nitrite reduction and acid production abilities, uninoculated controls, and two encapsulated acids, were evaluated in a 2X2 factorial design using 2 fermented sausage formulations, differing in their carbohydrate content, and 2 repetitions. Mutton was dropped from the study due to an early analysis of variance which

showed mutton and beef, both with added carbohydrate, were the same in their mean pH and residual nitrite values produced by bacterial treatments. A more precise statistical model used after this research was complete showed that these two model meat systems were not the same.

Analysis of variance tables for pH and residual nitrite are shown in Appendix E, Tables 26 and 27. Since the strains showing the best acid production and nitrite reduction were selected for this study, the differences in their abilities to reduce pH was found insignificant. As a population, bacterial mean pH values were not found to be different, nor was the variation due to added carbohydrate level (dextrose level), time, or any and all interactions of these variables (Table 26).

The variation in residual nitrite was significantly affected by the differences in means of bacterial treatment, added carbohydrate level, time, and all interactions (Table 27). Therefore, bacterial treatments are not all the same in their nitrite reducing abilities even though their mean pH values are similar.

Nitrite depletion and pH were highly correlated ($r=.95$, $p=.0001$) in the sausages containing added carbohydrate while only slightly less ($r=.87$, $p=.0001$) in the sausages formulated without. Although the mean pH levels of bacterial treatments was found not significant, the terminal pH levels (Table 19) of sausages without added carbohydrate

Table 19--Terminal pH and residual nitrite values for bacterial and chemically acidified treatments in 2 finished summer style sausage formulations containing 156 ppm nitrite and beef with and without added carbohydrate

Treatment	Beef with Carbohydrate		Beef without Carbohydrate	
	pH	Nitrite (ppm)	pH	Nitrite (ppm)
Initial value[a]	5.85	156	5.84	157
Control (uninoc.)	5.63	46	5.70	56
lactic acid (encap.)	5.15	9	5.14	10
glucono delta lactone	5.13	14	5.14	15
Beef LBS 1-3	5.24	10	5.44	16
Streptococcus faecalis[e]	5.14	6	5.47	14
Mutton MRS 5-5	5.23	5	5.46	10
Pediococcus pentosaceus[c]	5.01	4	5.47	9
Lactobacillus leichmannii[b]	5.00	1	5.42	11
Lactobacillus plantarum[d]	5.00	4	5.39	11
Beef LBS 3-2	5.03	2	5.49	15

[a]Taken at time of inoculation

[b]ATCC

[c]Trumark Inc.

[d]ABC Research Corp.

[e]pork isolate

fall in a range (pH 5.39-5.49) decidedly above that stated by Kramlich et al. (1973) as acceptable for a commercial fermented product (pH 4.8-5.0). This, combined with terminal residual nitrite levels below 20 ppm (Table 19), may make a product containing a bacterial culture but no added fermentable carbohydrate suspect as to its botulinal safety.

L. leichmannii, S. faecalis (pork isolate), and beef isolates Beef LBS 1-3 and Beef LBS 3-2, appear to have abilities to reduce nitrite (Table 20, Fig 1) and pH (Fig. 2) similar to L. plantarum and P. pentosaceus, two commercial starter cultures already in use. These four species are not currently used commercially but may be deserving of further research, including sensory evaluation of the products they produce.

Table 20--Duncan's multiple range test comparing mean residual nitrite for bacterial and chemically acidified treatments in summer style sausages formulated with and without added carbohydrate, containing 156 ppm nitrite and incubated at 38° C with determinations taken at 0, 6, 12, 18, 24, 30, and 36 hours

Treatment	Mean Residual Nitrite (ppm)[f]/Duncan Grouping[a]	
	Beef with carbohydrate	Beef without carbohydrate
Control[g]	90.0	97.4
lactic acid (encap.)	129.1 A	129.4 A
glucono delta lactone	128.6 A	129.0 A
Beef LBS 1-3	55.9 B	61.4 B
Streptococcus faecalis[e]	52.3 B	61.0 B
Mutton MRS 5-5	48.9 B	47.1 C
Pediococcus pentosaceus[c]	48.2 B	56.2 B C
Lactobacillus leichmannii[b]	47.3 B	45.4 C
Lactobacillus plantarum[d]	38.6 C	48.3 B C
Beef LBS 3-2	34.2 C	54.0 B C

[a]Means with the same letter are not significantly different at p = .05. Duncan value = 13.256.

[b]ATCC

[c]Trumark Inc.

[d]ABC Research Inc.

[e]pork isolate

[f]Analysis for nitrite is accurate to 1 ppm. Fractional values are due to mean computation (14 data points) and are necessary for Duncan procedure.

[g]Not given a Duncan grouping since not included in analysis of variance.

Fig. 1--Nitrite depletion in summer-style sausages inoculated with 10^7 bacterial cells per g of meat, containing 156+/-2 ppm added nitrite and 2% added carbohydrate and processed at 38^0 C.

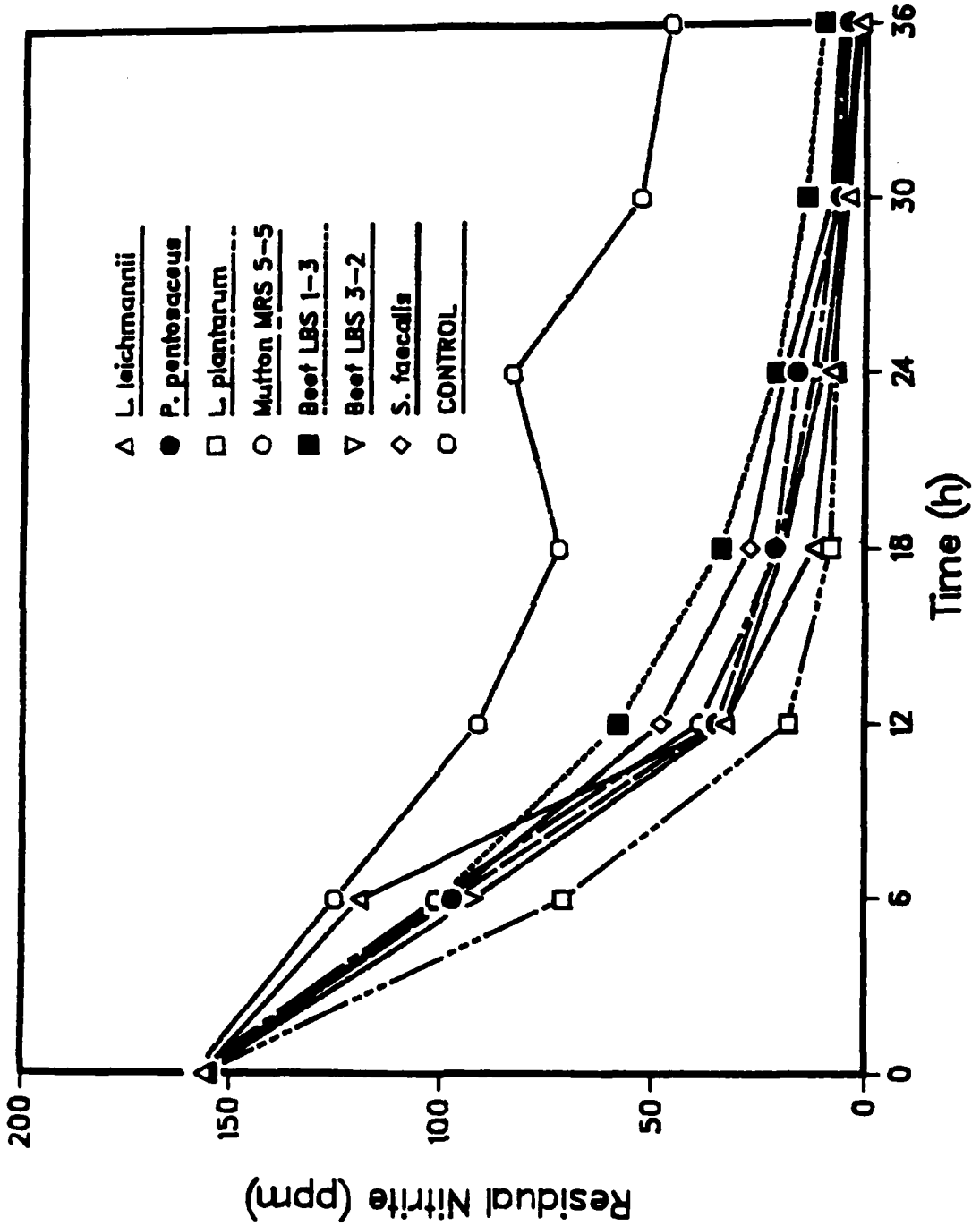
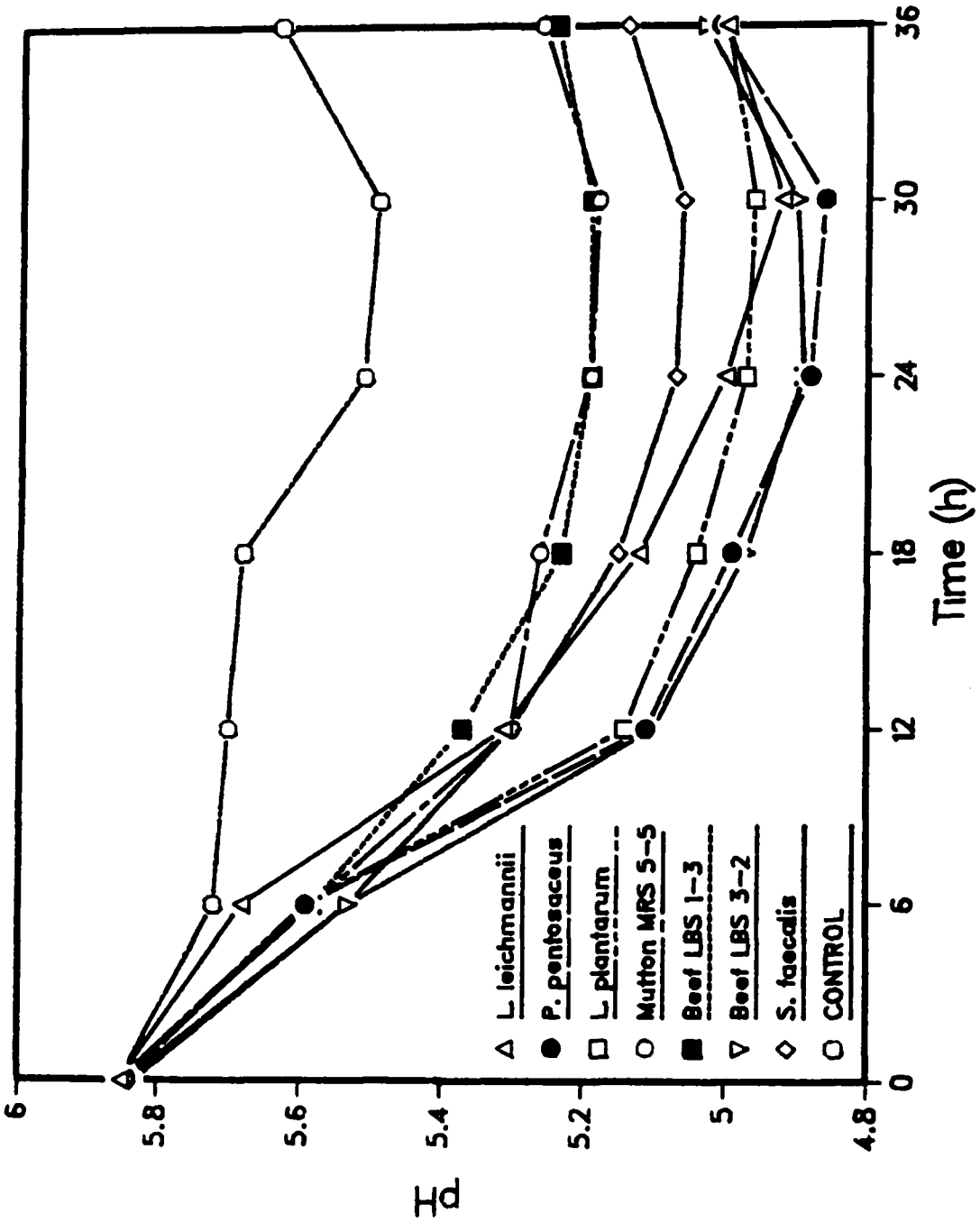

Residual Nitrite (ppm)
200
150
100
50
0
0
6
12
18
24
30
36
Time (h)
L. leichmannii
P. pentosaceus
L. plantarum
Mutton MRS 5-5
Beef LBS 1-3
Beef LBS 3-2
S. faecalis
CONTROL

Fig. 2-7 Changes in pH in summer-style sausages inoculated with 10^7 bacterial cells per g of meat, containing 156+/- 2 ppm added nitrite and 2% added carbohydrate and processed at 38^0 C.


pH
6
5.8
5.6
5.4
5.2
5
4.8
0
6
12
18
24
30
36
Time (h)
L. leichmannii
P. pentosaceus
L. plantarum
Mutton MRS 5-5
Beef LBS 1-3
Beef LBS 3-2
S. faecalis
CONTROL

CONCLUSION

Nitrite depletion and acid production are affected by the factors of time, incubation temperature, pH, and bacterial action independent of pH. Temperatures greater than 15^{o} C were more effective in increasing the rate of acid production and nitrite depletion. These rates were also shown to increase over time.

Nitrite depletion and acid production are highly correlated in all systems where any fermentable carbohydrate was present to lower pH values. Decreasing pH values enhanced significantly the rate of nitrite depletion in all media used for bacterial growth.

In basal broth where fermentable carbohydrate was negligible, L. leichmannii, S. faecalis, P. pentosaceus, L. plantarum, and three atypical lactobacilli isolated from fresh beef and mutton showed an ability to reduce nitrite irrespective of pH. In meat systems containing no added carbohydrate, this ability is masked by decreasing pH values apparently due to fermentable substances naturally in the meat.

Individual bacterial species affect the amount of nitrite reduced through their ability to lower pH and through a system independent of pH. When the pH levels produced by several bacteria were statistically the same, their rates of nitrite depletion were found to be different.

L. leichmannii and S. faecalis were inhibited somewhat in their nitrite reduction abilities in basal broth by carbon monoxide gas, an efficient enzyme inhibitor. These bacteria need to be looked at more closely to determine the exact nature of their possible nitrite reductase enzyme systems.

Three bacterial strains not used commercially in fermented meat products showed abilities to produce acid and reduce nitrite as well as two starter cultures presently used by industry. Although not subjected to sensory evaluation, L. leichmannii, S. faecalis, and an atypical lactobacillus isolated from beef produced summer-style sausages with similar pH and residual nitrite levels and should be examined for their commercial application.

REFERENCES

Anon. 1980. Does nitrite impart color? Meat Industry. June:42.

Association of Official Analytical Chemists. 1980. "Official Methods of Analysis," 13th Ed. Washington, D.C.

Bard, J. 1977. Collaborative USDA, FDA, and AMI studies on sodium nitrate and sodium nitrite in cured meat products. Presented to the Expert Panel on Nitrites and Nitrosamines. June 27. USDA, Washington, D.C.

Bartholomew, D.T., and Blumer, T.N. 1980. Inhibition of Staphylococcus by lactic-acid bacteria in country-style hams. J. Food Sci. 45:420.

Binkerd, E.F., and Kolari, O.E. 1975. The history and use of nitrate and nitrite in the curing of meat. Food Cosmet. Toxicol. 13:655.

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

Christiansen, L.N. 1980. Factors influencing botulinal inhibition by nitrite. Food Technol. 34(5):237.

Christiansen, L.N., Johnston, R.W., Kautter, D.A., Howard, J.W., and Aunan, W.J. 1973. Effect of nitrite and nitrate on toxin production by Clostridium botulinum and on nitrosamine formation in perishable canned communited meat. Appl. Microbiol. 25:357.

Christiansen, L.N., Tompkin, R.B., and Shaparis, A.B. 1978. Fate of Clostridium botulinum in perishable canned cured meat at abuse temperatures. J. Food Prot. 41:354.

Christiansen, L.N., Tompkin, R.B., Shaparis, A.B., Keuper, T.V., Johnston, R.W., Kautter, D.A., and Kolari, O.J. 1974. Effect of sodium nitrite on toxin production by Clostridium botulinum in bacon. Appl. Microbiol. 27:733.

Collins-Thompson, D.L., and Rodriguez Lopez, G. 1981. Depletion of sodium nitrite by lactic acid bacteria isolated from vacuum-packed bologna. J. Food Prot. 44:593.

Crosby, N.T., and Sawyer, R. 1976. N-nitrosamines: A review of chemical and biological properties and their estimation in foodstuffs. Adv. Food Res. 22:1.

Cross, C.K., and Ziegler, P. 1965. Comparison of the volatile fractions from cured and uncured meat. J. Food Sci. 30:610.

Deibel, R.H., Wilson, G.D., and Niven, C.F., Jr. 1961. Microbiology of meat curing IV. A lyophilized Pediococcus cerevisiae starter culture for fermented sausage. Appl. Microbiol. 9:239.

DeMan, J.C., Rogosa, M., and Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23:130.

Dethmers, A.E., Rock, H., Fazio, T., and Johnston, R.W. 1975. Effect of added sodium nitrite and sodium nitrate on sensory quality and nitrosamine formation in thuringer sausage. J. Food Sci. 40:491.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. 11:1.

Emi-Miwa, M., Okitani, A., and Fujimaki, M. 1976. Comaprison of the fate of nitrite added to whole meat, meat fractions and model systems. Agr. Biol. Chem. 40:1387

Fiddler, W., Piotrowski, E.G., Pensabene, J.W., Doer, R.C., Wasserman, A.E. 1972. Effect of sodium nitrite concentration on N-nitrosodimethlyamine in frankfurters. J. Food Sci. 37:668.

Fournaud, J., and Mocquot, G. 1966. Etude de la reduction de l'ion nitrite par certains lactobacilles. C. R. Acad. Sci. Paris. 262:230.

Fox, J.B., Jr. 1966. The chemistry of meat pigments. J. Agr. Food Chem. 14:207.

Fox, J.B., Jr., and Ackerman, S.A. 1968. Formation of nitric oxide myoglobin: Mechanisms of the reaction with various reductants. J. Food Sci. 33:364.

Fox, J.B., Jr., and Nicholas, R.A. 1974. Nitrite in meat: Effect of various compounds on loss of nitrite. J. Agr. Food Chem. 22.302.

Fujimaki, M., Emi, M., and Okitani, A. 1975. Fate of nitrite in meat-curing model systems composed of myoglobin, nitrite and ascorbate. Agr. Biol. Chem. 39:371.

Gardner, G.A., Carson, A.W., and Patton, J. 1967. Bacteriology of prepacked pork with reference to the gas composition within the pack. J. Appl. Bacteriol. 62:321.

Goodfellow, S.J. 1979. Acidulants. Proc. Meat Ind. Res. Conf., Amer. Meat Inst Found. Chicago, Ill. p. 87.

Gray, J.I., and Randall, C.J. 1979. The nitrite/N-nitrosamine problem in meats: An update. J. Food Prot. 42:168.

Griffith Laboratories. 1971. Rapid and official analytical methods for the meat industry. The Griffith Laboratories. Chicago, IL.

Grindley, A.S. 1929. The influence of potassium nitrate on the action of bacteria and enzymes. Studies in Nutrition, Univ. of Illinois, Urbana, Ill., 2:359.

Haldane, J. 1901. The red color of salted meat. J. Hyg. Camb. 1:115.

Herring, H.K. 1973. Effect of nitrite and other factors on the physico-chemical characteristics and nitrosamine formation in bacon. Proc. Meat Ind. Res. Conf., Amer. Meat Inst. Found., Chicago, Ill. p. 47.

Hoagland, R. 1908. The action of saltpeter upon the color of meat. USDA Bureau of Animal Industry. 25th Ann. Rept. p. 301, Washington, D.C.

Hustad, G.O., Cerveny, J.G., Trenk, H., Deibel, R.H., Kautter, D.A., Fazio, T., Johnston, J.W., and Kolari, O.E. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in weiners. Appl. Microbiol. 26:22.

Ingram, M. 1975. The lactic acid bacteria--a broad view. In "Lactic acid bacteria in beverages and food." J.G. Carr, C.V. Cutting, and G.C. Whiting (Eds.). p. 209. Academic Press, London.

Jensen, L.B. 1954. " Microbiology of meats." 3rd ed. The Garrard Press. Champaign, Ill.

Jones, H.B., and Grendon, A. 1976. Nitrosamine carcinogenisis and latency. Proc. Meat Ind. Res. Conf., Chicago. Ill.

Kerr, R.H., Marsh, C.T.N., Schroeder, W.F., and Boyer, E.A. 1926. The use of sodium nitrite in curing meat. J. Agric. Res. 33:541.

Kitchell, A.G., and Shaw, B.G. 1975. Lactic acid bacteria in fresh and cured meats. In "Lactic acid bacteria in beverages and food." J.G. Carr, C.V. Cutting, and G.C. Whiting (Eds.), p. 209. Academic Press, London.

Kramlich, W.E., Pearson, A.M., and Tauber, F.W. 1973. "Processed Meats", AVI Publishing Co., Inc. Westport, Conn.

Kubberod, G., Cassens, R.G., and Greaser, M.L. 1974. Reaction of nitrite with sulfhydryl groups of myosin. J. Food Sci. 39:1228.

Kueper. T.V., and Trelease, R.D. 1974. Variables affecting botulinum toxin development and nitrosamine formation in fermented sausages. Proc. Meat Ind. Res. Conf., Amer. Meat Inst. Found., Chicago, Ill. p. 69.

Lechowich, R.V., Brown, W.L., Diebel, R.H., and Somers, I.I. 1978. The role of nitrite in the production of canned cured meat products. Food Technol. 32(5):45.

Lee, S.H., Cassens, R.G., Winder, W.C., and Fennema, O.R. 1978. Factors affecting the formation of nitrate from added nitrite in model systems and cured meat products. J. Food Sci. 43:673.

Lijinsky. W. 1976. Health problems associated with nitrite and nitrosamines. Ambio. 5:67.

Magee, P.N. 1971. Toxicity of nitrosamines: Their possible human health hazards. Food Cosmet. Toxicol. 9:207.

Magee, P.N., and Barnes, J.M. 1956. The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine. Brit. J. Cancer. 10:114.

Marriot, N.G., Lechowich, R.V., and Pierson, M.D. 1981. Use of nitrite and nitrite-sparing agents in meats: A review. J. Food Prot. 44:881.

Mottram, D.S., and Rhodes, D.N. 1974. Nitrite and the flavor of cured meat. p. 161. In B. Krol, and B.J. Tinbergen (eds.) Proc. of the Intern. Symp. on Nitrite in Meat Products. Pudoc, Wageningen, The Netherlands.

Nitrite Safety Council. 1980. A survey of nitrosamines in sausages and dry-cured meat products. Food Technol. 34(7):45.

Nordin, H.R. 1969. The depletion of added sodium nitrite in ham. Can. Inst. Food Sci. Technol. J. 2:79.

Olsman, W.J., and Krol, B. 1972. Depletion of nitrite in heated meat products during storage. Proc. 18th Europ. Meet. Meat Res. Workers Conf. Guelph, Ontario, Canada. p. 409.

Ostle, B., and Mensing, R.W. 1975. "Statistics in Research" 3rd ed. The Iowa State University Press, Ames, Iowa.

Payne, W.J. 1973. Reduction of nitrogenous oxides by microorganisms. Bacteriol. Rev. 37:409.

Payne, W.J., Riley, P.S., and Cox, C.D. 1971. Separate nitrite, nitric oxide, and nitrous oxide reducing fractions from Pseudomonas perfectomarinus. J. Bacteriol. 106:356.

Perigo, J.A., Whiting, E., and Bashford, T.E. 1967. Observations on the inhibition of vegetative cells of Clostridium sporogenes by nitrite which has been autoclaved in a laboratory medium discussed in context of sub-lethally processed cured meats. J. Food Technol. 2:377.

Reddy, D., Lancaster, J.R., and Cornforth, D.P. 1983. Nitrite inhibition of Clostridium botulinum: Electron spin resonance detection of iron-nitric oxide complexes. Science. 221:769.

Reuter, G. 1975. Classification problems, ecology and some biochemical activities of lactobacilli of meat products. In "Lactic acid bacteria in beverages and food." J.G. Carr, C.V. Cutting, and G.C. Whiting (Eds.) p. 221. Academic Press, London.

Roberts, T.A., and Ingram, M. 1966. The effect of sodium chloride, potassium nitrate and sodium nitrite on the recovery of heated bacterial spores. J. Food Technol. 1:147.

Rubin, L.J. 1977. Nitrites and nitrosamines in perspective. Can. Inst. Food Sci. Technol. J. 10:A11.

Scanlan, R.A. 1975. N-Nitrosamines in foods. C.R.C. Critical Rev. Food Technol. 5:363.

Shank, J.L., Silliker, J.H., and Harper, R.H. 1962. The effect of nitric oxide on bacteria. Appl. Microbiol. 10:185.

Sofos, J.N., and Busta, F.F. 1980. Alternatives to the use of nitrite as an antibotulinal agent. Food Technol. 34(5):244.

Sofos, J.N., Busta, F.F., and Allen, C.E. 1979. Botulism control by nitrite and sorbate in cured meats: A review. J. Food Prot. 42:739.

Tarr, H.L.A. 1941a. The action of nitrates on bacteria. J. Fish. Res. Bd. Can. 5:265.

Tarr, H.L.A. 1941b. Bacteriostatic action of nitrites. Nature. 147:417.

Tarr, H.L.A. 1942. The action of nitrites on bacteria; further experiments. J. Fish. Res. Bd. Can. 6:74.

Thauer, R.K., Jungerman, K., and Decker, K. 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41:100.

Tompkin, R.B., Christiansen, L.N., and Shaparis, A.B. 1978a. Effect of prior refrigeration on botulinal growth in perishable canned cured meat when temperature abused. Appl. Environ. Microbiol. 35:863.

Tompkin, R.B., Christiansen, L.N., Shaparis, A.B. 1978b. Causes of variation in botulinal inhibition in perishable canned cured meat. Appl. Environ. Microbiol. 35:886.

Tompkin, R.B., Christiansen, L.N., and Shaparis, A.B. 1978c. The effect of iron on botulinal inhibition in perishable canned cured meat. J. Food Technol. 13:521.

United States Department of Agriculture. 1908. Regulations governing the meat inspection. Bureau of Animal Industry, Order 150, as amended, effective May 1. p. 33.

United States Department of Agriculture. 1925. Bureau of Animal Industry. Service and Regulatory Announcements. p. 102.

United States Department of Agriculture. 1975. Nitrates, nitrites and salt. Fed. Regist. 40:52614.

United States Department of Agriculture. 1978. Nitrates, nitrites and ascorbates (or isoascorbates) in bacon. Fed. Regist. 43:20992.

United States Department of Agriculture. 1979. Acid producing microorganisms in meat products for nitrite dissipation. Fed. Regist. 44:9372.

Vahabzadeh, F., Collinge, S.K., Cornforth, D.P, Mahoney, A.W., and Post, F.J. 1983. Evaluation of iron binding compounds as inhibitors of gas and toxin production by Clostridium botulinum in ground pork. J. Food Sci. 48:1445

Wasserman, A.E., and Talley, F. 1972. The effect of sodium nitrite on the flavor of frankfurters. J. Food Sci. 37:536.

White, J.W., Jr. 1975. Relative significance of dietary sources of nitrate and nitrite. J. Agr. Food Chem. 23:886.

Widdus, R., and Busta, F.F. 1982. Antibotulinal alternatives to the current use of nitrite in foods. Food Technol. 36(12):105.

Woolford, G., Cassens, R.G., Greaser, M.L., and Sebranek, J.G. 1976. The fate of nitrite: Reaction with protein. J. Food Sci. 41:585.

Youatt, J.B. 1954. Denitrification of nitrite by a species of Achromobacter. Nature (London). 173:826.

APPENDICES

Appendix A

Basal MRS Broth Medium

(DeMan et al., 1960)

Tryptone	10 g
Yeast extract	5 g
Polyoxyethylene sorbitan mono-oleate (Tween-80)	1 ml
Potassium phosphate, dibasic (K_2HPO_4)	2 g
Sodium acetate ($CH_3COONa.3H_2O$)	5 g
Triammonium citrate ($(NH_4)_3C_6H_5O_7$)	2 g
Magnesium sulfate ($MgSO_4.7H_2O$)	200 mg
Manganese sulfate ($MnSO_4.4H_2O$)	50 mg
Distilled water	1 l

1. Combine the ingredients and heat to boiling to dissolve completely.
2. Sterilize for 15 minutes at 15 pounds pressure (121^{o} C).
3. The pH generally falls between 6.0 and 6.5 after sterilizing (6.2 to 6.6 before).

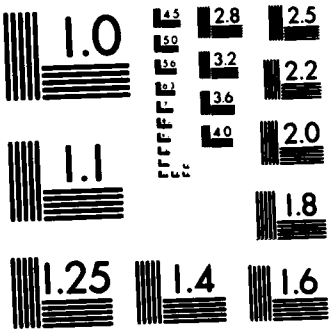
AD-A145 426 EFFECTS OF LACTIC ACID BACTERIA ON RESIDUAL NITRITE IN A SUMMER STYLE SAUSAGE(U) UTAH UNIV SALT LAKE CITY B L WOODBURY 1884 2/2

UNCLASSIFIED F/G 6/13 NL

END

FILMED

DTIC



Appendix B

Determination of Residual Nitrite (Cured Meats)

(AOAC, 1980)

Reagents and Apparatus

1. NED reagent. Dissolve 0.2 g N-(1-naphthyl) ethylenediamine.2HCl in 150 ml 15% (v/v) acetic acid. Filter if necessary and store in a brown glass bottle
2. Sulfanilamide reagent. Dissolve 0.5 g sulfanilamide in 150 ml 15% acetic acid. Filter if necessary and store in a brown glass bottle.
3. Nitrite standard solutions. Stock solution-1000 ppm sodium nitrite. Dissolve 1.000 g sodium nitrite in distilled water and dilute to 1 liter. Intermediate solution-100 ppm sodium nitrite. Dilute 100 ml stock solution to 1 liter with distilled water. Working solution-1 ppm sodium nitrite. Dilute 10 ml intermediate solution to 1 liter with distilled water.
4. Filter paper. Test for nitrite contamination by analyzing 3-4 sheets, at random, throughout the box. Filter about 40 ml water through each sheet. Add 4 ml sulfanilamide reagent, mix let stand 5 minutes, add 4 ml NED reagent, mix, and wait 15 minutes. If any sheets are positive, discard entire box.

Determination

5. Weigh 5 g finely comminuted and thoroughly mixed sample into a 50 ml beaker. Add about 40 ml water heated to 80° C. Mix thoroughly with a glass rod, taking care to break up all lumps, and transfer to a 500 ml volumetric flask. Add enough hot water to bring the volumn to approximately 300 ml, transfer flask to steam bath, let stand 2 hours, shaking occasionally. Cool to room temperature, dilute to volumn with water, and remix. Filter, add 2.5 ml sulfanilamide reagent to an aliquot containing 5-50 micrograms sodium nitrite in a 50 ml volumetric flask, and mix. After 5 minutes, add 2.5 ml NED reagent, mix, dilute to volumn, mix, and let color develop 15 minutes. Transfer portion of solution to a photometer cell and observe absorbance at 540 nanometers against a blank of 45 ml water, 2.5 ml sulfanilamide reagent and 2.5 ml NED reagent.

6. Determine nitrite present by comparison with a standard curve prepared as follows: Add 10, 20, 30, 40 ml nitrite working standard solution to 50 ml volumetric flasks, add 2.5 ml sulfanilamide reagent, mix, and proceed as above, beginning "After 5 minutes,..." in step 5 above. The standard curve is a straight line to 1 ppm sodium nitrite in the final solution.

Appendix C

Modified Babcock Rapid Fat Determination

(Griffith Laboratories, 1971)

1. Tare paley bottle on balance.
2. Weigh 9 g of meat into paley bottle.
3. Add 10 ml warm water into paley bottle through wide opening.
4. Stopper wide opening and shake sample to disperse meat.
5. Carefully and slowly add 15-17 ml of concentrated sulfuric acid down the reading tube (3-5 ml at a time). Mix or swirl sample until it is digested (dark purplish-black color) (no lumps or particles remain).
6. Add hot water sufficiently to bring fat up to the 45% mark on the reading neck.
7. Balance the preheated centrifuge and centrifuge sample for 2-3 minutes.
8. Remove bottle from centrifuge and read fat column from bottom of lower layer to top of highest layer. The difference from top to bottom is the fat content in percent.

Note: If sample contains more than 40% fat, use 4.5 g instead of 9 g and multiply fat reading by 2. Centrifuge will remove air bubbles and other material so a clear, clean fat column is produced.

Appendix D

Rapid Moisture Determination on Meat Using the CENCO Moisture Balance

(Griffith Laboratories, 1971)

The method utilizes the CENCO Moisture Balance, Model 26680, which measures the moisture content of materials by driving off the water by infrared radiation and relating weight loss to percent moisture.

1. Place aluminum pan into heating chamber.
2. Close heating chamber and tare aluminum pan by the following steps:
   a. Turn dial that regulates the % moisture scale until the 100 value is aligned with the center line.
   b. Turn dial on left of the Cenco Balance and adjust arrow to coincide with the 100 setting and the center line.
   c. Readjust the % moisture scale back to zero by turning the dial on the right side.
3. Weigh exactly 5 g of finely ground beef onto the tarred aluminum pan. This is done by adding small portions of meat to the pan until the red indicator on the moisture graduated scale is aligned with the zero point and center line.
4. Important: Set heating dial to 90. This is essential in that a higher setting will cause splattering and will give a false moisture value.

5. Close cover, turn on 250 watt infrared bulb and allow the heating to proceed for 15 minutes.

6. When 15 minutes have elapsed, realign the graduated scale with the red indicator and the center line by turning the dial on the right of the Cenco Moisture Balance.

Note: In determining the moisture of a meat emulsion, the length of time that the meat sample is submitted to heating should be extended from 15 to 20 minutes.

Appendix E

Tables of Statistical Analysis

Table 21--Analysis of variance of residual nitrite (ppm) in basal MRS media, containing no added carbohydrate, produced by bacterial treatments

sources of variation	Df	M.S.	F	alpha[a]
Bacteria (B)	19	2248.628	395.65	.0001
Time (TI)	1	59724.150	10508.65	.0001
Temperature (TE)	2	59303.285	10434.60	.0001
B X TI	19	223.974	39.41	.0001
B X TE	38	1578.555	277.75	.0001
TI X TE	2	3252.612	572.31	.0001
B X TI X TE	38	136.187	23.96	.0001
Error	120	5.683		
Corrected Total	239			

[a]Significantly different at p = .05

Table 22--Analysis of variance for pH of MRS (Difco) media, containing 2% added carbohydrate, produced by bacterial treatments

sources of variation	Df	M.S.	F	alpha[a]
Bacteria (B)	19	.914	1778.33	.0001
Time (TI)	1	6.137	11936.99	.0001
Temperature (TE)	2	35.188	68437.80	.0001
B X TI	19	.026	51.29	.0001
B X TE	38	.572	1112.70	.0001
TI X TE	2	.240	468.20	.0001
B X TI X TE	38	.054	105.92	.0001
Error	120	.001		
Corrected Total	239			

[a]Significantly different at p = .05

Table 23--Analysis of variance for residual nitrite (ppm) in MRS (Difco) media, containing 2% carbohydrate, produced by bacterial treatments

sources of variation	Df	M.S.	F	alpha[a]
Bacteria (B)	19	3127.899	1169.31	.0001
Time (TI)	1	21281.667	7955.76	.0001
Temperature (TE)	2	387057.460	99999.99	.0001
B X TI	19	239.640	89.59	.0001
B X TE	38	1757.019	656.83	.0001
TI X TE	2	8785.629	3284.35	.0001
B X TI X TE	38	301.510	112.71	.0001
Error	120	2.675		
Corrected Total	239			

[a]Significantly different at p = .05

Table 24--Analysis of variance of pH in 3 model meat systems composed of mutton and beef with and without added carbohydrate

sources of variation	Df	M.S.	F	alpha[a]
Rep (R)	1	.000063	1.39	.05
Bacteria (B)	11	.203319	4508.18	.0001
Meat (M)	2	.533011	11820.53	.0001
B X M	22	.015380	341.01	.0001
Error(a)	35	.000045		
Time (T)	3	8.145455	209394.72	.0001
Error(b)	3	.000039		
B X T	33	.051528	1463.85	.0001
M X T	6	.423156	12021.49	.0001
B X M X T	66	.011748	333.74	.0001
Error(c)	105	.000035		
Corrected Total	287			

[a]Significantly different at p = .05

Table 25--Analysis of variance of residual nitrite (ppm) in 3 model meat systems composed of mutton and beef with and without added carbohydrate

sources of variation	Df	M.S.	F	a'pha[a]
Rep (R)	1	6.490	7.66	.025
Bacteria (B)	11	956.673	1129.48	.0001
Meat (M)	2	449.436	530.62	.0001
B X M	22	174.091	205.54	.0001
Error(a)	35	.847		
Time (T)	3	336158.330	3777059.90	.0001
Error(b)	3	.089		
B X T	33	256.204	498.87	.0001
M X T	6	927.521	1773.46	.0001
B X M X T	66	88.713	169.62	.0001
Error(c)	105	.523		
Corrected Total	287			

[a]Significantly different at p = .05

Table 26--Analysis of variance of pH in 2 fermented summer style sausage formulations composed of beef with and without added carbohydrate

sources of variation	Df	M.S.	F	alpha
Rep (R)	1	138.514	1.12	NS[a]
Bacteria (B)	6	110.505	.89	NS
Dextrose level (D)	1	114.417	.93	NS
B X D	6	109.772	.89	NS
Error(a)	12	123.567		
Time (T)	6	122.797	.98	NS
Error(b)	6	125.489		
B X T	36	114.074	.91	NS
D X T	6	120.584	.96	NS
B X D X T	36	113.828	.91	NS
Error(c)	72	125.375		
Corrected Total	188			

[a]Not significantly different at = .05

Table 27--Analysis of variance of residual nitrite in 2 fermented summer style sausage formulations composed of beef with and without added carbohydrate

sources of variation	Df	M.S.	F	alpha[a]
Rep (R)	1	168.339	1.28	NS[b]
Bacteria (B)	6	918.209	7.01	.005
Dextrose level (D)	1	2168.681	16.55	.005
B X D	6	392.042	3.00	.05
Error(a)	12	131.042		
Time (T)	6	77710.537	2616.25	.0001
Error(b)	6	29.703		
B X T	36	164.455	5.53	.0001
D X T	6	78.175	2.63	.025
B X D X T	36	64.437	2.17	.005
Error(c)	72	29.713		
Corrected Total	188			

[a]Significantly different at p = .05

[b]Not significantly different at p = .05

VITA

Bruce L. Woodbury

Candidate for the Degree of

Master of Science

Thesis: Effects of Lactic Acid Bacteria on Residual Nitrite in a Fermented Summer Style Sausage

Major Field: Nutrition and Food Science

Biographical Information:

Personal Data: Born at Logan, Utah, 27 April 1951, son of LaVar D. and Lora A. Woodbury; married Patricia J. Berardy 11 January 1974; children--Angela, Ben, and Ryan.

Education: Attended elementary and junior high school in Logan, Utah; graduated from Logan High School in 1969; received Bachelor of Science Degree in Nutrition and Food Science from Utah State University in Logan, Utah in 1976; completed U.S. Army Quartermaster Food Management Course at the Quartermaster School, Fort Lee, Virginia in 1976.

Experience: 1970-1972, completed a mission to England for The Church of Jesus Christ of Latter-day Saints; 1976, commissioned in the Regular Army and called to active duty as a Quartermaster Officer; 1976-1984, served in several food-related assignments including Subsistance (Food Management) Staff Officer and Company Commander attaining the rank of Captain.