Processing factors that influence the quality of Braunschweiger liver sausage

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Liver sausage and Braunschweiger liver sausage are mixtures of meats consisting of pork liver, pork trimmings, a mixture of spices and salt. In addition to these, sodium nitrite, sugar, milk powder and perhaps other extenders are added to the meat. Production of liver sausage and Braunschweiger liver sausage has a unique and important role in the processed meat industry. It is a method that maximizes the utilization of unpopular raw meats such as organ meats and meat byproducts and converts these less expensive raw materials into highly nutritious products.

Little attention has been given to the quality of these products and the desirable characteristics and properties that attract consumers are not well defined. The composition of the product, methods of processing, cooking and handling — which have a great influence on the quality of the finished product — vary with each sausage maker. All these factors can directly or indirectly influence the popularity of liver sausage and Braunschweiger in the U.S.A. The production of liver sausage was about 600 million pounds in 1967 (Kramlich, 1971) yet declined to only 100 million pounds in 1977 (Statistical Summary, 1977).

The purposes of this research project were to (1) compile information from various sources including unpublished service manuals, meat technologists, literature, regulations and a survey of meat processors to define and

characterize the desirable attributes for Braunschweiger liver sausage, and (2) investigate the influence of processing variables encountered in the processed meat industry on the organoleptic and microbiological quality of Braunschweiger liver sausage.

The processing factors examined included liver pretreatments, nitrite levels, cooking treatments, addition of calcium-reduced dried skim milk and fat levels. Various liver pretreatments such as freezing, scalding at 93°C for 2 min, and soaking in 5% brine for 1 hr are practiced by meat processors. The use of fresh liver for manufacturing of Braunschweiger liver sausage is usually preferred because most processors are of the opinion that it produces a superior flavor. Many manufacturers have to use frozen liver due to the limited availability of fresh liver. Scalding of the liver has been used as a means of controlling and/or reducing the microbial load of the liver before processing. Soaking of the liver is believed to leach bitter flavors from the liver. All of the reasons mentioned, however, have little experimental data to support them.

The influence on the quality of Braunschweiger liver sausage of different nitrite levels (0, 50, 100, and 156 ppm) incorporated in the formulation was investigated. Nitrite has been used for centuries in the curing of meat. Cured meat develops a pink color and cured flavor which have come to be accepted as normal and desirable. The antimicrobial properties of nitrite

are specifically of concern because of its role in protecting the consumer against hazard of botulism. However, the prospect of banning or reducing nitrite in meat products is under consideration because of its potential role in carcinogenesis.

Cooking temperature plays an important role on the quality of Braunschweiger liver sausage. In general, lower cooking temperatures produce more desirable palatability and appearance than higher cooking temperatures but low cooking temperatures are detrimental to the shelf life of the finished product. Addition of calcium-reduced dried skim milk is thought to inhibit the deleterious effects of high cooking temperatures on the organoleptic quality of the product. Commercially available Braunschweiger liver sausages contain a wide range of fat levels. Therefore, the effects of three levels of fat (20, 30 and 35%) on the sensory quality of sausage were examined.

II. REVIEW OF LITERATURE

A. Liver Sausage and Braunschweiger — General Aspects

1. Standard of identity for liver sausage and Braunschweiger

Liver sausage is a mildly seasoned, cooked mixture of liver and meat. In Europe, many varieties of liver sausages are made which may vary in liver content from 15% to 50% (Brouwer et al., 1976). Related products are Braunschweiger, liver cheese and liver loaf. To assure uniformity and quality of liver sausage and Braunschweiger, the U. S. Department of Agriculture has published a standard of identity for these products in the Federal Register of November 5, 1976. The standard of identity is summarized as follows:

1. Liver sausage and Braunschweiger are cooked sausages made from fresh and/or frozen pork and pork livers and/or beef livers and may contain cured pork, beef, and veal and pork fat.
2. Liver sausage, but not Braunschweiger, may also contain beef and pork byproducts, pork skins, sheep livers and goat livers.
3. These products must contain not less than 30% of liver computed on the weight of fresh liver.
4. The finished products may contain not more than 3.5% of the permitted binders and extenders which include cereal, vegetable starch, starchy vegetable flour, soy flour, soy protein concentrate (3.0% of this extender is equal to 3.5% of others), isolated soy protein (2.0% of

this extender is equal to 3.5% of others), nonfat dry milk and calcium-reduced skim milk or dried milk.

5. No phosphates are allowed in the formulation except those from uncooked pork cured with phosphate (0.5% phosphate in cured meat).
6. These products may contain no more than 10% of added water in the finished product.

2. Manufacture of liver sausage and Braunschweiger

a. Composition Pork liver and pork (especially pork and/or pork trimmings) are the two most commonly employed meat ingredients in the formulation of liver sausage and Braunschweiger. A 1:1 ratio of pork liver to pork jowls as the main meat ingredients of liver sausage and Braunschweiger is usually recommended to assure the correct percentages of fat, liver and natural gelatin. This mixture produces a firm sausage with good spreading qualities and rich flavor (Rust, 1976).

Salt is the most critical ingredient other than meat in sausage manufacture. The three important functions of salt in sausage making are to (1) enhance the flavor of the finished product (2) retard microbial growth and (3) extract the myofibrillar protein of meat which serves as the binding agent for a good, stable emulsion (Tauber, 1957; 1974). The amount of salt used in liver sausage or Braunschweiger formulation is about 2.5% to 3.0%.

Sugars, such as dextrose and corn syrup, at levels of 0.5% are commonly

used in liver sausage and Braunschweiger primarily for flavoring and to help mask the salt flavor when a high salt level is used (Tauber, 1974).

The distinct, attractive odor and flavor of the product, enhanced through proper use of spices and flavorings, plays an important role in palatability and acceptability. The addition of a smoked flavor to liver sausage can be accomplished by the incorporation of bacon ends and/or smoked jowls in the formulation or by the addition of liquid smoke to the product at the rate of 56 to 114 ml (2 to 4 fluid oz) per 45.5 Kg (100 lb) of product (Rust, 1976).

The addition of nitrite to meat products is believed to have four functions: (1) to prevent growth and toxin formation of pathogenic organisms such as Clostridium botulinum and Staphylococcus aureus (Steinke and Foster, 1951d; Johnston et al., 1969; Buchanan and Solberg, 1972; and Greenberg, 1972), (2) to develop a desirable pinkish color (Kisskalt, 1899; Lehmann, 1899), (3) to give the characteristic cured meat flavor (Brooks et al., 1940; Barnett et al., 1965; Cho and Bratzler, 1970; Wasserman and Talley, 1972; Simon et al., 1973; Bailey and Swain, 1973; Herring, 1973; MacNeil and Mast, 1973; Mottram and Rhodes, 1973), and (4) to serve as an antioxidant stabilizing the product against oxidative rancidity (Younthan and Watts, 1959; Zipser, et al., 1964; Love and Pearson, 1971). Traditionally, liver sausage has little or no nitrite but Braunschweiger receives a full nitrite cure. The nitrites, sodium and potassium, are permitted at 7.1 g (0.25 oz) per 45.5 Kg (100 lb) of chopped meat. Although current regulations permit up to 78.1 g (2.75 oz) of sodium or

potassium nitrate per 45.5 Kg (100 lb) of chopped meat, most of the meat industry except for dry-cured ham and fermented sausage, in general, has discontinued the use of nitrate. Nitrate is used as a source of nitrite because it can be reduced by the meat constituents or microbes to form nitrite (Polenske, 1891).

A further restriction is that 200 ppm nitrite is the maximum allowable level in any finished cured meat product (U.S.D.A., 1976a). Recently, the U.S.D.A. Expert Panel on Nitrites and Nitrosamines, suggested some preliminary recommendations for changes in curing regulations as follows (Greenberg, 1975):

1. Eliminate nitrate wherever possible
2. Standardize in-going nitrite at 156 ppm (except bacon and cured primal meats, pending more information)
3. Reduce residual nitrite maximal level (dependent on specific product category) to
    a. 50 ppm in sterile products
    b. 100 ppm in cooked sausage products
    c. 125 ppm in canned and pickle-cured products

Dried skim milk is used as an ingredient in many sausage products. It serves primarily as an extender to reduce formulation costs, to improve processing yields and/or to improve emulsifying capacity (Smith et al., 1973). It also contributes to other desirable sensory properties (Carpenter et al., 1977)

such as flavor and firmness of the product. However, the main purpose of the addition of this extender to the liver sausage formulation containing a high amount of hog skins is to prevent the formation of undesirable gelatin or jelly pockets during the cooking process (Rust, 1976). Calcium-reduced dried skim milk, in which up to 70% of the calcium has been replaced with sodium, is preferred as a binder for sausage emulsions (Kramlich, 1971). Dried skim milk contains approximately 36% protein, of which close to 80% is casein. Most of the casein is combined with calcium to form calcium caseinate which makes this protein almost insoluble in water. For proteins to serve as emulsifiers, they must be dispersed (Hansen, 1960). The ability of dried skim milk to emulsify fat is limited because only a small amount of the protein is readily dispersable. To increase dispersability, part of the calcium can be replaced with sodium.

b. Manufacturing procedures Little information is available about the commercial manufacturing procedures for liver sausage and Braunschweiger. However, manufacturing practices for Braunschweiger are summarized by Rust (1976) as follows:

1. Fresh and/or frozen pork liver are used and pretreatment such as scalding or soaking in cold salt brine may be applied;
2. The pork is ground through a 4.8 mm (3/16 in.) plate;
3. Livers are chopped in a silent cutter with salt, seasoning and nitrite until the mixture becomes bubbly;

4. Pork is added to the mixture and chopped until it reaches 12°C (55°F);
5. Dried skim milk is added to the batter and chopping is continued until it reaches 15-18°C (60-65°F);
6. The mixture is stuffed into a moisture-proof fibrous casing;
7. The sausages are cooked in a water vat at 73.8°C (165°F) until internal product temperature reaches 68.3°C (155°F);
8. After cooking, the product is chilled in a vat containing cold water;
9. After chilling, the sausages are stored in a cooler.

The above manufacturing method is similar to the "conventional method" mentioned by Brouwer et al. (1976) of Holland. The European meat research workers describe the conventional method as chopping the liver with 1.8% salt and nitrite mixture till the beginning of the well-known phenomenon of bubble formation. Then, hot broth which is the cooking water from cooking the minced fat in water at 90°C for 30 min, is added, followed by precooked fat; this mixture then is chopped to a homogeneous product. Finally the balance of salt and nitrite mixture is added. Also, there is another difference between U.S.A. and Holland in the casing used for stuffing of liver sausage. In the U.S.A., the raw mixture of liver sausage made commercially is often stuffed into either a moisture-proof fibrous casing (Rust, 1976) or a Saran casing (Steinke and Foster, 1951b) and water cooked until internal product temperatures reach 68.5°C (155°F) (Rust, 1976) or lower (Kramlich, 1971; Steinke and Foster, 1951d). However, the European meat research workers report that the

processing of liver sausage is done in a can and the heat treatment is such that the center of the can is held at 80°C for 10 min (Grever, 1973) or at 85°C for one hour (referred to as pasteurization) or at 115°C for 45 min (referred to as sterilization) (Brouwer et al., 1976).

Another production method referred to as the "emulsion method" described by Brouwer et al. (1976) has been strongly recommended to liver sausage manufacturers. They reported that the range in which the raw material portion can be varied to obtain a stable liver sausage with acceptable appearance and texture is much greater for the Emulsion Method than for the Conventional Method. The principal difference between these two methods is that in the Emulsion Method the livers and cooked fat-water mixture are chopped separately and then mixed together.

In general, there are two common categories of commercial Braunschweiger available in the supermarket. The first category is the "traditional type" which is manufactured in a long cylindrical stick. The stick is in a moisture-proof fibrous casing, 6-7 cm (2.5 in.) in diameter, 60-70 cm (24-27 in.) in length, weighing about 2.3 to 2.7 Kg (5 lb) with the two ends clipped shut. A great deal of this type of product is cut into small portions and vacuum packaged at the processing plant before transport to the store. Another popular handling approach is that the whole, long sticks are received by the store then separated into small portions that are wrapped with Saran or polyethylene film.

The second category of commercial product is a Braunschweiger raw mixture stuffed into a short transparent heavy Saran type casing (which is known for its low moisture and oxygen permeability) with two ends clipped shut, after which the product is cooked and cooled. This product (the saran chub or small self-service package) is transported to the store with a quality control date stating that the freshness of the product is not guaranteed after that time. Most of the time, the shelf life expectancy is three or four months at refrigerated temperature, without opening the casing. The second category mentioned here is referred to as a "modern type" which has many different sizes such as 114 g (4 oz), 227 g (8 oz), 284 g (10 oz) and 454 g (1 lb). The lighter weights of Braunschweiger are stuffed into a much narrower casing (2.5 to 3.5 cm in diameter) than that of the 454 g.

In addition to the two common types mentioned above, there is another type of Braunschweiger called the "fancy type." Most often the raw batter of this type of product receives little or no nitrite cure and is stuffed into a natural animal casing such as a hog bung, cooked, then smoked at a low temperature (32°C) for 4 to 6 hr. Special care has to be taken to successfully produce this delicate type. The higher price as well as a short shelf life explain why it is not commonly available in most supermarkets. This special type is most often found in localized areas in delicatessens.

3. Desirable characteristics of liver sausage and Braunschweiger

Consumption of unprocessed pork liver is not as high as the consumption of beef liver. Pork is the chief source of livers used in the production of liver sausage and Braunschweiger. Manufacturers make use of this low-demand, strong-tasting byproduct and convert it into a high quality product with great consumer appeal.

The desirable characteristics of Braunschweiger can be considered in terms of composition, color, flavor, texture and homogeneity (with no fat, air or jelly pockets). It is well-known to meat technologists that sausage formulation and composition, production methods, production temperatures, etc. have a great influence on the characteristics of the finished product.

A useful summary (Table 1) of the role of common ingredients of sausage on the characteristics of the finished products has been assembled by Tauber (1957, 1974).

The analysis of the macrocomponents of seven brands of commercial Braunschweiger indicated that the starting formulae varied. The ranges of the percentage of fat, protein, water and salt in these samples were 24-35%, 12-14%, 46-56%, and 2.4-2.9%, respectively (Klement, 1975). Birdsall (1975) also reported a wide range of fat content (26.0-37.8%) in twenty different brands of commercial liver sausage and suggested the need for nutritional labeling and possible standard nutrition

Table 1. The role of some of the ingredients of sausage in color, flavor, texture, yield and nutritive value of the finished sausage (Tauber, 1957; 1974)

	Color	Color development	Color stabilizer	Flavor	Texture	Yield	Added nutritive value
Beef	v[a]	-[b]	-	v	v	v	v
Pork	v	-	-	v	v	v	v
Ice	-	-	-	-	v	v	-
Salt	-	-	-	v	v	-	-
Sodium nitrite	-	v	v	-	-	-	-
Sodium nitrate	-	-	v	-	-	-	-
Spices	v	-	-	v	-	-	-
Sugar and syrups	-	-	v	v	-	-	v
Sodium ascorbate	-	v	v	-	-	-	-
Sodium isoascorbate	-	v	v	-	-	-	-
Binders	-	-	-	-	v	v	v

[a] yes.

[b] no.

information per serving for processed meat items including liver sausage. For cooked sausages such as frankfurters and bologna, there is a limitation of 30% fat content specified in the federal meat inspection regulations (U.S.D.A., 1976b). Liver sausage and Braunschweiger belong to the category of ready-to-serve luncheon meat items which do not have fat specifications. Limitation of fat content in this product may be needed in two respects: (1) to correct unfair criticism by consumers who consider liver sausage and Braunschweiger to be one of the foods containing high levels of fat (2) to attain a better, more uniform quality of liver sausage and Braunschweiger among different brands.

Appearance is an important consumer requirement, color being the most important attribute. The normal color of liver sausage and Braunschweiger may range from a white or gray product (sometimes known as white for fresh liver sausage) to a deep red product generally known as Braunschweiger. The color of the finished product depends on several factors (Anon.,1965):

1. Amount of liver and lean skeletal meat;
2. Residue of blood in liver;
3. Degree of scalding of livers;
4. Levels of curing salts;
5. Percent conversion of total pigment in emulsion to cured meat pigment;
6. Heat processing temperature.

Meat color is determined by the percentage of myoglobin and hemoglobin in combination with muscle, fat and connective tissue. Pork liver and jowl

meat both have quite high color ranking compared to that of other meat ingredients such as pork trimmings and back fat trimmings (Kramlich et al., 1973). In general, a higher liver percentage in the formulation will result in a more desirable color. Increasing the amount of pork trim in a formulation usually results in a lighter colored sausage. The different types and amount of fat in the formulation also play an important role in the color of finished product (Brouwer et al., 1976).

Discoloration of liver sausage and Braunschweiger is a serious problem and a defect which can result in rejection by consumers of this highly nutritious product. Discoloration is not necessarily caused by microbiological growth. Tauber (1971) reported that liver sausage with a low content of curing agents is susceptible to greenish discoloration or brown and black areas. He indicated that one of the factors in discoloration associated with the curing of Braunschweiger may be due to the highly unsaturated fatty acids in liver fat which are subject to oxidation. It is strongly emphasized in Tauber's report that levels of 50 ppm to 120 ppm of residual nitrite in finished Braunschweiger is necessary for both achieving and stabilizing the pink color. In order to achieve a high residual nitrite level in the finished product, the Braunschweiger had to be cooked at water temperature not above 73.8°C (165°F). The internal product temperature is critical and should not be above 62.2°C (145°F). Kramlich (1966) also reported an

approximate fourfold decrease in residual nitrite when the final internal product temperature of the sausage was increased from 62.2°C (145°F) to 68.3°C (155°F). He studied the effect of sodium erythorbate, vacuum chopping and cooking temperature on liver sausage color and found that liver sausage processed at 73.8°C (165°F) to an internal product temperature of 68.3°C (155°F) was a deeper shade of red than sausage processed to 62.2°C (145°F). The pigment conversion throughout 21 days storage period showed the former had 67.7% pigment conversion compared to 62.6% pigment conversion in the latter. However, in the case of both temperatures, there was no difference in color between products with or without erythorbate. Sausage chopped under a full vacuum was slightly darker (redder) than either the nonvacuum or the partial-vacuum chopped products. Similar comments about vacuum chopping in emulsion preparations having the potential to extend product shelf life (in terms of color) have been reported by other research workers, also. Fox et al., (1967) stated that vacuum chopping improved the initial frankfurter color and improved color stability during storage. Ertle (1969) reported that vacuum-chopped, cooked bologna showed more intense cured meat colors than did those receiving nonvacuum chopping. Pigment conversion was 87% for vacuum-chopped and 83% for nonvacuum-chopped bologna. Also the more intense colors of vacuum-chopped bologna were found to be less critically demanding as to the character of the packaging film (oxygen transmission rate) than those of

nonvacuum-chopped bologna.

Flavor is probably the most important single sensory property of sausage. Each sausage product has a characteristic flavor made to appeal to a certain segment of the population. In liver sausage and Braunschweiger as well as other kinds of sausage, the use of salt and spices is necessary and important for development of flavor attributes. In general, the use of fresh liver is recommended for obtaining a better flavor when compared to frozen liver; also a stronger taste is achieved with increasing amounts of liver in the formulation. At a fixed liver concentration, higher amounts of fat incorporated will contribute a richer taste than will low amounts of fat. With conventional manufacturing, the use of belly fat and flare fat in the liver sausage gave a greasy and undesirable taste. In contrast to the above, the use of cheek fat was greatly preferred (Brouwer et al., 1976).

Kramlich (1965) studied three formulations representing three different types of liver sausage commonly produced by manufacturers of liver sausage. He applied the variations of cook water temperature and internal temperature of the product and found that increasing the temperature of the cook water from 73.8°C (165°F) to 79.4°C (175°F) and above resulted in finished products with decreased taste panel scores. He reported that regardless of internal meat temperature attained, average taste panel scores were slightly lower for product cooked at 79.4°C (175°F) and 85°C (185°F) than for product cooked at 68.3°C (155°F) and 73.8°C (165°F).

Smooth, creamy and homogeneous texture is desirable in all of the spreadable, sliceable or sliceable and spreadable kinds of liver sausage. Crumbly, grainy and nonuniform texture is considered to be unacceptable. It is realized that sausage texture is influenced by the kinds and amounts of water, salt and binders. Variations in processing conditions also result in different textures of the finished product. Kramlich (1966) reported the application of different degrees of vacuum during chopping resulted in textural differences. Sausage chopped under a full vacuum showed the smoothest and most dense texture when compared to those products made under partial or nonvacuum treatments. Ertle (1969) also reported that vacuum chopping served to reduce the quantity of air entrapped in the cooked sausages and provided a more compact, dense texture. Vacuum-chopped bologna had a density greater than 1.00 while nonvacuum-chopped products had densities of about 0.95. Brouwer et al. (1976) indicated that higher chopping temperatures promoted spreadability and lower chopping temperatures promoted the sliceability of the finished products. Temperature of the cook water and internal product temperature attained in the liver sausage are considered as the most critical factors for emulsion stability by many sausage technologists (Kramlich, 1965; Tauber, 1971). In general, as cook water temperature is increased, emulsion stability decreases. Sausage cooked at lower cook water temperature and with lower internal temperature showed fewer defects including fat separation, jelly formation and air pockets.

Kramlich (1965) concluded that disregarding other considerations such as shelf life and formulation, the most stable liver sausage was produced when the sausage was cooked at 68.3°C (155°F). The internal temperature of this liver sausage should not exceed 62.2°C (145°F).

4. Nutritional information

There is very little difference in the macrocomponents of pork liver and pork lean meat (Watt and Merrill, 1963; Kiernat et al., 1964). The fat and protein content in both of them are about the same: 3-5% fat and 18-20% protein. In general, pork liver has a slightly higher water content (73%) compared to that of lean pork (70%). However, the use of variety meat and by-products is a common practice for liver sausage manufacturing because these items are less expensive than the more popular cuts. The proximate composition and energy value of fresh lean meats, livers and various common meat cuts and byproducts are shown in Table 2.

Table 3 shows the macrocomponents of liver sausage and frankfurters, the most popular sausage product, representing about 29% of all sausage sold in the United States (Watt and Merrill, 1963; Birdsall, 1975). In general, liver sausage has a slightly higher protein content compared to that of frankfurters. Both products have a similar fat content, on the average about 27-30% but liver sausage has much wider range in fat content.

As shown in Table 4, meat is a very good dietary source of several of the B vitamins. Pork, especially, has a high thiamine level and represents an

Table 2. Composition of fresh lean meat, livers and variety meat and byproducts

Product description	Protein (%)	Water (%)	Fat (%)	Ash (%)	Carbohydrates (%)	Cal/100 g
Pork liver[a]	20.6	71.6	3.7	1.5	2.6	131
Beef liver[a]	19.9	69.7	3.8	1.3	5.3	140
Lamb liver[a]	21.0	70.8	3.9	1.4	0	140
Pork, carcass — thin[b] separable lean-raw	18.3	70.7	8.6	2.4		156
Pork, carcass — thin[b] (53% lean, 47% fat) raw	11.2	41.1	47.0	0.6		472
Regular pork trim[c]	9.7	34.3	55.0			
Lean pork trim[d]	15.2	55.9	28.0			
Pork cheek[d]	16.2	58.9	24.0			
Pork fat[e]			66.0			
Soft belly fat[f]		15.0				
Cheek fat[f]		25.0				
Flare fat[f]		6.0	66.0			
Back fat[g]		13.0	85.0			

[a]Kiernat, et al., 1964.

[b]Watt and Merrill, 1963.

[c]Cook, 1974.

[d]Carpenter, 1974.

[e]Anonymous. 1973.

[f]Brouwer et al., 1976.

[g]Carpenter, et al., 1977.

Table 3. Macrocomponents of some sausages

Product description	Cal/100 g	Water (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Braunschweiger[a]	319	52.9	14.8	27.4	2.3	2.9
Liver sausage[b]	346	ND[c]	13.9	30.9	ND	ND
Frankfurter[a]	304	57.3	12.4	27.2	1.6	1.5
Frankfurter[b]	319	ND	11.3	29.0	ND	ND

[a]Watt and Merrill, 1963.

[b]Birdsall, 1975.

[c]No data.

Table 4. The vitamin content of fresh meats and livers (mg/100 g)

	Product Description				
	Medium Grade[a] Beef Loin	Medium Grade[a] Pork Loin	Beef Liver[b]	Pork Liver[b]	Lamb Liver[b]
Thiamine	0.10	0.800	0.250	0.300	0.40
Riboflavin	0.13	0.190	3.260	3.030	3.28
Nicotinic acid	4.60	4.300	13.600	16.400	16.90
Vitamin B_6	ND[c]	0.500	0.740	0.510	0.37
Pantothenic acid	ND	2.000	7.300	6.600	8.10
Biotin[d]	ND	5.500	100.000	85.000	130.00
Folic acid[d]	ND	0.007	0.081	0.074	ND
Vitamin B_{12}[d]	ND	ND	65.000	23.000	35.00
Vitamin C	ND	ND	31.000	23,000	33.00
Vitamin A[e]	ND	ND	43.900	10.900	50.50

[a]Watt and Merrill, 1963.

[b]Kiernat, et al., 1964.

[c]No data.

[d]µg/100 g

[e]IU/100 g

outstanding source of thiamine in the diet. The nutritional value of liver deserves special attention because of its relatively high content of a number of vitamins. Vitamin A and Vitamin C (ascorbic acid) are not found in significant amounts in muscle meat or in most other organs but are present in significant amounts in liver. Thus, nutritionally, liver is an excellent source of these trace nutrients.

Most of the vitamins in meat are relatively stable to processing procedures. The thiamine present in meat is partially destroyed in the course of such processes as curing, smoking, cooking, canning, etc. The extent of the retention of thiamine averages about 75% in cooked and processed meat and meat products and appears to be related to the severity of the heat processing methods employed (Rice, 1971). Vitamin B_6 was reported more stable and, in general, will show only about half as much heat loss as thiamine (Noble, 1964, 1965; Meyer et al., 1969). Riboflavin and niacin, as well as other B vitamins, are stable to cooking and standard processing techniques. Refrigerated and frozen storage have little or no effect on vitamin levels in meat unless rancidity or bacterial action develops (Rice, 1971). Table 5 shows the mineral and vitamin content of some sausages and canned meat items. It is realized that meat products are low in calcium. However, liver sausage is an excellent source of vitamin B_{12}, riboflavin, niacin, and iron. Birdsall (1975) reported that liver sausage contains as much as 20 times, 10 times, 3 times, and 6 times of the above

Table 5. Mineral and vitamin content of some sausages and canned meats (mg/100 g)

	Product Description						
	Canned Baby Food			Sausage			
	Liver[a] (strained)	Beef[a] (strained)	Pork[a] (strained)	Braunschweiger[a]	Liver[b] sausage	Frankfurters[a]	Frankfurters[b]
Ca	6.00	8.00	8.00	10.00	ND[c]	5.00	ND
P	182.00	127.00	130.00	245.00	ND	102.00	92.00
Fe	5.60	6.00	1.50	5.90	8.60	1.50	1.30
Na	253.00	228.00	223.00	-	ND	-	ND
K	202.00	183.00	178.00	-	ND	-	ND
Thiamine	0.05	0.01	0.19	0.17	0.24	0.15	0.23
Riboflavin	2.00	0.16	0.20	1.44	1.31	0.20	0.14
Niacin	7.60	3.50	2.70	8.20	7.20	2.50	2.30
Vitamin A[d]	24,000.00	-[e]	-	6,530.00	13,317.00	-	ND
Vitamin B_{12}[f]	ND	ND	ND	ND	20.80	ND	1.30
Vitamin C	10.00	0[g]	-	-	12.30	-	ND

[a]Watt and Merrill, 1963.

[b]Birdsall, 1975.

[c]No data.

[d]IU/100 g.

[e]No reliable data, the amount is believed to be negligible.

[f]μg/100 g.

[g]No detectable amount.

specific microcomponents as compared to frankfurters. He also suggested the possible standard nutritional information per serving of liver sausage and meat frankfurters as shown in Table 6.

5. Survey of the industry

In order to gain insight into some of the variables encountered in the manufacturing of Braunschweiger, a portion of the meat processing industry was surveyed. The detailed information as shown in Table 7 indicated that variations exist in the formulation and manufacturing process for liver sausage and Braunschweiger. Both fresh and frozen pork livers were used in the formulation, although fresh liver seemed to be preferred due to flavor aspects. Liver content of the product varied from 30% to 55% which could affect the texture and flavor of the product. Some pretreatment such as scalding or soaking of the livers was done before the livers were mixed with other ingredients during the processing. The pretreatments, especially scalding of the livers, could significantly reduce the microbial load of the livers and, consequently, play an important role in the microbial quality of the finished product. Soaking the livers is believed to leach bitter flavors from the liver. Pork jowls, pork trim and bacon ends are common fat sources used in the formulation of liver sausage. The cooking of the liver sausage was done mostly in hot water; however, cooking in smokehouses was indicated by some manufacturers. The processing temperatures chosen

Table 6. Nutritional information per serving of liver sausage and frankfurters (Birdsall, 1975)

	Liver Sausage	Frankfurters
Serving Size	28.4 g (1 oz)	45 g (1 link)
Calories	100.0	140
Protein g	4.0	5
Carbohydrate g	1.0	1
Fat g	9.0	13
Percentage of U.S.R.D.A.		
Protein	8.0	10
Vitamin A	v	*
Vitamin C	v	v
Thiamine	4.0	6
Riboflavin	20.0	2
Niacin	10.0	4
Vitamin B_6	4.0	6
Vitamin B_{12}	100.0	8
Folic acid	2.0*	*
Pantothenic acid	8.0	*
Calcium	*	*
Phosphorous	4.0	4
Iron	12.0	2
Zinc	4.0	4
Magnesium	*	*
Copper	2.0	*
Biotin	4.0	ND

*: Contains less than 2% of the U.S.R.D.A. of these nutrients.

v: Variation due to formula differences.

ND: No data.

Table 7. Information concerning Braunschweiger liver sausage obtained from a survey of some meat processors

Variables		Company								
		A	B	C	D	E	F	G	H	I
Liver	Type	Fh[a]	Ff[b]	Fh	Fh	Fh	Ff	Fh	Fr[c]	Fr
	%[d]	50	30-40	45-55	C[e]	40	50	40	50	50
	Pretreatment	NO[f]	SK[g]	NO	NO	NO	SC[h]	SC	NO	NO
Type of pork fat		PJ[i]	PJ	PJ	PT[i]	PT	PT	PJ	PJ	PT
Casing		Sa[k]	MP[l]	MP	MP	Sa	Sa	Sa	MP	MP
Cooking	Equipment	W[m]	W	SM[n]	SM	W	W	W	W	W
	Water temperature (°C)	74	77	74	C	—[o]	88	—	71	C
	Internal temperature (°C)	68	66	70	C	68	70	71	65	C
Additional handling or packaging		NO	CV[p]	CV	CV	NO	NO	NO	NO	NO

Shelf life (days)	>90	≥35	≥60	28-42	___	>90	≥60	___	≥40

[a]Fresh liver.

[b]Fresh liver mostly, frozen liver if necessary.

[c]Frozen liver.

[d]% liver in the formulation.

[e]Confidential.

[f]None.

[g]Soaking.

[h]Scalding.

[i]Pork jowl.

[j]Pork trimming.

[k]Saran.

[l]Moisture-proof.

[m]Water vat.

[n]Smoke house.

[o]No data.

[p]Cut into chunks then vacuum packed.

to cook the products have a wide range of 71° C (160° F) to 88° C (190° F) for water temperature and 65° C (150° F) to 70° C (158° F) for internal product temperatures. The different heat processes of the products would influence organoleptic and microbiological qualities of finished products. Moisture-proof casings (MP casing) and Saran tubes were used by different manufacturers for stuffing the product. Products were shipped as wholesale long sticks (with MP casing), small chunks (with MP casing and Cry-O-Vac packaging) or clipped chubs (with Saran casing). The different methods of handling the product results in a shelf life range of 28 to more than 90 days.

Variables of particular interest to the respondents in the survey were:

1. The effect of fresh vs. frozen liver on fat and water binding capacity.
2. The effect of liver pretreatments on organoleptic and microbiological quality of Braunschweiger liver sausage.
3. The effect of fat content of finished product on product stability, organoleptic quality and economic value.
4. The use of nitrite in Braunschweiger with or without ascorbates.
5. The influence of added water on the quality of finished product.
6. The addition of salt at the beginning or the end of chopping.
7. The use of precooked fat ingredients.
8. The causes of jelly pockets in the liver sausage.

9. The microbial content of the liver and a reasonable specification.

10. The effect on quality of Braunschweiger of fat temperature before addition to the liver.

B. Microbiology of Braunschweiger Liver Sausage and Related Meat Products

1. Introduction

The groups of microorganisms associated with fresh meats are quite different from those associated with processed meats. The predominant flora of raw meat is comprised of Pseudomonas, Aeromonas, Flavobacterium, Alcaligenes, Gram positive sporeformers, Corynebacterium, Arthrobacter, Microbacterium, Enterobacteriaceae and chromogenic micrococci (Ayres, 1951; 1963; 1975; Vanderzant and Nickelson, 1969). However, these organisms are not the most common on processed meats. Ayres (1975) divided the principal microbial growth on processed meats into two categories. The first category, the most frequently isolated microorganisms referred to as the primary offenders, included Micrococcus, Staphylococcus, Streptococcus, Lactobacillus, Microbacterium, and Aspergillus. The secondary category, which included Sarcina, Neisseria, Leuconostoc, Pediococcus, lactobacilli (catalase positive), Torulopsis and Rhodotorula, was isolated often also.

Sausage is one of the oldest forms of processed food and may be one of the first processed meat products. Fundamentally, all sausage is comminuted and seasoned meat and it is best classified according to the manner in which it is processed. Meat processing, in the broad sense, includes curing, smoking, canning, cooking, freezing, dehydration, production of intermediate moisture products and use of certain additives such as chemicals and enzymes (Rust, 1976).

Sausage products can be divided into two classes: one is the form which requires cooking before serving and the other form is a ready-to-serve type which requires no further cooking. The former includes fresh sausage, uncooked smoked sausages, and the latter includes dry and semidry sausages, cooked smoked sausages, cooked sausages, luncheon meats and jelly products.

The specific processing method of each sausage product results in complex physical, chemical and bacteriological changes and gives each product easily recognizable characteristics. With the exception of dry and semidry sausages, however, sausages included in the class of fully cooked and ready-to-serve form have some similarity in their microbiological aspects. These sausages are generally composed of mixtures of meats, salt, sugar, sodium nitrite and spices and then are mildly heated or pasteurized which aids in the development of a cured color; the finished products require refrigeration.

2. Microflora associated with ready-to-eat sausage products

Frankfurters and bologna are examples of cooked, smoked sausages with the characteristics of cured meats; they are comminuted, seasoned, stuffed into casings, smoked and fully cooked and do not require further cooking; some, however, are heated before serving. These are the most popular sausages. In 1977, the production of frankfurters and bologna was calculated to be 34% and 20%, respectively, in the total sausage production of the U. S. (Statistical Summary, 1977).

Many papers have reported on the bacteriological aspects of frankfurters. Ayres (1951) reported that Micrococcus, Sarcina, Lactobacillus, Microbacterium and Bacillus are the most common organisms found in frankfurters. Because of the inhibitory or bactericidal effects of curing agents, smoking and heating, the flora of frankfurters is restricted. The bacteria present on frankfurters are predominantly Gram positive types (Drake et al., 1958). Hall and Angelotti (1965) isolated Clostridium perfringens from 19% of market samples of frankfurters and other processed meats which required mild cooking. Heiszler et al. (1972) reported that the initial count of aerobic bacteria on freshly made frankfurters was approximately 10^4 organisms/g of sample and the incidence of yeast and mold was quite low, in the hundreds per gram. They indicated, also, that 5% (6 out of 120) of the samples tested contained Clostridium perfringens; Staphylococcus aureus occurred in 1% of the samples.

Shank and Lundquist (1963) used Saran-type film with and without vacuum sealing and studied the microflora of refrigerated frankfurters. They found that after 21 days of refrigerated storage vacuum packaged frankfurters had a mild, acid taste and the predominant spoilage flora consisted of lactic acid bacteria including lactobacilli and streptococci. Aerobically spoiled frankfurters had a yeasty, alcoholic odor and not only had the same numbers of lactic acid bacteria as the vacuum packaged product but also had high numbers of yeasts and molds. In both packaging systems, low numbers of Bacillus (10^3/g) and high numbers of micrococci (10^7/g) were recovered.

From a survey of 254 vacuum packaged frankfurters, Brown (1972) reported that 75%, 26%, 4%, and 0.4% of the samples had a microbial load of $< 10^3$/g, $< 10^4$/g, $< 10^5$/g, and $< 10^6$/g, respectively. Most of the products attained a microbial load in the range of 10^6/g to 10^7/g within two weeks storage. The total plate count provides useful information concerning the total numbers of aerobic organisms that are present in or on the meat products and as a consequence it gives some indication of the length of the shelf life of the products.

Liver sausage (which was about 3% of the total sausage production in 1977) has received less attention from bacteriologists than have certain other types. Steinke and Foster (1951a,c) reported that commercial, freshly-made liver sausage had an aerobic, mesophilic count in the

range of 10^3 organisms/g to 10^4 organisms/g of sample; the anaerobic mesophilic count was found lower than that of the aerobes and was estimated to be in the hundreds per gram of sample. They reported that the predominant microorganisms in the freshly-made product were species of Micrococcus and Bacillus which were strict aerobes and did not grow in sausage held at 5° C. The liver sausage they examined was stuffed in a Visking casing and then covered with a Pliofilm envelope. Visking casing, a transparent cellulose derivative, is gas and moisture permeable; Pliofilm, a plastic film, is less permeable to water vapor and gases than is Visking. After several weeks of storage at 5° C, these liver sausages were spoiled by Gram positive, nonsporeforming, catalase negative, microaerophilic rods and some facultative yeasts. These spoilage organisms were located mainly adjacent to the casing. They recommended the replacement of the Visking casing for packaging liver sausage products by Saran casing. They concluded that liver sausage stuffed into Saran casing, cooked and stored at 10° C or less would have a shelf life beyond 12 wk, and that no microbial growth or no change in appearance would be evident during this time.

Ertle (1970) reported that liver sausage can remain acceptable for more than four weeks. The liver sausage she observed was cooked at 71.1°C (160°F) in a water bath until the internal product temperature reached 67.1° C (153° F); the final product was stored intact in a moisture-proof

fibrous casing at 4°C (40°F). Identical liver sausage samples were sliced with a knife and subjected to additional handling during packaging in an oxygen-permeable film; the shelf life in this instance was reduced to less than one week. Slime was found on the product and the total bacterial count was 5×10^6 per gram of core. A two-year bacteriological survey of chopped liver products from eight establishments showed that at the time of manufacturing, the majority of samples had a reasonable microbial load; that is, the aerobic plate count was in the range of 10^3/g to 10^4/g. Only 8.6% of samples contained Escherichia coli; one unit was positive for Staphylococcus aureus; and all units (209) were negative for salmonellae (Surkiewicz et al., 1977). The results indicated that good sanitation conditions and practices as well as prompt chilling of cooked ingredients and finished products enhanced the microbial quality of the finished product.

3. Spoilage organisms

Microorganisms which cause food spoilage are distributed throughout our environment. They are in the raw materials used for sausage, on the processing equipment as well as on production personnel and their clothing. Microbial spoilage encountered in frankfurters, bologna, liver sausage, and luncheon meats can be classified as follows: (a) sliminess (b) moldiness (c) souring and gassy packages (d) green cores (e) green rings (f) surface greening and (g) putrefaction (Ertle, 1970).

a. Sliminess Slime is caused by the abundant growth of microbes on the outer surfaces of the sausage. Accumulation of cells of bacteria and yeasts usually results in a white or yellowish slime. Processing temperatures are generally sufficient to destroy most forms of vegetative cells on the surface of a processed meat item; contamination of the product after processing is the main source of organisms causing this type of spoilage. Since cellulosic casing acts as a barrier to microbial contamination, it follows that meat products processed in cellulosic casing are reasonably well protected from sliming organisms until the casings are removed. On nonvacuum packaged products, slime appears in the form of characteristic beads, sticky to the touch and having an off-odor sometimes described as yeast-like. Drake et al. (1958) reported that commercially produced Cellophane-wrapped frankfurters stored under refrigeration showed slime formation. They found that the slime consisted primarily of lactic acid bacteria, yeasts and micrococci; and among 353 isolates, 275 isolates were bacteria and 78 isolates were yeasts. Vacuum packaging of the frankfurters removed available oxygen and the low oxygen tension favored the multiplication of facultative anaerobic yeasts and heterofermentative lactic acid bacteria. The yeasts produced a whitish liquid while the lactobacilli produced swells because of the formation of CO_2 (Lechowich, 1971).

Products packaged in a casing such as bologna and liver sausage may spoil due to the microbial growth between the casing and the meat (Steinke

and Foster, 1951a). Microbial growth can occur most readily at this interface since moisture accumulation occurs there during processing if the casing is penetrable to water (Lechowich, 1971). Steinke and Foster (1951a) demonstrated a heavy "slime" made up of Gram positive micrococci and a few long Gram negative rods; this slime developed between the layers of casing and liver sausage after storage under refrigeration for several weeks. This growth resembled slime observed on weiners. Allen and Foster (1960) studied the spoilage of four types of sliced processed meats including bologna, chopped pressed ham, smokie patties, and P & P loaf; these meats were vacuum packed and stored under refrigeration. They reported that lactic acid bacteria (Gram-positive, catalase-negative rods) were responsible for spoilage which consisted of slime formation and development of atypical flavor. Putrefaction did not occur.

Warnecke et al. (1966) and Ertle (1970) reported that cooking meat emulsions to a proper internal temperature (71.1°C) is an effective procedure for reducing microbial contamination levels in sausages. Studies on bacterial and water permeability of casings have indicated that the pore diameter of cellulosic casings prevents the passage of microbes into the product (Hartman et al., 1963; Ronsivalli et al., 1966). It was concluded that no microbial slime will occur on properly cooked sausage with an intact cellulose casing. However, recontamination by microbes during peeling of

the product and packaging can serve as an inoculum for the development of the slime.

b. Moldiness Most molds and their spores are killed by moist heat at 60°C (140°F) in 5 to 10 min although some species are more heat resistant (Frazier, 1967). Molds are generally destroyed by the amount of heat employed in the manufacturing of most cured products. Molds are air-borne contaminants; therefore, they normally occur on cooked products as the result of post-processing contamination (Kramlich et al., 1973). Application of modern vacuum packaging has greatly reduced this type of spoilage because molds need both oxygen and headspace for growth. Although mold spoilage of table-ready meats is not common, it can and does occur under favorable conditions. When the products are moist and stored under conditions of high humidity, they tend to undergo bacterial and yeast spoilage. Mold spoilage is likely to occur when the surface becomes dry or when the products are stored under conditions which do not favor bacteria or yeasts (Jay, 1970).

c. Souring and gassy packages Bacteria can grow within sausages upon long term refrigeration or during shorter periods at temperature above 10°C (50°F) (Steinke and Foster, 1951c). Some of the genera encountered in spoilage of bologna and liver sausage are bacilli, micrococci, lactobacilli, and leuconostocs. Anaerobic metabolism of carbohydrates in meat products by

lactobacilli, leuconostocs, and streptococci can result in various fermentation products, primarily organic acids. The principal acid formed is lactic acid; its presence causes lowering of the pH of the product and development of a sour flavor. The bacteria that produce acidity usually, but not always, form gas which produces a gassy package. The souring spoilage is not due to aerobic bacteria, yeasts or molds but to anaerobic or facultative bacteria (Kramlich et al., 1973). Insufficient heat treatment of cured-type sausage can also allow the survival of salt tolerant Streptococcus faecium and other lactic acid bacteria such as Lactobacillus viridescens and Leuconostoc, which can cause both souring and swelling (Lechowich, 1971).

d. Green cores The occurrence of green cores is usually associated with large sausages such as bologna, liver sausage and luncheon meats. It develops in meat in which the emulsion has been contaminated and improperly cooked; that is, the internal temperature of the product has not reached 66.5°C (152°F). The greening organisms which are very common in sausage plants can survive mild heat treatment. Cooking processed meat products to an internal temperature of 66.5°C to 68.3°C (152°F to 155°F) destroys greening organisms (Niven et al., 1954). However, on rare occasions, when the contaminating level is quite high, it is necessary to cook to an internal temperature of 71.1°C (160°F) (Kramlich et al., 1973). Greening of the meat pigments does not occur until the sausages are cut and exposed to air. Greening bacteria, (heterofermentative species of lactobacilli and Leuconostoc,

principally Lactobacillus viridescens) produce hydrogen peroxide which degrades the meat pigments and results in a green or grayish green color (Niven, 1951; Niven and Evans, 1957; Niven et al., 1949). The reaction may occur rapidly within an hour, or it may take as much as 24 hr. Discoloration begins as a small area and soon covers the complete surface. This phenomenon of spreading enables one to distinguish microbiological greening from metallic or chemically induced greening (Kramlich et al., 1973).

e. Green rings The occurrence of green rings in sausage is very rare. These rings appear at varying depths beneath the sausage surface and can be seen as soon as the sausage is cut. They usually develop 12 to 36 hr after processing even under adequate refrigeration (Ertle, 1970) even though bacteria may have been present throughout the sausage. The most frequently accepted theory for the development of the discoloration as a ring is that in this zone the oxygen tension is conducive to pigment oxidation (Ertle, 1970; Kramlich et al., 1973). The spoilage problems of both green core and green ring are concluded to be due to unusually high numbers of greening bacteria in the sausage emulsion with subsequent undercooking of the sausage. These problems are rarely encountered by modern processors.

f. Surface greening Surface greening is one of the most common types of discoloration associated with sausages and smoked meats. Surface

greening caused by greening bacteria usually is not noticeable until at least 5 days after processing and often appears after a couple of weeks. In contrast to the green core and green ring problems, surface greening has been reported as due to recontamination with greening bacteria after processing.

g. Putrefaction Putrefaction is spoilage that is caused by certain proteolytic bacteria. These bacteria attack meat protein, peptides and free amino acids very rapidly and produce degradation products that emit, in some instances, putrid and foul odors (Cavett, 1962). This type of reaction occurs rapidly in bacon stored at 30°C. At 20°C, proteolysis and lipolysis occur slowly and other compounds can be detected organoleptically.

4. Factors affecting the microbiological quality of ready-to-eat sausage products

a. Incidence of microorganisms in raw materials (ingredients) The healthy, inner flesh of muscle is normally free of microorganisms, although bacteria have been found in lymph nodes, bone marrow and even flesh (Lepovetsky et al., 1953, Vanderzant and Nickelson, 1969). Meat is contaminated by organisms from hides and feet of the animals, air, water, soil and sawdust with which the carcass comes in contact, the hands of workers, knives, saws, brushes and swab cloths (Ayres, 1963). Under proper slaughter and sanitary processing, fresh meat trimmings, a main ingredient of

sausage formulae, may have a microbial load of 10^4-10^5 bacteria/g (Dockerty et al., 1970; Brown, 1972). There have been numerous reports in the literature on the microbial flora of surfaces of carcasses, fresh meat cuts, and processed meats (Jay, 1961; Jay, 1964; Vanderzant and Nickelson, 1969; Brown and Hoffman, 1972; and Rey et al., 1976); these reports conclude that sanitation of the packing plant, handling and washing, packaging material, and storage temperatures and time are the decisive factors affecting the numbers and kinds of microbes growing in the raw meat (Halleck, et al., 1958a; 1958b; Dockerty et al., 1970).

Very little work has been done on the microbiology of organ meats. Jay (1961) has reported that beef livers from the retail market have a total count of 10^8 organisms/g and the count of coagulase positive staphylococci is about 10^5/g. Jay (1970) indicated that beef liver generally contains large numbers of microbes because such organs filter out organisms from the circulating blood and, in part, because of the generally higher pH of these organs. The higher pH of livers favors microbial growth at a more rapid rate than in meat which has a lower pH. Fresh beef has a pH value $\leq$ 5.8 (Shelef, 1974) whereas fresh beef liver has a pH value $\geq$ 6.3 (Shelef, 1975). Fresh pork has a pH value of 5.9 (Rey et al., 1976) whereas we have measured the pH of fresh pork liver to be 6.2. Gardner (1971) stated that the microbiological contamination of fresh porcine liver occurs predominantly on the surface with a microbial load of 10^2-10^4 mesophilic

aerobes/cm^2. The microflora associated with fresh and frozen pork liver are those listed below in decreasing order of occurrence: Micrococcus, Flavobacterium, coryneforms, lactic streptococci, Leuconostoc, Acinetobacter, and Microbacterium thermosphactum. However, under aerobic, refrigerated storage for 7 days, the porcine liver was spoiled mainly by Pseudomonas spp. Shelef (1975) investigated the microflora of refrigerated beef livers and found that fresh beef liver harbored a mixed population (c. 1×10^5 organisms/g) of Gram positive cocci, chromogens and nonchromogens, sporeformers, presumptive coliforms and Gram negative rods. After 7 days storage at 5°C in a beaker with an aluminum cover, beef liver became sour; lactic acid bacteria predominated in the spoilage flora.

Some spices such as cinnamon, cloves, horseradish, garlic, onion, and mustard have all been shown by various investigators to possess antimicrobial activities to varying degrees (Fabian et al., 1939; Foter and Gorlick, 1938; Jay, 1970). However, in the concentrations used in meat products, the bacteriostatic effect of the spices is negligible (Lechowich, 1971). Most spices that do not contain antimicrobial compounds such as bay leaves, thyme, curry, paprika, pepper, etc., tend to have a high load of microbes (c. 10^7 organisms/g). The microflora of spices consists of aerobic spores, anaerobic spores, coliforms, yeasts and molds (Karlson and Gunderson, 1965). Palumbo et al. (1975) reported that commercially available spices,

especially black pepper, red pepper and allspice, have a very high microbial content (c. 10^7 organisms/g) and that the predominant component of the flora of these spices is Bacillus subtilis. Treatment of dried spices with propylene oxide greatly reduces the microbial content and is the treatment used when spices with a guaranteed low number of organisms are purchased (Lechowich, 1971).

The addition of milk solids to sausages may be a source of lactic acid bacteria and yeasts (Jay, 1970). Water or ice used in certain meat products may also add bacteria. Chlorination of the plant's water supply or municipal supplies is useful for controlling this problem (Lechowich, 1971).

Selection of raw materials of high microbiological quality is necessary to ensure an acceptable finished product. Niven (1951) reported that high levels of contamination of raw material with lactobacilli may result in green core development. Warnecke et al. (1966) demonstrated that microbial growth in raw materials prior to and during processing had little effect on the subsequent growth of microbes in cooked, packaged bologna; a high level of contamination of the raw material, however, did affect the flavor and texture of the finished product.

b. Effect of salt Of all the food preservatives, salt is probably used most and has the longest history of usefulness (Ingram and Kitchell, 1967). Individual microorganisms have different degrees of tolerance to salt; Pseudomonas species, which are the predominant cause of putrefaction of chilled pork, and

coliforms are very sensitive to salt. A "brine concentration" ($\frac{\%\ \text{salt}}{\%\ \text{water in meat product} + \%\ \text{salt}}$) of 5% - 7% will easily exclude the above two organisms, but not micrococci, Bacillus species and fecal streptococci (Ingram and Kitchell, 1967; Sherman, 1937; Ingram and Dainty, 1971).

All of the lactic acid bacteria which were responsible for the refrigerated spoilage of four processed meats, were able to grow in APT broth containing 6.5% salt and all tolerated at least 8.5%. Two-thirds of the cultures even grew in the presence of 9.5% to 10.0% salt (Allen and Foster, 1960). The four meats spoiled in approximately the same order as they increased in brine concentrations. The brine concentrations in the 4 meats were as follows: P & P loaf - 4.2%, smokie patties - 4.6%, bologna - 5.0%, and pressed ham - 5.8%. Cavett (1962) reported that the initial salt content of bacon exerts a selective effect on bacterial development. During storage at 20°C, bacon with a normal salt content of 5-7% (w/v) in the aqueous phase spoiled as a result of the multiplication of micrococci, group D streptococci, lactobacilli, pediococci and leuconostoc, whereas, bacon with the highest salt content of 8-12% was spoiled mainly by micrococci, leuconostoc and yeast.

Staphylococcus aureus, a food-poisoning mesophile, is very salt resistant and can tolerate up to 15% or occasionally 20% salt (Fagraeus, 1949). Few foods can remain palatable and contain enough salt to prevent the growth of

staphylococci; nevertheless, they are not common on raw cured meat products because they are not good competitors compared to other indigenous microorganisms (Ingram, 1960; Cavett, 1962; Bryan, 1968a). It is when harmless organisms are removed that the development of food poisoning staphylococci becomes likely, e.g. in cooked ham. Most cooked meat products have salt levels (% in water phase) of 3-5% (Spencer, 1966). However, a salt concentration of 5% is the highest level of salt that permits the production of staphylococcal enterotoxin.

The microbiological effect of salt in food probably depends on osmotic withdrawal of water; the addition of salt results in a decrease of water activity in the food. The water activity (A_w) of fresh meat is about 0.99 and the A_w of frankfurters and related sausage products is in the range of 0.90 to 0.94 (Ayres, 1973). The minimum A_w for the growth of slime producing microorganisms, Pseudomonas, E. coli, Bacillus, Clostridium, and Salmonella is reported as 0.99, 0.97, 0.96, 0.95, 0.95, and 0.95, respectively (Scott, 1957; Hansen and Riemann, 1962).

The salt sensitivity of microorganisms is also dependent on other existing environmental factors such as temperature, pH, availability of oxygen and composition of the medium or food. The lethal action of salt on organisms decreases as the temperature is decreased. Salmonella (a pathogenic organism) and Escherichia coli (an indicator organism) can

survive for weeks in a cold brine but are rapidly destroyed in such brine at normal temperatures (Ingram and Kitchell, 1967). The greatest tolerance of molds and sporeforming bacteria exists at the optimal temperature for growth (Tomkins, 1930; Heintzeler, 1939; Ohye and Christian, 1967).

The influence of pH on growth of microorganisms supplements that of salt. A general rule is that as acidity increases less salt is needed to prevent growth of bacteria and yeasts (Ingram and Kitchell, 1967). This supplemental inhibition by pH on microorganisms is worthy of emphasizing because the current tendency is to produce cured meat products with lower salt concentrations.

The effect of salt on growth and toxin production of Clostridium botulinum has been studied extensively. Tanner and Evans (1933) found that for lightly heated spores, up to 12% salt was needed to prevent growth and toxin production. However, when the spores had been strongly heated as in canning, between 3.5% and 1.5% of salt actively inhibited the germination of spores (Gross et al., 1946; Silliker et al., 1958; Abrahamsson et al., 1966). The spores of other bacteria are inhibited by comparatively low concentrations of salt if they have been heated (Roberts and Ingram, 1966), and it is believed that this is an important reason for the stability, under commercial conditions, of many canned products known to contain viable spores (Spencer, 1966).

c. Effect of nitrite The antimicrobial function of nitrite in cured meats has been studied by many research workers. The first definite work on the inhibition of microorganism by nitrite was shown by Tarr (1941a, 1941b). He reported that several genera of bacteria, including Achromobacter, Aerobacter, Escherichia, Flavobacterium, Micrococcus and Pseudomonas, were inhibited by 0.02% nitrite at pH 6.0. More recent work has indicated that C. perfringens (Labbe and Duncan, 1970) and Streptococcus faecalis (Stoychev and Djejeva, 1971) as well as Pseudomonas, Enterobacter and Micrococcus (Spencer, 1971) are inhibited by nitrite. Pathogenic, Gram negative organisms such as Salmonella spp. have also been shown to be inhibited by commercial concentrations of nitrite (Leistner et al., 1973).

Several workers demonstrated and suggested that nitrite is most bacteriostatic under acid conditions. That the bacteriostatic effect of nitrite is increased approximately tenfold as the pH is lowered one unit was clearly demonstrated by Castellani and Niven (1955) with Staphylococcus aureus; the same effect was later confirmed by Eddy and Ingram (1956) using Bacillus species and by Perigo et al. (1967) and Duncan and Foster (1968a, 1968b, 1968c) using Clostridium sporogenes PA3679. Jensen (1954) and Shank et al. (1962) suggested that the formation of nitrous acid (HNO_2) is mainly responsible for the antimicrobial effect. Nitrite in the slightly acid environment of cured meats (pH 5.5 to 6.5) exists in equilibrium with

nitrous acid. At pH 5.0 to 5.5, nitrous acid is produced and is responsible for the development of maximum bactericidal activity.

Several explanations have been offered for the bacteriostatic properties of nitrite such as interference with the sulfur nutrition of the microorganisms (Castellani and Niven, 1955), the formation of nitrosothiols from nitrite and SH compounds (Mirna and Hofmann, 1969); and the formation of an iron-nitrosyl complex (van Roon, 1973). Because of the rather wide range of tolerance toward nitrite among different microorganisms, it is likely that the mechanism of bacteriostasis varies from one organism to another.

The prospect of banning nitrite from cured meat has renewed interest in its antimicrobial properties, especially its role in protecting the consumers against the hazard of botulism. That C. botulinum spores are widely distributed in nature is well-established (Foster and Duncan, 1974). Nevertheless, it is also generally agreed that botulinal spores exist in low numbers in meat products (Greenberg, 1972). This food-poisoning organism has been found in low numbers in commercially processed, table-ready cured meats: 1 of 10 samples of frankfurters and 0 of 50 samples of other cured meat (Insalata et al., 1969); 5 of 100 samples of ham and 1 of 41 samples of smoked turkey (Abrahamsson and Riemann, 1971).

Steinke and Foster (1951d) observed the marked antibotulinal effect of nitrite in liver sausage inoculated with spores of C. botulinum. Pivnick et al. (1967) showed a direct relationship between nitrite concentrations and the

time required for botulinal toxin formation in processed meats. Recent studies to evaluate the importance of nitrite in cured meats showed that nitrite plays a critical role in preventing growth of C. botulinum in various types of cured meat products (Christiansen et al., 1973; Christiansen et al., 1974; Christiansen et al., 1975; Hustad et al., 1973; Bowen et al., 1974). The extent to which nitrite inhibits growth of clostridia depends on other factors such as pH, salt concentration, degree of thermal processing, and number of cells (Roberts, 1974). Grever (1973) examined emulsions of cooked sausages and liver sausage for the amount of nitrite required to prevent growth of clostridia after pasteurization at 80°C and storage for 5 weeks at 24°C. He concluded that 200 ppm nitrite must be added to pasteurized products provided the maximum pH is 6.2. The pH range found in cooked, cured meat products is between 5.8 and 6.8. The growth of clostridia is best at the higher pH values.

Inoculated sausage emulsions heated at Fo = 0.5 often show inhibition without nitrite; this inhibition may have resulted from the action of salt on spores damaged by heat. Grever (1973) concluded that for pasteurized meat products containing 3.5% brine concentration and heated to an Fo = 0.5, addition of 100 ppm sodium nitrite is sufficient to prevent growth of clostridia. He also observed that more nitrite is required to prevent growth of bacilli than of clostridia. Fo is defined as the time in minutes required to destroy a specified number of spores at 121°C (250°F) when z

is equal to 18. Z is defined as the degrees Fahrenheit required to reduce the thermal death time tenfold. A z value of 18 is usually assumed for C. botulinum when thermal death time determinations have not been made in the product under consideration (National Canners Association Research Laboratories, 1968).

The mechanism by which nitrite inhibits clostridia in cured meats was investigated by Roberts and Ingram (1966) and they concluded that as heating occurs during the processing of cured meat products, bacterial spores are rendered more sensitive to nitrite, and these heat-injured spores are less able to grow out in the presence of nitrite than are unheated spores. Duncan and Foster (1968a) confirmed the above finding and showed that commercially acceptable levels of nitrite inhibited division of cells newly emerged from germinated spores but did not interfere with germination of spores. Labbe and Duncan (1970) made similar observation with C. perfringens.

The Perigo factor, a powerful anticlostridal factor, was discovered by Perigo et al. (1967) and Perigo and Roberts (1968) when they heated nitrite in a bacteriological culture medium. However, in heated meat suspensions containing nitrite, inhibition by the Perigo factor did not occur (Johnston et al., 1969). The work of Johnston et al. (1969) and Ashworth and Spencer (1972) led to the conclusion that the Perigo factor is of little

or no consequence in explaining the role of nitrite in the safety of commercially produced canned cured meat. However, other so called "Perigo-type factors" may be formed when nitrite is heated in the presence of meat (Chang et al., 1974; Pivnick and Chang, 1973; Ashworth and Spencer, 1972). In conclusion, the role of inhibitory factors produced by heating nitrite in meats has yet to be resolved.

d. Effect of heat processing Heat processing works in two ways to extend shelf life of meat and meat products. First, it destroys considerable numbers of microbes in the raw meat and results in a lower microbial content of the finished product. Second, heating decreases the water content of raw meat, especially on the surface, which in turn, lowers the A_w and enhances the keeping quality of the finished product (Rogers et al., 1967). Thermal treatment is the most widely used method of killing spoilage and potentially pathogenic microorganisms in meat and meat products (Heiszler et al., 1972). The effect of cooking on the numbers of microorganisms in meats is to generally reduce coliforms, staphylococci, and Gram negative bacteria either to very low levels or to completely destroy them. Sporeformers, coryneforms, lactic acid bacteria, enterococci, and micrococci sometimes survive pasteurization in varying numbers (Huber et al., 1958; Gardner, 1968).

The thermal resistance of bacteria is influenced by a number of factors. Although it is difficult to make an accurate appraisal of the factors affecting thermal resistance from a survey of the literature, Hansen and Riemann (1963) have outlined some of these factors and have made the following conclusions:

1. Heat resistance of microbial cells increases with decreasing humidity. Superheated steam acts as dry air at 140-150°C and has less killing effect than wet steam at 100°C (Precht et al., 1955).
2. Many salts influence the heat resistance of microbial cells in several ways but it is almost impossible to predict the effect. Divalent cations such as Ca^{++} and Mg^{++} may increase the heat resistance of microorganisms by linking proteins together to give a strong complex (Precht et al. 1955). High concentrations of soluble salts may decrease the A_w and thereby increase the heat resistance of bacterial cells by a mechanism which is similar to the effect of drying.
3. High concentrations of soluble carbohydrates decrease the A_w of the heating menstruum and result in increased heat resistance of yeasts and bacteria.
4. Acid and alkaline reactions increase susceptibility of protein in cells to heat denaturation and also cause a decrease in heat resistance of bacteria (White, 1963).

5. Fat and protein in the heating menstruum have a protecting effect on microorganisms (White, 1952; Precht et al.,1955).
6. Unknown protective substances in the heating menstruum may increase heat resistance; that is, some molecules other than proteins and amino acids may combine with proteins and provide protection (Precht et al., 1955).
7. The presence in the heating menstruum of any lethal or inhibitory substance must be expected to cause a reduction in heat resistance. However, the effect of inhibitory compounds on heat resistance is difficult to predict quantitatively because of the interactions between the compound and the heating temperature or other factors. Greenberg and Silliker (1961) found that 100 ppm of nitrite had no effect on the numbers of surviving Streptococcus faecalis and S. faecium cells at 64.7°C (148.5°F) but at 68.3°C (155°F) and 70.3°C (158.5°F) there was a hundred to five hundredfold decrease in the survivors compared with the control.
8. Dense populations in a cell suspension increase heat resistance. Vas and Prostzt (1957) have hypothesized that specific substances excreted by cells contribute to this type of resistance.
9. Heat resistance changes during the various growth phases of the microorganism. Cells in the log phase generally are less heat

resistant than cells in the maximum stationary phase of growth (White, 1953; Lemcke and White, 1959).

10. Growth temperature may have an influence on the heat resistance of microorganisms (White, 1963). Heat resistance of many bacteria increases with an increase in growth temperature.

Meat products, a complicated food system, contain many variables already mentioned above and also other unrecognized factors. The recommendation of an optimum heat processing, which has to fit the requirement of desirable palatability of the specific meat product and to insure the complete destruction of organisms, is a very difficult task. However, the need for a minimum heat treatment among precooked meat is necessary for the protection of public health. Principal public health hazards are Salmonella spp., Staphylococcus aureus, Clostridium perfringens and C. botulinum.

In general, to insure the destruction of salmonellae, foods must be heated to an internal temperature of 71.1°C (160°F) or 73.9°C (165°F), but if these temperatures cause undesirable changes in products, lower temperatures and longer times can be effective in many instances (Bryan, 1968b).

Castellani et al. (1953) concluded that a temperature of 73.9°C (165°F) in the center of a stuffed turkey during roasting is sufficient to kill enterococci, staphylococci, and salmonellae and to allow a modest margin of safety. Angelotti et al. (1961) inoculated custard, ham salad and chicken

a la king with 10^7 salmonellae per gram; when these products were heated to 65.6°C (150°F) and held for 12 minutes, the organisms were reduced to nondetectable levels. The same level of destruction occurred when these foods were heated at 60°C (140°F) and held for 83 min. Bryan et al. (1968) reported that loads of more than 10^6 salmonellae per cm^2 were reduced to nondetectable levels when the internal temperature of the turkey rolls reached at least 65.6°C (150°F). Furthermore, prolonged heating at any temperature above 50°C (122°F) gradually destroyed these organisms. U.S.D.A. regulations (1976c) state that cured and smoked poultry rolls must reach an internal temperature of at least 68.3°C (155°F), and all other poultry rolls must reach an internal temperature of at least 71.1°C (160°F).

Heat processing employed in the production of most cured meat products is at moderate heating levels which is in contrast to the severe heating applied to canned meat items other than canned cured meats. In recent years, however, the final internal temperature reached in practically all cooked sausages has gradually increased. Higher internal temperatures contribute to an increased shelf life of the product. Heiszler et al. (1972) reported that increasing the internal temperature of frankfurters from 60°C to 76.8°C was slightly effective in reducing aerobic bacteria but during subsequent storage of the frankfurters at 5°C, that there was an inverse relationship between internal temperature of smoking and the capability of bacteria to multiply. In addition, Solberg and Elkind (1970) have observed

that the rate at which frankfurters are heated to an internal temperature of 68-69°C affects the survival of aerobes and anaerobes. Sausages cooked in a smoke house generally reach an internal temperature in the range of 68-72°C (154 to 162°F). Those sausages cooked within a moisture-impermeable casing or metal container in water or steam at 68 to 76°C (154 to 169°F), such as liver sausage and various loaf items, are more subject to emulsion breakdown and are therefore cooked to lower internal temperatures, usually between 66 to 68°C (151 to 154°F) (Kramlich, 1971). Therefore, modern heat processing of cooked, cured meats should be adequate to kill salmonellae that might be present.

The effectiveness of thermal processing in controlling staphylococcal intoxications depends on the destruction of S. aureus cells that have an opportunity to produce enterotoxin, which is heat stable (Bryan, 1968a). A temperature of 75°C (167°F) is lethal to staphylococci. In custard and chicken a la king, more than ten million cells of food poisoning strains of S. aureus were reduced to nondetectable levels at temperatures of 60°C (140°F) in 53 min and at 65.6°C (150°F) in 6 min (Angelotti et al., 1961). However, enterotoxin remaining in food is not destroyed by conventional methods of cooking or heat processing of foods. Crude enterotoxin in food has been shown to be active after heating at 100°C (212°F) for one hour and at 121°C (250°F) for as much as 30 min (Bryan, 1968a).

The moderate heating applied to cured, precooked sausages does not kill bacterial spores (Tanner and Evans, 1933; Gough and Alford, 1965; and Solberg and Elkind, 1970). In pasteurized, comminuted meat products counts of about 100 Bacillus and 10 Clostridium spores per gram are frequently encountered (Ingram, 1973). However, a heat treatment which does not necessarily result in complete inactivation of spores caused enough damage to prevent growth and multiplication under the conditions tested; this is generally thought to be the case in the canning of cured meats (Roberts and Ingram, 1966; Duncan and Foster, 1968a; Labbe and Duncan, 1970). Although heat-injured spores are more sensitive to the unfavorable conditions existing in cured meat products, the surviving C. perfringens in frankfurters may become a source of food poisoning if contaminated with significantly high numbers of this organism before or during processing and under subsequent storage temperatures above 12°C (Solberg and Elkind, 1970).

Spores of C. botulinum are quite heat resistant, and an equivalent heating of at least 2.5 min at 121°C (250°F) is recommended to assure safety in canned foods that might support germination and outgrowth of botulinum spores (Lechowich, 1971). Greenberg (1972) stated that canned, cured meat products have enjoyed an unblemished botulism safety record in the United States. It is generally agreed that the public health record of shelf-stable canned cured meat is due to interdependent combinations (or interacting effects)

of salt, nitrite, mild heat processing and low incidences of botulinal spores in the raw materials. Spencer (1966) reviewed all the investigations dealing with the safe system of heat processed, cured meats and indicated that the major inhibitory factor is salt, with some supplementary effect of nitrite. Also, the supplementary effect of heating to the order of Fo = 0.1 to 1.0 is of critical importance. Numbers of organisms are also critical in evaluating the effectiveness of heat processes; for example, meats containing 1 - 10 spores per gram fail when challenged with numbers of organisms 100 to 1000 times greater.

e. Effect of packaging and storage conditions The storage life of meat products depends on the numbers and types of contaminating bacteria and on the effects of physical and chemical environment of storage on their metabolism and rate of growth. The cooked cured meat products, which started with raw materials of good microbial quality (with an expected total aerobic count of 10^4 to 10^6 per gram) and proper heat processing, generally, will have a low microbial content (total count in hundreds per gram) (Warnecke et al., 1966). After heat processing the only place contamination of product surfaces can occur is during peeling or slicing and packaging, that is when the product is taken out of one protective container and placed in another. Ertle (1969) suggested that the practical ways to eliminate contaminants in the peeling and packaging areas are: (1) exercise great

care in the sanitation of equipment, (2) encourage good sanitary practices by personnel and (3) maintain low temperatures in the range of 5 to 9°C (41 to 48°F).

Fowler et al. (1977) conducted a survey of retail outlets and concluded that little difficulty should be experienced with guidelines which restrict the Standard Plate Count (SPC) in luncheon meats to 1×10^6 microorganisms per gram. Total coliforms do not appear to be a significant problem in these products. Hill et al. (1976) reported that a microbiological standard of 10^6 SPC would assure a shelf life of luncheon meats of 24 to 28 days after manufacture. Development of a total bacterial population of 10^8 per gram with corresponding decrease in sensory quality did not occur until 46-60 days after manufacture.

Packaging materials, packaging techniques, and storage temperatures have been considered as decisive factors for establishing the shelf life of cured meat products (Ertle, 1969; Ingram and Dainty, 1971). Because table-ready meats are handled and packaged under sanitary but not aseptic conditions, it is understood that such a product is not sterile; hence, refrigeration to minimize microbial development is necessary. Allen and Foster (1960) demonstrated that 4 commercially available luncheon meats had significantly longer storage lives at 1.1°C than at 7.2°C. The spoilage of the meats was definitely retarded by the lower storage temperature.

Refrigeration equipment, in general, is expected to operate between 5 and 10°C; nevertheless, many home refrigerators are operated at temperatures approaching 15°C (Solberg and Elkind, 1970). Steinke and Foster (1951c) reported that liver sausage and bologna when packaged in Saran and held under fair (10°C) to good (5°C) conditions of refrigeration had a shelf life of more than 13 weeks and concluded that these types of products were very stable. However, when these products were poorly refrigerated (16°C, 61°F), the shelf life was less than 8 wk. Both types of sausages spoiled rapidly within 3 days when held at or near room temperature.

The use of low-oxygen permeable film along with the vacuum packaging techniques has greatly extend the shelf life, both chemically and microbiologically, of table-ready meats (Urbain and Ramsbottom, 1948; Shank and Lundquist, 1963). In the vacuum packaging system, there are two main factors for extending the shelf life of cured meat products. One is the transmission rate of oxygen through the film which is measured as cubic centimeters of oxygen passing through 100 square inches of film during a 24 hr period; measurements are made at room temperature and atmospheric pressure in a Dow cell. The other is the residual oxygen in the package (Ertle, 1969). There are some claims made, however, that vacuum per se has little significance in packaging. Therefore, it is believed that the quantities of air left inside and the degree to which ultimate physical contact

between film and meat are achieved are the important considerations (Hannan, 1962).

Ertle (1969) reported that for maximum pigment stabilization that bologna be packaged with the best oxygen barrier (a packaging material with oxygen transmission rate of 1 cc) under a vacuum of 29 in.of Hg. At this specific vacuum, the package would result in an initial residual oxygen content of 1%. Similar findings were observed by Kraft and Ayres (1954). However, for frankfurters a vacuum pressure of 27 to 28 in.of Hg and initial residual oxygen content of 2% or less was recommended. Ertle (1969) stated that cured meat pigments in frankfurters containing smoking ingredients were less sensitive to oxygen-caused and light-catalyzed discoloration than that of the pigments in the inner core of bologna. Packaging frankfurters at higher vacuum pressures such as 29 in.of Hg resulted in the expression of more juice and more distortion of the products than that of packaging at low vacuum pressures such as 27 in.of Hg.

In a vacuum package, the initial low residual oxygen content, the consumption of oxygen by cured meat at a substantial rate, and the subsequent utilization of oxygen and production of CO_2 from the respiration of indigenous bacteria sustain the anaerobic condition which favors growth of anaerobes and facultative organisms (Solberg, 1967). Shank and Lundquist (1963) reported that the spoilage of vacuum-packaged, table-ready meats such as

sliced, cooked ham, frankfurters and bologna, involved lactic acid bacteria almost exclusively; whereas,the spoilage pattern of nonvacuum-packaged cured meats also included large numbers of yeasts and molds. They stated that, generally speaking, anaerobic spoilage is defined as "sourness" whereas aerobic spoilage of cured meats is characterized as "yeasty." These definitions certainly correlate with the respective flora shown to dominate in the different extremes of packaging.

III. MATERIALS AND METHODS

A. Experimental Design

Five variables were observed for their effect on the quality of Braunschweiger liver sausage. These five variables were pretreatment of liver, nitrite levels, processing temperatures, addition of calcium-reduced dried skim milk and fat content. These variables were divided into four major studies in order to properly manage the determinations and observations. Study I involved the effect of liver pretreatments (fresh liver, frozen liver, liver scalded at 93°C for 2 minutes and liver soaked in 5% NaCl solution at 0°C for one hr) on the quality of Braunschweiger. Four levels of sodium nitrite (0, 50, 100 and 156 ppm) in the formulation of Braunschweiger were the variables examined in Study II. Study III was a two factorial experimental design which included the addition of calcium-reduced dried skim milk (CRDSM) (Savortex from Western Dairy Products, San Francisco, CA) and four different sets of processing temperatures. The percentage of Savortex used in the product was 3.5% computed on the basis of total weight of meat ingredients. The four different sets of processing temperatures were: T1, 68°C/63°C; T2, 74°C/68°C; T3, 79°C/74°C; and T4, 85°C/74°C. The first temperature is the water vat temperature during cooking and the second temperature is the internal temperature of the finished product. T1 is the processing temperature recommended for liver sausage by Kramlich (1966), T2 is the processing

temperature of liver sausage commonly used in the meat industry in the U.S.A. (information obtained from a survey of some meat processors, Table 7), while T3 is used in Europe (J. Schut, Meat Research Laboratory, D. M. V. Veghel, The Netherlands, personal communication). T4 is a short time-high temperature treatment. In Study IV, the fat content was varied to include 20%, 30% and 35% fat.

B. Sample Preparation

1. Formulation

A basic formulation as shown in Table 8 was used for the first three studies. Some deviations from this formulation were used in Study I, one treatment used frozen, thawed liver instead of fresh liver. In the nitrite experiment, four levels of nitrite (0, 50, 100 and 156 ppm) were used; and in Study III, half of the samples containing 3.5% CRDSM computed on the basis of the weight of meat ingredients while the other half contained no CRDSM. Each of these experiments had two replications which were conducted on two consecutive days. In Study IV, all products had a formulation of pork liver, 50%; pork trim containing different levels of fat, 50%; bacon ends, 0.6%; CRDSM, 3.5%; spice-salt mixture, 3.3%; sodium chloride, 0.8%; sodium nitrite, 156 ppm; and char oil, 60 ml/45.5 Kg.

Table 8. Braunschweiger liver sausage formulation

Ingredients	% (w/w)[a]
Fresh pork liver	50
Fresh pork trim (50/50)	50
Salt and spices mixture[b]	3.3
Char oil[c]	60 ml/45.5 Kg
Sodium nitrite[d]	156 ppm

[a]Computed on the basis of total meat ingredients.

[b]Griffith's RTU Braunschweiger liver sausage seasoning.

[c]Solution of natural wood smoke flavors in vegetable oil, Red Arrow Products Company, Manitowoc, WI.

[d]Baker's Analytical Reagent grade.

2. Manufacturing procedures

a. Preparation Fresh pork liver (27.2 Kg per cardboard box) was purchased from Wilson Packing Company (Des Moines, IA). Upon arrival at the ISU Meat Laboratory within 2 hr after slaughter, the temperature of the fresh pork liver was 30-32°C. Individual fresh pork livers weighed 1-2 Kg and the color varied greatly from brick red to darkish purple to slight green or grey. A total of 45.5 Kg of fresh pork liver was selected and weighed, then each individual liver was cut into 8 parts. Each part was distributed to one of each of 8 batches. This liver

preparation was thought necessary to assure a homogeneous source of raw liver materials in each batch to eliminate variations due to individual liver differences. After separation and distribution, the livers were stored in a plastic container at 0°C and were used the next day.

Liver pretreatment in Study I included thawing the frozen liver, and scalding and soaking the fresh liver. The frozen liver, purchased from Wilson Packing Company (Des Moines, IA), was thawed at 10°C for 3 hr until the temperature rose to 0°C. For scalding, 5.7 Kg of fresh livers/batch (2 batches were made on two consecutive days) were scalded at 93°C (200°F) for two minutes, drained, and then chilled at 0°C. For soaking, fresh livers were soaked in a 5% salt solution at 0°C for one hour, then washed with cold tap water and stored at 0°C until used.

Fresh pork trim was ground through a 0.95 cm (3/8 in) plate using a Hobart grinder and then mixed thoroughly with an electric paddle mixer. A 5.91-Kg (13 lb) sample was taken for estimating fat content using the Anyl-Ray fat testing machine model M-201 (Anyl-Ray Corporation, Waltham, MA). The fat content of ground pork was adjusted by addition of fat or lean meat as necessary.

b. Chopping Fresh pork liver was put into a silent cutter type VSM 65 equipped with six blades (Kramer Grebe, Randoph, MA). Chopping started at low speed for the first four revolutions and then continued at high

speed for 20 revolutions (about 1.5 min) until the liver macerate showed bubbles. Ground pork was added to the bubbling liver and chopped some more with the addition of the salt-spice mixture, nitrite, char oil and CRDSM (if used) until 100 revolutions were completed. The total chopping time was about 5.5 min and the temperature of the mixture was about 16-18°C (60-65°F).

c. Stuffing The emulsion was immediately stuffed into 5.5 cm x 42 cm MP (moisture-proof) casings (Union Carbide) using a Vemag stuffer, Robot 1000 s type 116 (Robert Reiser & Co., Inc. Boston, MA). The casings were soaked in cold water before stuffing. Two clips were used for the first casing closure and after stuffing a third clip was used for the second closure. Each sample had a diameter of 5.5 cm and a length of 10 cm. The weight of each sample was about 170 g (6 oz). After stuffing, the outsides of the casings were washed with cold tap water and dried. The samples were then vacuum packaged in a Fresh-tuff bag (American Can Company, Neenah, WI) using a Multivac pouch machine AG 800 (Sepp Haggeümuller KG, West Germany). All the processes, including preparation, chopping, stuffing and vacuum packaging were carried out in a 10°C (50°F) room.

d. Cooking After vacuum packaging, all the products were cooked in water held at 74°C (165°F) until the internal temperature reached 68°C (155°F). Exceptions were the products of Study III in which different processing

temperatures were used as variables. The internal temperature of the product was measured by inserting a metal thermometer through the end of the sample into the geometric center of the sample. The cooking time required to reach 68°C internal temperature was about 28-30 min. After cooking, the sausages were chilled immediately by immersing in cold water (10°C) for 30 min after which they were drained and stored at 0°C.

3. Storage conditions

In general, most products were stored at 5°C after 2 days of chilling at 0°C. The products with different levels of nitrite were stored in an open-top display case at 7°C. Exceptions were those products in the study on temperature effects for which variable temperatures were used.

C. Panel Evaluation and Statistical Analysis

A sensory panel of 15 members evaluated the product characteristics for palatability attributes (texture, flavor, bitterness and overall acceptability), general appearance (product stability and color), and textural properties (sliceability and spreadability). The panel was made up of staff members and graduate students in Meat Science and Food Technology at Iowa State University. Panelists were given an orientation on evaluating these characteristics during which they were asked to do some preliminary judgements.

An eight-point hedonic scoring system (Figures 1 and 2), with 8 being the most desirable score and 1 being the least desirable, was used for the evaluation of all characteristics. The taste panel was conducted in the sensory evaluation room of the Meat Laboratory, Iowa State University. Five samples (with one commercial sample) were evaluated at each session. The evaluation period consisted of two parts. During the first part, the panelists evaluated the palatability attributes and spreadability of the samples under green illumination. The green illumination in the panel booth was used to mask any visual differences that might occur among the samples made with different processing variables. Two small pieces (1 cm x 1 cm) of each sample were tasted by the panelist for the palatability score and one piece of the same size sample was spread by the panelist on a 9 cm^2 area of a paper plate using a paring knife.

During the second part of the evaluation, the panelists judged other product characteristics including stability, sliceability and color under cool white fluorescent light having a light intensity of 40 foot candles. Three pieces of each sample (4.5 cm in diameter, 8 cm in length, about 130 g in weight) were placed on a white paper plate. Panelists judged the product stability by visual observation and the sliceability by using a paring knife to slice the samples.

For the panelists to judge the color score of the sample, fresh sliced samples (2 cm in thickness) were wrapped with a Saran film and displayed on

Figure 1. Score sheet for panel evaluation of texture, flavor, bitterness, overall acceptability and spreadability of Braunschweiger liver sausage

Judge _____

Date _____

TEXTURE (desirable as creamy and/or smooth) (undesirable as crumbly and/or grainy)		FLAVOR		BITTERNESS		OVERALL	
Extremely creamy and/or smooth	8	Extremely desirable	8	Imperceptible	8	Like extremely	8
Very creamy and/or smooth	7	Very desirable	7	Slightly perceptible	7	Like very much	7
Moderately creamy and/or smooth	6	Moderately desirable	6	Moderately perceptible	6	Like moderately	6
Slightly creamy and/or smooth	5	Slightly desirable	5	Perceptible	5	Like slightly	5
Slightly crumbly and/or grainy	4	Slightly undesirable	4	Slightly pronounced	4	Dislike slightly	4
Moderately crumbly and/or grainy	3	Moderately undesirable	3	Moderately pronounced	3	Dislike moderately	3
Very crumbly and/or grainy	2	Very undesirable	2	Very pronounced	2	Dislike very much	2
Extremely crumbly and/or grainy	1	Extremely undesirable (Rancid)	1	Extremely pronounced	1	Dislike extremely	1

SAMPLE	TEXTURE SCORE	FLAVOR SCORE	BITTERNESS SCORE	OVERALL SCORE

Spreadability (the performance of spreading the product with a knife on a solid surface)

Extremely well (very smooth and creamy)	8
Very well (smooth spread, slightly sticky)	7
Moderately well (smooth spread, moderately sticky)	6
Slightly well (can be spread, some sticky)	5
Slightly difficult (crumbles or slightly hard)	4
Moderately difficult (some crumbliness or hardness)	3
Very difficult (often crumbly or hard)	2
Extremely difficult (very hard or very coherent texture)	1

SAMPLE	SPREADABILITY SCORE

Figure 2. Score sheet for panel evaluation of product stability, sliceability and color of Braunschweiger liver sausage

Judge _____

Date _____

Product Stability
(desirable as homogeneous, uniform appearance undesirable as the incidence of air, fat, or jelly pockets)

Extremely desirable	8
Very desirable	7
Moderately desirable	6
Slightly desirable	5
Slightly undesirable	4
Moderately undesirable	3
Very undesirable	2
Extremely undesirable	1

Sliceability (the possibility to cut slices of 1 cm thickness at 4°C)

Extremely well (very easy to cut, no stickiness)	8
Very well (easily cut, slightly sticky)	7
Moderately well (less easily cut, moderately sticky)	6
Slightly well (can be sliced, somewhat sticky)	5
Slightly difficult (crumbles or deforms slightly, adhers or breaks occasionally)	4
Moderately difficult (some crumbliness or deformation, some breakage)	3
Very difficult (crumbles or deforms often, much breakage of slices)	2
Extremely difficult (distortion and breakage of the slice)	1

SAMPLE	PRODUCT STABILITY SCORE	SLICEABILITY SCORE

Color

Dark redish pink	8	Very little pink to slightly gray	4
Reddish pink	7	Pale gray	3
Pink	6	Moderately brownish gray	2
Pale pink	5	Brown, green abnormal color	1

SAMPLE	COLOR SCORE

a sheet of dove grey paper. Also, eight Munsell color discs mounted on another sheet of dove grey paper were used as color references. These discs were 10 cm in diameter and glossy. The nominal notations of the color discs were 7.5R 5/6 for color score 8, 7.5R 5/8 for color score 7, 7.5R 6/6 for color score 6, 10R 7/4 for color score 5, 5 YR 7/4 for color score 4, 10 YR 7/4 for color score 3, 10 YR 6/4 for color score 2 and 5 YR 7/4 for color score 1 (Munsell Book of Color—Glossy Finish Collection, 1970).

Analysis of variance was used to analyze the data collected from the panel evaluation (Snedecor and Cochran, 1967). The panel evaluation data were based on the hedonic scale of 1 to 8 with the numerical difference of 1 between each pair of numbers on the scale. However, panelists do not distinguish or assign equal differences between numbers at the extreme ends of the scale as compared to numbers at the middle of the scale. There is a psychological tendency to score the middle numbers closer than the numbers at either end of the scale (Warren et al., 1969). Thus a score of 1 versus 2 represents greater product differences than does a score of 4 versus 5. Therefore, the data were transformed. The panel scores before and after transformation are shown in Table 9.

Table 9. Transformation of the taste panel scores[a]

Taste Panel Score	Difference between each pair of two consecutive scores	Transformed taste panel score	Difference between each pair of two transformed, consecutive scores
1		-1.42	
	1		.57
2		-0.85	
	1		.38
3		-0.47	
	1		.32
4		-0.15	
	1		.30
5		0.15	
	1		.32
6		0.47	
	1		.38
7		0.85	
	1		.57
8		1.42	

[a]Transformed by the equation: $z = \frac{xi - \bar{x}}{s}$

z = transformed taste panel score

xi = individual taste panel score

$\bar{x}$ = mean of taste panel score $\bar{x} = \frac{1+2+3+4+5+6+7+8}{8} = 4.5$

s = standard deviation

$$s = \sqrt{\frac{\sum (xi - \bar{x})^2}{n-1}} = \sqrt{\frac{4.75}{7}} = 2.44.$$

D. Chemical Analysis

1. Nitrite determination

Nitrite concentrations of the samples were determined spectrophotometrically by using the method described by the Association of Official Analytical Chemists (AOAC, 1970) in sections 24.014 and 24.015.

a. Modified Griess Reagent Five-tenths of a gram of sulfanilic acid (Fisher Certified A. C. S.) was dissolved in 150 ml of 15% (v/v) glacial acetic acid (Fisher Reagent). One-tenth g α-naphthylamine (Baker Chemical) was boiled in 20 ml deionized, distilled water under a hood until dissolved and then poured, while hot, into the sulfanilic acid solution. This mixture was transferred to a brown glass bottle with a tight screw cap and stored under refrigeration.

b. Standard curve A 0.1514 g portion of sodium nitrite (Baker Analyzed Reagent which contained 99.1% sodium nitrite) was dissolved in deionized distilled water in a 100-ml volumetric flask. One ml of this solution was transferred into a 1-liter volumetric flask. Each ml of the above solution contained 1 μg of nitrite which was equal to a concentration of 1 ppm nitrite. Aliquots of 0, 3.0, 5.0, 10.0 and 20.0 ml of the above preparation were pipetted into separate 50 ml volumetric flasks and then diluted with deionized distilled water to 50 ml. The nitrite concentration in each of these flasks was 0, 0.06, 0.1, 0.2, and 0.4 ppm, respectively. Two ml of Griess Reagent

was added to each flask and mixed well, then held at room temperature for one hr for the development of color. A suitable amount of solution (about 7 ml) was transferred to a cuvette and the absorbance measured at a wavelength of 520 nm on the Spectronic-20 spectrophotometer (Bausch and Lomb Inc., Rochester, NY).

c. Determination of nitrite in meat preparations A 5 g-sample was removed from each sausage and blended with 50 ml deionized, distilled water at a temperature of 80°C with a Waring blendor model FC 114, for two minutes. The slurry was transferred into a 500-ml volumetric flask and the blendor jar was rinsed with 250 ml of deionized, distilled water (80°C). The flask containing the slurry and the rinse water was then put in a steam bath for two hours, during which the flasks were shaken occasionally. After steaming, 5 ml of saturated mercuric chloride solution (Analytic Reagent, Mallinkrodt Chemical Work) was added to the hot mixture, mixed and cooled to room temperature then diluted with deionized, distilled water. The mixture of 500 ml was slowly filtered through Whatman #1 filter paper. A suitable amount of filtrate was transferred into a 50-ml volumetric flask and diluted with deionized, distilled water up to the mark and then, 2 ml of Griess Reagent were added. After a one-hour period for color development, 7 ml of the above solution were transferred into a cuvette and the absorbance of the solution was measured with a Spectronic-20 spectrophotometer at 520 nm. The nitrite concentration was determined by comparing the above reading with the standard curve.

2. Thiobarbituric acid (TBA) test for rancidity

The 2-thiobarbituric acid (TBA) test (Tarladgis et al., 1960) is highly desirable for measuring oxidative rancidity of unsaturated fatty acid in meat and meat products (Zipser et al., 1964). The TBA test was used to measure malonaldehyde, a lipid oxidation product that can be distilled from a sample of liver sausage. Since nitrite interferes in this test, a modified procedure (Zipser and Watts, 1962) was used. In both procedures, duplicate determinations were made and the average was reported as the TBA number, which is defined as milligrams of malonaldehyde per 1,000 grams of meat (Sinnhuber and Yu, 1958). The TBA tests were performed as follows.

a. Reagents

1. TBA Reagent. 0.02M 2-thiobarbituric acid (Eastman Kodak Co.) in 90% glacial acetic acid.
2. Sulfanilamide Reagent. 0.5% sulfanilamide (Eastman Kodak Co.) in 20% hydrochloric acid (HCl) solution (v/v).
3. HCl solution. One part of concentrated HCl to two parts of distilled water (approximately 4 N).
4. AF 72. Antifoam emulsion, food grade. (General Electric Co.)

b. Procedure

1. Analyses of samples without nitrite A 10-g sample of liver sausage and 50 ml of deionized distilled water were blended in a Waring

blendor for two minutes. The slurry was poured into a 500 ml distillation flask which contained two glass beads, 1 ml of antifoam and 2 ml of 4N HCl. The blendor was then rinsed with 48 ml deionized distilled water, which were transferred to a distillation flask. The flask was placed in a distillation unit equipped with an electric heater set at 280° C, cool water condenser, and a 50 ml volumetric collection flask. After collecting 50 ml of distillate the aliquot was well-mixed and, 5 ml of the aliquot was pipetted into a screw cap test tube containing 5 ml of TBA reagent. The above mixture was mixed and steamed in a steam bath for 35 min along with another tube containing 5 ml deionized distilled water and 5 ml TBA reagent; the latter tube served as a reagent blank. After steaming, the tube was cooled in tap water for 10 minutes. A 7 ml portion of the solution was transferred into a cuvette and the absorbance of the sample was read at a wavelength of 538 mu. The TBA number was calculated by multiplying the absorbancy reading by 7.8 (Taladgis et al., 1960).

2. Analysis of samples with nitrite A 10 g-sample of Braunschweiger liver sausage was blended with 49 ml of distilled water and 1 ml of sulfanilamide reagent in a Waring blendor for two minutes. The slurry was poured into a 500-ml distillation flask which contained two glass beads, 1 ml of antifoam and 2 ml of 4N HCl. The procedures described previously were followed thereafter.

3. Salt determination

Salt concentrations (residual sodium chloride) of the samples were determined by the volumetric method of Volhard (AOAC, 1970).

a. Reagents

1. Ferric alum indicator. Saturated aqueous solution of reagent grade $FeNH_4(SO_4) \cdot 12\ H_2O$.
2. Silver nitrate ($AgNO_3$). 0.100 N.
3. Potassium thiocyanate (KCNS). 0.100 N.
4. Potassium permanganate ($KMnO_4$). 5% aqueous solution (w/v).
5. Nitrobenzene (reagent grade).

b. Procedure A three-gram sample was transferred into a 300-ml Erlenmeyer flask which contained 25 ml of 0.1 N $AgNO_3$ solution. The flask was swirled to achieve intimate contact of the contents. Then, 15 ml of concentrated nitric acid was added and the mixture boiled until the meat dissolved. A 10-ml aliquot of 5% $KMnO_4$ solution was then added to oxidize any organic matter not disposed of by the nitric acid. After that, 25 ml of deionized distilled water was added to the contents and boiled for 5 min, cooled, and diluted to ca. 150 ml with deionized distilled water. At last, 1 ml nitrobenzene and 2 ml ferric alum indicator were added to the above mixture and shaken vigorously to coagulate the precipitated AgCl. The excess $AgNO_3$ was titrated with the KCNS solution to a

permanent light brown end-point. The percentage of salt was calculated as shown in the following equation:

$$\% \, N_aCl = \frac{(25 \text{ ml} - \text{ml KCNS used in the titration}) \, (0.1N) \, (5.85)}{\text{sample weight}}$$

4. Proximate analyses

Water and fat contents were determined following the procedures described in AOAC (1970).

a. Moisture determination A 10-g sample of the Braunschweiger liver sausage was transferred into a pre-dried and weighed cellulose extraction thimble and dried for 24 hr in a vacuum oven with a 25 p.s.i. (172.37 KPa) vacuum at a temperature of 100°C. The thimble was allowed to cool to room temperature in a desiccator for 30 min before weighing. The weight loss was expressed as percent moisture of the initial sample weight.

b. Fat analysis The pre-dried sample from the moisture determination was transferred to a modified Goldfisch fat extractor and the fat extracted using 50 ml of hexane (Skelly B). The extraction was performed for 24 hr and the samples were then dried in a vacuum oven for 1 hr and cooled to room temperature in a desiccator for 30 min and weighed. The loss in weight of the contents of the cellulose extraction thimble (solvent-extractable fat) was computed as percent of initial sample weight.

5. pH measurement

A fifty-gram sample of Braunschweiger liver sausage and 50 ml of distilled water were mixed in a Waring blendor for two min. The pH of the homogenate was measured using a Beckman pH meter.

6. Color measurement

Objective color measurements ofsamples were made on a Photovolt photoelectric reflection meter (model 610), using the standard search unit 610Y with amber, green, and blue tristimulus filters. A standard white enamel plaque was used to standardize the instrument. The search unit containing the amber or green or blue tristimulus filter was first placed on a calibrated white enamel plaque and the needle was set on the calibration value of the plaque (75.0, 75.5, and 76.5 for the amber, green and blue filters, respectively). Then, the search unit was placed on the surface of the sample, the needle indicated the reflectance value directly. The reflectance value of a surface is its reflection expressed in percentage of the reflection of magnesium oxide.

The tristimulus values, X, Y, and Z were calculated from the following equations: $X = 0.8A + 0.18B$; $Y = G$, and $Z = 1.18B$, where A, G, and B are respectively the reflectance values obtained by measuring with amber, green and blue filters. The chromaticity coordinates were calculated as follows (Judd, 1952):

$$x = \frac{X}{X+Y+Z}$$

$$y = \frac{Y}{X+Y+Z}$$

$$z = \frac{Z}{X+Y+Z}$$

7. Water activity (A_w) measurement

A_w of equilibrated samples was measured with a hygrometer (Model 15-3050, American Instrument Co., Silver Spring, MD). Each of the 20-g samples (4.5 cm in diameter, c. 1 cm in heighth) was cut diagonally into eight equal parts and then placed in Mason jars with a sensor-fitted cap screwed on top of each for an air-tight fit. The hygrosensors (type TH-3) consisted of a strip of paper coated with a hygroscopic material (lithium chloride), contained within an aluminum case. Sensors were calibrated to an accuracy of ± 1.5% relative humidity and ± 1.1°C. The closed system was allowed to come into equilibrium at room temperature (28°C) for approximately three hr. The equilibrium relative humidity (E.R.H.) readings and the temperature readings were recorded and converted to A_w values.

E. Microbiological Study

1. Microbial content and flora

Raw ingredients (liver, pork trim, and the salt-spice mixture), the

raw mixture as stuffed into casings, the cooked and freshly-made and the stored Braunschweiger liver sausage were examined microbiologically. Except for liver, 30 g samples of the ingredients were blended with 270 ml of 0.1% peptone (Difco) water in sterile Mason jars for one minute each at low and high speeds. Serial dilutions were made into either 99 ml or 9 ml of sterile 0.1% peptone water.

Livers were sampled by swabbing in which a moistened cotton swab was rolled three times over a 10 cm^2 area. The cotton portions of the swab sticks were then snapped off into bottles containing 100 ml of 0.1% sterile peptone water.

Counts were determined for the following organisms: total aerobic mesophilic bacteria, total anaerobic mesophilic bacteria, enterococci and aciduric bacteria. Media, plating techniques and incubation conditions for this study are listed in Table 10. Triplicate samples of each treatment were examined. Representative colonies were selected and characterized to determine the proportion of types present.

2. Storage-temperature study

One batch of Braunschweiger liver sausage in Study I was manufactured using all the same materials and methods; however, the batch was divided into halves designated P and Q for cooking because of space limitations. Cooking of each of these portions was done on the same day. The product was cooked in water held at 74°C until the internal temperature reached

Table 10. Methods employed for enumeration of bacteria

Determination	Growth Medium	Plating Technique	Incubation
Total aerobic mesophilic count	Trypticase Soy Agar (TSA) (BBL)[a]	Spread plate	30°C/2 days
Total anaerobic mesophilic count	TSA + 0.5% sodium thioglycollate (Difco)[b] (TT)	Spread plate	30°C/3 days anaerobic (Nitrogen atmosphere)
Enterococcal count	KF streptococcus Agar (KF) (Difco)	Spread plate	35°C/2 days
Aciduric bacteria count	LBS agar (BBL)	Spread plate with overlay	30°C/5 days

[a]BBL division of BioQuest, Cockeysville, MD.

[b]Difco Laboratories, Detroit, MI.

68°C. The formulation of these products (using fresh liver) is given in Table 8. The cooked products were stored at 15°C and 22°C. The microbial content, flora, pH and odor were determined after storage for 3, 6, and 21 days at 22°C and 6, 14 and 21 days at 15°C.

3. Identification of microorganisms

For the identification of the isolated cultures the following tests were used: Gram stain, catalase test, carbohydrate fermentation tests, motility test, nitrate reduction, gelatin liquefaction, indole formation, hydrogen sulfide production, casein agar digestion, starch hydrolysis, MR-VP tests and reaction on litmus milk. The procedures followed are described by Durand et al. (1973). Additional tests that were made included the OF test (Evans and Kloos, 1972), arginine hydrolysis (Collins and Lyne, 1970), 40% bile resistance (Facklam, 1972), pyruvate fermentation (Gross et al., 1975), glycerol anaerobic fermentation (Gunsalus and Sherman, 1943), 0.04% tellurite tolerance (Cavett, 1963), growth on LBS agar (Cavett, 1963) and growth on KF medium (Kenner et al., 1961).

4. Inoculation study with spoilage organisms

a. Spoilage organisms Two cultures obtained from spoiled Braunschweiger liver sausage held under refrigeration (5-7°C) were used in this work. One culture was isolated from spoiled samples emitting a unique

perfumy odor; this culture was designated M. The other culture, designated N, was isolated from spoiled samples with a low pH of 5.5-5.8 as compared to the pH of normal Braunschweiger liver sausage with a pH of 6.1 to 6.3. Inocula for these cultures were prepared in APT broth (Difco) incubated at 35°C for 24 hr.

b. Inoculated samples and methods

1. Sterilized samples Braunschweiger liver sausages which were processed by cooking in water held at 74°C until an internal temperature of 68°C was reached, were used to prepare sterilized samples. A sample of 50 g of this product was transferred into a quart size blender jar, blended for a few seconds, covered with a screw-cap and autoclaved. Five ml of a 24-hr cell suspension of the organisms were pipetted into the sterile samples and aseptically mixed using an alcohol-flamed spatula. After mixing, a small portion of the inoculated sample was aseptically removed into a small beaker for determining the pH. The inoculated samples were incubated at 30°C for 3 days. A control was prepared by mixing 5 ml of sterile APT broth into the sterile sample. After incubation, the pH and odor of the samples were observed and recorded.

2. Pasteurized samples The Braunschweiger liver sausage used in this part of the inoculation studies were those samples cooked in a water vat at 79°C until the internal temperature reached 74°C (same

as the T3 treatment described previously). These samples were chosen because they had a low indigenous microbial content of 2.1×10^3/g; Bacillus spp. predominated under these conditions.

A suitable dilution and size of inoculum was used to achieve about 10^6 cells/g in the preparation; thus, the organisms added in the inoculum would predominate and be able to successfully compete with any other organisms present.

A 100-g portion of Braunschweiger liver sausage was aseptically transferred into a sterile, quart-size blender jar and blended for a few seconds with 10 ml of the inoculum until well mixed. A control was also prepared. After blending, the initial pH of the inoculated sample was recorded. Inoculated samples were stored at 30°C for 2 days, 15°C for 10 days and 10°C for 20 days. After this period of incubation, pH and odor of the inoculated samples were recorded. Total aerobes were enumerated on APT agar (Difco) incubated at 30°C for 48 hours.

Another set of experiments in which Braunschweiger liver sausage was inoculated with these same organisms was done under anaerobic conditions. A 30-g portion of Braunschweiger liver sausage was aseptically weighed, chopped and placed into a sterile plastic petri dish. One ml of cell suspension containing about 10^7 cells/ml was evenly distributed into the sample with a sterile micropipette dropper. After inoculation the petri dish half containing the inoculated sample was inserted into a Fresh-tuff bag and vacuum packaged

using a Multivac pouch machine. After storage at 22°C for 5 days, the pH and odor of the inoculated samples were recorded. Total anaerobic count was determined using TT medium (TSA + 0.5% sodium thioglycollate) incubated in a nitrogen atmosphere in an anaerobic incubator at 30°C for 72 hr. Enterococci were enumerated on KF streptococcus medium (Difco) incubated at 35°C for 48 hr and aciduric bacteria were counted on LBS medium (BBL) incubated at 30°C for 5 days. These counts were done using the spread plate technique.

5. Heat resistance studies using spoilage organisms

Cell suspensions of cultures of M and N containing about 10^9 cells/ml were diluted with 0.1% sterile peptone water to obtain appropriate cell concentrations. Inocula for these suspension were 24-hr cultures grown in APT broth. A sterile micropipette (145 mm in length with 1 mm diameter width at the tip end) was used to drop the cell suspension into the bottom of screw-capped sterile vials (17 x 60 mm). Twenty drops of the cell suspension (approximately equal to 0.5 ml in volume) were carefully placed in the center part of the bottom of the vial to avoid any contact of the suspension with the side of the vial. The vials containing cell suspensions were transferred to water baths at 60°C and 65°C. After certain time intervals (60°C for 30 and 60 min; 65°C for 3 and 5 min), the heated vials were immediately immersed in a cold ice-water bath (2-4°C) for 5 min and then 5 ml sterile

APT broth was poured into the vial. The mixtures were incubated at 35°C for 2 days. The presence of turbidity indicated the survival and growth of the heat-treated organisms. Each trial included 5 vials. In each trial, when turbidity developed in at least 4 out of 5 tubes the trial was recorded as a "survived" response. Two replications in each trial were done.

6. Prediction of microbial quality of Braunschweiger liver sausage

After the microbial platings of fresh, cooked samples, the homogenate or slurry which consisted of 30 g of sample and 270 ml of sterile peptone water was incubated at 30°C for 2 days. Total anaerobic mesophilic count and enterococci counts were made after incubation to determine the predominant microflora. Some of the nonmicrobial changes such as pH value, odor and slurry behavior were recorded before and after incubation in order to observe any relationships between microbial and nonmicrobial changes in the homogenate. The slurry was observed for separation of water and meat residues in the homogenate after shaking and then standing for 30 min. After the first incubation, some samples with pH $\geq$ 5.1 were incubated for an additional 3 days at 22°C for further observations of nonmicrobial changes.

IV. RESULTS AND DISCUSSION

A. Organoleptic Quality of Braunschweiger Liver Sausage

1. Influence of liver pretreatments

The results of sensory evaluation of liver sausage using different pretreatments of liver as the tested variables are shown in Table 11. Liver pretreatment had a significant effect ($P < 0.05$) on the color of liver sausage. The color of all liver sausage made in this study was acceptable since the color scores graded as pale pink to pink. The pinkish color intensity decreased in the following order: frozen-thawed liver, fresh-soaked liver, fresh-scalded liver, fresh liver.

Pretreatment of liver had no statistically significant effect on the overall palatability of the finished products. However, the results indicated that the panelists had the strongest preference for liver sausage made of fresh-soaked liver and least preference for the liver sausage made of fresh liver. Comparisons among liver sausages revealed a slightly higher score in flavor and overall palatability for liver sausages made from frozen-thawed liver than those liver sausages made from fresh liver. These results were in contrast to the report of Brouwer et al. (1976) in which they stated that liver sausage made from fresh liver had a higher taste quality than that made from frozen liver. Also, the information we obtained from the survey

Table 11. Relationships between taste-panel scores for Braunschweiger liver sausage and pretreatment of pork liver used in the sausage

Pretreatment of pork liver	Sensory Evaluation Scores[1,2]							
	Texture	Flavor	Bitterness	Overall Acceptability	Spreadability	Product Stability	Sliceability	Color
No pretreatment — fresh	0.64	0.51	0.68	0.50	0.64	0.69	0.65	0.22a
Frozen and thawed	0.50	0.64	0.75	0.58	0.51	0.73	0.73	0.36b
Scalded	0.58	0.62	0.63	0.59	0.55	0.64	0.78	0.24ac
Soaked	0.68	0.63	0.71	0.65	0.55	0.74	0.78	0.32bc

[1]There were 26 observations in each cell of the first two pretreatments and 27 observations in each cell of the last two pretreatments.

[2]Means in the same column with different letters are significantly different ($P < 0.05$). Panel scores have been transformed (See Table 9).

of some meat processors indicated that fresh liver was preferred due to flavor advantages. The reason for the conflicting results could be because the frozen liver used in the industry is commonly stored for a long period of time. In contrast, the frozen liver used in our study was only stored for one day. Fresh frozen meats are susceptible to lipid oxidation which causes a deterioration in flavor quality of meats (Keskinel et al., 1964). The amount of phospholipids in meat is relatively small about 0.5% to 1% (Hornstein et al., 1961); but the high content of unsaturated fatty acids in phospholipids causes it to be extremely susceptible to lipid oxidation (Keskinel et al., 1964). Hemoglobin and nonheme iron components are active catalysts of lipid peroxidation (Kwoh, 1971). Ascorbic acid may also function as a prooxidant in meat (Love and Pearson, 1971). Pork liver contains substantial amounts of phospholipids (Rice, 1971) and the fatty acids in liver fat are highly unsaturated and subject to oxidation (Tauber, 1971). In addition to that, liver contains significant amounts of hemoglobin, iron and ascorbic acid (Watt and Merrill, 1963; Kiernat et al., 1964). Wattiaux and de Duve (1956) showed that freezing of rat liver ruptured lysosomal membranes, thus liberating hydrolytic enzymes and making them accessible to their substrates. Lawrie (1963) reported that at -20°C, there was a slow breakdown of proteins to amino acids in pork liver. Therefore, it is likely that degradation of liver can occur during long term storage resulting in an undesirable flavor. On

the other hand, the fresh liver used in our study was chilled in a 0°C cooler until used the next day. Apparently this slow chilling did not decrease the temperature of the fresh liver rapidly enough to minimize the undesirable lipid oxidation in the liver. If this is true, then rapid chilling of warm liver (30°C) before storage at 0°C could be an important practice for the assurance of good flavor in liver sausage made of fresh liver.

Liver sausage made from fresh-soaked liver was found to be the most desirable among the four groups of liver sausage according to the scores for sensory traits. This group had the highest score in texture, overall, product stability and spreadability; very desirable scores for flavor, lack of bitterness and color; and a good score for sliceability. A possible explanation for these high scores could be related to the slight difference in composition. Liver sausage made of fresh-soaked liver contained a slightly higher water content (57%) and salt content (2.31%) than those of the liver sausage made from fresh liver which had a water content of 55% and a salt content of 2.20%. An increase in water and salt contents in a meat product will increase the emulsifying capacity of protein resulting in better product stability (Swift and Sulzbacher, 1963). The differences of the water and salt contents could result from the carry-over effect of the soaking and washing pretreatment of the livers. These results imply that the addition of salt and water in the formulation of liver sausage may be a profitable practice

for enhancing the organoleptic quality of liver sausage. However, the question arises if such small changes as encountered in our work could be responsible for the level of enhanced acceptability observed.

The product stability of liver sausage made of fresh-scalded liver had the lowest score. This indicates that scalding of liver decreased the emulsion stability of the product. This instability could result from the denaturation of some of the protein on the surface of the liver during scalding. Although the product stability of liver sausage made of scalded liver graded as moderately desirable, this observation implies that scalding of liver might result in a serious problem when liver sausage is made with formulation of a high fat content because our product was formulated to contain 25% fat. Product stability of liver sausage made of fresh-soaked liver had the highest score indicating that soaking liver in 5% salt brine at 0°C following with washing increased the emulsion stability of the product.

2. Influence of nitrite levels

Panel evaluation of liver sausage containing no nitrite or 156 ppm nitrite showed very little difference in overall taste acceptability by the panelists (Table 12). Among the samples containing nitrite, however, desirability in flavor, bitterness and overall acceptability increased as nitrite level increased. The importance of nitrite for cured flavor in liver sausage cannot be evaluated in this study. The reason is that the panelists were asked

Table 12. Influence of nitrite levels on organoleptic quality of liver sausage as evaluated by a trained taste panel

Nitrite content[1] (ppm)	Sensory Evaluation Scores[2,3]							
	Texture	Flavor	Bitterness	Overall Acceptability	Spreadability	Product* Stability	Sliceability	Color**
0	0.70	0.71	0.81	0.57	0.49	0.31a	0.57	-0.40a
50	0.59	0.48	0.73	0.45	0.43	0.55ab	0.70	0.34b
100	0.54	0.57	0.82	0.53	0.46	0.63b	0.72	0.34b
156	0.57	0.64	0.86	0.60	0.40	0.57b	0.62	0.34b

[1]Nitrite content at time of formulation.

[2]18 observations in each cell.

[3]Means in the same column with different letters are significantly different. Panel scores have been transformed (See Table 9).

*Significant $P < 0.05$.

**Significant $P < 0.01$.

whether they liked or disliked the product. They were not asked to distinguish the difference in flavor among the samples.

Cho and Bratzler (1970) reported that nitrite is responsible for the development of typical cured meat flavor in the pork loin. Simon et al. (1973) found all-meat frankfurters made with stepwise increases in nitrite (39-156 ppm) resulted in higher scores for flavor acceptability. Their report also indicated that all-beef frankfurters were acceptable even in the absence of nitrite. Hustad et al. (1973) found through expert panel evaluations that the flavor quality of liquid smoke-treated weiners containing 50 to 300 ppm nitrite was judged significantly higher than weiners made without nitrite. Similar results were reported by Sebranek et al. (1977). They reported that evaluation by a consumer taste panel of frankfurters cured with 0, 26, 52 and 156 ppm of nitrite showed that color, flavor and overall acceptability decreased with decreasing nitrite concentration. Frankfurters without nitrite were scored undesirable or unacceptable for all sensory traits. Wasserman and Talley (1972) reported that the role of nitrite in frankfurter flavor is complex. In triangle tests with both smoked and unsmoked frankfurters, they found significant differences between products prepared with or without 156 ppm nitrite. When unsmoked frankfurters were scored, a highly significant difference in flavor was produced by the addition of nitrite. When the smoked frankfurters were scored; however, there was essentially no difference in the scores for untreated and

nitrite-treated franks. It appears from these results that smoking masks some of the difference in flavor of frankfurters caused by nitrite, at least for the first 24 hr following processing.

The fact that liver sausage prepared without nitrite is acceptable also appeared in the results of a 34-member consumer panel (Table 13). The results in Tables 12 and 13 indicate that there is no difference in the taste desirability for samples without nitrite or with different levels of nitrite. However, in both tables, the means of transformed scores of the consumer panel for all the sensory traits were lower than those of the regular panel. This may be explained by the fact that 10% of the consumer panelists showed a dislike for liver sausage and rejection of the product was due to bitterness. However, 90% of the consumer panelists did not indicate any objection due to bitterness associated with liver sausage.

After the first cutting of the product, the color of the liver sausage prepared without nitrite was a pale pink which is quite different from the color of other meat products prepared without nitrite. Dymicky et al. (1975) stated that elimination of nitrite from cured meat products results in a beige or tan color of hemochrome, the ferrous form of denatured myoglobin and hemoglobin. Wasserman and Talley (1972) described the interior color of smoked-nonnitrite treated samples as grey. Hustad et al. (1973) reported that characteristic cured meat color was absent in nitrite-free weiners.

Table 13. Influence of nitrite levels on organoleptic qualities of liver sausage as evaluated by a consumer panel

Nitrite Content[1] (ppm)	Sensory Evaluation Scores[2,3]				
	Texture	Flavor	Bitterness	Overall	Spreadability
0	0.55a	0.32	0.27	0.31	0.20
50	0.55a	0.32	0.44	0.37	0.32
100	0.41a	0.39	0.44	0.41	0.45
156	0.38a	0.35	0.39	0.29	0.18
Commercial A	0.13b	0.26	0.32	0.27	0.37

[1]Nitrite content at time of formulation.

[2]34 observations in each cell.

[3]Means in the same column with different letters are significantly different ($P < 0.01$). Panel scores have been transformed (See Table 9).

Fox (1966) reported that the principal source of meat color in meat products is myoglobin, the muscle heme pigment, although hemoglobin, the blood pigment, will comprise 20 to 30% of the total pigment present. He also added that most of the reactions of the two pigments are identical; however, several reactions of importance in meat color, such as autoxidation, reaction with nitrite, and denaturation, have different rates for the two pigments. The pale pink color found in liver sausage prepared without nitrite could be due to the high hemoglobin content of the raw mixture since liver represented 50% of the meat ingredients. Furthermore, the significant content of nicotinic acid, vitamin C and iron in liver compared to muscle could play an important role in the formation of a pale pink color in liver sausage prepared without nitrite. It has been observed that niacin or nicotinic acid reacts with reduced myoglobin under anaerobic conditions resulting in formation of hemochrome, a red reaction product (Coleman et al., 1949; Kendrick and Watts, 1969). Also, Brown and Tappel (1957) ascribed pink pigment of cooked tuna to hemochrome formation with nicotinamide derived from nicotinamide adenine dinucleotide.

During color evaluation, the sample was cut and wrapped with a Saran film and evaluated by the panelists under a white fluorescent light. The results as shown in Table 12 indicate that the color of the product without nitrite was significantly different from the other products with different levels

of nitrite. Furthermore, it was observed that the initial pale pink color of liver sausage prepared without nitrite changed rapidly to a pale grey color during the evaluation period. Apparently the pale pink color of liver sausage prepared without nitrite was extremely susceptible to light and oxygen.

The color of samples prepared with 50, 100 and 156 ppm nitrite had a characteristic cured pink color and there was no difference in color intensity among those samples. This result is similar to reports for other meat products. Hustad et al. (1973) reported that weiners prepared with 50 to 300 ppm nitrite had a characteristic cured color and no difference in color was observed between groups. Eakes and Blumer (1975) reported that 70 ppm of nitrite produced acceptable color and flavor in country style ham. In contrast to the above, Sebranek et al. (1977) reported that the color score of frankfurters prepared with 156 ppm nitrite was higher than that of frankfurters prepared with 52 ppm nitrite.

3. Influence of processing temperatures and the addition of calcium-reduced dried skim milk (CRDSM)

Cooking temperature had a great influence on many of the sensory traits of liver sausage including texture, flavor, overall taste quality, spreadability, product stability and color (Table 14). There was a significant difference ($P < 0.01$) among the four cooking treatments for the texture of liver sausage. The texture of liver sausage cooked in 68°C water to an internal temperature

Table 14. Analysis of variance of taste-panel scores of liver sausage cooked at different temperatures and with or without CRDSM[1]

Source of Variation	Sensory Evaluation Traits[2]							
	Texture	Flavor	Bitterness	Overall Acceptability	Spreadability	Product Stability	Sliceability	Color
Replication		**					**	
Cooking temperature (CT)	**	*		**	*	**		Δ
CRDSM		*						
CT x CRDSM						**		
Judge	**	**	**	**	**	**	**	**

[1]Calcium-reduced dried skim milk.

[2]There were 200 observations.

*P < 0.05.

**P < 0.01.

Δ P = 0.06.

of 63°C graded as very creamy and/or smooth and was the most desirable texture among all. Increasing cooking water temperature and internal product temperature resulted in a decrease of texture score which is very well-demonstrated in Table 15. The texture of liver sausage cooked in 85°C water to an internal temperature of 74°C had the lowest texture score, described as slightly creamy and/or smooth.

Cooking temperature had little effect on the bitterness of the product. There was a significant difference ($P < 0.05$) between cooking temperatures 1, 2 and 4 for flavor while the differences in flavor between cooking treatments 1, 2 and 3 or 3 and 4 were not significant. There was also a significant difference ($P < 0.01$) between treatments 1, 2, 3 and 4 for overall taste acceptability. This indicates that flavor was the most important taste trait and greatly influenced the decision of overall taste acceptability. The correlation between flavor and overall acceptability, texture and overall acceptability, bitterness and overall acceptability was 0.80, 0.55 and 0.61, respectively.

Spreadability differences between treatments 1 and 3, 4 were significant ($P < 0.05$) but not between treatments 1 and 2, also differences between 2 and 3, 4 were not significant. Product stability of liver sausage processed using cooking treatment 4 was slightly desirable, using cooking treatments 2 and 3 was moderately desirable and using cooking treatment 1 was very

Table 15. Effect of cooking temperatures on organoleptic quality of Braunschweiger liver sausage

Cooking Treatment	Cooking Water Temperature (0°C)	Internal Product Temperature (0°C)	Total Cooking Time (Minutes)	Sensory Evaluation Scores [1,2,3]							
				Tex	Fla	Bit	Over	Spr	Pr S	Sli	Col
1	68	63	40	1.02a	0.67a	0.76	0.67a	0.58a	0.88a	0.74	0.44a
2	74	68	37	0.77b	0.70a	0.77	0.67a	0.49ab	0.68b	0.67	0.34ab
3	79	74	40	0.55c	0.64ab	0.80	0.59a	0.37b	0.73b	0.65	0.34ab
4	85	74	33	0.32d	0.56b	0.69	0.46b	0.36b	0.36c	0.70	0.30b

[1]50 observations in each cell.

[2]Tex = Texture; Fla = Flavor; Bit = Bitterness; Over = Overall Acceptability; Spr = Spreadability; Pr S = Product Stability; Sli = Sliceability and Col = Color.

[3]Means in the same column with different letters are significantly different ($P < 0.05$). Panel scores have been transformed (See Table 9).

desirable. Evidently, the lower cooking water temperature improved the product stability of the liver sausage. However, the slower cooking rate demanded a longer cooking time to reach the desired internal temperature. The panel score also indicated that the intensity of pink color of the liver sausage was higher in the one cooked at cooking treatment 1 than that of the liver sausage cooked at treatment 4.

Cooking treatments are concluded to be of critical importance for the organoleptic quality of liver sausage. The taste panel rated liver sausages cooked to an internal temperature of 63°C more desirable than those cooked to 68°C and 74°C. The taste panel, in general, accepted the liver sausage cooked in 74°C water to an internal temperature of 68°C as well as the liver sausage cooked in 79°C water to an internal temperature of 74°C. The formation of some fat pockets in the liver sausage cooked in 85°C water to an internal temperature of 74°C was a critical defect which could be magnified in a product with high fat content and may result in the rejection of the product by consumers regardless of the acceptable taste.

The addition of 3.5% calcium-reduced dried skim milk (CRDSM, Savortex) to the sausage formulation resulted in a higher taste panel rating for flavor in the product. The mean of transformed flavor scores was 0.68 for the liver sausage prepared with CRDSM and was 0.60 for the liver sausage prepared without CRDSM. This difference was significant ($P < 0.05$).

These results indicate that addition of CRDSM to liver sausage improved the flavor of the product yet had no effect on other sensory traits.

There was a significant interaction ($P < 0.01$) between cooking treatments and addition of CRDSM (Table 14) for product stability. This interacting effect as shown in Figure 3 demonstrated that addition of CRDSM exerted little difference on product stability of liver sausage cooked at cooking treatments 1, 2 and 3. However, the addition of CRDSM to liver sausage cooked with cooking treatment 4 greatly improved the product stability. This result implies that the function of CRDSM on product stability is to stabilize the product when some unfavorable or detrimental processing variables simultaneously exist. Processing temperature affects on emulsion capacity and stability of meat products has been very well-demonstrated by Saffle et al. (1967) in frankfurters and by Kramlich (1965) in liver sausage.

4. Influence of fat content and frozen storage

Fat content had a highly significant effect ($P < 0.01$) on the texture, sliceability and color of Braunschweiger liver sausage (Table 16). Increase in fat content of liver sausage resulted in a more creamy and smooth texture. Among all the samples those containing 30% fat were the most preferred according to the panel scores for flavor and overall quality. Products containing 35% fat had the best spreadability and sliceability yet the

Figure 3. Comparison by taste panel evaluation of product stability of Braunschweiger liver sausage with or without CRDSM[1] and cooked at different temperatures.[2] (Each point represents the average of two replications. Each replication contained 12 observations for a total of 24 observations)

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[1]CRDSM refers to calcium-reduced dried skim milk (Savortex).

[2]Cooking treatment 1 represents cooking in 68°C water to an internal temperature of 63°C; cooking treatment 2, cooking in 74°C water to 68°C; cooking treatment 3, cooking in 79°C water to 74°C; and cooking treatment 4, cooking in 85°C water to 74°C.

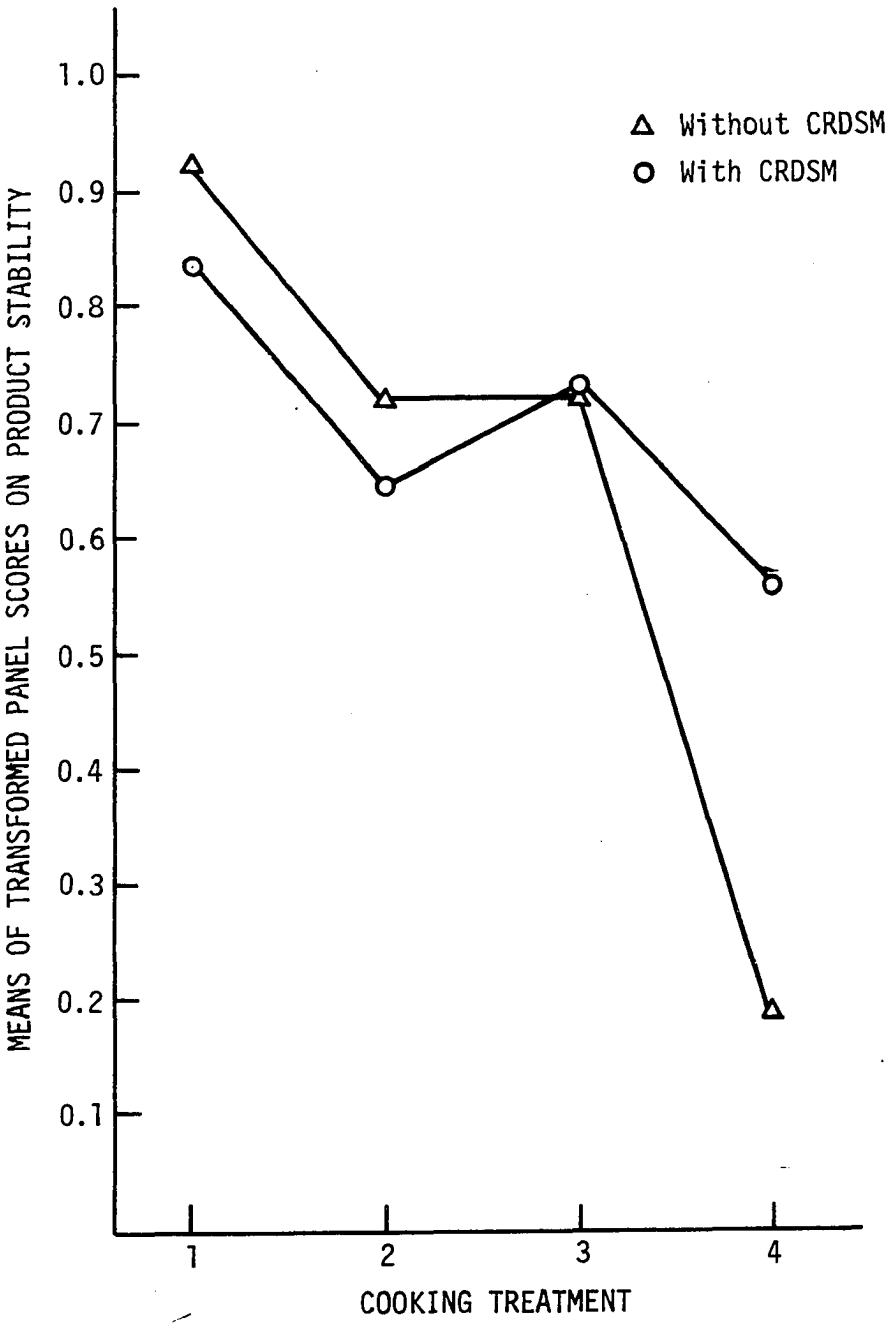

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
MEANS OF TRANSFORMED PANEL SCORES ON PRODUCT STABILITY
Without CRDSM
With CRDSM
1
2
3
4
COOKING TREATMENT

Table 16. Influence of fat content on acceptability of Braunschweiger liver sausage

Fat Content (%)	Sensory Evaluation Scores[1,2]							
	Texture**	Flavor	Bitterness	Overall Acceptability	Spreadability	Product Stability	Sliceability**	Color**
20	0.40a	0.72	0.90	0.70	0.33	0.63	0.65ab	0.46a
30	0.60b	0.84	0.95	0.77	0.31	0.64	0.51b	0.25b
35	0.80c	0.72	0.84	0.71	0.49	0.42	0.74a	0.28b

[1]14 observations in each cell.

[2]Means in the same column with different letters are significantly different ($P < 0.01$). Panel scores have been transformed (See Table 9).

**$P < 0.01$.

product stability was least desirable due to the formation of fat pockets at the ends of the samples. Panel scores for color of liver sausage were influenced by the fat content. Significant differences ($P < 0.01$) were noted only between the product containing 20% fat and the others. No difference in color was found between product containing 30% fat and 35% fat.

The difference in palatability and spreadability of liver sausage stored under refrigeration at 5°C or at -29°C in a freezer for one week was compared. Before the taste panel evaluation period, the frozen samples were thawed in a refrigerator overnight. The panel evaluation (Table 17) showed that frozen storage had little influence on the palatability or spreadability of the product. Significant differences ($P < 0.01$) for spreadability of liver sausage was noted only between the products containing the highest and the lowest fat content.

5. Subjective and objective color measurements

Preliminary studies showed that the scores for subjective color evaluation on the same Braunschweiger liver sausage based on descriptive terms of color (Figure 2) given by panelists varied widely. This observation agrees with the statement of Billmeyer and Saltzman (1966). They concluded that since people differ in their individual response to visual scores, they will also differ in their interpretations of subjective, defined terms for color. Therefore,

Table 17. Relationships between organoleptic qualities of liver sausage and different levels of fat and different storage conditions

Treatment	Sensory Evaluation Scores[1,2]				
	Texture	Flavor	Bitterness	Overall Acceptability	Spreadability
Stored at 5°C for one week	0.60	0.76	0.89	0.73	0.38
Stored at -29°C for one week	0.60	0.75	0.90	0.66	0.45
20% fat	0.51a	0.76	0.89	0.70	0.30a
30% fat	0.56a	0.76	0.94	0.70	0.39ab
35% fat	0.73b	0.73	0.86	0.68	0.54b

[1]There were 42 observations for each variable in liver sausage stored at 5°C; 39, for liver sausage stored at -29°C; and 27 for liver sausage containing different fat contents.

[2]Means in the same column with different letters are significantly different ($P < 0.01$). Panel scores have been transformed (See Table 9).

eight Munsell color discs were chosen in our study as color references.

Objective measurements of color with a Photovolt-610 reflectance meter are compared with visual color scores in Table 18. Variations in color of Braunschweiger liver sausages are evident from the three tristimulus values; the chromaticity coordinates x, y and z, however, remain more or less constant. The simple correlation coefficients presented in Table 19 indicate that objective measurements of color with a photovolt were significantly related to the visual color scores ($P < 0.05$). Comparisons made among the tristimulus values of the reddish-pink and pink samples showed an increase in tristimulus value was indicative of a lighter pink color. Based on our results, for practical purposes, it is safe to compare the characteristic cured color by any one of the three tristimulus values; however, since Y = G and G is the reflectance value obtained directly from measuring with the green filter, it is more convenient to use the direct reflectance value.

B. Chemical Analysis

1. Water, fat, salt content and brine concentration

The water, fat, and salt content and brine concentration of the finished products are presented in Table 20. Among the first three studies, the common formulation (Table 8) resulted in finished products with a consistent composition. They had an average water content of 54.9% with a range of 52.9 to 57.0%, an

Table 18. Tristimulus values and chromaticity coordinates for the color shades of Braunschweiger liver sausage

Braunschweiger liver sausage in		Tristimulus values			Chromaticity coordinates			Mean of transformed taste-panel score	Color visual grading
Study	Variables	X	Y	Z	x	y	z		
I	Liver								
	Fresh	31.7	30.0	21.9	0.38	0.36	0.27	0.22	Pink 1+
	Frozen-thawed	30.6	28.1	21.9	0.38	0.35	0.27	0.36	Dark reddish pink
	Fresh-scalded	32.5	30.3	23.7	0.38	0.35	0.28	0.24	Pink 1+
	Fresh-soaked	32.1	30.0	23.4	0.38	0.35	0.27	0.32	Pink 2+
II	Nitrite								
	0 ppm	33.4	29.5	24.5	0.38	0.34	0.29	0.20	Pale pink
	50 ppm	30.4	26.5	21.2	0.39	0.34	0.28	0.34	Pink 2+
	100 ppm	27.7	25.6	21.2	0.39	0.33	0.28	0.34	Pink 2+
	156 ppm	26.3	24.6	20.1	0.38	0.33	0.28	0.37	Pink 2+

III	Processing[a] Temperature								
	T1	27.4	24.8	18.1	0.39	0.35	0.26	0.44	Reddish pink
	T2	28.6	26.0	19.2	0.39	0.35	0.26	0.34	Pink 2+
	T3	30.0	27.5	20.7	0.38	0.36	0.27	0.34	Pink 2+
	T4	30.0	27.3	20.5	0.38	0.35	0.26	0.30	Pink 2+
IV	Fat Content								
	20%	24.7	23.0	17.4	0.39	0.35	0.26	0.46	Reddish pink
	30%	28.8	27.0	21.2	0.37	0.35	0.28	0.25	Pink 1+
	35%	27.8	26.0	19.5	0.38	0.35	0.27	0.28	Pink 1+

[a]T1 represents cooking in 68°C water to an internal temperature of 63°C; T2 represents cooking in 74°C water to an internal temperature of 68°C; T3 represents cooking in 79°C water to an internal temperature of 74°C; and T4 represents cooking in 85°C water to an internal temperature of 74°C.

Table 19. Simple correlation coefficients of tristimulus values with panel visual evaluation scores

	Y	Z	Visual Evaluation Score
X	0.96*	0.91*	-0.74*
Y		0.91*	-0.78*
Z			-0.79*

*Significant $P < 0.05$.

average fat content of 24.9% with a range of 22.6 to 25.8% and an average salt content of 2.24% with a range of 2.15 to 2.33%. During the preparation of the raw materials, an Anyl-Ray fat analyzer was used to determine the fat content in the pork trim (50/50) to assure the desired level of fat in the finished product. The fat content of the pork trim (50/50) purchased from the meat industry varied from 30% to 70%.

The average salt content of the finished product in Study IV was 2.86% with a range of 2.81 to 2.92%. The salt content of the products in Study IV was higher than that in the first three studies; the product made in the first three studies was formulated using recommended levels of a commercially available salt-spice mixture. For the last study, the product was formulated using both commercially available salt-spice mixture plus additional amounts of salt. Therefore, a difference in the salt content resulted.

Table 20. Composition of Braunschweiger liver sausage manufactured in the different studies

Braunschweiger liver sausage in		%			
Study	Variable	H_2O	Fat	Salt	Brine[a] Concentration
I	Liver				
	Frozen-thawed	55.0	24.9	2.15	2.69
	Fresh	55.0	24.5	2.20	2.76
	Fresh-scalded	55.0	24.7	2.24	2.81
	Fresh-soaked	57.0	22.6	2.31	2.90
II	Nitrite				
	0 ppm	55.5	23.2	2.29	2.91
	50 ppm	55.7	23.4	2.20	2.78
	100 ppm	55.0	24.2	2.28	2.88
	156 ppm	55.5	23.3	2.24	2.84
III	Processing Temperature[b]				
	T1	53.7	25.2	2.19	2.78
	T2	53.0	25.8	2.20	2.79
	T3	52.9	25.6	2.33	2.97
	T4	55.1	23.4	2.28	2.90
IV	Fat Content				
	20%	57.5	19.5	2.81	3.65
	30%	48.7	30.0	2.92	3.71
	35%	44.7	35.4	2.85	3.56

[a]Brine concentration calculated as $= \frac{\%\text{ salt in sausage}}{\%\text{ water in sausage} + \%\text{ salt}}$.

[b]T1 refers to cooking at 68°C water until an internal temperature of 63°C; T2 refers to cooking at 74°C water until an internal temperature of 68°C; T3 refers to cooking at 79°C water until an internal temperature of 74°C; and T4 refers to cooking at 85°C water until an internal temperature of 74°C.

An increase in fat content in raw materials resulted in a decrease of water content in the finished products (Figure 4). This can be explained by the fact that pork fat has a water content of about 14% (Brouwer et al., 1976; Carpenter et al., 1977). In contrast to that, lean meat contains about 70% water (Watt and Merrill, 1963). Examining the results in Figure 4, the relationship between water content and fat content in the Braunschweiger liver sausage (made of pork liver:pork = 1:1) shows a linear trend. This linear curve could serve as a simple, useful reference for the quality control of the finished products.

2. Residual nitrite

The residual nitrite contents in the raw mixture and cooked Braunschweiger liver sausage formulated with 156 ppm nitrite and stored for 1/4, 1, 2, and 3 days at 5°C are presented in Figure 5. The results show that there is a drastic reduction of added nitrite as a result of contact with the raw mixture. The raw mixture contained pork liver, pork trim, salt-spice mixture and char oil. The residual nitrite content of the raw mixture, which was collected after chopping and after stuffing and storaged at 5°C for 6 hr, demonstrated that approximately 80% of the added nitrite was lost. The raw mixture after chopping was collected in a screw-capped jar and stored at 5°C, while the mixture after stuffing was stuffed into an MP (moisture-proof) casing and stored at 5°C. The cooked product was

Figure 4. Composition of Braunschweiger liver sausage containing different levels of fat. (Each point represents the average of two samples from one replication)

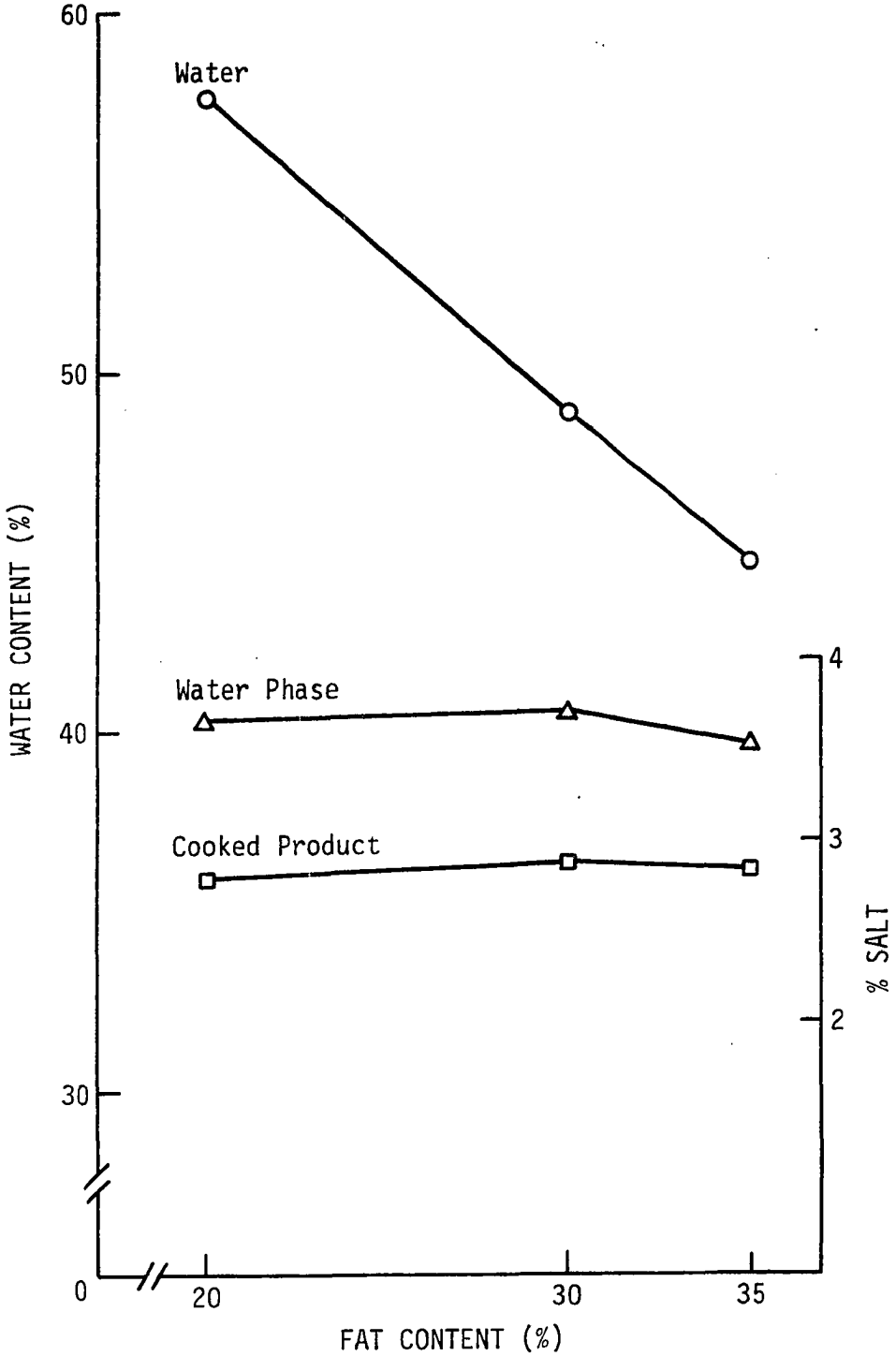
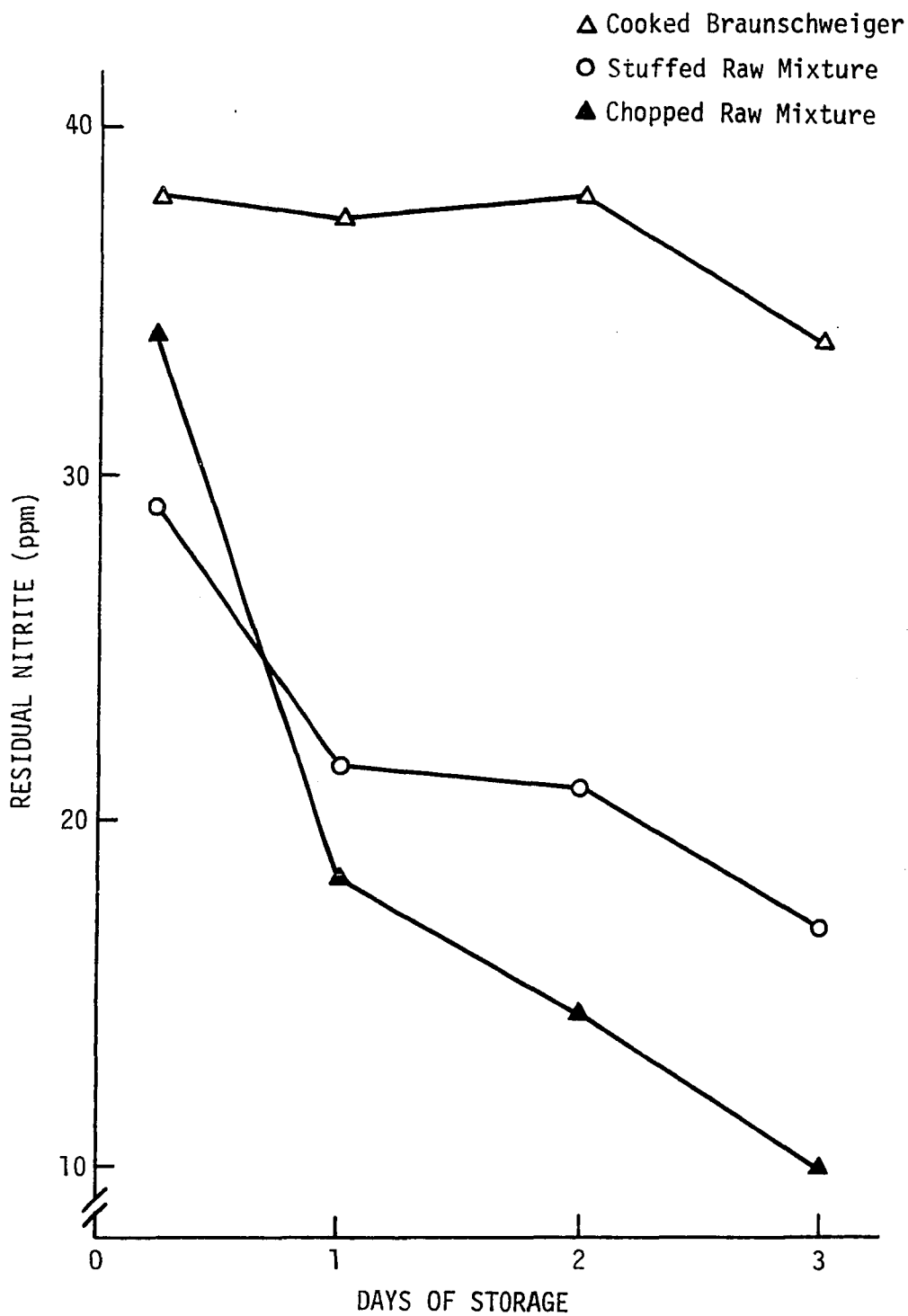

60
50
40
30
0
Water
Water Phase
Cooked Product
WATER CONTENT (%)
4
3
2
% SALT
20
30
35
FAT CONTENT (%)

Figure 5. Nitrite depletion in chopped, stuffed raw mixtures and cooked Braunschweiger liver sausage prepared with 156 ppm nitrite and stored at 5°C. (Each point represents the average of three replications. Each replication contained two samples for a total of six samples)


△ Cooked Braunschweiger
○ Stuffed Raw Mixture
▲ Chopped Raw Mixture
RESIDUAL NITRITE (ppm)
40
30
20
10
0
1
2
3
DAYS OF STORAGE

stuffed into MP casings, vacuum packaged with Fresh-tuff film then cooked in 74°C water to an internal temperature of 68°C and stored at 5°C for 6 hr before the nitrite determination was made. About 76% of the added nitrite was lost in the cooked product. The difference between residual nitrite content in the raw mixture and the cooked product was not significant. However, during storage for 1, 2 and 3 days, the raw mixture had a faster depletion of residual nitrite as compared to that of the cooked product. Therefore, the raw mixture had a much lower level of residual nitrite content than the cooked product.

The depletion of added nitrite in raw meat has been reported to be about 25% to 50% after formulation (Greenberg, 1972). The best elucidated reaction of nitrite has been that with meat pigments and this can account for about 10% of the nitrite (Sebranek et al., 1973). Thus a significant amount of added nitrite is converted to undetectable forms. Nitrite is a very reactive ion. When it is added to a complex biological system such as meat, it has the ability to participate in a wide variety of chemical reactions (Bard and Townsend, 1971). Many postulated possibilities have been proposed such as the reaction with α-amino acids (Bard and Townsend, 1971); the formation of nitric oxide and nitrous oxide (Sebranek et al., 1973); reactions with tyrosine (Knowles et al., 1974), SH groups (Mirna and Hofmann, 1969), sorbic acid (Nagata and Ando, 1971), ascorbic acid

(Mirna, 1972), sugars (ten Cate, 1963 a, b) and secondary amines (ten Cate, 1963a, b). None of these reactions have been evaluated quantitatively, however.

Our results show that the degree of nitrite loss in Braunschweiger liver sausage raw mixture is much higher than in other raw meat products. The possible reason for such a low recovery (20%) of added nitrite in Braunschweiger liver sausage raw mixture could be due to the liver components. Liver differs from muscle in many aspects (Shelef, 1975). The higher content of carbohydrate, hemoglobin, vitamins and minerals in liver could result in a more complicated, wider variety of chemical reactions by nitrite in the product. The lower residual nitrite content in the raw mixture after storage at 5°C for 3 days compared to that of the cooked product was also observed in Braunschweiger liver sausage formulated with 50 or 100 ppm nitrite (Figure 6).

An increase in the added nitrite resulted in a higher residual nitrite content in the cooked Braunschweiger liver sausage (Figure 7). Similar findings were reported for ham products by Greenberg (1972). The decrease of residual nitrite in cooked Braunschweiger liver sausage under refrigeration between the 11th day and 33rd day was not significant in the products formulated with 156 ppm nitrite and almost no change in the products formulated with 50 or 100 ppm nitrite.

Figure 6. Residual nitrite in Braunschweiger liver sausage at different processing stages and prepared with different levels of nitrite. (Each bar represents the average of two replications. Each replication contained two samples for a total of four samples)

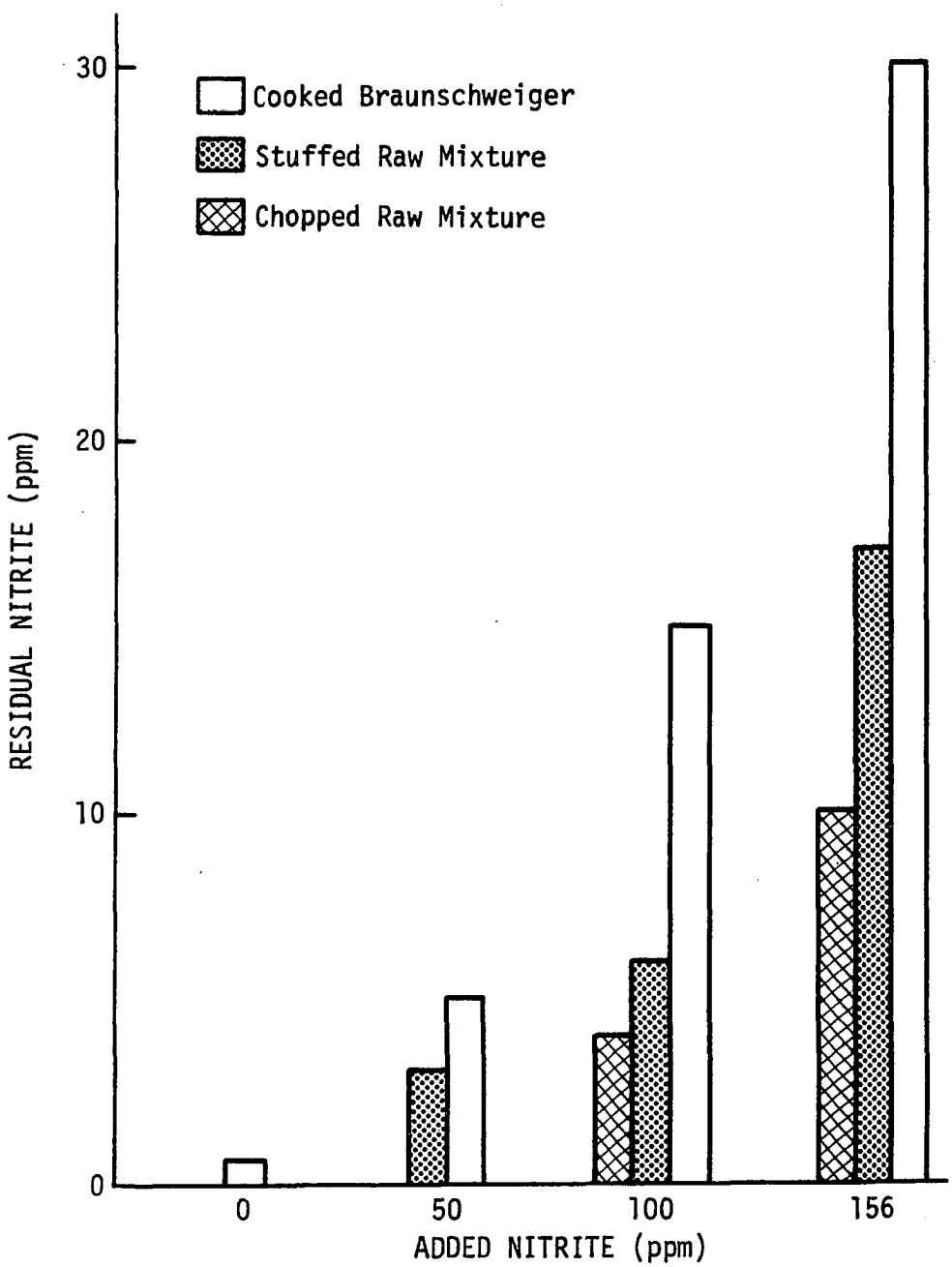
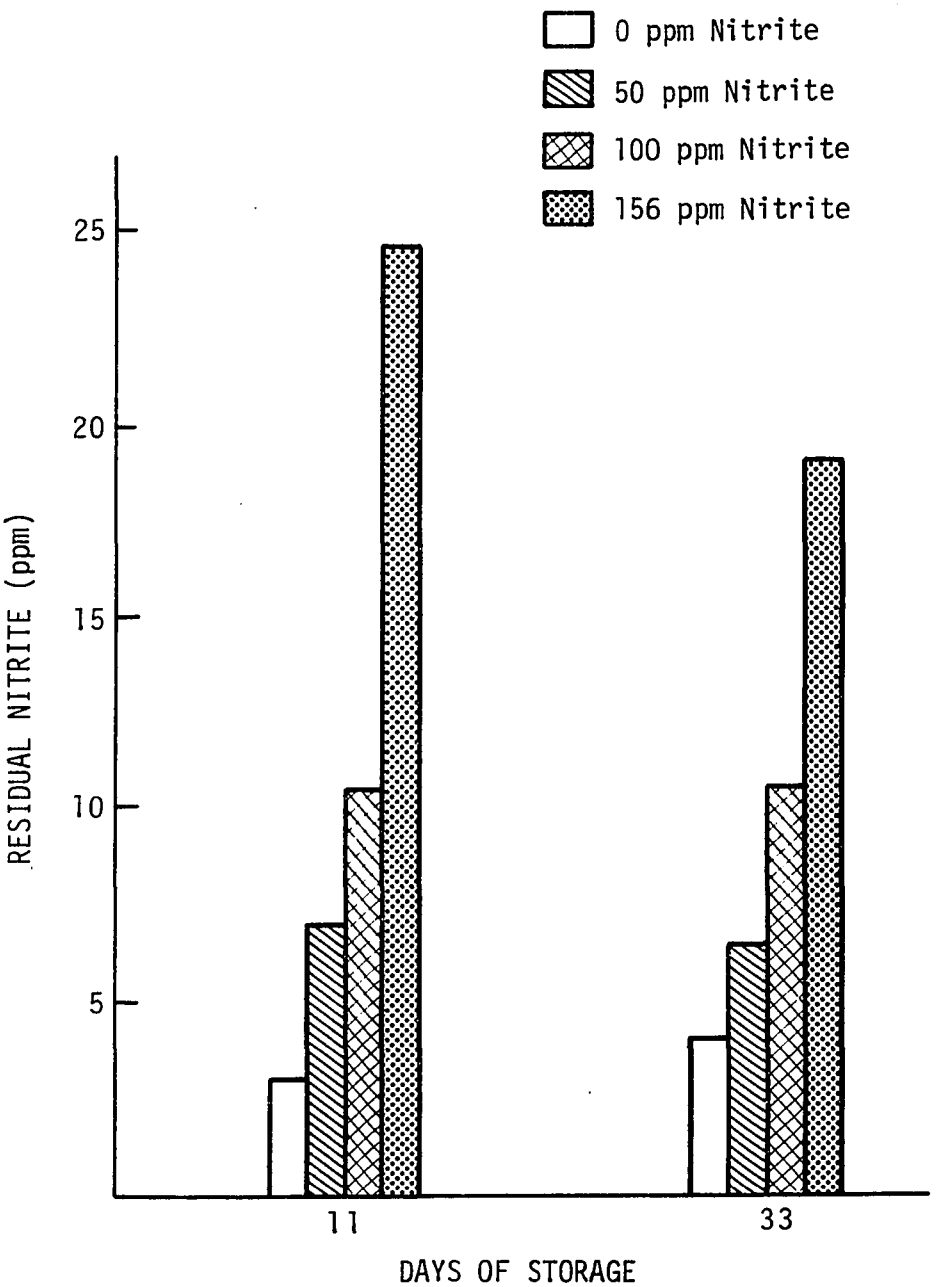

30
20
10
0
RESIDUAL NITRITE (ppm)
Cooked Braunschweiger
Stuffed Raw Mixture
Chopped Raw Mixture
0
50
100
156
ADDED NITRITE (ppm)

Figure 7. Residual nitrite in cooked Braunschweiger liver sausage prepared with different levels of nitrite and stored at 5°C. (Each bar represents the average of two replications. Each replication contained two samples for a total of four samples)


0 ppm Nitrite
50 ppm Nitrite
100 ppm Nitrite
156 ppm Nitrite
RESIDUAL NITRITE (ppm)
25
20
15
10
5
11
33
DAYS OF STORAGE

The degree of nitrite loss among the samples containing different fat levels varied little as a result of cooking (Figure 8). In fact, approximately an 80% loss of the analyzable nitrite occurred during cooking. Nitrite levels decreased slowly during refrigeration of cooked samples. The reduction rate in the early stages of storage was faster than during the later period. After refrigeration for 100 days, the residual nitrite content in the sample was about 50% of that in the freshly cooked sample.

3. TBA values

Cooked Braunschweiger liver sausages prepared to contain different fat contents were sliced into pieces of 1 cm thickness, vacuum packaged in transparent Fresh-tuff bags using a Multivac pouch machine. Samples were stored in an open-top display case at 7°C under a soft white fluorescent light. The effect of 3 different fat contents on TBA (thiobarbituric acid) numbers are shown in Table 21. The results indicate that increasing the fat content from 20% to 35% in Braunschweiger liver sausage did not make any significant difference in the TBA numbers of the products. During 33 days of refrigeration, no increase in TBA numbers occurred in any of the vacuum-packaged samples.

Vacuum packaged samples stored at 22°C for 5 days showed no sign of change in TBA value, residual nitrite, pH, odor and color (Table 22). However, growth of indigenous microorganisms caused spoilage of the

Figure 8. Nitrite depletion in cooked Braunschweiger liver sausage prepared with 156 ppm nitrite but containing different levels of fat. (Each point on the curve of 25% fat represents the average of six replications. Each replication contained two samples for a total of twelve samples. Each point on the curves of 20, 30 and 35% fat represents the average of two samples from one replication)

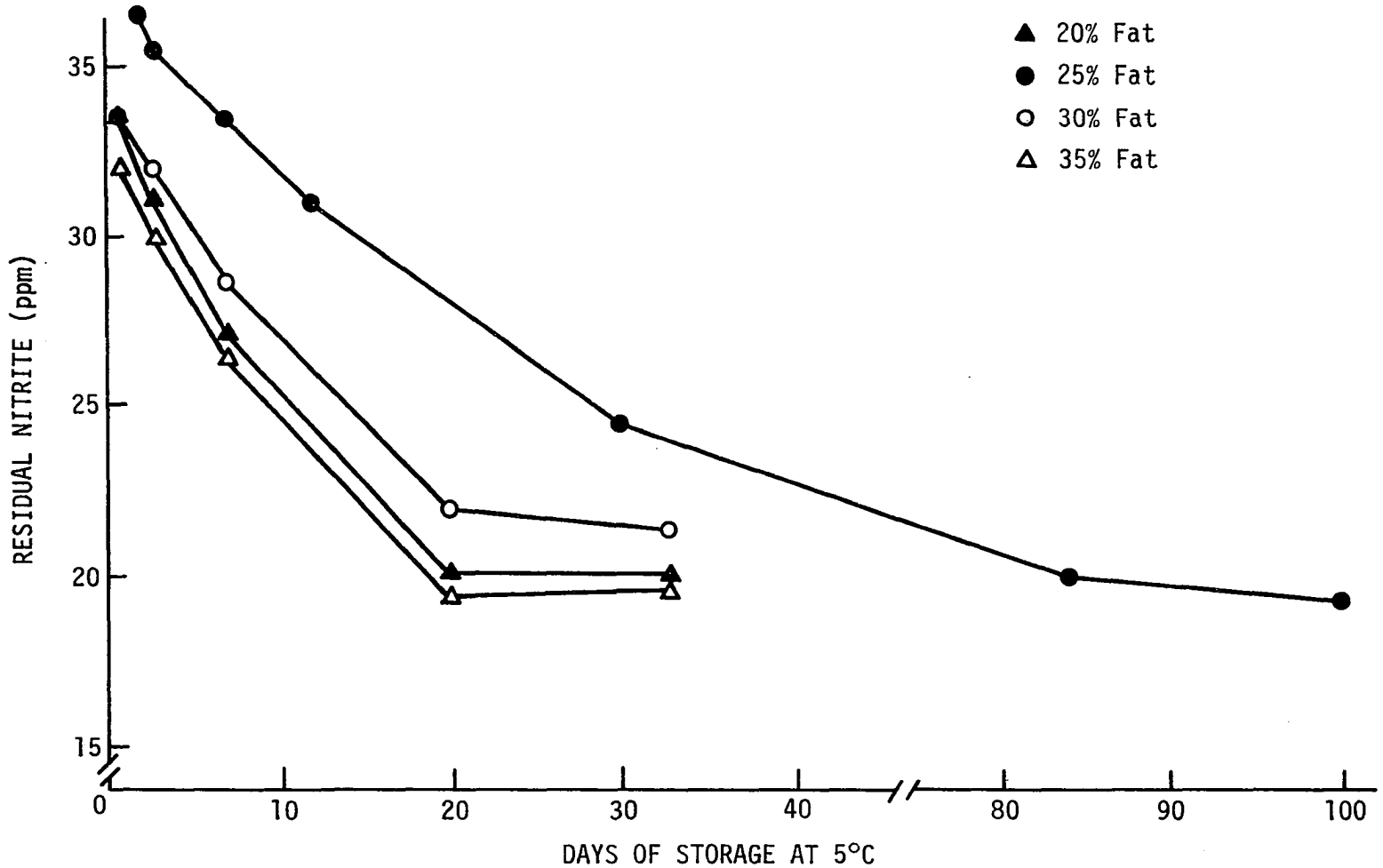

▲ 20% Fat
● 25% Fat
○ 30% Fat
△ 35% Fat
RESIDUAL NITRITE (ppm)
35
30
25
20
15
0
10
20
30
40
80
90
100
DAYS OF STORAGE AT 5°C

Table 21. Changes in TBA numbers[a] of vacuum-packaged Braunschweiger liver sausages under refrigeration

Storage Time (days)	Types of Braunschweiger Liver Sausage		
	20% fat	30% fat	35% fat
0	0.30	0.32	0.27
5	0.37	0.23	0.23
15	0.24	0.23	0.24
33	0.23	0.30	0.23

[a] mg of malonaldehyde in 1000 g of sample.

sample. This microbial spoilage could not be detected by visible means.

The effect of 3 different packaging materials on TBA numbers are presented in Table 23. Samples were vacuum-packed, Saran wrapped or heat sealed in LSAD cellophane pouches (a moisture and oxygen permeable film). The latter two types of packaging materials are commonly used to wrap Braunschweiger liver sausage in super markets or at home. The results indicate that after refrigeration for 5 days, samples of liver sausage packaged in different films varied little in TBA values; color differences among the samples were evident, however. Vacuum-packaged samples maintained a pinkish color; Saran wrapped samples had an uneven color, a tan color on the edge

Table 22. TBA number[a], residual nitrite, pH, odor and microbial content of vacuum-packaged Braunschweiger liver sausage[b] stored at 22°C

Days of Storage	Measurement	Types of Sample		
		20% fat	30% fat	35% fat
0 day	TBA number	.23	.30	.23
	Residual Nitrite (ppm)	21.50	21.40	19.40
	pH	6.37	6.43	6.41
	Odor	Typical	Typical	Typical
	Anaerobic Mesophiles (No. of microorganisms/g)	2.1×10^3	3.4×10^3	1.9×10^3
5 day	TBA number	.33	.23	.29
	Residual Nitrite (ppm)	21.80	21.30	20.30
	pH	6.38	6.45	6.43
	Odor	Typical	Typical	Typical
	Anaerobic Mesophiles (No. of microorganisms/g)	3.2×10^7	2.5×10^7	6.8×10^6

[a]mg of malonaldehyde in 1000 g of sample.

[b]Prepared with 156 ppm nitrite.

Table 23. Change in TBA numbers[a] of Braunschweiger liver sausages wrapped in different packaging materials and held at 7°C

Types of Sample	Packaging[b] Materials	Storage Time (days) 0	5	15
20% fat	VP	.37	.29	.24
	SR	.30	.29	.46
	LS	.32	.23	.65
30% fat	VP	.23	.23	.23
	SR	.25	.29	.60
	LS	.28	.38	.67
35% fat	VP	.23	.23	.65
	SR	.27	.31	1.00
	LS	.24	.39	1.22

[a] mg of malonaldehyde in 1000 g of sample.

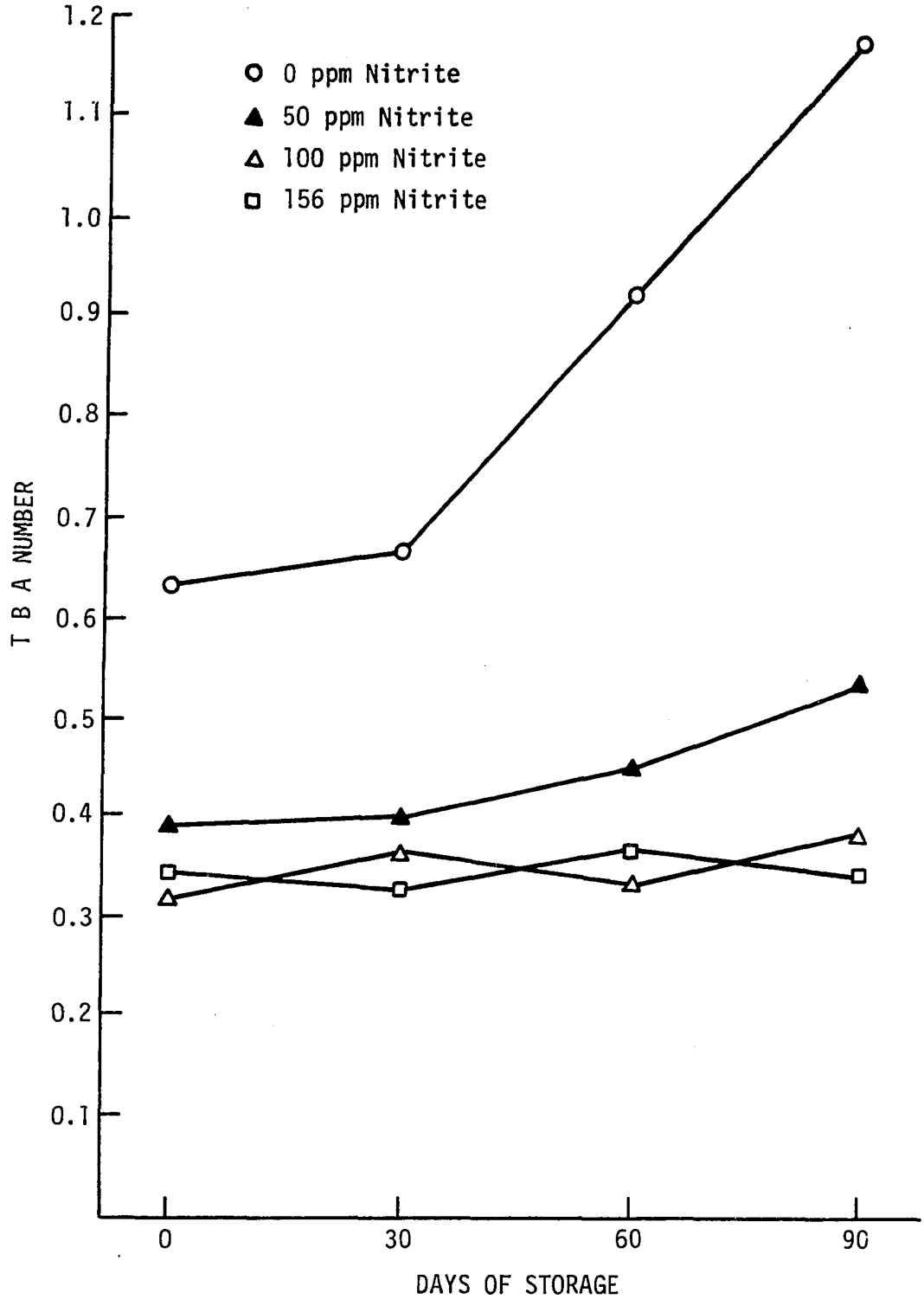
[b] VP, vacuum-packaged; SR, Saran wrapping; LS, LSAD pouch.

and a pinkish color at the center of the surface; the samples in the LSAD film had a tan color. However, after 15 days of refrigeration, samples packed in Saran and LSAD film had a significant increase in TBA number and a rancid odor was noticed. Also, samples containing higher fat (30 or 35%) had a greater increase in TBA number compared to those containing 20% fat. These results indicate that lipid oxidation, the oxidative decomposition of unsaturated fatty acid, resulting in rancidity and off-flavor in the Braunschweiger liver sausage was a function of storage time, packaging material, fat content and temperature of storage. The characteristic pinkish color, absence of a rancid odor and low

TBA number associated with the vacuum-packaged samples indicates that vacuum packaging of cured meat products will extend the shelf life of the product.

TBA numbers of freshly cooked Braunschweiger liver sausage initially containing 156 ppm nitrite, in general, were in the range of 0.21 to 0.37. Pretreatment of the liver and cooking temperatures had no influence on TBA numbers in the products. Nitrite content had considerable influence on the TBA number of the Braunschweiger liver sausage (Figure 9). Freshly cooked Braunschweiger liver sausages prepared with 0, 50, 100 and 156 ppm nitrite had a TBA number of 0.62, 0.39, 0.34 and 0.37, respectively. These results agree with the report of Younathan and Watts (1959). They reported that uncured cooked meat had a higher TBA number than cured cooked meat shortly after cooking. They suggested that the ferric form of the meat pigment is the active catalyst in meat rancidity. A pale, pinkish color was associated with liver sausage without nitrite cure. Addition of nitrite at levels of 50, 100, 156 ppm produced a desirable pinkish color that did not vary for the different levels. After 2 months of refrigeration, a slightly greenish color at the meat-casing interface was observed in Braunschweiger liver sausage without nitrite and with 50 ppm nitrite. However, there was no color change in those sample cured with 100 ppm or 156 ppm nitrite after 3 months refrigeration. The difference in TBA

Figure 9. Change in TBA number with storage time of Braunschweiger liver sausages prepared with different nitrite levels, vacuum packaged and stored at 7°C. (Each point represents the average of two replications. Each replication contained two samples for a total of four samples)


1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
T B A NUMBER
0 ppm Nitrite
50 ppm Nitrite
100 ppm Nitrite
156 ppm Nitrite
0
30
60
90
DAYS OF STORAGE

number between fresh, noncured and cured Braunschweiger liver sausage demonstrated the antioxidant effect of nitrite. Furthermore, the dark-green color at the meat-casing interface, the drastic increase of TBA numbers and the evident rancid odor were associated with the samples without nitrite or with 50 ppm nitrite cure after 3 months refrigeration. Evidently, the time factor magnifies the antioxidant effect of the nitrite cure.

4. pH values and water activity (A_w)

The pH values and water activity (A_w) of Braunschweiger liver sausages manufactured in the different studies are presented in Table 24. The results of Study I show that raw or cooked Braunschweiger liver sausage containing frozen-thawed liver had slightly higher pH and A_w value than those of the others. Liver pretreatments such as scalding or soaking did not influence the pH and A_w values of the sample. Samples containing different levels of nitrite had almost the same pH and A_w value. Processing temperatures had no effect on the pH and A_w of the samples.

Freezing of meat causes some denaturation of proteins including a decrease in solubility of sarcoplasmic proteins (Lawrie, 1968), a decrease in titratable SH groups (Khan and van den Berg, 1964) and a pH increment (van den Berg, 1961). Therefore, the higher pH and A_w values of Braunschweiger liver sausage using frozen-thawed liver could be explained on the basis of denaturation of certain liver proteins.

Table 24. pH values and water activity (A_w) of Braunschweiger liver sausages manufactured in different studies

Braunschweiger liver sausage in Study	Variables	pH		A_w of Cooked Sausage
		Raw Mixture	Cooked Sausage	
I[a]	Liver			
	Frozen-thawed	6.08	6.32	0.97
	Fresh	5.98	6.20	0.95
	Fresh-scalded	5.99	6.19	0.95
	Fresh-soaked	5.96	6.21	0.95
II[a]	Nitrite			
	0 ppm	5.95	6.15	0.97
	50 ppm	5.98	6.19	0.97
	100 ppm	5.98	6.18	0.97
	156 ppm	5.98	6.21	0.97
III[a]	Processing Temperature[b]			
	T1	5.94	6.20	0.96
	T2	5.94	6.20	0.95
	T3	5.94	6.20	0.96
	T4	5.94	6.20	0.96

III[c]	Processing Temperature[b]			
	T1	6.04	6.36	0.95
	T2	6.04	6.34	0.94
	T3	6.04	6.37	0.95
	T4	6.04	6.37	0.95
IV[c]	Fat Content			
	20%	6.17	6.42	0.97
	30%	6.24	6.50	0.96
	35%	6.25	6.50	0.95

[a]Without calcium-reduced dried skim milk.

[b]T1 refers to cooking at 68°C water until an internal temperature of 63°C; T2 refers to cooking at 74°C water until an internal temperature of 68°C; T3 refers to cooking at 79°C water until an internal temperature of 74°C; and T4 refers to cooking at 85°C water until an internal temperature of 74°C.

[c]With calcium-reduced dried skim milk.

Comparison of the data in Studies I, II, and III with the exception of data for frozen-thawed liver, indicates that little variation occurred in pH and A_w values in Braunschweiger liver sausage made with the same formulation (Table 8) but in different lots. The average pH for the raw batter was 5.96 with a range of 5.94 to 5.99; for the cooked product, the average pH was 6.19 with a range of 6.15 to 6.21. The average A_w for the cooked sausages was 0.96 with a range of 0.95 to 0.97. When the formulation was changed by addition of 3.5% calcium-reduced dried skim milk (Savortex) the pH increased to 6.04 for the raw batter and 6.36 for the cooked product; A_w decreased to 0.95. Samples containing 3.5% CRDSM and a salt level of 2.86% had much higher pH values.

Cooking, in general, resulted in an increase in pH. Similar findings were reported by Fox et al. (1967). They reported that the pH changes in frankfurters during cooking rose from an initial value of 5.45 to a constant value of 5.90 when cooked to an internal temperature of 65.6°C (150°F). Hamm and Deatherage (1960) determined the acidic and basic groups in muscle protein after heating at different temperatures. They concluded that there was little or no change in basic groups but a stepwise decrease of the acidic groups of muscle protein during cooking resulted in an increase of pH. However, Forrest et al. (1975) stated that when muscle proteins are subjected to heat a slight upward shift in pH (approximately 0.3 unit) is believed to result from the

exposure of a reactive group on the amino acid, histidine.

The addition of 3.5% CRDSM resulted in an increase of pH in the raw batter and cooked sausage. This observation agreed with the report of Rongey and Bratzler (1966). They found that including 3.5% dried skim milk in the formulation of bologna increased the pH of the finished product from 6.2 to 6.4.

Water activity (A_w) is a measure of free water in a multicomponent system (Corry, 1973). When the water in a system becomes associated with solute molecules in the system, less water is available for chemical reactions necessary to support life. Our results show practically no decrease in A_w in Braunschweiger liver sausage when the formulation (Table 8) contained 3.5% CRDSM. Dried skim milk is composed of 51% lactose (Kramlich, 1971). Lactose can act as a humectant in food systems (Bone, 1973). On the other hand, the decrease in A_w in the high fat content samples would be associated with the decrease in water content (Table 20).

C. Microbiological Study

1. Microbial content and flora

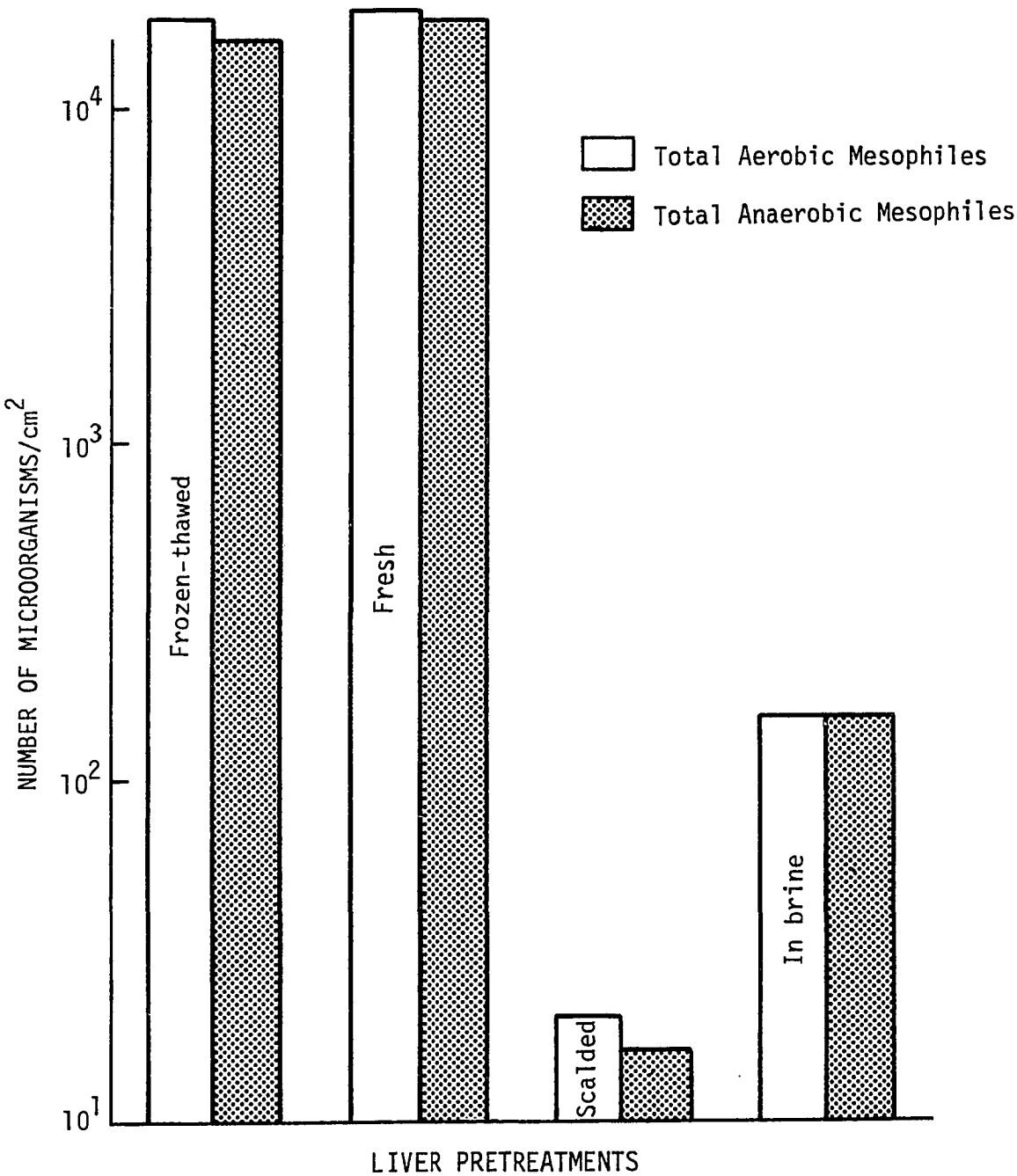
a. Influence of liver pretreatments The effect of pretreatment of livers on the microbial reduction on the surface of raw livers was determined

by swabbing a known surface area. The results are shown in Figure 10. The surface of the fresh liver had a count of 2.8×10^4 total aerobic mesophiles/ cm^2. A survey of these organisms revealed that the microflora consisted of enterococci, Lactobacillus, Pediococcus, Bacillus, Micrococcus and coryneform bacteria. The total aerobic mesophilic count was slightly higher than the total anaerobic count. The microbial content and microflora on the surface of fresh pork liver are comparable to the findings of Gardner (1971). He reported that the surface of fresh pork liver harbored 6.4×10^4 aerobes/ cm^2 and the microflora consisted of Micrococcus, Flavobacterium, coryneform bacteria, Acinetobacter, lactic streptococci and Leuconostoc. Also, Shelef (1975) recovered low numbers of aerobes from ground beef liver (about 1.0×10^5 aerobes/g). Gram positive cocci, lactic acid bacteria, enterococci and Gram negative rods were the main isolates in this instance.

Comparison of the microbial loads on frozen-thawed liver to fresh liver showed that freezing and thawing influenced the count very little. Soaking in brine and washing of the liver resulted in a reduction of almost one log cycle on the initial microbial load. The scalding treatment was found to be the most efficient pretreatment for reducing the microbial load on the surface of raw liver.

At different stages of manufacture, the pH values and bacterial counts of the raw mixture using liver exposed to different pretreatments were

Figure 10. Effect of liver pretreatments on the microbial load of raw liver. (Each bar represents the mean of two replications. Each replication contained three samples for a total of six samples)


Total Aerobic Mesophiles
Total Anaerobic Mesophiles
NUMBER OF MICROORGANISMS/cm2
10^4
10^3
10^2
10^1
Frozen-thawed
Fresh
Scalded
In brine
LIVER PRETREATMENTS

determined. Raw mixtures containing frozen-thawed liver had a slightly higher pH value (0.1 unit) when compared to that of the others (Table 25). The microbial count of the stuffed raw mixtures containing different livers exposed to different pretreatments varied little. The highest total anaerobic mesophilic count was 7.2×10^4 organisms/g for the sample containing fresh liver while the lowest total anaerobic mesophilic count was 1.5×10^4 organisms/g for the sample containing fresh-scalded liver. These results were not compatible with the previous findings indicating an efficient microbial reduction on the surface of scalded liver. In the Braunschweiger liver sausage formulation (Table 8), the main ingredients which would affect the microbial load of the final product were pork liver, pork trim and spice-salt mixture. The pork trim, which constitutes 50% of the sausage ingredients, had a total anaerobic mesophilic count of 2.4×10^3 organisms/g and was judged to be of good microbial quality. The spice-salt mixture, which is added at a level of 3.3% (w/w) in the formulation, contained a total anaerobic mesophilic count of 1.5×10^5 organisms/g and is probably one of the causes of the increased number of microorganism in the raw mixture containing scalded liver. All the microorganisms isolated from the spice-salt mixture belonged to the genus Bacillus.

Another possible source of high numbers in the raw mixture containing scalded liver could be due to the growth of microbes in the deep tissue

Table 25. pH and microbial counts of the raw mixture containing liver exposed to different pretreatments[a]

Liver type	Raw mixture sampled at manufacturing stage after	Total aerobic mesophiles (No. of organisms/g)	Total anaerobic mesophiles (No. of organisms/g)	pH
Frozen-thawed	Chopping	3.0×10^4	3.0×10^4	6.07
	Stuffing	3.7×10^4	2.5×10^4	6.09
Fresh	Chopping	8.9×10^4	9.3×10^4	5.98
	Stuffing	7.5×10^4	7.2×10^4	5.98
Fresh-scalded	Chopping	2.2×10^4	1.6×10^4	5.99
	Stuffing	2.4×10^4	1.5×10^4	5.98
Fresh-soaked	Chopping	4.4×10^4	5.2×10^4	5.97
	Stuffing	3.6×10^4	2.6×10^4	5.95

[a]Each result is the mean of 2 replications. Each replication contained 3 samples for a total of 6 samples examined.

of scalded liver. After the scalding treatment of 93°C for 2 min, the internal temperature of the scalded liver was found to be in the range of 50-60°C; it required 8 to 9 hr for such liver to cool to 10°C when held in a plastic container at 0°C. This slow chilling process would maintain a temperature zone favorable for growth of any microorganism in the tissues. Gardner (1971) used a technique (Gardner and Carson, 1967) which prevented any transfer of contamination from the surface of fresh pork liver to examine

the numbers and types of microorganisms that might be present in the deep tissues. He reported that the deep tissue of fresh pork liver contained about two hundred microorganisms per gram and the microflora consisted of Flavobacterium, coryneform bacteria, Micrococcus, lactic streptococci and Leuconostoc. He also found that after refrigeration at 5°C for 7 days, both lactic streptococci and Leuconostoc grew well (about 5.6×10^6 organisms/g) and predominated in the deep tissue of the liver.

Cooked Braunschweiger liver sausage had pH values in the range of 6.19 to 6.32 (Table 26). Cooking usually resulted in an approximate increase

Table 26. pH of Braunschweiger liver sausage before cooking and after cooking[a] and storage at 5°C

Liver Type	Type of Braunschweiger				
	Before Cooking[b]	Cooked and after storage for			
		2 wk	5 wk	10 wk	16 wk
Frozen-thawed	6.08[c]	6.32	6.34	6.44	6.3
Fresh	5.98	6.20	6.23	6.37	6.21
Fresh-scalded	5.99	6.19	6.20	6.32	6.23
Fresh-soaked	5.96	6.20	6.21	6.35	6.25

[a]Cooked in water at 74°C to an internal temperature of 68°C.

[b]Raw mixture refers to the raw emulsion after being stuffed into casings and prior to cooking.

[c]Each result is the mean of 2 replications. Each replication contained 3 samples for a total of 6 samples examined.

in pH of 0.2 unit. During storage for 2 to 10 wk, the pH values tended to increase. Since cooking destroys activities of proteolytic enzymes in meat proteins (Khan and van den Berg, 1964) and our results show no sign of microbial growth in the products, the increase of pH during this refrigeration period is interpreted as a result of chemical changes in the product. After 16 wk of storage the pH values of samples were lower than those observed after 10 wk but higher than those after 2 wk and 5 wk. The decrease in pH during 10 to 16 wk could be due to the growth and production of lactic acid by enterococci in the sample containing scalded liver.

Comparison of the microbial load of the Braunschweiger before and after cooking showed that cooking in water at 74°C to an internal temperature of 68°C reduced the microbial population by more than 90% (Table 27). All cooked Braunschweiger, stored at 5°C for 2 wk, had a total anaerobic count in the range of 1.1×10^3 organisms/g to 2.2×10^3 organisms/g. Identification of the isolates from total anaerobic plates indicated that facultative Bacillus (< 20%) were the main survivors in freshly made Braunschweiger. These numbers were slightly lower than the total aerobic mesophilic count (Table 27). Identification of the isolates from aerobic plates indicated that the difference between the total aerobic mesophiles and total anaerobic mesophiles was mainly due to the inability of the aerobic Bacillus spp. and Micrococcus spp. to grow under anaerobic conditions. Aerobic bacilli and micrococci

Table 27. The effect of cooking on microbial numbers in Braunschweiger liver sausage containing different types of liver[a]

Liver type	Raw mixture[b] Total anaerobic mesophiles/g	Cooked sausage stored at 5°C for 2 wk		% microbial reduction
		Total aerobic mesophiles/g	Total anaerobic mesophiles/g	
Frozen-thawed	2.5×10^4	2.6×10^3	2.2×10^3	91.2
Fresh	7.2×10^4	2.5×10^3	1.5×10^3	98.0
Fresh-scalded	1.5×10^4	2.2×10^3	1.4×10^3	90.7
Fresh-soaked	2.6×10^4	1.7×10^3	1.1×10^3	95.8

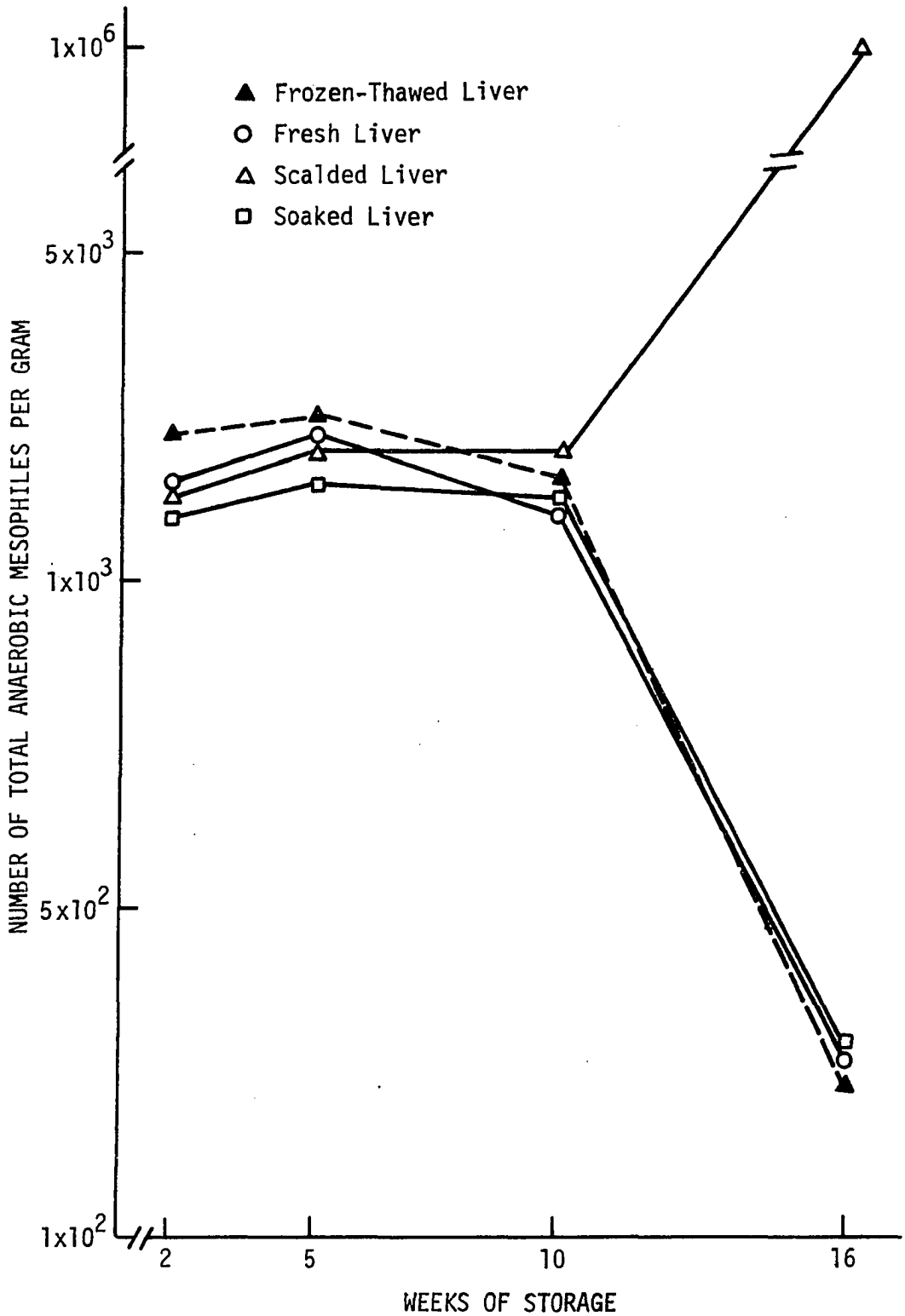
[a]Cooked in water at 74°C to an internal temperature of 68°C.

[b]Raw mixture refers to the raw emulsion after being stuffed into the casings and prior to cooking.

previously have been reported as being unable to grow in liver sausage stored for 12 wk at 5°C (Steinke and Foster, 1951a). For this reason, in the latter part of the study on refrigerated storage only the total anaerobic mesophilic count was recorded.

Little change occurred in the numbers of anaerobic mesophiles during storage for 2 to 10 wk at 5°C (Figure 11). After 16 wk of storage, all samples except those containing scalded liver had a much lower count compared to those of previous time intervals. Evidently no growth and/or the death of the

Figure 11. Change in microbial load of Braunschweiger liver sausage containing liver exposed to different pretreatments and stored at 5°C. (Each point is the mean of two replications. Each replication contained three samples for a total of six samples)


1x10^6
5x10^3
1x10^3
5x10^2
1x10^2
Frozen-Thawed Liver
Fresh Liver
Scalded Liver
Soaked Liver
NUMBER OF TOTAL ANAEROBIC MESOPHILES PER GRAM
2
5
10
16
WEEKS OF STORAGE

surviving microorganisms (Bacillus and enterococci) in the Braunschweiger liver sausage occurred during long term storage at 5°C. Samples containing scalded liver showed growth of surviving enterococci during the 10th wk to 16th wk (Table 28). In each of the two replications, two out of three samples had a total anaerobic mesophilic count of 3.9×10^5 and 5.3×10^6/g; the other sample showed no growth of surviving organisms. A similar observation was reported by Steinke and Foster (1951a). They assumed that the variability was due to the nonhomogeneous distribution of the organisms in the freshly made sausage, which was magnified after growth occurred.

Our results, in general, show some similarity with the findings of Steinke and Foster (1951a, 1951c). They reported that within a single lot of sausage the distribution of anaerobic organisms was reasonably uniform not only in individual sausages but also between sausages. The numbers of anaerobic organisms in freshly made liver sausage were uniformly low (in hundreds) in all of the lots of liver sausage examined (Steinke and Foster, 1951a). They also reported that all of the anaerobic organisms were unable to grow in liver sausage held at 5°C (1951c); however, they did not identify the anaerobes. They observed that the aerobic flora in liver sausage consisted of Bacillus and Micrococcus which were unable to grow at 5°C; the spoilage organisms were Gram positive, catalase negative microaerophilic rods. Our results (Table 27) showed that freshly made

Table 28. Numbers of enterococci per gram of Braunschweiger liver sausage containing different types of liver stored at 5°C

Liver type	Replication[a]	Storage in Weeks		
		5	10	16
Frozen-thawed	A	1.4×10^2	< 50	< 50
	B	7.5×10^1	1.0×10^2	< 50
Fresh	A	3.5×10^2	< 50	< 50
	B	5.0×10^1	2.0×10^2	< 50
Fresh-scalded	A	5.6×10^2	7.0×10^2	3.9×10^5 [b]
	B	3.3×10^2	2.0×10^2	5.3×10^6 [b]
Fresh-soaked	A	1.0×10^2	2.0×10^2	< 50
	B	< 50	< 50	< 50

[a]Each replication contained 3 samples.

[b]Average of 2 samples, the third sample contained < 50 enterococci/g.

liver sausage contained about 1.5×10^3 anaerobes per gram which consisted of facultative bacilli and enterococci. In the freshly made sausage, a close agreement between number of organisms in individual sausages from the same lot was observed. After 16 wk of storage at 5°C, all samples appeared normal and all the samples, except the samples containing scalded liver, showed no indication of microbial growth.

Growth in the samples containing scalded liver and not in the other samples is difficult to rationalize. One possibility is the growth of organisms

in the deep tissue of scalded liver before chopping; as was mentioned previously these scalded livers cooled very slowly during refrigeration. Another possibility is that the scalding treatment increased the internal temperature of the liver activating enzymatic reactions which resulted in products capable of combining with proteins and providing protection against thermal damage of organisms (Precht et al., 1955) or may aid in the reactivation of damaged cells (Hansen and Riemann, 1963).

b. *Influence of nitrite levels* Pork liver and pork trim used in Study II, which entailed the examination of the effect of nitrite levels on the quality of Braunschweiger liver sausage, contained a total anaerobic mesophilic count of 5.3×10^5 organisms/cm^2 and 9.0×10^3 organisms/g, respectively. The number of enterococci, determined by using KF medium, indicated that pork liver was the ingredient contaminated with high numbers of enterococci ($3.5 \times 10^3/cm^2$); pork trim contained about 30 enterococci/g. The various batches of raw mixture containing the different levels of nitrite contained about the same numbers of bacteria/g (Table 29).

During storage for 2 wk at 5°C, the different raw mixtures showed an increase in numbers of total anaerobic mesophiles and enterococci of one or more log cycles (Table 29). The growth of the microbes in the samples resulted in a slight decrease in pH value. Spoilage was not evident at

Table 29. pH and microbial count per gram of the raw mixture[a] stored at 5°C

Nitrite levels (ppm)	Determinations	Storage in Weeks		
		0	1	2
0	Anaerobic mesophiles	8.4×10^5	1.5×10^6	3.9×10^6
	Enterococci	4.1×10^4	1.2×10^5	5.1×10^5
	pH	5.95	5.94	5.92
50	Anaerobic mesophiles	7.9×10^5	4.9×10^6	1.6×10^7
	Enterococci	7.4×10^4	2.3×10^5	7.4×10^5
	pH	5.98	5.92	5.89
100	Anaerobic mesophiles	7.5×10^5	1.1×10^6	1.1×10^7
	Enterococci	5.1×10^4	2.2×10^5	9.7×10^5
	pH	5.98	5.94	5.88
156	Anaerobic mesophiles	1.5×10^5	6.5×10^5	1.7×10^6
	Enterococci	6.3×10^4	2.2×10^5	9.9×10^5
	pH	5.98	5.95	5.90

[a]Raw mixture refers to the raw emulsion after being stuffed into casings but previous to cooking.

the end of this time.

The microbial content of the cooked Braunschweiger liver sausage containing different levels of nitrite was determined after storage at 7°C for 1, 4, 8 and 12 wk in a display case. Although a much higher microbial content was found initially in the raw mixture of Study II (Table 30) than in

Table 30. Effect of cooking[a] and storage time on the pH and microbial content of Braunschweiger liver sausage using different nitrite levels in the formulation

Nitrite level (ppm)	Determination	Raw mixture[b]	Cooked sausage stored at 7°C for	
			1 week	4 weeks
0	pH	5.95	6.15	6.32
	Anaerobic mesophiles	8.4×10^5	3.7×10^3	3.6×10^3
	Enterococci	4.1×10^4	0-100	0-200
50	pH	5.98	6.19	6.32
	Anaerobic mesophiles	7.9×10^5	2.5×10^3	4.2×10^3
	Enterococci	7.4×10^4	0-50	0-100
100	pH	5.98	6.18	6.31
	Anaerobic mesophiles	7.5×10^5	2.9×10^3	3.0×10^3
	Enterococci	5.1×10^4	0-150	0-100
156	pH	5.98	6.21	6.35
	Anaerobic mesophiles	7.5×10^5	2.2×10^3	3.1×10^3
	Enterococci	6.3×10^4	50	0-200

[a]Cooked in water at 74°C to an internal temperature of 68°C.

[b]Raw mixture refers to the raw emulsion after being stuffed into casings but previous to cooking.

that of Study I (Table 27), the initial microbial content of the freshly cooked sausage from the two mixtures was similar (Table 30, Table 27). Also, cooking increased the pH value by 0.21 unit (Table 30). During 4 wk of storage at 7°C, the microbial content in the samples containing different nitrite levels changed little; however, an increase in pH value was found in all of the samples.

The pH continued to increase in the samples during storage from 4 wk (Table 30) to 8 wk (Table 31) despite variation in the microbial content of the samples (Table 31). After eight weeks, the results, as shown in Table 31, indicated that no growth (10^3 organisms/g), initiation of growth (10^4 organisms/g) and abundant growth (10^5 organisms/g) of the indigenous microorganisms had occurred in the different samples.

Variation in numbers of total anaerobic mesophiles among the samples in the same group was prominent (Table 31). When the count was high, the predominant organisms were always enterococci. When the count was low bacilli predominated. Also, the enterococcal counts, using KF medium, were comparable to those of the total anaerobic mesophilic counts. Most of the samples with low nitrite levels (0 or 50 ppm) showed either initial growth or abundant growth of enterococci, while the majority of the samples formulated with 100 ppm or 156 ppm nitrite gave evidence of no growth or very little growth of the surviving enterococci. Thus, nitrite could be instrumental in

Table 31. pH and total anaerobic mesophilic count in Braunschweiger liver sausage containing different nitrite levels[a] after storage at 7°C for 8 weeks

Nitrite level[a]	Replication[b]	Number of samples containing the following counts per gram				pH
		10^3	10^4	10^5	10^6	
0	A	0	0	3	0	6.43
	B	1	2	0	0	6.48
50	A	2	0	0	1	6.45
	B	1	2	0	0	6.43
100	A	2	1	0	0	6.50
	B	3	0	0	0	6.43
156	A	2	1	0	0	6.45
	B	3	0	0	0	6.45

[a]Nitrite content refers to the nitrite added in the formula.

[b]Three random samples examined in each replication.

inhibiting growth of the surviving enterococci or at least extend the lag phase of their growth.

As storage was extended to 12 wk, all the samples stored at 7°C showed growth of enterococci. As shown in Table 32, a difference in the amount of growth occurred in each group of samples. Samples without nitrite had the most abundant growth among all the groups followed by the

Table 32. pH and total anaerobic mesophilic count in Braunschweiger liver sausage containing different nitrite levels after storage for 12 weeks at 7°C

Nitrite[a] level (ppm)	Replication[b]	Number of samples containing the following count per gram					pH
		10^4	10^5	10^6	10^7	10^8	
0	A	0	1	1	1	0	6.40
	B	0	0	1	0	2	6.32[c]
50	A	1	0	2	0	0	6.37
	B	0	1	1	1	0	6.31
100	A	0	0	3	0	0	6.39
	B	0	1	2	0	0	6.31
156	A	2	0	1	0	0	6.41
	B	2	0	1	0	0	6.37

[a]Nitrite content refers to the nitrite added in the formula.

[b]Three samples examined in each replication.

[c]The first sample contained 10^6 anaerobic mesophiles/g and had a pH of 6.32; the second sample contained 10^8 anaerobic mesophiles/g and had a pH of 6.32; the third sample contained 10^8 anaerobic mesophiles/g and had a pH of 6.08.

samples containing 50 or 100 ppm nitrite. Samples containing 156 ppm nitrite in the formula had the least growth. In other words, the higher the nitrite content used in the sample the slower the growth of enterococci.

The pH values of the cooked samples refrigerated for 12 wk were slightly lower than those stored for 8 wk. The pH values in samples containing the same levels of nitrite were similar despite variations in the microbial content; that is, growth of enterococci in the refrigerated samples did not result in a significant decrease of the pH. Therefore, pH value is not a useful index for an indication of the growth of enterococci in refrigerated Braunschweiger liver sausage.

Two samples containing no nitrite contained the greatest number of microorganisms after storage for 12 weeks. One had a total anaerobic mesophilic count of 1.0×10^8 organisms/g and a pH value of 6.32. This sample had a unique perfumy odor. The fact that numbers of enterococci recovered on KF medium equaled those of total anaerobic mesophiles led to the conclusion that enterococci predominated in the sample and, in fact, may have been the only microorganisms. The other one with the same perfumy odor had a total anaerobic mesophilic count of 2.2×10^8 organisms/g and pH value of 6.08. Identification of the isolates indicated that both enterococci and pediococci grew in about equal numbers in the latter sample.

Castellani and Niven (1955) reported that commercially acceptable concentrations of nitrite may interfere with the growth of bacterial cells. Labbe and Duncan (1970) demonstrated that addition of 100 to 200 ppm of nitrite resulted in an inhibition of outgrowth of heat-injured spores. Our study showed that when other variables, including raw materials, salt content and heat treatment, are the same, the use of nitrite in liver sausage demonstrated an inhibitory effect on the growth of surviving enterococci. Also, the addition of increasing amounts of nitrite to liver sausage cause an increasing level of inhibition on bacterial growth. These results agree with the work of Stoychev and Djejeva (1971). They studied the metabolic activity of Streptococcus faecalis in a laboratory medium and showed that sodium nitrite in concentrations applied at levels used in cured meat products exhibit an inhibitory action which increases as the concentration is increased.

c. Influence of processing temperatures and addition of calcium-reduced dried skim milk (CRDSM) The initial microbial loads of the raw mixture after being chopped and stuffed into the casings in Study III were the highest one among all the three studies (Tables 25, 29 and 33). Identification of isolates from the total anaerobic mesophilic plates showed that the microflora consisted of enterococci (92%), Bacillus (3%), and Lactobacillus plus Pediococcus (5%). Comparison of the pH values and

Table 33. The effect of storage time on the pH and bacterial count of raw mixture[a] of Braunschweiger during 5°C storage

Weeks in Storage	Sample[b]	pH	No. of organisms/g		
			Total Anaerobic Mesophiles	Enterococci	Aciduric Bacteria
0	A	5.94	2.1×10^6	1.1×10^5	ND[c]
	B	6.03	1.7×10^6	1.2×10^5	ND
2	A	5.90	6.0×10^7	1.1×10^5	ND
	B	5.98	4.3×10^7	1.2×10^5	ND
4	A	4.95	1.1×10^9	1.5×10^5	1.0×10^9
	B	5.10	1.4×10^9	7.5×10^4	1.5×10^9

[a]Raw mixture refers to the raw emulsion after being stuffed into the casings but previous to cooking.

[b]A represents the raw mixture not containing CRDSM. B represents the raw mixture containing CRDSM.

[c]ND: Not detected, perhaps due to the lack of overlaying the spread plates of LBS agar.

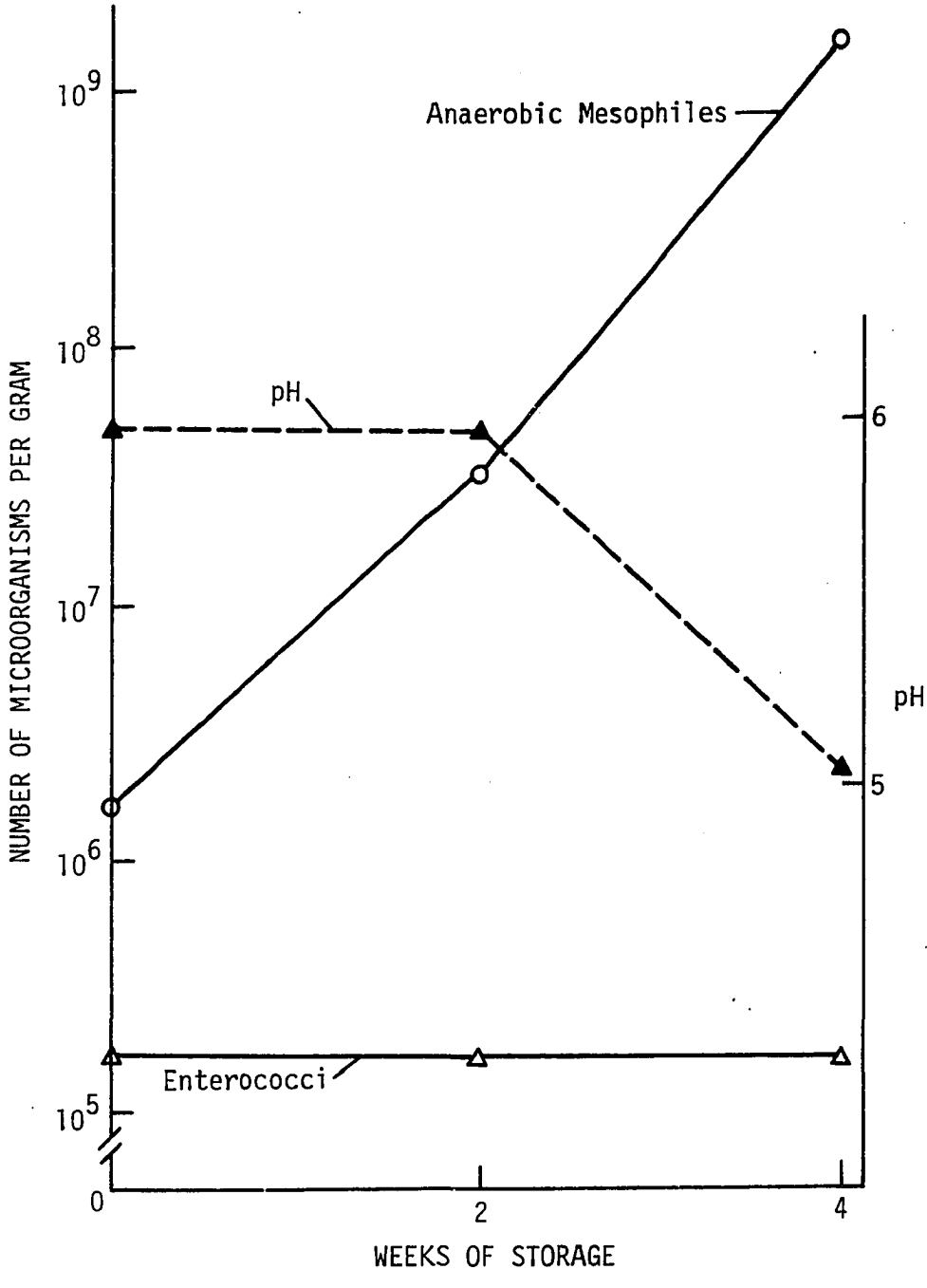
microbial loads in the raw mixtures with or without CRDSM (Table 33) showed that the addition of 3.5% CRDSM to the sample influenced the population very little, if any. However, the pH values of the samples containing CRDSM were slightly higher (0.1 unit) than those of the others.

After two weeks of storage at 5°C, the total anaerobic mesophilic count of the raw mixture of Braunschweiger increased about 20 fold. The microflora

at this time included: Pediococcus (80%), Lactobacillus (10%) and enterococci (10%). The initiation of multiplication by aciduric bacteria within the first two weeks did not change the pH value of the sample; however, the rapid and accumulative growth of the aciduric bacteria in the raw mixture of Braunschweiger during the latter two weeks resulted in a decrease of 1 pH unit in the sample. Both lactobacilli and pediococci, found as the most predominant microorganisms in the spoiled sample, are classified as lactic acid bacteria and characteristically produced lactic acid from carbohydrates (Sharpe et al., 1966). The souring of the mixture of Braunschweiger likely resulted from the utilization of carbohydrates and the production of acid by these organisms.

Enterococci, initially, were found to be the most predominant microorganisms in the sample. However, the number of enterococci appearing on the total anaerobic mesophilic plates was about 16 times higher than on the KF plates (Figure 12). This observation was interpreted as indicating that the majority of the enterococci in the sample was sensitive to selective agents in the KF medium; only 6% of the enterococci in the sample were capable of initiating growth on KF plates. During the first two weeks of storage at 5°C, these enterococci could only survive but could not grow in the sample. The salt and nitrite content in the sample also might have contributed to this specific inhibitory effect on

Figure 12. Changes in pH and microbial count of raw mixtures of Braunschweiger liver sausage during storage at 5°C. (Each point is the mean of two replications. Each replication contained three samples for a total of six samples)


Anaerobic Mesophiles
pH
Enterococci
NUMBER OF MICROORGANISMS PER GRAM
10^9
10^8
10^7
10^6
10^5
0
2
4
6
5
pH
WEEKS OF STORAGE

the growth of enterococci. Stoychev and Djejeva (1971) reported that sodium nitrite in concentrations applied in the curing and production of meat items exhibits an inhibitory action upon the metabolism of Streptococcus faecalis.

The effect of cooking temperatures on the reduction of microbial loads in the raw mixture of Braunschweiger is shown in Table 34. The effiency of killing of microorganisms with different cooking treatments was in the order of T3 $\geq$ T4 > T2 > T1. The heat processing temperatures not only affected the residual microbial content of the cooked Braunschweiger liver sausage but also played a decisive role in the kinds of survivors.

The T1 cooking treatment represents the cooking of Braunschweiger liver sausage in hot water at 68°C to an internal temperature of 63°C. The heat processing curve (Figure 13) shows that products receiving the T1 treatment received the lowest amount of heat although the total cooking time was the longest, 40 min. After the heat treatment of T1, the products contained predominantly Gram positive cocci as the survivors.

While enterococci account for 73% of the total anaerobic mesophiles, only 8×10^3 organisms/g or 4% could be cultivated on KF medium in contrast to a calculated number of 2.0×10^5/g. The calculated number was obtained by multiplying the number of the total anaerobic count by 73%. This observation indicated that 96% of the enterococci which survived in

Table 34. Effect of cooking temperatures on the pH and bacterial counts of Braunschweiger liver sausage

Sample	pH	Total Anaerobic Mesophilic Count				Enterococci/g (on KF plates)
		No. of organisms/g	Microflora (%)			
			Bacillus	Enterococci	Aciduric Bacteria	
Raw Mixture[a]	5.94	2.1×10^6	3	92	5	1.1×10^5
T1[b]	6.17	2.8×10^5	0	73	27	8.0×10^3
T2[c]	6.23	6.5×10^4	5	85	10	2.5×10^2
T3[d]	6.28	1.5×10^3	100	0	0	< 50
T4[e]	6.28	2.7×10^3	83	17	0	< 50

[a]Raw mixture refers to the raw emulsion after being stuffed into casings but previous to cooking.

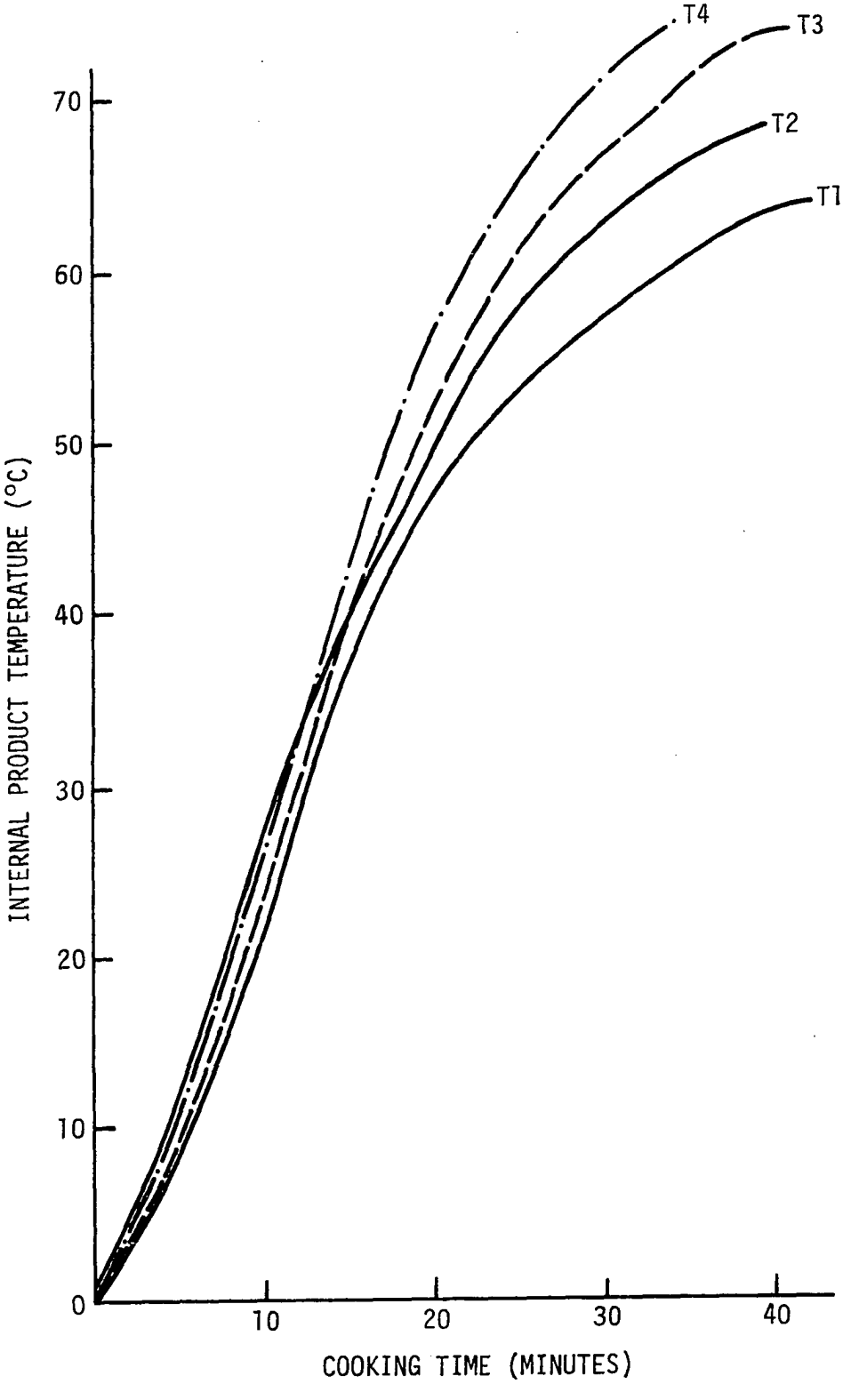
[b]T1 cooked in 68°C water to an internal temperature of 63°C.

[c]T2 cooked in 74°C water to an internal temperature of 68°C.

[d]T3 cooked in 79°C water to an internal temperature of 74°C.

[e]T4 cooked in 85°C water to an internal temperature of 74°C.

Figure 13. Heat processing curves for Braunschweiger liver sausages receiving different cooking treatments. T1 represents cooking in water at 68°C to an internal temperature of 63°C; T2, cooking in water at 74°C to 68°C; T3, cooking in water at 79°C to 74°C; T4, cooking in water at 85°C to 74°C. (Readings were taken at 1 minute intervals)


T4
T3
T2
T1
70
60
50
40
30
20
10
0
10
20
30
40
INTERNAL PRODUCT TEMPERATURE (°C)
COOKING TIME (MINUTES)

the sample were heat injured. Clark et al. (1968) reported that the exposure of Streptococcus faecalis R57 to a sublethal heating (60°C for 15 min) produced a temporary change in the salt tolerance and growth of the organism. After the sublethal heat treatment, manifestations of injury occurred such as (1) increase in sensitivity to salt, sodium azide, bromcresol purple and 0.1% methylene blue (2) extension of lag phase of growth (3) inability to grow at 10°C and 45°C and (4) decrease in ability to grow at pH 9.6. Duitschaever and Jordan (1974) demonstrated that heating of Streptococcus faecium at 55°C for 15 min produced injury. The injured population was sensitive to 2.5% NaCl but regained its tolerance when incubated in a recovery medium. In our study, the heat injured enterococci were unable to grow on selective KF medium or required a longer incubation time. The latter was confirmed through extended incubation of plates as shown in Table 35. One additional day of incubation showed a 2 to 3 fold increase in plate count; in fact, counts continued to increase up to 5 days and then remained constant. The variation in sensitivity of heat injured cells to KF medium may indicate varying degrees of injury among the cells in the sample. Through the use of the hot water cooking method, the geometric center of the product is exposed to the final temperature for a shorter period of time than are other parts. van Schothorst and van Leusden (1975) showed the need for longer periods of time for the recovery of severely damaged salmonellae

Table 35. Effect of incubation time on the numbers of colonies of heat-exposed enterococci developing on KF medium[a]

Incubation Days	Sample[b]		
	A	B	C
2	1.8×10^1	2.7×10^1	1.6×10^1
3	3.1×10^1	6.5×10^1	5.7×10^1
5	3.4×10^1	8.1×10^1	6.7×10^1

[a]Numbers of enterococci/g of Braunschweiger cooked in water held at 68°C to an internal temperature of 63°C.

[b]A, B and C represent three random samples.

cells and concluded that the damage to the cells in the population could be classified into categories of 1st, 2nd and 3rd degree injuries. They found that the degree of injury was one of the most important parameters influencing recovery time.

The T2 cooking treatment which represents cooking in hot water at 74°C to an internal temperature of 68°C resulted in a 97% reduction of microbes whereas the T1 treatment represented a reduction of 87% in the initial microbial load. In samples from the T2 treatment, enterococci constituted 85% of the total anaerobic mesophiles. Only 0.5% of the surviving enterococci (computed on the basis of the calculated number)

formed colonies on KF plates: this figure was obtained by comparing the recovery on KF medium as opposed that on Trypticase Soy Agar (plus 0.5% Na-thioglycolate). In other words 99.5% of the surviving enterococci were heat injured. The greater total microbial reduction and the higher percentage of injured enterococci in the T2 treated sample than in the T1 treated sample indicated that the T2 treatment is a more efficient heat process for microbial control of the sample. During the cooking period, the temperature at the geometric center of the sample with T2 treatment was higher than that of the T1 treatment (Figure 13).

The products treated with the T3 and T4 treatments showed much lower microbial loads than did T1 and T2 treatments. The products treated with T3 and T4 variations contained Bacillus as the predominant microflora. The T3 treatment represents cooking in 79°C hot water to an internal temperature of 74°C, and the T4 treatment represents cooking in 85°C hot water to an internal temperature of 74°C. Both T3 and T4 treatments had the same finishing temperature - an internal temperature of 74°C. Examining the heat processing curve (Figure 13) for the T3 and T4 treatments showed that during the first 15 min of cooking time, both products had similar internal temperatures but after 16 min of cooking, the rate of heat penetration was faster for T4 than for T3. The products in the T4 treatment reached the required internal temperature in 33 min while the T3 treated samples required 40 min. The survival of enterococci (Table 34) in T4 treated

sample compared to the survival of enterococci in the T3 treated sample indicated that the T3 treatment was more efficient for the destruction of enterococci in the raw mixture of Braunschweiger liver sausage. In other words, the treatment with lower heat penetrating rate and longer cooking time resulted in better microbial quality of Braunschweiger liver sausage.

In general, canned hams, a pasteurized product, are processed to an internal temperature of 66°C which is similar to the heat treatment used in T1 and T2. Kafel and Ayres (1969) examined 4241 commercially canned hams and were able to recover enterococci from 45.6% of them. Thus, it is not unusual to observe the survival of enterococci in mildly heat processed meats.

The microbial quality of Braunschweiger liver sausage treated at different cooking temperatures and stored at 5°C is shown in Table 36. All the samples with the exception of the T1 samples showed no growth within 12 wk of refrigeration. In fact, T3 samples showed a decrease in the numbers of facultative bacilli which existed in the sample during storage time.

During 8 wk of storage at 5°C, T1 samples had no change in their microbial content. However, the number of enterococci appearing on the KF medium of T1 samples at the 5th and 8th wk was approximately 5 times and 4 times higher, respectively, compared to that of the 2nd wk. Evidently

Table 36. The effect of storage time on pH and microbial quality of Braunschweiger liver sausage stored at 5°C

Storage Time	Determination	Sample[a]			
		T1	T2	T3	T4
2 weeks	pH	6.17	6.23	6.28	6.28
	Anaerobic mesophiles/g	2.8×10^5	6.5×10^4	1.5×10^3	2.7×10^3
	Enterococci/g	1.6×10^4	2.5×10^2	≤ 50	≤ 50
5 weeks	pH	6.17	6.23	6.28	6.28
	Anaerobic mesophiles/g	2.0×10^5	6.0×10^4	1.4×10^3	2.0×10^3
	Enterococci/g	7.6×10^4	1.6×10^3	≤ 50	≤ 50
8 weeks	pH	6.21	6.23	6.26	6.27
	Anaerobic mesophiles/g	1.4×10^5	4.3×10^4	3.0×10^2	2.4×10^3
	Enterococci/g	6.3×10^4	2.5×10^3	≤ 50	≤ 50
12 weeks	pH	6.18	6.27	6.27	6.23
	Anaerobic mesophiles/g	Table 37	1.5×10^4	1.0×10^2	1.3×10^3
	Enterococci/g	Table 37	1.5×10^3	< 50	< 50

[a]T1 cooked in 68°C water to an internal temperature of 63°C; T2 cooked in 74°C water to an internal temperature of 68°C; T3 cooked in 79° water to an internal temperature of 74°C; and T4 cooked in 85°C water to an internal temperature of 74°C.

some of the heat injured enterococci had recovered during the 2nd to 8th wk and, as a consequence, were now able to multiply and form colonies in KF medium.

After the 12th wk (Table 37), in one replication, all samples showed similar numbers of total anaerobic mesophiles, but much higher numbers of enterococci appeared on KF medium compared to those observed at the previous time intervals. Equal numbers of total anaerobic mesophiles and enterococci indicated extensive recovery of the heat injured enterococci. In another replication, one sample yielded results similar to those cited

Table 37. Microbial quality of Braunschweiger liver sausage cooked to an internal temperature of 63°C and stored at 5°C for 12 weeks

Replication	Total anaerobic mesophiles/g	Enterococci/g recovered on KF medium
I	2.9×10^5 [a]	3.3×10^5 [a]
II	2.5×10^4 2.4×10^5 3.0×10^7	2.3×10^4 3.0×10^5 2.0×10^7

[a] The average count of 3 samples.

in the previous example. The second sample contained lower numbers of total anaerobic mesophiles but higher percentages of enterococci compared to those of the previous time intervals. The third sample was spoiled due to the growth of the enterococci. Evidently the surviving enterococci had recovered from any heat injury they may have had and were not sensitive to the KF medium; or the injured cells could initiate multiplication after an extensive lag period. These results agree with the findings of Kafel and Majewska (1961). They reported that enterococci were not only tolerant of the curing salts in pasteurized ham but also were able to grow at relatively low temperatures and could multiply during refrigerated storage.

The number of aciduric bacteria in T1 and T2 samples decreased with storage. The number of the aciduric bacteria in the T1 sample after the 5th wk was three thousand per g but after the 12th wk was one hundred per g. In the T2 sample, after 5 wk the count was about two hundred aciduric bacteria per g but none after the 12 wk. Evidently, a gradual death of the surviving cells was occurring during refrigerated storage of the Braunschweiger liver sausage.

d. Comparison of Braunschweiger made with common treatment in different studies Among the studies for determining the effect of different variables on the microbial content and flora of Braunschweiger

liver sausage, one common treatment on each study can be observed. This common treatment is the one in which formulation of the sausage consisted of fresh pork liver containing 156 ppm sodium nitrite and cooked in 74°C water to an internal temperature of 68°C.

Comparison of all the results in these common treatments are shown in Table 38. The results indicate that the microbial load of the raw mixtures were significantly different from each other. These differences were mainly caused by variation in the contamination of the pork livers since the microbial contamination in the other ingredients was similar among the different studies.

Under our formulation, using the ratio of pork liver:pork trim of 1:1 the raw mixtures had an average pH of 5.97. The freshly made Braunschweiger liver sausage had a pH of 6.22, cooking resulted in an increase in pH value of 0.25 units. The pH of refrigerated sausages gradually increased during storage for 8-10 wk, then decreased after that time. The change in pH may have been caused by chemical changes since most of the samples showed little, if any, microbial multiplication.

Although all cooking was done in 74°C water to an internal temperature of 68°C, small variations occurred in the length of time to reach the required internal temperature. An examination of the

Table 38. Comparisons of different lots of Braunschweiger liver sausage receiving similar treatment[a]

Sample in Study	Raw mixture[b]		Cooked Braunschweiger liver sausage storaged					
	Anaerobic mesophiles/g	pH	Temperature (0°C)	1-2 weeks[c]		8-10 weeks[c]		12-16 weeks[c]	
				Anaerobic mesophiles/g	pH	Anaerobic mesophiles/g	pH	Anaerobic mesophiles/g	pH
I	7.2×10^4	5.98	5	1.5×10^3 (2)	6.20 (2)	4.0×10^3 (10)	6.37 (10)	2.2×10^3 (16)	6.21 (16)
II	7.5×10^5	5.98	7	2.2×10^3 (1)	6.21 (1)	2.9×10^3 (8)	6.45 (8)	$10^3 - 10^6$ (12)	6.37 (12)
III	2.1×10^6	5.94	5	6.5×10^4 (1)	6.23 (1)	4.3×10^4 (8)	6.27 (8)	1.5×10^4 (12)	6.27 (12)

[a]Similar treatment refers to the same formulation and heat processing in 74°C water to an internal temperature of 68°C.

[b]Raw mixture refers to the raw emulsion after being stuffed into casings but previous to cooking.

[c]Number in parentheses shows the weeks of storage when samples were examined.

heat processing curves (Figure 14) indicates that the difference in the temperature of the raw mixture before cooking could affect heat penetration. Factors influencing the temperature of the raw mixture during manufacturing could include length of handling time for chopping, stuffing, and especially setting time at 0°C before cooking. The difference in cooking time also could be caused by the amount of raw mixture cooked in one lot.

Despite the small variation in cooking in each study, the efficiency of the cooking for the destruction of microorganisms in raw mixture was higher than 97%. Cooked sausage stored at 7°C had a shorter shelf life than those stored at 5°C. The growth of enterococci is affected more by differences in refrigeration temperature than by the initial numbers of surviving enterococci in the cooked products.

2. Storage-temperature study

An examination of the heat processing curves for liver sausages from the same batch but cooked at different sessions on the same day and designated as batch P and batch Q in Figure 15 shows a difference in the initial temperatures of the respective raw mixtures. This difference, no doubt, resulted from the longer holding of the Q raw mixture at 0°C before cooking. Likely the lower initial temperature of the Q mixture prolonged the attainment of the desired internal temperature (36 min cooking time for the Q product as compared to 26 min for the P product).

Figure 14. Comparisons of heat processing curves for Braunschweiger liver sausage determined at monthly intervals. These curves were recorded for product heated to an internal temperature of 68°C in water at 74°C. (Readings were taken at 1 min intervals)

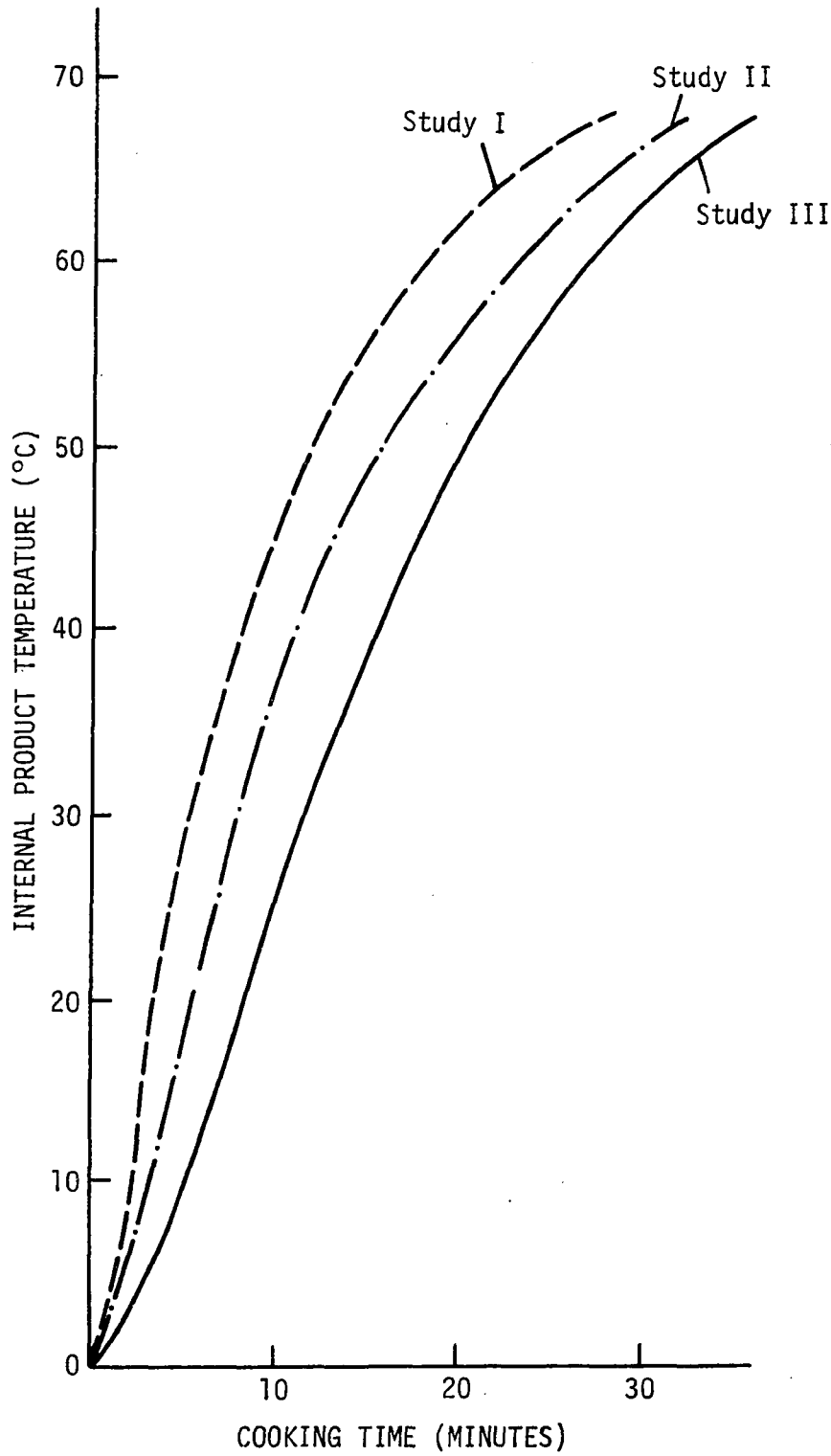
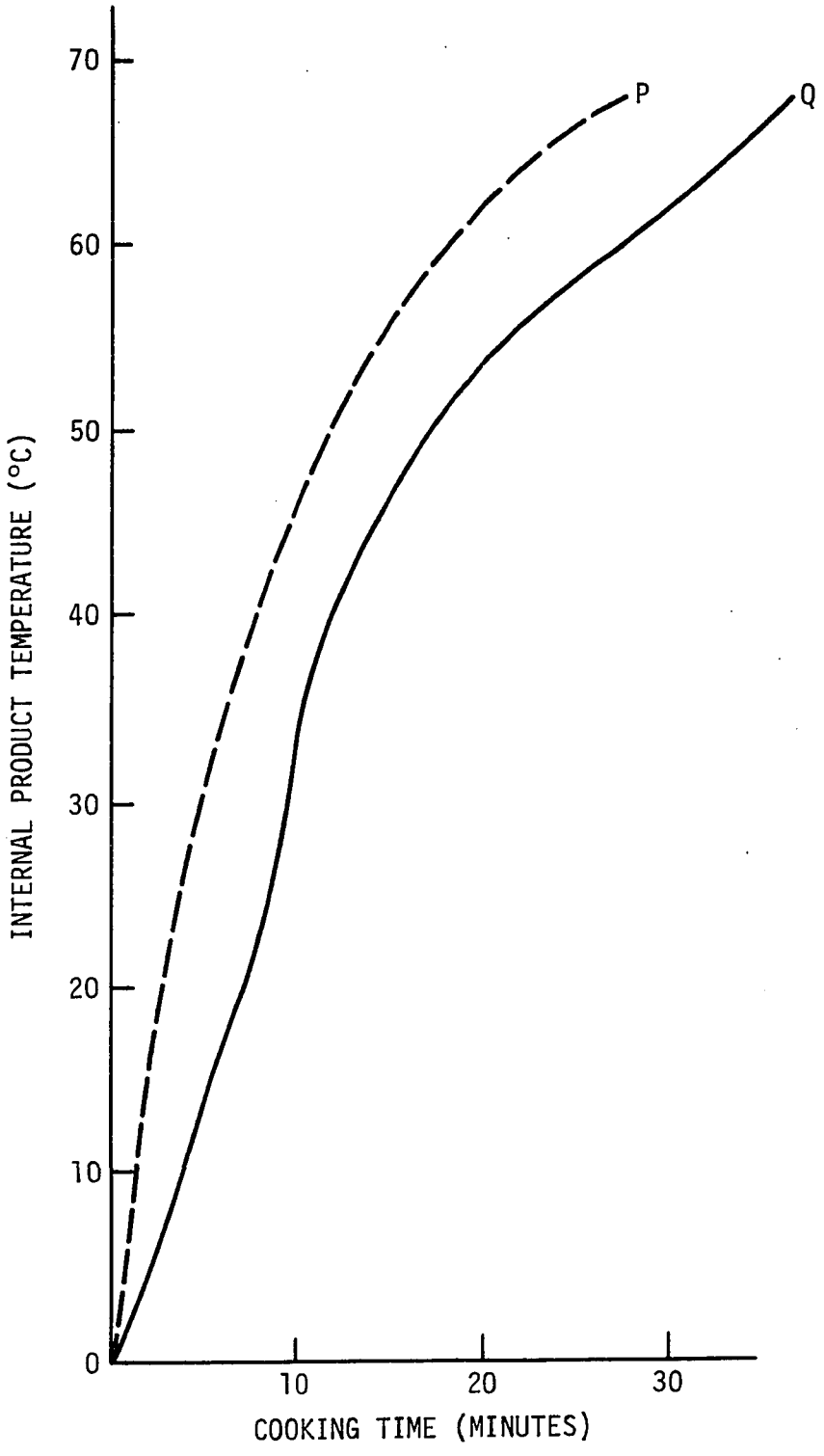

70
60
50
40
30
20
10
0
INTERNAL PRODUCT TEMPERATURE (°C)
Study I
Study II
Study III
10
20
30
COOKING TIME (MINUTES)

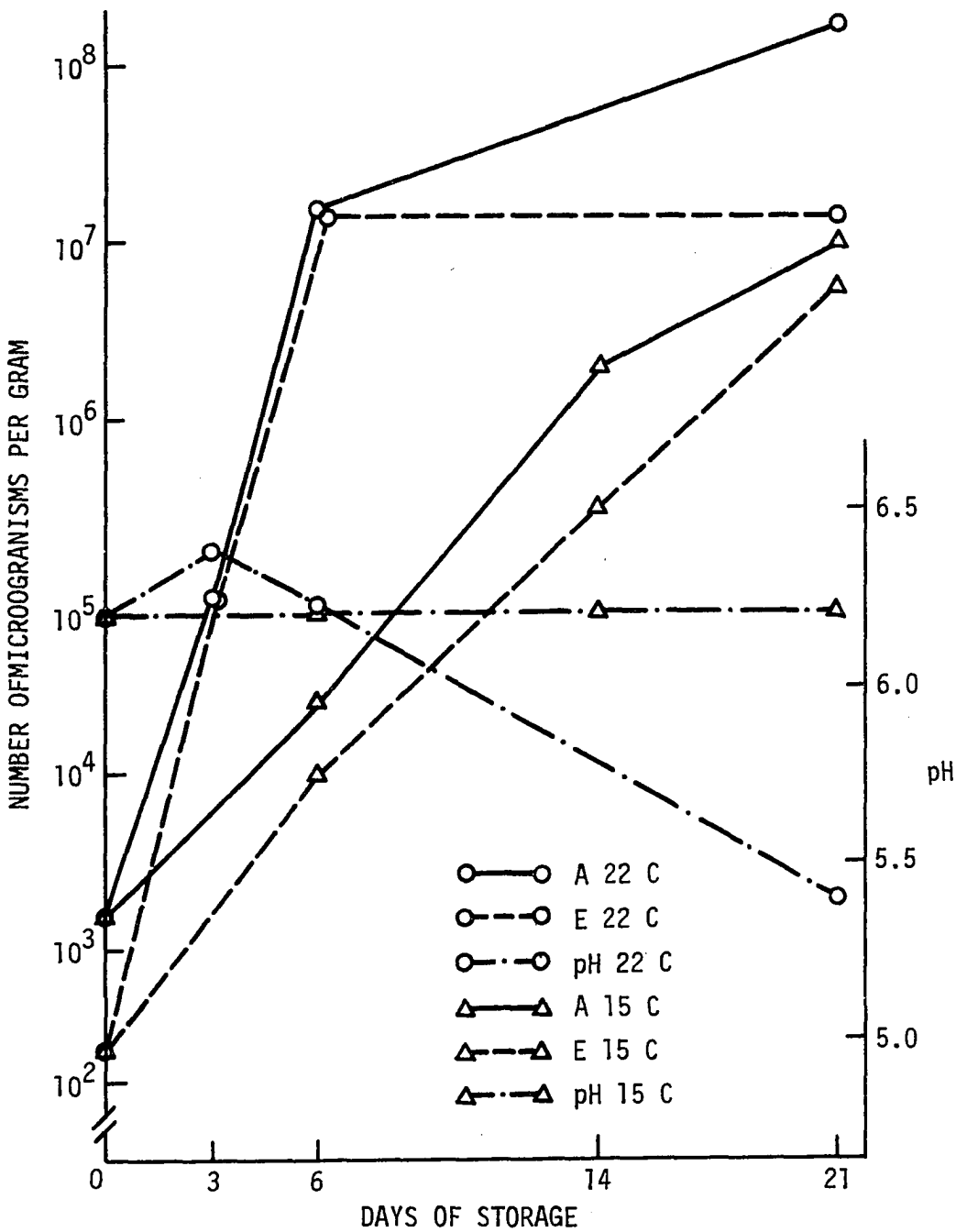
Figure 15. Heat processing curves for Braunschweiger liver sausages containing raw mixture from the same batch but cooked at two consecutive sessions. The two lots are designated P and Q. (Readings were taken at 1 min intervals)


70
60
50
40
30
20
10
0
INTERNAL PRODUCT TEMPERATURE (°C)
P
Q
10
20
30
COOKING TIME (MINUTES)

Both freshly cooked products contained equal numbers of anaerobic mesophiles; lot P contained 2.0×10^3 microorganisms/g and lot Q contained 3.0×10^3 microorganisms/g. The predominant organisms in each product differed, however. The microflora of P liver sausages consisted of enterococci (66%) and Bacillus (33%); the Q liver sausages consisted of Bacillus (95%) and enterococci (5%). About 2.0×10^2 enterococci/g in P liver sausage were recovered on KF medium; but none of the enterococci in Q liver sausage could be cultivated on KF medium.

The microbial content of vacuum packaged P liver sausage stored at 22°C for 3 days showed a two log cycle increase over the initial numbers (Figure 16). The sample showed a slight increase in pH; the color and odor remained normal. After storage for 6 days, a unique perfumy odor of the sample was detected and about 20 million microorganisms per gram were recovered from the product. The pH of the sample did not change during this time. The enterococcal counts on KF medium of P product after 3 and 6 days were comparable to the numbers of anaerobic mesophiles. This observation was interpreted to mean that the growth of enterococci was responsible for spoilage. Prolonged storage of the P liver sausage at 22°C for 21 days did not result in any increase in the number of enterococci. Furthermore, the

Figure 16. Changes in the anaerobic mesophiles, enterococci and pH of liver sausage (Lot P) stored at 22°C and 15°C. (Each point represents the mean of three samples in one replication. A stands for anaerobic mesophiles and E for enterococci)

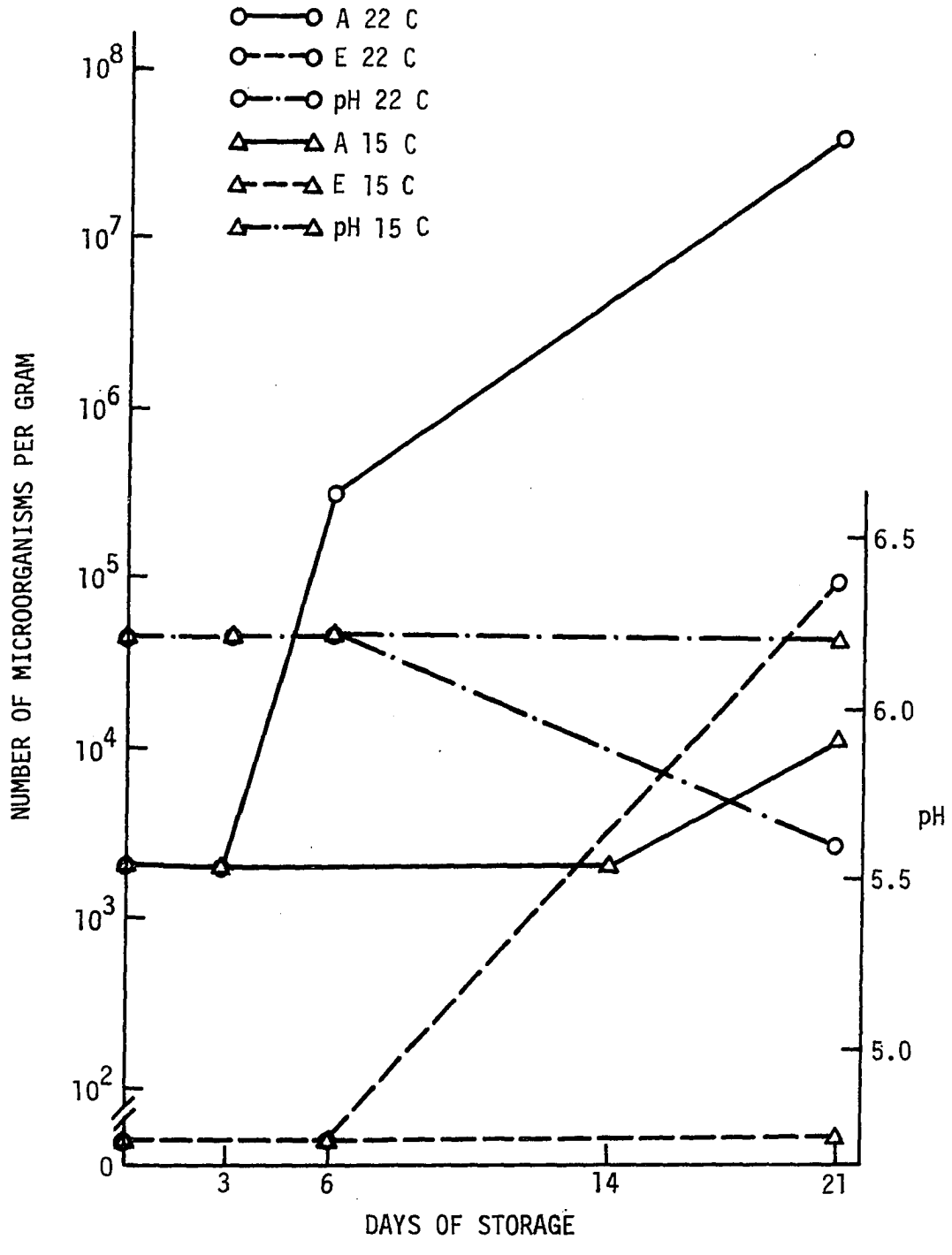

NUMBER OFMICROOGRANISMS PER GRAM
10^8
10^7
10^6
10^5
10^4
10^3
10^2
6.5
6.0
5.5
5.0
pH
A 22 C
E 22 C
pH 22 C
A 15 C
E 15 C
pH 15 C
0
3
6
14
21
DAYS OF STORAGE

predominant microorganism in the P liver sausage was Pediococcus sp. (90%); these organisms produced a pH of 5.4 in the product. Storage at 15°C controlled growth of the microorganisms to a greater extent than did storage at 22°C (Figure 16). Although sausage stored at 15°C for 21 days contained 1.0×10^7 anaerobic mesophiles/g, there was no change in color, odor and pH of the sample. The number of enterococci in the sample was about 7.5×10^6/g.

The anaerobic mesophilic count of Q liver sausage stored at 22°C for 3 days showed no increase over the initial numbers. At the 6th day, the anaerobic mesophiles had increased by 2 log cycles over the initial numbers (Figure 17). The product had no sign of change in color, odor and pH value, however. Enterococci in Q product initiated growth after the 6th day. After storage at 22°C for 21 days, Q liver sausage had a gassy, soft, sticky and slimy appearance and also a very putrid odor. The sample had a microbial count of 6.0×10^7 anaerobic mesophiles/g, the microflora consisted of bacilli (99%) and enterococci (1%) and the sausage had a pH of 5.6. After storage of the Q liver sausage at 15°C for 14 days, no microbial growth in the sample was observed. Slight growth of Bacillus was found after 21 days storage.

Our results demonstrated that storage temperature and the predominant microorganisms affect the shelf life of the liver sausage. Liver sausages

Figure 17. Changes in the anaerobic mesophiles, enterococci and pH of liver sausage (Lot Q) stored at 22°C and 15°C. (Each point represents the mean of three samples in one replication. A stands for anaerobic mesophiles and E for enterococci)


A 22 C
E 22 C
pH 22 C
A 15 C
E 15 C
pH 15 C
NUMBER OF MICROORGANISMS PER GRAM
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
6.5
6.0
5.5
5.0
pH
3
6
14
21
DAYS OF STORAGE

had a shorter shelf life when stored at 22°C compared to that of 15°C. Under the same storage temperature, liver sausages containing the same microbial load but different kinds of predominant organism manifested different types of spoilage. Enterococci, the predominant survivors in the P sample, grew rapidly at 22°C and produced a unique perfumy spoilage of the sample by the 6th day. On the other hand, bacilli as the main survivors in sample Q grew at a slower rate and caused a putrefactive spoilage by the twenty-first day. The initiation of growth of surviving enterococci in Q liver sausage after storage at 22°C for 6 days indicated that a long recovery time was involved before multiplication could start. It is likely that the slow heating rate and long cooking time in Q liver sausage caused more extensive injury of the surviving enterococci than did the more rapid heat penetration and short cooking time.

3. Identification of microorganisms

All the microorganisms isolated from pork liver, pork trim, salt-spice mixture, raw mixture and cooked Braunschweiger liver sausage were Gram positive. Preliminary separation into genera of these Gram positive isolates was done according to the scheme presented in Table 39. According to the 8th edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974), identification of the

Table 39. Scheme for classifying Gram positive isolates

Morphology	Catalase	Presumptive groups	Scheme and methods employed for detailed classification
Rods and visible spores	+	Bacillus spp.	None
Rods and cocoid form pleomorphic	+	Coryneform groups of bacteria	None
Rods or cocci	-	Lactic acid bacteria	Sharpe et al. (1966)
Cocci	+	Micrococcaceae	Evans and Kloos (1972)

genera in Corynebacteriaceae should not be attempted but a working concept of a coryneform group of bacteria to include Corynebacterium, Arthrobacter (with the related genus Brevibacterium and Microbacterium as genus incertae sedis), Cellumonas and Kurthia is preferable.

Lactobacillus isolates were recovered only from the raw mixture of ingredients before cooking. This indicated that these organisms did not survive the cooking temperatures. The isolates we obtained and identified as homofermentative Lactobacillus had the following characteristics: Gram positive, catalase negative, nonmotile rods; nitrate reduction negative, gelatin not liquefied, casein not digested, indole and hydrogen sulfide not produced, no pigment, growth at temperatures of 5°C and 50°C,

tolerate 8% NaCl, growth on LBS agar, metabolism fermentative, acid from glucose broth but no gas.

Cooked and refrigerated Braunschweiger liver sausage were spoiled mainly by Gram positive, catalase negative cocci which belong to the lactic acid bacteria group and further identification showed that the cocci belonged to two genera Streptococcus and Pediococcus. Attempts were made to identify the isolates of the above two genera to the species level. The results are shown in Table 40.

Identification of organisms belonging to Streptococcaceae to the genus level using biochemical tests is not easy (Garvie, 1960, 1974; Whittenbury, 1965). The main keypoints according to the 8th edition of Bergey's Manual (Buchanan and Gibbons, 1974) for placing the Gram positive, catalase negative cocci into the genus Pediococcus were: (1) cocci occurring in pairs or tetrads; (2) nonmotile, endospores not formed; (3) metabolism fermentative; (4) acid but no gas from glucose, fructose and mannose; (5) sorbitol and starch not fermented; (6) gelatin not liquified, nitrate not reduced to nitrite. According to Garvie (1960) acid and gas production from glucose broth is the main character for differentiating Leuconostoc from both Pediococcus and Streptococcus. Bergey's Manual (8th edition, Buchanan and Gibbons, 1974) relies heavily on cell arrangement to differentiate between Streptococcus and

Table 40. Characteristics[a] of isolates referred to as Streptococcus faecalis and Pediococcus pentosaceus

Property	Streptococcus faecalis	Pediococcus pentosaceus
Gram stain and morphology	Gram positive cocci, ovoid, in pairs short chains or irregular packets	Gram positive cocci, ovoid, in pairs, in tetrads, or irregular packets
Catalase	-	-
Growth at 5°C and 45°C	+	+
Growth at 50°C	+	-
Growth at pH 5	-	+
pH 7	+	+
pH 8, 9, 9.6	+	-
Growth in NaCl 6.5%, 8%	+	+
OF of glucose (Fermentation)	+	+
Arginine hydrolysis	+	+
Starch hydrolysis	-	-
Gelatin liquefaction	-	-
Nitrate reduction	-	-
Indole production	-	-
Motility test	-	-
Hydrogen sulfide production	-	-
MR test	+	+
VP test	-	-
40% bile resistance	+	-
0.04% tellurite tolerance	+	+
Growth on LBS agar (35°C, 5 days)	-	+

Growth and characteristics on KF medium (35°C, 3 days)	+ (Pinkish colony with red in center)	+ (Pinpoint size transparent, grey colony)
Casein agar digestion	+	-
Gas from glucose	-	-
Acid from Sorbitol	+	-
Raffinose	+	-
Arabinose	+	-
Glucose	+ (final pH = 4.4)	+ (final pH = 3.9)
Lactose	+	+
Sucrose	+	+
Glycerol fermentation (anaerobic)	G[b]	G[b]
Pyruvate fermentation	G[b]	G[b]
Litmus milk reaction clot	+	-
reduction	+ (slow)	-
acid	+	-

[a]+ for positive, - for negative.

[b]Growth but no acid production.

Pediococcus. Streptococcus spp. have cell division in one plane resulting in pairs and chains while Pediococcus spp. have cell division in two plans resulting in pairs and tetrads. However, Mundt et al. (1969) reported that the occurrence of two-dimensional tetrads may be rare; this characteristic varied with individual cultures and culture media. They concluded that the most readily recognized character of the Pediococcus spp. was the ability to initiate growth in liquid media which were acidified to pH 5.0 and contained 1.5% sodium acetate. They suggested that confirmatory characteristics for the genus Pediococcus include fermentation of glucose broth to a final pH 3.6 to 4.3, ability to initiate growth at 45°C, production of ammonia from arginine, dissimilation of malate and fermentation of arabinose.

The properties and tests used in this study to identify the isolate as Pediococcus pentosaceus were compiled by Garvie (1974) from many published data. The characteristics of species of P. pentosaceus and P. acidi-lactici are quite similar but differentiated only by one character that the former one does not initiate growth at 50°C while the latter one initiates growth at 50°C (Nakagawa and Kitahara, 1959). Other workers studying these same bacteria do not agree that they form two species (Whittenbury, 1965; Mundt et al., 1969) but suggested that they were only one species and resided only on plants.

P. pentosaceus originally was isolated from malt mash; it is widely distributed in fermenting materials such as sauerkraut, pickles, silages and cereal mashes (Mundt et al., 1969; Whittenbury, 1965). The isolates identified here as P. pentosaceus were cultivated from the cooked Braunschweiger liver sausages without nitrite cure under refrigeration for 12 wk and the Braunschweiger liver sausages with full nitrite cure stored at 22°C for 21 days. In both cases, the samples containing P. pentosaceus as the predominant organism had a low pH value.

The other culture was isolated from raw pork livers and freshly cooked Braunschweiger liver sausages. The isolates were capable of growing in the refrigerated liver sausage with no indication of a change in appearance or pH in the sample; however, the sample of liver sausage developed a perfumy odor when it contained more than 10 million cells of the isolate. This culture had most of the characteristics attributed to Streptococcus faecalis with few exceptions (Table 40). Colonies cultivated anaerobically for 3 days at 30°C on plates containing Trypticase Soy Agar plus 0.5% sodium thioglycolate were circular, entire, convex and 2 mm in diameter. Colonies had a snow-white opaque color. The isolate could tolerate 0.04% tellurite, reduce tetrazolium, clot litmus milk and ferment sorbitol. These four characteristics are included as the most conclusive tests to differentiate S. faecalis from S. faecium

(Deibel, 1964; Facklam, 1972). Certain reactions were typical of S. faecium and led to some difficulty in the final identification. These characters included fermentation of arabinose, growth at 50°C and slow reduction in litmus milk. However, the latter two tests are listed as less conclusive tests than the previously mentioned tests (Deibel, 1964). Also, Facklam (1972) reported that 4% of the S. faecalis of human origin fermented arabinose. Donnelly and Hartman (1978) suggested that both pyruvate and glycerol fermentation tests were useful keytests to differentiate S. faecalis from S. faecium. They reported that S. faecalis produced acid from pyruvate and glycerol fermentation while S. faecium did not. The isolates identified here as S. faecalis could grow well in both pyruvate and anaerobic glycerol fermentation tubes yet did not produce acid.

A common source of S. faecalis is feces of humans and warm-blood animals (Cooper and Ramadam, 1955; Bartley and Slanetz, 1960; Kjellander (1960). However, Deibel (1964) has emphasized that a factor which tends to discount enterococci (S. faecalis and S. faecium) as fecal indicators in some food products is their ability to grow in environments far removed from the original source of fecal contamination. He concluded that S. faecalis is common in food products and often unrelated to direct fecal contamination.

4. Inoculation studies with spoilage organisms

Spoilage of inoculated samples was undertaken to determine whether or not the suspected organisms produced specific changes in the sample during refrigerated storage and to observe what influence different environmental conditions had on the type of spoilage that occurred.

a. Inoculation of sterilized samples The initial pH and odor of sterile samples of Braunschweiger, with or without inoculum, were similar. The pH of the sterile control, the Streptococcus faecalis (M) inoculated sample and the Pediococcus pentosaceus (N) inoculated sample was 6.1, 6.0 and 5.9, respectively. The 5 ml of APT broth containing about 10^9 cells/ml did not make any significant change in the pH of the inoculated sample even though the pH of the broth containing the organisms was in the range of 4.4 to 4.8. The pH of the sterile APT broth was 6.5. All the samples had a very pleasant odor described best as caramel; this odor can be attributed to the overheating of the sample during sterilization. After incubation at 30°C for 3 days, the pH of the control sample, S. faecalis-inoculated and P. pentosaceus-inoculated sample was 6.2, 5.1 and 4.5, respectively. A decrease in pH and development of a soft texture and a loss of granular appearance of the meat particles were manifestations of the growth of each specific inoculum during incubation. The odor did not change in any of the

samples; that is, the caramel odor predominated.

b. Inoculation of pasteurized samples The result of the inoculation study in pasteurized samples under aerobic incubation are shown in Table 41. Under aerobic conditions, the Braunschweiger liver sausage was a good medium for the growth of the inoculum. Change in pH is a useful tool indicating the multiplication of the inoculated organisms in the sample. Lowering of the pH of the inoculated sample correlated well with the rapid growth of the inoculum. At all the incubation temperatures studied, pH and odor seemed to have a close relationship; that is, all the samples with a pH at or below 4.8 had a sour smell.

Table 42 shows that under anaerobic storage at 22°C for 5 days, both enterococci and aciduric bacteria initiated growth and predominated in the flora of the control sample; neither of these types could be isolated from the freshly cooked sample. This observation indicated to us that some heat-injured enterococci and pediococci were able to resuscitate in the refrigerated control sample. These injured cells were sensitive to the selective media; however, under favorable conditions (22°C temperature, rich nutrients from the sample) they probably started to multiply and the healthy descendants appeared on the selective medium. The initial growth of both organisms in the control sample caused no change in pH. Also, anaerobic storage

Table 41. Changes in pH, microbial content and odor in samples of Braunschweiger liver sausage inoculated with organisms isolated from spoiled sausage. Samples were held under aerobic conditions

Incubation Conditions	Control			Inoculated with *Streptococcus faecalis*			Inoculated with *Pediococcus pentosaceus*		
	pH	Odor	Total aerobes/g	pH	Odor	Total aerobes/g	pH	Odor	Total aerobes/g
0 time	6.2	Typical	2.1×10^3	6.2	Typical	1.0×10^6	6.2	Typical	1.0×10^6
30°C, 2 days	6.1	Off odor, slightly putrid	ND[a]	4.8	Sour and rancid	ND	4.7	Sour and rancid	ND
15°C, 10days	6.2	Typical	1.0×10^{7}[b]	4.4	Sour	1.0×10^{10}	4.4	Sour	1.0×10^{10}
10°C, 20days	6.2	Typical	1.5×10^3	4.8	Sour	9.0×10^8	4.7	Sour	4.0×10^9

[a]No data.

[b]Which contained 90% cocci and 10% bacilli.

Table 42. Changes in pH, odor and microbial flora in samples of Braunschweiger liver sausage inoculated with organisms isolated from spoiled sausage. Samples were vacuum packaged and stored at 22°C for 5 days.

Inoculum	pH	Odor	Total Anaerobes/g	Enterococci/g	Aciduric Bacteria/g
Control (before storage)	6.4	Typical	7.0×10^{2} [a]	< 50	< 50
Control (after storage)	6.4	Typical	1.0×10^{5} [b]	5.0×10^{4}	5.0×10^{4}
Pediococcus pentosaceus	4.4	Typical	2.5×10^{8}	< 50	1.5×10^{9}
Streptococcus faecalis	5.1	Very Perfumy	2.3×10^{8}	2.3×10^{8}	1.5×10^{4}
P. pentosaceus + *S. faecalis* (1:1)	4.6	Typical	5.0×10^{8}	5.0×10^{7}	5.0×10^{8}

[a] *Bacillus* spp. only.

[b] Contained 98% cocci and 2% bacilli.

conditions did not favor growth of Bacillus spp. although they were the predominant flora in the refrigerated control sample.

Samples inoculated with P. pentosaceus or a mixture of P. pentosaceus and S. faecalis decreased rapidly in pH. The predominance of aciduric bacteria in these samples indicated that the multiplication of P. pentosaceus was the major cause of acid production.

The detection of a unique perfumy odor associated with the S. faecalis-inoculoted sample only under anaerobic conditions indicates that a somewhat different metabolic pathway may be involved compared to the aerobic growth which resulted in souring spoilage only. Another possibility is that the compound or compounds responsible for the perfumy odor could be oxidized or masked by other odors under aerobic conditions.

5. Heat resistance studies with spoilage organisms

Isolated cultures of Streptococcus faecalis and Pediococcus pentosaceus from the finished product and refrigerated, spoiled products indicated that both organisms were capable of surviving the heat processing and eventually multiplying in Braunschweiger liver sausage at refrigerated storage. Therefore, these organisms were exposed to heat to determine how well they might survive. The heat treatments chosen for these heat resistance studies for both spoilage organisms were 65°C for 3 min, 65°C for 5 min, 60°C for 30 min and 60°C for 60 min. These heat treatments were chosen for two reasons. First,

the survey of the industry indicated that an internal temperature of 65°C was the one most commonly used in the manufacturing of Braunschweiger liver sausage. Second, in general, a lower internal temperature was preferred due to the assurance of good product stability which is especially critical in products formulated with a high fat content.

The results in Table 43 show that the culture isolated from fresh and spoiled Braunschweiger liver sausage identified as S. faecalis survived all the heat treatments used in the study. The heat resistance of S. faecalis has been the subject of much investigation and discussion. White (1953) studied the influence of age and incubation temperature of the culture on heat resistance. He reported that cells in the maximum stationary phase are more heat resistant than cells in the log phase and he also observed that the higher the temperature of incubation, the greater the heat resistance. White (1963) also studied the effect of variation in pH on the heat resistance of 3 strains of S. faecalis. He reported that S. faecalis was much more susceptible to heat at both low and high pH than at pH values approaching neutrality. The most critical range of pH was between pH 5.0 and 7.0 with maximal resistance at pH 6.6. Similar findings were reported by Bagger (1926). The heat resistance, thermal injury and recovery of S. faecalis R 57 has been extensively examined by Clark et al.(1968). They reported that heating a

Table 43. Survival[a] of *Streptococcus faecalis* and *Pediococcus pentosaceus* after exposure to various heat treatments

Cell Concentration	*Streptococcus faecalis*				*Pediococcus pentosaceus*			
	65°C		60°C		65°C		60°C	
	3 min	5 min	30 min	60 min	3 min	5 min	30 min	60 min
10^8	+	+	+	+	+	+	±	-
10^6	+	+	+	+	+	±	±	-
10^5	+	+	+	+	+	±	-	-
10^3	+	+	+	+	+	-	-	-

[a] + = Survival and growth in all trials; - = No survival and growth in any trial; ± Variable survival and growth.

suspension of 10^8 cells/ml in a phosphate buffer of pH 6.8 for one hour at 60°C reduced the number of viable cells by two log cycles. With further heating for 2 hr a great number of cells was killed yet there were still about 10^3 cells/ml surviving. Although all of the surviving cells could grow on TSA medium, none of them produced colonies on TSA containing 6% salt. They concluded that heat-injured cells were sensitive to salt. In addition, the heated cells displayed a sensitivity to incubation temperature, pH and 0.1% methylene blue.

Our results (Table 43) also show that S. faecalis survives heating in the range of 60°C to 65°C. It probably would survive the temperature to which it might be exposed during processing of Braunschweiger but our evidence does not substantiate this assumption. However, meat as a heating menstrum which contains fat and protein has been shown to have a protecting effect on microorganisms (White, 1952; Precht et al., 1955). Also, Jensen (1954) states that the heat resistance of streptococci is greatly increased when oils or fats are used as the suspending medium. Therefore, the high resistance of these organisms to heat makes them particularly likely to survive the pasteurization treatment used for Braunschweiger liver sausage.

Very little information has been reported about the heat tolerance of Pediococcus pentosaceus. Gunther and White (1961) stated that P. pentosaceus was killed at 65°C in 8 min. Whittenburry (1965) observed that survival of P. pentosaceus

at 63°C for 30 min varied among different strains. Our results show that P. pentosaceus isolated from the soured samples tolerated heat treatment at 65°C for 3 min; however, survival for 5 min was inconclusive and influenced by the cell populations. Dense populations in a cell suspension produced an increase in heat resistance at both 60°C and 65°C. No survival of P. pentosaceus after heating at 60°C for 60 min was observed. S. faecalis is obviously more heat resistant than P. pentosaceus. Therefore, the possibility exits that after cooking of the Braunschweiger liver sausage, few P. pentosaceus survived or at least, has sustained more extensive injury than did enterococci. This could be an explanation for the less frequent occurrence of P. pentosaceus than of enterococci in spoiled Braunschweiger liver sausage.

6. Prediction of microbial quality of Braunschweiger liver sausage

The most frequently encountered microorganisms in Braunschweiger liver sausage after heat pasteurization include bacilli, enterococci and pediococci. Bacilli, as the major predominant survivors in Braunschweiger, are not of particular concern in the shelf life of the product since anaerobic conditions in the product and refrigeration temperatures restrict growth of many of them in Braunschweiger. On the other hand, when the product contains either enterococci or pediococci or

both as the major survivors, the shelf life of the product can be expected to be short because these cocci can grow in vacuum-packaged Braunschweiger during refrigeration. In order to obtain organoleptically satisfactory Braunschweiger liver sausage, the common practice is to cook this product to an internal temperature of not above 68°C (155°F). Attainment of the minimal numbers of bacteria in the final cooked product can be achieved only if the raw mixture does not contain excessive numbers of bacteria.

The predominance of either bacilli or cocci in freshly cooked Braunschweiger liver sausage can serve as a predicter of the microbial quality and type of spoilage that might occur in the product. Previous results indicate that a unique perfumy spoilage in Braunschweiger was correlated with the abundant growth of enterococci; putrefactive spoilage, on the other hand, results from the degradation of meat proteins and is correlated with the multiplication of bacilli. Therefore, an incubation of the homogenate of freshly cooked sausage at 30°C for 2 days which results in rapid growth of the predominant microbes was conducted to observe the relationship between microbial and nonmicrobial changes. Homogenates in water of freshly cooked sausages had a typical odor, pH of 6.2 to 6.3 and a very homogeneous appearance. After incubation at 30°C for 2 days, the odor, pH and slurry behavior of the incubated homogenates could be divided into two categories (Table 44). Category A included a perfumy

Table 44. Microbial count, odor, pH and slurry behavior of Braunschweiger liver sausage homogenates after incubation at 30°C for 2 days[a]

Sample[b]	Anaerobic Mesophiles/g	Enterococci/g on KF plates	Odor	pH	Slurry Behavior[c]	
					Water Band (mm)	Separation Description
T1	4.3×10^9	1.2×10^9	Very perfumy and sour	4.3-4.6	0-20	Blurred
T2	3.0×10^9	1.2×10^7	Perfumy and sour	4.4-4.7	0-20	Blurred
T3	1.5×10^7	$\leq 10^2$	Putrid and gassy	5.1-5.3	30-45	Distinct
T4	6.3×10^7	$\leq 10^2$	Putrid and gassy	5.1-5.3	30-45	Distinct

[a]Average or range obtained from 2 replications. Each replication contained 3 random samples.

[b]T1 cooked in 68°C water to an internal temperature of 63°C; T2 cooked in 74°C water to an internal temperature of 68°C; T3 cooked in 79°C water to an internal temperature of 74°C; and T4 cooked in 85°C water to an internal temperature of 74°C.

[c]Slurry behavior refers to the separation of water and meat residues.

odor, pH range of 4.3 to 4.7 and there was little and blurred separation of water and meat residue in the homogenate. Microbial plating revealed that enterococci predominated when these changes occurred. Category B included a putrid odor, pH about 5.2 and a distinct separation of water and meat residue in the homogenate. Bacilli predominated when this type of spoilage occurred.

A scented odor has been detected also in vacuum-packed bacon after storage at 20°C for 15 days by Cavett (1962). He correlated the growth of lactic acid bacteria including Group D streptococci, lactobacilli, pediococci and lueconostoc to this characteristic odor. He stated that the heterofermentative lactic acid bacteria and pediococci which were encountered on bacon are known to produce volatile substances during fermentation (Rogosa and Sharpe, 1959; Garvie, 1960). These substances, together with the slowly accumulating products of lipolysis, probably give rise to the characteristic scented-sour off odor in bacon.

The changes in the incubated homogenate are interpreted as resulting from growth of the specific organisms. Enterococci are able to use carbohydrates in the homogenate as a source of energy for growth. The utilization of sugars accompanied by some volatile substances and acid production resulted in the perfumy-sour odor and a low pH in the slurry. On the other hand, many species of Bacillus are proteolytic bacteria; proteolysis is associated with the production of strong putrid odors which

could mask other spoilage odors. In addition, the anaerobic metabolism of carbohydrates in meat and meat products by Bacillus spp. result in production of lactic acid, acetic acid and CO_2 (Lechowich, 1971). Both proteolytic (alkaline production) and carbohydrate degradations (acid production) of the substrates by Bacillus spp. in homogenate result in much higher pH (5.2) than the homogenate containing mainly enterococci (pH 4.6). pH has a great effect on the water retaining capacity of meat protein (Pedersen, 1971). A minimum water retaining capacity of meat protein occurs around pH 5.0-5.1 (Grau et al., 1953) which corresponds approximately to the isoelectric point of the fibrillar proteins in the normal ionic environment of meat. Therefore, it is conceivable that the behavior of the different slurries was influenced by pH values produced by the growth of specific organisms. Prolonged incubation of the putrid slurry for an additional 3 days resulted in a stronger putrefactive odor and a pH of 5.6 to 5.8; the slurry had a blurred separation between the water and meat homogenate of 15 to 20 mm.

Comparison of the results in Tables 34 and 44 enables the correlation of changes in the incubated homogenate with the organisms predominating in freshly cooked liver sausage. Thus, it is feasible to use these changes in the incubated homogenate to predict the predominant survivors, either bacilli or cocci, in the sausage. Also, it is well-demonstrated in Tables 36 and 37 that longer shelf-life is expected for the products in which bacilli

predominate but a shorter shelf-life for products with cocci predominating. These observations can be useful to some extent to evaluate the microbial quality of Braunschweiger liver sausage.

V. SUMMARY

Desirable characteristics for Braunschweiger liver sausage include homogeneous appearance, a normal pink color, delicious taste, spreadability and sliceability, acceptable fat content and long microbial shelf-life. Therefore, quality of Braunschweiger liver sausage has to be evaluated from both organoleptic and microbiological aspects. Chemical and microbiological determinations on the product can serve as a means of evaluating these characteristics.

Five processing variables encountered in the processed meat industry were observed for their effects on quality of the product. The variables investigated included liver pretreatments, nitrite levels, cooking temperatures, addition of calcium-reduced dried skim milk (CRDSM) and fat content. Organoleptic quality of Braunschweiger liver sausage was examined by taste panel evaluation; microbiological shelf-life of the product was determined using microbial counts and flora as criteria.

Pretreatment of liver had no statistically significant effect on the overall palatability of the finished products. However, Braunschweiger liver sausage made from soaked liver (soaked in 5% brine at 0°C for 1 hr) was preferred by the panel in overall acceptability, product stability and sliceability. Braunschweiger liver sausage made from scalded liver (scalded in water held at 93°C for 2 min) had the lowest score in product stability indicating that scalding of liver decreases the emulsion stability

of the product. Liver pretreatment had a significant effect ($P < 0.05$) on the color of Braunschweiger. The pinkish color intensity of Braunschweiger made from liver receiving different pretreatments decreased in the order of frozen-thawed liver, fresh soaked liver, fresh scalded liver and fresh liver.

Results of panel evaluation by both trained and consumer panels showed a significant difference in color but not in taste desirability between samples with or without nitrite. Freshly cut Braunschweiger containing no nitrite had a pale pink color which is quite different from the grey color of other meat products prepared without nitrite. The pale pink color of Braunschweiger containing no nitrite is very susceptible to oxygen and light.

Cooking treatments had a great influence on many of the sensory traits of liver sausage including texture, flavor, overall taste quality, spreadability, product stability and color. Liver sausage cooked to an internal temperature of 63° C is more desirable than those cooked to 68° C and 74° C. The product instability of Braunschweiger cooked in 85° C water to an internal temperature of 74° C was a critical defect.

The addition of 3.5% CRDSM resulted in a more desirable flavor in liver sausage. Also, a significant interaction ($P < 0.01$) between cooking temperature and the addition of CRDSM on product stability was observed.

The addition of CRDSM to liver sausage cooked at high temperature greatly improved product stability.

Fat content had a significant effect on texture, sliceability and color of Braunschweiger. Samples containing 30% fat were preferred by the panelists due to flavor and overall palatability. Samples containing 35% fat had the smoothest texture and best spreadability but least product stability and a light pink color. Samples containing 20% fat had good overall taste quality and reddish pink color. Frozen storage of Braunschweiger for 1 week made little difference in organoleptic quality when compared to the refrigerated sample. Objective measurements of color with a photovolt reflectance meter were highly correlated with visual color scores.

Product from the different studies in which the formulation was constant had a consistent composition of 54.9% water, 24.9% fat and 2.3% salt. An increased amount of fat in the raw material resulted in a decrease of water content in the finished product.

A drastic reduction (80%) in added nitrite occurred in both raw Braunschweiger mixture and cooked Braunschweiger; however, during storage at 5°C the depletion of residual nitrite was faster in the raw than in the cooked product. An increase in nitrite added to the formulation resulted in a higher residual nitrite content in the cooked

product. Residual nitrite decreased slowly during refrigeration of Braunschweiger. The addition of nitrite had considerable influence on the TBA number of Braunschweiger. No development of rancidity occurred in vacuum packed Braunschweiger prepared with 156 ppm nitrite under refrigeration for 3 months. However, when samples were packaged in Saran or LSAD film, rancidity occurred within 15 days and increased with increasing amounts of fat.

The main ingredients affecting the microbial load of the raw mixture for manufacturing Braunschweiger liver sausage included pork liver, pork trim and spice-salt mixture. Pork trim contained about 10^3 microorganisms/g and spice-salt mixture harbored about 10^5 bacilli/g. Pork liver was the most critical source and caused great variation in microbial content (10^4–10^6/g) in the raw mixture manufactured in the different studies.

Pretreatment had a significant effect on the reduction of the microbial load on the surface of pork liver. Scalding of the liver was the most efficient, followed by soaking the liver in brine and washing. On the other hand, freezing and thawing had no effect.

In general, Braunschweiger raw mixture had a pH of 6.0 and contained a microbial load in the range of 10^4 to 10^6 microbes/g. The microflora consisted of enterococci, bacilli, lactobacilli and

pediococci. At 5°C, the raw mixture underwent a souring within 4 weeks due to the growth of lactobacilli and pediococci.

Cooking resulted in an increase in pH of 0.2 unit and a decrease of microbial content of greater than 90% of the initial load in the raw mixture. The efficiency of cooking on the microbial destruction is affected by the microbial load of the raw mixture and the specific cooking temperature used. In general, the lower the microbial load in the mixture and the higher the internal temperature of cooking, the fewer the numbers of microorganisms survived. When the internal temperature was the same, the treatment with lower heat penetration rate and longer cooking time resulted in better microbial quality of the cooked product.

Freshly cooked Braunschweiger liver sausage had a pH of 6.2 and contained 10^3–10^5 anaerobic mesophiles/g. The most frequently recovered bacteria were bacilli, enterococci and pediococci. When the count was low, bacilli predominated; and when the count was high, cocci predominated. The shelf-life of pasteurized Braunschweiger is governed by many factors. During storage at 5°C, the product made from raw mixture with good microbial quality (contained about 10^4 microorganisms/g) had a shelf-life greater than 16 weeks. Not only the numbers but also the kinds of predominating survivors in cooked Braunschweiger played a decisive role in the shelf-life of the product.

In Braunschweiger liver sausage stored at refrigerated temperatures (5° C and 7° C), facultative bacilli did not grow in the product but both enterococci and pediococci multiplied and spoiled the sample. Differences in the refrigerating temperature (5° C as compared to 7° C) exerted a greater influence on the growth of enterococci and ultimate shelf-life of the product than did the differences in initial numbers.

When other variables, including raw materials, salt content, and heat treatment were the same, the use of nitrite in liver sausage demonstrated an inhibitory effect on the growth of surviving enterococci in Braunschweiger stored at 7° C. Also, the addition of increasing amounts of nitrite (50, 100 and 156 ppm) to liver sausage caused an increased inhibition on bacterial growth.

Storage of Braunschweiger at elevated temperatures (15° C and 22° C) shortened the shelf-life of the product. Braunschweiger liver sausage stored at 22° C had a shorter shelf-life than that stored at 15° C. Liver sausage that had the same microbial content but differed in the kinds of microbes predominating manifested different types of spoilage. Liver sausage in which bacilli predominated had a putrid spoilage while liver sausage in which enterococci predominated had a perfumy spoilage. The incubation of homogenate of freshly-cooked Braunschweiger produced changes in pH, odor and slurry behavior that correlated with the kind of organisms predominating.

VI. CONCLUSIONS

The following conclusions can be made in regard to the effect of liver pretreatment, nitrite level, cooking temperature, addition of calcium-reduced dried skim milk (CRDSM) and fat content on the quality of Braunschweiger liver sausage.

1. Liver pretreatment such as scalding, soaking in brine or freezing had a significant effect ($P < 0.05$) on the color of liver sausage but not on the overall palatability of the finished products.

2. No significant differences are evident in flavor of Braunschweiger liver sausage made with or without nitrite; however, the color of Braunschweiger liver sausage manufactured without nitrite is significantly different from that made with nitrite.

3. The cooking temperature greatly affects the organoleptic quality of Braunschweiger. A low cooking temperature produces a product with a creamy, smooth texture; high cooking temperatures are detrimental to product stability.

4. The addition of calcium-reduced dried skim milk (CRDSM) to the formulation enhances the flavor of liver sausage.

5. Addition of CRDSM stabilizes the product when other unfavorable or detrimental processing variables exist simultaneously.

6. Fat content has a highly significant effect ($P < 0.01$) on the texture, sliceability and color of Braunschweiger. High fat content in the product favors a creamy, smooth texture and improved sliceability, but results in a lighter pink color than in a product of low fat content.

7. Frozen storage (1 week) has little influence on the palatability of Braunschweiger.

8. An increase in nitrite added to the formulation results in a higher residual nitrite content in the cooked product.

9. Residual nitrite decreases slowly during refrigeration of cooked Braunschweiger.

10. Development of rancidity in Braunschweiger liver sausage is a function of storage time, packaging material, fat content and storage temperature.

11. Nitrite can act as an antioxidant and color-stabilizer in Braunschweiger.

12. The most frequently recovered microorganisms in cooked Braunschweiger liver sausage are bacilli, enterococci and pediococci.

13. Facultative bacilli do not grow in Braunschweiger liver sausage held at refrigeration temperatures (5° C to 7° C).

14. The predominant organisms in raw Braunschweiger are enterococci, bacilli, lactobacilli and pediococci.

15. Enterococci are common contaminants of pork liver; bacilli occur in high numbers in the spice-salt mixture.

16. In general, when the microbial count in cooked Braunschweiger is low, bacilli predominate; when the count is high, cocci predominate.

17. Refrigerated, raw Braunschweiger liver sausage usually undergoes spoilage within a few weeks due to acid production by lactobacilli and pediococci; enterococci do not compete well under these conditions.

18. During refrigeration of cooked Braunschweiger, a perfumy odor develops when enterococci reach levels of 10^7/g.

19. Pediococci are less heat tolerant than enterococci but some may survive cooking and produce a souring of Braunschweiger liver sausage.

20. Pasteurized Braunschweiger made from raw materials of good microbial quality has a shelf-life in excess of 16 weeks.

21. The presence of nitrite in Braunschweiger inhibits the growth of enterococci.

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