

INDICATORS OF SPOILAGE IN THURINGER SAUSAGE

by

SIRISOPIT SUBSOONTORN, B.S.

A THESIS

IN

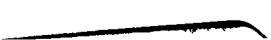
FOOD TECHNOLOGY

Submitted to the Graduate Faculty  
of Texas Tech University in  
Partial Fulfillment of  
the Requirements for  
the Degree of

MASTER OF SCIENCE

Approved

December, 1985



AC  
805  
TB  
1985  
NOV 9  
COP. 2

#### ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. R. D. Galyean for his guidance during the early part of my graduate program. Sincere appreciation is also extended to Dr. J. E. McCroskey, chairperson of this committee, for the helpful ideas which he contributed throughout this study.

Special thanks are extended to the other members of the committee, Dr. G. W. Davis, Dr. C. B. Ramsey and Dr. R. M. Miller, without whose help, support and enthusiasm this study could not have been completed. Their suggestions and recommendations throughout the process strengthened this thesis considerably.

Sincere gratitude is extended to Dr. J. R. Clark for his help with the final statistical analyses. Appreciation is also extended to Mr. L. J. Celentano for helping with the statistical analysis of the data.

The consistent and never ending support of my close friends throughout this study has been deeply appreciated.

Finally, I would like to dedicate this thesis to my parents and family. Their guidance, sacrifices and love enabled me to attain this goal.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.	. . . . .	ii
LIST OF TABLES.	. . . . .	iv
LIST OF FIGURES.	. . . . .	v
I. INTRODUCTION	. . . . .	1
II. REVIEW OF LITERATURE	. . . . .	4
Sausage Manufacturing Industry	. . . . .	4
Government Regulations	. . . . .	6
Factors Affecting Palatability in Sausage Products	. . . . .	8
Characteristics of Thuringer Sausage	. . . . .	17
Indicators of Spoilage	. . . . .	20
III. INDICATORS OF SPOILAGE IN THURINGER SAUSAGE	. . . . .	34
Summary	. . . . .	34
Introduction	. . . . .	35
Materials and Methods	. . . . .	37
Results and Discussion	. . . . .	42
Conclusions	. . . . .	66
LIST OF REFERENCES	. . . . .	67
APPENDICES	. . . . .	77
A. ANALYSIS OF VARIANCE OF THURINGER SAUSAGE TYPE, STORAGE TIME AND STORAGE TEMPERATURE ON PH PERCENT MOISTURE, FAT, ASH AND PROTEIN	. . . . .	78
B. ANALYSIS OF VARIANCE OF THURINGER TYPE, STORAGE TEMPERATURE AND STORAGE TIME ON TBA VALUES AND AEROBIC PLATE COUNTS	. . . . .	79

## LIST OF TABLES

1. COMPOSITION OF DRY AND SEMI-DRY SAUSAGES. . . . .	18
2. ANALYSIS OF VARIANCE OF THURINGER SAUSAGE FORMULATION AND FERMENTATION TIME ON PH AND TBA VALUES. . . . .	43
3. CHANGES IN PROXIMATE COMPOSITION OF THURINGER SAUSAGE DURING STORAGE. . . . .	50
4. POTATO DEXTROSE PLATE COUNTS (COLONIES/G) FOR THURINGER SAUSAGE DURING STORAGE. . . . .	62

## LIST OF TABLES

1. COMPOSITION OF DRY AND SEMI-DRY SAUSAGES . . . . .	18
2. ANALYSIS OF VARIANCE OF THURINGER SAUSAGE FORMULATION AND FERMENTATION TIME ON PH AND TBA VALUES . . . . .	43
3. CHANGES IN PROXIMATE COMPOSITION OF THURINGER SAUSAGE DURING STORAGE . . . . .	50
4. POTATO DEXTROSE PLATE COUNTS (COLONIES/G) FOR THURINGER SAUSAGE DURING STORAGE . . . . .	62

## LIST OF FIGURES

1. Changes in pH of Thuringer sausage during fermentation. . . . .	44
2. Changes in TBA of Thuringer sausage during fermentation . . . . .	45
3. Changes in pH of Thuringer sausage during storage. . . . .	47
4. Sausage pH as affected by formulation, time and temperature during storage. . . . .	49
5. Moisture content of Thuringer sausage as affected by formulation, time and temperature during storage. . . . .	51
6. Ash content of Thuringer Sausage as affected by formulation, time and temperature during storage. . . . .	53
7. Protein content of Thuringer sausage as affected by formulation, time and temperature during storage . . . . .	54
8. Lipid content of Thuringer sausage as affected by formulation, time and temperature during storage . . . . .	56
9. Changes in TBA of Thuringer sausage during storage. . . . .	57
10. MA content of Thuringer sausage as affected by formulation, time and temperature during storage. . . . .	59
11. The log mean aerobic plate counts (APC) for Thuringer sausage during storage. . . . .	61
12. PE content of Thuringer sausage as affected by formulation, time and temperature during storage. . . . .	65

## CHAPTER I

### INTRODUCTION

Food wastage is currently a major problem and is likely to remain so in the foreseeable future. Also, spoilage is a main cause of food wastage. Most foods under normal conditions of storage eventually undergo a series of reactions or changes which result in the food becoming unacceptable. Food spoilage can be regarded as any change in the nature of a fresh or processed food material whereby changes in chemical, physical or organoleptic properties of the food occur, leading to its rejection as acceptable food by the consumer.

Fermented sausage has been produced, initially as a means of preservation and more recently for the particular flavor effects achieved, without real knowledge of, or any rational effort to control, the microbiology involved. Today many producers of fermented sausage use either a lyophilized or frozen concentrate starter culture as Pediococcus acidilactici, originally described as Pediococcus cerevisiae, to achieve a much greater degree of uniformity of flavor, appearance and texture in about one-tenth the time required for the traditional process (Everson et al., 1970).

However, less than 50% of meat processors use starter cultures to produce fermented sausages (Anon, 1977), although it is probable that, on a volume basis, more fermented sausages are produced with starter culture than without. Many manufacturers of fermented sausages still use natural inoculation techniques such as "black-slopping" adding

meat reserved from a previous successful fermentation to the sausage mix) or enriching for fermentative microorganisms by aging salted meats at low temperatures. Both techniques can be successful as long as the desired microbial types are the predominant flora. However, failures may occur if nonfermentative microorganisms or heterofermentative lactic acid bacteria predominate. Addition of large numbers ( $10^4$  to  $10^8/g$ ) of desirable microorganisms will inhibit growth of undesirable species, thereby preventing or reducing fermentation failures.

A systematic investigation into the several aspects of changes occurring in fresh and stored sausages revealed that most of the contaminating bacteria were lipolytic, proteolytic or both, and proliferated rapidly in the course of spoilage. The constituent lipids of sausage can deteriorate because of atmospheric oxygen and microbial degradation (Bacus, 1984a). During storage conditions, variation in lipid composition may contribute to differences in oxidative rancidity and could have a significant influence on the stability of sausage products.

Thuringer sausage is a product requiring production of acid for safety and quality. The appropriate use of starter cultures appears to offer clear advantages for increasing the quality, productivity and safety of fermented Thuringer sausage. However, little or no research data is available to indicate the effects on pH development of the finished product.

Thus, the objectives of this study were (1) to determine if pH levels have any effect on the rate of lipid oxidation and (2) to evaluate the storage stability of Thuringer sausage (with and without starter

cultures) by determining the change in the rate of oxidative rancidity, lipid profiles and microbial analyses under storage conditions.

## P4APTER II

### '-REYTEW OF LITERATURE

*SAGU*

#### aus^ege^giamrfäctijring Industry

#### Historfcal áhd 'Trésént Status

Sausage is one of the oldest forms of processed foods. Records indicate that sausage was a popular food item during Grecian and Roman eras. Independent of the European practices, the American Indian is known to have prepared a rudimentary sausage consisting of chopped dried meat mixed with dried berries and pressed into a cake (Miller, 1975).

By the Middle Ages, sausage making was extensively practiced on a commercial scale in many places throughout Europe. Climatic conditions of some geographic areas also were responsible for further differences in sausage varieties.

Due to popular acceptance of some of the sausage varieties, many operations grew into substantial businesses. As markets grew, some products became associated with the village or city from which they originated. *For Exampl.* Berlin became known for its Berliner sausage, the province of Thuringia for its Thuringer sausage, etc. *KQliobuyy-and Qy'awfitonf-IQGQ'.*

Preparation and spicing of various sausages became an art in these Mediterranean countries and later in upper Europe. These meat processing operations grew rapidly and have led to the development of our current semi-dry and dry sausages. Nowadays, both fermented dry and semi-dry sausages represent a relatively small portion of the sausage

consumed in the United States. In 1981, the USDA stated that total sausage manufactured was about 2.3 billion kilograms. In Qdditi-^r^IZ^ ^att4-43^:(>f^TnijhityTrtn^T >gQm&A«em ilry .aa^e»n^dry sausages, respectively." (AMT-195^d).

Fresh sausage, cooked immediately prior to consumption, accounts for 0-AO bTlH-^Hl'Wlugi'mii-er 21.6% of the sausage manufactured in USDA inspected establishments. Cooked sausage that is generally sold "ready to eat" is the largest category at approximately H4&-térHfû"kt1ograms a¥ 63.7%. Cooked specialties and nonspecific loaves amount to slightly less than-^feJJ-miTron-kr1ogi^Qff& or 7.6%. Fresh, smoked sausage is cured, uncooked sausage and accounts for 6'Q^inifHon-kito^i'aiiji-8ii the remaining 0.26%. However, the dried and/or fermented sausages still are popular and represent a growing market in the United States ^ftffctr, ~~10045~~.

### Characteristics of Sausage

Sausages may be loosely classified into three general categories: fresh, cooked or smoked, and dry. Some sausages do not fit any of these categories ^{•S^Msbcrry^nd C¥mpton, 1960). The dry and semi-dry types depend on bacterial fermentation for tangy flavor. ARI'(I:9-82b) has ^deffned-the-€€^eferies—for-tfry--af i s «mi-4i::y-s «w^ges:

Dry sausages are defined as chopped or ground meat products that, as a result of bacterial action, reach a pH of 5.3 or less and are then dried to remove 25 to 50% of the / 'A moisture to have a moisture to protein ratio no greater than 1.2.3 to 1.0.^ /

Semi-dry, fermented sausages are defined as chopped or ground meat products that, as a result of bacterial action, reach a pH of 5.^3 or less and undergo up to 15% removal of moisture^during the fermentation and heatin^ processes. In general, the semi-dry sausages are not subs&quently dried in a dry room, but they are packaged soon after fermentation/

heatingz-process is completed. They are normally smoked during the fermentation cycle and have moisture to protein ratios no greater than 3.7 to r."0. ^

in-the-nase of Thuringer sausage, it is smoked, but it is not Cooked other than by the heat applied in the smokehouse (Salisbury and C-ranvptcmi 1960). **H#evert** Both dry and semi-dry sausages are manufactured by formulating the meat, spice and cure components at cold temperatures, stuffing into the proper casings, incubating the meat at higher temperatures (21 to 43 C) for fennentation, and subsequently drying the products at temperatures of 10 to 21 C (**Duuwri- IQ QHL**). Btii\*4fí§-the irret\*fe€i^4en-s\*a^-,-lactic acid microorganisms reduce the meat product pH-fjcamS^WZ to 4.8-5.3. -This fermentation encourages the product subsequently to release moisture more uniformly and rapidly. Furthermore, the lactic acid serves to denature the meat protein, resulting in a firmer texture. Due to inadequate fermentation of the sausage product, "case hardening," "ring" and "coUaping" of surface, product spoilage, off-flavors, and a potential health hazard may result.

## **Government Regulations**

The Congress directed the Secretary of Agriculture to provide for the enforcement of such standards and to establish standards of

wholesomeness. Federal inspection of the preparation of all meat and meat food products in interstate commerce was established under the Meat Inspection Act approved by Congress in 1906 and amended in 1907. Also, most state laws regulating the manufacture of sausage are based on the Federal Pure Food and Drugs Act, and frequently they incorporate much of the phraseology and many of the provisions as set out in the Act or in the standards and definitions issued by the Secretary of Agriculture under the authority of the Meat Inspection Act.

To guard against adulteration; the USDA published federal regulations to dictate the formulation and processing parameters of meat-products (USDA 1970). The USDA also recognized sausage having a moisture/protein ratio of 3.1 or less, and a pH of 5.0 or less, as not requiring refrigeration^ (USDA 1970).

Using specific microorganisms for fermentation, perishable foods such as rice, wheat, sorghum, corn, soybeans, black gum, -eas^ va, -t rrtr; milk, eggs, fish and meat can be converted to products with more desirable nutritive value, digestibility, appearance and shelf-life than original raw materials. Future research should include more work with the fermentation of indigenous foods to permit higher consumption. The United Nations World Hunger Program confirmed at a recent workshop that food fermentation could become more important as means of food preservation and food conservation^ (Xi^, 1979)."

(Unfortunately, little or no government regulation is available to qualify the fermented sausage products, especially dry and semi-dry sausages. The cafGty-mQrgit, irøwøvbir, dffmted vifi cuitu^ed meat fermervtation' Kas-beea-well documented (Bacus and Brown, 1981) and-if^in not tye discussed in^ Tl.

*include form r/x'~~~~~>>-^  
for purchasing sterilized  
res for quantity, bulk packages, indiv., etc.  
\*(\*\* ^ V^A refrig. or freez*

## Factors Affecting Palatability in Sausage Products

### Animal Tissues

Most sausages, except for English and pork sausages, contain about 85 to 90% (or higher) meat (including fat) content (Wilson et al., 1981). Many of the different animal tissues also will vary in their moisture-to-protein ratio and fat-to-lean ratios and in their relative amounts of pigment. Furthermore, they will vary in their water holding capacity (WHC). Skeletal tissues from the beef animal include bull meat, shank meat, chucks and boneless cow meat which are some examples of meats with high WHC. An excellent detailed review of the WHC was published by Acton et al. (1983). These researchers further said that the presence of salt (2.0 to 3.0% addition) enhanced protein hydration. Generally, the greater the salt concentration of the aqueous phase, the greater the amount of water held within the unit mass. The greater the degree of comminution (the process of reducing a material to a fine particulate state), the greater the amount of protein extraction and/or surface area created.

Bacus (1984c) stated that the relationship between WHC and pH affected meat characteristics and stability. He also said that the lactic acid generated through fermentation provided product stability and flavor while it allowed the meat tissue to release moisture more readily and uniformly because the lower pH was closer to the isoelectric point of meat protein. According to Forrest et al. (1975), in fresh meat the minimum WHC is observed around; pH 5.0-5.4, which is approximately the isoelectric point of the myofibrillar proteins in meat. Therefore, the myofibrillar proteins have maximum hydrophility. At pH values above the isoelectric point, the myofibrillar proteins develop excess negative charges. On

the other hand, the myofibrillar proteins develop excess positive charges at pH values below the isoelectric point. Both conditions result in repulsions of the filament, leaving more space for the water molecules. The initial meat pH is essential to subsequent fermentation time and final product pH. Also, meat with higher pH values will require more acid production to achieve the same end point (Acton et al., 1977).

The microbial flora of the raw meat materials may influence subsequent fermentation through either direct microbial interactions and/or indirect chemical changes in the raw material. For example, a high initial number of certain yeasts can compete successfully with the lactic acid microorganisms and retard pH drop because most yeasts are acid tolerant. According to De Ketelaere et al. (1974), a high oxygen content in the meat may induce a microbial oxidation of a portion of the available carbohydrate with the production of carbon dioxide, water, alcohols and carbonyl compounds. These situations can contribute to flavor problems.

#### Curing Ingredients

The common curing ingredients for sausage are sodium chloride ( $\text{NaCl}$ ), sodium nitrite and/or nitrate, sugar and/or phosphate. Salt is the most common and the most important nonmeat ingredient of sausage. Its functions include flavoring, preservation, and production of the proper texture by solubilization of muscle proteins, especially myosin. In sausage processing, comminution is conducted with meat in the presence of a salt concentration sufficient to give an ionic strength to induce swelling, water binding and partial extraction of the myofibrillar protein component (Acton et al., 1983). The result is the formation of a

fibrous, tacky protein sol that initially functions as a water binder and later as a fat stabilizer. Following formation of the fat dispersion within the protein sol matrix, and with the application of thermal energy during heat processing, protein-protein aggregation occurs. The aggregated filamentous network is suitably structured to entrap both water and fat.

Bacus (1984a) reported that NaCl addition increased the WHC and the swelling of meat on the alkaline side of the isoelectric point. Also, NaCl addition produces a shift in the isoelectric point of the myofibrillar proteins to a lower pH value, creating a larger net negative charge at the existing pH from the ionizable carboxyl groups of the protein. Repulsion between these negatively charged groups causes the protein to open up its spatial arrangement and an increase in hydration results (Hamm, 1960). Hydrated chloride ions are strongly attracted to the positively charged groups of the proteins, thus breaking the inter- and intra-protein salt (ionic) bridges (Schut, 1976). Therefore, increase of the effective negative charge enhances greater water attraction and binding.

Moreover, salt not only allows the lactic acid bacteria to predominate but also inhibits most undesirable microorganisms. In general, fermented sausages are formulated with 2.0 to 3.5% salt. Also, Zaika et al. (1978) said that no major differences in fermentation rate were observed up to 3% salt. He also stated that sausages containing 2 and 3% salt had desirable texture, flavor and fermentation. In addition, salt concentrations in excess of 3% lengthen fermentation time because fermentation is inhibited by increasing amounts of salt. According to Irvine

and Price (1961), more microorganisms are inhibited by salt concentration of less than 1%, whereas others can grow in saturated brines. For example, a sausage formulation with 4% salt may favor any contaminating bacterial growth, such as Staphylococcus, over the lactic acid microorganisms (Bacus, 1984a).

Sugar (dextrose and/or sucrose) frequently is used in curing to enhance flavor, a concentration level of 0.5-1.0% in many sausage products is" considered the most acceptable (Acton et al., 1977). Bacus (1984a) also stated that the type and amount of sugar could directly affect the ultimate pH of product. At the initial meat pH 6, 1% dextrose should be added to promote an adequate drop in pH (Acton et al., 1977). When fermentation was completed, these researchers further noted that the final pH was directly proportional to the initial dextrose level, up to about 0.5%. However, excess concentrations of added carbohydrate (>2%) also can reduce the rate of fermentation through the binding of the available water (Bacus, 1984a).

Nitric oxide myoglobin, a bright red color, is present in the cured product before heat processing for color development. According to Forrest et al. (1975), the color is stabilized by the denaturation of the protein part of myoglobin. Heating is the process that is used in sausage manufacture. The resulting pigment is nitroso-hemochromogen, a bright pink color. These researchers further said that nitric oxide was produced by the reduction of nitrate or nitrite. For nitrate reduction, nitrite is added directly to the cure. Moreover, many mechanisms, in which nitrite can be converted to nitric oxide, occur in animal tissues. For example, at a meat pH of 5.5 to 6.0, a portion of the nitrite that

is present as nitrous oxide decomposes to nitric oxide. Another example is nitrite being reduced to nitric oxide by the natural reducing activity of postmortem muscle tissue. However, the presence of rancid fat in sausage also can result in color instability. Furthermore, excessive amounts of nitrite can cause a greening of cured meat pigment, called nitrite burn. Nitrite not only is a precursor for cured meat pigment but also inhibits the germination of Clostridium botulinum spores, and prevents toxin production in cured meat products that are heat-pasturized during processing and require post-processing refrigeration (Bacus, 1984a).

When properly used, phosphates can be beneficial in sausages. According to Bacus (1984c), certain phosphates stabilize meat emulsions due to three functional properties. First, pyrophosphates can separate the meat protein, actomyosin, into actin and myosin. In cases of emulsification and "bind," myosin is more essential than actomyosin. Secondly, ionic strength improves water binding and is increased by phosphate anions. These anions are attracted to the positively charged protein sites in the meat tissue. The total negative charge of the proteins increases, causing the proteins to repel each other. Therefore, the proteins open up their structure, allowing more water to be contained. Finally, alkaline phosphates raise the pH of meat products, increasing the WHC and protein solubility. Phosphates may cause more uniform distribution of fat particles in the emulsion due to the increased negative charge on the protein. They also chelate or bind divalent cations (calcium, iron, magnesium) which may assist water binding and retard oxidation. Moreover, phosphates can decrease emulsion

viscosity, lowering the temperature increase during chopping and/or emulsifying. In some fermented sausages, the addition of certain phosphates (typically, sodium tripolyphosphates or STP) can markedly affect the observed pH decline. Since phosphates raise the initial meat pH, more lactic acid is required to lower the pH to the desired end-point, and the time required will also be extended. Although the final pH in a fermented sausage containing 0.5% STP may be higher than a corresponding control, the actual titratable acidity is greater, indicating more acid has been produced.

Also, Wilson et al. (1981) stated that polyphosphates were widely used in curing to improve fat and water retention. In addition, these researchers said that both the viscous and nonviscous types of commercial blends of polyphosphates were used in the sausage industry. They normally are added in the dry form at a rate of about 0.3%. However, the action of polyphosphates is to produce a dispersion of myofibrillar components rather than a splitting of the actomyosin (Bendall, 1954; Lewis and Jewell, 1975).

### Seasonings

Seasonings is an inclusive term that can be applied to any ingredient which improves the flavor of a meat product. Furthermore, seasonings function as preservatives and antioxidants (Atkinson et al., 1947; Chipault et al., 1956). Seasonings includes spices such as black pepper, paprika, mace and cinnamon; herbs such as garlic and onion; and other substances such as monosodium glutamate (Salisbury and Crampton, 1960). Cardamon, coriander and mustard also are classified as spices. Spices are one of the factors that affect the rate of fermentation by

stimulating acid production in the bacteria. This stimulation does not result from any contaminating microflora in the spices and is not accompanied by an increase in the bacterial population. All spices, which are added, stimulate acid production to varying degrees depending on the concentration and the culture type. According to Zaika et al. (1978), sterile or nonsterile spices enhanced fermentation to the same extent. Moreover, combinations of certain spices often can yield a shorter fermentation time than individual spices alone. Not only spices but also manganese can affect acid production (Zaika and Kissinger, 1982). Fermented sausages without spices, but with added manganese (10 M), developed a similar level of activity to those sausages with added spices.

#### Process

Processing is a parameter which controls the safety and quality of sausage products. Time, temperature, humidity and smoke are four factors involved in processing. The product temperature will determine the metabolic activity of microorganisms present (Bacus, 1984a). For example, the Pediococcus strains generally are preferred for higher fermentation temperature (37 C), while the Lactobacillus starters are preferred for lower temperature processes (32 C).

Slower fermentation rates will be observed as the product temperature deviates from the optimum. Lower temperatures are utilized to control undesirable, pathogenic microorganisms. Also, slower fermentations at lower temperatures are more desirable in controlling ultimate pH and developing flavor, color and other product characteristics. However, American processors prefer more rapid fermentations at higher

temperatures, yielding a higher acidity for tangy flavor and a lower final pH (4.6 to 5.1) according to Bacus and Brown (1981). Furthermore, a higher level of humidity contributes not only to the rate of fermentation but also to uniform quality traits such as texture.

Like curing, the smoking of meats improves the appearance and imparts a taste to the sausage product (Salisbury and Crampton, 1960). Also, smoking partially preserves the sausage product by the application of bacteriostatic substances on the surface. According to Wilson et al. (1981), woodsmoke contains a wide range of chemicals such as phenols, aldehydes, acetic acid and a range of polycyclic hydrocarbons. However, all woodsmokes appear to contain less of the aldehydes, phenols and alipatic acids than the products of destructive distillation of wood (Miller, 1975). Therefore, the preservative action of woodsmoke on meat is due to the condensates containing aldehydes, phenols and aliphatic acids. He also said that sawdust from soft woods was avoided since its smoke imparted an unpleasant taste to the meat. Usually, the product is smoked until the internal temperature of the product, especially pork, has reached at least 58 C to cause destruction of possible live trichinae.

Miller (1975) and Wilson et al. (1981) stated that the amount of nitrite at the beginning of the smoking operation was reduced during the heating of the meat in the smokehouse because of inhibition of nitrate-nitrite reduction. Miller (1975) further said that the disappearance of the nitrite was due to the reaction between the nitrite and aliphatic amino group of the protein. Thus, no change in the nitrate content of the meat occurs during the smoking process. According to Jensen (1945), the residual aldehydes from smoke are not effective as mycostats.

Moreover, the curing agents are not particularly effective against mold growth. Mold, therefore, grows readily on smoked sausage products when the conditions for its growth are favorable.

Casing diameter also is essential to control ultimate pH and predict fermentation time. According to Bacus (1984a), a larger diameter product will generally exhibit a lower pH than the identical formulation processed in smaller diameter casings. Although the fermentation may initially proceed slower in a larger diameter product, due to slower heat penetration, the fermentation is more difficult to stop with subsequent heat treatment or drying.

Therefore, formulation and process uniformity determine fermentation consistency. Uniform distribution of formulation ingredients is essential to maintain a constant micro-environment within each batch and from batch to batch. Variation also can result from product "positioning" within the smokehouse and cool room.

#### Culture

Starter culture is a key element in structuring a consistent fermentation process. According to Deibel and Niven (1957), commercial cultures of Pediococcus cerevisiae (later reclassified as P. acidilactic) and Micrococcus aurantiacus were introduced to meat industries of the United States and Europe, respectively. The Pediococcus strains were added for their lactic acid production while the micrococci were employed as a controlled mechanism to reduce nitrates to nitrites to effect the curing reaction. Moreover, early researchers concluded that the predominant organoleptic characteristics of semi-dry type products, aside from the "tangy" flavor, arose from the individual

spicing, salt, sugar and meat components. Flavor contributions due to secondary microbial actions appear to be insignificant.

Most semi-dry sausage products are fermented/smoked at higher temperatures which favor the Pediococcus strains. Normally, meat culture "activity" refers to the relative ability of the starter to produce lactic acid in a designated meat system. The pH rate decline in meat increases with increasing culture activity.

According to Bacus (1984a), an effective culture addition can be achieved when fewer total batches and "culture units" are involved. It is important to avoid direct contact between the viable microbial culture and the curing components (i.e., salt and nitrite) which may reduce the culture viability and activity. Usually, cultures are available in a concentrated form and these are diluted with water to obtain uniform distribution to maximize the benefit of employing a starter culture. Initially, the available starter culture had an optimum growth temperature (35 to 40.6 C), higher than was traditionally employed in the fermentation phase of the process. As a result, the fermentation temperature was accompanied by a rapid acid production. He also said that the rapid fermentation could yield a strong acid flavor and odor.

#### Characteristics of Thuringer Sausage

##### Proximate Composition

In 1928, Singler published the composition of samples collected by the inspectors in meat packing plants under government inspection. The meat products also can be characterized, and indirectly are regulated in the United States, by final moisture content and/or moisture to protein ratio (Terrell et al., 1978). These researchers noted that Lebanon

bologna was unique in that it had a high moisture content (55 to 60%), very low pH, and high sugar and salt content. Moreover, both Thuringer Cervelat and summer sausage may have the same composition (table 1).

Many factors affect the variability in composition of sausage products. Type of animal, preslaughter treatment, prerigor processing, and postrigor treatment are related to meat quality (Priestley, 1979). The handling of meats prior to the manufacture of sausages may further contribute to differences in moisture content. Of course, all meats that are chilled lose water during the process. Later storage for longer periods also will allow water to be lost. Trimmings not properly protected may lose moisture. Furthermore, frozen meats have poorer binding qualities than meats that are not frozen (Moulton and Lewis, 1970).

TABLE 1  
COMPOSITION OF DRY AND SEMI-DRY SAUSAGES<sup>1</sup>

Component	Thuringer Cervelat. summer sausages	Genoa salami	Lebanon bologna
Moisture, %	50	36	56
Fat, %	24	34	16
Protein, %	21	22	22
Salt, %	3.4	4.8	4.5
Sugar, %	0.8	1.0	4.1
pH	4.9	4.9	4.7
Total acidity	1.0	0.79	1.3
Yield, %	90	70	93

<sup>1</sup>From Terrell et al. (1978).

Using *lactobacillus*-*Micrococcus* and *Lactobacillus* starter cultures, respectively, Dierick et al. (1974) and Eskeland and Nordal (1980) showed that the level of free amino acids in European-style sausages increased during ripening. Eskeland and Nordal (1980) further found an increase in the digestibility of the meat proteins after fermentation and ripening. It was not clear if the changes found in dry sausages are the result of starter culture activity or other factors because these workers did not simultaneously study nonfermented sausages or sausages containing inactivated starters. However, Niinivaara et al. (1964) concluded that the increased free amino acid level found in dry sausages was produced by the action of meat tissue enzymes since the free amino acid pattern was similar in the sausages produced with or without a *Micrococcus* starter culture. Proximate composition of the sausage chubs, however, was not affected by air changes during fermentation, nor were any differences detected, either before or after heat processing, in proximate composition between starter culture and natural flora sausage chubs (Townsend et al., 1983).

Although the direct contribution of microbial cultures to the nutritive value of meat products has not been extensively studied, the prolonged stability of fermented meats certainly allows for greater consumption of a perishable raw material. This natural preservation system also precludes alternative means of preservation such as extreme heat and chemicals and that may reduce nutritive value. For example, cooking also causes fat hydrolysis (Harris and Lindsay, 1972) and reduction in phosphorus content of phospholipids (Lee and Dawson, 1976). Moreover, the effect of heat on protein (include thermal denaturation)

was explained by Tanford (1968). According to Priestley (1979), the predenaturational change that myoglobin undergoes at temperatures below the denaturation temperature partially exposes the hematin group and allows it to react with other proteins to form the brown "cooked-meat hemoproteins" of cooked meat. However, proteins may undergo further reactions following excessive heating, and Bjarnason and Carpenter (1970) made a study of some of these reactions. After heating proteins (containing about 14.1% water) at 115 C, the only changes noted were an appreciable loss in cystine content and some loss of lysine with concomitant evolution of hydrogen sulphide and ammonia. In the case of fresh or cured meat, a relatively mild heat treatment may be employed to pasteurize (Priestley, 1979). Not only heat process but also drying process affected the protein value. Kiernat et al. (1964) also reported that the higher protein value of many fermented meats generally resulted from the drying process that was consistently achieved through controlled fermentation.

#### Indicators of Spoilage

##### Composition of Meat Lipids

Normally, lipids in meat can be divided into three categories—subcutaneous (depot fat), intramuscular (tissue lipids), and intermuscular (Love and Pearson, 1971). All skeletal muscles contain fat depots and the amount and composition of fat varies quite markedly with the type of animal. However, most of the lipids in the depots are neutral triglycerides, i.e., glycerol esters of fatty acids. The most abundant triglyceride probably contains one palmitic (C16:0) and two oleic (C18:1) fatty acid residues and the next most abundant contains one

residue each of oleic, palmitic and stearic acid (C18:0) (Priestley, 1979). According to Kuchmak and Dugan (1965), the saturated fatty acids usually were found at the alpha position in the phosphoglycerides.

Structural fat, mainly phospholipids, glycolipids and cholesterol, form an integral part of biological membranes, which act as barriers between one body compartment and another and also are the sites of production of many biochemical substances important in metabolism. The triacylglycerols are stored in specialized cells, the fat cell, in adipose tissue (Gurr, 1984). Moreover, the tissue lipids exist in close association with protein and contain a large percentage of total phospholipids (Watts, 1962).

Also, most of the lipid material in the cell is associated with the membranes and these materials are rich in phospholipids. The phospholipid content of a membrane varies from one membrane to another. About 90% phospholipid in the lipid fraction of mitochondria and about 50% phospholipid in the lipid fraction of plasma membrane were reported (Fleischer and Rouser, 1965; Kidwai et al., 1973). The phospholipid content of various meats and muscle tissues has been published by Hornstein et al. (1965), O'Keefe et al. (1968) and Priestley (1979). Hornstein et al. (1961) also said that pork muscle contained about 0.7 to 0.9% phospholipid, while beef muscle contained about 0.8 to 1.0%. However, the amount of total lipids and neutral lipid are more variable than the amount of phospholipid (Hornstein et al., 1967; O'Keefe et al., 1968; Priestley, 1979).

The major phospholipids are phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin), phosphatidyl serine, phosphatidyl inositol, and some of the acidic glycerol phosphatides.

especially cardiolipin (Hultin, 1976). Moreover, lecithin and cephalin were found in greatest quantity in beef, pork, chicken and fish muscle (Kuchmak and Dugan, 1963; Shuster et al., 1964; Keller and Kinsella, 1973; Lee and Dawson, 1976).

Mainly, the fatty acid composition of phospholipids, not neutral fat, contained two or more double bonds (Hornstein et al., 1961). Igene and Pearson (1979) further stated that 27.5% of phospholipid fatty acids contained two or more double bonds compared to 2.1% of triglycerides. O'Keefe et al. (1968) reported that fatty acids C20:3 and C20:4 were present in bovine muscle phospholipids but had no counterparts in the neutral fat. Similarly, C14:1 was present in the neutral fat fractions, but it was absent from the phospholipids.

In chicken muscle, phospholipids have highly unsaturated fatty acid content and high levels of some free fatty acids, especially arachidonic and linoleic acids (Lee and Dawson, 1973). Thus, the differences in fatty acids between the phospholipid and neutral lipid fractions may result in varying rancidity.

As well as producing fat of undesired appearance and texture, an overabundance of unsaturated fat limits the storage life of meat by oxidative breakdown of unsaturated fatty acids, leading to the production of peroxides and eventually to rancidity (Gurr, 1984).

#### Lipid Oxidation in Meats

Lipids become rancid by slow autoxidation during storage. Undesirable odors and flavors result from the oxidation of lipids, frequently accompanied by the discoloration of pigments. Oxidation has been one of the major problems in maintaining high market quality.

This is the case with pork, particularly in excessively lean meat, because of its larger proportion of polyunsaturated fatty acids that are readily susceptible to oxidative breakdown (Gurr, 1984).

The accumulation of unesterified (free) fatty acids (FFA) during storage is a significant meat quality problem. FFA may undergo oxidation and cause off-flavors or off-odors, cause concurrent destruction of some vitamins and amino acids, and the carbonyls may react with proteins, resulting in changes in texture and impairing WHC of the muscle proteins (Cummings and Mattill, 1931; Burr and Barnes, 1943; Siedler and Schweigert, 1954; Kummerow, 1962; Lea, 1962; Gurr, 1984).

According to Anderson and Steinberg (1964), Dyer (1968), and Love and Elerian (1974), the FFA may interact directly with muscle protein causing denaturation and adversely affecting the WHC and texture of fish muscle. Moreover, Watts (1961) showed that the fatty acids of the phospholipids were the principal sources of oxidative rancidity in mullet. Olley and Lovern (1960) and Olley and Watson (1962) also demonstrated that, in cod and herring during frozen storage, a close relationship existed between the decrease of phospholipids and the accumulation of FFA. Hydrolysis of phospholipids accounted for roughly 75 and 45% of the FFA in white and dark muscle of herring, respectively, and lecithin was the principal phospholipid hydrolyzed (Bosund and Ganrot, 1969).

The oxidative deterioration in freeze-dried meats was postulated by Harper and Tappel (1957). However, deteriorative reactions occurring in freeze-dried meats may not be the oxidative rancidity reactions typical of fresh and frozen meats (Tuomy et al., 1969). These researchers also stated that the correlations between oxygen uptake and flavor

deterioration indicated the desirability of low levels of oxygen in the storage atmosphere of freeze-dried meat items.

Lower levels of lipid oxidation have been observed in cooked, cured meat than in uncured meat. Lipid oxidation occurs *very* slowly when the meat pigment is in the pink, cured, ferrous form. However, the pigment is converted to the brown ferric form, accelerating oxidation and resulting in increased thiobarbituric acid (TBA) values (a measure of rancidity) during storage of cured meat (Younathan and Watts, 1959).

Treatments which retard lipid oxidative are effective in preventing pigment breakdown. According to Zipser and Watts (1967), frozen, cured, cooked pork samples may be protected from salt-catalyzed lipid oxidation with sodium tripolyphosphate and 0.108% sodium ascorbate. These researchers further found that increases in lipid oxidation were related to the loss of ascorbate in the stored samples.

The off-flavor which occurs following the cooking of meats generally is called "warmed-over" (WOF), according to Tims and Watts (1958). An excellent detailed review of WOF was published by Pearson et al. (1977). WOF usually is accepted as being due to oxidation of the intramuscular phospholipid fatty acids. Oxidation proceeds at a very rapid rate following heating of the meat due to denaturation of the heme proteins and destruction of cellular structure thereby permitting intimate mixing of cellular constituents. The reaction probably is catalyzed by both heme and nonheme iron. Recent research indicates that nonheme iron is of substantially greater importance to WOF development in most meat products than the heme catalysts.

However, hematin-catalyzed lipid peroxidation has been demonstrated as an essential deteriorative reaction in unsaturated fat (Tappel, 1955) and precooked meats (Younathan and Watts, 1960). Also, inorganic ferrous ion is a catalyst of unsaturated lipid peroxidation in mitochondria and microsomes as well as in pure unsaturated lipids (Ottolenghi, 1959). The pro-oxidant contribution of metals in meat and meat products has not been widely investigated.

Wills (1966) presented evidence that both types of catalysts, heme-protein and nonheme iron, functioned in rat tissue. The two types could be differentiated by their relative activities at different pH and in the presence of chelating agents, ascorbic acid and thiol compounds. Moreover, Wills (1965) noted that the catalytic role of iron and ascorbic acid was an important nonenzymic mechanism for lipid oxidation in tissue. He further indicated that sufficient iron was present in tissue particulates to bring about such catalysis. Ethylene diamine-tetraacetate (EDTA), however, inhibited lipid oxidation during storage, presumably by its demonstrated effect on nonheme iron catalysis.

Ascorbic acid also inhibited lipid oxidation, probably indirectly by keeping the heme pigment in the catalytic inactive ferrous state. The possible involvement of enzymatic catalysts in lipid oxidation of fermented sausages is eliminated, because animal tissues lack lipoxygenase, the only known enzyme catalyzing the direct reaction of unsaturated fats with oxygen (Tappel, 1953). It has been suggested that oxidative deterioration of the lipids in meat has been caused by the catalysis of hematin compounds, namely, MetMb (metmyoglobin). Metals have been investigated as pro-oxidants in meats. Moskovits and

Kielsmeier (1960) stated that the contaminating iron in sausage exerted a powerful pro-oxidant activity.

Another factor which influences the oxidative rancidity in meat is pH. Keskinel et al. (1964) indicated an inverse relationship between the pH of meat samples and the TBA number. Other investigators (Wills, 1966; Liu, 1970) established that nonheme iron catalyzed oxidation was pH sensitive and most active at acidic pH, with an optimum pH of 5.5. No catalysis was observed above pH 6.4. On the other hand, hemeprotein catalyzed oxidation is most active at alkaline pH. Liu (1965) found a highly negative correlation between Metmyoglobin Reducing Activity (MRA) and malonaldehyde (MA) formation in raw meats. Presumably, at higher pH, the reducing enzymes are in a much more active state. Oxygen is utilized by way of the electron transport system and any MetMb is reduced. In fresh meat, not in postmortem meat, MRA activity is low and is completely lost upon cooking, thus permitting hematin catalyzed autoxidation (Greene, 1971; Kwoh, 1971). Hematin catalysis also is decreased in cured meat by maintaining a reduced state through formation of a stable ferrous nitric oxide hemochromogen (Ziper and Watts, 1967). This pigment gives cured meat its characteristic red color even after cooking (Younathan and Watts, 1960; Greene, 1971). Furthermore, heme compounds are known to inhibit rather than accelerate lipid oxidation when they are present in high concentrations relative to the unsaturated fatty acids (Lewis and Wills, 1963). However, these researchers reported that hemoglobin and other hemoproteins were active catalysts of lipid peroxide formation in solution more dilute than 10  $\mu\text{m}$ , but inhibited peroxide formation in more concentrated solutions.

Emanuel' and Lyaskovskaya (1967) found that a mixture of tocopherol, ascorbic acid and citric acid was 80 times more effective in inhibiting oxidation than ascorbate alone or in combination with citric acid in the tissue fats of fish. Several condensed phosphates had antioxidant activity in cooked meat (Tims and Watts, 1958). Again, ascorbic acid acted synergistically with phosphates to protect against rancidity in meats. The inhibition mechanisms of the chelating agents, citric acid and phosphates and the reducing agent, ascorbic acid, have not been discussed.

Little information is available on what mechanisms of NaCl are responsible for oxidative changes in cured meat. Watts (1962) reported efforts to identify NaCl catalyzing the oxidation of the stored triglycerides. Moreover, the effect of NaCl on fat oxidation depends on the level of free moisture content in the system (Chang and Watts, 1950). However, the effect of NaCl on oxidation has been attributed to the action of the reactive chloride ion on lipids, or to the modification of hemoprotein catalysts of lipid oxidation (Ellis et al., 1968). Also, apparently the effect is due to an activating influence on pro-oxidants already present, rather than a direct effect (Mabrouk et al., 1960).

#### Microbiological Quality

Sausage, being finely ground meat rather high in moisture, is an excellent medium for the growth of bacteria. Anon (1975) noted that unsanitary production practices might be reflected in the total aerobic plate count of a product, but that a high aerobic plate count did not necessarily mean insanitation. However, the Center for Disease Control in the U.S. analyzed the foodborn disease data for meats (Thatcher and Clark, 1968). These data suggested that, to prevent diseases due to

meat products, a reasonable means of control is health education directed to the food service industry and the homemaker. Also, this approach, when combined with active foodborn disease surveillance, may be considered a more effective means of control than requiring microbiological standards for these meat products. The total counts on nutrient agar for possible microbiological standards in sausages was reported by Wilson et al. (1981). These researchers also stated that freshly-made sausages should have a total viable bacterial count not exceeding  $1 \times 10^8$  organisms per gram, and preferably less than one-half of this number.

An interesting aspect of starter culture use is the extension of the shelf life of meats, particularly fresh meats. Fetlinski et al. (1979) found that addition of a 50/50 mixture of Pediococcus cerevisiae and Lactobacillus plantarum extended the shelf life of both cooked and raw mechanically-deboned poultry meat as well as that of ground chicken by inhibiting growth of various spilage Pseudomonas species (Raccach and Baker, 1978; Raccach and Baker, 1979; Raccach et al., 1979). Moreover, other investigators have shown that the shelf life of ground beef and beef steaks was prolonged by treatment with various lactic acid bacteria (Reddy et al., 1970; Daly et al., 1972; Gilliland and Speck, 1975; Reddy et al., 1975).

Inoculation of fresh meats with lactic acid bacteria may be desirable from a microbiological point of view because they inhibit gram negative psychrotrophic spoilage microorganisms; however, their effect on meat quality and sensory characteristics may preclude their use. Moon et al. (1982) have reported that, while strains of Streptococcus

*lactis* and *Lactobacillus casei* inhibit growth of the spoilage *Pseudomonas* species in culture media, they were not effective in preventing spoilage of shrimp during storage in ice.

Normally, aging of meat occurs at low temperatures to control undesirable bacterial growth on the carcasses. Spraying beef carcasses with *Thamnidium elegans* prevented growth of bacteria and allowed tenderization by meat proteases to take place at elevated temperatures and humidities (Anon, 1978). However, Kotula (1982) reported that the use of *Thamnidium elegans* on carcasses stored at 4 C did not tenderize the meat. Further studies indicated that little or no proteolytic enzyme was produced by the mold at 4 C (Kotula et al., 1982).

#### Nitrosamines

The ability of meat starter cultures containing *Lactobacillus plantarum* to lower the pH of sausage meats containing a fermentable sugar, coupled with its nitrate reducing capacity, could create conditions that would lead to nitrosamine formation from secondary amines and nitrite (Smith and Palumbo, 1978). However, dry and semi-dry fermented sausages did not contain detectable levels of volatile nitrosamines (Anon, 1980). Dethmers et al. (1975), using a mixed starter culture (*Pediococcus cerevisiae* and *Lactobacillus plantarum*) with various nitrite-nitrate cures (0 to 150 ppm nitrite; 0 to 1500 ppm nitrate), did not find volatile nitrosamines in Thuringer sausages.

During smoking and subsequent processing of bacons, the lactic acid bacteria grow and use the sugar present in the cure, thereby reducing the pH of the meat. The lowered pH aids in dissipating the nitrite. In addition, such acidulated bacon (*Lactobacillus plantarum* plus > 0.5%

sucrose) did not support growth of Clostridium botulinum upon temperature abuse even though the nitrite content was low (Tanaka et al., 1980).

### Mycotoxins and Mold

The predominant flora on sausage casings in Penicillium or Penicillium and Scopulariopsis species (Bullerman et al., 1969). However, no penicillic acid was detected (at 2, 4 and 10 wk), even though the molds produced the toxin in vitro, when Ciegler et al. (1972) used pure cultures of various Penicillium species to inoculate the surface of sausages.

Undesirable organisms developing on the surface of mold-ripened salamis are members of the genus Aspergillus (Bullerman et al., 1969). To prevent growth and aflatoxin formation by Aspergillus flavus, Bullerman and his coworkers incubated sausages at low temperatures (15 C or below) and low relative humidities (below 70%). Also, smoking the salamis was an aid in decreasing aflatoxin formation. These investigators further said that the level of aflatoxin produced by Aspergillus flavus in salamis incubated at 20 C and 75% to 80% humidity was low (1 to 3 yg/g) in contrast to the level produced on rice (330 to 480 yg/g). Similarly, Strzelecki (1973) found very low levels of aflatoxin metabolites in salamis inoculated with Aspergillus flavus and stored at a variety of temperatures. Therefore, meat does not appear to be a good substrate for aflatoxin production.

### Viruses

The fate of viruses present in meat used for fermented sausage has received little attention. During the fermentation of contaminated Thuringer sausage, the concentration of coxsackie virus decreased from

7.5X10<sup>3</sup> to 1.1X10<sup>3</sup> (Hermann and Cliver, 1973). According to Kantor and Potter (1975), the starter organism, a Lactobacillus species, increased in number from 4.0X10<sup>3</sup> to approximately 10<sup>5</sup> with a concomitant decrease in pH from 6.02 to 4.8. He further observed virtually no destruction of echovirus or poliovirus during starter culture fermentation (Lactobacillus plantarum plus Pediococcus cerevisiae) and subsequent low temperature storage of both cervelat and dry fermented salami. However, the data presented by McKercher et al. (1978) indicated variable destruction of viruses during sausage fermentation.

#### Foodborn Bacteria

Normally, the undissociated lactic acid molecule is inhibitory to microorganism growth (Jay, 1978). Thus, lactic acid bacteria contribute to microbial food safety in fermented meat products because most, if not all, food poisoning bacteria are susceptible to acidic conditions (Rubin and Vaughan, 1979).

Pepperoni undergoes a shorter fermentation period at 35 C than Lebanon bologna so that neither Salmonella dublin nor Salmonella typhimurium was eliminated during sausage fermentation regardless of the fermentation method (1-day fermentation with starter culture or 2-day with natural flora fermentation). No destruction of Salmonella dublin in dried nonfermented pepperoni (final pH 5.7) occurred. According to Smith et al. (1975), destruction of Salmonella typhimurium in nonfermented pepperoni was similar to that found in natural flora-fermented pepperoni, which suggests that drying alone was effective in eliminating Salmonella typhimurium. Destruction of Salmonella dublin, however, was achieved by a combination of acid conditions and drying. Also, complete

destruction of salmonellae in pepperoni could only be assured by heating the sausages (after fermentation but before drying) to an internal temperature of 60 C.

In summer sausage, Masters et al. (1981), observed that Salmonella newport and Salmonella typhimurium were eliminated completely by use of a Lactobacillus plantarum starter culture. The rapid reduction of pH produced by the starter culture enhanced salmonellae destruction and shortened the processing time.

An enteropathogenic strain of Escherichia coli was resistant to processing conditions (fermentation by Pediococcus cerevisiae followed by drying) used for the manufacture of dry, fermented turkey sausage (Baran and Stevenson, 1975). These researchers further studied the fate of Clostridium perfringens during fermentation and subsequent drying of fermented turkey sausage using Pediococcus cerevisiae as the starter culture. They also showed that Clostridium perfringens did not grow and the numbers of viable cells decreased during processing. However, a small population of Clostridium perfringens persisted in the final product. Whether the decrease in Clostridium perfringens population depended on a lowered pH brought about by the starter culture (the designated pH of the sausages was not given), or was due to drying or to a combination of the two processes was not known. In general, the acid conditions found in fermented sausages appear to limit growth and toxin production by clostridial species.

At a ratio of Pediococcus cerevisiae to Staphylococcus aureus of 10<sup>4</sup>:101, Barber and Deibel (1972) found a limited amount of staphylococcal growth at the surface of summer sausages, but no staphylococci were found in the core of the sausages. Moreover, Baran and Stevenson

(1975) found that the numbers of Staphylococcus aureus increased during processing of a dry fermented turkey sausage when Pediococcus cerevisiae was used as a starter culture.

According to Niskinen and Nurmi (1976), the Micrococcus species was responsible for inhibition of enterotoxin production. Staphylococcal counts of  $10^8/g$  or less would not be expected to result in enterotoxin production; Barber and Deibel (1972) reported that counts must be  $10^8$  to  $10^9/g$  for detectable enterotoxin production.

## CHAPTER III

### INDICATORS OF SPOILAGE IN THURINGER SAUSAGE

#### Summary

Thuringer sausages (with and without starter cultures) were produced from a beef-pork formulation. These sausages were tested during fermentation and during storage for overall spoilage indicators at two temperatures (4 and 22 C). They also were examined after 14, 24, 34 and 44 days of storage.

The inverse relationship between the pH and the TBA number was investigated during fermentation. The proximate analyses data indicated that storage conditions affected both types of fermented Thuringer sausages. The percent ash ( $P<.05$ ) and protein ( $P<.01$ ) for those of Thuringer sausages were linear contrasts and changing as days in various temperature storage. The quadratic contrasts ( $P<.05$ ) among formulation, time and temperature were found for moisture and lipid.

Thuringer sausage type, storage temperature and storage time caused significant differences in TBA values but not in microbial content. No significant changes in PC content were found. However, significant changes occurred in PI values during storage. Increases significantly in PE content among the three treatments (formulation, time and temperature) were observed.

In this study, TBA values showed the development of oxidative rancidity in Thuringer sausages (with and without starter cultures). The

changes in polar lipid components, especially PE, could be due mainly to the activity of all molds.

### Introduction

Fermentation is the oldest method of food preservation. As the technology of sausage fermentation has developed, the responsible lactic acid microorganisms have been isolated and reintroduced as pure starter cultures for the manufacture of these fermented sausages (Bacus, 1984b). One of the results for the expanded use of starter cultures is that the initial type and number of microorganisms inoculated into sausages has become more consistent.

According to Genigeorgis (1976), essential components of the environment are: 1) initial sausage formulation and eventual changes in pH, brine, Aw, redox potential and nitrite level; 2) temperature, relative humidity, casing type, and rate of physical and chemical changes in each storage condition; 3) the numbers of competing microflora in the sausage formulation including any starter culture; and 4) the initial numbers and types of pathogens.

Bacus and Brown (1981) stated that the specific formulation and processing conditions, as well as the type and activity of the culture employed, directly influenced the rate of fermentation and the ultimate pH of the sausage product. The initial meat pH is important in determining fermentation time and final product pH. Also, meat with higher pH values will require more acid production to achieve the same endpoint (Acton et al., 1977). Both dry and semi-dry varieties of fermented sausages do not receive extensive heating during preparation and processing.

Therefore, the safety and quality of the product depends on the rate and extent of acid production.

The aged flavor associated with many traditional cured meats is a combination of microbial fermentation, proteolysis, and lipolysis. As compared to Thuringer sausage containing nitrite and nitrate, fresh, fried or baked Thuringer sausage containing neither nitrite nor nitrate was judged most rancid and of poorest flavor and appearance quality (Dethmers et al., 1975). Oxidation is accelerated by grinding or cooking, which causes disruption of the tissue membranes (Pearson et al., 1977). Scalan (1975) reported that malonaldehyde (MA) content increased following prolonged storage. Although fat and protein degradation is associated most often with meat spoilage, the controlled development of unique, meat product organoleptic characteristics is dependent on the control of many of these degradative reactions via processing formulations, and parameters and/or specific microbial starter cultures (Bacus and Brown, 1981).

Considerable research concerning the influence of microorganisms on meat products has involved primarily starter cultures and their spoilage of the product. Little work has been done using bacteria in the development of new, safe and appealing meat products, especially Thuringer sausages which have acceptable keeping qualities.

Thus, this study was designed to: (1) determine whether pH levels have any effect on the rate of lipid oxidation, and (2) evaluate the storage stability of Thuringer sausages (with and without starter cultures) by determining the change in the rate of oxidative rancidity, lipid profiles and microbial analyses under storage conditions.

## Materials and Methods

### Experimental Design

Two batches of Thuringer sausages were used. Each experiment was established using a completely randomized block design with a factorial arrangement of treatments. The steps of experiments were divided into two parts. One part was set during fermentation. Each batch was analyzed for pH and TBA values at the same sausage fermentation time. The second part was storage. In this part, sources of variation consisted of: 1) batch types (with and without starter cultures), 2) temperature of storage (4 and 22 C), and 3) length of storage (14, 24, 34 and 44 days).

Samples of both types of sausages were analyzed for pH, percent moisture ash, protein, and lipid, and TBA value. Furthermore, the different types of phospholipids under each storage condition were investigated.

Using the Statistical Analysis System of SAS Institute (1982) and the mean separation technique of orthogonal polynomials, significance was determined by the F-test. Significant differences were accepted at the 5% level of probability.

### Sample Preparation

Thuringer sausage used was fabricated in the Meats Laboratory of the Animal Science Department at Texas Tech University. The laboratory temperature was maintained at about 7 C.

The meats (beef and pork) were allowed to partially thaw and were ground once through a 0.95-centimeter plate and thoroughly mixed with

all the other ingredients except the starter culture. The ingredients are shown below:

THURINGER SAUSAGE FORMULATION<sup>A</sup>

---

2.53 kg cow beef

1.27 kg 80/20 pork trim (certified Trichina free)

0.42 kg 50/50 pork trim (certified Trichina free)

0.12 kg salt

0.09 kg dextrose

0.09 kg cane sugar

0.01 kg coarse ground black pepper

0.0026 kg whole mustard seed

0.0053 kg ground coriander

0.0013 kg ground nutmeg

0.0013 kg ground allspice

0.0007 kg sodium nitrate

0.0003 kg sodium nitrite

Pediococcus acidilactici starter culture

---

<sup>A</sup>From Bacus (1984a).

The starter culture then was added and mixing continued for 3-4 minutes.

Both control Thuringer without starter culture and Thuringer containing starter culture were reground separately through a 0.32-centimeter plate and stuffed into pork casings. These two types of sausages were fermented at room temperature (22 C) to a 5.3 pH level. Each type was analyzed for pH and TBA values during fermentation. When the pH reached 5.3, the sausages were heavily smoked until their internal

temperature attained 57 C. Approximately 3 hr of smoking were required. About 14 kg of each type of sausage were prepared. After being smoked, sausages were allowed to stand at 7 C for 4 to 6 hr and then were chilled at 3 C.

#### Experimental Storage

The two types of sausages were stored at two temperatures (4 and 22 C) and examined after 14, 24, 34 and 44 days. At each designated day, sufficient numbers of subsamples were randomly selected, separately packed in air-tight polypropylene bags and kept at -20 C until analyzed.

Two subsamples of both types of sausages were analyzed for pH, percent moisture, ash, protein, and lipid, and TBA value. Neutral lipids and polar lipids were extracted, and a microbiological evaluation was performed.

#### Characterization of Thuringer Sausages

Percentages of protein, lipid, moisture and ash were determined by AOAC (1980) methods. TBA tests were performed by the method of Tarladgis et al. (1960). This method measures the malonaldehyde production arising from the lipid oxidation.

The pH values were determined using a Beckman Model 2500 pH meter (Beckman Instruments, Inc, Irvine, CA) with an epoxy body combination electrode (Corning Glass Works, Corning, NY). Five grams of sausage (1:10 ratio, meat to distilled water) were blended at high speed (10-15 sec) to make a smooth slurry at room temperature (22 C).

Neutral and polar lipids were isolated by solvent elution on dry columns composed of a tissue sample, anhydrous sodium sulfate, and

celite 545 diatomaceous earth ground together. Some modifications to the extraction method of Marmer and Maxwell (1981) were employed. The chromatographic column with sintered glass bottom was used for this method. Neutral and polar lipids were extracted by using dichloromethane and dichloromethane-methanol (9:1) mixtures, respectively. Solvent was removed from extracted lipid fractions in a 457 mm Hg vacuum chamber at 37±1 C.

Samples of polar lipid residues were redissolved in 5 ml of chloroform and stored at -20 C in sealed vials under nitrogen gas to protect them from oxidation until analyzed (after 1 mo) using High Performance Liquid Chromatography (HPLC).

Phospholipids in polar lipids were analyzed using a method modified from Hax and Geurts Van Kessel (1977) and Geurts Van Kessel et al. (1977). Phospholipid components were determined by using a Tracor Model HPLC with a Tracor Model 955 chromatographic pump (Traco Instrument, Austin, TX). The detector for phsopholipid components was a Tracor Model 970 variable wavelength absorbance instrument.

Samples were filtered through Whatman No. 1 filter paper (Whatman Ltd., Maidstone, Kent, UK) to remove nonlipid components. Polar lipid samples were injected using hypodermic syringes (Marshall Electronics Inc, Skokie, IL) with flat-edge needles via a 20-vl sample loop to an injection port (Rheodyne Inc., Cotati, CA). The recording system was a Hewlett-Packard 3390 A Integrator (Hewlett Packard, Avondale, PA). The column selected for this work was a y-Porasil column (30 cm X 4 mm id) which was packed with silica of 10  $\mu$  diameter particle size (Waters Assoc, Milford, MA). The mobile phase consisted of hexane/isopropanol/

water (6:8:1.3, V/V/V). The hexane and isopropanol were HPLC grade solvents (Fisher Scientific, Fair Lawn, NJ). The solvents were distilled and mixed. The solvent mix was vacuum filtered through a 0.2  $\mu$ m membrane filter disc (Micro Filtration Systems, Dublin, CA). Mobile phase flow-rate for the analysis was 1.0 ml/min. Column effluent was monitored by ultraviolet absorption spectroscopy. The wavelength of 206 nm was used for this experiment. To prevent precipitation, the system was flushed with hexane/isopropanol (9:1, V/V) after each use.

The compounds (PC, PE, and PI) were identified by comparison of retention times of each standard. The following phospholipid standards were purchased from Sigma Chemical Co., St. Louis, MO: L-a-phosphatidyl choline (PC) No. P5763, L-a-phosphatidyl ethanolamine (PE) No. P4513, i and L-a-phosphatidyl inositol (PI) No. P5766. Each peak area was integrated using the Hewlett-Packard 3390 A Integrator. The quantitative determination was estimated by comparison to the peak area of a known amount of the standard. Results were expressed as mg/100 g of sample weight.

Cross-sectional slices of sausages (50 g) were microbiologically analyzed at 14, 24, 34 and 44 days of storage at 4 and 22 C. Total aerobic plate count (TPC) was determined on Plate Count Agar (PCA) incubated 48 hr at 37 C. Fungi were determined on potato dextrose agar (PDA, pH 3.5) incubated at room temperature (22 C) for 3 days. All techniques were performed using the procedure described by Speck (1976). Serial dilutions of the sample were plated in duplicate on plate count agar and potato dextrose agar to enumerate the total viable bacteria and fungi, respectively.

### Statistical Analysis

Data were analyzed by the general linear model procedure and orthogonal polynomials using the SAS method (SAS Institute, 1982). Analyses of variance for a 2 X 5 factorial design were performed to study the main effects (formulation and time) during fermentation. The main effects (formulation, temperature and time) of storage were analyzed in a 2 X 2 X 4 factorial design.

### Results and Discussion

#### Characteristics of Thuringer Sausage during Fermentation

##### Changes in pH and TBA Values

Eighteen hours of fermentation were required to reach the designated pH of 5.3 in the sausage containing the starter culture. The rate of the pH decline in meat products depends on several factors including species, genetic characteristics, and stress to the animal prior to and during slaughter (Bacus, 1984a). In fermented sausages, the decrease in pH values is due to microbial activity (Acton and Keller, 1974). Bacus (1984a) further said that pork consisted of higher levels of niacin and thiamine, resulting in more rapid fermentation. He also noted that pork and beef products fermented at a faster rate than their all-beef counterparts. In addition, beef generally will have a higher initial pH and greater buffering capacity than pork.

Table 2 shows the analysis of variance summary table for the effects of sausage formulation and fermentation time on pH and TBA values. Both pH and TBA values were affected by sausage formulation, fermentation time and the interaction of sausage formulation with fermentation time ( $P < .01$ ).

TABLE 2

ANALYSIS OF VARIANCE OF THURINGER SAUSAGE FORMULATION  
AND FERMENTATION TIME ON PH AND TBA VALUES

Item	Source of variation <sup>a</sup>		
	S	T	S X T
pH	0.08(1)***	0.13(4)**	0.03(4)**
TBAb	0.39(1)**	0.40(4)**	0.04(4)**

<sup>a</sup>Mean squares (degrees of freedom) for S = Sausage type (with and without starter cultures) and T = fermentation time (6, 9, 12, 15 and 18 hr).

<sup>a</sup>TBA = thiobarbituric acid value.

..  
P<.01.

As shown in figure 1, formulation and time interval changes had a significant effect on the rate of pH reduction. A cubic contrast was observed between the pH values and the fermentation times of both sausage types ( $P<.05$ ).

The rate of decrease in pH was significantly greater for Thuringer sausages containing the starter culture (Formulation 1) than those containing only the natural flora ( $P<.05$ ). Initial pH of Thuringer sausages with starter culture was 5.79 which decreased to 5.27 after 18 hr of fermentation. The initial pH of Thuringer sausages without starter culture was 5.80 and decreased only to 5.60 after 18 hr of fermentation time. Thus, pH of Thuringer sausages with starter culture decreased 0.52 unit while that of Thuringer sausages without starter culture decreased 0.20 unit.

TBA values increased during fermentation at 22 C (figure 2), indicating that oxidation occurred. A significant quartic contrast was observed between the TBA values and the hours of fermentation time ( $P<.05$ ). According to Cross et al. (1978), the threshold value at which

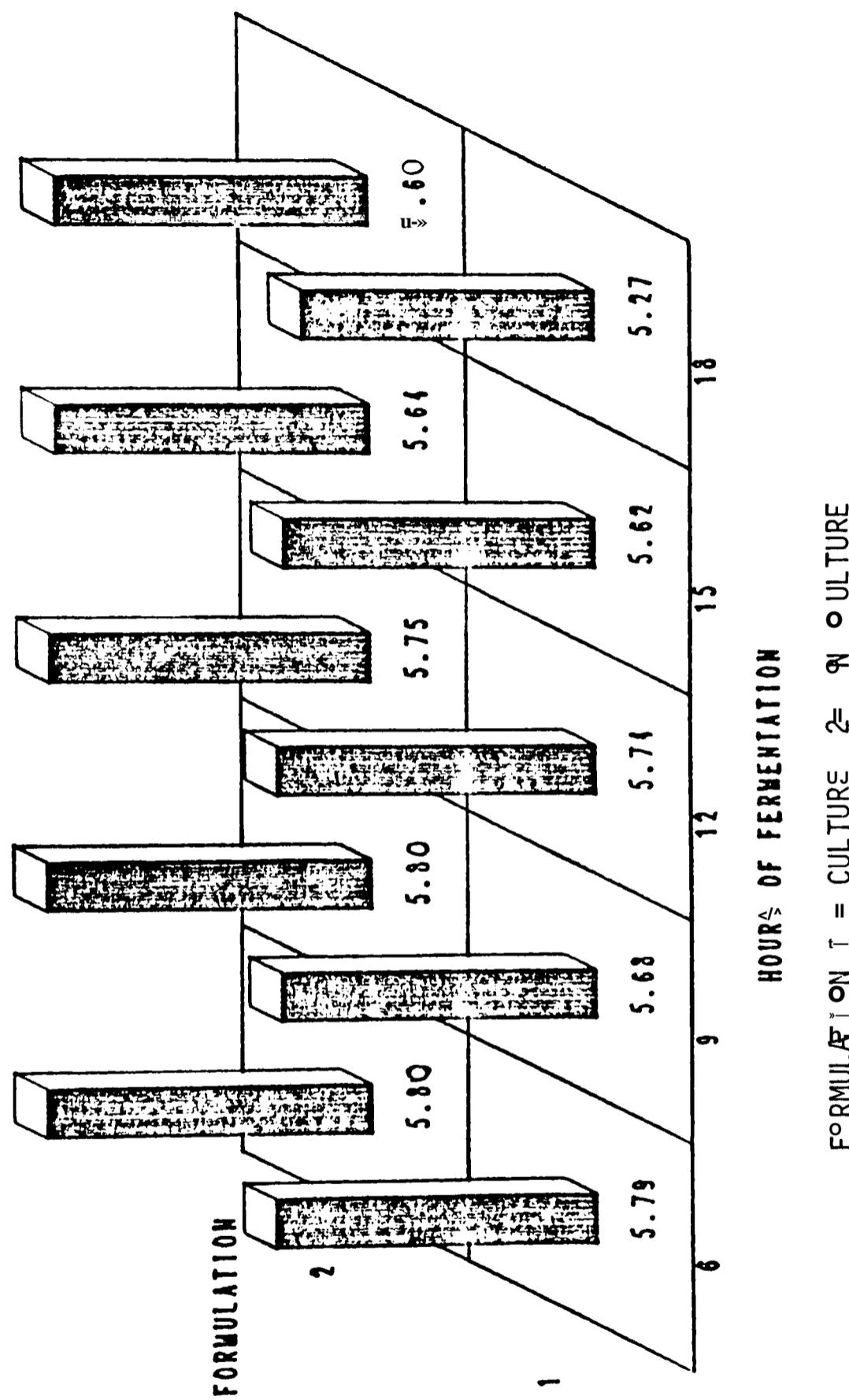


Fig. 1. Changes in  $\text{pH}$  of two cultures during fermentation.

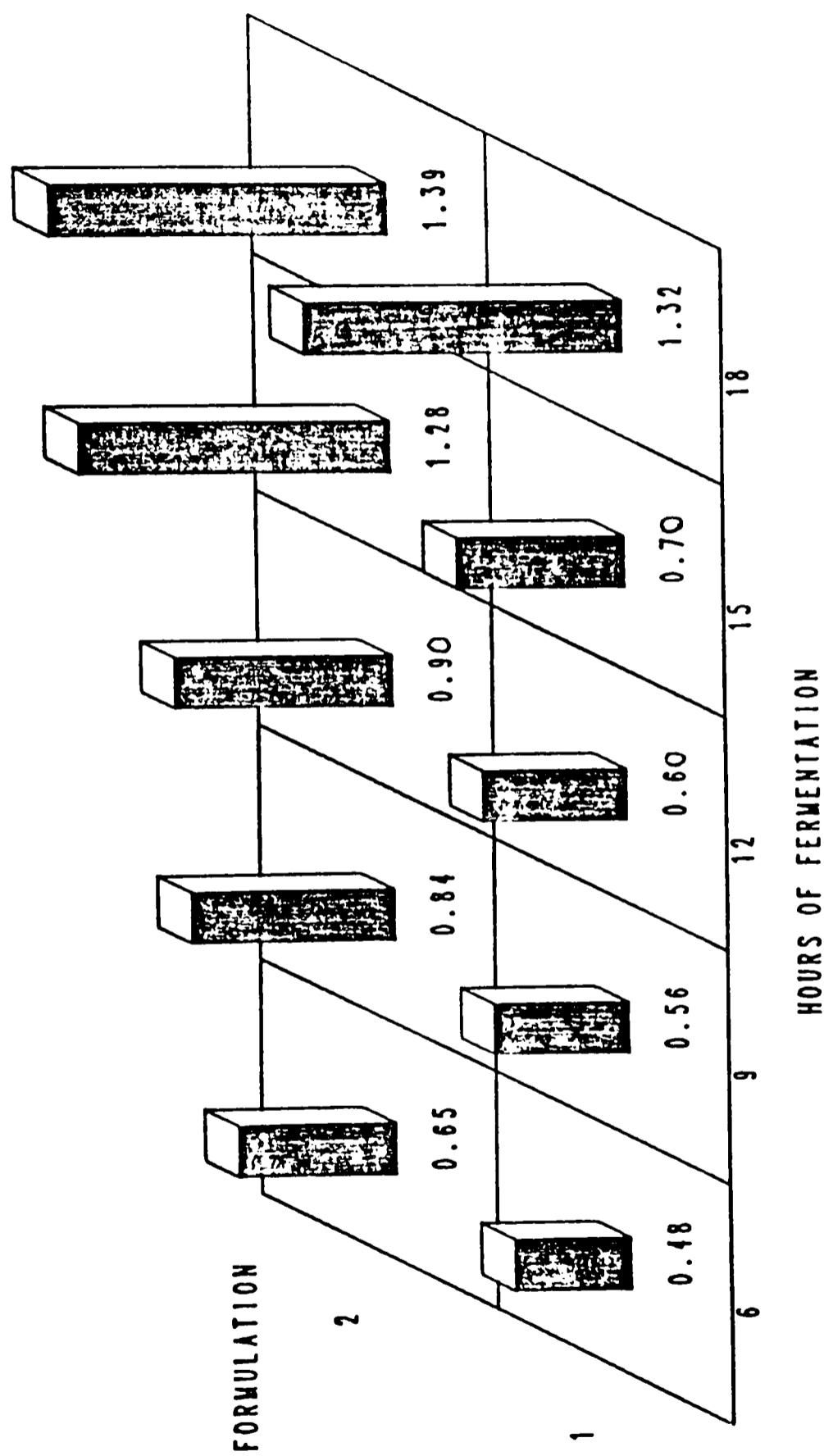


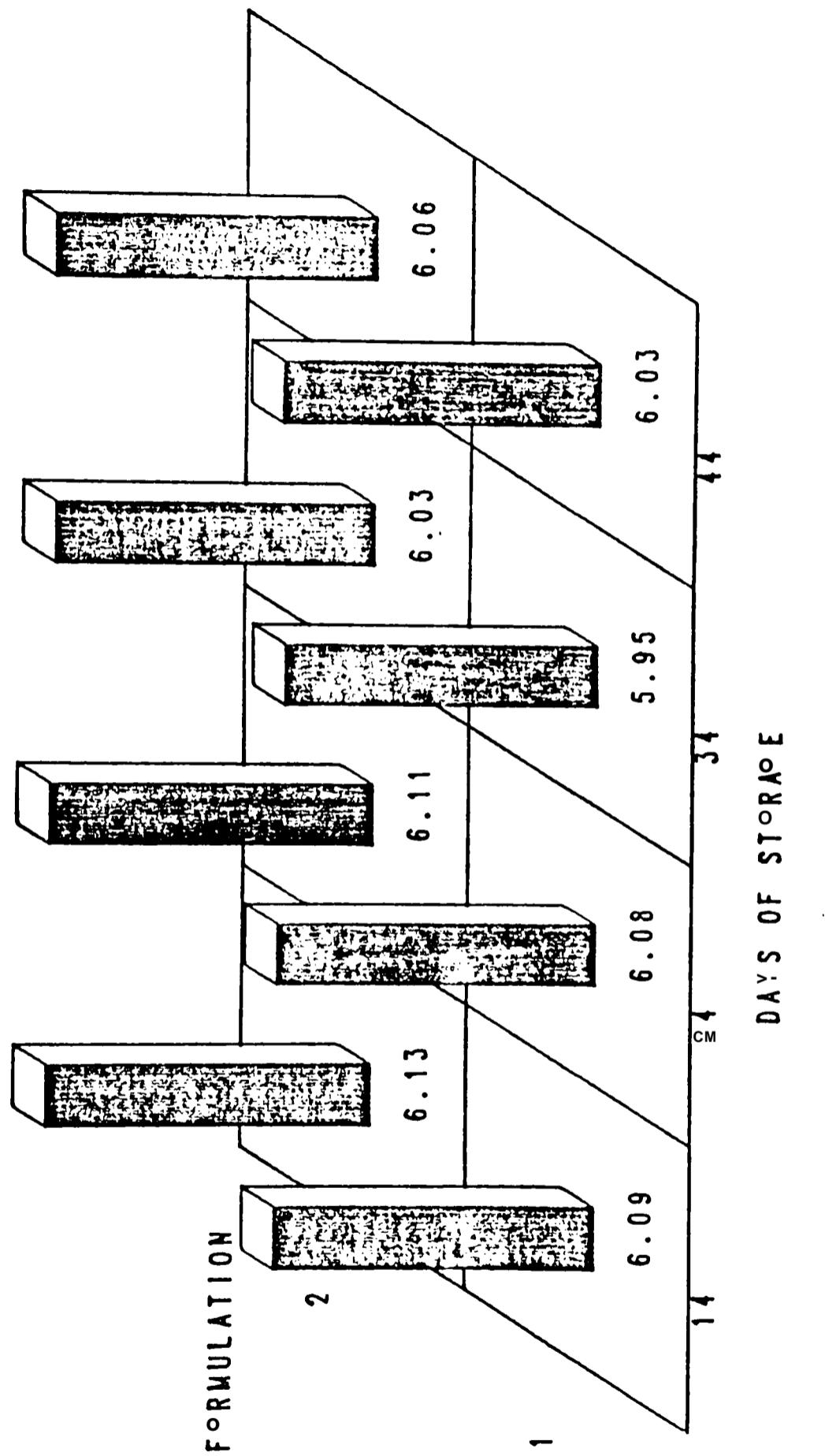
Fig. 2. *Concentration of Thuringer culture during fermentation.*

rancidity could be organoleptically detected is normally a TBA value of 1.0. The TBA values of Thuringer sausages containing natural flora had a tendency to reach the threshold value after 12 hr of fermentation time, whereas in Thuringer sausages containing starter culture, the TBA values did not reach the threshold value until after 15 hr of fermentation time. The initial TBA value for Thuringer-sausages without starter culture was higher (0.65) than that for Thuringer sausages with starter culture (0.48). The pH of both sausages rose only about 0.2 unit from 6 to 15 hr of fermentation. However, during the same time, TBA value increased 0.63 unit in the sausage without starter culture but only 0.22 unit in the sausage with starter culture. Therefore, oxidative rancidity occurred more rapidly in Thuringer sausages without starter culture than in Thuringer sausages with starter culture. Both sausages reached similar TBA values after 18 hr of fermentation.

#### Characteristics of Thuringer Sausage during Storage

##### Changes in pH

As shown in figure 3, after 14 days of storage the pH of Thuringer sausages containing starter culture was 6.09, which decreased to 6.03 after 44 days of storage time. In the Thuringer sausages containing only natural flora, the initial pH was 6.13 which decreased to 6.06 after 44 days of storage time. Therefore, these decreases of less than 0.1 pH unit support the work of Acton and Keller (1974) who noted that no increase could take place in lactic acid content of the sample after heat processing or smoking because of the great destruction of microbial activity between 49 and 60 C. William et al. (1983) said that the reason for loss of lactic acid during heat processing was unknown.



FORMULATION 1 = CULTURE 2 = NO CULTURE

Fig. 8. Observations in pH of Thuringean soil stored during storage.

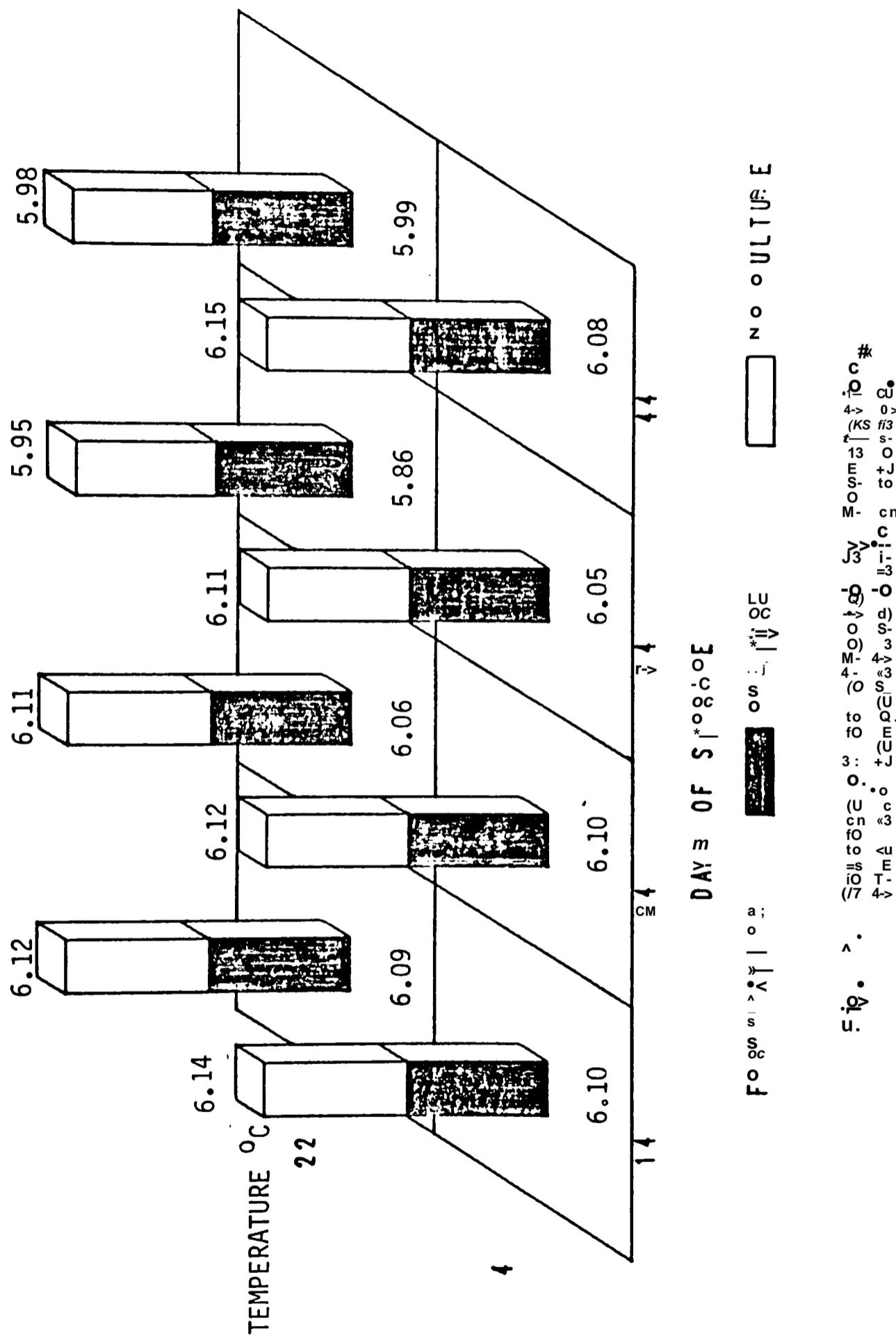
Moreover, Bacus (1984a) concluded that most yeasts were acid tolerant, the loss of lactic acid could occur after fermentation had been completed, resulting in a pH increase and the development of off-flavors during drying. He further stated that Micrococci present in the early stages of fermentation could be responsible for the complete conversion of carbohydrate. Therefore, either of these situations can result in a higher pH than expected.

Although the differences are small, their consistency caused a significant difference ( $P<.01$ ) between pH values of each sausage type (figure 3). At each time of measurement, the sausage with starter culture had a slightly lower pH than the sausage without starter culture. A significant formulation, temperature and time interaction was found.

As summarized in Appendix A, the pH values for Thuringer sausages showed a cubic contrast ( $P<.01$ ) for the main effects of storage temperature and storage time. Also, a cubic contrast was observed between the pH values and the storage time of Thuringer sausages ( $P<.01$ ). As shown in figure 4, interactions among formulation, temperature, and time indicated a quadratic contrast ( $P<.01$ ). Regardless of whether the Thuringer sausage contained starter culture or no starter culture, pH remained almost constant over time at 4 C and declined with time at 22 C. Thuringer sausage with starter culture had lower pH at 4 C, but at 22 C Thuringer sausages with and without starter cultures had the same pH at 44 days of storage.

#### Proximate Analyses

The means for proximate composition of Thuringer sausages during storage are presented in table 3. As compared to the results of changes



in proximate composition of Thuringer sausage with starter culture after 14 days of storage, Thuringer sausage with starter culture had a lower moisture, and higher ash, protein and lipid contents after 44 days of storage. Thuringer sausages without starter culture had unsteady changes in proximate composition during storage.

TABLE 3  
CHANGES IN PROXIMATE COMPOSITION<sup>A</sup> OF THURINGER  
SAUSAGE DURING STORAGE

Type	Item	Storage time (days)			
		14	24	34	44
Thuringer with starter culture	Moisture, %	29.53	28.16	26.44	25.73
	Ash, %	4.35	5.38	4.62	5.40
	Protein, %	27.29	28.18	28.62	28.95
	Lipid, %	30.74	31.20	32.11	33.59
Thuringer without starter culture	Moisture, %	28.87	27.44	28.11	27.72
	Ash, %	5.16	5.38	4.68	5.02
	Protein, %	26.08	27.43	27.71	26.70
	Lipid, %	29.31	32.41	32.26	33.08

<sup>A</sup>Expressed as percentage of wet sample weight.

For moisture, cubic contrasts between time and temperature ( $P<.01$ ) and between formulation and time ( $P<.05$ ) were found. A three-way interaction existed among formulation, time and temperature (figure 5, Appendix A) which showed a quadratic contrast ( $P<.05$ ). The loss in moisture for both types of Thuringer sausages was higher for the treatments held at 22 than 4 C. At 14 days of storage, moisture content for Thuringer

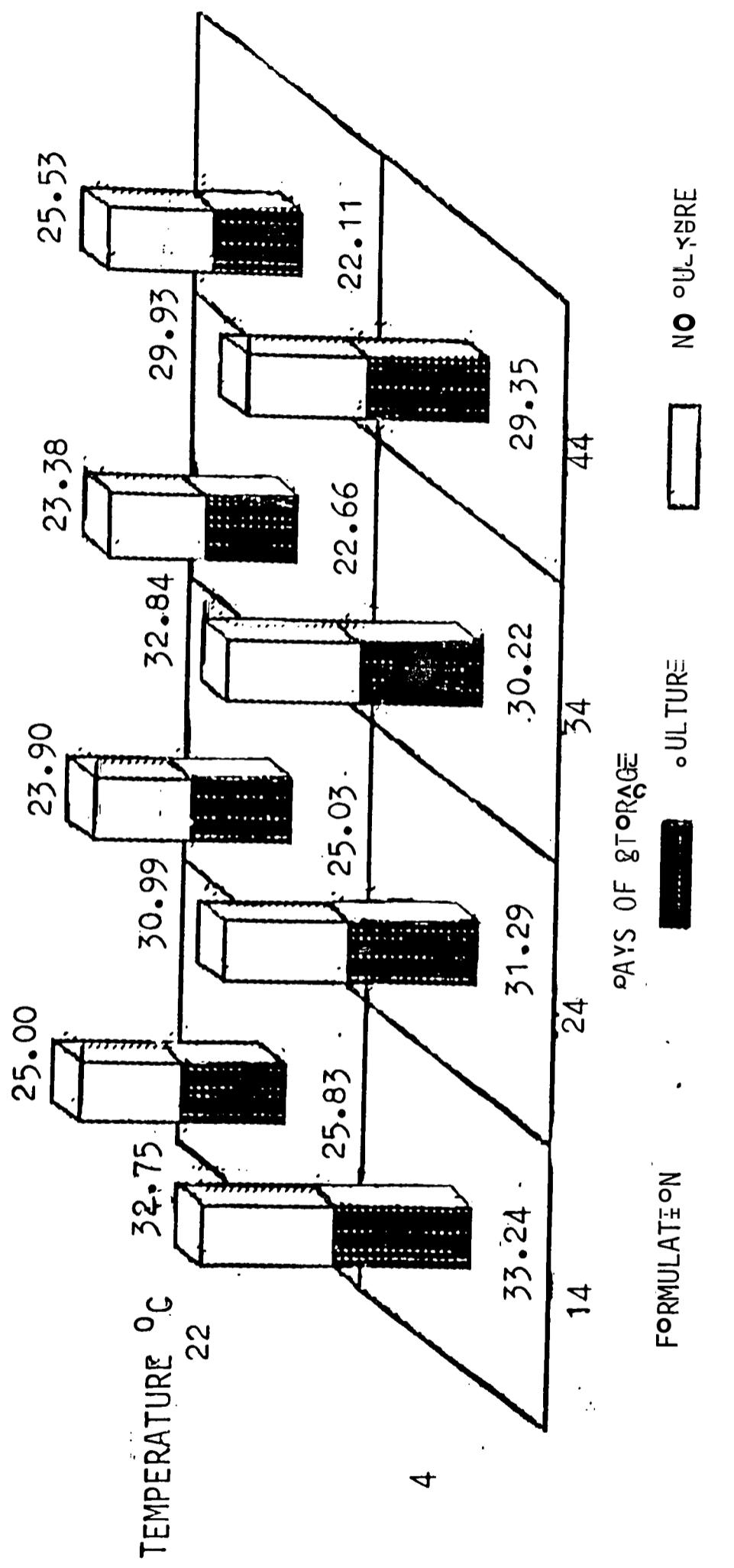


Fig. 5. No culture count of *T. niger* stored at various temperatures for different days and different formulation.

sausage with starter culture decreased from 4 (33.24%) to 22 C (25.83%). Also, moisture content for Thuringer sausage with starter culture still declined from 4 (29.35%) to 22 C (22.11%) after 44 days of storage. Thuringer sausage without starter culture had a tendency toward decreased moisture content with increasing temperature. However, Thuringer sausage without starter culture showed an increase in moisture content from 23.38% to 25.53% at 22 C during 34 and 44 days of storage.

No significant ( $P>.05$ ) two-way interactions between temperature and time and between formulation and time were found. A linear contrast ( $P<.05$ ) for ash existed among formulation, time and temperature (figure 6, Appendix A). The means for percent ash ranged between 4.40 to 5.52%. At 14 days at 4 C, Thuringer sausage without starter culture had higher ash content (6.15%) than Thuringer sausage with starter culture (4.06%). As compared to the Thuringer sausage without starter culture, the higher ash content was observed for Thuringer sausage with starter culture at the higher temperature after 44 days.

A significant interaction between temperature and time ( $P<.01$ , linear contrast) was reported (Appendix A). Also, a linear contrast ( $P<.01$ ) was exhibited among formulation, time and temperature (figure 7, Appendix A). The means for percentage protein ranged from 25.33 to 30.31%. At 14 days of storage, protein content for Thuringer sausage with starter culture had a tendency to increase from 4 (26.37%) to 22 C (28.21%). Furthermore, protein content at 44 days of storage for Thuringer sausage with starter culture showed an increase from 4 (27.36%) to 22 C (30.53%). During storage, Thuringer sausage without starter culture indicated an increasing protein content with increasing temperature.

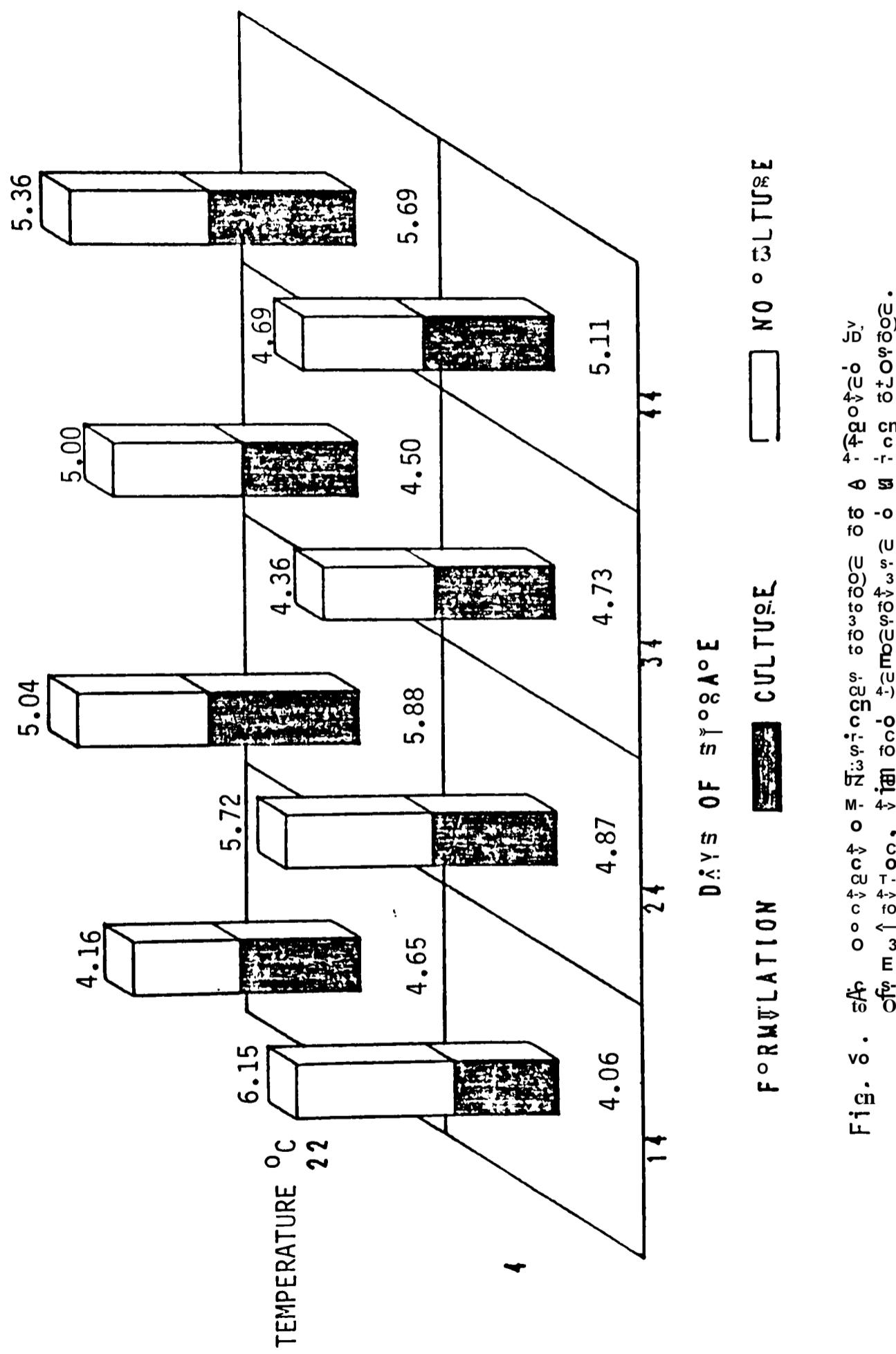


Fig. 6. Effect of temperature on the stability of the formulation of *Aspergillus* sp. at  $22^{\circ}\text{C}$ . The temperature was maintained at  $22^{\circ}\text{C}$  for 14 days, after which it was increased to  $4^{\circ}\text{C}$  for 10 days, followed by a return to  $22^{\circ}\text{C}$  for another 10 days.

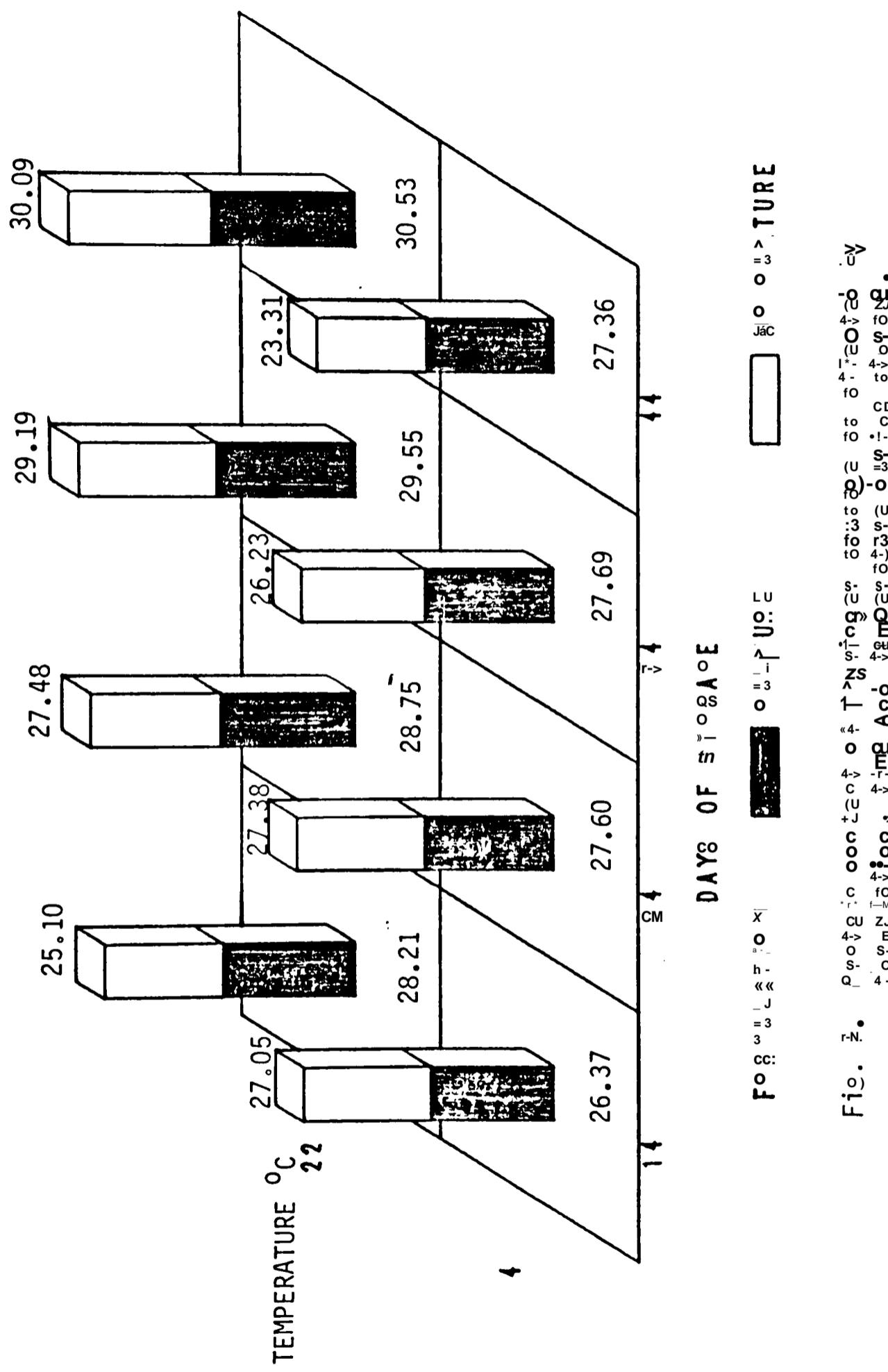


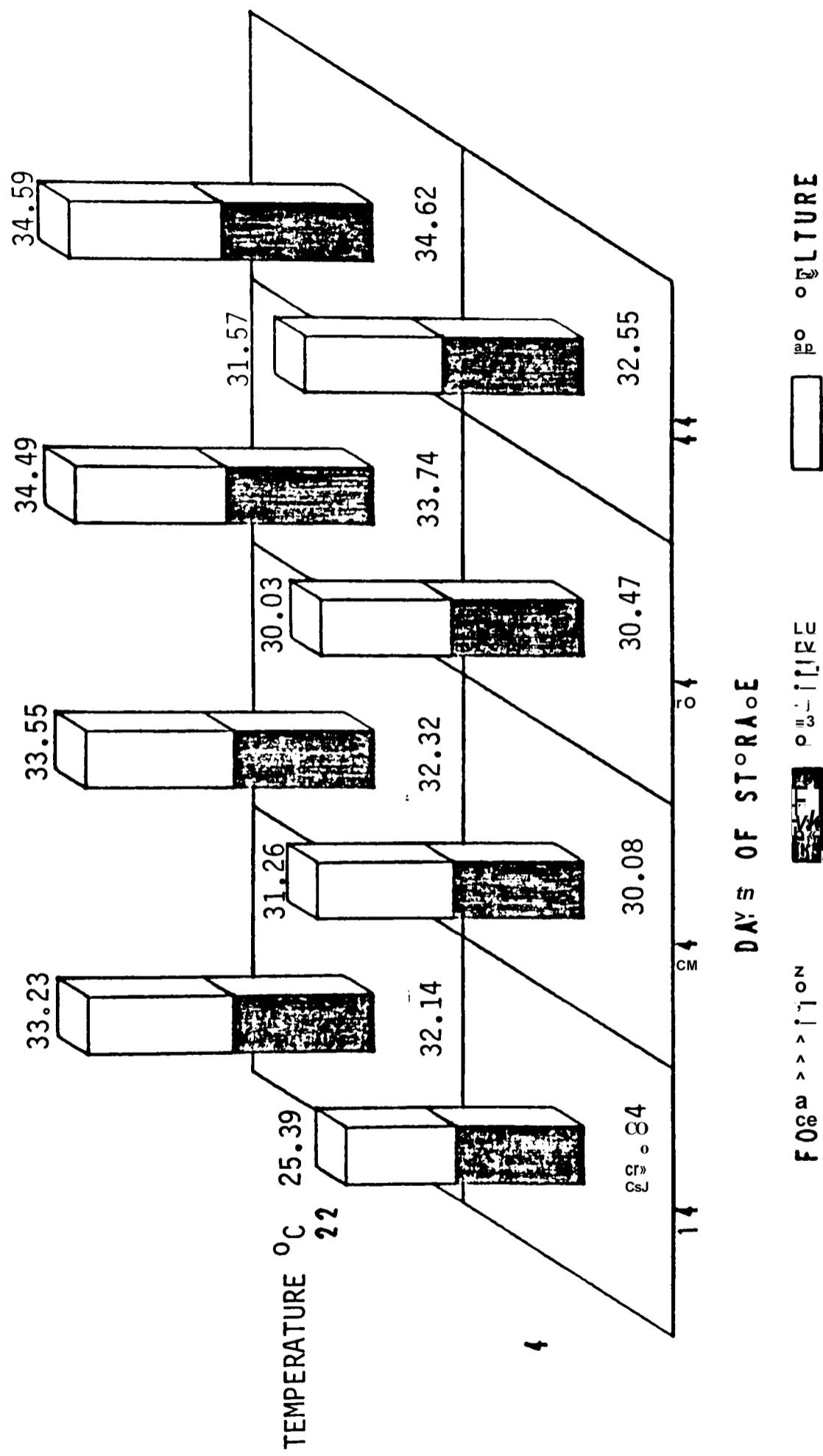
Fig. 2. Temperature distribution in the soil profile at different days of the year.

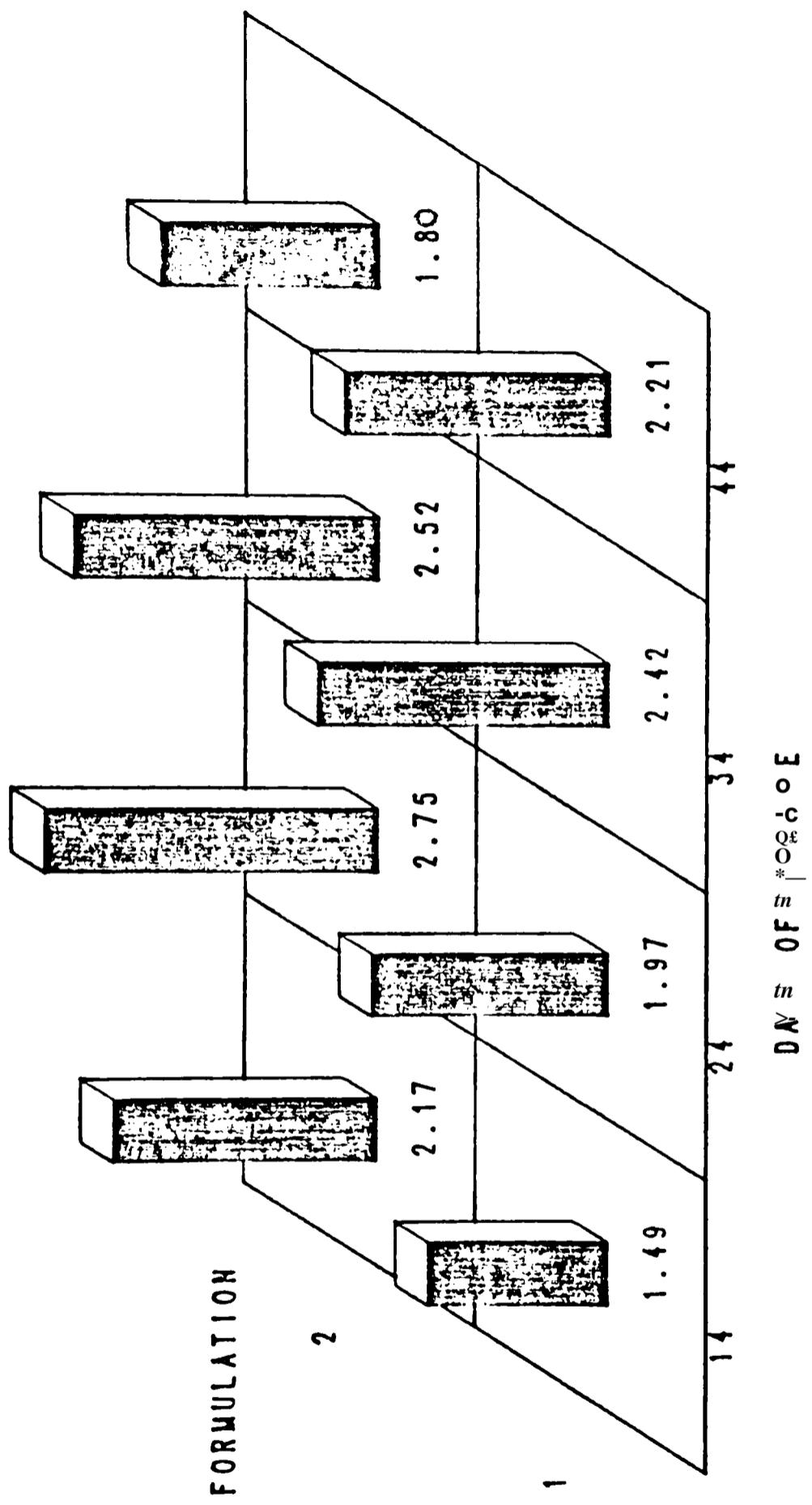
The percent lipid showed a significant two-way interaction between temperature and time ( $P<.01$ , cubic contrast) as reported in Appendix A. A quadratic contrast ( $P<.01$ ) between formulation and time was found for lipid content. Figure 8 shows a three-way interaction ( $P<.05$ , quadratic contrast) among formulation, time and temperature as indicated in Appendix A. The means for percent lipid ranged between 27.36 and 34.60%. Lipid content for Thuringer sausage with starter culture at 14 days showed an increase from 4 (29.34%) to 22 C (32.14%). Also lipid content for Thuringer sausage with starter culture at 44 days of storage still indicated an increase from 4 (32.55%) C to 22 C (34.62%). The results for Thuringer sausage without starter culture during storage showed an increasing lipid content with increasing temperature. At 44 days at 22 C, Thuringer sausage with starter culture had higher lipid content (34.62%) than Thuringer sausage without starter culture (34.59%) had.

#### Indicators of Spoilage during Storage

#### Oxidative Rancidity and Microbiological Aspects

The mean TBA values are presented in figure 9. The results showed that Thuringer sausages containing only natural flora gave higher TBA values than those containing starter culture at 14, 24 and 34 days. This supports the work of Anon (1978) who noted that rancidity was suppressed by the culture organisms. The TBA value for Thuringer sausages containing only natural flora was higher (2.17) than that for Thuringer sausages containing starter culture (1.49) after 14 days of storage. Therefore, oxidative rancidity occurred more rapidly in Thuringer sausages containing natural flora than in Thuringer sausages containing





FORMULATION =  $\sigma_{33}^{\text{LFSR}}$  2 = NO PULTRUDE

Fig. 7. Changes in  $\sigma_{33}$  of Thixotropic storage during aging.

starter culture. During 34 and 44 days of storage, TBA values of Thuringer sausages containing starter culture decreased 0.21 unit (from 2.42 to 2.21) while that of Thuringer sausages containing only natural flora decreased 0.72 unit (from 2.52 to 1.80). The results agree with the work of Bidlack et al. (1972) who said that free MA was present initially, but decreased with storage time as revealed by the TBA test on cooked pork.

As shown in Appendix B, the experimental treatments (formulation and time) were significantly ( $P < .05$ ) different for TBA value. In addition, interactions between temperature and time and between formulation and time showed the quadratic and linear contrasts, respectively. Figure 10 indicated a quadratic contrast ( $P < .05$ ) of the three-way interaction among formulation, time and temperature. The means for TBA values ranged from 1.46 to 2.77. TBA values for Thuringer sausages with starter culture after 34 to 44 days of 4 C showed a decrease from 2.73 to 2.51 while that of TBA values for Thuringer sausages without starter culture had an increase from 2.11 to 2.59. However, at 22 C after 24 to 44 days of storage, the values for TBA decreased from 2.29 to 1.92 for Thuringer sausages with starter culture and from 3.26 to 1.01 for Thuringer sausages without starter culture. Thus, unsteady decreases in TBA values on Thuringer sausages (with and without starter cultures) were found.

However, in the concentrations present in distillates from meats, MA does not contribute significantly to the value for total carbonyls. The formation of carbonyl addition products would possibly account for the apparent loss in MA during storage at various temperatures as mentioned by Chang et al. (1961). The rate of reaction between  $\alpha$ -amino acids of myosin and MA also depends on the optimum temperature (Buttkus,

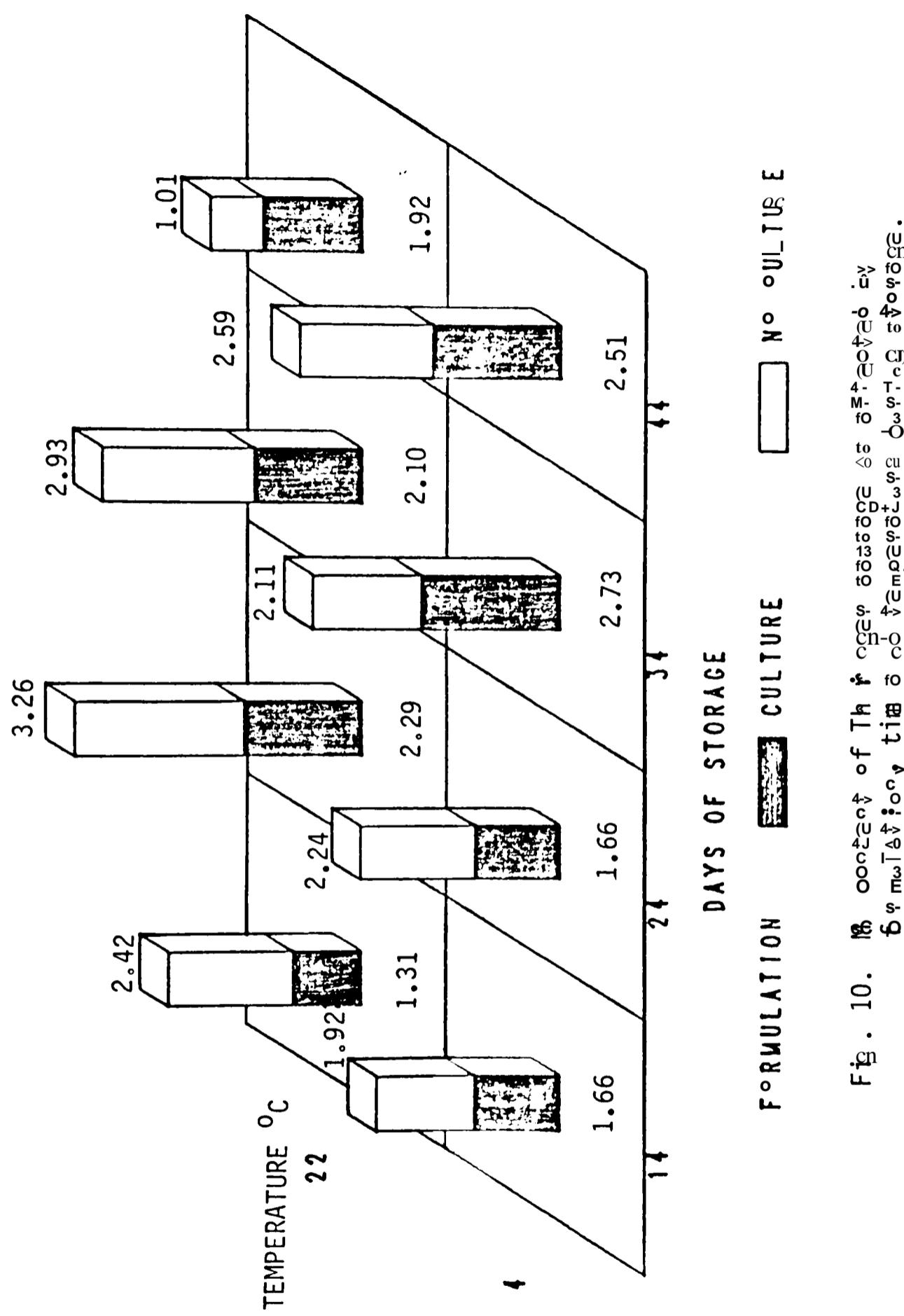


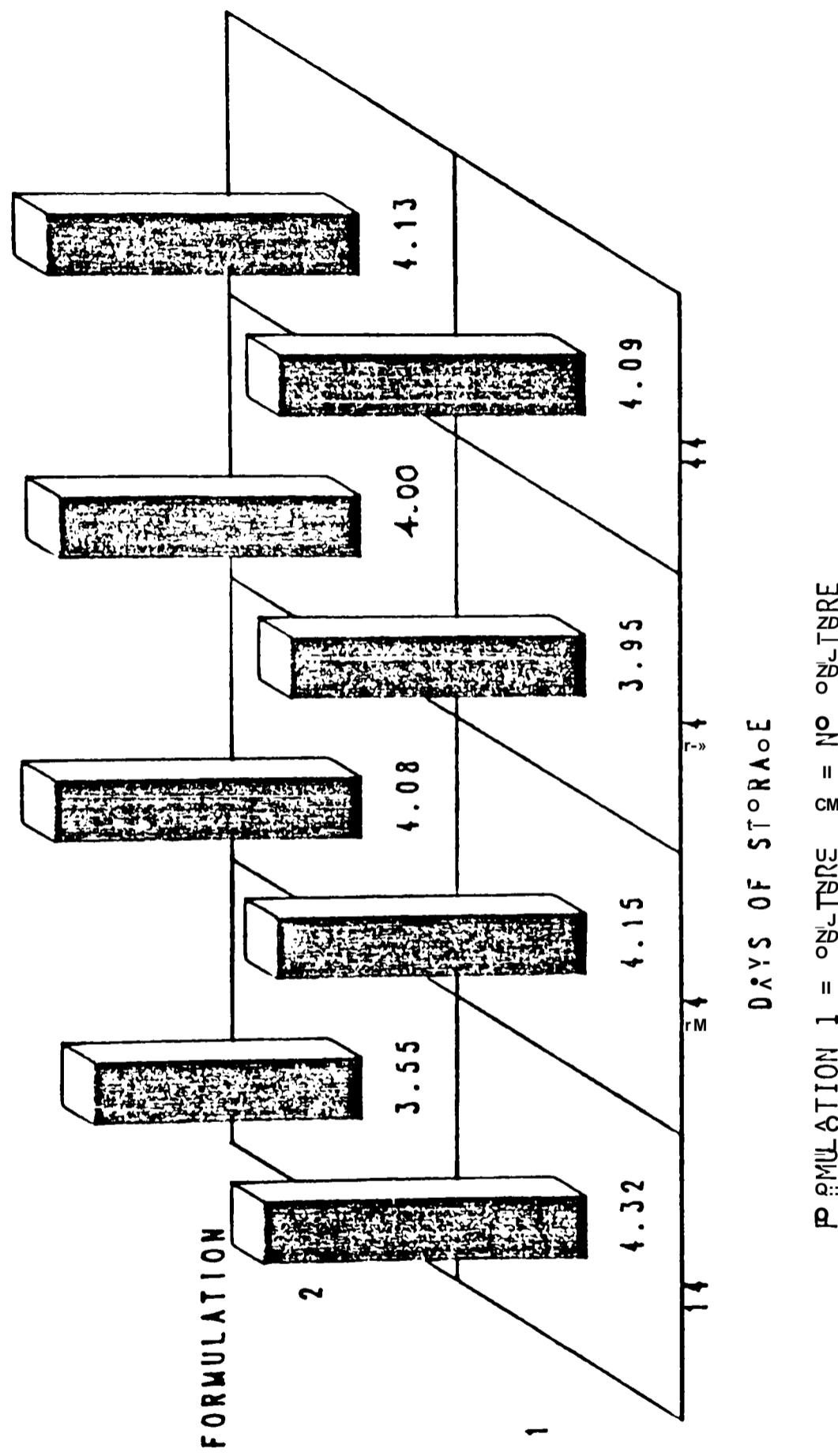
Fig. 10. The viscosity of Thriocetyl gel stored at 22°C to 26°C by month; time of sample collection.

1967). Loss of MA could occur through its reaction with guanidine to form nonvolatile derivatives (King, 1966). Several researchers (Kwon, et al., 1965; Crawford, et al., 1966; Buttkus, 1967) concluded that MA can react with amino acids, proteins, glycogen and other food constituents to form products in which the MA exists in bound form.

Figure 11 shows the log mean aerobic plate counts (APC) for both types of Thuringer sausages. Sausages containing only natural flora had a lower bacterial population than those containing starter culture during the first period of storage. From 24 to 34 days of storage, the aerobic plate counts in both types of Thuringer sausages had a tendency to decrease. However, the log mean aerobic plate counts after 34 to 44 days of storage fluctuated from 3.95 to 4.09 for Thuringer sausages containing starter culture and from 4.00 to 4.13 for Thuringer sausages containing only natural flora.

A surface slime developed on both types of Thuringer sausages kept at 22 C after 34 days of storage: whereas, no slime developed on Thuringer sausages kept at 4 C with air movement. This supports the results of Townsend et al. (1983) who reported that slime developed only on fermented sausages without air movement. Temperature and humidity are also important to the microorganism activity, especially mold growth. Bacus (1984a) stated that yeast or heterolactic contamination could be found after processing because of inadequate smoke concentration at Thuringer sausage surfaces. Green coloration on sausage products was observed since the sausage products had higher pH than expected (Bacus, 1984a).

After 34 to 44 days of storage, the population of mold in Thuringer sausages containing starter culture had a tendency to increase rapidly



FORMULATION 1 =  $\text{O}_2\text{N}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{O}-\text{C}_2\text{H}_5$   
 FORMULATION 2 =  $\text{O}_2\text{N}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}_2\text{H}_5$

Fig. 11.  $\text{O}_2\text{N}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{O}-\text{C}_2\text{H}_5$  (Formulation 1)  
 $\text{O}_2\text{N}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}_2\text{H}_5$  (Formulation 2)

at 22 C (table 4). Large numbers of mold colonies could interfere with the aerobic bacterial growth in fermented Thuringer sausages. Bacus (1984a) also noted that many molds produced antibiotics which might affect the bacterial microflora. Generally the mycelial development is less sensitive to environmental conditions and can proliferate during typical, subsequent storage processes.

No significant ( $P>.05$ ) changes in aerobic plate counts were observed during storage for time and temperature of Thuringer sausages. However, formulation significantly affected ( $P<.05$ ) the total aerobic plate count in Thuringer sausages. A cubic contrast between the various storage times and temperatures for the aerobic plate counts ( $P<.05$ ) was observed. However, all these effects were modified by the significant two-way interaction between formulation and time (quadratic contrast;  $P<.05$ ). No significant differences ( $P>.05$ ) among formulation, time and temperature were observed.

TABLE 4

POTATO DEXTROSE PLATE COUNTS (COLONIES/G)  
FOR THURINGER SAUSAGE DURING STORAGE

Formulation	Time (d)	Temperature (C)	
		4	22
Thuringer with starter culture	14	<10 Est <sup>a</sup>	<10 Est
	24	<10 Est	530
	34	<10 Est	16,000
	44	<10 Est	18,000
Thuringer without starter culture	14	<10 Est	<10 Est
	24	<10 Est	20 Est
	34	20 Est	<100 Est
	44	<10 Est	11,000 Est

<sup>a</sup>Estimated.

Appendix B is an analysis of variance summary table indicating the effects of Thuringer sausage type, storage time and storage temperature on TBA values and microbiological quality. The TBA values were affected by the Thuringer sausage type ( $P<.05$ ) and storage time ( $P<.01$ ). However aerobic plate counts were affected by the Thuringer sausage type ( $P<.01$ ). Unlike aerobic plate counts, TBA values were affected ( $P<.01$ ) by the interaction among storage temperature, Thuringer sausage type and storage time.

Thus, the indicators of spoilage in both Thuringer sausage types for this study were affected by the TBA more than the bacterial growth.

#### Changes in Polar Lipids in Thuringer Sausage

Since total phospholipids were major contributors to development of WOF in cooked meat, it was considered imperative to evaluate the contributions of PC and PE. PE exerted a much greater pro-oxidant effect than PC (Corliss and Dugan, 1970; Tsai and Smith, 1971), although PC constitutes the bulk of total phospholipid in meat (Kuchmak and Dugan, 1963; Shuster et al., 1964; Keller and Kinsella, 1973; Lee and Dawson, 1976; Gokalp et al., 1981). PC levels decreased with storage time in ground beef and mechanically separated beef (Lee, 1983).

No significant ( $P>.05$ ) changes in the concentration of PC were observed during storage conditions of Thuringer sausages (with and without starter cultures). Thuringer sausages (with and without starter cultures) might contain sphingomyelin content that is difficult to separate from PC using eluting solvent (n-hexane/2-propanol/water mixture) as described by Guerts Van Kessel et al. (1977) and Hax and Guerts Van Kessel (1977).

Significant changes ( $P < .05$ ) in PI values were found during storage. Also, the interaction of storage temperature with storage time showed a cubic contrast ( $P < .05$ ). No significant ( $P > .05$ ) differences were observed in PI contents among the three treatments (formulation, time and temperature).

PE values exhibited linear contrasts between storage time and temperature and between storage time and formulation ( $P < .05$ ). A quadratic contrast ( $P < .05$ ) among formulation, time and temperature was shown in PE value (figure 12). As compared to Thuringer sausage without starter culture at 14 days of storage, the lower PE content was found from Thuringer sausage with starter culture at a lower temperature. However, Thuringer sausage with starter culture had a higher PE content (132.87 mg/100g) than Thuringer sausage without starter culture (15.07 mg/100g) at 44 days of 22 C.

Thus, PC, PI and PE from different samples did not consistently behave in the same manner during storage. The changes in the component phospholipids may be due to either autoxidation, hydrolytic decomposition, lipid browning reactions or lipid-protein co-polymerization as outlined by researchers (Lea, 1957; Lee and Dawson, 1976). In addition, microbial systems that produce peroxide will catalyze the chemical oxidation of the unsaturated fatty acids in meat. Bacus (1984a), however reported that all molds reduced rancidity development by preventing oxygen penetration into meat since they had catalase activity and formed a coating over the surface.

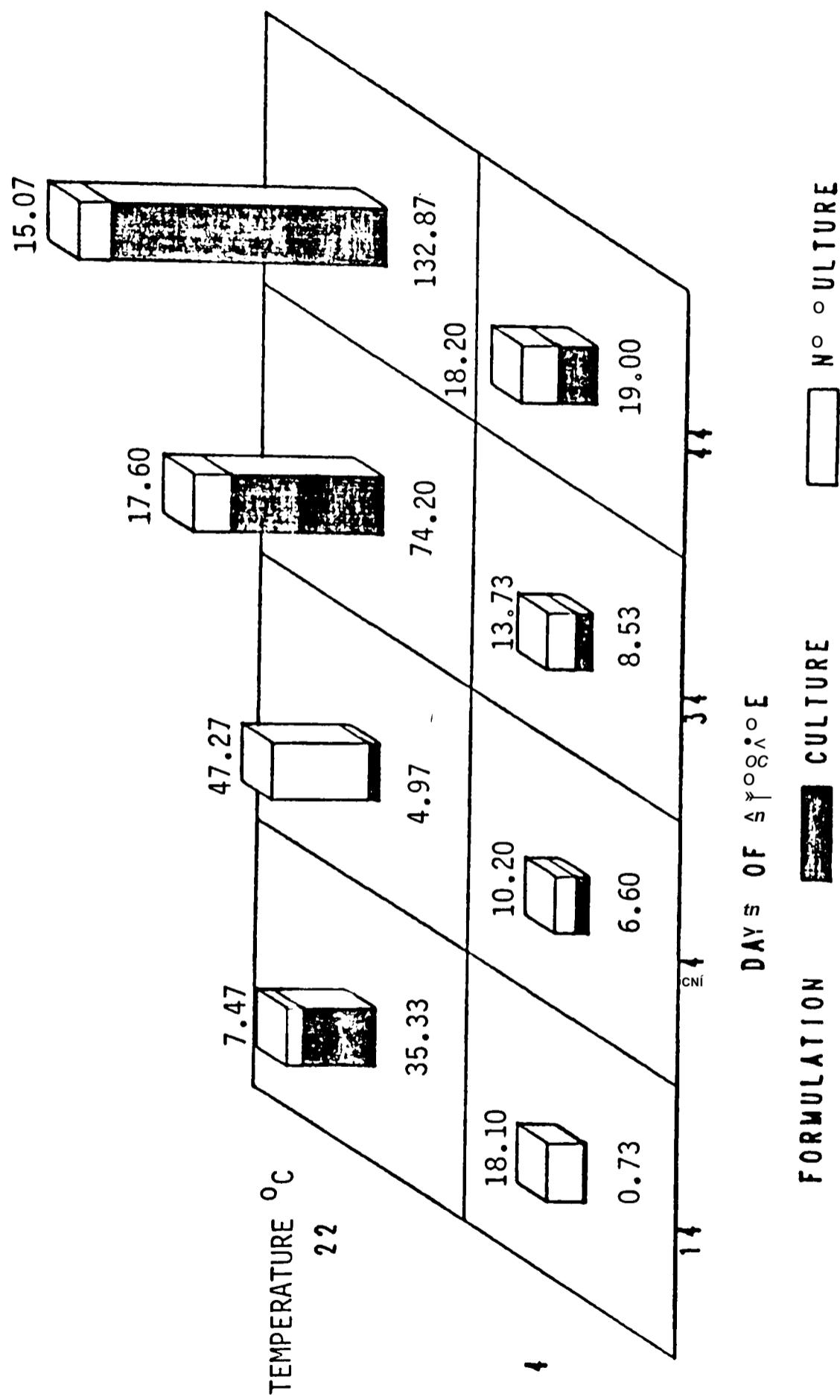


Fig. 12. የፌዴራል ማመራሪያ በትኩረት ንብረቶ ስርዓት ማመራሪያ መሆኑን ተስተካክለ ተደርጓል.

### Conclusions

During fermentation, oxidative rancidity occurred more rapidly in Thuringer sausages without starter culture than Thuringer sausages with starter culture. Both pH and TBA values were affected by sausage formulation, fermentation time, and the interaction of sausage formulation with fermentation time ( $P<.01$ ). An inverse relationship between pH and TBA values was shown. As moisture decreased, percentage of ash, protein and lipid increased during storage. It could be concluded that the increases in protein content during storage were probably due to the drying process as also was reported by Kiernat et al. (1964). Microbial content did not clearly affect the development of oxidative rancidity.

No significant ( $P>.05$ ) changes in the concentration of PC were investigated during storage conditions of Thuringer sausages. Unlike PI content, PE content was significantly ( $P<.05$ ) affected by all three treatments (formulation, time and temperature).

The rate of reduction in rancidity development in Thuringer sausages, especially Thuringer with starter culture, could probably be attributed to the catalase activity of all molds (Bacus, 1984a).

## LIST OF REFERENCES

- Acton, J. C., Dick, R. L. and Norris, E. L. 1977. Utilization of various carbohydrates in fermented sausage. *J. Food Sci.* 42:174.
- Acton, J. C. and Keller, J. E. 1974. Effect of fermented meat pH on summer sausage properties. *J. Milk and Food Technol.* 37:570.
- Acton, J. C., Ziegler, G. R. and Burge, D. L. 1983. Functional ity of muscle constituents in the processing of comminuted meat products. CRC Press, Inc, Boca Raton, Florida. *Critical Reviews in Food Science and Nutrition.* 18(2):101.
- AMI. 1953. "Sausage and Ready-to-Serve Meats." Amer. Meat. Inst., Chicago, IL.
- AMI. 1982a. "Meat Facts: A Statistical Summary about America's Largest Food Industry." Amer. Meat Inst., Washington, DC
- AMI. 1982b. "Good Manufacturing Practices, Fermented Dry and Semi-Dry Sausages." Amer. Meat Inst., Washington, DC
- Anderson, M. and Steinberg, M. 1964. Effect of lipid content on linolenate in fish muscle homogenates. *J. Food Sci.* 29:327.
- Anon. 1975. Food Chem. News (USA). Filed Jun. 16.
- Anon. 1977. The staphylococcal enterotoxin problem in fermented sausages. Task Force Report, Food Safety and Quality Service, U.S. Dept. Agriculture, October.
- Anon. 1978. Fast aging with mold spray. *Meat Ind.* 24(3):51.
- Anon. 1980. A survey of nitrosamines in sausages and dry-cured meat products. *Food Technol.* 34(7):45.
- AOAC 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC
- Atkinson, I., Cecil, S. R. and Woodroof, J. G. 1947. Extending keeping'quality of frozen pork sausage. *Food Ind.* 19:1198.
- Bacus J. N. 1984a. "Utilization of Microorganisms in Meat Processing: Å Handbook for Meat Plant Operators." John Wiley and Sons, Inc, New York.
- Bacus, J. N. 1984b. Update: Meat fermentation 1984. *Food Technol.* 38(6):59.

- Bacus, J. N. 1985Cv "Fermented Sausage-Modern Approaches to Ancient Products." The National Provisioner, Inc, Chicago.
- Bacus, J. N. and Brown, W. L. 1981. Use of microbial cultures: Meat products. Food Technol. 35(1):74.
- Baran, W. L. and Stevenson, K. E. 1975. Survival of selected pathogens during processing of a fermented turkey sausage. J. Food Sci. 40: 618.
- Barber, L. E. and Deibel, R. H. 1972. Effect of pH and oxygen tension on staphylococcal growth and enterotoxin formation in fermented sausage. Appl. Microbiol. 24:891.
- Bendall, J. R. 1954. The swelling effect of polyphosphates on lean meat. J. Sci. Food and Agric 5:468.
- Bidlack, W. R., Kwon, T. W. and Snyder, H. E. 1972. Production and binding of malonaldehyde during storage of cooked pork. J. Food Sci., 37:664.
- Bjarnason, J. and Carpenter, K. J. 1970. Brit. J. Nutr. 24:313.
- Bosund, I. and Ganrot, B. 1969. Lipid hydrolysis in frozen baltic herring. J. Food Sci. 34:13.
- Bullerman, L. B., Hartman, P. A. and Ayres, J. C 1969. Aflatoxin production in meats. II. Aged dry salamis and aged country cured hams.. Appl. Microbiol. 18:718.
- Burr, G. O. and Barnes, R. H. 1943. Non-caloric function of dietary fats. Physiol. Rev. 23:256.
- Buttkus, H. 1976. The reaction of myosin with malonaldehyde. J. Food Sci. 32:432.
- Chang, I. and Watts, B. M. 1950. Some effects of salt and moisture on rancidity in fats. Food Res. 15:313.
- Chang, P. Y., Younathan, M. T. and Watts, B. M. 1961. Lipid oxidation in pre-cooked beef preserved by refrigeration, freezing, and irradiation. Food Technol. 15:168.
- Chipault, J. R., Mizuno, G. R. and Lundberg, W. O. 1956. The antioxidant properties of spices in foods. Food Technol. 10:209.
- Cieqler, A., Mintzlaff, H. J., Weisleder, D. and Leistner, L. 1972. Potential production and detoxification of penicillic acid in mold-fermented sausage (salami). Appl. Microbiol. 24:114.

- Corliss, G. A. and Dugan, L. R., Jr. 1970. Phospholipid oxidation in emulsions. *Lipids*. 5:486.
- Crawford, D. L., Yu, T. C and Sinnhuber, R. O. 1966. Reaction of malonaldehyde with glycine. *J. Agric. Food Chem.* 14:182.
- Cross, H. R., Kotula, A. W. and Nolan, T. W. 1978. Stability of frozen ground beef containing mechanically deboned beef. *J. Food Sci.* 43:281.
- Cummings, N. J. and Mattill, N. A. 1931. The autoxidation of fats with reference to their destructive effect on vitamin E. *J. Nutr.* 3:421.
- Daly, C, Sandine, W. E. and Elliker, P. R. 1972. Interactions of food starter cultures and food-borne pathogens: Streptococcus diacetylactis versus food pathogens. *J. Milk Food Technol.* 35:349.
- Deibel, R. H. and Niven, C F., Jr. 1957, Pediococcus cerevisiae: A starter culture for summer sausage. *Bacteriol. Proc* 14-15.
- De Ketelaere, A., Demeryer, D., Vanderkerckhove, P. and Vervaeke, J. 1974. Stoichiometry of carbohydrate fermentation during dry sausage ripening. *J. Food Sci.* 39:297.
- Dethmers, A. E., Rock. H. and Johnston, R. W. 1975. Effect of added sodium nitrite and sodium nitrate on sensory quality and nitrosamine formation in Thuringer sausage. *J. Food Sci.* 40(3):491.
- Dierick, N., Vandekerckhove, and Demeyer, D. 1974. Changes in nonprotein nitrogen compounds during dry sausage ripening. *J. Food Sci.* 39:301.
- Dyer, W. J. 1968. "Low Temperature Storage of Foods," Pergamon Press, London.
- Ellis, R., Gurrie, G. T., Thornton, F. E., Bollinger, N. C and Gaddis, A. M. 1968. Carbonyls in oxidizing fats 11. The effect of the pro-oxidant activity of sodium chloride on pork tissue. *J. Food Sci.* 33:555.
- Emanuel', N. M. and Lyaskovskaya, Yu. N. 1967. "The Inhibition of Fat Oxidation Processes," p. 12. Pergamon Press, New York.
- Eskeland, B. and Nordal, J. 1980. Nutritional evaluation of protein in dry sausages during the fermentation process with special emphasis on amino acid digestibility. *J. Food Sci.* 45:1153.
- Everson, C W., Danner, W. E. and Hammes, P. A. 1970. Bacterial starter cultures in sausage products. *J. Agric Food Chem.* 18(4):570.

- Fleischer, S. and Rouser, G. 1965. Lipids of subcellular particles. J. Am. Oil Chem. Soc 42:588.
- Forrest, J. C, Aberle, E. D., Hedrick, H. B., Judge, M. O. and Merkel, R. A. 1975. "Principles of Meat Science." W. H. Freeman and Company, San Francisco, CA.
- Genigeorgis, C A. 1976. Quality control for fermented meats. J. Vet. Med. Assoc 169(11):1220.
- Geurts Van Kessel, W. S. M., Hax, W. M. A., Demel, R. A. and DeGier, J. 1977. High performance liquid chromatographic separation and direct ultraviolet detection of phospholipids. Biochim. Biophys. Acta. 486:524.
- GiUiland, S. E. and Speck, M. L. 1975. Inhibition of psychrotropic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. J. Food Sci. 40:903.
- Gokalp, H. Y., Ockerman, H. W., Plimpton, R. F. and Peng, A. C 1981. Qualitative alternation of phospholipids in beef patties cooked after vacuum and nonpackaged, frozen storage. J. Food Sci. 46:19.
- Greene, B. E. 1971. Oxidations involving the heme complex in raw meat. J. Am. Oil Chem. Soc 48:637.
- Gurr, M. I. 1984. "Role of Fats in Food and Nutrition." Elsevier Applied Science Publishing Co., Inc, New York, NY.
- Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10:355.
- Harper, J. C and Tappel, A. L. 1957. "Advances in Food Research," E. M. Mrak and G. F. Stewart (Ed.). Academic Press, Inc, New York.
- Harris, N. D. and Lindsay, R. C 1972. Flavor changes in reheated chicken. J. Food Sci. 37:19.
- Hax W M. A. and Geurts Van Kessel, W. S. M. 1977." High-performance liquid chromatographic separation and photometric detection of phospholipids. J. Chromatography. 142:735.
- Hermann, J. E. and Cliver, D. O. 1973. Enterovirus persistence in sausage and ground beef. J. Milk Food Technol. 37:426.
- Hornstein, I., Crowe, P. F. and Heinberg, M. J. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci. 26:581.
- Hornstein, I., Crowe, P. F. and Hiner, R. 1967. Composition of lipids in some beef muscles. J. Food Sci. 32:650.

- Hultin, H. O. 1976. Characteristics of muscle tissue. In "Principles of Food Science. Part 1: Food Chemistry," O. R. Fennema (Ed.), p. 590. Marcel Dekker, Inc, New York, NY.
- Igene, J. O. and Pearson, A. M. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *J. Food Sci.* 44:1285.
- Irvine, D. M. and Price, W. V. 1961. Influence of salt on the development of acid by lactic starters in skim milk and in cured submerged in brine. *J. Dairy Sci.* 44:243.
- Jay, J. M. 1978. "Modern Food Microbiology," 2nd ed. D. Van Nostrand Co., New York, NY.
- Jensen, L. B. 1945. "Microbiology of Meats," 2nd ed. The Garrard Press, Champaign, IL.
- Kantor, M. A. and Potter, N. N. 1975. Persistence of echovirus and poliovirus in fermented sausages. Effects of sodium nitrite and processing variables. *J. Food Sci.* 40:968.
- Keller, J. D. and Kinsella, J. E. 1973. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. *J. Food Sci.* 38:1200.
- Keskinel, A., Ayres, J. C and Snyder, H. E. 1964. Determination of oxidation changes in raw meats by the 2-thiobarbituric acid method. *Food to chnol.* 18:101.
- Kidwai, A. M., Radcliffe, M. A., Lee, E. Y. and Danual, E. E. 1973. *Biochim. Biophys. Acta.* 298:593.
- Kiernat, B. H., Johnson, J. and Siedler, A. 1964. "A Summary of the Nutrient Content of Meat," Bulletin No. 57, Am. Meat Inst. Found. Chicago, IL.
- King, T. P. 1966. Selective chemical modification of arginyl residue. *Biochem.* 5:3454.
- Kotula, A. W. 1982. Role of government in research on meat microbiology.\* *J! Food Prot.* 45:1165.
- Kotula, A. W., Campano, S. G. and Kinsman, D. M. 1982. Proteolytic and lipolytic activity of molds isolated from aged beef. *J. Food Prot.* 45:1242.
- Ku-hmak, M. and Dugan, L. R., Jr. 1963. Phospholipids of pork muscle tissue. *J. Am. Oil Chem. Soc* 4:734.

- Kuchmak, M. and Dugan, L. R., Jr. 1965. Composition and positional distribution of fatty acids in phospholipids isolated from pork muscle tissues. *J. Am. Oil Chem. Soc* 42:45.
- Kummerow, F. A. 1962. Toxicity of heated fats. In "Symposium on Foods: Lipids and Their Oxidation," H. W. Schultz, E. A. Day and R. O. Sinnhuber (Ed.). AVI Publishing Co., Westport, CT.
- Kwoh, T. L. 1971. Catalysts of lipid peroxidation in meats. *J. Am. Oil Chem. Soc* 48(10):550.
- Kwon, T. W., Menzel, D. B. and Olcott, H. S. 1965. Reactivity of malonaldehyde with food constituents. *J. Food Sci.* 30:308.
- Lea, C H. 1957. Deteriorative reactions involving phospholipids and lipoproteins. *J. Sci. Food and Agric* 8:1.
- Lea, C H. 1962. The oxidative deterioration of food lipids. In "Symposium on Foods: Lipids and Their Oxidation," H. W. Schultz, E. A. Day and R. O. Sinnhuber (Ed.), AVI Publishing Co., Westport, CT.
- Lee, C 1983. Storage stability of frozen mechanically separated beef, M. S. Thesis, Texas Tech University, Lubbock.
- Lee, W. T. and Dawson, L. E. 1973. Chicken lipid changes during cooking in fresh and reused cooking oil. *J. Food Sci.* 38:1232.
- Lee, W. L. and Dawson, L. E. 1976. Changes in phospholipids in chicken tissues during cooking in fresh and reused cooking oil, and during frozen storage. *J. Food Sci.* 41:598.
- Lewis, D. F. and Jewell, G. G. 1975. Leatherhead Food RA Research Report No. 212.
- Lewis, S. E. and Wills, E. D. 1963. Inhibition of the autoxidation of unsaturated fatty acids by hematin proteins. *Biochem. Biophys. Acta.* 70:336.
- Liu, H. P. 1970. Catalysts of lipid peroxidation in meats. 1. Linoleate peroxidation catalyzed by MetMb or Fe(II)-EDTA. *J. Food Sci.* 35:590.
- Liu, T. H. 1965. Thesis, The Florida State University.
- Love J. D. a"<sup>^</sup> Pearson, A. M. 1971. Lipid oxidation in meat and meat products-a review. *J. Am. Oil Chem. Soc* 48:548.
- Love, R- ^- ^"^ Elerian, M. K. 1974. Protein denaturation in frozen fish 8. The temperature of maximum denaturation in cod. *J. Sci. Food Agric* 15:805.

- Mabrouk, A. F. and Dugan, L. R. 1960. A kinetic study of the autoxidation of methyl linoleate and linoleic acid emulsions in the presence of sodium chloride. *J. Am. Oil Chem. Soc* 37:486.
- Marmer, W. N. and Maxwell, R. J. 1981. Dry column method for the quantitative extraction and simultaneous class separation of lipids from muscle tissue. *Lipids*. 16(5):365.
- Masters, B. A., Oblinger, J. L., Goodfellow, S. J., Bacus, J. N. and Brown, W. L. 1981. Fate of Salmonella newport and Salmonella typhimurium inoculated into summer sausage. *J. Food Prot.* 44:527.
- McKercher, P. D., Hess, W. R. and Hamdy, F. 1978. Residual viruses in pork products. *Appl. Environ. Microbiol.* 35:142.
- Miller, A. R. 1975. "Meat Hygiene." Lea and Febiger, Philadelphia.
- Moon, N. J., Beuchat, L. R., Kinkaid, D. T. and Hays, E. R. 1982. Evaluation of lactic acid bacteria for extending the shelf life of shrimp. *J. Food Sci.* 47:897.
- Moskovits, V. G. and Kielsmeier, E. W. 1960. Proceedings of the 12th Research Conference of the American Meat Institute Foundation, Chicago.
- Moulton, C. R. and Lewis, W. L. 1970. "Meat Through The Microscope," 3rd ed. Institute of Meat Packing, The University of Chicago, Chicago, IL.
- Niinivaara, F. P., Pohja, M. S. and Komulainen, S. E. 1964. Some aspects about using bacterial pure cultures in the manufacture of fermented sausages. *Food Technol.* 18(2):25.
- Niskinen, A. and Nurmi, E. 1976. Effect of starter culture on staphylococcal enterotoxin and thermonuclease production in dry sausage. *Appl. Environ. Microbiol.* 31:11.
- O'Keefe, P. W., Wellington, G. H., Mattick, L. R. and Stouffer, J. R. 1968. Composition of bovine muscle lipids at various carcass locations. *J. Food Sci.* 33:188.
- Olley, J. and Lovern, J. A. 1960. Phospholipid hydrolysis in cod flesh stored at different temperatures, *J. Sci. Food Agric* 11:644.
- Olley, J. and Watson, H. 1962. Phospholipase activity in herring muscle. in "First International Congress of Food Science and Technology," Vol. 1. Gordon and Breach Science Publishers Ltd., London.
- Ottolenghi, A. 1959. Interaction of ascorbic acid and mitochondrial lipides. *Arch. Biochem. Biophys.* 79:355.

- Pearson, A. M., Love, J. D. and Shorland, F. B. 1977. Warmed-over flavor in meat, poultry and fish. *Adv. Food Res.* 23:1.
- Priestley, R. J. 1979. "Effects of Heat on Foodstuffs." Applied Science Publishers, Ltd., London.
- Raccach, M. and Baker, R. C. 1978. Lactic acid as an anti-spoilage and safety factor in cooked, mechanically deboned poultry meat. *J. Food Prot.* 41:703.
- Raccach, M. and Baker, R. C. 1979. The effect of lactic acid bacteria on some properties of mechanically deboned poultry meat. *Poultry Sci.* 58:144.
- Raccach, M. and Baker, R. C., Regenstein, J. M. and Mulnix, E. J. 1979. Potential application of microbial antagonism to extended storage stability of a flesh type food. *J. Food Sci.* 44:43.
- Reddy, S. G., Chen, M. L. and Patel, P. J. 1975. Influence of lactic cultures on the biochemical, bacterial, and organoleptic changes in beef. *J. Food Sci.* 40:314.
- Reddy, S. G., Henrickson, R. L. and Olson, H. C 1970. The influence of lactic cultures on ground beef quality. *J. Food Sci.* 35:787.
- Rubin, H. E. and Vaughan, F. 1979. Elucidation of the inhibitory factors of yogurt against Salmonella typhimurium. *J. Dairy Sci.* 62:1873.
- Salisbury, G. W. and Crampton, E. W. 1960. "The Science of Meat and Meat Products." W. H. Freeman and Company, San Francisco, CA.
- SAS Institute. 1982. "SAS User's Guide: Statistics." Statistical Analysis Systemlnstitute Inc , Cary, NC
- Scanlan, R. A. 1975. N-nitrosamines in foods. *Crit. Rev. Food Technol.* 5:357.
- Schut, J. 1976. Meat emulsions. In "Food Emulsions," Friberg, S. (Ed.). Marcel Dekker, New York.
- Shuster, C Y., Froines, J. R. and Olcott, R. 1964. Phospholipids of tuna white muscle. *J. Am. Oil Chem. Soc* 41:36.
- Siedler, A. J. and Schweigert, B. S. 1954. Effect of added stabilized animal fats on stability of vitamin A in feeds. *J. Agric Food Chem.* 2:193.
- c-înniû/- P. A 1928. Detection and determination of add moisutre in 'slûsage.' *J. of Assoc Offi. Agric Chem.* 6:112.

- Smith, J. L., Huhtanen, C N., Kissinger, J. C. and Palumbo, S. A. 1975. Survival of salmonellae during pepperoni manufacture. *Appl. Microbiol.* 30:759.
- Smith, J. L. and Palumbo, S. A. 1978. Reduction of nitrate in a meat system of Lactobacillus plantarum. *J. Appl. Bacteriol.* 45:153.
- Speck, M. L. (Ed.). 1976. "Compendium of Methods for the Microbiological Examination of Foods." American Public Health Association, Washington, DC
- Strzelecki, E. L. 1973. Behavior of aflatoxins in some meat products. *Acta Microbiol. Polonica, Ser. B;* 5:171.
- Tanaka, N., Traisman, E., Lee, M. H., Cassens, R. G. and Foster, E. M. 1980. Inhibition of botulinum toxin formulation in bacon by acid development. *J. Food Prot.* 43:450.
- Tanford, C 1968. Protein denaturation. *Adv. Protein Chem.* 23:121.
- Tappel, A. L. 1953. Linoleate oxidation catalysts occurring in animal tissues. *Food Res.* 18:104.
- Tappel, A. L. 1955. Unsaturated lipid oxidation catalyzed by hematin compounds. *J. Biol. Chem.* 217:721.
- Tarladgis, B. G., Watts, B. M. and Younathan, M. T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc* 37:44.
- Terrell, R. N., Smith, G. C and Carpenter, Z. L. 1978. Practical manufacturing technology for dry and semi-dry sausage. In Proc 20th Ann. Meat Sci. Inst., Athens, GA.
- Thatcher, F. S. and Clark, D. S. 1968. "Microorganisms in Foods." University of Toronto Press, Canada.
- Tims, M. J. and Watts, B. M. 1958. Protection of meats with phosphates. *Food Technol.* 12(5):240.
- Townsend, W. E., Leroy, C B., Ruel, L. W. and James, E. T. 1983. Effect of air movement during fermentation on certain properties of natural flora and starter culture-fermented sausage. *J. Food Prot.* 46(11):982.
- Tsai, Lee-Shin and Smith, L. M. 1971. Role of the bases and of phosphoryl bases of phospholipids in the autoxidation of methyl linoleate emulsions. *Lipids.* 6:196.
- Tuomy, J. M., Hinnergardt, L. C and Helmer, R. L. 1969. Effect of oxygen on quality of cooked, freeze-dried combination foods. *J. Agric Food Chem.* 17:1360.

- UN. 1979. Workshop-Research and Development Needs in the Field of Fermented Foods. International Symposium of Microbiological Aspects of Food Storage, Processing and Fermentation in Tropical Asia. Bogor, Indonesia, Dec. 14-15.
- USDA. 1973. Meat and Poultry Inspection Regulations. U.S. Government Printing Office, Washington, DC
- USDA. 1977. The Staphylococcal Enterotoxin Problem in Fermented Sausage. Task Force Report. F.S.Q.S., Washington, DC
- Watts, B. M. 1961. The role of lipid oxidation in lean tissues in flavor deterioration of meat and fish. Proc Flavor Chem. Symp., p. 83. Campbell Soup Co., Camden, NJ.
- Watts, B. M. 1962. Autoxidation in foods: Lipids and Their Oxidations," H. W. Schultz, E. A. Day and R. O. Sinnhuber (Ed.), p. 202. AVI Publishing Co., Westport, CT.
- William, T. E., Leroy, B. C, Ruel, W. L. and James, T. E. 1983. Effect of air movement during fermentation on certain properties of natural flora and starter culture-fermented sausage. J. Food Prot. 46(11): 983.
- Wills, E. D. 1965. Mechanisms of lipid peroxide formation in tissue: Role of metals and hematin proteins in the catalysis of oxidation of unsaturated fatty acids. Biochem. Biophys. Acta. 98:238.
- Wills, E. D. 1966. Mechanisms of lipid peroxide formation in animal tissues. Biochem. J. 99:667.
- Wilson, N. R. R., Dyett, E. J., Hughes, R. B. and Jones, C R. V. 1981. "Meat and Meat Products: Factors Affecting Quality Control." Applied Science Publishers, Inc, New Jersey, USA.
- Younathan, M. T. and Watts, B. M. 1959. Relationship of meat pigments to lipid oxidation. Food Res. 24:728.
- Younathan, M. T. and Watts, B. M. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25:538.
- Zaika, L. L. and Kissinger, J. C 1982. Fermentation enhancement by spices: Identification of active component. In Proc 42nd Ann. IFT Meeting, Las Vegas.
- Zaika, L. L., Zell, T. E., Palumbo, S. A. and Smith, J. L. 1978. Effect of spices and salt on fermentation of Lebanon bologna type sausage. J. Food Sci. 43(1):196.
- Zipser, M. W. and Watts, B. M. 1967. Ascorbate and tripolyphosphate in cured, cooked, frozen pork. J- Agric Food Chem. 15:80.

## A P P E N D I C E S

## APPENDIX A

### ANALYSIS OF VARIANCE OF THURINGER SAUSAGE TYPE, STORAGE TIME AND STORAGE TEMPERATURE ON PH, PERCENT MOISTURE, FAT, ASH AND PROTEIN

Source <sup>a</sup> of Variation	Item				
	pH	Moisture	Fat	Ash	Protein
T	0.12(1)**	816.74(1)**	195.93(1)**	0.09(1)	63.12(1)**
S	0.03(1)**	5.26(1)*	0.33(1)	0.24(1)	26.11(1)**
T X S	0(1)	0.01(1)	13.03(1)**	2.75(1)	0(1)
D	0.05(3)**	54.24(3)**	30.11(3)**	1.99(3)	6.66(3)**
T X D	0.02(3)**	16.10(3)**	7.85(3)**	1.23(3)	20.18(3)**
S X D	0(3)**	25.72(3)**	4.93(3)*	0.98(3)	1.81(3)
T X S X D	0(3)**	12.21(3)*	4.89(3)*	2.51(3)	9.90(3)**
<hr/>					
Contrast <sup>b</sup>					
T	0.12(1)**	816.74(1)**	195.93(1)	0.10(1)	63.12(1)**
S	0.03(1)**	5.26(1)*	0.33(1)	0.24(1)	26.11(1)**
D L <sup>^</sup>	0.07(1)**	50.67(1)**	84.95(1)**	0.32(1)	11.47(1)**
D Q <sup>^</sup>	0.02(1)**	2.93(1)	1.61(1)	0.01(1)	8.51(1)*
D C <sup>^</sup>	0.05(1)**	0.65(1)	3.75(1)	5.62(1)*	0(1)
T X D L <sup>^</sup>	0.05(1)**	2.39(1)	9.00(1)*	3.22(1)	56.95(1)**
T X D Q <sup>^</sup>	0(1)**	3.18(1)	3.03(1)	0.20(1)	3.57(1)
T X D C <sup>^</sup>	0.02(1)**	10.53(1)**	11.52(1)**	0.28(1)	0.02(1)
S X D L <sup>^</sup>	0(1)	21.52(1)**	0.59(1)	2.43(1)	2.13(1)
S X D Q <sup>^</sup>	0(1)*	0.15(1)	10.89(1)**	0.13(1)	3.22(1)
S X D C <sup>^</sup>	0(1)**	4.04(1)*	3.30(1)	0.36(1)	0.06(1)
S X T X D L <sup>^</sup>	0(1)**	3.61(1)*	6.20(1)*	5.56(1)*	29.66(1)**
S X T X D Q <sup>^</sup>	0.01(1)**	6.80(1)*	5.65(1)*	0.71(1)	0.01(1)
S X T X D C <sup>^</sup>	0(1)	2.04(1)	2.82(1)	1.26(1)	0.04(1)

<sup>a</sup>Mean squares (degrees of freedom) for S = Thuringer sausage type, D = storage time, and T = storage temperature.

<sup>b</sup>Using orthogonal polynomials.

<sup>^</sup>L = Linear contrast, Q = Quadratic contrast, and C = Cubic contrast.

\*P<0.05.

• \*V<0.01.

## APPENDIX B

### ANALYSIS OF VARIANCE OF THURINGER TYPE, STORAGE TEMPERATURE AND STORAGE TIME ON TBA VALUES AND AEROBIC PLATE COUNTS

Source <sup>a</sup> of Variation	Item	
	TBA°	Aerobic plate counts (log/g)
T	0(1)	0.04(1)
S	1.32(1)*	0.28(1)**
T X S	0.72(1)*	0.01(1)
D	1.42(3)**	0.07(3)
T X D	2.50(3)**	0.48(3)**
S X D	1.23(3)**	0.30(3)**
T X S X D	1.08(3)**	0.02(3)
Error	0.16(48)	0.02(16)

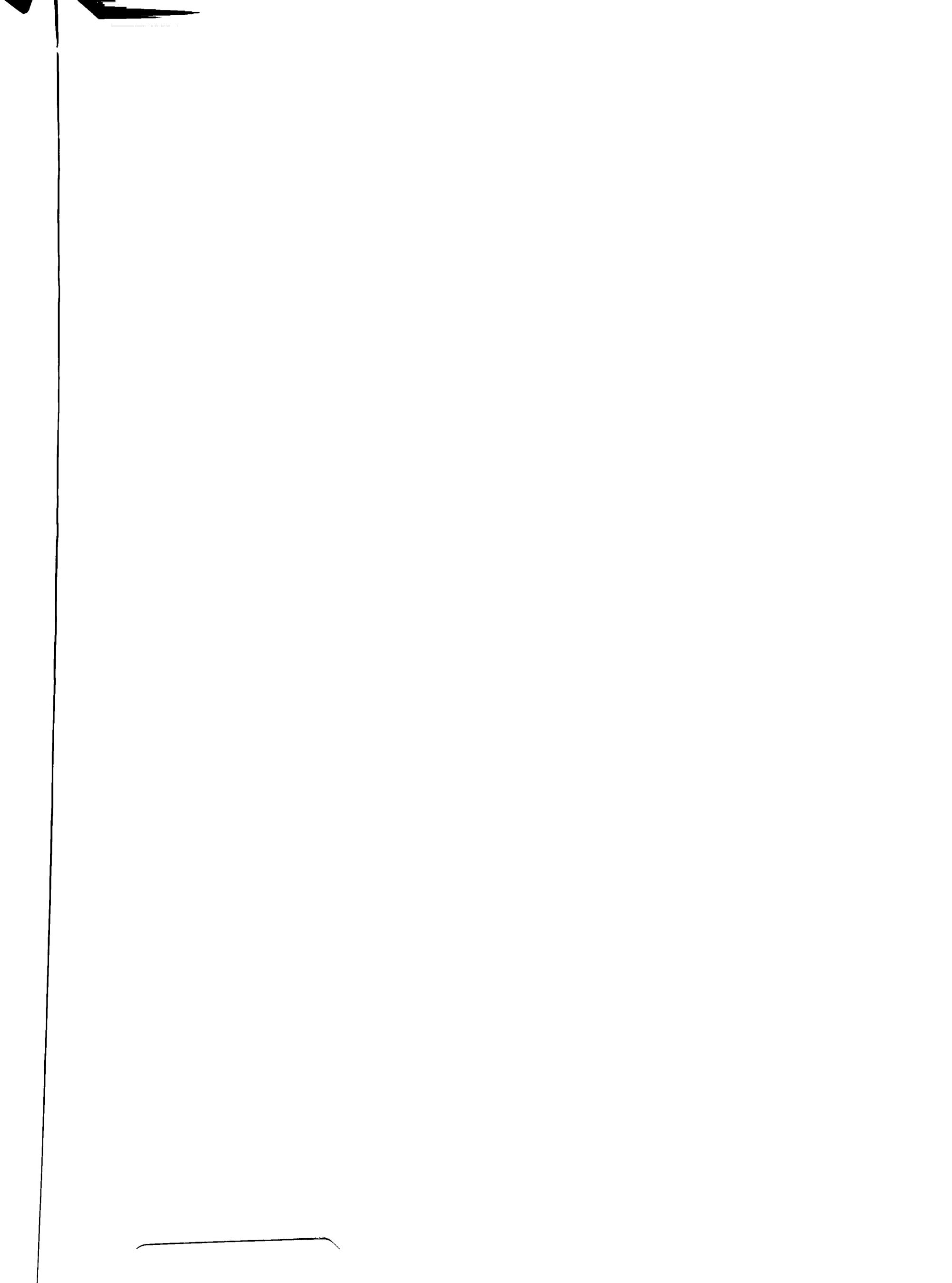
<sup>a</sup>Mean squares (degrees of freedom) for S = Thuringer sausage type, D = storage time, and T = storage temperature.

<sup>b</sup>TBA = thiobarbituric acid value.

\*P<0.05.

<sup>c</sup>kit

P<0.01.



**H ff ^**