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BROWNING AND AROMA DEVELOPMENT DURING HEATING OF BOVINE MUSCLE SYSTEMS AS AFFECTED BY LIPID COMPOSITION OF THE MUSCLE

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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DEDICATION

This dissertation is dedicated to Dr. Robert A. McGrath of the U.T. Counseling Center, who by his patience, guidance and professional expertise is helping me to stop pushing against doors that open inward.

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ABSTRACT

Browning and aroma development were studied in heated lyophilized meat systems of differing lipid composition. To achieve variation in lipid content of the meat systems, bovine muscle was obtained from animals that were fed differently as to both ration and overall plane of nutrition. These included grass-fed, limited grain-fed and full grain-fed animals. Chloroform-methanol extracts of lyophilized meat from grass-fed, limited grain-fed and full grain-fed animals were analyzed for phospholipid content by phosphorus analysis. The extracted lipid was fractionated, and methyl esters of the neutral lipid and phospholipid fractions were analyzed by gas-liquid chromatography.

Four lyophilized meat systems from beef of each dietary treatment were heated for browning and aroma assessment. The four systems were treated before lyophilization as follows: (1) no treatment, (2) added glucose (4% by weight), (3) glucose oxidase treatment and (4) glucose oxidase treatment plus added glucose (4% by weight). Hunter L, a and b color values were obtained for the heated lyophilized systems. Aroma of heated systems was assessed by means of aroma profiling by a trained sensory panel of seven members.

There was a significant effect of animal diet on neutral lipid ($P < 0.01$) and phospholipid ($P < 0.05$) content of steaks used for the preparation of lyophilized systems. Beef from grass-fed animals had higher concentrations of polyunsaturated fatty acids than did beef from grain-fed animals.

Dietary treatment of the animals had a significant effect on Hunter L values of heated systems ($P < 0.01$). The addition of glucose at 4% had a significant effect on both Hunter L ($P < 0.001$) and b (yellowness) values ($P < 0.01$). Systems with added glucose were visibly much browner than systems with no added glucose. The removal of intrinsic glucose by enzymatic treatment, however, had little or no effect on browning.

Panel assessments of meat system aroma in terms of 14 standard aromas indicated that both dietary treatment of the beef animal and system modification imposed by the researcher affected panelists' responses to most aroma notes. Grass and ammonia aromas were more often used to characterize heated systems containing grass-finished beef than those containing grain-finished beef. Rancid fat and beef aromas were more characteristic of heated systems containing grain-finished beef. Dominant aroma notes expressed for systems containing added glucose were brown sugar, toast and burnt paper.

Although lipid analysis in this investigation indicated significant dietary influences on proportions and fatty acid composition of neutral lipid and phospholipid, the browning data suggest that the addition of reducing sugar was a more important factor in meat browning than was lipid composition as affected by animal diet. Similarly, although diet affected both lipid composition of the meat and panelists' perception of most aroma notes in the heated systems, a direct relationship between lipid composition and aroma development was not observed. Possibly the added glucose obscured aroma differences that might have been attributed to diet.

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CHAPTER I

INTRODUCTION

For the biochemist lipid-protein interactions in biological material encompass wide areas of cell-membrane and enzyme mechanics, blood coagulation, and serum lipoprotein transport (Dillard and Tappel, 1976). For the food scientist lipid-protein interactions are of interest in such diverse areas as lipovitellin in egg yolk (Kamat et al., 1975), lipid binding during dough formation in bread making (Chung and Tsen, 1975), and the post mortem stability of tissue lipids (El-Gharbawi and Dugan, 1965). A particular interest in polar lipids of skeletal beef tissue has stemmed from observed deterioration of freeze-dried meat upon storage and the apparent contribution of phospholipids to off-flavor development in processed meats (Dugan, 1967).

Extensive reported research has involved interactions of free amines, peptides, amino acids and protein with reducing sugars to form carbonyl-amine precursors responsible for brown pigment formation. Less has been reported on the possible involvement of nitrogen-containing phospholipid moieties or on the involvement of oxidative products of highly unsaturated fatty acids, which are concentrated in phospholipids, in carbonyl-amine browning and flavor development.

In this laboratory nonoxidative interactions involving phospholipids, proteins, free amino acids, reducing sugars and other meat components have been studied in cooked meat, in drip lipids and in prepared reaction systems. The most recent work, reported herein, has

been with possible phospholipid-reducing sugar interactions and their influence on two important quality attributes, browning and browning-associated aroma, of lyophilized meat heated with added glucose.

Earlier work in this laboratory (Campbell and Turkki, 1967) showed the possibility of either selective reactions involving phospholipids or partitioning of phospholipid classes between meat and drip lipids during heating. McCord (1974) attempted to implicate phospholipids in the browning of meat to which reducing sugar was added before heating. However, the drip lipids presented analytical problems that prevented detection of changes in phospholipids. Conventional methods for cooking meat allow separation of drip lipids from the meat. In addition to the problems of analyzing drip lipids, a further result of the separation might be that conditions do not exist throughout the entire heating process to allow all possible reactions between lipid components and other muscle constituents. Many commercial meat formulations are processed in such a way that drip separation does not occur during heating. These formulations also are a type of meat product to which a reducing sugar frequently is added. Thus for the purpose of the present investigation, a combination model system-meat system in which all available lipid could be retained in the heated material was designed.

Meat of varied lipid/phospholipid characterization, made possible by controlling dietary treatment, was selected for preparation of the reaction systems. Selection of meat representing a wide spectrum of lipid characteristics seemed important in that meat with a high ratio of phospholipid to neutral lipid might prove to be especially reactive in such systems. Further, it was thought that levels of polyunsaturation

in phospholipid fatty acid moieties of meat used in reaction systems might influence browning and aroma development. Phospholipids are a significant factor in quality attributes of excessively lean meat (Dugan, 1967) because phospholipid concentration is relatively high in lean meat. Recent feed management research has focused on the production of lean beef because of the recognized need to reduce human intake of saturated animal fat.

Carbonyl-amine reactions can occur during the application of heat to any food system containing both aldehyde and amine reactants. Under given conditions these reactions can be responsible for the pleasant flavors and desirable color of roasted, baked and toasted foods. Under some conditions, however, they can bring about unwanted discoloration, off-flavor, loss of solubility and/or nutritive value of protein and adverse textural effects on many foods. Amino compounds (free amines, amino acids, peptides, proteins) are known to react with free or liberated aldehyde to produce carbonyl intermediates capable of undergoing a series of degradation, condensation and polymerization steps leading to the production of highly colored, fluorescent, macromolecular pigments (Reynolds, 1965). These dark brown pigments, melanoidins, are responsible for the browning and flavor of many cooked foods.

Voluminous research exists on model systems for the study of carbonyl-amine reactions (Hodge, 1953; Wasserman and Spinelli, 1970; and Warmbier et al., 1976), and often the system employs a solution as the reaction medium. Previous research in this laboratory was concerned with carbonyl-amine browning reactions in solution (Armitage, 1976). Since in actuality the moisture content of food systems in which carbonyl-amine

reactions are most important is low, the need to prepare a low-moisture meat system for the present investigation seemed evident.

Zaika (1969) found reducing sugar to be the limiting reagent in carbonyl-amine reactions in meat. The addition of excess sugar in the form of reagent grade glucose might serve to enhance browning and flavor reactions. McCord (1974) assessed browning of meat to which glucose was added at 2% and 4% levels. In formulated meat products, glucose is a common ingredient, as, for example, the use of dextrose syrups in frankfurter emulsions (Kramlich et al., 1973).

For the purposes of the present investigation, a lyophilized meat system that allowed no separation and loss of lipid during heating and to which glucose could be added was designed. The use of meats that differ in their concentrations of phospholipid and polyunsaturated fatty acids might result in varying levels of interaction between lipid and reducing sugar during heating. The phospholipids could conceivably contribute to carbonyl-amine browning and aroma development in the heated meat in two ways: by providing free amino nitrogen from polar moieties and by providing carbonyl groups from the autoxidative products of the polyunsaturated fatty acids.

CHAPTER II

REVIEW OF LITERATURE

I. MEAT LIPID STUDIES: PROGRESS TO DATE

Prior to the present investigation, a number of researchers in this laboratory have been concerned with characterizing meat lipids, particularly phospholipids, and monitoring the reactions that occur in heated bovine muscle and are responsible for quality attributes of cooked meat. Earlier studies were focused upon overall phospholipid to neutral lipid content and/or phospholipid class distribution in raw and cooked meat and drip. The method of expressing phospholipid concentration was found to be important when interpreting data. Using lean meat, Roberts (1966) was able to show the proportion of phospholipid in total lipid extracted from bovine muscle to remain unchanged during heating. Campbell and Turkki (1967), on the other hand, used ground beef with a higher fat content and observed considerable loss of neutral lipid in the drip during heating, resulting in higher phospholipid concentration in cooked meat than in raw meat when phospholipid was expressed as a percentage of total lipid. Lackey (1973) did not find significant differences between phospholipid content of raw and cooked muscle when concentration was expressed as a percentage of total lipid, but when expressed on the basis of percentage of muscle, phospholipid content of cooked meat was higher than that of raw meat. Further, she found evidence of partitioning, drip lipids having significantly higher cephalin and lower lecithin content than raw or cooked lipids.

In addition to meat lipid characterization, attempts have been made to elucidate relationships between meat phospholipids and other muscle constituents. Turkki (1965) worked with two bovine muscles representing different degrees of usage by the animal and analyzed total lipid, total lipid phosphorus, total nitrogen fractions and DNA in the unheated muscle tissue. She found expression of phospholipid concentration in relation to concentrations of other components to provide valuable information concerning interactions between these muscle constituents.

Morrison and Campbell (1971) found the portion of pork semitendinosus muscle having a high white fiber content to have higher total protein and lipid than the portion with a high proportion of red fibers. The fact that the portion of the muscle with predominating red fibers had higher concentrations of phospholipid and DNA than the light portion suggested that phospholipid was more closely related to metabolism than to exercise.

In an investigation involving a combination of muscle components of heated samples, Hall (1974) used extensor, semitendinosus and gastrocnemius muscle. She was unable to show a quantitative relationship between phospholipid and extractable myoglobin of extensor muscles heated to three end point temperatures.

Since simple system data are generally recognized to be predictive of reactions occurring in heated food systems, the preparation of model systems containing phospholipid as well as other muscle components has been undertaken in this laboratory as a means of examining important reaction mechanisms. Although phospholipids are not water-

soluble, their polar nature is responsible for their emulsifying capacities. Melton (1973) studied phospholipid-sarcoplasmic protein emulsions and found that heating caused a decrease in free lipid and in lecithin. She reported phospholipid autoxidation and a decrease in free amino groups that suggested the formation of carbonyl-amine compounds during holding at elevated temperatures. Melton's simple system data indicated phospholipid-myoglobin interactions. Armitage (1976) subjected phospholipid emulsions and phospholipid-free dispersions containing glucose and free amino acids to heat treatments of 25, 55 and 77°C. She reported that the presence of phospholipid was accompanied by a significant degree of browning and loss of free amino acids.

McCord (1974) studied reducing sugar and phospholipids in relation to color of cooked ground beef. She found cooked meat to exhibit lower reducing sugar levels than raw meat, and she observed a significant increase in browning of drip when glucose was added to the meat.

The observations cited above stimulated the researcher's interest in bovine lipids and laid the groundwork for the present investigation of phospholipid-reducing sugar interactions in meat systems.

II. PHOSPHOLIPIDS

Whereas free neutral lipids can be easily extracted from muscle tissue with nonpolar fat solvents, a large portion of the lipid in metabolically active beef tissue is bound to protein and/or carbohydrate and is less accessible. Phospholipids are one class of these conjugate lipids exhibiting a polar nature. The specific biological role of

phospholipids is not clear in that individual phospholipids may have independent metabolic roles. Phospholipids are the storage form of phosphate and the source of choline for nerve tissue. They are intermediate in the transport, absorption and metabolism of fatty acids and important to the utilization of sodium and potassium by the cells (Terrell and Bray, 1969).

The close proximity of phospholipids to carbohydrate and protein cellular components suggests that interactions between cell constituents occur. Since interactions of this nature are known to occur in biological systems, similar interactions in post-mortem muscle seem likely. The difficulty encountered in extracting phospholipid is indicative of these strong tendencies for complex formation with other tissue components.

Karel (1973) discussed several phospholipid-protein bonds responsible for lipid-protein interactions. These include electrostatic interactions between negatively charged phosphate groups and positively charged protein groups, salt bridges involving divalent metals and hydrophobic bonding.

Free fatty acids are also able to form insoluble complexes with protein. An increase in fatty acids during the storage of frozen fish paralleled a decrease in protein solubility. Isolated muscle protein upon reaction with linoleic and linolenic acid became partially insoluble (Lea, 1957). Several researchers have looked at complexes that form between oxidized fatty acids and certain proteins. Free radicals that form during lipid autoxidation have been found capable of initiating protein polymerization and bringing about a reduction in concentrations of certain amino acids, notably methionine, lysine, histidine and

cysteine. Further, cephalins were able to form unsaturated aldehydes and to increase in affinity for protein upon the application of heat (Reynolds, 1969).

Rzhekhin and Krasil'nikov (1965) reported a reaction between phosphatides and soluble protein from soybean. Ikeda and Taguchi (1968) found increased stability of actomyosin in the presence of lecithin, presumably because of decreases in soluble protein-phospholipid complexes.

Changes in the phospholipid fraction of meat during heating have been shown to be an important factor in the subsequent color and flavor development in heated meat. Phospholipids contribute to the production of carbonyl precursors by virtue of their nitrogen moieties, their relatively high proportion of unsaturated fatty acids and their close association with protein in meat systems (Dugan, 1967). The highly unsaturated fatty acid moieties of polar lipids plus other possible propensity for reactivity in polar portions of the molecules introduce processing and shelflife concerns. Even though the amount of phospholipid may be small, the unique contribution it makes to the quality characteristics of meat, especially excessively lean meat, is large.

Wilson et al. (1976) concluded that phospholipid played a major role as compared to neutral lipid in influencing organoleptic characteristics of heated meat and suggested the need for further investigation into the exact nature of the various polyunsaturated fatty acids of meat phospholipids in the development of meat off-flavor. They pointed out that the detection of warmed-over flavor in beef seemed to increase as the proportion of phospholipid to total lipid increased, and they

implicated the possible involvement of myoglobin-catalyzed lipid oxidation. Acosta et al. (1966) showed that a relatively high ratio of phospholipid to total lipid brought about a notable susceptibility to rancidity in meat due in part to differences in the kinds and number of polyunsaturated fatty acid moieties in the phospholipid fractions. In addition to the propensity for autoxidation resulting from unsaturation, El-Gharbawi and Dugan (1965) cite as another factor in the oxidative instability the close association between muscle lipids and the iron-containing heme compounds, which are able to act as prooxidants. They reported that as freeze-dried beef was stored, phospholipids were oxidized, and free amino acid groups decreased, having a significant effect on the subsequent browning and flavor development in heated systems. Mabrouk et al. (1969) emphasized the importance of polar lipids in skeletal beef tissue to the deterioration of freeze-dried meat upon storage.

It has been well established that certain oxidation-related quality factors in beef are attributable to the phospholipids. Phospholipid fractions of meat lipids have been found to have larger amounts of linoleic and linolenic acids than have neutral fractions (Hornstein et al., 1961), and the existence of substantial quantities of arachidonic acid in polar lipids from meat tissue has been substantiated (Anderson et al., 1975).

Terrell et al. (1968) found several undefined fatty acids in phospholipid fractions of beef lipids and by subsequent microhydrogenation prior to further gas liquid chromatography determined these unknowns to be unsaturated. In bound fractions of beef lipid from

freeze-dried meat, Giam and Dugan (1965) found several saturated odd-carbon-number fatty acids corresponding to C_{13}, C_{15} and C_{17} as well as three unknown unsaturates thought to be oxidative products.

Terrell et al. (1968) found that the long chain unsaturated fatty acid moieties of phospholipids in intact tissue of the live animal enhance membrane permeability, increasing reactivity and thus enhancing energy efficiency. Therefore, it seems logical to assume that this same tendency for reactivity may be important in post-mortem muscle. Lea (1957) reported that oxidative products of polyunsaturated fatty acids of phospholipids or phosphoglycerides and/or reactions involving specific nonlipid moieties of the various phospholipids could contribute to the role of phospholipids in determining quality of fresh and processed meat.

Hornstein and Crowe (1960) reported that both total lipid and phospholipid fractions of beef and pork developed odors associated with rancidity when exposed to air. The development of the dark color in phospholipid fractions was found to closely parallel the increase in TBA values indicating autoxidative changes. Since phospholipids are associated with the lean, it is not surprising that Younathan and Watts (1960) found TBA values of lipid from lean tissue to be significantly greater than those of depot fat. Further, lipid fractionation and analysis by classes showed that TBA values of neutral lipid were lower than those of polar lipid classes. It might be concluded from this that a varying tendency for rancidity and off-flavor development among species, among animals of a given species and even among muscles of a given animal may be related to varying concentrations of the phospholipid and thus of polyunsaturated fatty acids.

III. EFFECT OF DIET ON MEAT LIPIDS: GRASS-FED/GRAIN-FED BEEF

Recent economic trends in beef cattle production brought about changes in cattle feeding practices that have led to the production of leaner beef. Increased use of forage for finishing beef animals is currently under investigation. Generally, when slaughtered on an age-constant basis, forage-fed animals produce beef with thinner exterior fat covering and lower marbling scores than their grain-fed counterparts. Furthermore, organoleptic assessment of grass-fed beef has been of concern, and low sensory ratings on some attributes have been cited. In an earlier diet management study at The University of Tennessee, Knoxville, for example, Meyer et al. (1960) found higher sensory ratings for flavor of grain-fed beef than for flavor of grass-fed beef. Bidner (1975) reported lower aroma and flavor scores for rib roasts from pasture-fed steers than for those from grain-fed steers.

The effect of diet on the fatty acid composition of meat lipids has been studied. Sumida et al. (1972) reported marked effects of feeding regimen on fatty acid composition of muscle lipids. In an earlier investigation of grass-fed beef lipids in this laboratory, Cook (1963) found that even though there appeared to be no effect of feed management on the fatty acids of neutral beef lipids in cooked or raw beef, the phospholipid fractions from grass-fed beef had several components not found in grain-fed beef. The retention times of these fatty acids suggested that they had odd-numbered chain lengths.

A definite order of deposition exists among specific body fat sites with visceral fat being deposited first, skeletal muscle lipid

later and marbling last (Zinn et al., 1970). Since phospholipids are growth related, it seems likely that the source of dietary energy would influence muscle phospholipid to a greater extent than neutral lipids. Link et al. (1970) pointed out that the percentage of total intramuscular lipid made up of phospholipid was greater in grass-fed beef than in grain-fed beef. Further, there was a marked influence on fatty acid composition of muscle lipids in animals fed a grass diet.

The importance of level of nutrition and rate of gain to lipid characteristics also has been investigated. Utley et al. (1975) showed that steers fed all-forage diets had less marbling, lower yield grades and less fat covering than steers fed high-energy diets. Jacobson and Fenton (1956) found that beef animals on a high plane of nutrition showed greater yield of lean and fat, better flavor, and greater tenderness than animals on a low plane of nutrition. Terrell and Bray (1969) reported that muscle lipids from fast-gaining steers on a high level of nutrition contained more saturated fatty acids, notably palmitic and stearic, and less linolenic and arachidonic than those of animals held on a low plane of nutrition.

These reports of marked lipid differences attributable to diet suggest a means of examining meat lipid involvement in the browning and flavor development in cooked meat. Dugan (1967) discussed the importance of phospholipids in the quality of fresh and processed meats and specifically mentioned problems associated with excessively lean meat. Thus utilization of dietary management to produce meat with varying levels of lipid, varying phospholipid/neutral lipid ratios and varying fatty acid profiles seems worthwhile. Studies cited here showed lipid differences

attributable to level of nutrition and to dietary source. These findings are the basis for the assumption that the meat from animals on different feeding regimens would provide varying lipid parameters in the systems used in this study.

IV. BROWNING REACTIONS IN MEAT

The carbonyl-amine reaction involves initially a reducing sugar such as glucose and a free amino group such as the potentially reactive one in lysine. Nitrogen groups associated with free amines, basic amino acids in proteins or phospholipids are important constituents of meat cells and could presumably react with meat sugars. Jarboe and Mabrouk (1974) found glucose, fructose and ribose to be present in sufficient levels in meat to allow their participation in carbonyl-amine browning reactions. Pearson et al. (1962) showed the development of browning during heating of fresh pork to be due to the presence of free amines and reducing sugars. Henrickson et al. (1955) implicated carbonyl-amine browning in dried meat. They found total monocarbonyls to decrease with increasing time. Tarr (1953) found similar carbonyl-amine browning to occur in freeze-dried fish.

The role of reducing sugars in these meat-related nonenzymatic browning reactions was established by Yu et al. (1969) in an evaluation of room-temperature storage of radiation-sterilized seafood. Because of serious acceptance problems brought about by brown discoloration after 12 months' storage of irradiated fish patties, they attempted to lower reducing sugar by a leaching process. They induced accelerated carbonyl-amine reactions in leached and unleached fish and reported that removing

reducing sugar by leaching prevented brown discoloration. Bowers et al. (1968) found that as reducing sugar content increased in beef, browning increased. Wood (1961) demonstrated considerable browning of this nature in ox muscle extracts and specifically related it to ribose from the ribose-5-phosphate nucleotides.

Although voluminous past research has assessed carbonyl-amine associated color changes in food systems containing reducing sugar and protein-related amine, the role of lipid in this regard has received less attention. Reynolds (1965) implicated phospholipid-associated amines in reactions with aldehydes and reducing sugars in meats. Dugan (1967) reported significantly more rapid browning by phospholipid preparations than by neutral lipid preparations during heat application. He demonstrated that fresh lecithin, which is colorless, changed to yellow and finally to brown when exposed to light and air. Under the same conditions, cephalin changed from colorless to a red-brown intermediate before becoming brown. Carbonyl-amine reactions were implicated in these color changes in phospholipid preparations.

Phospholipids have been found to brown even under ideal storage conditions because of the high degree of unsaturation in their fatty acid moieties and the resulting tendency for autoxidation to occur. Lea (1957) was one of the earliest researchers to observe that in the presence of nitrogenous groups supplied by protein, amino acids or free amines, browning and off-flavor development in phospholipid preparations was pronounced. Lea attributed this to complexes that form between free amines and oxidative fragments from unsaturated fatty acid chains. The monocarbonyls produced by mildly oxidized esters of oleic, linoleic and

linolenic acids have been shown to be important in furfural formation and subsequent production of carbonyl-amine compounds. Using rendered fat from pork, beef and lamb, Ellis et al. (1961) demonstrated the production of three aldehydes capable of carbonyl-amine participation. Thus substantial documentation seems to exist relating losses in food quality to processing and storage conditions that allow accelerated carbonyl-amine reactions. Investigation of these reactions in meats, especially in meats known to represent lipid differences, would be useful.

V. FLAVOR DEVELOPMENT IN HEATED MEAT

The importance of water-soluble components of meat systems to the development of meat flavor has been established. Hornstein and Crowe (1960) found evidence that the flavor characterizing all lean meat regardless of species was due to the free sugar and amino components associated with the lean but that flavor differences among species and differences within a species due to the diet of the animal may be attributable to meat lipids. More specifically, other investigators have cited changes in the phospholipid fraction of meat lipids during heating as important factors in subsequent flavor development in heated meat. Hornstein et al. (1960) observed the development of strong fishy odors upon heating the cephalin extracted from beef and pork as well as strong liver-like odors from heating lecithin. El-Gharbawi and Dugan (1965) reported that during storage of freeze-dried beef, oxidation of phospholipid occurred, and a decrease in free amino groups paralleled flavor development in the heated meat.

Wilson et al. (1976) discussed metmyoglobin-catalyzed lipid oxidation as a factor in observed susceptibility of tissue lipids to oxidative deterioration. They concluded that phospholipids played a major role as compared to neutral lipids in influencing organoleptic characteristics in heated meat suggesting the need for further investigation into the exact nature of the various polyunsaturated fatty acids of meat phospholipids involved in the development of meat off-flavor. Mabrouk et al. (1969) emphasized the relation of polar lipids in skeletal beef tissue to the deterioration of freeze-dried meat upon storage. Important flavor and aroma changes occurring when phospholipids are exposed to air seem to be accelerated by heat and low moisture levels. These conditions are optimal for carbonyl-amine reactions. While fatty acid characteristics as discussed above are one factor in this increased reactivity, the nitrogen moiety and reactions involving associated phosphoglycerides and plasmalogens have also been implicated in flavor development (Lea, 1957). The role of phospholipids in meat flavor led Younathan and Watts (1960) to distinguish between "tissue rancidity" and traditional oxidative rancidity of lipid material in the flavor that was imparted to heated meat by lipid changes. Hornstein et al. (1967), attempting to determime the effects of phospholipids on meat flavor, found that cephalin, characteristically high in arachidonic acid, produced fishy aroma when heated while triglycerides heated in the same manner produced a fat-like aroma.

CHAPTER III

MATERIALS AND METHODS

I. OVERVIEW OF EXPERIMENTAL PLAN

The purpose of this study was to determine to what extent the nature of the lipid present in meat samples influences the browning and aroma produced in that meat upon heating. The larger project of which this investigation is a part deals with the interaction of phospholipids and other muscle components during heating. Therefore, an attempt was made to select meat samples from animals maintained on diets that brought about differing phospholipid-to-total-lipid ratios and differing fatty acid composition and to use this meat in heated reaction systems both with and without reducing sugar.

II. HISTORY OF ANIMALS

Beef for the present investigation was obtained from animals fed for Agricultural Experiment Station research at The University of Tennessee Blount Farm. For the comprehensive research project, 39 steers were grouped into 13 trios on the basis of breed, weight and similarities in body type. Eighteen Angus, 6 Hereford, 12 Charolais and 3 Angus x Hereford crosses were represented in this comprehensive feeding management trial. All steers were wintered together on silage at The University farm, but in the spring, when pasture grass was sufficient to allow grazing, animals were randomly assigned to three feeding treatments in a completely randomized block experimental design.

Thirteen steers, representing the 13 trios, grazed ad libitum on a pasture of orchard grass, fescue and clover. Thirteen steers were placed in a dry lot and allowed to consume grain (ground corn-milo concentrate) ad libitum. The remaining 13 animals were placed in a dry lot but fed the grain ration to an extent that allowed only 1.4-1.5 pounds weight gain per day, restricting their gain to the standard values for spring pasture gains. All steers remained on their respective feeding regimens approximately 110 days.

At the end of the summer, the cattle were slaughtered on an age-constant basis in three groups over a 10-day period at The University of Tennessee abattoir. The final weights ranged from 460 to 680 pounds. Care was taken to slaughter and analyze trios simultaneously in an attempt to remove external variables other than diet. Carcasses were chilled for 72 hours (1.6°C), and the left side of each carcass was divided into closely trimmed retail cuts by The University of Tennessee Meat Laboratory personnel.

III. SELECTION OF MEAT

A loin steak (second rib chop) from each of the 18 fullbred Angus animals was purchased for the present investigation. The 18 steaks representing six trios were wrapped tightly in freezer paper and placed in -20°C frozen storage for 1 year.

IV. PREPARATION AND BROWNING OF LYOPHILIZED MEAT

Each of the 18 steaks was taken through the entire browning and lyophilization procedure separately. Each steak was thawed overnight in

the refrigerator, and cut weights were recorded for intact steaks prior to trimming and grinding. Trimmed muscles were fed through the meat grinder attachment of a Hobart mixer, Model N-50. Plates with holes of 9.5, 6.4 and 3.2 mm diameters were used successively. The grinder and bowl were maintained at 0°C by means of an ice bath, and a blanket of nitrogen was maintained over the apparatus during grinding. Four lots (40 g each) were removed so as to allow for the lyophilization of two whole meat portions and two glucose-free portions. Approximately 15 g of the raw meat were set aside at this point for moisture determination (AOAC, 1975). The glucose-free system was prepared by combining 0.4 g glucose oxidase dissolved in 2 ml distilled water with 40 g meat and incubating at room temperature for 15 min according to the method of Pearson et al. (1966). Reagent grade glucose (1.6 g) was added to one of the 40 g whole meat portions and to one of the 40 g glucose oxidase-incubated portions. The four meat portions were then firmly pressed into thin sheets to line the bottoms and sides of four 500 ml VirTis freeze-drying flasks. Samples were frozen in the flasks before being connected to a VirTis freeze dryer, Model B-10. Lyophilization required 12 or 16 hours depending on the particular modification of the system. It was necessary to use the longer lyophilization time for the glucose-loaded systems in order to arrive at comparable final moisture levels among modified systems. Lyophilized samples were removed from the freeze-dryer ports, quickly pulverized by grinding with a pestle against the sides of the flasks and immediately transferred to sieves. Ground powders were passed successively through #40, #100 and #200 sieves. At this point four variations of lyophilized powders were available for heating and/or

lipid extraction, light reflectance measurements and aroma analysis by a sensory panel:

1. Whole meat
2. Whole meat plus glucose
3. Glucose-free meat
4. Glucose-free meat plus added glucose

A 5 g portion of the lyophilized unheated whole meat powder was set aside for lipid extraction. A 2.5 g sample of the sieved powder from each of the four variations was placed in a 125 ml Erlenmeyer flask, flushed with nitrogen and stoppered with a ground glass stopper. The four samples were heated simultaneously for 15 min at 149°C in an electric Despatch rotary oven. Samples were cooled and light reflectance readings were taken on the heated lyophilized systems. Then the portions were transferred to labeled vials, flushed with nitrogen and stored at -20°C for later olfactory assessment.

V. LIGHT REFLECTANCE

The Kollmorgan Color Eye Colorimeter, Model D-1, was used for obtaining, X, Y, Z and X' values on the heated lyophilized powders. Powder was packed tightly into a round receptacle that was selected to fit the aperture of the instrument and covered with clear plastic film. Reflectance measurements were obtained against a standard white vitrolite tile and were converted to Commission Internationale de l'Eclairage (C.I.E.) tristimulus values, X, Y and Z. The Hunter system of color coordinates is in more common usage for meat color analysis; therefore,

Hunter L, a and b values were calculated from the C.I.E. values by the following equations:

$$\text{Lightness} = 10\sqrt{Y_{CIE}}$$

$$\text{Hunter a} = \frac{(X_{CIE} - Z_{CIE})}{X_{CIE} + 2Y_{CIE} + Z_{CIE}} \times 100$$

$$\text{Hunter b} = \frac{0.4(Y_{CIE} - Z_{CIE})}{X_{CIE} + 2Y_{CIE} + Z_{CIE}} \times 100$$

A program for the Olivetti Desk Computer was used to calculate these values.

VI. LIPID EXTRACTION

Lipid was extracted from both unheated and heated lyophilized meat according to the procedure of Ostrander and Dugan (1961). This method involves homogenizing the rehydrated tissue with chloroform and methanol in such proportions that an immiscible system will form in the presence of water. A homogenate of this nature separates into two layers upon standing, and an extract of the available lipid can be obtained by isolating the chloroform layer. The procedure for lipid extraction is outlined in Appendix A.

VII. PHOSPHOLIPID CONCENTRATION IN TOTAL EXTRACTED LIPID

Phospholipid concentration in total extracted lipid was determined by a modification of the original method of Fiske and Subbarow (1925). This involves the wet oxidation of an aliquot of total lipid extract

containing 1-8 μg phosphorus. The phosphate formed is measured by the reduction of the phosphomolybdate complex, and the calculation of phospholipid concentration is based on the assumption that the phosphorus content is 4% of the phospholipid present (Appendix B).

VIII. FRACTIONATION OF EXTRACTED LIPID

Phospholipids were separated from total lipid by the slurry method of Murty et al. (1960). Details of this procedure are given in Appendix C.

IX. PREPARATION OF METHYL ESTERS AND FATTY ACID ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

Fatty acids were freed and converted to methyl esters according to the procedure described by Hornstein et al. (1960). A Bendix GLC, Model 2500, equipped with a flame ionization detector, a Dohrman recorder and a digital electronic integrator were used for separation and analysis of the methyl esters. The procedure for preparation of esters and analysis of fatty acids is given in Appendix D.

X. SENSORY ANALYSIS--SELECTION OF AROMA STANDARDS

A set of aroma descriptors was needed in the present study to simulate possible aroma notes detectable in the meat systems by panel members. Harper (1975) reported a method of odor description in which pure chemical stimuli were used to represent standard food aromas. He used valeric acid, for example, to represent the odor of rancid fat. Dravnieks et al. (1973) modified this procedure to include a group of

natural food substances rather than pure chemicals to represent aroma notes. Preliminary attempts to arrive at standards by both approaches suggested the approach of Dravnieks et al. to be the more easily adaptable to the evaluation of the meat systems in the present study. Therefore, 14 standard substances considered to represent both pleasant and unpleasant aroma notes in meat were selected for use in screening and training an aroma profiling panel and in the sensory evaluation of the lyophilized meat systems. In each case an aroma standard was chosen because it represented aroma notes detected by the researcher in the lyophilized meat systems during preliminary work or because it represented an aroma reported in the current literature to be useful as a standard in meat flavor analysis. Food-related standards were prepared from commercial extracts, spices or food condiments such as onion salt, butter flavoring and dried soup mixes. These items were standard foodstuffs purchased at a local food market. Several nonfood standards were included in order to provide a more complete aroma profile of the meat systems. Aroma standards included such diverse items as: caramel, toast, butter, rancid fat, onion, fish, grass, ammonia, beef by-products, roast beef, burnt paper, bacon, brown sugar and acrolein.

Caramel candy was selected to represent the expected caramel aroma derived from a carbonyl-amine reaction in a moist medium. Herz and Shallenberger (1960) reported that amino compounds heated in the presence of butyric acid and glucose produced a distinctive caramel-like aroma. Toast, in which carbonyl-amine browning results in a somewhat different aroma, was used as another standard.

Wasserman and Gray (1965) reported an oleaginous or buttery aroma developing upon heating a water extract of ground beef. Butter was selected as a standard in the present investigation in an attempt to relate diacetyl or possibly short chain fatty acids to aroma in meat.

Odors reminiscent of oniony, fishy or grassy characteristics could possibly be related either to phospholipids or to unsaturation in fatty acids of meat lipids in the various meat systems analyzed; thus these aromas were chosen as standards. Onion flavor notes in meat were detected by Schulte and Koenders (1972). They reported a fresh onion flavor in the headspace of cooked meat and implicated the possible involvement of sulfur-containing amino acids. Fish-like aroma notes have been linked to phospholipids in heated model systems. Hornstein et al. (1960) observed the development of strong fishy odors upon heating cephalin from beef and pork. Likewise Dugan (1967) reported cephalin, which is characteristically high in arachidonic acid, to produce fishy aromas upon heating.

The presence of grassy, hay-like odors and flavors in leaf protein concentrate was found by Betschart and Kinsella (1975) to be related to the oxidation of the lipid fraction associated with the protein. It might be possible that similar interactions producing these aroma characteristics occur in meat lipids.

Based on literature reports, the inclusion of some of the more unpleasant aroma standards in the present study was felt to be justified. Henrickson et al. (1955) reported ammonia to be present in volatile fractions of stored dehydrated pork. Hornstein and Crowe (1960) reported small amounts of ammonia as well as hydrogen sulfide in the volatiles

from cooked beef. Persson and vonSydow (1973) reported a distinct retort flavor in canned beef as a result of the high heat treatment necessary for sterilization. In an attempt to find possible indications of "heat processing aroma" in the lyophilized samples, a canned beef product was used as a standard. To present a contrast to the canned beef product, freshly prepared roast beef also was used as a standard.

Borton et al. (1974) found that freshly slaughtered beef absorbed off-flavors such as exhaust fumes and cigarette smoke during storage and transport. Burnt paper was used as a standard in the present investigation to simulate a nonfood-related combustion aroma. Bacon, brown sugar and acrolein were selected as standards as a result of the researcher's own evaluation of the systems during preliminary work.

XI. AROMA PROFILING

Sensory analysis of the odor characteristics of the (1) whole meat, (2) whole meat plus glucose, (3) glucose-free meat and (4) glucose-free meat plus added glucose systems was performed by a seven-member, trained panel who had been screened and trained in descriptive odor evaluation using the selected aroma standards. Prior to selection of the seven panelists, 30 faculty, staff or graduate student volunteers from the departments of Food Science, Nutrition and Food Systems Administration or Food Technology and Science were screened for accuracy in aroma detection of standard food substances. The panelists selected for training participated in two training sessions at which time standard aromas were identified and discussed, and descriptive terms for these aroma notes were agreed upon by panel members.

In a series of eight testing sessions, lyophilized meat systems were presented in individual booths to trained panel members. On each test day nine modified meat systems were placed in foil-covered vials, and cheesecloth was fitted down into vials. To check the accuracy of panel members' evaluations, three hidden standards were prepared and presented in the same manner at each testing session. All samples and standards were coded with three-digit numbers, and order of presentation was completely randomized. Odors in the test samples were developed by reheating the lyophilized powders from each system in a sand bath maintained as closely as possible at 149°C, the temperature used in the initial heating of the meat powdors. Panelists were instructed to uncap vials one at a time and sniff. Presented along with the samples was a response sheet (Appendix E) on which panelists could indicate the presence of any of the standard aroma notes or any additional ones they might detect. Instructions stated that vials were to be uncapped, sniffed and recapped quickly and that samples were not to be tasted.

XII. STATISTICAL ANALYSIS

Because the six trios of cattle represented by the 18 loin steaks used in the study had been assigned to the various trios on the basis of breed, weight and similarities in body type, nonsignificant effects for trio in an analysis of variance would indicate that the six steaks for each dietary treatment could be considered replications. Moisture and cut weights were determined on the 18 steaks prior to the preparation of the lyophilized systems. Total lipid and phospholipid data were

obtained from extracts of unheated lyophilized whole meat powders. Relative percentages of the fatty acids present in methylated lipid samples were obtained for both neutral and polar fractions of extracted lipid. For each of the above determinations, an analysis of variance was performed to test for the effects of diet and trio as shown in Table 1. When means were found to be significantly different, Duncan's multiple range test was applied (Duncan, 1955).

Table 1--Analysis of variance for measurements on unheated samples

Source of variation	Degrees of freedom
Diet	2
Trio	5
Error	10
Total	17

Hunter L, a and b values and sensory aroma data were collected on the whole, whole-plus-glucose, glucose-free and glucose-free-plus-added glucose systems prepared from the lyophilized meat. Analysis of data for the four heated systems was performed by analysis of variance as shown in Table 2. Duncan's multiple range test was applied when means were significantly different (Duncan, 1955).

Frequencies of panel responses indicating perception of each of the aroma standards were tabulated, and means for the six replications were compared among diet and among system modification. Panel responses for aroma notes detected in the hidden standards were used to assure the

Table 2--Analysis of variance for measurements on heated systems

Source of variation	Degrees of freedom
Diet	2
Trio	5
Diet x trio	10
System	3
Diet x system	6
Error	45
Total	71

researcher of the accuracy and repeatability of panel member responses.

In much of the previous work involving attempts to evaluate the effects of diet on muscle characteristics, animal histories were not known and/or sample size was too small to allow extensive and reliable statistical analysis. However, the comprehensive feeding project from which meat was obtained for the present investigation was planned and conducted for a specific experimental design.

CHAPTER IV

RESULTS AND DISCUSSION

I. CUT WEIGHT AND MOISTURE CONTENT OF RAW MEAT USED FOR MODIFIED SYSTEMS

On the basis of previous data from the comprehensive feeding study, it was known that slaughter weights and thus individual cut weights from animals on grass-fed or limited grain-fed diets were significantly lower than those from animals on full-grain diets (Lusby, 1977). Mean weights of corresponding steaks used for preparation of the lyophilized systems are given in Table 3. The analysis of variance (Table 4) shows that there was a significant ($P < 0.001$) effect of dietary treatment, but not of trio, on cut weights. Steaks from grass-fed and limited grain-fed animals were smaller than steaks from full grain-fed animals (Table 3).

There were no significant differences in percentage moisture among the steaks used for preparation of the lyophilized systems (Table 4). This was true among dietary treatments, as well as among trios.

II. TOTAL LIPID AND PHOSPHOLIPID IN LYOPHILIZED MEAT

It is generally recognized that high energy grain concentrates fed ad libitum to beef cattle will produce meat with higher levels of marbling and subcutaneous fat than will pasture grasses. Through the use of meat from animals fed rations that represented both high and low

Table 3--Mean values for 18 steaks used for preparation of meat systems: cut weight (g); moisture (%)

Diet	N	Cut weight (g)	Moisture (%)
Grass	6	361.8[a]	76.0
Limited grain	6	433.0[a]	75.6
Full grain	6	555.8[b]	75.2

Trio	N	Cut weight (g)	Moisture (%)
1	3	484.3	74.2
2	3	450.8	77.0
3	3	479.5	75.6
4	3	438.8	74.9
5	3	362.7	75.7
6	3	485.1	76.3

[a,b]Means with the same superscript do not differ significantly at the level $P < 0.05$.

Table 4--Mean square values and significance of F-ratios for cut weight and moisture

Source of variation	df	Mean squares	
		Cut weight	Moisture
Total	17		
Diet	2	57832.9***	1.0^{NS}
Trio	5	6613.8^{NS}	3.1^{NS}
Error	10	2631.9	3.4

***P < 0.001

levels of caloric intake; it was hoped that insight into the role of lipid in aroma and browning of meat systems might be gained. Table 5 contains means for percentage of total lipid and phospholipid extracted from lyophilized meat. As could be expected, total lipid, expressed as a percentage of total muscle, was affected significantly ($P < 0.01$) by ration (Table 6). Phospholipid extracted from lyophilized meat was also affected significantly ($P < 0.05$) by diet (Table 6). The phospholipid mean values (Table 5) reflect the previously mentioned differences in marbling that resulted from the dietary treatments. It is to be expected that a muscle sample from a well-marbled steak would have a higher concentration of total intramuscular lipid and a correspondingly lower concentration of phospholipid than a sample from a steak having scant marbling. As with cut weights and moisture values, total lipid and phospholipid concentrations did not differ significantly among trios (Table 6).

III. FATTY ACID COMPOSITION OF NEUTRAL AND POLAR LIPID FRACTIONS

Active biohydrogenation in the rumen of beef cattle is responsible for a relatively stable fatty acid composition of bovine lipid regardless of diet. Nevertheless, the role of diet in lipid composition in ruminants cannot be entirely discounted. Gann (1977) analyzed ground beef lipids from animals on this dietary program and found the highest unsaturation in lipids from grass-fed animals. The effect of unsaturation of beef lipid fatty acids on the aroma and flavor in the heated meat was of interest in this investigation.

Table 5--Mean values for total lipid and phospholipid extracted from lyophilized meat (expressed as a percentage of original muscle on a wet weight basis)

Diet	N	Total lipid	Phospholipid
Grass	6	5.18[a]	1.92[a]
Limited grain	6	6.14[a]	1.46[b]
Full grain	6	8.69[b]	1.20[b]
Trio	**N**	**Total lipid**	**Phospholipid**
1	3	7.32	0.90
2	3	7.51	1.32
3	3	5.35	2.02
4	3	6.00	1.56
5	3	7.10	1.63
6	3	6.74	1.76

[a,b]Means within a column with the same superscript do not differ significantly at the level $P < 0.05$.

Table 6--Mean square values and significance of F-ratios for total lipid and phospholipid

Source of variation	df	Mean squares	
		% lipid	% phospholipid
Total	17		
Diet	2	19.68**	79.77*
Trio	5	5.24^{NS}	44.66^{NS}
Error	10	1.85	17.66

*P < 0.05

**P < 0.01

Extensive work with protected lipid supplementation, in which a deliberate attempt is made to prevent microbial hydrogenation, has suggested a "polyunsaturated flavor" associated with beef high in unsaturated fat (Park et al., 1975). It would seem, therefore, that beef with a relatively high degree of unsaturation resulting either from an overall low plane of nutrition or from a low-fat forage ration might be expected to exhibit, upon heating, aromas distinguishable from those of highly finished grain-fed beef.

Mean percentages for the fatty acid concentrations in neutral lipid fractions are reported in Table 7. As seen in Table 8, dietary variations resulted in significant differences in concentrations of caprylic (C_8), capric (C_{10}), lauric (C_{12}), myristic (C_{14}), pentadecanoic (C_{15}), palmitic (C_{16}), oleic ($C_{18:1}$), linolenic ($C_{18:3}$), arachidic (C_{20}) and arachidonic ($C_{20:4}$) acids. There were no significant differences due to diet for myristoleic ($C_{14:1}$), palmitoleic ($C_{16:1}$), heptadecanoic (C_{17}), stearic (C_{18}) or linoleic ($C_{18:2}$).

Table 9 is a compilation of average concentrations of total saturates, total monoenes and total polyenes in neutral lipid from samples representing grass-fed, limited grain-fed and full grain-fed animals. Lipid from grass-fed animals showed a threefold concentration of polyenoic fatty acids (linoleic, linolenic and arachidonic) over that of lipid from full grain-fed animals.

Fatty acid means for polar lipids of beef, presented in Table 10, differed significantly due to dietary influence for capric (C_{10}), myristic (C_{14}), myristoleic ($C_{14:1}$), pentadecanoic (C_{15}), palmitic (C_{16}), heptadecanoic (C_{17}), stearic (C_{18}) and oleic ($C_{18:1}$) acids

Table 7--Mean values for percentages of fatty acids in neutral lipid

Diet	N	C_8	C_{10}	C_{12}	C_{14}	$C_{14:1}$
Grass	6	0.50^a	0.00^a	1.00^a	1.00^a	1.41
Limited grain	6	0.50^a	0.40^b	0.50^b	4.00^b	1.60
Full grain	6	0.00^b	0.00^a	0.00^c	3.00^b	1.00

Diet	N	C_{15}	C_{16}	$C_{16:1}$	C_{17}	C_{18}
Grass	6	3.00^a	19.00^a	6.00	3.00	12.00
Limited grain	6	0.50^b	28.17^b	4.50	1.00	14.00
Full grain	6	0.00^b	28.00^b	4.00	0.00	15.00

Diet	N	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	$C_{20:4}$
Grass	6	42.00^a	4.00	4.00^a	2.00^a	1.00^a
Limited grain	6	41.00^a	2.00	2.00^b	0.00^b	0.00^b
Full grain	6	46.00^b	2.00	1.00^b	0.00^b	0.00^b

^{a,b,c}Means within a fatty acid grouping with the same superscript do not differ significantly at the level $P < 0.05$.

Table 8--Mean squares and significance of F-ratios for neutral lipid fatty acids

Source of variation	df	Mean squares			
		C_8	C_{10}	C_{12}	C_{14}
Total	17				
Diet	2	0.5000**	0.3200***	1.5000***	14.0000*
Trio	5	0.0533^{NS}	0.0067^{NS}	0.0900^{NS}	0.4334^{NS}
Error	10	0.0413	0.0067	0.0306	3.4333

Source of variation	df	Mean squares			
		$C_{14:1}$	C_{15}	C_{16}	$C_{16:1}$
Total	17				
Diet	2	0.5672^{NS}	15.5000*	165.0556***	6.5000^{NS}
Trio	5	0.1006^{NS}	1.6307^{NS}	9.5222^{NS}	1.3813^{NS}
Error	10	0.7806	1.8107	2.5222	2.1613

Source of variation	df	Mean squares			
		C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$
Total	17				
Diet	2	14.0000^{NS}	14.0000^{NS}	42.0000**	8.0000^{NS}
Trio	5	3.3667^{NS}	0.8000^{NS}	2.6667^{NS}	7.3334^{NS}
Error	10	0.1667	2.4000	6.2667	4.5333

Source of variation	df	Mean squares		
		$C_{18:3}$	C_{20}	$C_{20:4}$
Total	17			
Diet	2	14.0000**	8.0000**	2.0000**
Trio	5	1.9000^{NS}	1.0066^{NS}	0.1667^{NS}
Error	10	1.5000	1.0667	0.1667

*P < 0.05 **P < 0.01 ***P < 0.0001

Table 9--Summary of mean concentrations: saturates, monoenes and polyenes in neutral lipid of beef

Diet[a]	Total saturates	Total monoenes	Total polyenes
1	41.5	49.4	9.0
2	49.1	47.1	4.0
3	46.0	51.0	3.0

[a]1 = grass-fed
2 = limited grain-fed
3 = full grain-fed

Table 10--Mean values for percentages of fatty acids in polar lipid

Diet	N	C_8	C_{10}	C_{12}	C_{14}	$C_{14:1}$
Grass	6	0.00	0.00[a]	0.00	4.00[a]	3.00[a]
Limited grain	6	0.13	0.48[b]	0.41	2.00[b]	0.16[b]
Full grain	6	1.36	0.36[b]	0.58	1.50[b]	0.83[b]

Diet	N	C_{15}	C_{16}	$C_{16:1}$	C_{17}	C_{18}
Grass	6	0.58[a]	22.00[a]	3.00	2.00[a]	8.00[a]
Limited grain	6	0.00[b]	29.00[b]	2.50	1.83[a]	10.00[b]
Full grain	6	0.00[b]	29.00[b]	1.50	0.16[b]	11.66[b]

Diet	N	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	$C_{20:4}$
Grass	6	28.00[a]	14.00	4.00	1.33	9.50
Limited grain	6	26.00[b]	15.00	2.91	0.83	8.66
Full grain	6	24.00[c]	16.00	3.58	0.16	8.33

[a,b,c]Means within a fatty acid grouping with the same superscript do not differ significantly at the level $P < 0.05$.

(Table 11). There were no significant differences due to diet in caprylic (C_8), lauric (C_{12}), palmitoleic ($C_{16:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), arachidic (C_{20}) or arachidonic ($C_{20:4}$) acids. Total saturates, monoenes and polyenes for the respective dietary treatments are summarized for polar lipids in Table 12. Total saturates and monoenes seemed to reflect dietary influence to a greater extent than did polyenes; this observation is confirmed by the analyses of variance for the individual fatty acids. The polyene concentrations were much higher in the phospholipid (Table 12) than in the neutral lipid fraction (Table 9, page 39).

There were no significant trio effects on fatty acid concentrations (Table 8, page 38, and Table 11).

IV. CARBONYL-AMINE REACTIONS

Mean Hunter L, a and b values by diet and by system modification are given in Table 13. The analysis of variance (Table 14) shows that L values differ significantly with both diet ($P < 0.01$) and system ($P < 0.001$). The mean L (lightness) values (Table 13) for systems to which glucose was not added are nearly double those for systems to which glucose was added. The samples containing added glucose were visibly much browner after heating than those that did not contain added glucose. Neither diet nor system affected a values (redness index), and only system affected b values (yellowness index). The mean b values for samples containing added glucose were lower than those without added glucose.

Table 11--Mean squares and significance of F-ratios for polar lipid fatty acids

Source of variation	df	Mean squares			
		C_8	C_{10}	C_{12}	C_{14}
Total	17				
Diet	2	3.4067^{NS}	0.3817**	0.5417^{NS}	10.5000**
Trio	5	1.6307*	0.0223^{NS}	0.1334^{NS}	2.4667^{NS}
Error	10	1.8493	0.0450	0.1750	1.0667

Source of variation	df	Mean squares			
		$C_{14:1}$	C_{15}	C_{16}	$C_{16:1}$
Total	17				
Diet	2	13.1667**	0.6805**	98.0000***	3.5000^{NS}
Trio	5	0.9334^{NS}	0.0806^{NS}	2.5332^{NS}	2.8000^{NS}
Error	10	1.5000	0.0806	4.3333	2.9000

Source of variation	df	Mean squares			
		C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$
Total	17				
Diet	2	6.1667*	20.6665*	16.6667***	6.0000^{NS}
Trio	5	1.0667^{NS}	9.0667^{NS}	3.0667^{NS}	3.2000^{NS}
Error	10	1.0333	4.9333	0.9333	1.6000

Source of variation	df	Mean squares		
		$C_{18:3}$	C_{20}	$C_{20:4}$
Total	17			
Diet	2	1.7917^{NS}	2.0556^{NS}	2.1665^{NS}
Trio	5	6.0334^{NS}	6.3000^{NS}	6.3000^{NS}
Error	10	2.9750	0.9222	2.7667

*P < 0.05 **P < 0.01 ***P < 0.001

Table 12--Summary of mean concentrations of saturates, monoenes and polyenes in polar lipid of beef

Diet[a]	Total saturates	Total monoenes	Total polyenes
1	34.3	34.0	27.5
2	42.9	28.7	26.6
3	44.6	26.3	27.7

[a]1 = grass-fed
2 = limited grain-fed
3 = full grain-fed

Table 13--Mean Hunter L, a and b values

Diet	N	L	a	b
Grass	24	3.55[a]	8.49	5.28
Limited grain	24	3.82[b]	9.32	5.77
Full grain	24	3.38[c]	8.96	5.28
System	**N**	**L**	**a**	**b**
Whole	18	4.64[a]	9.28	6.59[a]
Whole/added glucose	18	2.43[b]	8.60	4.16[b]
Glucose-free	18	4.82[a]	8.77	6.44[a]
Glucose-free/added glucose	18	2.44[b]	9.05	4.58[b]

[a,b,c]Means within a treatment grouping with the same superscript do not differ significantly at the level $P < 0.05$.

Table 14--Mean squares and significance of F-ratios for Hunter L, a and b values

Source of variation	df	Mean squares		
		L	a	b
Total	71			
Diet	2	1.1916**	4.1633^{NS}	1.8989^{NS}
Trio	5	0.2861^{NS}	1.2062^{NS}	0.5340^{NS}
Diet x trio	10	0.5687^{NS}	5.5478^{NS}	1.4456^{NS}
System	3	31.8605***	1.6067^{NS}	28.1020***
System x diet	6	0.1679^{NS}	5.0980^{NS}	1.0924^{NS}
Error	45	0.2232	3.6676	0.9516

**P < 0.01

***P < 0.001

Previous analysis of free sugars in meat from animals on this comprehensive feeding study indicated lower levels of intrinsic reducing sugar in ground beef from grass-fed and limited grain-fed animals than in that from full grain-fed animals (Brown et al., 1978). However, in the preparation of lyophilized systems for this investigation, incubation with glucose oxidase had little or no effect on L, a or b color values.

A food product that is acceptable in flavor, in texture and in nutritive value may still be rendered valueless in market appeal if undesirable color changes accompany the heat processing necessary for its production. Henrickson et al. (1955) were able to extend the storage life of dehydrated pork by reducing the glucose content by fermentation. It seems apparent, therefore, that increasing the glucose content of a meat system prior to drying, as was done in the present investigation, would enhance the potentiality for carbonyl-amine-related deterioration. Further, it seemed important to observe these reactions in a low moisture system. Carbonyl-amine browning and associated flavor development reach a maximum in low moisture foods containing both free amines and reducing sugars thus making it a potential problem in dried meats with added glucose.

Glucose is often used as a sweetener or as part of the cure mixture in processed meats. Further, the heat treatment necessary for sterilization of a canned meat product is even more severe than that of a normal cooking procedure for fresh meat. To compound this effect, the product is sealed in the can, and carbonyl-amine flavor volatiles

that may form during the retorting process remain in contact with the product.

For the purposes of this study, insight into dietary influences on the lipid composition of the meat used to prepare the glucose-loaded or glucose-free lyophilized systems was felt to be valuable. Based on current trends in beef cattle production, it is likely that scantly marbled, lean carcasses such as those resulting from low-energy feeding regimens will be marketed for use in processed meats, whereas highly marbled Prime and Choice beef from animals fed energy-rich grain concentrates will be marketed as retail cuts. The lipid analyses did show that the diets influenced the proportions and fatty acid content of neutral lipids and phospholipids. However, browning data for the modified systems suggest that the addition of reducing sugar was a more important factor in meat browning than was lipid composition as affected by animal diet (Table 13, page 44). Thus, the greatly enhanced carbonyl-amine browning reaction in a processed meat product with added glucose might serve to obscure browning differences resulting from phospholipid components of excessively lean meat.

V. AROMA ANALYSIS

The perceived flavor of a food depends on aroma, taste and mouthfeel characteristics, but for most foods odor is the most important and distinctive aspect of flavor. Therefore, olfactory assessment of the heated meat systems by a trained sensory panel was used to characterize meat aroma due to diet and due to system modification. Panel members indicated the perception of given aroma notes in the meat

systems by placing check marks in the appropriate columns on the sensory response sheet; thus, panel assessments of meat system aromas could be obtained in terms of each of the 14 standard aroma notes. The judges consistently identified the hidden standards, though they occasionally indicated the presence of additional aroma notes. Trio means, presented in Table 15, seem to suggest that both the dietary treatment of the beef animal and the system modification imposed by the researcher affected panelists' responses to most of the aroma notes. Grass and ammonia aromas were more frequently noted in heated systems containing grass-finished beef than of those containing grain-finished beef, although the general magnitudes of the aroma ratings indicate that grassy and ammonia aromas were not among the dominant aromas overall. Rancid fat and beef aromas, on the other hand, were more frequently noted in heated systems containing grain-finished beef. The aroma notes that were affected most by the addition of glucose were rancid fat, grassy, bacon and acrolein aromas, which were less noticeable in the presence of glucose and brown sugar, toast and burnt paper aromas, which were enhanced in the presence of glucose.

Table 15--Frequencies of panel responses[a] to heated meat systems in terms of 14 aroma standards; classification by diet

Diet	Caramel	Onion	Rancid fat	Grass	Brown sugar	Bacon	Beef by-products
Grass	2.13	0.29	2.33	0.63	2.62	1.67	1.92
Limited grain	3.71	0.71	3.04	0.51	3.44	2.73	2.34
Full grain	2.17	0.46	4.32	0.34	2.13	2.00	2.54

Diet	Fish	Toast	Butter	Burnt paper	Roast beef	Acrolein	Ammonia
Grass	0.88	2.50	0.07	2.13	1.38	2.25	1.75
Limited grain	1.50	4.21	0.19	3.34	1.63	1.02	0.59
Full grain	0.61	3.88	0.04	4.65	2.82	2.43	0.07

[a]Each value represents the average number of responses for the six trios. The total possible frequency in each case is 7.

Table 16--Frequencies of panel responses[a] to heated meat systems in terms of 14 aroma standards; classification by system modification

System	Caramel	Onion	Rancid fat	Grass	Brown sugar	Bacon	Beef by-products
Whole	1.44	0.47	4.61	0.64	0.83	4.17	2.61
Whole/added glucose	4.25	0.45	1.17	0.28	4.28	0.31	1.75
Glucose-free	2.58	0.61	5.45	0.75	2.00	3.47	2.58
Glucose-free/ added glucose	2.39	0.42	1.70	0.17	3.78	0.59	2.11

System	Fish	Toast	Butter	Burnt paper	Roast beef	Acrolein	Ammonia
Whole	1.05	1.34	0.03	1.87	2.48	2.84	1.03
Whole/added glucose	1.20	5.48	0.03	3.98	2.56	0.56	0.50
Glucose-free	1.03	2.32	0.00	2.19	1.11	3.50	0.71
Glucose-free/ added glucose	0.70	5.06	0.32	5.71	3.29	0.82	0.82

[a]Each value represents the average number of responses for the six trios. The total possible frequency in each case is 7.

CHAPTER V

SUMMARY

The amino acid and the carbohydrate components of beef muscle have long been surmised to be vital to the potentiality for browning and aroma development in heated meat. Based on known reaction pathways for carbonyl-amine involvement, however, the lipid components of muscle might also be involved in interactions resulting in browning and aroma development during heating. It was the intent of this investigation to determine to what extent the nature of the lipid present in meat samples would influence these characteristics of heated meat. The experimental plan involved selection of meat samples from animals maintained on diets that brought about differing phospholipid/total lipid ratios and differing fatty acid compositions and the use of this meat in heated systems both with and without added glucose.

There was a significant effect of dietary regimen ($P < 0.001$) on cut weight, and steaks from grass-fed and limited grain-fed animals had visibly less marbling and trim fat than did steaks from full grain-fed animals. Analysis of neutral lipid, phospholipid and fatty acid composition of the lipid extracted from lyophilized systems containing meat from grass-fed, limited grain-fed and full grain-fed beef cattle indicated significant influences of animal diet on lipid composition. The proportion of total lipid that was phospholipid and the degree of unsaturation in fatty acid moieties were greater in the meat from grass-fed animals than in that from grain-fed animals.

Although lipid analysis in this investigation indicated significant dietary influences on proportions and fatty acid composition of neutral lipid and phospholipid, the browning data suggest that the addition of reducing sugar was a more important factor in meat browning than was lipid composition as affected by animal diet. Similarly, although diet affected both lipid composition of the meat and panelists' perception of most aroma notes in the heated systems, a direct relationship between lipid composition and aroma development was not observed. Possibly the added glucose obscured aroma differences that might have been attributed to diet.

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APPENDIXES

APPENDIX A

LIPID EXTRACTION (Ostrander and Dugan, 1961)

1. To rehydrate lyophilized sample, disperse 5 g in 49.5 ml distilled water for 2 min at high speed with the voltage regulator set at 70-80.

2. Blend 5 min with 130 ml absolute methanol with voltage regulator set at approximately 35.

3. Add 65 ml chloroform and reblend 5 min with voltage regulator set at approximately 35.

4. Add another 65 ml chloroform and reblend 20 sec (voltage regulator set at approximately 35).

5. Add 65 ml distilled water containing 1.5 g zinc acetate and blend 10 sec as above.

6. Transfer to No. 3 Buchner funnel and filter through Whatman No. 1 filter paper with suction. Maintain a blanket of nitrogen over the funnel.

7. Transfer residue to blender jar rinsing funnel with 100 ml chloroform and reblend for 2-1/2 min.

8. Filter as above, rinsing blender jar into the funnel with 75 ml chloroform.

9. Transfer into a 500 ml graduated bylinder with 25 ml methanol for rinsing.

10. Let stand until a sharp interface is seen between the chloroform and water-methanol layers.

11. Read the volume of the chloroform extract (lower layer). Record.

12. Transfer contents of cylinder to 500 ml separatory funnel. Drain off clear chloroform layer into an evaporating flask.

13. Mix thoroughly and immediately pipet triplicate 10 ml portions into preweighed 30 ml beakers for determination of total lipid concentration.

14. Leave beakers under the hood until lipid appears dry and then complete evaporation of solvent to constant weight in a vacuum desiccator under reduced pressure.

15. Determine percent lipid by the following:

$$\% \text{ lipid (wet wt basis)} = \frac{10 \text{ (ml total chloroform ext. x g lipid/10 ml)}}{\text{g muscle rep. by wt of powder extracted}}$$

16. Concentrate remaining extract on a rotary evaporator to a volume of 30-50 ml. Store under nitrogen at -20°C until analyzed.

APPENDIX B

PHOSPHOLIPID CONCENTRATION IN TOTAL EXTRACTED LIPID

1. Prepare standard phosphorus solution.[1]

2. Label micro-Kjeldahl flasks so as to allow for two duplicate blanks, two duplicate lipid samples and two duplicates each of phosphorus solutions diluted to contain 1, 3 and 5 μg of phosphorus.

3. Into each flask place 2 glass beads.

4. Into flasks for sample pipet 0.5 ml lipid extract (which has been diluted to contain 2-16 μg P/ml) and 2.0 ml water.

5. Into flasks for phosphorus standards, pipet 0.5, 1.5 and 2.5 ml standard phosphorus solution and 2, 1 and 0 ml water, respectively.

6. Into flasks for blanks, pipet 2.5 ml water.

7. Pipet 1.2 ml 70% perchloric acid into each flask.

8. Digest 30 min on a micro-Kjeldahl digestion rack.

9. Cool. Add 7 ml distilled water, then 1.5 ml 2.5% ammonium molybdate solution.[2] Mix well.

10. Add 0.2 ml Fiske-Subbarow reagent.[3]

11. Heat flasks in boiling water for 7 min.

---

[1]Stock solution is prepared by bringing 0.2196 g monopotassium phosphate (KH_2PO_4) to 1 liter volume with distilled water. Standard solution is prepared by diluting 5 ml stock solution to 100 ml. The standard stock solution contains 2.5 μg P/ml.

[2]Ammonium molybdate solution is made by bringing 6.25 g ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$ to 250 ml volume in distilled water.

[3]Fiske-Subbarow reagent is prepared by bringing 7.5 g sodium bisulfite ($NaHSO_3$) to 50 ml volume in distilled water. Add 0.125 g 1-amino-2-naphthol-4-sulfonic acid and 0.25 g anhydrous sodium sulfite (Na_2SO_3). Filter and store at 4°C. Make fresh weekly.

12. Cool 20 min in cold water. Pour into cuvettes and read absorbance at 830 nm with red filter and red-sensitive phototube. Read against the reagent blank.

13. Assume phospholipids to contain 4% phosphorus. This allows phosphorus value to be multiplied by 25 to obtain phospholipid.

14. Calculate phospholipid concentration in total lipid by the following:

<u>Phosphorus in sample:</u>

$$\mu g \text{ P in 0.5 ml extract} = \frac{\text{abs. of sample} \times \mu g \text{ P in std.}}{\text{abs. of standard}}$$

<u>Phospholipid concentration in total lipid:</u>

$$\% \text{ p.l. in total lipid} = \frac{\mu g \text{ p.l. in 0.5 ml extract}}{\text{mg lipid/ml extract}}$$

APPENDIX C

FRACTIONATION OF EXTRACTED LIPID--SLURRY METHOD
(Murty et al., 1960)

1. Transfer a 25 ml sample of concentrated lipid extract with 50 ml chloroform to a slurry of 50 g silicic acid (dried) and 100 ml chloroform in a 500 ml Erlenmeyer flask.

2. Mix for 10 min on a magnetic stirrer.

3. Transfer the slurry to a sintered glass funnel and filter with suction.

4. Wash the silicic acid in the funnel with five 50 ml portions of chloroform. During each washing, release the suction and stir the chloroform into the silicic acid in the funnel.

5. The filtering flask will now contain the nonphosphorus-containing lipids dissolved in chloroform. Transfer this to a 500 ml cylinder. A 10 ml aliquot of this extract should contain sufficient lipid for methylation. Place this aliquot in a 15 ml centrifuge tube, flush with nitrogen, stopper and store at -20°C for later methylation.

6. Transfer the silicic acid mixture remaining in the funnel back to the 500 ml flask with 100 ml methanol and stir on the magnetic stirrer for 10 min.

7. Filter the slurry into a clean flask using 150 ml methanol in four portions for washing.

8. When filtering is complete, transfer, with 50 ml methanol, the extract containing the phospholipids to a rotary evaporator. In order to obtain adequate concentration for analysis, evaporate to about 100 ml before taking a 10 ml sample for methylation.

APPENDIX D

PREPARATION OF METHYL ESTERS AND FATTY ACID ANALYSIS BY GAS-LIQUID CHROMATOGRAPHY

1. Place an amount of lipid extract containing approximately 100-200 mg of lipid into a 15 ml conical centrifuge tube.

2. Evaporate the solvent under nitrogen.

3. Add 5 ml 1.1 N HCl in methanol.

4. Cover tube with a marble and set into a water bath at 70-75°C for 4 hours.

5. Stir the contents of the tube with glass rod at intervals during the heating.

6. Cool to room temperature and decant into a 30 ml separatory funnel.

7. Add 6 ml of petroleum ether and 2 ml of distilled water. Mix.

8. Drain off the bottom layer leaving the petroleum ether extract of methyl esters.

9. Wash with two additional 2 ml portions of water, discarding the bottom layer each time. Discard generously the last time.

10. Dry the stem of the separatory funnel with a pipe cleaner and run the petroleum ether extract into a 15 ml centrifuge tube containing 0.5 g sodium sulfate and 0.5 g dried silicic acid. Mix.

11. Flush with nitrogen, stopper and store overnight in refrigerator.

12. Decant into another tube the next day and if not analyzed immediately, store at -20°C under nitrogen. Add solvent during storage if necessary; lipids should not be permitted to get dry.

13. Inject 4 to 6 microliters of methyl ester solution with a 10 microliter syringe into a Bendix GLC Model 2600. A 2 m by 4 mm inside diameter glass U-tube column packed with 15% diethylene glycol succinate on 60/80 mesh acid washed chromosorbW (Applied Science) can be used to separate the esters.

14. Maintain GLC conditions at: nitrogen flow rate, 40 ml/min; hydrogen flow rate, 40-50 ml/min; air flow rate, 400-500 ml/min; injection temperature, 245°C; detector temperature, 240°C; column temperature, 195°C.

15. Prepare methyl ester standards from reference mixtures obtained from Applied Science Laboratories containing known percentages of the fatty acids, C_8 - $C_{20:4}$.

APPENDIX E

SENSORY RESPONSE SHEET

NAME: _____

DATE: _____

DIRECTIONS: Please evaluate these twelve aromas based on your training in olfactory assessment. Uncap vials one at a time and sniff the contents. If you perceive any of the aroma notes listed, place a check mark (√) in that column. A sample may have several of the listed aroma notes and/or several additional ones present. Feel free to mark as many as you wish and to specify under "other" any additional odors you detect. This set of twelve vials includes meat model systems as well as some pure "hidden" standards. A set of labeled standards accompanies your samples and you may refamiliarize yourself with standard aromas if necessary. DO NOT TASTE SAMPLES OR STANDARDS. Recap vials tightly and return tray through hatch before leaving. Thank you very much.

Caramel	Onion	Rancid Fat	Grass	Brown Sugar	Bacon	Beef-By-Products	Fish	Toast	Butter	Burnt Paper	Roast Beef	Ammonia	Acrolein	Other (Specify)

VITA

Janna Dea Harris was born in Cliffside, North Carolina, on January 7, 1950. She attended Cliffside Elementary School and graduated from Chase High School, Forest City, North Carolina, in 1967. In May 1971 she was graduated magna cum laude from Western Carolina University, Cullowhee, with a Bachelor of Science in Home Economics Education. She taught home economics at T. W. Andrews High School in High Point, North Carolina, for 2 years before beginning graduate study. In August 1975 she received the Master of Science degree with a major in Foods and Nutrition from the University of North Carolina, Greensboro.

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