March 6, 1963

To the Graduate Council:

I am submitting herewith a thesis written by Anne Looney Cook entitled "The Effect of Method of Finishing and Length of Time of Aging on the Lipids of Cooked and Uncooked Beef." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Foods.

Ada Marie Campbell
Major Professor

We have read this thesis and recommend its acceptance:

Ruth Buckley

John T. Smith

Accepted for the Council:

Dean of the Graduate School

THE EFFECT OF METHOD OF FINISHING AND LENGTH OF TIME OF AGING ON THE LIPIDS OF COOKED AND UNCOOKED BEEF

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Anne Looney Cook

March 1963

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ACKNOWLEDGMENT

To express adequately my appreciation for the help and encouragement received is difficult. Many thanks are due to Dr. Ada Marie Campbell whose support from the inception to the completion of the project was invaluable. Thanks are due also to Miss Ruth Buckley and Dr. John T. Smith for their suggestions and criticisms throughout the study.

In addition to my academic advisors, I wish to express gratitude for the inspiration and faith in me given by my family--my parents, husband, and son, Christopher, whose arrival interrupted the study but intensified the need for completing it.

A. L. C.

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CHAPTER I

INTRODUCTION

Since the late 1920s the meat industry has been concerned with the market value of beef produced by pasture feeding with or without dry lot supplementation. Feeding management programs have been begun at several experiment stations to define the influence of rations on the quality of marketable beef. In addition to feeding management, the ripening, or aging, of the carcass has been studied as a possible determinant of consumer acceptability. At the University of Tennessee research in this area began about 1955. Emphasis has been given to the methods of feeding management and carcass aging of beef steers as these factors relate to palatability, vitamin content, and cooking losses of the meat. The fat has been evaluated in terms of amount, color, and flavor, adipose tissue having been the primary source.

Early studies at the University of Tennessee indicated that grass-fed beef was less acceptable in flavor than grain-fed beef, and that with both types of finish flavor acceptability declined still further with carcass aging. In 1958 Stames studied peroxide formation and free acidity of adipose tissue as possible causal factors in the

deterioration of palatability of grain- and grass-fed beef. The peroxide values and percentages of free fatty acids did not approach the values established by Younathan and Watts (1960) as being organoleptically detectable. While results of Stames' palatability studies showed a consistent preference for the flavor of fat from grain-fed beef, the flavor of fat from both types of beef decreased in acceptability with aging.

Current interest in the field of lipid metabolism focuses upon the relationship between ingested fats and the organism's ultimate deposition of specific lipid constituents. The fat studied by Stames was external fat of roasts. Younathan and Watts (1960) advanced a theory of "tissue rancidity" whereby organoleptic changes occur with reactions involving protein bound lipids, specifically phospholipids. It is known that muscle tissue contains more phospholipids than does adipose tissue. Because phospholipids are labile components in foods, they are highly subject to deteriorative reactions during processing and storing; it seems desirable, therefore, to study phospholipids separately from other lipid components. The purpose of this study was to compare the composition of muscle tissue lipids of beef steers finished on different feeding regimens. The fatty acid content of phospholipids and non-phosphorous-containing lipids of ground beef samples from grain-finished and grass-finished steers subjected to varying amounts of carcass aging was investigated.

CHAPTER II

REVIEW OF LITERATURE

In the studies made to date with grain- and grass-fed animals, the emphases have been on palatability, vitamin content, and color and amount of depot fat. Black, et al. (1931) found that grain-supplemented cattle increased their gains approximately 22% over those of grass-fed cattle, and this increased total weight reflected chiefly fat accumulation. Reviewing the literature, one finds a concensus that after an animal reaches chemical maturity (about 5 months), the fat content of the edible portion increases at a more rapid rate than does the protein. Grain-fed animals show higher fat content per edible portion than do grass-fed animals; and conversely, grass-fed animals show a higher proportion of lean per edible portion than do grain-fed animals (Black, et al., 1931; Black, et al., 1940; Hammond, 1940; Bull, et al., 1941; Hopper, 1944; Hunt, et al., 1953; Meyer, et al., 1960a; Luttingh, 1962).

With the greater fatness in the grain-finished animals, more marbling in the muscle tissue has been found than in grass-finished animals (Hopper, 1944; Meyer, et al., 1960a, b). As a result of the increased fattening and marbling, the carcass grades have been

consistently higher for the grain-fed cattle (Bull, et al., 1941; Wanderstock and Miller, 1948; Meyer, et al., 1960a; American Livestock Journal, 1961). Carcass grades also are influenced by color of the fat. Grass-fed animals tend to deposit a yellow-tinged fat which is less acceptable than the cream-colored fat of the grain-fed animals (Black, et al., 1940; Hammond, 1940; Bull, et al., 1941; Wanderstock and Miller, 1948; Hunt, et al., 1953).

Results of subjective tests for palatability in the studies comparing grain- and grass-fed animals have not always been in agreement. In an early study Black, et al. (1931) showed no significant difference between the two feeding regimens in their effect on flavor, juiciness, texture, and aroma of roasted rib samples. In a later study, Black, et al. (1940), correlating subjective tenderness scores with shear tests on rib cuts, again reported insignificant differences due to feeding regimen. Wanderstock and Miller (1948), on the other hand, observed that grain-produced beef was higher in quality and palatability than that produced from pasture alone and that difference in quality apparently was due to difference in fatness. More recently, Stames (1958) and Meyer, et al. (1960a) also have reported that grain-finished beef is significantly more palatable than grass-finished beef. Perhaps a clue to the varying results lies in the proposal of Hunt, et al. (1953) that, other things being equal, quality is the same from

either grain-fed or grass-fed beef, provided both are from cattle of equal fatness.

In many of the studies concerned with feeding management, the length of time of carcass aging also has been considered. Beef usually is ripened about three weeks before retailing in order to improve quality and perhaps tenderness (Hoagland, et al., 1917). Stames' results (1958) with external fat showed a downward trend for flavor acceptability when the meat was ripened longer than 21 days. Griswold and Wharton (1941) found that meat stored 37 days at 34°F had stronger flavor and aroma and slightly less juice than meat stored 9 days. Barbella, et al. (1942) found that when beef carcasses were ripened at 33-38°F for average periods of 15.7 days and 50 days the rates of ripening were not influenced significantly by the grain versus grass feeding. Rate of ripening was studied by means of moisture, fat, and amino and non-protein nitrogen determinations.

Explanations for palatability differences, when shown, have been sought in various quarters and several hypotheses have been advanced. Barbella, et al. (1942) found a higher sulfhydryl content in grass-fattened beef than in grain-fattened beef. This finding was ascribed to a higher sulfhydryl content in grass than in grain. However, they did not find a correlation between sulfhydryl content and palatability. Stames (1958) found that ripening appeared to increase

the susceptibility of fat to hydrolysis. She found definite increases in free acidity in both types of beef with ripening. Feeding management, on the other hand, did not appreciably affect the susceptibility of fat to hydrolysis in Stames' study. It was postulated that the inferior flavor of fat in grass-fed beef might be due to absorption of some unknown flavor constituents peculiar to the grass.

The kind of fat an animal deposits has been studied under many circumstances and with many experimental animals. When conditions are normal and a mixed diet is fed, an animal deposits a fat characteristic of the species. It is probable that in addition to sex hormones and other hormones, environmental conditions also influence the nature of this fat (Deuel, 1955). There is general agreement that dietary fats affect adipose tissue of some species. Rats and pigs show a quick response to kinds of ingested fats, and references to studies with these animals are extensive. Beadle, et al. (1943) found that trienoic fatty acids, especially linolenate, were elevated in depot fat of rats and pigs by the feeding of high levels of linseed oil. Ostwald, et al. (1952) showed that on a given diet rats tend to maintain adipose tissue of constant physical properties and that during low dietary supply some of the linoleate stored in adopose tissue can be released. The chicken is another animal measurably responsive to dietary fat. Feigenbaum and Fisher (1959) noted that the body fat of

chickens could be influenced by either saturated or unsaturated fatty acids in the feed. The egg fat, however, seemed to be influenced only by the level of dietary unsaturated fatty acids.

According to Deuel (1955) the ruminant has little ability to deposit any of the dietary fat as ingested. Fatty acids of the ingested fats are broken down by bacteria in the rumen before assimilation by the animal. The depot fat, therefore, largely mirrors the composition of fat synthesized in the body from carbohydrates and protein. Reiser and Reddy (1956) concurred with Deuel's theory when they found in the depot fat of ruminants larger amounts of stearic acid than could be accounted for by diet. They stated that this fat is much less affected by dietary fat than is the depot fat of non-ruminants and suggested that hydrogenation of dietary fats occurs through action of the rumen microorganisms.

As indicated previously, adipose tissue has been used in most studies of composition of meat fat. Because flavor changes affecting palatability have been found and because depot fat of both grain- and grass-fed beef animals has been shown to be affected by hydrolytic rancidity, the possibility of tissue lipids also undergoing changes should be considered. The data concerning fatty acid composition of muscle tissue lipids are rather sparse. In contrast to the usually visible and separable adipose tissue, largely triglycerides,

the lipids of muscle tissue are not arranged in large globules within the cells. Watts (1962) discusses these lipids as being integral parts of various cellular structures, including cell wall, myofibrils, mitochondria, and microsomes, and as being at least partially associated with proteins. She considers the intracellular fatty substances to be primarily phospholipids which are relatively constant in amount, containing a high proportion of polyenes. According to Watts, the composition of these lipids can be influenced somewhat by diet, but apparently a high degree of unsaturation is maintained even on fat free or hard fat diets. Watts suggests that glycerides and phospholipids can oxidize quite independently of each other, possibly because of differences in the distribution of these two classes of lipids.

Additional information concerning tissue lipids comes from Younathan and Watts (1960), who investigated cooked pork in an effort to characterize oxidative rancidity contributable to off odors and flavors. Studying whole muscle tissues rather than just adipose fat, they found a high correlation between organoleptic changes and chemical tests for rancidity. They interpreted their results to indicate that the phospholipids were contributing to the reactions responsible for rancid odors and flavors in cooked meats. These workers reported that phospholipids from a particular tissue often

have been found to be appreciably more unsaturated than triglycerides from the same source. Thus, the phospholipids would be more susceptible to oxidation. Such oxidation would lead to deteriorative reactions and to strongly flavored degradative products at an early stage. Younathan and Watts termed the oxidation of phospho- and proteolipids as "tissue rancidity," differentiating it from neutral fat oxidation. It was concluded that the nonglyceride fraction not only oxidizes more rapidly in cooked meats, but also produces off odors which are distinctly different in quality from those associated with neutral fat oxidation.

With refinements in extraction, separation, and fatty acid identification, the study of food lipids became less cumbersome and new approaches becamefeasible. Hornstein, et al. (1961) studied the fatty acid composition of beef and pork muscle as well as the possible effect of phospholipids on meat flavor. The lipids of lean muscle tissue were fractionated into three groups, triglycerides, cephalins, and lecithins and sphingomyelins. These fractions were subjected to gas liquid chromatography for analysis, and the results were expressed as per cent of total fatty acids. Unsaturated acids with two or more double bonds made up 10% of the triglyceride fraction and about 50% of the phospholipid fraction. The absolute amounts of these acids contributed by the two fractions were similar since the ratio of

triglycerides to phospholipids was about 4:1 in beef and about 8:1 in pork.

Callow (1962) studied breed and level of nutrition as possible contributors to percentages of fat in fatty and muscular tissues of steers and to composition of the fat as indicated by iodine number. He theorized that there is a small but constant proportion of phospholipids in all the cells of muscular tissue and that these phospholipids have a high iodine number. The iodine number of muscle fat thus should increase toward a high but approximately constant value as the over-all percentage of fat decreases, causing the relative proportion of phospholipids to increase. Callow's data showed an inverse relationship between the iodine number and percentage of fat in various joints of animals finished on grain and grass. The grass-finished animals had the higher iodine numbers and the difference was attributed to the greater degree of fattening in the grain-fed animals.

It can be seen that in recent years efforts have been made to study the composition of muscle tissue lipids, notably the phospholipids, apart from the composition of adipose tissue lipids. The advent of gas liquid chromatography for the separation and identification of fatty acids has accelerated the investigation. Younathan and Watts (1960) concluded that phospholipids contribute to off flavors and odors by development of tissue rancidity. Hornstein, et al. (1961)

fractionated the phospholipids generally occurring in beef muscle and studied the fatty acid composition of two major classes. Callow (1962) illustrated the concept of other studies that grain-fed beef is fatter than grass-fed beef by means of iodine numbers as a measure of proportion of lean to fat. It remains to be seen whether methods of finish and length of time of aging can influence the amounts and fatty acid composition of phospholipids in beef muscle tissue. The following study was carried out as an initial approach to this area of lipid investigation.

CHAPTER III

PROCEDURE

After procurement of the meat and preparation of individual samples, the general procedure involved extracting lipid materials from the meat, reducing the extract, and fractionating the total lipid into two separate classes. Fatty acids of these fractions then were converted into methyl esters and subjected to gas liquid chromatography.

Various solvents and methods are used for lipid extraction. When it is important to remove total lipids from undried tissue with as little change in the fatty acids as possible, the use of a mixture of a polar and a relatively non-polar solvent is indicated. Methanol and chloroform constitute such a mixture. Also desirable is evaporation of solvent under nitrogen or under reduced pressure by the use of a rotary evaporator.

The use of silicic acid for lipid fractionation is reported frequently in the literature. The silicic acid slurry method devised by Murty, et al. (1960) was employed here for the separation of total lipid into glycerides and sterols as one fraction and phospholipids as a second fraction. From a chloroform extract of lipid material the

silicic acid adsorbs the phospholipids, which then can be eluted with methanol.

Preparation of lower boiling derivatives of fatty acids in order to expedite gas chromatographic analysis is common practice. Methyl esters usually are prepared either by saponification followed by methylation or by the more direct procedure of heating with methanol and a mineral acid. The latter method was found satisfactory by Hornstein (1960) in his work with meat lipids and was used in this study.

The emergence of gas liquid chromatography as an established method of analysis has accelerated lipid research. Since the initial and successful efforts of James and Martin (1952), both methodology and instrumentation have improved. Notable advances in gas liquid chromatography of fatty acid esters have been brought about through the use of polar liquid phases (Orr and Callen, 1958; Lipsky, et al., 1959). Now it is possible to achieve complete separation of the commonly known fatty acids, including the polyunsaturates, in a reasonable length of time.

Source of Meat

Meat for this study came from two pairs of Hereford steers. The animals had been paired as closely as possible with

regard to genetic background and age. They were all approximately 2 years old when slaughtered. Pertinent information is provided in Table I. All four animals were pasture fed to some extent before finishing periods began. The one of each pair selected for grain finishing then was full fed on dry lot up to time of slaughter. The grass finished steers were pasture fed their entire lives except for 2 or 3 weeks when pasture was unavailable. Animal #31 received some dry lot supplement which was considered insufficient to change his basic "grassy" character. The second pair of steers, #32 and #33, both received grass silage supplemented with 2 lb. corn, 1 lb. cottonseed meal, and 3 lb. mixed hay for approximately 6 months until spring pasture became available. This feeding regimen was prior to the periods when methods of finish were imposed.

Pasture for all the animals was predominantly orchard grass and Ladino clover. Slight amounts of Bermuda and other native grasses were present. The dry lot feed was 25% roughage (hay) and 75% concentrates (64% corn, 8% cottonseed meal, 3% molasses, 0.5% salt, and 0.5% Dical).

For study of effect of carcass ripening, samples of the longissimus dorsi and semimembranosus muscles were taken at four different times of aging: 0, 7, 21, and 42 days. Carcasses were chilled immediately after slaughter and aging proceeded at 33$\pm$3^{0}F

TABLE I

INFORMATION CONCERNING STEERS FROM WHICH MEAT WAS OBTAINED

Animal	Finish	Weight When Method of Finish Began	Time for Total Grain Finishing	Slaughter Weight	USDA Grade
		lb.	mo.	lb.	
#30	Grain	610	4.5	975	High Good
#31	Grass	575[a]	...	775	Low Good
#32	Grain	745	3.0	985	High Good
#33	Grass	745	...	858	Low Good

[a]Estimated

(relative humidity approximately 85%). Samples were removed from one side of a carcass for the first two periods and from the other side for the last two periods. The meat was aged as sides until the 21st day, samples being removed as needed. On the 21st day the remaining sections of loin and round were removed, covered with wax, and held thus until the 42-day ripening period was completed.

There was a definite, noticeable difference in the color and texture of the meat as it progressed from 0 to 42 days of aging. At 0 days the meat was bright red, crumbly, very moist, and resistant to formation of a meat patty. By 42 days the meat was a dark, dull, reddish brown, very sticky, and easy to shape into a patty. Paul, et al. (1944) reported similar findings, with the amount of drip also varying with the ripening period. They found roasts to be dry on the surface on the first day, but quite moist after 2 to 4 days storage. After 18 days' storage, roasts were sticky rather than wet.

Selection of the above-named two muscles was made for a previous study of B-vitamin content (Meyer, et al., 1960b). The meat was stripped of all separable fat, then ground and frozen until assays could be made. The meat remaining after completion of the vitamin studies was held at -20°C until used for the current study.

Preparation of Sample

Because the supply of several samples was quite limited, it was necessary to do some combining. Method of finishing and length of aging time were considered the most important factors for study; therefore, for each of four aging periods for each of the two methods of finishing, a sample of ground beef was prepared by combining equal portions of the ground longissimus dorsi and semimembranosus muscles from each of two animals. This procedure resulted in eight samples, one for each treatment combination studied.

The frozen meat was allowed to thaw in the refrigerator before the samples were combined as described above. Equal portions of the ground meat were weighed, and those to be combined were blended together by hand as one would in preparing a meat loaf. Approximate portions for the separate ensuing analyses were wrapped in waxed paper for easy separation. The whole sample then was wrapped securely in heavy duty aluminum foil, labeled, and refrozen at -20°C. Samples for analysis were removed as needed and thawed overnight in a refrigerator.

The program for analysis involved extraction of the lipid material from the meat in its raw state, its cooked state, and its accumulated drippings. Moisture determinations were carried out in duplicate for both the raw and the cooked samples by the A.O.A.C.

method (1960).

Samples for cooking were 113 g. portions of raw meat shaped into patties approximately 3 in. in diameter and 3/4 in. in thickness. These were cooked on a rotating hearth in a Despatch electric oven. Oven temperature was regulated at 350°F, and the meat patties were cooked to an internal temperature of 75°C which required about 25 minutes' cooking time. The meat patties were cooked separately on small wire racks resting on shallow glass baking dishes. In this manner the drippings were collected from individual patties.

The cooked meat patties were allowed to cool on their cooking racks to room temperature, requiring about 20 minutes. To effect homogeneity of the sample, each patty was homogenized in a Waring Blendor at low speed for 1 minute. The cooked, ground sample then was removed and a 50 g. portion was weighed for lipid extraction. Duplicate 5 g. portions also were removed at this time for moisture determinations.

The accumulated drippings were washed from the collecting dish with sufficient quantities (< 1/2 cup) of hot distilled water and collected in a small glass custard cup (Chang and Watts, 1952). This collection of drippings and water was refrigerated overnight in an effort to coalesce the fat into a solid cake for simple removal.

However, the amount of fat in the drippings was so small that no such solid layer formed. It was necessary, therefore, to subject the drippings to the same type of extraction as was used for the raw and cooked meat samples.

Extraction

The method of extraction followed generally the procedure of Folch, et al. (1957) as modified by Bligh and Dyer (1959) and adapted to the equipment available for the present study. A total volume of 450 ml. chloroform:methanol mixture, 2:1 (v/v), was used for the extraction of each sample. Thirty g. samples of raw meat and 50 g. samples of cooked meat were extracted.

The extraction procedure involved placing the meat sample in a blender jar and slurrying with 80 ml. of the 450 ml. chloroform: methanol mixture at a very slow speed. Speed for blending was controlled by an Adjust-A-Volt rheostat. After 2 minutes of blending, the slurry was filtered on a Büchner funnel under reduced pressure.[1] The meat was returned to the blender and again slurried two minutes with 80 ml. of the solvent mixture and again the slurry was filtered.

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[1]Glass fiber filter paper, no. 934-AH, 9.0 cm. diameter, was used.

This process of slurrying and filtering was repeated an additional two times. A fifth 30 ml. volume of solvent was used to rinse the blender jar and lid and the filter funnel.

The total lipid extract was transferred from the suction flask into a graduated cylinder, rinsing being accomplished with the final 50 ml. of the total 450 ml. of extraction solvent. To this mixture was added 0.2 volume of distilled water. The mixture was left standing overnight in a refrigerator to allow for a clean-cut separation of the mixture into two phases.

The addition of water caused the mixture to look cloudy, but any emulsification was very temporary. The water attracted the methanol, drawing it away from the chloroform. The upper phase, therefore, was a solution of distilled water and methanol, and the lower phase was the chloroform containing the extracted lipid. After separation, a thin, partial layer of meat fibers was always present at the interface. Sometimes fibers were also visible adhering to the side of the cylinder in the upper phase. These meat fibers readily clung together and in no way interfered with the reading of the total volume of the chloroform extract. This volume was recorded.

The separation of the two phases was achieved by drawing off the upper phase with gentle suction. Because of the necessity of removing meat fibers, a pipette with a broken tip was used with a

water aspirator. To insure complete removal, the upper phase was drawn off generously. Duplicate 10 ml. aliquots of the chloroform extract were taken immediately. Sodium sulfate, approximately 5 g., was added to the chloroform extract remaining in the cylinder. The mixture was allowed to stand at least one hour to remove water remaining in the extract.

Evaporation

Two methods were used for the evaporation of solvent from the lipid material. The 10 ml. aliquots of total lipid were evaporated in tared 10 ml. Erlenmeyer flasks placed in a warm water bath (50°C). A gentle stream of nitrogen was directed at each sample during evaporation. When evaporation seemed complete, the flasks were held in a vacuum desiccator until they reached constant weight. The weighed samples of total lipid were given a protecting stream of nitrogen gas, covered tightly with aluminum foil, and stored at -20°C. No immediate evaluation other than per cent total weight was planned for these aliquots.

The remaining chloroform extract was decanted quantitatively from the sodium sulfate into an evaporating flask. The flask was attached to a rotary evaporator which removed the solvent under reduced pressure. For fractionation, the lipid material was removed

from the flask with 100 ml. chloroform in 3 or 4 portions.

Silicic Acid Fractionation

The chloroform extract was added to 25 g. silicic acid in a 500 ml. Erlenmeyer flask. The mixture was stirred 10 minutes with a magnetic stirrer at the lowest setting. The dispersion then was filtered through a fine sintered glass funnel under slightly reduced pressure. When the silicic acid appeared fairly dry in the funnel, the vacuum line was broken and the silicic acid was washed in the funnel with 50 ml. chloroform. After filtration, the washing was repeated.

The collected chloroform extract and washings, containing the non-phosphorus lipids, were transferred quantitatively to a graduated cylinder for a recording of total volume. Duplicate aliquots of 10 ml. were transferred immediately to tared 15 ml. centrifuge tubes. The aliquots were evaporated under nitrogen as described above.

The silicic acid, with adsorbed phospholipids, was transferred to a 500 ml. Erlenmeyer flask and stirred 10 minutes with 50 ml. redistilled anhydrous methanol. The magnetic stirrer again was used on its lowest setting. The dispersion was filtered through the fine sintered glass funnel under reduced pressure. The silicic acid was rinsed twice in the funnel with 50 ml. of redistilled methanol each

time. The methanol-phospholipid extract was transferred quantitatively to a graduated cylinder and a volumetric measurement was made as with the chloroform extract. Duplicate aliquots of 20 ml. of the phospholipid solution were transferred to tared 100 ml. evaporating flasks. Solvent was removed on the rotary evaporator. The aliquots were dried to constant weight in a vacuum desiccator.

The remaining volumes of extracts, chloroform containing glycerides and sterols and methanol containing phospholipids, were evaporated separately on the rotary evaporator. After the removal of solvent, the lipid material was rinsed from the sides of the flask with a minimum of petroleum ether, 5 to 15 ml. The material was pooled in the bottom of the flask, and the petroleum ether was evaporated under nitrogen. This pooling of lipid made for easy removal of samples for methylation. After such samples were taken, the remaining lipid fraction was rinsed from the flask with petroleum ether, gassed with nitrogen, and stored in the freezer for any possible future evaluation.

Methylation

To a weighed sample of lipid, 0.05-0.1 g., in a 15 ml. centrifuge tube, 3 ml. of 1% sulfuric acid in methanol were added. The tube was capped with a marble and placed in a sand bath in a

heating mantle. Temperature of the sand bath was maintained at 70°C by a rheostat setting of 21. The sample was refluxed 4 hours; it was stirred with a glass rod every 10-15 minutes during the first 2 hours and every 30 minutes thereafter.

At the end of the refluxing period, any visible lipid was removed from the tube by a gentle touch with the tip of a pipette. To the methylated sample were added 5 ml. of redistilled petroleum ether and 2 ml. of distilled water. The petroleum ether served as a solvent for the methyl esters. The water served to remove the methanol and sulfuric acid. A biphasic system resulted, and the lower phase (aqueous) was removed by pipette and gentle suction applied with a rubber bulb. The aqueous phase was discarded. The addition of petroleum ether and distilled water was repeated, and again the lower phase was removed and discarded. For further removal of water, 0.5 g. anhydrous sodium sulfate was added to the sample. This was allowed to stand at least one hour or was stored overnight under refrigeration.

Prior to gas liquid chromatography the petroleum ether was poured off the sodium sulfate into another 15 ml. centrifuge tube. The solvent was evaporated under nitrogen, leaving a small pool of fatty acid methyl esters.

Chromatography

Gas liquid chromatographic analysis was performed with a Barber-Colman instrument, Model 61C, equipped with an argon ionisation detector with radium source. An 8 ft. coiled, copper column (1/4 in. inside diameter) was used. This column was packed with a solid support coated with diethylene glycol succinate polyester (15.4% by weight on 80-100 mesh chromosorb W). Flowing through the column was argon gas. The column was preconditioned approximately 30 hours at operating temperature, 185°C, before it was connected to the detector. A sample of 2 µl. of the methyl esters was diluted with 10 µl. of acetone. From this dilution, a 2 µl. portion was injected into the column.

Operating conditions included:

Flash Heat Temperature. . . . . 215°-218°C

Cell Temperature . . . . . .210°-212°C

Split Temperature . . . . . .225°-229°C

Cell Voltage 900

Gas Pressure . . . . . .25 psi

Attenuator Setting 16

Sensitivity Setting 10

The attenuator was changed only if the sample size seemed too large and, therefore, required less sensitive detection.

Major components were identified by comparison of experimental chromatograms with those of a standard fatty acid methyl ester mixture. Under a given set of operating conditions, the peak for a specific fatty acid always appears the same number of minutes after sample injection. Peaks for shorter chains appear before those for longer chains. With a column having a polar liquid phase, the fatty acid methyl esters of a given chain length come off in order of increasing number of double bonds. The number of minutes elapsing between sample injection and attainment of maximum height of a component peak is referred to as the retention time of the component. Retention times for all the days on which gas chromatography was carried out are shown in Table IX in the Appendix.

For ascertaining amounts of fatty acids, a base line was drawn under each peak. Peak "area" was calculated by the equation used by Hornstein, et al. (1961):

Area = Height of peak x Width of peak at half height x Attenuation factor.

The percentage of a specific fatty acid in a sample was calculated by the equation:

$$\% \text{ FA in sample} = 100 \times \frac{\text{Area of peak}}{\text{Total area under all peaks in the chromatogram}}.$$

CHAPTER IV

RESULTS AND DISCUSSION

As would be expected from the muscles selected for study and from the fact that they were stripped of separable fat, the samples were relatively low in total lipid. Values for fat content on wet and dry bases are presented in Table II. Neither type of finish nor length of time of aging appears to have affected fat content appreciably.

When the extracted lipids were fractionated, the total weight of fractions obtained did not equal the weight of lipid subjected to the fractionation procedure. Calculation of proportions of the two fractions as per cent of lipid fractionated gave values with no meaning. When yield of each fraction was expressed as per cent of total weight of the two fractions the values appeared to provide a better basis for comparison. These are shown for the phospholipids in Table III. Because of the difficulty in making a quantitative fractionation, it would be unwise to draw conclusions from these data; however, it might be pointed out that the fat in raw meat from grass-fed animals apparently was higher in phospholipids than that from grain-fed animals. Fat from raw samples consistently had a higher concentration of phospholipids than did fat from cooked samples. These differences were

TABLE II

PER CENT FAT IN RAW AND COOKED MEAT FROM STEERS OF TWO TYPES OF FINISH AND FOUR PERIODS OF CARCASS AGING[a]

Days of Aging	Raw Samples						Cooked Samples					
	Wet Weight Basis			Dry Weight Basis[b]			Wet Weight Basis			Dry Weight Basis[b]		
	Grain	Grass	Ave.	Grain	Grass	Ave.	Grain	Grass	Ave.	Grain	Grass	Ave.
0	3.82	3.04	3.43	13.86	11.18	12.52	5.44	4.30	4.87	14.68	11.94	13.31
7	4.11	2.88	3.50	15.12	11.16	13.14	5.16	4.27	4.72	13.93	11.94	12.93
21	3.55	3.58	3.57	13.25	13.80	13.53	5.56	5.02	5.29	14.98	14.00	14.49
42	3.24	3.44	3.34	11.45	12.74	12.10	5.71	5.70	5.71	15.83	16.70	16.27
Ave.	3.68	3.24		13.42	12.22		5.47	4.82		14.87	13.65	

[a]Each value is an average for 2 determinations.
[b]Moisture values are given in Table X in Appendix.

TABLE III

PROPORTION OF PHOSPHOLIPID IN FAT[a]

Days of Aging	Grain		Grass		Ave.	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
0	31.8	16.7	56.3	18.8	44.1	17.8
7	44.5	15.8	64.5	23.2	54.5	19.5
21	40.9	15.7	62.6	18.8	61.8	17.3
42	30.8	26.0	50.8	13.0	40.9	19.5
Ave.	37.0	18.6	63.6	18.5	50.3	18.5

[a]Expressed as per cent of total weight of the glyceride and phospholipid fraction. In each case, then, the per cent of glyceride fraction is the difference between 100 and the value for phospholipid.

large enough and consistent enough so that they might possibly represent real differences in spite of the shortcomings of the data. Apparent effects of aging are neither large nor consistent. If the fractionation procedure can be made more nearly quantitative, it might be worthwhile to study further the effect of these factors on relative proportions of phospholipids and glycerides in muscle tissue.

The lipid content of the drippings from the cooked meat was too small to permit fractionation; therefore, it was methylated as total lipid. Although peaks in the chromatograms were small, they were measurable for the major components. The composition of the drippings fat for the treatments studied is shown in Table IV in terms of total saturates and total monoenes. Data for individual fatty acids may be found in Table XI in the Appendix. As might be expected, the percentages closely resemble those of the same components in the glyceride fraction, to be discussed later. Trace amounts of C_{12}, $C_{14:1}$, $C_{18:2}$, and $C_{18:3}$ fatty acids were detected. Apparently neither finish nor carcass aging affected the fatty acid composition of the lipids in the drippings.

The two fractions of the lipid extracted from meat were quite different in their fatty acid composition, as is shown in Table V and Figure 1. In Table V averages are given for all aging periods, both finishes, and raw and cooked meat. In Figure 1 chromatograms

TABLE IV

FATTY ACID COMPOSITION OF TOTAL LIPIDS IN THE DRIPPINGS[a]

Aging Periods	Total Saturates[b]			Monoenes[c]		
da.	Grain	Grass	Ave.	Grain	Grass	Ave.
0	58.4	52.7	55.6	41.6	47.3	44.5
7	58.3	56.9	57.6	41.7	43.1	42.4
21	55.7	60.2	58.0	44.3	39.7	42.0
42	55.8	56.9	56.4	44.2	42.8	43.5
Ave.	57.1	56.7		43.0	43.2	

[a]Per cent of total fatty acids.

[b]Including C_{14}, C_{16}, C_{18}.

[c]Including $C_{18:1}$, $C_{18:1}$.

TABLE V

COMPARISON OF FATTY ACID COMPOSITION OF GLYCERIDE AND PHOSPHOLIPID FRACTIONS[a]

Fatty Acids	Glycerides			Phospholipids[b]		
	Sat.	Monoene	Diene	Sat.	Monoene	Diene
C_{14}	2.6			1.9		
C_{16}	37.1			24.7		
$C_{16:1}$		4.6			2.6	
C_{18}	12.0			16.3		
$C_{18:1}$		42.6			33.6	
$C_{18:2}$			0.9			16.6
Total	51.7	47.2	0.9	42.9	36.2	16.6

[a]Per cent of total fatty acids. Averages for all aging periods, both finishes, raw and cooked meat.

[b]Not shown here: 4.3% of the phospholipid fatty acids, which have retention times corresponding to those of dienes (See page 39).

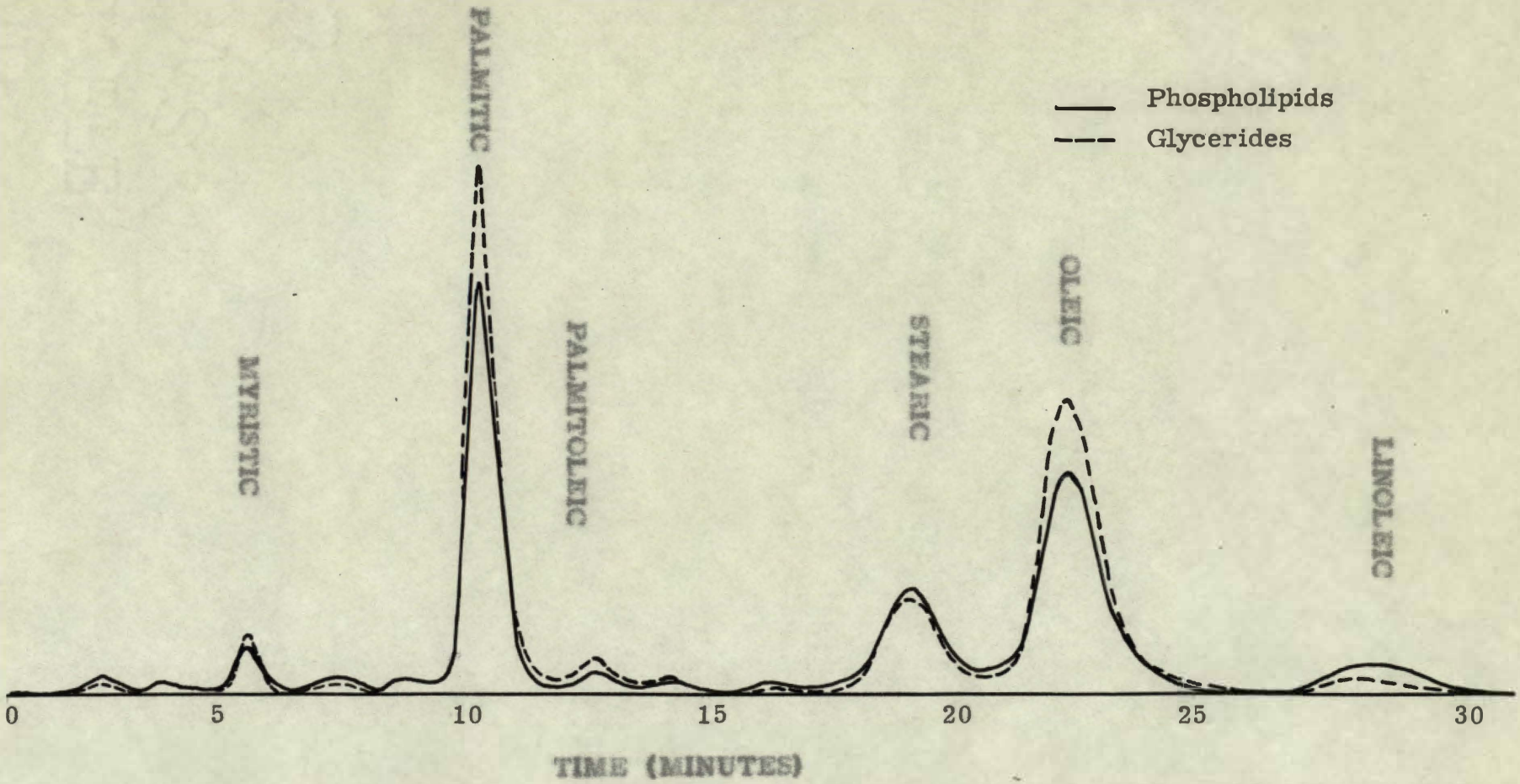

Figure 1. Comparison of chromatograms for the two fractions of a single raw sample: Grain, 21 days aging.

are compared for the two fractions of a single lipid sample (Grain, raw, 21 days aging). The most striking difference is the much larger proportion of linoleic acid in the phospholipids. This is reflected particularly in the smaller quantity of saturates, primarily palmitate, and of the monoene oleate. Other workers (Younathan and Watts, 1960; Hornstein, et al., 1961) also have reported that the phospholipids of muscle tissue have a much higher degree of polyunsaturation than do the glycerides.

The types of finish and periods of carcass aging did not appear to affect the fatty acid composition of the glycerides in beef muscle tissue. This can be seen in Table VI, in which values for total saturates, monoenes, and dienes are presented. Values for raw and cooked meat samples also did not differ appreciably. The data for individual fatty acids are included in Table XII in the Appendix.

The glyceride fraction consistently showed traces of several components which were not present in the fatty acid standard mixture used for identification. Frequently unknown components may be identified by the plotting of retention times against chain length on semi-logarithm paper. Members of a homologous group, such as the saturated components, lie on a straight line when thus plotted; monoenes, dienes, trienes, etc. lie each on a separate parallel

TABLE VI

COMPOSITION OF GLYCERIDE FRACTIONS IN TERMS OF SATURATED, MONOENOIC, AND DIENOIC FATTY ACIDS[a]

Aging Time	Finish	Saturated[b]		Monoenoic[c]		Dienoic	
Days		Raw	Cooked	Raw	Cooked	Raw	Cooked
0	Grain	52.8	55.8	46.8	44.4	0.5	tr.
0	Grass	52.8	50.0	46.7	49.6	0.7	tr.
Ave.		52.8	52.9	46.8	47.0	0.6	tr.
7	Grain	52.5	49.9	46.7	50.2	0.9	tr.
7	Grass	51.0	48.1	47.3	50.5	1.8	1.6
Ave.		51.8	49.0	47.0	50.4	1.4	0.8
21	Grain	50.9	51.9	48.1	46.2	1.2	1.9
21	Grass	50.8	51.5	48.6	48.2	0.9	0.4
Ave.		50.8	51.7	48.4	47.2	1.1	1.2
42	Grain	52.0	52.9	46.4	46.5	1.7	0.8
42	Grass	52.2	52.5	48.9	47.2	1.1	0.2
Ave.		52.1	52.7	46.7	46.9	1.4	0.5
Overall average		51.9	51.8	47.2	47.9	1.1	0.8

[a]Per cent of total fatty acids.

[b]Including C_{14}, C_{16}, C_{18}.

[c]Including $C_{16:1}$, $C_{18:1}$.

line (James and Martin, 1952; Orr and Callen, 1958; Fontell, et al., 1960; Hornstein, 1960). Several plots were made from the chromatograms in this study and were nearly identical. In Figure 2 a representative plot for a glyceride chromatogram is shown. Retention times for the identified saturates, C_{14}, C_{16}, and C_{18}, of a glyceride sample were plotted and the points were connected to form line S. Three other retention times, measured from the chromatogram, are located at points a, b, and c and mark the times at which C_{12}, C_{15}, and C_{17} saturated acids would emerge. In addition, if the line is extended, the measured retention time of 2.3 minutes is found to be that of the C_{10} saturate. The measured retention times for the identified monounsaturates, $C_{18:1}$ and $C_{18:1}$, were plotted to form the parallel line M. Intersections of this curve at d and e indicate retention times for $C_{14:1}$ and $C_{17:1}$ fatty acids almost identical to the measured times of 7.22 and 15.4 minutes. Although structure cannot be assigned definitely from the gas chromatographic results, Hornstein (1960) and Hornstein, et al. (1961) also have reported the apparent presence of C_{10}, C_{12}, C_{15}, $C_{14:1}$, and $C_{17:1}$ in meat lipids.

The effects of feeding management on fatty acid composition seemed to occur in the phospholipid fraction. In Table VII the distribution of fatty acids in the phospholipids from raw samples is

	Measured Retention Time	Point On Curve
	minutes	
	2.30	
	3.53	/ a
C_{14}*	5.85	.
	7.22	/ d
	7.55	/ b
C_{16}*	10.00	•
$C_{16:1}$*	11.90	o
	13.20	/ c
	15.40	/ e
C_{18}*	17.45	.
$C_{18:1}$*	20.22	o
$C_{18:2}$*	25.32	+

*Identified by use of standard mixture.

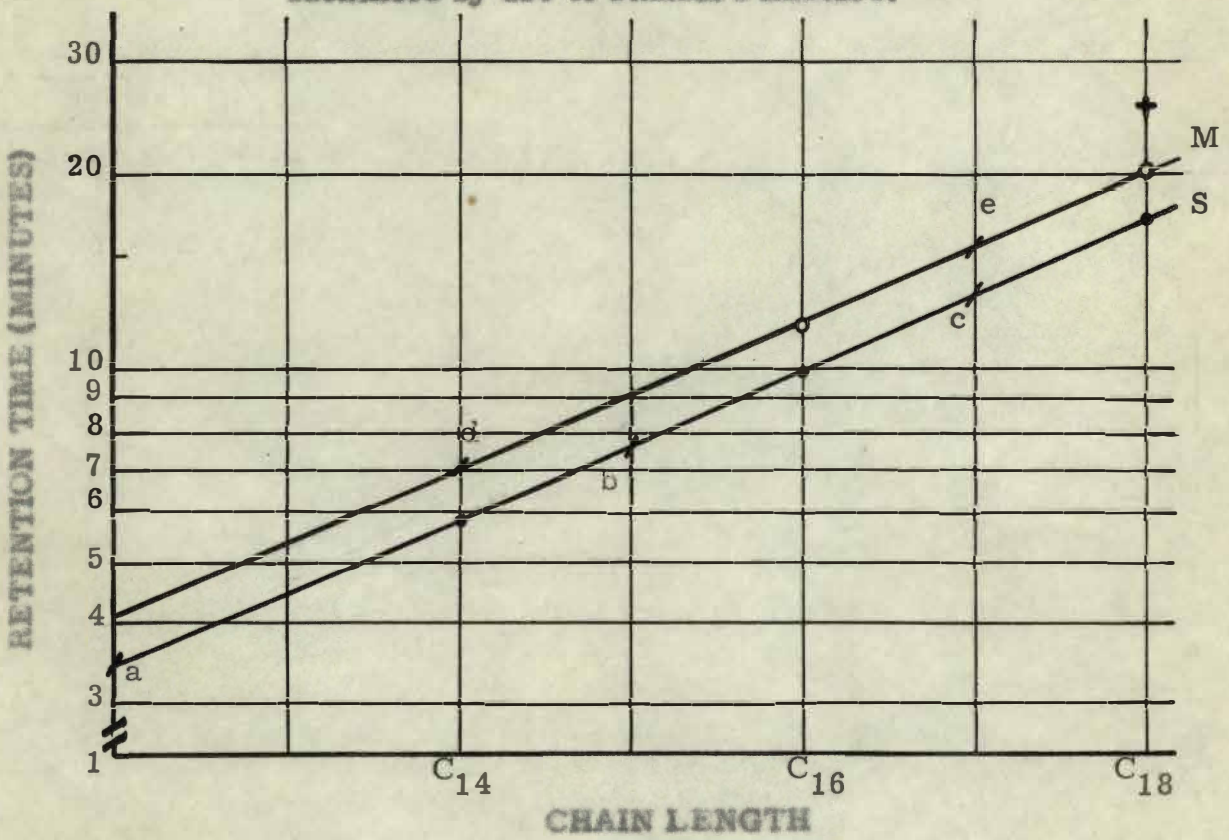

Figure 2. Retention times of components of a glyceride fraction.

TABLE VII

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM RAW SAMPLES[a]

Finish and Aging	?[b]	C_{14}	?[b]	C_{16}	$C_{16:1}$	?[b]	C_{18}	$C_{18:1}$	$C_{18:2}$	Total Saturates	Total Monoenes	Total ?
Grain												
0 da.	tr.	2.2	tr.	27.8	2.3	tr.	17.0	38.3	12.5	47.0	40.6	tr.
7	tr.	2.5	tr.	24.2	3.1	tr.	17.6	38.2	14.4	44.3	41.3	tr.
21	tr.	2.7	tr.	32.5	1.9	tr.	14.9	39.6	8.5	50.1	41.5	tr.
42	tr.	2.1	tr.	27.0	2.4	tr.	16.9	37.8	13.8	46.0	40.2	tr.
Ave.	tr.	2.4	tr.	27.9	2.4	tr.	16.6	38.5	12.3	46.9	40.9	tr.
Grass												
0 da.	0.6	1.7	1.7	26.3	2.0	0.9	15.4	39.5	12.1	43.4	41.5	3.2
7	1.8	2.2	4.0	21.2	1.2	2.6	15.8	36.3	14.9	39.2	37.5	8.4
21	1.6	2.2	3.2	22.7	1.9	2.2	13.9	34.9	17.5	38.8	36.8	7.0
42	1.9	1.5	5.3	22.3	1.5	0.8	12.6	38.0	16.1	36.4	39.5	8.0
Ave.	1.5	1.9	3.6	23.1	1.7	1.6	14.4	37.2	15.2	39.5	38.8	6.7

[a]Per cent of total fatty acids. Averages for 2 determinations.

[b]Components having retention times corresponding to those of dienes (See page 39).

shown for the two types of finish. The columns headed by question marks indicate the appearance of components which were not present in the standard mixture. Their presence in somewhat larger amounts in phospholipids from grass-fed animals than in those from grain-fed animals seems to indicate an influence of ration. The main effect of ration on major fatty acids was a lower proportion of saturates in the grass-fed beef than in the grain-fed beef. If average per cents of C_{14}, C_{16}, and C_{18} are totaled for the two finishes, the saturated fatty acids comprised 46.9% of the phospholipid fatty acids from grain-fed animals as compared with 39.5% for the phospholipids from grass-fed animals.

If the unidentified components happened to be saturated fatty acids, the difference in total saturates due to method of finish would disappear; therefore, an attempt at identification by the method previously described for the glycerides was made. It showed these components to be different from any found in the glyceride fraction. In Figure 3 line S was obtained as in Figure 2. Point a, corresponding to the measured retention time of linoleic acid, then was located and line D was drawn through point a, parallel to line S. The extrapolated retention times of 5.0, 8.9, and 15.6 minutes at points b, c, and d, are nearly identical to the measured retention times of 4.9, 9.0, and 15.7 minutes and are those of C_{12}, C_{14}, and C_{16} dienes, if such exist.

	Measured Retention Time	Point On Curve
	minutes	
	4.90	/ b
C_{14}*	6.15	.
	9.00	/ c
C_{16}*	10.60	.
$C_{16:1}$*	12.60	o
	14.15	/ e
	15.70	/ d
C_{18}*	18.45	.
$C_{18:1}$*	21.48	o
$C_{18:2}$*	27.00	+ a

*Identified by use of standard mixture.

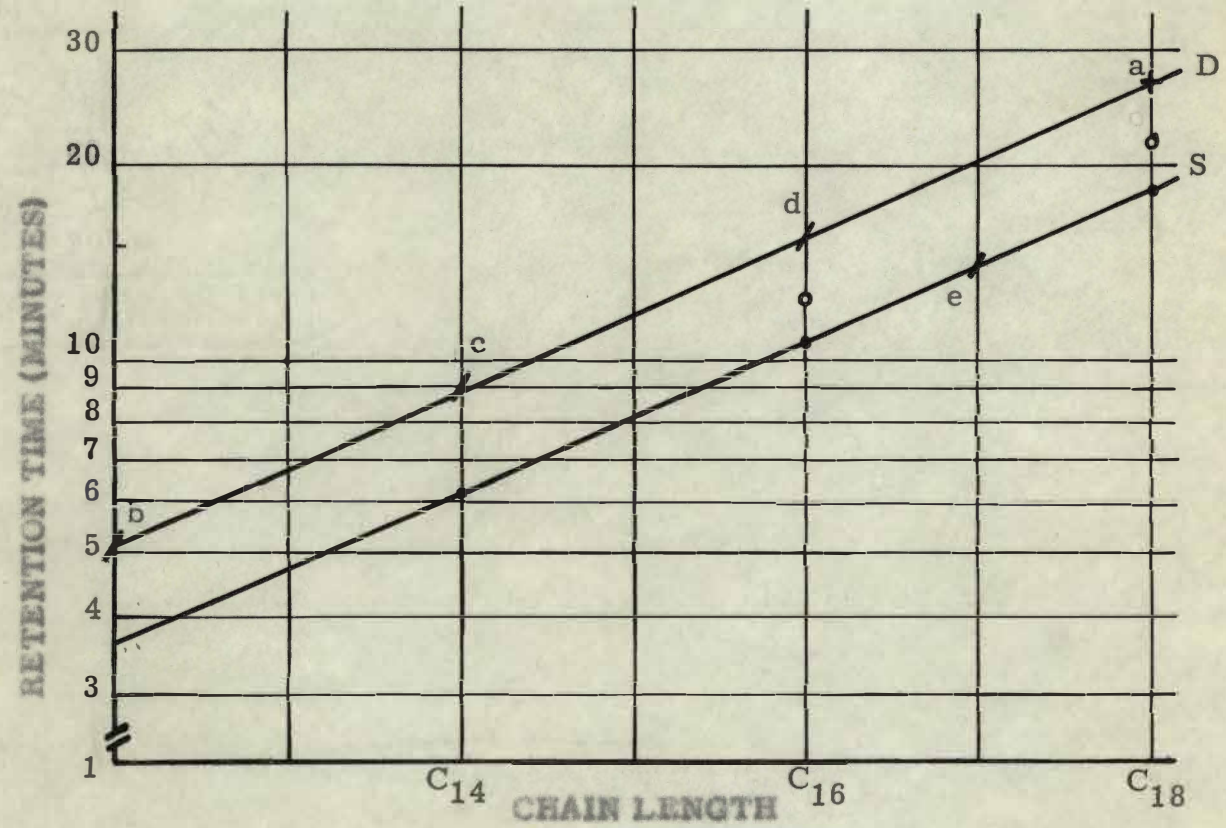

Figure 3. Retention times of components of a phospholipid fraction.

Actual identification of the components would require study of structure by other methods and was not within the scope of this study. The apparent existence of polyunsaturation in a medium-chain fatty acid is not unprecedented. Among the fatty acids to which Hornstein et al. (1961) tentatively assigned structures in their study of beef fat was a tetradecadienate.

It seems possible that β-oxidation played a part in the occurrence of the unknown fatty acids. Hornstein (1960) and Hornstein, et al. (1961) reported fatty acids of more than 18 carbons and more than two double bonds in beef fat. During carcass aging, the enzyme action resulting in β-oxidation would have an opportunity to occur. During cooking of the meat, the enzyme action would cause further β-oxidation before destruction of the enzyme by heat. It is conceivable that shortening of the carbon chain by β-oxidation could occur prior to saturation of the double bonds, giving rise to medium-chain, polyunsaturated fatty acids in the muscle tissue lipids of beef.

If it is assumed that the three components are indeed dienes, then the total average diene contents of the phospholipid fatty acids were 12.3% with grain feeding and 21.0% with grass feeding. Feeding management would seem to have had an appreciable effect on the fatty acid composition of the phospholipids of raw samples.

Carcass aging did not seem to produce progressive changes in the phospholipid fatty acids, as may be seen in Table VII. Perhaps worthy of note, however, is the apparent tendency of aged samples from the grass-finished animals to contain a higher proportion of the possible C_{12}, C_{14}, and C_{16} dienes than did the unaged samples.

In Table VIII the fatty acid composition of phospholipids from cooked samples is presented. In view of the fact that the study was carried out largely in an attempt to find an explanation for results of earlier palatability studies, probably the most interesting data for the cooked samples are those for the possible C_{12}, C_{14}, and C_{16} dienes. When these three values are totaled for each aging period of each finish and the totals for the two finishes are averaged, the average total percentage of these components is 3.7 for the unaged samples, followed by 5.5, 6.4, and 7.3 for the succeeding aging periods. An effect of aging that is suggested by the data for raw samples seems more pronounced and more clear-cut where fat from the cooked samples is concerned. With the cooked samples, though to a lesser extent than with the raw samples, there was a higher concentration of the possible C_{12}, C_{14}, and C_{16} dienes in the phospholipid from grass-fed beef than in that from grain-fed beef. It seems possible, therefore, that these components might provide the explanation for the fact that in the previous palatability studies, flavor of fat from grass-finished steers was inferior to that from grain-finished steers and

TABLE VIII

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM COOKED SAMPLES[a]

Finish and Aging	?[b]	C_{14}	?[b]	C_{16}	$C_{16:1}$	?[b]	C_{18}	$C_{18:1}$	$C_{18:2}$	Total Saturates	Total Monoenes	Total ?
Grain												
0 da.	1.2	1.4	2.2	26.5	3.5	0.7	13.4	26.1	20.9	46.3	29.6	4.1
7	0.6	1.1	1.7	26.4	3.0	1.4	15.2	27.9	19.7	45.7	30.9	3.7
21	1.6	1.6	3.0	25.2	2.2	1.0	16.2	26.5	22.9	43.0	28.7	5.6
42	1.9	1.8	4.3	21.4	5.0	0.2	14.0	32.8	15.8	37.2	37.8	6.4
Ave.	1.3	1.5	2.8	24.9	3.4	0.8	15.7	28.3	20.8	43.1	31.8	4.9
Grass												
0 da.	tr.	1.2	2.1	23.1	1.5	1.1	15.8	36.0	16.3	43.1	37.5	3.2
7	1.2	2.1	3.4	22.1	4.2	2.6	15.5	32.3	16.0	40.7	36.5	7.2
21	2.0	1.5	3.5	22.8	2.3	1.7	15.5	28.2	19.7	42.8	30.5	7.2
42	1.0	2.0	5.0	24.2	3.6	2.2	15.1	25.4	20.8	42.3	29.0	6.2
Ave.	1.6	1.7	3.5	23.0	2.9	1.9	17.5	30.5	18.2	42.2	33.4	5.4

[a]Per cent of total fatty acids. Averages for 2 determinations.

[b]Components having retention times corresponding to those of dienes (See page 39).

that with both types of finish, flavor deteriorated with aging.

With cooking, the variation in proportion of saturates with type of finish appears to have disappeared, as has the difference in proportion of polyunsaturates. Cooking appears to have brought about an actual increase in the percentage of polyunsaturated fatty acids.

During some of the first analyses carried out, some difficulty was encountered with sample size and the peaks in the chromatograms were very small. The linoleic acid peaks were particularly low and broad and thus difficult to measure. The problem was most acute with the raw samples, and it seems possible, therefore, that the phospholipids of the raw samples actually had higher concentrations of linoleic acid than the results indicated and that the increase with cooking was not real.

If the concentration of linoleic acid really did increase with cooking, an explanation might be sought in the decrease of another component. Chromatograms occasionally showed traces of linolenic acid. As stated previously, methyl esters from the raw meat phospholipids were available in very small quantities; this coupled with the fact that linolenate emerges from the column so late as to be rather diffuse, could have permitted appreciable quantities of linolenic acid to be undetected. A decrease in linolenic acid occurring with cooking possibly could give rise to isolinoleic acids through the hydrogenation

of one double bond. Under the conditions of this study, differentiation between normal linoleic acid and an isomer with one of the double bonds located differently is not likely; thus an increase in linoleate would be apparent. Such a finding would not be out of line with that of Chang and Watts (1952) who investigated effects of cooking on fatty acid composition. Although separate fractions were not analyzed, the results indicated that only a small amount of polyunsaturated fatty acids was lost with ordinary methods of cooking.

The findings of this study, while far from conclusive, suggest some interesting possibilities for further work concerning the effect of finish and carcass aging on meat lipids. In future studies identification of the phospholipid components which appear to be medium-chain dienes should be pursued. It might be worthwhile to study different classes of phospholipids separately in relation to their content of the fatty acids in question.

CHAPTER V

SUMMARY

The effect of method of finishing and time of carcass aging on the lipids of beef was studied. Lipids were extracted from raw and cooked samples of grain- and grass-finished beef with aging periods of 0, 7, 21, and 42 days. The extracted lipids were fractionated into phospholipids and glycerides, and the fatty acid composition of the fractions was studied by gas liquid chromatography.

In both raw and cooked samples the fatty acids of the glyceride fraction were unaffected by feeding management and carcass aging. The most striking effect of the treatments on the composition of the phospholipid fraction was the occurrence of three components which had retention times corresponding to those of C_{12}, C_{14}, and C_{16} dienes. Their identity could not be established positively by available means. It is thought that medium-chain dienes possibly could arise from the β-oxidation of long-chain polyunsaturates. Because these components tended to increase in concentration with aging and were present in greater concentration in fat from grass-fed beef than in that from grain-fed beef, they might provide the explanation for results of previous studies concerned with flavor.

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APPENDIX

TABLE IX

RETENTION TIMES OF THE FATTY ACIDS AS MEASURED IN MINUTES FROM THE CHROMATOGRAMS

Date	C_{10}	C_{12}	?	C_{14}	?	?	C_{15}	C_{16}	$C_{16:1}$	?	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$
July 3	2.30	3.80		6.00		8.70	7.45	10.30	12.20	15.80	13.60	18.00	20.80	26.15
5		3.80	4.90	6.15		9.00	7.60	10.60	12.60	15.70	14.15	18.45	21.48	27.00
6		3.75	4.95	6.20		9.05	7.65	10.70	12.70		14.10	18.60	21.55	27.00
9		3.55	4.75	5.88		8.80	7.50	10.25	12.25	15.35		17.90	20.80	26.10
11		3.70	5.00	6.10		8.60	7.50	10.50	12.48	15.70	14.00	18.25	21.20	26.60
Oct. 1	2.30	3.58	4.75	5.85	7.22	8.25	7.55	10.00	11.90	15.40	13.20	17.45	20.22	25.32
2	2.22	3.65	5.10	5.85	7.20	8.63	7.60	9.90	11.18	15.30	12.90	17.47	20.20	25.30
3	2.25	3.60	4.70	5.85	7.00	8.59	7.80	10.00	11.80	14.90	13.30	17.60	20.45	25.55
4	2.15	3.60	4.75	5.82	7.20	8.25	7.80	9.98	11.75	14.75	13.20	17.35	20.05	25.15

TABLE X

MOISTURE DETERMINATIONS FOR RAW AND COOKED SAMPLES[a]

Aging Period	Grain				Grass			
Days	Raw		Cooked		Raw		Cooked	
	Ave. % Moisture	Ave. % Solids	Ave. % Moisture	Ave. % Solids	Ave. % Moisture	Ave. % Solids	Ave. % Moisture	Ave. % Solids
0	72.44	27.56	62.92	37.08	72.80	27.20	63.99	36.01
7	72.82	27.18	63.14	36.86	74.19	25.81	64.25	35.75
21	73.21	26.79	62.85	37.15	74.02	25.98	64.12	35.88
42	71.76	28.24	64.08	35.92	72.96	27.04	65.84	34.16
Ave.	72.56	27.44	63.25	36.75	73.49	26.51	64.55	35.45

[a]Averages for 2 determinations.

TABLE XI

FATTY ACID COMPOSITION OF TOTAL LIPIDS IN THE DRIPPINGS[a, b]

Finish and Aging	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$
Grain					
0	4.2	37.9	3.6	16.3	38.0
7	4.7	37.9	3.7	15.7	39.0
21	3.4	34.8	4.3	17.5	40.0
42	2.5	36.9	2.7	16.5	41.5
Ave.	3.7	36.9	3.3	16.5	39.6
Grass					
0	3.3	32.7	4.5	16.7	42.8
7	2.5	37.9	5.1	16.5	38.0
21	2.9	39.0	2.3	18.3	37.4
42	4.2	33.0	2.7	19.7	40.1
Ave.	3.2	35.7	3.7	17.3	39.6

[a]Per cent of total fatty acids.

[b]Trace amounts of C_{12}, $C_{14:1}$, $C_{18:2}$, $C_{18:3}$ were detected.

TABLE XII

FATTY ACID COMPOSITION OF GLYCERIDE FRACTIONS[a]

Finish and Aging	C_{14}	? $C_{14:1}$[b]	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	Total Sat.	Total Mono-Unsat.
Grain-Raw									
0	1.7		37.6	2.2	13.5	44.6	0.5	52.8	46.8
7	1.8		38.6	2.9	12.1	43.8	0.9	52.5	46.7
21	2.6		37.3	3.7	11.0	44.4	1.2	50.9	48.1
42	2.7		39.5	3.4	9.8	43.0	1.7	52.0	46.4
Ave.	2.2		38.3	3.1	11.6	44.0	1.1	52.1	47.0
Grass-Raw									
0	1.6		36.4	4.4	13.8	42.3	0.7	52.8	46.7
7	1.9		34.0	3.7	15.1	43.6	1.8	51.0	47.3
21	2.4		35.3	3.8	12.9	44.8	0.9	50.6	48.6
42	2.2		36.0	3.1	14.0	43.8	1.1	52.2	46.9
Ave.	2.3		35.4	3.8	14.0	43.6	1.1	51.7	47.4
Grain-Cooked									
0	3.0	1.0	40.1	4.5	12.7	38.9	Tr	55.8	44.4
7	2.7	1.0	37.0	9.0	10.2	40.2	Tr	49.9	50.2
21	3.7	1.1	38.5	4.6	9.7	40.5	1.9	51.9	46.2
42	2.3	0.8	37.2	5.4	13.4	40.3	0.8	52.9	46.5
Ave.	2.9	1.0	38.2	5.9	11.5	40.0	0.7	52.6	46.8
Grass-Cooked									
0	3.0	0.7	33.6	9.5	13.4	39.4	Tr	50.0	49.6
7	2.9	0.8	35.3	4.0	9.9	45.7	1.6	48.1	50.5
21	3.3	0.8	37.5	5.3	10.7	42.1	0.4	51.5	48.2
42	3.2	1.0	40.1	4.1	9.2	42.1	0.2	52.5	47.2
Ave.	3.1	0.8	36.6	5.7	10.8	42.3	0.6	50.5	48.9

[a] Per cent of total fatty acids. Averages for 2 determinations.

[b] The only tentatively identified minor component present in measurable quantities; included in values for total mono-unsaturates.