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To the Graduate Council:

I am submitting a thesis written by Sarah Margaret Nutt entitled "Effect of Finish and Aging Time on the Phospholipid Constituents of Beef." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Foods.

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EFFECT OF FINISH AND AGING TIME ON THE PHOSPHOLIPID
CONSTITUENTS OF 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

by
Sarah Margaret Nutt

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CHAPTER I

INTRODUCTION

Recent advances in micro-techniques have made possible more extensive and thorough study of lipids and related compounds than was possible previously. Two of the most outstanding developments have been gas-liquid chromatography and thin layer chromatography. Gas-liquid chromatography has contributed a quantitative method for fatty acid determinations while thin layer chromatography has produced an accurate and rapid method for separating various classes of lipids. A combination of these methods has opened the door to investigations which had been deferred because of inaccurate and time consuming methodology.

The role of lipids in meat flavors has been of interest for many years. Studies at the University of Tennessee have indicated definite flavor differences in the fat of cooked meat from grass-fed and grain-fed beef. Length of aging period also has been found to affect flavor of beef fat. Attempts to find explanations for the effects of finish and aging have not been conclusive.

After Stames (1958) found no correlation between flavor scores and free fatty acid content or peroxide values of the external fat, Cook (1963) undertook an investigation of the composition of internal fat. Cook's work indicated that the phospholipid fraction of beef

fat tends to be more affected by aging time and by finish than does the glyceride fraction. Some unidentified constituents, the concentration of which appeared to be affected by the type of finish and by aging time, were found in the phospholipids. It seemed desirable, therefore, to study the different classes of phospholipids in grain- and grass-fed beef aged for different periods of time.

CHAPTER II

REVIEW OF LITERATURE

Results of studies dealing with effects of finish and ripening on beef flavor have been equivocal. Early studies (Black et al., 1931; Black et al., 1940) showed no difference in flavor between grain-fed and grass-fed beef although more tender meat was obtained with a grain-feeding regime. In 1941 Bull et al. reported higher palatability scores with grain finish than with grass finish, attributing their findings to a lower level of fatness in the grass-fed than in the grain-fed beef. Similar results were reported by Wanderstock and Miller (1948). All of these beef samples were scored acceptable. More recent studies (Meyer et al., 1960; Stames, 1958) have shown significantly better flavor scores for grain-fed beef than for grass-fed beef.

Harrison et al. (1949) reported very little variation in the aroma and flavor scores for beef aged one to 20 days. The samples aged only 10 days showed a slightly higher palatability score than did those aged for shorter or longer times. With 20 or more days of aging, a downward trend in acceptability of flavor was shown. Musty odors and high flavors were descriptive terms given to the meat aged for the longer periods. Slight differences were found also in beef aged for periods of 9 to 37 days at 34°F (Griswold and Wharton, 1941). Although differences reported were not great, the aroma, the flavor of

the fat, and the flavor of the lean were shown to be more intense with the longer aging periods. Stames (1958) found decreasing flavor scores for beef fat when the meat had been ripened 21 days or longer.

Meyer et al. (1960) studied the effects of limited and extensive ripening on the palatability of beef from grass- and grain-finished steers. Results showed that ripening afforded little or no improvement in the flavor of the lean of grass-finished animals, but tended to improve the flavor of the grain-finished lean up to 21 days. Scores for the flavor of the lean decreased significantly between 21 and 42 days of ripening, the decrease being especially marked for the grain-finished beef. Even greater differences were observed in the fat, the fat from the grain-finished animal receiving significantly higher flavor scores than that from the grass-finished animal. As with the lean, increasing ripening time from 21 to 42 days caused decreases in the scores for fat flavor for both types of beef.

Stames (1958), determining free fatty acids in exterior fat from grass- and grain-finished beef, found no difference in the susceptibility to hydrolysis. However, with both types of finish an increase in free fatty acids was found with aging. Stames postulated that the inferior flavor of fat in the grass-fed beef might be due to absorption of flavor constituents of the grass. Cook (1963) separated the neutral lipids and the phospholipids in grass- and grain-finished beef muscle aged 0, 7, 21, 42 days and analyzed the two fractions for fatty acid content. No appreciable differences were found in the fatty acid composition of the neutral lipids from beef of the two types of finish or the four

ripening periods. The phospholipid fraction of the beef muscle fat showed some effect of aging and of finish. Some unidentified constituents appeared in greater concentration in the grass-fed than in the grain-fed animals and tended to increase with aging time.

Phospholipids have been implicated increasingly in flavor deterioration. Younathan and Watts (1960) postulated that bound lipids contribute markedly to the reactions responsible for rancid odors and off flavors in cooked meats. In their study the oxidation reaction in rancid samples of beef was much more intense in the phospho- and proteolipid phase than in the neutral phase, even though the conjugated lipids represented only a small proportion of the total weight of the lipid. Watts (1962) stated that phospholipids are almost always more unsaturated than the glycerides from the same sample and that their presence renders the fat more susceptible to oxidation. Deuel (1955) indicated that muscle phospholipids contain a relatively high ratio of unsaturated to saturated fatty acids. Snider (1936) found the ratio of unsaturated acids to saturated in muscle phospholipids to be 73:24, which is much higher than the 55:40 ratio reported for liver (Snider and Bloor, 1933).

Hornstein et al. (1961) in studying the effects of phospholipids on meat flavors, separated the beef muscle lipids into triglycerides, cephalins, and a mixture of lecithins and sphingomyelins. After heating freshly isolated fractions in air, they concluded that the phospholipids do not contribute to desirable meat flavor, but in excessively lean meat may contribute to poor flavor.

The phospholipids have been found to be associated with other compounds, particularly in heart tissue. Klenk and co-workers (1952) stated that in addition to the lipids normally found in other tissues, several aldehydes also are contained in muscle. From the phospholipids of horse muscle and beef heart, they isolated a mixture of C_{14} , C_{16} , and C_{18} aldehydes in the form of dimethyl acetals, as well as Δ^9 octadecenal. Plasmalogens have been found to be present in as large concentrations as 6.5 per cent of the phospholipids of swine heart (Gomori, 1949). More recent studies by Klenk and Gehrman (1953) have shown that the plasmalogens of beef heart are concentrated in the lecithin fraction and that only relatively small proportions are present in the cephalin fraction.

Lipid composition of various mammalian species has been studied. Bloor (1936) listed the average phospholipid content of skeletal muscles of mammals as 4.59 per cent. Hornstein et al. (1961) found the phospholipids to make up 0.8 to 1.0 per cent of beef muscle as compared with 0.7 to 0.9 per cent in pork muscle. Bloor (1927) reported that lecithin and cephalin are present in mammal skeletal muscle in approximately equal amounts, lecithin being somewhat higher. Hornstein et al. (1961) obtained similar results with beef muscle, lecithin comprising 40 to 45 per cent of the phospholipids and cephalins 40 to 45 per cent. Sphingomyelin comprised 10 to 15 per cent of the crude phospholipid.

Investigations with rats and also with beef have shown the effects of muscular activity on the muscle lipids. Reed et al. (1930) found

that forced activity and voluntary nocturnal activity increased the proportion of intermuscular lipids in the rat. Bloor and Snider (1934) reported a correlation between the extent of activity of beef muscle and its phospholipid content. The higher the degree of activity, the higher was the level of phospholipids.

Lea (1957) reviewed the deteriorative reactions involving phospholipids. He concluded that the presence of phospholipids not only increases the susceptibility to oxidation of the lipid containing them, but in addition appears to lead to the formation of strong flavored decomposition products in a relatively early stage of the autoxidative process.

CHAPTER III

PROCEDURE

Meat Samples

Meat for this study was taken from a pair of Hereford steers, one finished on grain and one on grass. The animals had been paired as closely as possible as to background and age. A mixture of 75 per cent concentrate (corn, cottonseed meal, and molasses) and 25 per cent mixed hay was fed the grain-finished steer the last four months prior to slaughter. The grass-finished animal was on orchard grass and Ladino clover the entire time.

After slaughter the carcasses were chilled and aged at $36^{\circ}\text{F} \pm 2^{\circ}$. Samples of the semitendinosus muscles of the two animals were taken at 4 different levels of aging: 0, 7, 21, 42 days. The 0- and 7-day samples were removed from the right side of the carcass and the 21- and 42-day samples from the left. After grinding, mixing, and packaging, each sample was frozen immediately in an air blast freezer. The meat was kept frozen at -20°C until the day before it was to be used, at which time it was transferred to the refrigerator for thawing.

Cooking

Portions of approximately 115 g of each sample were weighed roughly on a spring scale, quickly wrapped in pre-weighed aluminum foil

and then weighed on an analytical balance. For shaping patties, each portion of meat was packed into a metal ring having a diameter of 8.4 cm. Each patty was placed on a wire rack in a low Pyrex baking dish* and cooked on the rotating hearth of a Despatch electric oven at 350°F to an internal temperature of 75°C (approximately 28 minutes). After one hour of cooling on the rack, the entire meat sample was blended in a Waring Blendor for one minute. Moisture determination and extraction of the total lipids followed immediately.

Moisture Determinations

Moisture determinations were carried out in triplicate on both the raw and cooked meat. Three to five grams of sample were placed in weighed moisture pans, covered, and weighed on an analytical balance. The pans were placed, uncovered, in a drying oven at $100^{\circ}\text{C} \pm 10^{\circ}$ for 18 hours with a raw sample and 16 hours with a cooked sample. After replacement of covers and removal from the oven, the pans and their contents were cooled in a desiccator until constant weights were obtained. Moisture content was expressed as per cent of original weight.

Extraction of Lipids

Duplicate lipid extractions of both raw and cooked samples were accomplished by the method of Ostrander and Dugan (1961). Approximately 30 g of meat were weighed onto a previously weighed

*The inverted cover of a casserole was used.

piece of aluminum foil on a spring scale, wrapped, and reweighed on an analytical balance. The sample was transferred quantitatively to a Waring Blendor jar with one Kim-Wipe and 130 ml absolute methanol, and blended for 5 minutes with the speed controlled by means of an Adjust-A-Volt regulator set at 26. Sixty-five ml chloroform were added and the mixture was blended again for 5 minutes at the same speed. An additional 65-ml portion of chloroform was added and the mixture was blended for 30 seconds at a setting of 30. The final addition, 65 ml distilled water containing 1.5 g zinc acetate, was blended with the sample for 10 seconds with the voltage regulator set at 30.

The extraction mixture was poured into a No. 3 Büchner funnel and filtered through Whatman No. 1 filter paper with suction. The residue, with filter paper, was transferred to a blender jar. The funnel was wiped with one Kim-Wipe and the residue, filter paper, and Kim-Wipe were reblended with 100 ml chloroform for 2½ minutes. The mixture was transferred to the funnel and refiltered with the use of 50 ml chloroform for washing.

The filtrate was transferred into a 500 ml graduated cylinder, with a small amount of chloroform used for rinsing. The volume of the chloroform layer was noted and the contents were poured into a 500-ml separatory funnel. The bottom layer, containing the lipid material, was collected in a 1000-ml evaporating flask. Two 10-ml aliquots were taken and put into weighed 25-ml Erlenmeyer flasks.

Most of the solvent was allowed to evaporate from the aliquots at room temperature under the hood. The samples were dried further to constant weight in a vacuum desiccator. Per cent lipid in each sample was calculated:

$$\text{Per cent lipid} = \frac{\text{g lipid in 10-ml aliquot} \times \text{ml CHCl}_3 \text{ extract} \times 100}{10 \times \text{g of sample extracted}}$$

The solvent in the remaining lipid solvent mixture was evaporated on a flash evaporator. The extracted lipids were transferred to a 50-ml beaker with chloroform, gassed with nitrogen, covered with aluminum foil and stored at -20°C for later analysis.

Separation of Neutral and Acidic Lipids

The phospholipids were separated from the neutral lipids by a modification of the slurry method of Murty et al. (1960). Approximately 35 g of silicic acid were dried 6 to 8 hours in a 250°F oven and cooled in a desiccator. The silicic acid was placed in a 500-ml Erlenmeyer flask with 50 ml chloroform. About 3 g of a sample of extracted lipids (weighed to the nearest 0.1 mg) were transferred quantitatively to the flask with 50 ml chloroform. A magnetic stirring bar was added and the mixture was slurried on a magnetic stirrer for 10 minutes.

The slurry was transferred quantitatively into a sintered glass funnel and filtered with suction. Six 50-ml portions of chloroform were used for the washing. When filtration was complete, the filtrate, containing the non-phosphorus lipids, was transferred quantitatively to a 1000-ml evaporating flask with 50 ml chloroform and evaporated on the flash evaporator.

The silicic acid mixture remaining in the funnel was transferred into a 500-ml flask with 100 ml redistilled methanol and stirred on the magnetic stirrer for 10 minutes. This slurry was filtered into a clean flask and washed with a total of 100 ml of redistilled methanol in three portions. When filtration was complete, the filtrate, containing phospholipids, was transferred quantitatively to a 1000-ml evaporating flask with 50 ml redistilled methanol. (In some cases, it was necessary to use methanol:chloroform, 1:1, v:v, to remove all of the sample.) The solvent was evaporated from the mixture on the flash evaporator.

After evaporation the contents of the flasks were transferred quantitatively to weighed beakers with redistilled petroleum ether and evaporated under a stream of nitrogen. A vacuum desiccator was used to get rid of any remaining solvent and to bring the beaker and contents to constant weight. Per cents of phospholipids and triglycerides in the extracted lipid were calculated.

Separation of Phospholipids into Classes

The phospholipids were separated into classes by a modification of the thin layer chromatography (TLC) procedure of Skipski et al. (1962). Fifteen g of silica gel with binder* and 1.5 g ammonium sulfate were mixed with 28 ml distilled water and applied to 100 x 200-mm glass plates in layers approximately 250 μ thick with the use of a Camag applicator.

*Anasil B (Analabs).

After 5 minutes of air drying, the plates were dried in a 250°F oven for 20 minutes. The phospholipid samples were dissolved in chloroform:methanol, 1:1, v:v, and applied to the plates with a micro-syringe in 5- μ l portions* in order to keep spot size small. About 30 μ l were applied per spot by repeated spotting. Four plates, each containing five sample spots, were prepared for each sample. Development of the plates was carried out in an enclosed chamber with troughs at the bottom containing a solvent mixture of chloroform: methanol:water, 80:3:2, v:v:v. At the end of a 50-minute period, the plates were removed, air dried for two minutes and sprayed with a 0.5 per cent aqueous solution of Rhodamine B indicator.** Identification of spots was made possible by a preliminary spotting of a plate with three known phospholipids: cephalin, sphingomyelin, and lecithin. Rf values were not completely reproducible, but because sphingomyelin always appeared between the other two phospholipid classes, it was necessary subsequently only to spot each plate with a sphingomyelin standard to assist in detection and separation. Ultraviolet light facilitated differentiation of the zones.

The three fractions were marked off and the silica gel containing each was scraped into a beaker containing chloroform:methanol, 80:30,

*Approximately 300-500 μ g/ μ l.

**Practical grade (Eastman Kodak Company).

v:v. For each fraction of each sample the scrapings from all four plates were combined in a single beaker. Each slurry was transferred into a sintered glass funnel and filtered under reduced pressure. Three 20-ml washings with methanol, each followed by filtration, completed the elution. The filtrate was transferred to an evaporating flask and the solvent was evaporated on the flash evaporator. Each phospholipid fraction was transferred quantitatively to a 15-ml centrifuge tube with redistilled petroleum ether. If methylation was not to be carried out immediately, the fraction was stored overnight in the freezer in this form. Just prior to methylation the petroleum ether was evaporated under a stream of nitrogen.

Although it has been stated that lipids are well protected against autoxidation in TLC (Mangold, 1961), a preliminary trial was carried out to determine whether highly unsaturated samples would be altered by the chromatography procedure. Corn oil was used because of its high content of linoleic acid and a sample that had not been subjected to TLC served as a control. A second sample was given the entire TLC treatment as described above except that the fractions were combined, and a third sample was treated similarly to the second except for omission of the Rhodamine B spray. The untreated corn oil and the samples eluted from the TLC plates were methylated. Gas-liquid chromatography of the methyl esters showed no appreciable differences in the fatty acid composition of the three samples (Appendix, Table VIII). This method of separating phospholipids apparently did not alter the fatty acids.

Methylation

For analysis of fatty acids by gas-liquid chromatography (GLC), the acids are converted to their methyl esters which have lower boiling points than do the acids themselves. Methylation may be preceded by saponification or may involve a more direct interesterification procedure. In preliminary work these two general methods produced esters giving nearly identical gas chromatograms, and the more rapid procedure, bypassing the saponification, was used throughout the study.

About 40 mg of sample were placed in a 15-ml centrifuge tube. Three ml of one per cent sulfuric acid in methanol (v:v) were added and the samples were refluxed 4 hours in a sand bath held at approximately 70°C by means of an Adjust-A-Volt regulator set at 25. The samples were stirred every 15 minutes to assure methylation of all the lipid. At the end of the reflux period, samples were cooled and 6 ml petroleum ether and 2 ml distilled water were added. The contents of each tube then were poured into a 30-ml separatory funnel and the bottom layer, containing sulfuric acid and water, was removed. Washing with water and removal of the bottom layer was repeated twice. The petroleum ether layer, containing the methyl esters, was poured into a clean centrifuge tube and 0.5 g sodium sulfate and 0.5 g silicic acid were added to remove traces of water and other contaminants. The mixture was allowed to stand at least one hour, after which the petroleum ether extract was decanted into another tube. If the methyl esters were to be used immediately, the solvent was evaporated under a stream of nitrogen, leaving the methyl esters in the tube. If they were to be

stored, the tubes containing the petroleum ether extract were stoppered and stored in the freezer.

Two finishes and four aging periods constitute 8 treatment combinations. Raw and cooked samples of each raise the number to 16 and phospholipids were obtained from duplicate samples. For each of the 16 samples one portion of total phospholipid was methylated, as well as the three fractions from the remainder of the total phospholipid, resulting in 64 methyl ester preparations.

Gas-Liquid Chromatography

A Barber-Colman, Model 61 C, Gas-Liquid Chromatograph was used to analyze the samples for fatty acid content. The chromatograph was equipped with an argon ionization detector with radium source and an argon gas supply. A packed column with a polar liquid phase was used. In the preparation of the column a 7-foot length of copper tubing ($\frac{1}{4}$ in. O. D.) was cleaned* and then packed with 80-100 mesh Chromosorb W coated with 15.4 per cent (by weight) diethylene glycol succinate polyester.** The packed column was coiled and installed except for the connection to the detector. Preconditioning at 195°C, with argon gas flowing, was carried out for 216 hours until no bleeding of liquid phase could be detected. The column temperature was lowered to 185°C, the connection

*Aqueous detergent solution, distilled water, methanol, acetone, methylene chloride, and benzene used in sequence.

**Packing prepared by Applied Science Laboratories, Inc.

to the detector was made, and the following additional operating conditions were established:

Flash Heat Temperature -----	248°C
Cell Temperature -----	208°C
Split Temperature -----	223°C
Cell Voltage -----	825
Gas Pressure -----	15 psi
Attenuator Setting* -----	16
Sensitivity Setting -----	10

Methyl esters were diluted with acetone just prior to injection into the chromatograph. Because of limitations in quantity of methyl esters, it was possible to obtain only one chromatogram for each of the 64 samples.

The major components were identified by comparison of retention times with those of a standard fatty acid methyl ester mixture. In order to identify minor components not present in the standard mixture, it was necessary to plot retention times for the known components against the carbon number on semi-logarithmic paper.

The concentrations of fatty acids present were found by first calculating the "area" under each peak. This was accomplished by multiplying the height of the peak by the width at half-height and correcting for any change in the attenuator, i.e., multiplying by

*The attenuator was changed only if the sample size seemed too large.

the attenuation factor. To find the percentage of a particular fatty acid (FA) in a sample, the following equation was used:

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

CHAPTER IV

RESULTS AND DISCUSSION

Values for lipid content are shown in Table I. Generalization must be limited because of variability due to the fact that some exterior fat was ground along with the muscle. However, values for the grain-finished animal were consistently higher than those for the grass-finished animal. From their consistency and their magnitude, these differences would seem to be real. Other workers (Hopper, 1944; Hunt et al., 1953) also have reported grain-finished animals to show a higher proportion of fat per edible portion than do grass-finished animals. Fat content appears to have increased somewhat during aging.

A comparison of per cent phospholipid in the lipid extracted is given in Table II. With the exception of the cooked samples from the meat aged 7 days, lipids from the grass-fed animal appear to have had higher concentrations of phospholipids than did those from the grain-finished animal. Cook's work (1963) indicated a similar tendency. Although the present study did not include sensory testing, this difference in phospholipid content of grain- and grass-fed beef might be considered as the basis for flavor differences observed by Mayer, et al. (1960). Further evidence in support of such a relationship might be seen in the increase in phospholipid concentration between the twenty-first and forty-second days, the interval during which Mayer, et al. found flavor deterioration.

TABLE I

PER CENT LIPID EXTRACTED FROM GRAIN- AND GRASS-FED BEEF AGED FOUR PERIODS OF TIME^a

Aging Period	Raw Samples				Cooked Samples			
	Wet Weight Basis		Dry Weight Basis ^b		Wet Weight Basis		Dry Weight Basis ^b	
Days	Grain	Grass	Grain	Grass	Grain	Grass	Grain	Grass
0	9.86	4.93	31.08	19.24	17.32	5.66	38.59	16.15
7	10.81	8.15	34.94	27.46	15.49	11.26	34.48	28.50
21	21.81	7.64	56.51	26.55	21.55	10.06	45.32	26.25
42	21.08	7.23	53.14	25.30	23.58	8.14	48.98	22.70

^aEach value is an average of two determinations.^bMoisture values used for calculations are given in Table IX, Appendix.

TABLE II

PER CENT PHOSPHOLIPID IN LIPIDS EXTRACTED FROM GRAIN- AND GRASS-FED
BEEF AGED FOUR PERIODS OF TIME^a

Days of Aging	Raw		Cooked	
	Grain	Grass	Grain	Grass
0	19.23	34.07	19.59	20.73
7	18.40	23.37	18.86	15.63
21	16.85	25.79	14.43	18.77
42	26.98	29.42	20.99	29.68

^aAverage of two determinations.

Cooking appeared to have no appreciable effect on proportions of fatty acids; therefore, the values for raw and cooked samples were averaged in the tables showing fatty acid composition of the total phospholipids and the fractions. The data for the total phospholipids are presented in Table III. Except for the final aging period, the phospholipids from grain-fed beef tended to have somewhat higher proportions of palmitate (C_{16}) and slightly lower proportions of oleate ($C_{18:1}$) and linoleate ($C_{18:2}$) than did the phospholipids of grass-fed beef.

Aging apparently produced greater differences in composition than did finish (Table III). Palmitate and stearate (C_{18}) tended to decrease and oleate to increase with aging time in phospholipids from both grain- and grass-finished beef. Cook (1963) found less effect on these components with aging. Another difference between the findings of this study and those of Cook is the tendency for linoleate to decrease slightly with aging in this study and to increase slightly in the work reported by Cook. Cook also found higher proportions of linoleic acid and of the unidentified components than were found in the total phospholipid in this study. Hornstein et al. (1961) reported rather large quantities of arachidonate, which was not detected at all in this study. Failure to find arachidonate may have been due in part to the operating conditions used for gas chromatography. Both the relatively low levels of linoleate and the apparent absence of arachidonate may have been due in part to the samples used. Phospholipids tend to vary with many factors, including the specific muscle. It should be noted also that

TABLE III
FATTY ACID COMPOSITION OF TOTAL PHOSPHOLIPIDS^a

Finish and Aging	C ₁₄	C _{14:1}	?	C ₁₆	C _{16:1}	?	C ₁₈	C _{18:1}	C _{18:2}	Total Saturates	Total Monoenes	Total ?
<u>Grain</u>												
0 da.	2.9	0.5	0.5	39.9	1.9	1.2	9.1	38.7	5.9	51.9	41.1	1.7
7	2.6	0.3	0.5	40.3	1.7	1.2	9.7	38.8	5.2	52.6	40.8	1.7
21	2.5	0.4	0.3	39.0	1.4	1.2	7.5	43.1	4.9	49.0	44.9	1.5
42	2.9	0.5	0.4	27.8	3.2	1.3	5.6	53.0	5.5	36.3	46.7	1.7
Ave.	2.7	0.4	0.4	36.8	2.1	1.2	8.0	43.4	5.4	47.5	45.9	1.7
<u>Grass</u>												
0 da.	2.0	0.6	0.5	33.0	1.9	1.3	9.7	40.8	10.4	44.7	43.3	1.9
7	2.1	0.8	0.9	32.8	2.4	0.9	9.5	42.9	7.8	44.4	46.1	1.8
21	2.0	1.0	0.6	31.3	2.3	1.8	8.6	46.9	5.7	41.9	50.2	2.4
42	1.8	0.7	0.6	27.6	2.6	1.4	8.2	51.6	5.0	37.6	54.9	2.0
Ave.	2.0	0.8	0.7	31.2	2.3	1.4	9.0	45.6	7.2	42.2	48.6	2.0

^aPer cent of total fatty acids.

Each value is an average of a raw and a cooked sample.

whereas Cook (1963) and Hornstein et al. (1961) analyzed muscle fat, some exterior fat was ground with the meat used in this study.

A comparison of the average composition of the total phospholipids and of the three fractions is presented in Table IV. The major difference among the fractions appears to have been a lower degree of saturation in lecithin than in cephalin or sphingomyelin. The lecithin fraction tended to be relatively low in palmitate and stearate and relatively high in oleate and linoleate. Lecithin is the only fraction in which linolenate ($C_{18:3}$) was detected and usually it was not measurable.

The lecithin fraction also contained higher proportions of the unidentified compounds than did the total phospholipids or the other two fractions. Finish apparently had an effect on the concentration of these constituents through the 7 day aging period, as shown by the fact that the amounts were greater in the grass-fed than in the grain-fed beef (Table V). Little difference was detected from 21 days on. Finish apparently had little effect on the major components of the lecithin fraction. Aging, on the other hand, resulted in a decrease in C_{18} and in $C_{18:2}$, along with a rather large increase in $C_{18:1}$. The unidentified components decreased with aging.

The cephalins were somewhat less saturated in the grass-fed than in the grain-fed beef (Table VI). The influence of aging on the cephalin fraction was different from that on the other fractions. Palmitate in the grass-fed beef remained fairly constant through 21 days and then dropped. Oleate remained fairly constant through 21 days and then rose.

TABLE IV

COMPARISON OF AVERAGE FATTY ACID COMPOSITION OF TOTAL PHOSPHOLIPIDS AND THREE FRACTIONS^a

Fraction	C14	?	C16	C16:1	?	C18	C18:1	C18:2
Total	2.4	0.6	34.0	2.2	1.3	8.5	44.5	6.3
Lecithin	1.8	2.4	29.3	2.1	2.5	9.4	45.8	7.2
Cephalin	2.2	1.4	33.7	1.1	0.9	15.7	39.8	5.6
Sphingomyelin	2.0	0.9	38.1	1.4	0.9	14.0	36.3	6.9

^aPer cent of total fatty acids.

TABLE V
FATTY ACID COMPOSITION OF LECITHIN FRACTION^a

Finish and Aging	C ₁₄	?	C ₁₆	C _{16:1}	?	C ₁₈	C _{18:1}	C _{18:2}	Total Saturates	Total Monoenes	Total ?
<u>Grain</u>											
0 da.	2.5	1.2	37.5	1.6	2.0	14.7	32.9	8.0	54.7	34.5	3.2
7	1.4	1.0	40.6	2.1	1.4	10.2	34.9	8.6	52.2	37.0	2.4
21	2.3	1.1	23.8	2.7	2.0	7.8	54.1	6.2	33.9	56.8	3.1
42	1.7	0.4	26.0	2.0	0.6	5.1	60.1	4.2	32.8	62.1	1.0
Ave.	2.0	0.9	32.0	2.1	1.5	9.5	45.5	6.8	43.4	47.6	2.4
<u>Grass</u>											
0 da.	1.9	9.3	24.4	1.3	5.5	15.3	33.0	9.5	41.6	34.3	14.8
7	1.5	3.8	24.7	2.0	4.6	9.7	45.8	8.1	35.9	47.8	8.4
21	1.8	1.1	38.6	2.2	2.1	6.7	50.0	7.6	37.1	52.2	3.2
42	1.1	0.5	28.6	2.5	1.6	5.3	55.7	5.1	35.0	58.2	2.1
Ave.	1.6	3.7	26.6	2.0	3.5	9.3	46.1	7.6	37.4	48.1	7.1

^aPer cent of total fatty acids.

Each value is an average of a raw and a cooked sample.

TABLE VI
FATTY ACID COMPOSITION OF CEPHALIN FRACTION^a

Finish and Aging	C ₁₄	?	C ₁₆	C _{16:1}	?	C ₁₈	C _{18:1}	C _{18:2}	Total Saturates	Total Monoenes	Total ?
<u>Grain</u>											
0 da.	3.3	0.3	39.8	1.1	tr	19.4	34.2	2.1	62.5	35.3	0.3
7	1.8	2.3	39.7	0.7	1.7	15.0	36.6	1.9	56.5	37.3	4.0
21	2.1	0.3	44.3	1.0	tr	11.9	37.3	3.2	58.3	38.3	0.3
42	0.9	5.3	29.6	1.3	3.2	13.9	39.6	6.4	44.4	40.9	8.5
Ave.	2.0	2.1	38.4	1.0	1.2	15.1	36.9	3.4	55.4	38.0	3.3
<u>Grass</u>											
0 da.	3.3	tr	30.4	1.5	tr	17.0	41.6	6.4	50.7	43.1	tr
7	3.0	1.0	31.0	0.9	1.8	13.3	40.5	8.6	47.3	41.4	2.8
21	1.7	2.1	33.7	1.3	tr	16.6	38.4	6.5	52.0	39.7	2.1
42	1.1	tr	20.7	0.6	tr	18.2	50.4	9.2	40.0	51.0	tr
Ave.	2.3	0.8	29.0	1.1	0.5	16.3	42.7	7.7	47.5	43.8	1.2

^aPer cent of total fatty acids.

Each value is an average of a raw and cooked sample.

In the grain-fed beef a rise in palmitate was seen between 7 and 21 days and a drop between 21 and 42 days.

The sphingomyelins appeared to be less affected by either finish or aging than were the other fractions (Table VII). No appreciable difference occurred as a result of finish. Aging perhaps produced a tendency for saturation to decrease

Although organoleptic testing was not carried out in this study, one might attempt to relate the information obtained in this work to the results of previous flavor studies. As was mentioned in the review of literature, Meyer et al. (1960) reported higher flavor scores for grain-fed than for grass-fed beef. They also found that flavor deteriorated with aging between the twenty-first and forty-second days. According to the data on fatty acid composition of the total phospholipids in this study (Table III), the flavor effects could be related to saturation. Stames (1958) found no correlation between flavor scores and peroxide values; however, with the method of extraction used it is unlikely that she extracted the phospholipids, which we now know to be more susceptible to oxidation than the glycerides. It would seem from the data in Tables III, V, VI and VII that the higher degree of saturation with grain finish than with grass finish noted in the total phospholipids in the present study largely reflects differences in the cephalin. The tendency toward decreased saturation with aging represents changes primarily in the lecithin and cephalin fractions. Thus the cephalin and/or lecithin fraction of the phospholipids would appear to be implicated in possible flavor changes.

TABLE VII
FATTY ACID COMPOSITION OF SPHINGOMYELIN FRACTION^a

Finish and Aging	C14	?	C16	C16:1	?	C18	C18:1	C18:2	Total Saturates	Total Monoenes	Total ?
<u>Grain</u>											
0 da.	1.4	1.0	44.3	0.7	1.7	10.8	32.8	7.5	56.5	33.5	2.7
7	2.7	0.4	47.4	0.6	0.5	10.6	34.0	3.8	60.7	34.6	0.9
21	1.1	tr	31.5	2.0	tr	18.0	40.2	6.3	50.6	42.2	tr
42	2.0	1.7	33.9	1.8	0.7	10.1	41.6	8.3	46.0	43.4	2.4
Ave.	1.8	0.8	39.3	1.3	0.7	12.4	37.2	6.5	53.5	38.4	1.5
<u>Grass</u>											
0 da.	2.3	0.1	40.6	1.7	0.4	13.6	36.2	5.5	56.5	37.9	0.5
7	2.0	1.3	35.3	1.8	1.7	15.5	33.1	9.5	52.8	34.9	3.0
21	3.1	0.5	36.5	1.5	1.3	16.3	35.4	5.5	55.9	36.9	1.8
42	0.9	1.6	34.8	0.8	0.9	16.9	36.4	8.3	51.6	37.2	2.5
Ave.	2.1	0.9	36.8	1.5	1.1	15.6	35.3	7.2	54.2	36.7	2.0

^aPer cent of total fatty acids.

Each value is an average of raw and cooked sample.

Further evidence for involvement of the lecithin fraction is the fact that it had a relatively high concentration of the unidentified components, which in Cook's study appeared to be related to the flavor changes reported by Meyer et al. (1960). The possible origin of these substances is of interest. Hornstein et al. (1961) reported the presence in beef phospholipids of a compound that he tentatively identified as a tetradecadiene. Identification was made by plotting retention time against chain length on semi-logarithmic paper. Such a plot renders the members of a homologous series in a straight line. The saturates fall in a line parallel to the monoenes, dienes, and trienes on separate lines. Cook (1963) observed the presence in beef phospholipids of three compounds that fell on the diene line of a plot similar to that used by Hornstein. The three components had retention times corresponding to those of a dodecadiene, a tetradecadiene, and a hexadecadiene. Plotting of retention times for the unidentified components found in the present study indicates that these compounds probably are the same as the two higher homologs that Cook found.

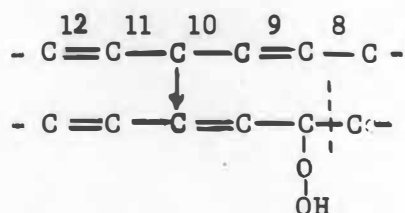
Identification of the unknown substances was not possible with the facilities available in this study; however, some clues may be found in the literature. Keeney (1962), in a symposium on lipids, stated that it is possible for compounds having the same retention times as certain fatty acids actually to be other compounds, as for example, semi-aldehydes, aldehydes and substituted fatty acids. In the discussion following Keeney's presentation, reference was made to a 9-carbon

aldehyde ester that comes shortly before palmitate in a gas chromatogram and to another compound that appears before myristate (C_{14}) in oxidized samples.

Although no aldehyde standards were available and it was not feasible to collect fractions during the chromatographic analysis, it would seem possible that the unidentified compounds of this study were aldehydes of a homologous series. At least two explanations for their occurrence might be advanced. Plasmalogens, which are phosphatides, contain vinyl ether structures which yield aldehydes upon mild acid hydrolysis (Keeney, 1962). According to Klenk and Gehrman (1953), the plasmalogens tend to be concentrated in the lecithin fraction. The fact that the unidentified components in this study were found chiefly in the lecithin, along with the fact that plasmalogens have been found to migrate with lecithins in TLC under the conditions used here (Skipski et al., 1962), would seem to support the explanation that the compounds in question originate from plasmalogens. From the relative concentrations of the unidentified compounds it appears that the phospholipids from grass-fed beef contained a higher percentage of plasmalogens than did those from grain-fed beef.

The above suggestion does not explain either the increase of the unidentified compounds found with aging in Cook's study or the decrease with aging in this study. It would appear that an hypothesis that could explain these quite different results would need to involve linoleic acid because in both studies the compounds in question tended to change with the linoleate.

Another possibility is that the postulated aldehydic components are breakdown products of higher fatty acids. A complex variety of hydroperoxides and aldehydes has been shown to be produced as secondary degradation products in autoxidation (Bading, 1960). Patton et al. (1959) found decadienal as an oxidation product of beef tallow linoleate when heat was applied. In their study of flavor thresholds of this compound, they found that it could be detected in as small an amount as one part per billion. The mechanism they thought to be involved was a shift of the double bond at position 9 to the 10 position and hydroperoxide attack of the 9 carbon followed by chain cleavage at the 8 position as shown below:



Aldehydes of different chain lengths were found as products of oxidation at different temperatures.

Another feature of the fatty acid analysis of the total phospholipids and lecithin fraction in this study was an increase of C_{18:1} with aging. In the cephalin oleate also appeared in higher concentrations in the grass-fed beef than in the grain-fed beef. If the hydroperoxide that formed before the cleavage in the above scheme appeared on a gas chromatogram as C_{18:1}, this could lead to an apparent increase in C_{18:1} when a higher fatty acid was being broken down and before the subsequent split to form an aldehyde. The increase in C_{18:1} might then

be an indication of oxidation taking place with a possible increase in formation of lower aldehydes that were too volatile to be detected under the conditions of gas chromatography used or that were oxidized to carboxylic acids.

The above mechanism involves autoxidation, some of which presumably could occur during storage and handling of the samples. However, there is no reason to suppose that the lecithin samples were exposed to conditions favoring autoxidation any more than were the other samples; and the explanation involving plasmalogens, therefore, seems more plausible. Perhaps both mechanisms are operative. If the increase in free acidity with aging observed by Stames (1958) occurred also in this study, one might expect liberation of bound aldehydes by mild hydrolysis accompanied by formation of free radicals. Then both the liberated aldehydes and the free radicals could participate in chain reactions of autoxidation.

The findings of this study suggest the need for further work with lecithins from grain- and grass-fed beef. Exterior fat should be excluded or studied separately from muscle fat. Identification of the postulated aldehydes might be pursued by changing the TLC procedure to separate plasmalogens from the lecithins and by the use of known aldehydes in gas chromatography. The effects of various storage and cooking procedures on the concentration of these compounds might be investigated in studies that include palatability tests.

CHAPTER V

SUMMARY

The effect of type of finish and the time of carcass aging on the phospholipid constituents of beef was studied. Lipids were extracted from raw and cooked samples of the semitendinosus muscle from grain- and grass-finished beef aged four periods of time: 0, 7, 21, and 42 days. Separation of the phospholipids from the extracted lipids was followed by further fractionation of the phospholipids by thin layer chromatography. Total phospholipids and three fractions, lecithin, cephalin and sphingomyelin, were analyzed for fatty acid content by gas-liquid chromatography.

The fat from grass-finished beef was found to have higher proportions of phospholipids than that from grain-finished beef. The phospholipids from grass-finished beef tended to be somewhat less saturated than those from grain-finished beef. Aging tended to decrease saturation in the phospholipids of both types of beef, though polyunsaturation tended to decrease also. Occurring in largest quantity in the lecithin fraction and tending to change in concentration with linoleate, were two unidentified compounds. Their relatively high concentration in the lecithin fraction suggests that they are aldehydes possibly formed from plasmalogens, known to be associated with lecithins. The plasmalogens could supply aldehydes and free radicals to participate in chain reactions of autoxidation.

The observed effects may relate to flavor deteriorations found in earlier studies.

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APPENDIX

TABLE VIII

COMPARISON OF FATTY ACID CONTENT OF UNTREATED CORN OIL, CORN OIL
SUBJECTED TO THE ENTIRE TLC PROCEDURE AND CORN OIL SUBJECTED
TO TLC PROCEDURE OMITTING RHODAMINE B SPRAY^a

	Fatty Acids			
	C16	C18	C18:1	C18:2
No Treatment	9.5	1.7	24.2	64.6
TLC (With Spray)	9.2	1.5	25.4	63.9
TLC (No Spray)	9.5	1.4	26.1	63.0

^aPer cent of total fatty acids. Each value is an average from two chromatograms of the methyl esters.

TABLE IX

PER CENT MOISTURE IN GRAIN- AND GRASS-FED BEEF SAMPLES,
RAW AND COOKED^a

Aging Period Days	Raw		Cooked	
	Grain	Grass	Grain	Grass
0	69.07	74.36	55.14	64.92
7	69.07	70.32	55.14	60.58
21	61.38	71.22	52.57	61.68
42	60.35	71.42	51.86	64.15

^aEach value is an average of three determinations.