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Comparison of Two Fractionation Methods in the Study of Muscle Lipids from Grain- and Grass-finished Beef

Laura Elizabeth Taylor

To the Graduate Council:

I am submitting herewith a thesis written by Laura Elizabeth Taylor entitled "Comparison of Two Fractionation Methods in the Study of Muscle Lipids from Grain- and Grass-finished Beef." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Ada Marie Campbell, Major Professor

We have read this thesis and recommend its acceptance:

Bernadine Meyer, John T. Smith

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

December 1, 1964

To the Graduate Council:

I am submitting herewith a thesis written by Laura Elizabeth Taylor entitled "Comparison of Two Fractionation Methods in the Study of Muscle Lipids from Grain- and Grass-finished 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:

Bernadine Meyer

John T. Smith

Accepted for the Council:

Dean of the Graduate School

COMPARISON OF TWO FRACTIONATION METHODS IN THE STUDY OF MUSCLE LIPIDS FROM GRAIN- AND GRASS-FINISHED 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

Laura Elizabeth Taylor

December 1964

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L. E. T.

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CHAPTER I

INTRODUCTION

Possible differences in composition of fat from grain- and grass-finished beef have been of interest at the University of Tennessee since Meyer et al. (1960) reported the fat of grain-finished beef to be superior in flavor to that of grass-finished beef. In later work the fat from animals of these two types of finish differed chiefly in the proportion of phospholipids and in the degree of saturation of the phospholipids (Cook, 1963; Nutt, 1963).

Observations of other workers also have focused attention on lipid fractions. Early studies showed a correlation between muscle activity and phospholipid content (Bloor, 1927; Bloor and Snider, 1934; Snider, 1936). More recently lipid fractions have been studied in attempts to explain flavor changes in foods (Lea, 1957; Younathan and Watts, 1960; Lee and Mattick, 1961; Hornstein et al., 1961). Results of these studies have implicated phospholipids in flavor deterioration.

In view of the interest in the study of specific fractions, methods of separating phospholipids from non-phosphorus lipids are important. Two general fractionation procedures have been used most extensively: (1) the slurry method described by Murty et al. (1960), and (2) the column method exemplified by the procedure of Hornstein et al. (1961). Both methods have been used in this laboratory but a comparison of their effectiveness had not been made. Therefore, a

study was planned for the purpose of making such a comparison and also of obtaining further information concerning the muscle lipids of grain- and grass-finished beef. Three different muscles were used on the assumption that they might represent a range with respect to phospholipid content.

CHAPTER II

REVIEW OF LITERATURE

Today gas-liquid chromatography is used in the identification and measurement of specific fatty acids in lipids. The availability of this method has expedited the study of lipids and has spurred interest in the techniques of separating lipid fractions.

Both of the methods that have been used most extensively for separating neutral lipids from acidic lipids make use of silicic acid as the adsorbant. Two solvents differing considerably in polarity, frequently chloroform and methanol, are used for removal of the lipid fractions. In the method used by Murty et al. (1960) the silicic acid and the solvent containing the sample are slurried and shaken. Suction filtration and numerous washings with the eluting solvents are used to remove the two lipid fractions. The column method, described by Hornstein et al. (1961), is the more time consuming because of the packing and washing of the column and the slow drip rate of the eluants.

As early as 1931, work was reported concerning the effect of grain and grass finishing of steers on quality. Black et al. (1931) concluded that the feeding of grain supplement was advisable and in later work (1940) they concluded that cattle fed on grass alone yielded the highest percent of rib-eye, total lean, and bone but the lowest percent of fat and total edible portion in the 9-10-11 rib cut. Bull et al. (1941) found that the color of the fat from grass-finished beef was yellow and

inferior to that of the grain-finished beef. The yellow color of fat in the carcasses was due to the transfer of carotene from pasture to body fat. Grass-finished carcasses graded lower than grain-finished carcasses because of lack of finish. Wanderstock and Miller (1948) also found grain-finished cattle to be graded higher than grass-finished cattle, largely due to a difference in fatness. Beef from both grain- and grass-finished animals was acceptable. Callow (1962) considered a difference between grain- and grass-finished beef with respect to saturation of fat to be due to the greater rate of fattening in grain-finished steers. Hunt et al. (1953) reported that beef produced from grass was equal in quality to beef produced from grain provided both were from cattle of equal fatness. Cook (1963) and Nutt (1963) both reported grain-finished beef to have a higher percent of muscle lipid than grass-finished beef. Meyer et al. (1960) reported palatability of fat and meat from grain-finished beef to be superior to that from grass-finished beef. Flavor differences were highly significant for both meat and fat.

Some of the work reported on animal lipids has dealt only with depot fat. In a study reported by Reed et al. (1930) more total depot fat was stored by rats ingesting rations rich in fat than by those on rations rich in carbohydrate and starch, but depot fat was distributed similarly for rats of the same sex, age, and weight regardless of the type of diet. Storage areas differed with sex. Forced activity or voluntary activity of the rats at night caused an increase in the proportion of intermuscular fat over that of the control group.

Some work has been concerned with relative amounts of intramuscular fat in different portions of the carcass. Lawrie (1961) stated that in beef the longissimus dorsi muscle in the lumbar region has a higher content of intramuscular fat than does the same muscle in the thoracic region regardless of age or overall level of fatness. The longissimus dorsi muscle of beef cattle was found to be higher in intramuscular fat than was that of dairy cattle. In reporting lipid content of six different muscles, Lowe and Kastelic (1961) found psoas major to have the highest percent lipid of all the muscles analyzed.

Composition of intramuscular and of depot fat has received some attention. Deuel (1951) listed palmitic, stearic, and oleic acids as the only fatty acids found in beef tallow in appreciable amounts. Ostrander and Dugan, Jr. (1962), working with beef, veal, pork, and lamb, showed the composition of intramuscular lipids and depot fats to be drastically different. They attributed this difference to the presence in intramuscular lipids of phospholipids which are a rich source of polyunsaturated fatty acids. Callow (1962) presented evidence that as the percent of fat in the muscle tissue decreased, the relative proportion of phospholipid increased. Hornstein et al. (1961) reported 0.8-1.0% of phospholipids for beef muscle as compared with 0.7-0.9% for pork muscle.

Variation of the phospholipid content with muscle location and/or with activity has been reported in several studies. In an early study Bloor (1927) correlated phospholipid content of five voluntary muscles of beef with their apparent metabolic activity. He stated that "the

more a muscle is called on to do, the higher is the phospholipid content." Bloor and Snider (1934) found that when laboratory rabbits were compared with wild rabbits, the thigh and front leg muscles of the wild animals were higher in phospholipid content than were the same muscles of the laboratory animals. In pigeons, owls, and roosters the pectoralis major, the main flying muscle of the breast, was higher in phospholipid content than was the pectoralis minor. The phospholipid content of both breast muscles was greatest in the pigeon and least in the rooster. These results seem to indicate a positive relationship between muscle activity and phospholipid content. Kuchmak and Dugan, Jr. (1963) reported variation of phospholipid content of pork with different carcass locations. A sample from the loin section was lowest in phospholipid content and a sample from the belly was the highest in phospholipid of the four samples studied. Values ranged from 0.4590 to 0.5819 g./100 g. fresh meat. Snider (1936) compared eight species of animals as to fatty acid composition of the muscle phospholipid and concluded that the fatty acid content of the phospholipids was similar in all the animals studied.

CHAPTER III

PROCEDURE

Meat Samples

Meat from a pair of Hereford steers, one finished on grain and one on grass, was used. Age and background were considered in pairing the animals as closely as possible. A mixture of 25% mixed hay and 75% concentrate (corn, cottonseed meal, and molasses) was fed the grain-finished steer the last four months prior to slaughter. Orchard grass and Ladino clover constituted the entire diet of the grass-finished steer. The grain-finished animal weighed 977 lb. at the time of slaughter and was graded high Good; the grass-finished steer weighed 914 lb. and was graded low Good.

The carcasses were chilled and aged at 37 ± 1° F. for 21 days after slaughter. The psoas major muscle and matched portions of the semitendinosus and gastrocnemius muscles were taken from the left side of each carcass. Each muscle or portion of muscle was ground and reground in a hand grinder with the finest blade. Each sample was mixed and packaged in heavy aluminum foil in amounts appropriate for later use. The packages were stored in a food freezer at -20° C. until the day before the meat was to be used, at which time they were transferred to a refrigerator for thawing.

Cooking

A pre-packaged sample weighing approximately 120 g. was quickly wrapped in pre-weighed aluminum foil and weighed on an analytical balance. For uniform shape, the meat sample was packed into a metal ring having a diameter of 8.4 cm. The patty was placed on a wire rack in a low Pyrex baking dish and cooked to an internal temperature of 75° C. (approximately 45 minutes) on the rotating hearth of a Despatch electric oven held at 350° F. After one hour of cooling, a wedge of approximately 15 g. was removed from the patty for determination of moisture. The wedge, shaped like a piece of pie, was taken in such a way as to avoid the hole left by the meat thermometer. Total lipids were extracted immediately from the remainder of the patty. The cooking procedure was carried out at two different times for each of the six meat samples.

Moisture Determinations

The wedge removed from the cooked patty was ground in a Waring Blendor for 30 seconds prior to sampling for moisture determination. Three portions of 3-5 g. each were placed in weighed moisture pans, covered, and weighed on an analytical balance. The pans were placed uncovered in a drying oven at 108 $\pm$ 1° C. for 16 hours. After replacement of the covers and removal from the oven, the pans were cooled for one hour in a desiccator and weighed. Moisture was expressed as percent of original weight. Along with each cooked sample a raw subsample was carried through the same procedure except that a drying period of 18 hours was used; preliminary work had shown 18 hours in the oven to be desirable to assure attainment of constant weight of raw samples.

Extraction Procedure

Lipid extractions of both the raw and cooked meat were carried out by the method of Ostrander and Dugan, Jr. (1961). A sample of approximately 50 g. was wrapped in a pre-weighed piece of aluminum foil and weighed on an analytical balance. The sample was transferred to a blender jar with the aid of a piece of facial tissue. A piece of dry ice approximately equivalent to a one-inch cube was added and the sample was blended with 130 ml. of absolute methanol for 5 minutes with the Adjust-A-Volt regulator set at 40. Sixty-five milliliters of chloroform were added and the mixture was blended for 5 minutes at a setting of 38. After the addition of 65 ml. of distilled water containing 1.5 g. zinc acetate, blending was continued for 10 seconds with the speed regulator set at 36.

The extraction mixture was poured into a No. 3 Büchner funnel and filtered through Whatman No. 1 filter paper with suction. A carbon dioxide blanket was kept on the residue through the filtering process by means of an inverted funnel connected to a separatory funnel which contained dry ice and water. The residue and the filter paper were transferred to the blender jar and the funnel was wiped with a piece of facial tissue. To the residue, facial tissue, and filter paper, 100 ml. of chloroform were added and the mixture was blended for 2½ minutes with the regulator set at 32. The mixture was transferred to the funnel in which a new Whatman No. 1 filter paper had been placed and again filtered. Fifty milliliters of chloroform were used for rinsing the blender jar and washing the solids.

The filtrate was transferred with a small amount of chloroform to a 500-ml. graduated cylinder and the volume of the chloroform layer was recorded. A 500-ml. separatory funnel was used to separate the lipid-containing chloroform extract from the methanol-water layer. The chloroform extract was collected in a 1000-ml. evaporating flask. After thorough mixing, two 10-ml. aliquots were taken and put into weighed 50-ml. beakers. 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 under reduced pressure in a vacuum desiccator. Percent lipid in each sample was calculated:

$$\% \text{ lipid} = \frac{\text{g. lipid in 10-ml. aliquot x ml. } CHCl_3 \text{ extract x 10}}{\text{g. of sample extracted}}$$

The solvent was evaporated from the remaining lipid extract on a flash evaporator. The extracted lipids were transferred with chloroform to a 50-ml. Erlenmeyer flask, after which the flask was flushed with nitrogen, stoppered, and stored at -20° C. until the sample was fractionated.

Fractionation of Extracted Lipids

Fractionation was carried out by the slurry method described by Murty et al. (1960) and by the column procedure of Hornstein et al. (1961). The amounts of extracted lipid were not sufficient for fractionation of each sample by each method. Therefore, the lipids from the duplicate samples were pooled and redivided for the two methods of fractionation. Each pooled sample was diluted with chloroform in a

100-ml. graduated cylinder to a volume that was adjusted so that 25 ml. contained 1-2 g. of lipid and so that at least 70 ml. of solution were available. After thorough mixing, a 25-ml. aliquot was removed for each of the two fractionation procedures. When a sample was not to be used immediately for fractionation it was stored under nitrogen in a stoppered 50-ml. Erlenmeyer flask at -20° C. Two 10-ml. aliquots were transferred from the remaining extract to weighed 50-ml. beakers, evaporated, and weighed for calculation of the weight of sample fractionated.

For each fractionation procedure 50 g. of silicic acid were used.* The silicic acid was activated by heating at 110° C. for 20 hours and was cooled for one hour in a desiccator prior to use. Reagent grade chloroform without any treatment was used. Reagent grade anhydrous methanol, dried on sodium sulfate and redistilled, was used for fractionation. All aliquots that were removed solely for obtaining weights were evaporated in air under the hood to apparent dryness and then evaporated to constant weight under reduced pressure in a vacuum desiccator. Samples dried in this way were discarded after the recording of their weight.

Slurry method. The 25-ml. sample aliquot was transferred quantitatively with 50 ml. of chloroform to a slurry of 50 g. silicic acid and 100 ml. of chloroform in a 500-ml. Erlenmeyer flask. A magnetic stirring bar was added and the slurry was mixed for 10 minutes on a magnetic stirrer. In this respect the procedure differed from that described by

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*Mallinckrodt AR 100 mesh.

Murty et al. (1960), who shook the slurry for 10 minutes. A nitrogen atmosphere over the mixture was provided by delivery of nitrogen through a funnel inverted over the sample flask.

The slurry was transferred quantitatively to a sintered glass funnel and filtered with suction. Five 50-ml. portions of chloroform were used for washing the silicic acid in the funnel. During each washing the suction was released and the silicic acid and chloroform were stirred.

The filtrate, containing the non-phosphorus lipids, was transferred quantitatively with chloroform to a 500-ml. graduated cylinder. The volume was recorded and two 25-ml. aliquots were transferred to weighed 50-ml. beakers. Solvent was evaporated from these subsamples and their weights were used for calculation of the weight of the non-phosphorus fraction:

$$\text{Total weight of fraction} = \frac{\text{weight in 25 ml. extract} \times \text{ml. } CHCl_3 \text{ extract}}{25}$$

A 10-ml. aliquot was removed from the remaining extract and put into a 15-ml. centrifuge tube. The tube was flushed with nitrogen, stoppered, covered with aluminum foil, and stored at -20° C. for later methylation.

The silicic acid mixture remaining in the funnel was transferred back to the 500-ml. flask with 100 ml. of methanol and stirred on the magnetic stirrer for 10 minutes. The slurry was filtered into a clean flask and 150 ml. of methanol in four portions were used for washing. When filtering was complete, the filtrate, containing the phospholipids, was transferred quantitatively with 50 ml. of methanol to a 500-ml.

graduated cylinder. The procedure of recording volume, removing aliquots, and calculating yield was the same as for the glyceride fraction except that before the 10-ml. portion was taken for methylation, the extract was concentrated on a flash evaporator to approximately 100 ml. This was necessary in order to obtain adequate samples for methylation. The yields of the phospholipid and glyceride fractions were calculated both as percent of lipid fractionated and as percent of lipid recovered.

Column method. Fifty grams of silicic acid were slurried with 100 ml. of chloroform-methanol (3:1) in a 250-ml. graduated cylinder and allowed to stand covered 30 minutes. Forty milliliters of the fine suspension were decanted and discarded. After the addition of 40 ml. of 3:1 chloroform-methanol mixture to the cylinder, the suspension was mixed well and poured into a 2.5 x 90-cm. chromatographic tube that was fitted with a sintered disc at the bottom. The stopcock below the disc was open during the preparation of the column and remained open except when collection flasks were changed. The sides of the tube were rinsed with additional solvent (3:1) and the air bubbles were removed by stirring the mixture with a long glass rod. The silicic acid was allowed to settle and slight nitrogen pressure was applied. When the solvent was about 10 cm. above the silicic acid-solvent interface, granular anhydrous sodium sulfate was added to give a layer approximately 2.5 cm. thick. When the solvent was only slightly above the sodium sulfate-solvent interface, washing was accomplished with 200 ml. of 20:1 chloroform-methanol followed by 300 ml. of chloroform. After these washings the column was

ready to use and was not permitted to go dry until fractionation had been completed.

When the chloroform wash barely covered the top of the column, the 25-ml. aliquot of known weight was transferred quantitatively to the column with the smallest possible amount of 20:1 chloroform-methanol. The sample was eluted with 300 ml. of 20:1 chloroform-methanol, followed by 200 ml. of 1:1 chloroform-methanol, and finally 300 ml. of methanol. Neutral lipids were collected in a 1000-ml. boiling flask. The receiver was changed when the second solvent front reached a level of 10 cm. above the bottom of the column. The second solvent front could be distinguished from the first because the column was translucent with the 20:1 chloroform-methanol mixture and became opaque with the 1:1 mixture. The last two eluates, containing phospholipids, were collected in a single 2000-ml. boiling flask. Flow rate was controlled at 2-4 ml./minute by application of nitrogen pressure at the top of the column.

The procedure described for the slurry method was used for quantitating the results.

Methylation

Prior to methylation, the 15-ml. centrifuge tubes containing the 10-ml. aliquots of sample were brought to room temperature and evaporated under a stream of nitrogen. To each tube 3 ml. of 1% sulfuric acid in methanol (v:v) were added and the tubes were placed in a water bath held at 70-75° C. for 4 hours. The contents of the tubes were stirred with glass rods at intervals during the heating. At the end

of the heating period the tubes were cooled and the contents of each were decanted into a 30-ml. separatory funnel. Six milliliters of petroleum ether and 2 ml. of distilled water were mixed with the sample. The bottom layer, containing water and sulfuric acid, was removed. Two additional washings with water in 2-ml. portions were carried out and each time the bottom layer was discarded. The stem of the separatory funnel was dried with a pipe cleaner and the petroleum ether layer, containing the methyl esters, was collected in a 15-ml. centrifuge tube containing 0.5 g. of sodium sulfate and 0.5 g. of silicic acid. The tube was flushed with nitrogen and stoppered. After standing overnight under refrigeration, the mixture was brought to room temperature and the petroleum ether extract was decanted into another 15-ml. centrifuge tube, gassed with nitrogen, stoppered, and stored at -20° C.

Gas-Liquid Chromatography

Fatty acid content of the lipid fractions was determined by gas chromatographic analysis of the methyl esters. The gas-liquid chromatograph used was a Barber Coleman, Model 61 C, equipped with an argon ionization detector with a radium source. The packed column consisted of 7 feet of coiled quarter-inch copper tubing containing 13% ethylene glycol succinate polyester on 80-100 mesh Gas Chrom P.* A column temperature of 175° C. was used and the following additional operating conditions were established:

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*Applied Science Laboratories, Inc.

Flash Heat Temperature	220° C.
Cell Temperature	216° C.
Split Temperature	219° C.
Cell Voltage	900
Attenuator Setting*	32
Sensitivity Setting	10
Gas Pressure	16 psi

At the time of analysis the methyl ester-petroleum ether solutions were brought to room temperature, evaporated under nitrogen and diluted with hexane.** Dilution of methyl esters and volume of solution used were adjusted as needed to produce chromatograms in which the greatest peak height was at least half-scale. Two chromatograms were obtained for each of the 48 methyl ester preparations. A standard mixture of fatty acid methyl esters was used for identification of the components.

In determining concentrations of the fatty acids present, the area under each peak was obtained by multiplying the height of the peak by the width at half-height. The area was corrected for change in the attenuation factor if necessary. The percentage of a specific fatty acid in a sample was calculated by the following equation:

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

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

**Matheson Coleman and Bell, Chromatoquality.

CHAPTER IV

RESULTS AND DISCUSSION

Percent lipid for both raw and cooked samples was found to be higher in the meat from the grain-finished animal than in that from the grass-finished animal, particularly in the psoas major and semitendinosus muscles (Table I). In the grain-finished animal the semitendinosus muscle had the highest percent lipid and the gastrocnemius muscle had the lowest percent lipid. In the grass-finished animal the psoas major muscle was highest in lipid concentration and the semitendinosus and gastrocnemius muscles were considerably lower and very similar in their lipid content. An apparent peculiarity of the semitendinosus sample from the grain-finished animal might have influenced the results. A rather large fat deposit was removed from the interior of the muscle prior to grinding. Although the visible fat deposit was removed, its presence might be an indication of an abnormality of this particular sample, which could have contributed to the large difference seen here between the lipid concentrations of the semitendinosus muscles of grain- and grass-finished beef. Such a possibility is suggested by a comparison of the results obtained here with those reported by Lowe and Kastelic (1961). The values compared are the lipid concentrations, dry weight basis, of raw samples. For the semitendinosus muscle, for which Lowe and Kastelic reported a value of 12.7%, in this study values of 32.5% and 10.6% were obtained for the grain- and grass-finished beef respectively. For the psoas major muscle, for which the value reported by Lowe and Kastelic

TABLE I

PERCENT LIPID EXTRACTED FROM THREE MUSCLES OF GRAIN- AND GRASS-FINISHED BEEF[a]

Muscle	Raw Samples				Cooked Samples			
	Wet Weight Basis		Dry Weight Basis[b]		Wet Weight Basis		Dry Weight Basis[b]	
	Grain	Grass	Grain	Grass	Grain	Grass	Grain	Grass
Psoas Major	5.96	4.60	22.00	17.74	9.56	7.56	22.37	17.80
Semitendinosus	9.95	2.68	32.54	10.61	14.68	4.26	30.69	10.11
Gastrocnemius	3.18	2.74	12.43	11.02	4.90	4.60	12.28	11.26
Average	6.36	3.34	22.32	13.12	9.71	5.47	21.78	13.06

[a]Each value is an average for two aliquots from each of two extractions.

[b]Based on moisture values presented in Table V, Appendix.

was 19.5%, the results here were 22.0% and 17.7% for the grain- and grass-finished beef respectively. Lowe and Kastelic did not work with the gastrocnemius muscle and they do not specify the method of finishing their animals. Judging by the above comparisons, the lipid concentration of the semitendinosus muscle from the grain-finished animal of this study would seem to be abnormally high. Study of samples from additional animals would be desirable.

In the lipid fractionations, the average percent recovery was 100.9 for the column method and 93.1 for the slurry method. A comparison of percent phospholipids in extracted lipid fractionated by the two methods is shown in Table II. Values calculated as percent of fat fractionated differed very little from those calculated as percent of fat recovered and only the results based on the fat recovered are presented here; those based on the fat fractionated are included in Table VI of the Appendix.

A greater percentage of phospholipids, along with a correspondingly lower percentage of glycerides, was found with the slurry method than with the column method of fractionation. The overall averages for percentage of phospholipids in the extracted lipid were 16.7 for the column method and 28.4 for the slurry method. From the evidence presented thus far, it would seem that the glycerides obtained from the column were contaminated with phospholipids and/or the phospholipids obtained from the slurry were contaminated with glycerides.

The apparent effect of finish differed for the two methods. For the column method of fractionation, the fat of grass-finished beef had a higher percentage of phospholipids than did that of grain-finished

TABLE II

PERCENT PHOSPHOLIPIDS IN LIPID EXTRACTED FROM THREE BEEF MUSCLES AND FRACTIONATED BY TWO METHODS[a]

Muscles	Raw			Cooked		
	Grain	Grass	Average	Grain	Grass	Average
Column method						
Psoas major	11.67	14.37	13.02	12.13	14.79	13.46
Semitendinosus	6.63	21.79	14.21	7.75	25.39	16.57
Gastrocnemius	20.65	22.90	21.78	20.36	21.98	21.17
Average	12.98	19.69		13.41	20.72	
Slurry method						
Psoas major	22.40	17.75	20.08	21.90	30.03	25.96
Semitendinosus	23.76	24.50	24.13	26.17	36.11	31.14
Gastrocnemius	32.78	33.46	33.12	38.11	33.48	35.80
Average	26.31	25.24		28.73	33.21	

[a]Calculated as percent of fat recovered.

beef, averaging 20.2% as compared with 13.2%. Percentages of phospholipids obtained by the slurry method did not show consistent differences between lipids of grain- and grass-finished beef. Nutt (1963) and Cook (1963), both of whom used the slurry method, reported the same tendency as was found with the column method in this study.

Neither fractionation method showed a consistent difference between raw and cooked samples with respect to phospholipid concentration of their fat.

Although the small number of samples makes generalization difficult, comparison of phospholipid concentration of the fat extracted from different muscles is interesting because of the rather large amount of evidence relating phospholipid content to activity (Bloor, 1927; Bloor and Snider, 1934; Snider, 1936). When phospholipid percentages are combined for all treatments for each muscle, the averages show some tendency for phospholipid concentration of the fat to vary among the muscles. The fat of psoas major muscle, which is generally assumed to be exercised the least of the three muscles used in this study, had the lowest percentage of phospholipids, averaging 18.1%. The fat of semitendinosus muscle averaged 21.5% phospholipid and that of gastrocnemius averaged 28.0% phospholipid. When the phospholipid percentages for the psoas major, semitendinosus, and gastrocnemius muscles are considered for each method they average 13.2, 15.4, and 21.5 respectively for the column method and 23.0, 27.6, and 34.5 for the slurry method. A similar comparison of the phospholipid values for the psoas major, semitendinosus,

and gastrocnemius muscles on the basis of finish gives averages of 17.0, 16.1, and 28.0% respectively for the grain finish and 19.2, 26.9, and 28.0% for the grass finish. The muscle phospholipid comparisons are influenced by the peculiar values obtained by the column method for both raw and cooked semitendinosus muscle of the grain-finished animal. It will be remembered that this was the piece from which the peculiar deposit of fat was removed and that it also seemed to yield an unexpectedly large amount of extracted lipids. Depot fat tends to be lower in phospholipid concentration than does muscle fat (Deuel, 1957; Ostrander and Dugan, Jr., 1962) and it seems possible that the piece of muscle included additional amounts of the depot fat in a less readily separable form than that which was removed prior to grinding. The fact that the fractions obtained by the slurry method did not show this peculiarity could represent a difference between the two methods in efficiency of separation.

As a means of comparing the two fractionation methods with respect to completeness of separation, eight of the fractions were selected for study by thin-layer chromatography. The eight fractions comprised four phospholipid samples and the corresponding glycerides obtained by the two methods from the raw semitendinosus and gastrocnemius muscles of the grain-finished animal. Selection of these samples was made on the basis of their including both a very low and a very high yield of phospholipids, as well as providing a basis for comparison of the fractionation methods. The techniques used were those described by Nutt

(1963). Silica gel G* was used as the adsorbant and a solvent system suggested by Vogel et al. (1962) was used for development. The solvent mixture contained chloroform-methanol-water, 80:25:3 (v/v). Although no attempt was made to elute the spots and quantitate the results of the thin-layer chromatography, approximately 280 μg. of each sample were applied to a thin-layer plate. It was evident after development of the plates that the glycerides were not in any case contaminated by phospholipids, regardless of method of fractionation. Glycerides were found in the phospholipid fraction from both samples obtained by the slurry method. The phospholipids obtained by the column method, on the other hand, showed no glycerides. It is apparent, therefore, that the high phospholipid percentages found by the slurry method are due to contamination with glycerides. It is apparent, also, that the very low values obtained by the column method for the phospholipid content of the lipid from the semitendinosus muscle of the grain-finished beef were not due to loss of phospholipids into the glycerides. Whether the two methods differed in their relative effectiveness because of some condition(s) peculiar to this study is not known. As was mentioned previously, the slurries were stirred rather than shaken. If the slurry method were to be used again in this laboratory, some preliminary study of the methodology would be desirable.

One further comparison with respect to the phospholipids is of interest. When the values are expressed as percent of the raw muscle,

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*Brinkmann Instruments, Inc.

dry weight basis, the psoas major and gastrocnemius muscles from animals of both finishes are found to have had phospholipid concentrations falling within the range of 2.54-2.58%. In view of the information presented previously, it is not surprising that the corresponding value for the semitendinosus muscle of the grain-finished animal is 2.16%. The fact that the raw semitendinosus muscle of the grass-finished animal had a phospholipid content of 2.30% on a dry weight basis clouds the picture somewhat and re-emphasizes the desirability of studying a larger number of samples. The apparent tendency for the phospholipid content to vary in terms of percent of fat but not in terms of dry tissue is in agreement with the work of Callow (1962).

A comparison of the fatty acids of the glyceride fraction is seen in Table III. Because there was no consistent difference found in individual fatty acid percentages or in total saturates and unsaturates between glycerides of raw and cooked samples, the values for raw and cooked samples have been averaged. The similarity of fatty acid composition for glycerides obtained by the two methods could be expected because the samples used for the two methods were from the same source and the thin-layer chromatography did not show contamination of the glycerides obtained by either method. Linoleate was found in more glyceride samples obtained by the column method than in those obtained by the slurry method but the amounts were so small in the glyceride fraction that the linoleate may not always have been detected.

Glycerides from grain- and grass-finished beef tended to differ somewhat in their relative proportions of the major fatty acid components.

TABLE III

FATTY ACID COMPOSITION OF GLYCERIDES OBTAINED BY TWO METHODS OF FRACTIONATION[a]

Method, Finish and Muscle	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	Total Saturates	Total Monoenes
Column method								
Grain								
Psoas major	3.6	47.2	0.6	14.8	33.8	--	65.6	34.4
Semitendinosus	2.2	44.0	1.4	7.6	44.5	0.2	53.8	45.9
Gastrocnemius	2.8	45.0	0.8	9.2	41.9	0.4	57.0	42.7
Average	2.9	45.4	0.9	10.5	40.1	0.2	58.8	41.0
Grass								
Psoas major	2.5	40.2	0.6	18.7	37.9	0.1	61.4	38.5
Semitendinosus	1.8	41.0	0.7	11.8	44.9	--	54.6	45.6
Gastrocnemius	2.7	37.6	1.1	9.7	48.3	0.3	50.0	49.4
Average	2.3	39.6	0.8	13.4	43.7	0.1	55.3	44.5
Slurry method								
Grain								
Psoas major	7.6	49.5	1.6	10.3	31.2	--	67.4	32.8
Semitendinosus	2.1	43.7	1.2	8.4	44.7	--	54.2	45.9
Gastrocnemius	2.7	43.1	1.0	10.5	42.6	--	56.3	43.6
Average	4.1	45.4	1.3	9.7	39.5	--	59.3	40.8
Grass								
Psoas major	2.6	40.2	0.8	19.4	36.9	--	62.2	37.7
Semitendinosus	2.6	37.6	1.4	10.9	47.4	0.1	51.1	48.8
Gastrocnemius	3.0	37.2	1.4	11.6	46.8	--	51.8	48.2
Average	2.7	38.3	1.2	14.0	43.7	--	55.0	44.9

[a]Percent of total fatty acids.

Each value is an average for two chromatograms from a raw sample and two chromatograms from a cooked sample.

Concentrations of palmitate, stearate, and oleate averaged 45.4%, 10.1%, and 39.8% respectively for the grain-finished beef, as compared with 39.0%, 13.7%, and 43.7% for the grass-finished beef. These differences, though not large, were quite consistent.

In the glycerides obtained by both methods, the fat of the psoas major muscle tended to be more saturated than did that from the other two muscles. This overall difference largely reflects differences in relative proportions of stearate and oleate. Psoas major glycerides averaged 15.8% stearate and 35.0% oleate, as compared with corresponding percentages of 9.7 and 45.4 for semitendinosus and 10.2 and 44.9 for gastrocnemius.

In Table IV are shown the fatty acid values for the phospholipids from the two fractionation methods. Cooking was found to have no effect on the fatty acid composition of the phospholipid fraction; therefore, in Table IV values for raw and cooked samples are averaged. The only striking and consistent differences are in the concentrations of oleate and linoleate. The phospholipids obtained by the column method were lower in oleate and higher in linoleate than were those obtained by the slurry method. Two reasons might be advanced for considering these results to be further evidence that the slurry method, as used in this study, separated the fractions less effectively than did the column method: (1) With respect to their content of oleate and linoleate, the phospholipids obtained by the slurry method were more similar to the glycerides than to the column phospholipids, and (2) the fatty acid

TABLE IV

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS OBTAINED BY TWO METHODS OF FRACTIONATION[a]

Method, Finish and Muscle	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	Total Saturates	Total Monoenes
Column method								
Grain								
Psoas major	1.4	46.5	tr	18.0	25.6	8.6	65.9	25.6
Semitendinosus	0.7	44.8	0.2	16.1	31.5	6.8	61.6	31.7
Gastrocnemius	1.1	41.0	0.2	15.2	30.0	12.6	57.3	30.2
Average	1.1	44.1	0.1	16.4	29.0	9.3	61.6	29.2
Grass								
Psoas major	2.2	41.4	0.6	16.4	25.4	14.0	60.0	26.0
Semitendinosus	3.0	45.6	0.7	9.5	25.5	16.0	58.1	26.2
Gastrocnemius	2.9	40.7	1.6	18.0	26.1	15.6	61.6	27.7
Average	2.7	42.6	1.0	14.6	25.7	15.2	59.9	26.6
Slurry method								
Grain								
Psoas major	3.1	44.8	0.9	11.7	35.3	4.3	59.6	36.2
Semitendinosus	2.8	41.2	1.8	7.7	45.8	0.6	51.7	47.6
Gastrocnemius	2.4	39.3	0.8	13.8	40.8	2.8	55.5	41.6
Average	2.8	41.8	1.2	11.1	40.6	2.6	55.6	41.8
Grass								
Psoas major	2.1	37.7	0.8	15.7	38.8	5.0	55.5	39.5
Semitendinosus	2.0	40.9	1.2	15.4	36.4	4.4	58.3	37.6
Gastrocnemius	1.6	36.5	1.1	12.0	45.4	4.4	50.1	46.6
Average	1.9	38.4	1.0	14.4	40.2	4.6	54.6	41.2

[a]Percent of total fatty acids.

Each value is an average for two chromatograms from a raw sample and two chromatograms from a cooked sample.

values for the phospholipids obtained by the column method in this study more nearly resemble those reported by workers in other laboratories (Hornstein et al., 1961; Ostrander and Dugan, Jr., 1962) for phospholipids than did those obtained by the slurry method.

Traces of linolenate and arachidonate, too small to measure, were present in the phospholipids from the column but neither was observed in the phospholipids from the slurry method.

Nutt (1963) found a higher degree of saturation in phospholipids from grain-finished beef than in those from grass-finished beef. The only consistent effect of finish found in this study was the lower concentration of linoleate in the phospholipids from the grain-finished animal than in those from the grass-finished animal. This was true for both methods. The phospholipids from the column method gave average linoleate percentages of 9.3 and 15.2 for grain- and grass-finished beef respectively, while corresponding averages for the slurry method were 2.6% and 4.6%.

Fatty acid composition of the phospholipids did not vary consistently with the muscle. Snider (1936) found fatty acid content to be similar in spite of differences in phospholipid concentration of muscle from different animals, exercised and unexercised.

CHAPTER V

SUMMARY

A comparison was made of two methods of fractionating muscle lipids of beef. Lipids were extracted from both raw and cooked samples of the psoas major, semitendinosus, and gastrocnemius muscles from grain- and grass-finished beef aged 21 days. The phospholipids and glycerides obtained by each method of fractionation were analyzed for fatty acid content by gas-liquid chromatography.

Under the conditions used in this study, the column method gave a better total recovery than did the slurry method. A difference in relative effectiveness of the two methods is indicated by the fact that the slurry method gave a higher percentage of phospholipids and lower percentage of glycerides than did the column method. Thin-layer chromatography indicated that the phospholipids from the slurry were contaminated with glycerides. The fatty acid composition of the fractions reflected the differences in fractionation. Glycerides obtained by the two methods were similar in their fatty acid content, whereas the phospholipids from the column had a higher percentage of linoleate and lower percentage of oleate than did those from the slurry.

No consistent effect of finish was found in fractions from the slurry method, while in fractions from the column method phospholipids constituted a lower proportion of the fat from the grain-finished animal than of the fat from the grass-finished animal. The glycerides from the

grain-finished beef were higher in palmitate and lower in oleate than were those from the grass-finished beef; the phospholipids from the grain-finished animal were lower in linoleate than were those from the grass-finished animal.

Phospholipid content, expressed as percent of the extracted fat, was lowest in the psoas major muscle, which is assumed to be the least active of those studied. In two of the muscles, both for the grain finish and for the grass finish, phospholipid concentrations were almost identical when calculated as percent of the muscle tissue, dry weight basis. The fatty acid composition of the phospholipids did not vary with the muscle.

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APPENDIX

TABLE V

PERCENT MOISTURE IN GRAIN- AND GRASS-FINISHED BEEF SAMPLES, RAW AND COOKED[a]

Muscle	Raw		Cooked	
	Grain	Grass	Grain	Grass
Psoas Major	72.88	74.10	57.26	57.52
Semitendinosus	69.44	74.81	52.15	57.86
Gastrocnemius	74.46	75.22	60.14	59.18

[a]Each value is an average of two determinations in triplicate.

TABLE VI

PERCENT PHOSPHOLIPID IN LIPID EXTRACTED FROM THREE BEEF MUSCLES AND FRACTIONATED BY TWO METHODS[a]

Muscles	Raw			Cooked		
	Grain	Grass	Average	Grain	Grass	Average
Column method						
Psoas major	11.54	14.83	13.18	12.20	15.03	13.62
Semitendinosus	6.69	22.04	14.36	7.97	25.91	16.94
Gastrocnemius	20.69	23.69	22.19	20.12	21.29	20.70
Average	12.97	20.19		13.43	20.74	
Slurry method						
Psoas major	21.36	18.26	19.81	20.76	27.74	24.25
Semitendinosus	24.03	20.18	22.10	25.05	31.19	28.12
Gastrocnemius	30.57	30.27	30.42	35.25	28.90	32.08
Average	25.32	22.90		27.02	29.28	

[a]Calculated as percent of fat fractionated.