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To the Graduate Council:

I am submitting herewith a thesis written by Carolyn Jean Lackey entitled "Phospholipid Content of Beef Biceps Femoris as Affected by Cooking." 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 Food Science.

Ada Marie Campbell
Major Professor

We have read this thesis
and recommend its acceptance:

Mary G. Bass

J. F. Collins

Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

PHOSPHOLIPID CONTENT OF BEEF BICEPS FEMORIS AS AFFECTED BY COOKING

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

Carolyn Jean Lackey

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ABSTRACT

Differences in phospholipid class distribution in raw and cooked meat and drip have been of interest to researchers at The University of Tennessee, Knoxville. The present research was performed to determine if such differences result from partitioning.

Drip lipid was found to have significantly higher cephalin content and lower lecithin content than raw or cooked biceps femoris muscle lipid. Correspondingly, cooked muscle lipid had significantly lower cephalin and higher lecithin content than raw muscle or drip lipids.

Evidence of partitioning was to be obtained through data conversion from percentage basis to absolute amounts. However, several developments possibly related to experimental procedure and/or heat induced complexes and interactions made such calculations ineffectual. More total lipid and phospholipid were present in the lipid extract of cooked muscle than were present in the raw muscle lipid extract. Therefore, conclusive evidence of partitioning could not be given. Suggestions for further study are made.

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CHAPTER I

INTRODUCTION

The development of formulated foods requires further knowledge of the role of food constituents in chemical reactions and interactions. Some food constituents that have received little research attention may have important functions in developing the sensory qualities that determine consumer acceptance of a food. One such group of food components may be the phospholipids of muscle tissue. Phospholipids are a fraction of muscle lipids that account for less than one perçent (0.5-1.0%) of the muscle tissue. Most phospholipids are diacyl esters of glycerol esterified at one terminal carbon to phosphoric acid. The phosphoric acid also is esterified either to a nitrogenous base, most commonly choline, ethanolamine or serine, or to inositol. Another phospholipid is sphingomyelin, a fatty acid amide of the base sphingosine esterified to phosphorylcholine (Price and Schweigert, 1971).

Research on phospholipids has been confined, for the most part, to their chemistry, metabolism and function in the living organism. The effect of processing, storing and cooking on all food components, including phospholipids, is of concern to the food industry, as well as to the consumer.

Muscle tissue generally receives heat treatment at some point in industrial processing or during preparation by con-

sumers. It is important then to expand the body of knowledge of heat effects on meat phospholipids.

Early investigators found that changes in phospholipids of food might occur during processing and storing. Lea (1957) reported autoxidation of phospholipids. Unsaturated fatty acids of phospholipid molecules were susceptible to atmospheric oxidation. Objectionable degradative products were produced. Results of studies by Younathan and Watts (1960) and Hornstein et al. (1961) suggested that the phospholipid fraction played a role in development of off odors and flavors in stored cooked meat tissue.

Traditional meat cookery methods accelerate the oxidation of meat lipids (Terrell et al., 1968). Research at the University of Tennessee, Knoxville focused on changes of phospholipid classes during heat treatment. Campbell and Turkki's (1967) data indicated higher percentages of phospholipids in cooked than in raw meat, either on a percent total lipid or meat basis. Roberts (1966) reported higher phospholipid percentages in cooked meat than raw meat when expressed as percent muscle, wet weight basis. The drip lipids from cooked meat had low concentrations of phospholipid in both studies. Lower loss of phospholipid than of neutral lipid may be explained by differences in the nature of phospholipids and neutral lipids. Neutral lipids are mostly intercellular whereas phospholipids are integral parts of the cell. Often phospholipids appear attached to

tissue proteins or carbohydrates by some unknown type of bonding (Dawson, 1957; Lea, 1957; Price and Schweigert, 1971). Such bonding may reduce susceptibility to phospholipid loss in the drip during cooking.

Results of investigations involving infrared analysis (Campbell and Turkki, 1967) and thin-layer chromatography (Roberts, 1966) suggested a partitioning among the three major phospholipid classes. Both the raw meat and the drip lipid had higher cephalin concentrations than the cooked meat lipid. However, neither investigation provided conclusive evidence of partitioning.

Therefore, the purpose of the present study was to investigate possible partitioning and/or degree of degradative changes affecting the three major phospholipid classes, lecithin, cephalin and sphingomyelin. Such information should provide explanation for differences in relative proportions of phospholipid classes in raw and cooked meat and drip.

CHAPTER II

REVIEW OF LITERATURE

Although indications of the occurrence of complex fatty compounds were reported by Fourcroy in 1793, the field of phospholipid research is still in its infancy. In 1812, Vauquelin succeeded in isolating phosphorus-containing fats from brain (MacLean and MacLean, 1927; Deuel, 1951). Thudicum is credited with making some early advances in the field of phospholipid research. He isolated and analyzed large numbers of lipid preparations from animal tissues (Ansell and Hawthorne, 1964). By the late nineteenth century phosphatidylethanolamine and sphingomyelin were fairly well characterized chemically and the chemical constituents of phosphatidylcholine were known. Most phospholipid research still involves isolation, chemical characterization and identification of biochemical functions.

In recent years improved analytical methods aided researchers in obtaining more information about structure and metabolism of phospholipids. No methods of extraction and/or fractionation prove entirely acceptable for study of phospholipids (Ansell and Hawthorne, 1964; Marinetti, 1962; Lovern, 1957). Extraction procedures are complicated by several factors. No known methods extract phospholipids selectively. The presence of neutral lipids must be noted and non-lipid contaminants must be removed from the extrac-

tant (Ansell and Hawthorne, 1964). Some lipid-protein and lipid-carbohydrate complexes are insoluble in fat solvents. Other lipids exhibit selective solubility in a limited range of fat solvents. Certain non-lipid constituents are soluble in some of the fat solvents used for extraction (Lovern, 1957). No one method is universally used in phospholipid research, thus complicating a study of heat treatment on meat phospholipids.

Very little information about cooking studies in relation to meat phospholipids is available. A brief review of other phospholipid research may lend an insight into the stated problem.

Tissue variation in phospholipid levels has been linked with physiological activity and degree of exercise of the tissue. More recently the quantity of tissue membranes has been related to phospholipid level variation.

As early as 1913, Mayer and Schaeffer suggested that phospholipid concentration was related to physiological activity. Among animals of the same species, phospholipid content was relatively constant for a given organ. When organs were ranked for phospholipid content, the order was the same for all species examined. Bloor and associates reported higher concentrations of phospholipids in tissues with greater metabolic activity, whether due to normal tissue function or to tissue hypertrophy as a result of increased exercise. Higher phospholipid levels were observed in some muscles of

wild animals than in laboratory animals of the same species. This relationship could be related to the increase in mitochondria in response to higher rates of metabolism in those muscles of wild animals (Bloor, 1927; Bloor and Snider, 1930; Bloor et al., 1930; Bloor, 1937). Kaucher et al. (1943) concluded that tissues with greater variety of physiological activity, i.e. brain and liver, had higher concentrations of phospholipids. Hanahan and Thompson (1963) stated that cells with prolonged activity, those with large numbers of mitochondria, have relatively high phospholipid levels.

Phospholipid levels in relation to exercise have been studied (Bloor, 1940). Shchesno (1965) experimented with forced exercise of red and white limb muscles of rabbits. The average phospholipid level of the exercised red muscles was not significantly different from that of control muscles. Results of measuring phospholipid levels of exercised white muscles indicated that half of the animals had considerably increased phospholipid levels while the others had small increases or even decreases in phospholipid levels. Masoro et al. (1964) performed similar experiments on soleus muscles of a monkey. Comparisons between exercised and non-exercised muscles gave large differences in some pairs and no differences in others. Therefore existing data do not provide conclusive evidence that exercise is directly related to higher phospholipid levels in tissues. A factor that complicates interpretation of the data is the frequency with

which phospholipid concentration has been expressed as percent of total lipid. Results expressed in this way may indicate a difference in phospholipid concentration when the tissue concentration actually does not differ between two samples.

More recent research shows organ phospholipids to be present almost exclusively as major components of the cell surface and subcellular particulate membranes. Each specific membrane appears to have a characteristic phospholipid class distribution with no significant species variation. Thus variation in phospholipid composition of an organ is determined by relative abundance of membrane types (Fleischer and Rouser, 1965; Simon and Rouser, 1969; Rouser et al., 1969).

The constant nature of phospholipids has been studied. In contrast to variable neutral lipid content of muscle tissues, phospholipid concentration is relatively constant for a given tissue. Bloor and Snider (1930) found that specific tissues have a characteristic constant phospholipid level and phospholipid composition. Any variation in phospholipid levels for the same muscle in different animals was thought to be caused by natural differences in activity of the animals (Bloor, 1927). Recent research again gave evidence to support the constant nature of phospholipid concentration in raw beef muscle (Link et al., 1967; Hornstein and Crowe, 1967). This constant nature of phospholipid does not in-

dicate static state. Phospholipids within individual animal tissues are in a state of dynamic equilibrium. They are continually broken down and resynthesized (Dawson, 1957).

Although the constancy discussed above is observed at a given level of total lipid, the phospholipid content of animal tissues has been shown to vary with total lipid present. Research results indicate that as the concentration of total lipid increases, the concentration of phospholipid, expressed as percent of total lipid, decreases (Callow, 1962; Taylor, 1964; Turkki, 1965; Campbell and Turkki, 1967).

The phospholipid content of animal tissues has also been studied with respect to variation caused by species, age, sex, weight and diet. Indications are that phospholipid concentration is relatively independent of species (Mayer and Schaeffer, 1913; Bloor and Snider, 1934; Gray and MacFarlane, 1961). Simon and Rouser's (1969) data show that among vertebrates, there is little or no species variability in phospholipid class distribution in muscle cell surface and subcellular particulate membranes. Link et al. (1967) reported that in preliminary work total extractable intramuscular lipid increased as animals increased in age and weight. The neutral lipid fraction increased while phospholipids remained constant on per gram muscle basis. Hornstein and Crowe (1967) found that variation in age and diet of Angus steers produced little change in phospholipid content of given muscles. In a study of porcine muscle lipids

of animals varying in weight and sex, Allen et al. (1967) found differences in fatty acid composition only in the neutral lipids. Fatty acid composition of phospholipids remained essentially constant. Terrell et al. (1968) studying bovine longissimus dorsi also found the effect of sex totally associated with neutral fatty acids with no effect upon phospholipid fatty acid composition.

Phospholipids have been compared to neutral lipids in fatty acid composition. Research has shown that phospholipids contain greater amounts of unsaturated fatty acids than neutral lipids in beef, veal, pork and lamb tissues (Ostrander and Dugan, 1962; Giam and Dugan, 1965). Hornstein et al. (1961) stated that unsaturated fatty acids containing two or more double bonds make up fifty percent of the total fatty acids. Campbell and Turkki (1967) found phospholipid fatty acids of beef and pork considerably more unsaturated than neutral lipid fatty acids. Terrell and Bray (1969) found larger quantities of C18:2, C18:3 and C20:4 fatty acids in phospholipids than in neutral lipids from three bovine muscles.

Fatty acids in various classes of phospholipids have been shown to vary in degree of unsaturation. Bloor (1927) measured higher cephalin fatty acid unsaturation in beef heart muscle phospholipids than in lecithin. Hornstein et al. (1961) reported higher linoleate and lower arachidonate levels in raw beef and pork lecithin and sphingomyelin

classes than in cephalin. Cephalins had more total unsaturation than the combined sphingomyelin-lecithin classes. Kuchmak and Dugan (1965) also found greater unsaturation in cephalin than in lecithin fatty acids in pork muscle phospholipids.

The amount of phospholipid present in raw muscle tissues has been reported. Only those studies reporting phospholipid levels on a wet weight percent meat basis will be cited. Hornstein et al. (1961) measured phospholipid contents ranging from 0.8 to 1.0 percent in beef muscles and 0.7-0.9 percent in pork muscle. Slightly lower values for pork were reported by Kuchmak and Dugan (1963), 0.46-0.58 percent. Taylor's (1964) phospholipid values for three muscles of grass and grain finished beef ranged from 0.58 to 0.69 percent. Turkki (1965) reported comparable values, 0.52-0.72 percent for two beef muscles. A range of 0.5-0.7 percent phospholipid content for raw beef muscles was reported by Roberts (1966). Campbell and Turkki (1967) recorded phospholipid values of 0.56 and 0.61 percent for raw ground beef.

The proportions of phospholipid classes in raw muscle tissues have been investigated. Hornstein et al. (1961) reported that the phospholipids of beef and pork lipids consist of 40-45, 40-45, and 10-15 percent lecithin, cephalins and sphingomyelin, respectively. Kuchmak and Dugan (1963) found pork phospholipids distributed as 61 percent lecithin,

36 percent cephalins and 3 percent sphingomyelin. Similar values were obtained in beef studies by Turkki (1965), 62 percent lecithin, 30 percent cephalin and 8 percent sphingomyelin. Roberts (1966) reported 60 percent lecithin, 33 percent cephalin and 7 percent sphingomyelin.

Few investigations of cooking effects on total phospholipid concentration and class distribution have been reported. Such studies might link phospholipids with flavor deterioration in cooked meats. Younathan and Watts (1960) in their study of refrigerated (7 day) cooked pork, reported more rapid autoxidation in cooked meat than in raw meat. More intense oxidation reactions were found in the phospholipid fraction than in neutral lipid. However, the effect cannot be attributed totally to cooking since the cooked meat was stored prior to analysis. Research on the phospholipid fraction could further define quality changes during cooking and storing of meat.

Cooking studies on total lipids have been reported. Paul (1965) stated that cooked muscle tissue has been reported to be higher in ether extractable material than raw muscle tissue. In an earlier study of low grade beef, Hood et al. (1955) found a lower fat content in cooked meat than in raw meat. Higher average fat content of cooked biceps femoris muscle as compared with raw muscle was found by Lowe and Kastelic (1961). Ritchey (1965) reported little change in percent fat of raw and cooked beef muscle

on a dry weight basis. However, when expressed on a wet weight basis cooked meat had a higher fat content. Researchers at the University of Tennessee, Knoxville also reported fairly constant lipid percents of raw and cooked meat expressed on a dry weight basis. Again when expressed on a wet weight basis, cooked meat contained higher total lipid concentrations (Nutt, 1963; Taylor, 1964; Roberts, 1966).

Only a few studies of changes in phospholipid content in cooked meat have been reported. Taylor (1964) and Roberts (1966) measured higher phospholipid contents in cooked meat when expressed as percent muscle, wet weight basis. However, if the same data were expressed as percent total lipid or percent muscle, dry weight basis, no consistent change attributable to cooking was found.

The cooking effects on relative proportions of phospholipid classes have been studied even less. Campbell and Turkki's (1967) evidence from infrared analysis indicated that drip phospholipid contained higher concentrations of cephalins than did raw or cooked meat phospholipids. Relatively high drip cephalin contents also were reported by Roberts (1966).

Studies reporting changes in fatty acids of cooked meat lipids have been reported. Chang and Watts (1952) found an increase in free fatty acids with cooking, drip having the most. They reported only a small loss of polyunsaturated fatty acids in the cooked meat. Cooking was shown to pro-

duce slight loss of arachidonic and docosahexaenoic acids in the total lipids of variety meats giving no significant total change in fatty acids (Siedler et al., 1964). Terrell et al. (1968) analyzed phospholipid fatty acids of cooked bovine longissimus dorsi. Percent of C8 was significantly greater in broiled meat than raw meat. Broiled meat contained lower levels of C14 and C15 than raw meat. Testing for changes in fatty acid distributions in several phospholipid classes, Roberts (1966) found cooking to result in loss of linoleic acid from cephalins. No other differences in fatty acid distributions were recorded. Campbell and Turkki (1967) reported no significant differences in phospholipid fatty acids of raw and cooked meat. Drip phospholipids were observed to be more saturated than phospholipids of raw or cooked meat.

CHAPTER III

PROCEDURES

Selection and Preparation of Meat Samples

Nine coded beef biceps femoris muscles were obtained from the Animal Science Department of the University of Tennessee, Knoxville. Animals with known histories were slaughtered when they reached a specific back fat thickness rather than specific age. Selection of six muscles was based on similar phospholipid concentrations (non-fat basis) determined in preliminary analyses. The muscles were from Charolais-Hereford Crossbreeds: three were three-quarters Charolais and one-quarter Hereford, two were half Charolais and half Hereford and one was one-quarter Charolais and three-quarters Hereford. All were heifers. Obvious external and intermuscular fat were removed from the muscles. Muscles were ground in the meat grinder attachment of a Hobart N-50 mixer. Discs with 3/8 and 3/16 inch openings were used once each and a disc with 1/8 inch openings was used for the two final grindings. An attempt was made to keep the meat temperature below 12^{o}C by setting meat containers in crushed ice and cooling the grinder with dry ice.

Two 1100 gram samples of each ground muscle were wrapped in double medium weight aluminum foil. Gaseous nitrogen was used for flushing samples during closure. Samples were stored at -20^{o}C.

Cooking

Frozen meat samples were thawed in the refrigerator (4°C) beginning 48 hours prior to analysis. Meat from each muscle was shaped into sixteen 125 gram patties with an 8.4 cm diameter metal ring. Patties, placed on a wire rack in a shallow pan were cooked to an internal temperature of 77°C in a Despatch rotating hearth oven (177°C). Cooked patties were cooled one hour. Weights of raw and cooked meat and drip were recorded. Four randomly selected patties were combined for analysis.

Moisture Determination

Moisture and dry matter were determined for the raw and cooked muscle and drip. Triplicate 3-5 gram samples of each, in weighed aluminum moisture pans, were dried 18 hours at 100°C in an air oven. Samples were covered, cooled one hour in a desiccator and reweighed.

$$\text{percent dry matter} = 100 \left(\frac{\text{dry weight of sample}}{\text{wet weight of sample}} \right)$$

Lipid Extraction

Lipids were extracted from 50 gram samples of the raw and cooked muscle and from the total drip for each muscle. The modified Bligh and Dyer method (Ostrander and Dugan, 1961) slightly altered by Turkki (1965) was used (Appendix, p. 40). After the extraction mixture in the graduated cylinder separated into layers, the volume of the lower layer,

and blanks. A Bausch and Lomb Spectronic 20 spectrophotometer, with a red filter and red sensitive phototube was used at a wavelength of 830 nm. For determination of phospholipid concentration as percent of total lipid, one-ml samples of concentrated extract were removed at the time of sampling for phosphorus analysis. These samples were dried and weighed by the procedures for determining total lipid. Procedure for determining percent phospholipid in extracted lipid is presented in the Appendix, p. 43.

Thin-Layer Chromatography (TLC)

Thin-layer chromatography separated phospholipids from neutral lipids and fractionated them into major classes. TLC plate preparation procedures appear in the Appendix, p. 44 (Wood and Kinsell, 1963).

Plates spotted with 10 µl of raw and cooked extract and 25 µl of drip extract were placed in a developing chamber saturated with chloroform-methanol-water solvent mixture in a ratio of 80:35:5 (v:v:v). The drip extract was further concentrated to 1/5 volume for this procedure. Application was made with a microliter syringe. Plates were developed approximately one hour, sprayed with 50% (v:v) sulfuric acid solution and heated 25 minutes at 180°C. Standard solutions of lecithin, cephalin and sphingomyelin also were spotted.

Phospholipid Classes

TLC plates were read by the Photovolt Varicord Densito-

meter equipped with an integrator. Pen strokes under the curves corresponding to lecithin, cephalin and sphingomyelin were counted.

$$\text{percent phospholipid class} = 100\left(\frac{\text{pen strokes under specific curve}}{\text{total pen strokes of curves examined for the sample}}\right)$$

Statistical Analysis

The t-test for paired comparisons was computed on the Olivetti Programma 101 and used to compare sample means. Rohlf and Sokal's (1969) "Statistical Tables" was used to determine significances of differences.

CHAPTER IV

RESULTS AND DISCUSSION

Few studies are available that report the effects of cooking on the phospholipid content of meat. The studies of Roberts (1966) and Campbell and Turkki (1967) will be used as the primary basis for comparison with the present research.

Dry Matter

Percents dry matter for the raw, cooked and drip samples are presented in Table I. Mean percents dry matter are 26.4 raw, 35.4 cooked and 20.8 drip. Dry matter is necessary for expressing lipid and phospholipid on a dry weight basis.

Total Lipid

Percents lipid of the raw and cooked muscle and drip are shown in Table II. On a wet weight basis raw muscle averaged 3.7 percent lipid and cooked muscle 5.4 percent. The difference in lipids extracted from raw and cooked muscle is significant ($P<0.001$). This difference in percent lipid of raw and cooked muscle agrees with findings of other studies (Nutt, 1963; Taylor, 1964; Roberts, 1966; Campbell and Turkki, 1967).

On a dry weight basis there is less difference in the percent lipid extracted from raw and cooked samples than on

TABLE I

PERCENT DRY MATTER: RAW AND COOKED BICEPS FEMORIS MUSCLE AND DRIP

	Dry Matter		
Sample	Raw	Cooked	Drip
031	26.0	33.6	18.1
311	27.7	37.2	23.0
141	25.3	34.6	18.9
481	26.5	36.6	19.8
711	26.6	35.8	24.4
541	26.6	34.6	20.7
Mean	26.4	35.4	20.8
Std. Dev.	0.8	1.4	2.4

TABLE II

PERCENT EXTRACTED LIPID: RAW AND COOKED BICEPS FEMORIS MUSCLE AND DRIP

	Wet Weight Basis			Dry Weight Basis[1]		
Sample	Raw	Cooked	Drip	Raw	Cooked	Drip
031	3.1	4.7	0.13	12.0	14.0	0.72
311	4.7	7.0	0.19	17.0	18.7	0.82
141	2.8	3.9	0.13	11.1	11.2	0.69
481	4.8	6.7	0.06	17.9	18.2	0.30
711	3.2	4.6	0.13	11.8	12.9	0.53
541	3.8	5.4	0.07	14.3	15.7	0.34
Mean[2]	3.7 [b]	5.4 [a]	0.12	14.0 [t]	15.1 [s]	0.57
Std. Dev.	0.9	1.2	0.05	2.9	3.0	0.21

[1] Based on moisture values--Table I.

[2] Means for raw and cooked muscle within each weight basis not followed by the same letter are significantly different; Wet weight basis, $P < 0.001$. Dry weight basis, $P < 0.05$.

a wet weight basis. The average percent lipid extracted from raw muscle, 14.0, differs from the mean 15.1 for cooked muscle only at the probability level $P<0.05$. Lowe and Kastelic (1961), Taylor (1964) and Roberts (1966) found no significant difference in percent lipid of raw and cooked meat expressed on a dry weight basis.

A very small proportion of lipid was lost in the drip. The average percent lipid extracted from drip was 0.12 on a wet weight basis.

Phospholipid

Phospholipid content has been expressed in several ways in various studies. Frequently phospholipid concentration is expressed as percent of total lipid. Researchers have shown that phospholipid so expressed decreases as percent total lipid increases (Taylor, 1964; Turkki, 1965; Roberts, 1966; Campbell and Turkki, 1967). As the percent lipid of raw meat varies, the percent phospholipid varies inversely and curvilinearly. Data from this study are plotted on a scatter diagram with those from Turkki's (1965) and Roberts' (1966) studies (Figure 1). Data from all three studies are in agreement.

Phospholipid content of raw muscle lipid averaged 16.6 percent and of cooked muscle lipid 17.6 percent (Table III). The means are not significantly different. Roberts (1966) obtained similar results, whereas Campbell and Turkki (1967) whose samples were much higher in lipids, found phospholipid

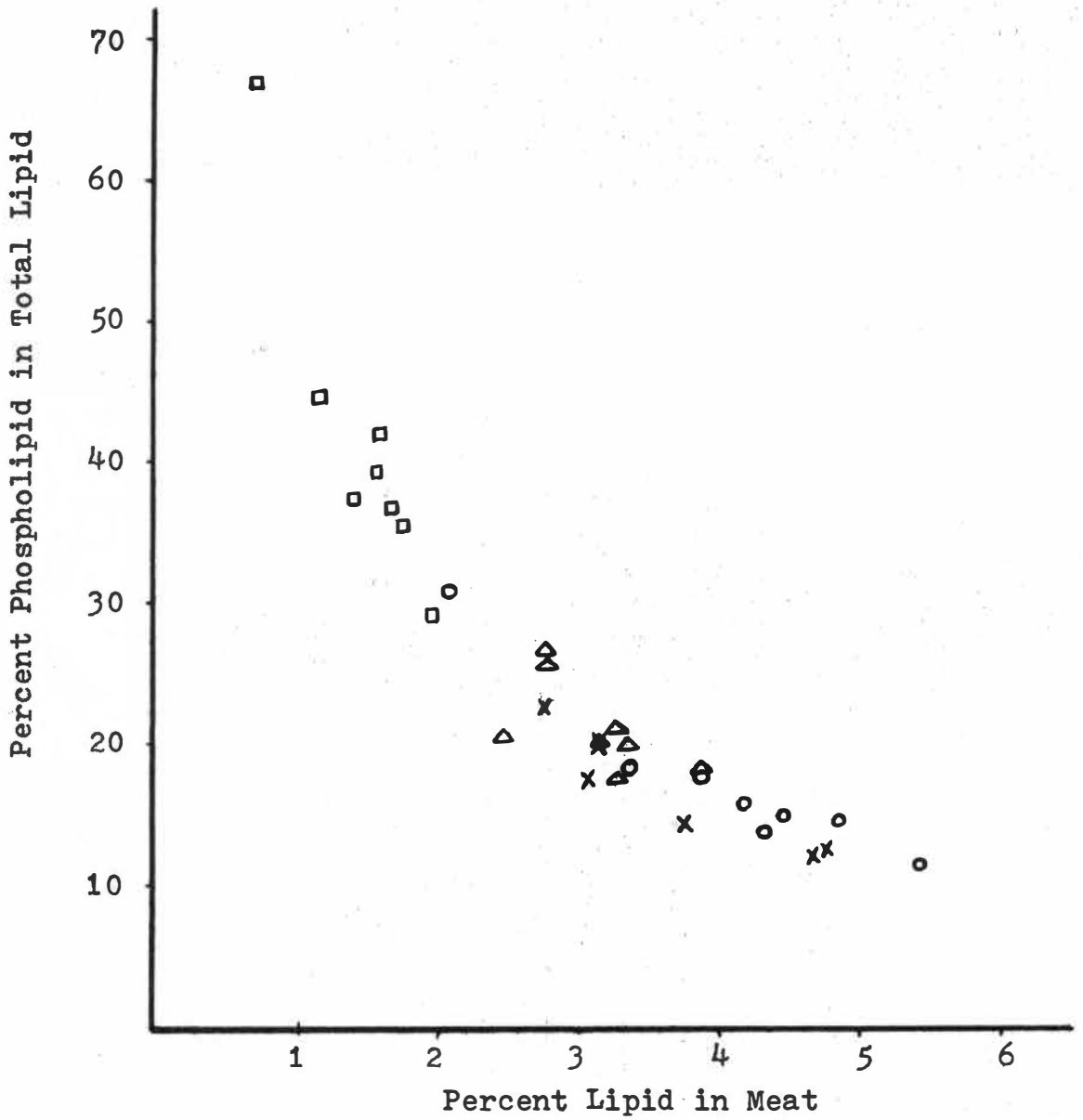

Figure 1. Relationship between lipid concentration in meat and proportion of phospholipid in total lipid. □ extensor carpi radialis (Turkki, 1965); O psoas major (Turkki, 1965); △ beef chuck (Roberts, 1966); X biceps femoris (This study).

TABLE III

PERCENT PHOSPHOLIPID IN EXTRACTED LIPID: RAW AND COOKED BICEPS FEMORIS MUSCLE AND DRIP

	Percent of Extracted Lipid			Percent of Muscle Wet Weight		Dry Weight	
Sample	Raw	Cooked	Drip	Raw	Cooked	Raw	Cooked
031	17.5	18.7	6.9	0.54	0.88	2.1	2.6
311	12.4	13.3	3.8	0.59	0.93	2.1	2.5
141	22.6	21.3	16.7	0.63	0.83	2.5	2.4
481	12.6	13.4	7.8	0.60	0.89	2.3	2.4
711	20.0	22.5	8.4	0.63	1.04	2.4	2.9
541	14.7	16.2	11.9	0.56	0.88	2.1	2.5
Mean[1]	16.6[a]	17.6[a]	9.2	0.59[g]	0.91[f]	2.2[z]	2.6[y]
Std. Dev.	4.1	3.9	4.5	0.04	0.07	0.18	0.19

[1]Means for raw and cooked muscle within each expression basis not followed by the same letter are significantly different; Wet weight basis, $P < 0.001$. Dry weight basis, $P < 0.05$.

concentrations to be higher in cooked than in raw meat lipids.

In studies involving muscle samples that vary considerably in phospholipid content expressed as percent of total lipid more nearly constant values may be found when phospholipids are expressed as percent of the muscle, wet or dry weight basis. When data of the present study are expressed as percent of muscle, differences among animals become much smaller as indicated by the standard deviations (Table III). The phospholipid concentration was significantly higher in cooked than in raw muscle, particularly when expressed on a wet weight basis ($P<0.001$).

The average phospholipid content of the drip lipid was small, 9.2 percent on a percent lipid basis. This amount is similar to the average 9.4 percent found by Roberts (1966). Campbell and Turkki's (1967) work on meat of a higher fat content again shows the relationship between lipid and phospholipid contents in meat. Their average percent phospholipid was 0.8. These results further emphasize that apparent cooking effects depend upon the level of total lipid in the raw muscle and the terms in which phospholipid are expressed.

Smith et al. (1972) suggested that perhaps the apparent increase in percent phospholipid in cooked meat is related more to changes in water and neutral lipid content than an actual quantitative change in phospholipid. Neutral lipids

are lost more readily than phospholipids during cooking. Meat with a higher total lipid content and therefore larger proportion of neutral lipids loses more lipid during cooking than meat of lower lipid content. The result is a change in relative proportions of neutral and phospholipids. When very lean meat is cooked there is little loss of lipid in the drip and essentially no change in proportions of phospholipid and neutral lipid.

Phospholipid Classes

The percent distribution of phospholipids among the three major classes is presented in Table IV. The raw muscle averages of 52.9, 38.5 and 8.7 percent for lecithin, cephalin and sphingomyelin, respectively are similar to the corresponding values of 60.1, 32.7 and 7.2 percent reported by Roberts (1966) for ground chuck. Values reported by Turkki (1965), 62.0, 30.2 and 7.8 percent, and Kuchmak and Dugan (1965), 61, 36, and 3 percent lecithin, cephalin and sphingomyelin, respectively, also follow this general pattern. These values vary considerably from the early work of Hornstein et al. (1961) who reported 40-45, 40-45 and 10-15 percent for the respective classes in beef and pork.

In this study the difference in percent lecithin in raw muscle (52.9) and cooked muscle (64.2) was significant ($P<0.001$). Roberts (1966) did not find a consistent change in percent lecithin in cooked meat. A significant difference in percent cephalin of raw (38.5) and cooked (28.4)

TABLE IV

DISTRIBUTION OF PHOSPHOLIPID AMONG LECITHIN, CEPHALIN AND SPHINGOMYELIN: RAW AND COOKED BICEPS FEMORIS MUSCLE AND DRIP[1]

	Lecithin			Cephalin			Sphingomyelin		
Sample	Raw	Cooked	Drip	Raw	Cooked	Drip	Raw	Cooked	Drip
031	53.2	65.9	37.1	35.3	28.8	53.6	11.3	5.3	9.4
311	50.7	63.1	35.8	39.5	29.0	52.9	9.9	7.9	11.3
141	50.6	62.1	34.1	44.9	30.7	55.8	4.5	7.2	10.0
481	56.9	66.4	39.1	36.8	25.3	52.2	6.4	8.3	8.7
711	52.6	64.0	38.3	35.8	28.4	50.8	11.6	7.7	10.9
541	53.3	63.9	39.2	38.3	28.4	51.4	8.4	7.7	9.4
Mean[2]	52.9 ^{b}	64.2 ^{a}	37.3 ^{c}	38.5 ^{g}	28.4 ^{h}	52.8 ^{f}	8.7 ^{xy}	7.4 ^{y}	10.0 ^{x}
Std. Dev.	2.3	1.6	2.0	3.5	1.8	1.8	2.8	1.1	1.0

[1]Values expressed as percent of total phospholipid analyzed.

[2]Means for raw, cooked and drip within each phospholipid class not followed by the same letter are significantly different. Lecithin and cephalin, $P < 0.001$. Sphingomyelin, $P < 0.01$.

muscle was noted ($P<0.001$). Roberts (1966) also reported a similar significant change in percent cephalin. In both this study and Roberts' (1966) there was no significant difference in the proportion of sphingomyelin in raw and cooked meat.

The higher lecithin and lower cephalin levels in cooked muscle were accompanied by lower lecithin and higher cephalin levels in the drip (Table IV). These results agree with those reported by Roberts (1966).

The relatively high percent cephalin in the drip has been of interest to other researchers (Campbell and Turkki, 1967; Roberts, 1966). This finding instigated the research reported here. In order to consider whether changes in proportions of the three main phospholipid classes result from selective partitioning of the classes or degree of susceptibility to heat degradation further calculations are necessary. These calculations are needed in order to express the results in absolute terms rather than percents for total lipid, phospholipid and phospholipid classes.

Calculations of Grams Lipid and Phospholipid

The calculations for one sample, 031, follow with an explanation of difficulties encountered at this stage of the research.

The grams of lipid in the total raw and cooked muscle and the drip were calculated.

g lipid = (wt raw or cooked muscle or drip) (% lipid)

For sample 031 the grams lipid determined from this

calculation were 62.0 raw, 65.9 cooked and 0.18 drip. These values indicate that more lipid appears to have been extracted from cooked muscle than was present in the raw muscle. This was true for four of the six samples.

Several possibilities may explain this result. The raw sample could have been incompletely extracted because of lipid-protein complexes that make extraction difficult. Cooking may have altered these complexes in such a manner that extraction from cooked muscle was more complete. Woolsey and Paul (1969) mention this possibility and also that of an alteration in muscle structure during cooking. They also suggest that fat loss may be masked by proportionately greater losses of other components. They considered the results of their study, involving two extraction methods as well as other experimental variables, to support the conclusion that heating makes muscle lipid more accessible to extraction.

Another possible explanation is that extraction from raw muscle was complete but that the cooked muscle lipid extract may have contained protein complexed with lipid in addition to free lipid. Finally the results may indicate that the chosen extraction method needed to be performed with more skill and exactness.

A possible area for further investigation would be a search into this problem. This might include varying the extraction procedure to see if ratio of solvents, time of

mixing or temperature of the mixture has an effect upon percent lipids extracted from raw and cooked muscle. The raw muscle might be re-extracted by different extraction procedures to determine whether incomplete extraction was obtained. One such procedure would be to freeze-dry the residue and then re-extract. If greater recovery is again recorded for cooked meat the lipid extracts might be subjected to chemical analysis, i.e. nitrogen determination, to detect the presence of contaminants in the extracts.

Further calculations were performed in the present study in an attempt to correct for the greater than one-hundred percent recovery of lipids. The correction procedure involved calculating the extracted cooked muscle lipid as percent of the total lipid extracted from the cooked meat plus drip. The proportion for drip also was determined. For sample 031 lipid extracted from cooked meat accounted for 99.73 percent of the total and drip for 0.27 percent. These percentages and the total lipid extracted from the raw muscle then were used to calculate corrected values for amount of lipid extracted from cooked muscle and drip. The corrected value for grams lipid from cooked muscle was 61.8 and that for drip, 0.2.

These corrected values for total lipid then were used in further determinations of total phospholipids and phospholipid classes. Grams total phospholipid were calculated by multiplying corrected grams lipid by percent phospholipid in the lipid. From these determinations, 10.8, 11.6 and

0.01 grams phospholipid were obtained for raw and cooked muscle and drip, respectively. As with total lipid the total grams of phospholipid in the cooked muscle plus drip exceeded the grams of phospholipid in the raw meat. With these results, further calculations of absolute amounts of lecithin, cephalin and sphingomyelin did not seem worthwhile.

It was disappointing that the data obtained did not provide an answer to the question of whether partitioning of phospholipids occurs during cooking of meat. However, further substantiation of a relatively high proportion of cephalin in the drip lipid provides motivation for additional study of what actually happens to meat phospholipids during cooking. Study of interactions and complexes in raw and cooked meat may answer questions posed by the processor of formulated foods. The knowledge of natural and heat induced interaction among food components is indeed limited.

CHAPTER V

SUMMARY

Phospholipid content of raw and cooked muscle and drip of biceps femoris was studied. Total lipids were extracted from the muscle samples. Phosphorus analysis was performed to determine the phospholipid content of the total lipid. Phospholipids were separated from neutral lipids and fractionated into three major classes by thin-layer chromatography. The percent of each class was determined by densitometry.

The method of expressing phospholipid values had an effect upon significant differences attributable to cooking. There was no significant difference between phospholipid content of raw and cooked muscle when expressed as percent of total lipid. On a percent muscle basis the cooked muscle had significantly higher phospholipid contents than raw muscle (dry weight basis, $P<0.05$; wet weight basis, $P<0.001$). Only a small proportion of phospholipid was lost into the drip.

Significant differences ($P<0.001$) were found in distribution of phospholipid classes. Cooked muscle had higher lecithin and lower cephalin percentages than raw muscle. There was no significant difference in sphingomyelin distribution in raw and cooked muscle. Drip phospholipid had lower lecithin and higher cephalin

percentages than either raw or cooked muscle. Drip had a higher sphingomyelin content than cooked muscle at the $P<0.01$ level of significance.

These results further substantiate previous reports of higher cephalin content in drip than in raw or cooked muscle.

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APPENDIX

APPENDIX

LIPID EXTRACTION - MODIFIED BLIGH AND DYER METHOD (OSTRANDER AND DUGAN, 1961) SLIGHTLY ALTERED BY TURKKI (1965)

1. Transfer weighed sample quantitatively to blender jar.
2. Blend 5 minutes with 130 ml absolute methanol.
3. Blend 5 minutes with 65 ml chloroform.
4. Blend 20 seconds with 65 ml chloroform.
5. Add 65 ml distilled water containing 1.5 g zinc acetate. Blend 10 seconds.
6. Transfer contents of blender to a No. 3 Büchner funnel covered with Whatman No. 1 filter paper. Filter under suction, maintaining a stream of nitrogen over the funnel.
7. Transfer residue, filter paper and 1/2 facial tissue used for wiping funnel to blender jar. Rinse funnel with 100 ml chloroform. Add rinse to blender jar.
8. Blend sample 2 1/2 minutes.
9. Filter. Rinse jar into the funnel with 75 ml chloroform.
10. Transfer filtrate to 500 ml graduated cylinder. Rinse flask into the cylinder with 25 ml methanol.
11. Cover. Place in cold (1°C) overnight or until two clear phases appear.

PHOSPHORUS ANALYSIS -- BARTLETT (1959) AS MODIFIED BY MARINETTI (1962)

1. Three samples of lipid extract, two samples of phosphorus standard and two blanks are analyzed simultaneously in acid-washed glassware.

2. Place the following in micro-Kjeldahl flasks.

Samples	0.2 ml diluted extract[a] 0.8 ml demineralized water 1.2 ml 70% perchloric acid glass beads
Blanks	1.0 ml demineralized water 1.2 ml 70% perchloric acid glass beads
Standards	1.0 ml standard phosphorus solution[b] 1.2 ml 70% perchloric acid glass beads

3. Digest all samples, blanks and standards for 30 minutes on micro-Kjeldahl digestion rack. Cool. Add 7 ml distilled water and 1.5 ml 2.5% ammonium molybdate solution.[c]

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[a]Diluted extract--for extract of raw and cooked meat 0.25 ml of concentrated extract was diluted to 10 ml in a volumetric flask. Drip extracts were not diluted.

[b]Standard phosphorus solution--dilute 5 ml stock solution to 1 liter. Stock solution is prepared by bringing 5.624 g K_2HPO_4 to 1 liter volume with distilled H_2O. Standard phos. solution contains 5 µg P/ml.

[c]Ammonium molybdate (2.5%)--Combine 5.0 g ammonium molybdate with 200 ml demineralized H_2O.

Mix. Add 0.2 ml Fiske Subbarow reagent[d]. Heat flasks in boiling water. Cool 20 minutes. Pour into cuvettes. Read absorbance at 830 nm.

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[d]Fiske Subbarow--combine 3.75 g sodium bisulfite with 25 ml demineralized water. Add 0.0635 g 1-NH_2 naphthol -4-sulfonic acid. Add 0.1250 g anhydrous sodium sulfite. Filter and store in refrigerator (4°C). Make fresh weekly.

CALCULATION OF PERCENT PHOSPHOLIPID IN EXTRACTED LIPID

1. Determine dilution of extract to be used for analysis.

2. Calculate µg phosphorus (P) in volume of extract used.

$$\mu g\ P \text{ in x ml extract} = \frac{(\text{absorbance of sample})(\mu g\ P/1\ ml\ \text{standard})}{\text{absorbance of standard}}$$

3. Convert µg P to µg phospholipid (PL).

$$\mu g\ PL \text{ in x ml extract} = (\mu g\ P)(25).$$

4. Convert to grams phospholipid.

$$g\ PL \text{ in x ml extract} = \frac{\mu g\ PL}{10^6}$$

5. Calculate g phospholipid per ml extract.

$$g\ PL \text{ in 1 ml extract} = \frac{g\ PL \text{ in x ml extract}}{\text{ml extract in the dilution}}$$

6. Percent phospholipid in total lipid.

$$\%\ PL \text{ in total lipid} = 100\left(\frac{g\ PL \text{ in 1 ml extract}}{g\ \text{lipid in 1 ml extract}}\right)$$

7. Conversion to phospholipids as percent of meat.

$$\text{PL as \% of meat, wet weight basis} = \frac{(\%\ \text{total lipid wet weight})(\%\ PL \text{ in lipid})}{100}$$

$$\text{PL as \% of meat, dry weight basis} = 100\left(\frac{\%\ PL \text{ in meat wet weight basis}}{\%\ \text{dry matter}}\right)$$

THIN-LAYER CHROMATOGRAPHY--WOOD AND KINSELL, MODIFIED (1963)

1. Acid wash 20x20 cm glass plates.
2. Mix 30 g silica gel G and 60 ml distilled water in a mortar. Coat plates with 0.3 mm layer of silica gel with a Camag applicator.
3. Air dry plates 10 minutes and activate by heating at 120°C one hour.
4. Store plates in a closed container with desiccant. Reactivate if not used within 48 hours.

VITA

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