

CHARACTERIZATION OF PORK FAT BY  
GAS-LIQUID CHROMATOGRAPHY

By

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## CHAPTER I

### INTRODUCTION

Lard is a purified rendered fat of the pig. It is a creamy white unctuous mass having a slight characteristic aroma, and a bland taste. It is a highly valued cooking and baking fat. Muslims prohibit the consumption of pork and pork by-products and they may not be eaten or included in any of their daily diets. Since lard is one of the fats used as a shortening in food preparation, it becomes the concern of many groups in the world to examine ingredients especially of manufactured food items.

Analysis of a food by gas-liquid chromatography can be used for the determination of fatty acid composition in food. This method is a rapid one using a very small sample in the determination of the relative fatty acid composition. It is frequently of interest, however, to calculate absolute levels of fatty acids in foods, but standard titrimetric or gravimetric procedures are difficult when assaying for microgram quantities. The method may be especially well-suited to fatty acid characterization considered essential by Muslims where pork fats in foods are prohibited.

Giam and Dugan (1965) reported that lamb and beef are similar in fatty acid composition and quite different from pork. The myristic and myristoleic acid content is higher in lamb and beef than in pork, but linoleic and arachadonic acid content of pork exceeds that of lamb



or beef.

The objectives of this study are:

- 1) To identify the major fatty acids in lard using a specific detecting column.
- 2) To estimate the quantitative fatty acid composition of lard.
- 3) To compare the fatty acids of lard with lamb and beef fat samples.
- 4) To determine the relative variation in the fatty acids in lard from different sources by gas-liquid chromatography determination as well as the variation associated with the method of determination.

## CHAPTER II

### REVIEW OF LITERATURE

#### Pork Fat (Lard)

Sakr (1974) reported that for many generations lard has been used in preparing doughs and batters. The current use of lard in the United States is relatively small as compared to its more widespread use in years past. During the past decade, the use of pure vegetable oil has increased dramatically. With the introduction of the process of hydrogenation in 1910, manufacturers of the all-hydrogenated oils (cottonseed oils) began to recognize the advantages of developing possible new substitutes for lard. The shortenings on the market today are far different from pure lard, as they have greater resistance to rancidity and are more effective as creaming or emulsifying agents in foods such as batter. Even today lard is processed in industry to resemble hydrogenated shortening.

Henrickson (1978) stated that pork fat used for lard should be relatively pure, i.e. free from meat and hide. Desirable lard can be made from fat trimmings such as the clear plate, fat back, leaf fat, intestinal fat and fat trimmings. The fat should be ground or cut into relatively uniform small pieces for maximum surface area and more rapid rendering. Preferably a steam jacketed kettle should be used. When rendering, it is essential that the lard not exceed 240°F.

It is important not to overcook the lard as discoloration will occur. Lard should be stored at a temperature of 40° or lower

#### Fatty Acid Composition of Lard

Hilditch and Williams (1964) reported that the composition data for pig depot fats are more numerous than for any other land animals, and the major component of depot fatty acids are palmitic, stearic and the unsaturated oleic acid which is the predominating fatty acid present. The minor component acids are linoleic, lauric, and myristic acid which comprise about one percent of the total fatty acids while palmitoleic appears to be fairly constant at about 2-3 percent.

Beare (1962) reported that gas-liquid chromatography was employed to investigate the nature of the fatty acids present in margarines, and samples of fat of pork meat showed that pork fat contain: myristic (1.8 percent), palmitic (25.9 percent), palmitoleic (3.4 percent), stearic (14.8 percent), oleic (43.7 percent), and linoleic (6.3 percent), and some trace amounts of other fatty acids.

Catchpole and Laurie (1972) studied porcine fat in muscle from six different locations using gas-liquid chromatography and reported that the major components of pig fat is oleic (40 percent), palmitic (25 percent), linoleic (10-20 percent) and stearic (10-20 percent). These workers observed no difference between fat muscle location in the fatty acids composition.

Wood (1973) reported that a pair-feeding experiment was conducted with large white and pietrain pigs to determine whether these differences could be explained by the lower voluntary feed intake of pietrains. The results showed that the large white continued to accumulate

relatively saturated fatty acids even at the lower level of feeding and both the inner and outer layers of pietrain backfat had higher concentrations of unsaturated fatty acids and lower concentrations of saturated fatty acids.

Martin et al. (1972) reported that fatter carcasses had higher values for percent myristic and palmitic acids and a lower percentage of linoleic acid. Fat from boars and barrows had a higher percentage linoleic acid content than gilts while fat from barrows had a greater percentage of the saturated myristic and palmitic acids than fat from gilts or boars.

Villegas et al. (1973) reported that diet can alter the fatty acid composition of pork fatback and leaf fat. Pigs that received whole roasted soybeans in their diet were found to contain more unsaturated fatty acids than pigs that received raw soybean meal in the diet.

Giam and Dugan (1965) employed gas chromatography to determine whether cooking changed the fatty acid content of free and bound lipid in freeze-dried pork, beef, and lamb. Cooking for 30 minutes in boiling water appeared to have no significant influence on the fatty acids of either class of lipids.

Campbell et al. (1967) reported that fatty acid patterns of the lipids were similar in raw and cooked meat except that the concentration of linoleate in the phospholipid fatty acids was higher in cooked pork than in raw pork.

#### Fat in the Daily Diet

The term "fat" includes all edible oils and solid fats extracted

from plant and animal sources used in food preparation. Sources of plant fats commonly used in the United States include corn, cotton-seeds, olives, soybeans, and peanuts; the common animal sources are beef, pork, lamb, chicken, and milk. These differ in flavor, consistency, and somewhat in color. Fat, one of the required nutrients in the daily diet, has just as important physiological functions as the more fascinating micro-nutrients, and is just as important to survival.

The uses of fat in nutrition include:

1. Provides a portion of the body needs for energy necessary in maintenance, growth, etc.,
2. Contributes the essential fatty acids needed for specialized cells and functions,
3. Serves as a carrier for fat-soluble nutrients,
4. Assists in filling the fat depots of the body for reserve energy supplies for cushioning and for temperature adjustment to environmental changes (Griffith, 1957).

In addition to the nutritive aspects, moderate amounts of fat improve palatability of certain foods, thereby influencing intake of other nutrients.

Fat provides approximately 9 calories per gram or 4000 cal per pound. The amount of refined fat purchased would indicate an average daily intake of one-fourth cup, or approximately 1000 cal per person per day.

The Food and Nutrition Board of the National Research Council has suggested a minimum intake of 20 to 25 percent of total calories from fat. This provides a condition favoring absorption of fat soluble vitamins and other substances. For active adolescents and

young people, the Food and Nutrition Board indicated that 30 to 35 percent of the calories may be derived from fat. These individuals may tolerate fat in quantities even greater than the recommended 30 to 35 percent, and it is estimated that the American armed forces are ingesting 40 percent of their total calories in the form of fat. Other active individuals requiring 4000 to 6000 calories per day often obtain an even higher proportion of calories from fat (Griffith, 1957).

### Essential Fatty Acids

Current interest in the health relationship of fat composition centers largely on linoleic acid arachadonic acids, and cholesterol.

The essentiality of linoleic acid in the diet of rats was first demonstrated in 1922 by Burr and Burr (1929), who found that this acid would prevent or cure a characteristic dermatitis observed in rats fed fat-free diets. Baldwin and Longenecker (1944) demonstrated that human milk had a different pattern of fatty acids than cows' milk. Hansen and his associates (1947) demonstrated the importance of linoleic glycerides in infant nutrition, as they influenced growth rate, skin condition, and efficiency of food utilization.

### The Analysis of Fatty Acids

Gas-liquid chromatography (G.L.C.) is a method for separating components of mixtures of volatile compounds, generally organics such as hydrocarbons, alcohols, esters, acids, amines, mercaptans, etc. In most applications, the separations are made in order to identify

and determine the quantity of each component of a sample of the mixture (Littlewood, 1970).

The basic requirement for a G.L.C. sample is that it be volatile, or that it can be volatilized if sufficient heat is applied when introduced into the chromatograph. The sample must not decompose in the process of being vaporized, and must remain stable (Ackman and Sipos, 1964).

In the simplest terms, the chromatographic system consists of four parts: (a) a source of gas (usually nitrogen or helium), called the carrier gas, which is the mobile phase; (b) a means of sample introduction, (c) a chromatographic column, and (d) a detector. The column and detector are normally housed in a constant temperature oven or heater. The output of the detector is fed to a millivolt recorder (Regis Chemical Company, 1976).

G.L.C. has found tremendous application in the petroleum industry for the analysis of various products. It is a commonly used tool in many research laboratories for the analysis of mixtures of organic compounds. In recent years, G.L.C. has become a popular method for analyzing for fatty acid composition in foods. Most of the work, however, is on the analysis of the higher fatty acids found in animals and plants. The fatty acids can be analyzed either as the free acids or as esters. Since the free acids are polar and interact strongly both with themselves and with most support materials, they are very likely to produce peaks which are badly "tailed." Decomposition can occur with free fatty acids. Thus, in the majority of routine analyses, acids are converted to volatile esters before chromatography (Littlewood, 1970).

Liquid phases have been developed that separate the fatty acids according to the degree of unsaturation. For example, the C-18 acids will be eluted in the order of no double bond, one double bond, and two double bonds; that is, the stearate, oleate, and linoleate, linolenate.

Two types of column paraffinic hydrocarbon and polyesters, have been useful for separating fatty acid mixtures. The non-polar paraffinic hydrocarbons, e.g. Apiezon L., have high heat stability but are not useful for separating C-18:2 from C-18:3 and C-20:3 from C-20:4 (Horning et al., 1964).

A method for preparing fatty acid methyl esters for G.L.C. should ideally be simple, rapid, and quantitative, and should give rise to no unwanted structural changes or side reactions.

Binder and Applewhite (1964) reported that methanolysis of lipids is normally accomplished with an alkaline or an acidic catalysis. Mason and Waller (1964) reported that the use of 2,2-dimethoxypropane (DMP) to drive transesterification to completion eliminates the need for elevated temperatures. 2,2-dimethoxypropane (DMP) reacts with glycerol to form isopropylidene glycerol (IPD) which chromatographs readily and serves as a convenient marker in determining retention times. This method requires only a few simple operations and is especially adaptable to routine analyses of large numbers of samples.



## CHAPTER III

### MATERIALS AND METHODS

#### Samples

The first step, to achieve the objectives of this study, is to obtain reliable information about the occurrence of different fatty acids in lard and their levels. These may depend on many factors. Age, breed and sex of the hogs greatly influence the fatty acids in lard and their levels. The environment and the feeds used also may have some influence. The manufacturing process employed to render fat is not identical in all companies. Some of these factors may introduce variation in the content of the different fatty acids in lard. These must be taken into consideration in deciding how lard samples should be selected to obtain basic data for the study.

The variation introduced by the companies could be studied by drawing lard samples from a number of different companies randomly. With this reasoning it was decided to collect random samples of fresh pork fat from four different companies (Schwab Meat Co., Wilson's Foods Industries, Farmland Industries, Wickham Packing Co.). In all selected companies pork fats were made available in boxes of nine cans each. The cans in any one box were not manufactured on a single day-- which could be verified by checking the manufacturing date on the cans. Actually, the dates were randomly scattered over a month. Hence

the cans in any one box could be taken to cover whatever variations may occur in the lard from a company when produced on different days.

The actual procedure employed to collect samples of lard is explained below.

From each of the four selected companies, one box of cans of lard was selected at random. From each of the four boxes, four out of the nine cans were selected at random. Thus the basic sample consisted of 16 cans of lard, four each from the four companies.

From each can, four samples were prepared for freezing at  $-20^{\circ}\text{F}$  where they were stored in polyethylene bags until such time as they could be analyzed for fatty acids content. Ten fat samples of beef and ten samples of lamb fat were obtained from the Oklahoma State University meat laboratory, and rendered at about  $150^{\circ}\text{F}$  in a water bath. These fat samples were stored in the manner described above for lard samples.

#### Preparation of Fatty Acid Methyl Esters from Triglycerides

The method as described by Mason and Waller (1964) was used to transesterify the fatty acids of the fats. These analytical methods require specific chemical reagents for accomplishing the analysis. The following is a description of these reagents:

Benzene, reagent grade - dried over sodium

2,2-dimethoxypropane, methanol super dry with less than 0.02%  $\text{H}_2\text{O}$

Methanolic  $\text{HCl}$  is normally prepared as follows: bubble dry  $\text{HCl}$  gas through cooled methanol until the concentration is in excess of 101 (w/v) as noted by titration with standard base.

The solution is stored in the cold over a desiccant and retitrated periodically to check its strength. A kit to prepare 100 ml of this reagent was also used to expedite the research.

### Procedures

Preparation of esters from fats. Approximately 0.200 gram of sample from each can were accurately weighed in a test tube. Reagents were added in the following order:

1. 4 ml of sodium dried benzene,
2. 0.4 ml of 2,2-dimethoxypropane then mixed,
3. 0.5 ml of methanolic HCl was added and mixed until the sample was one phase.

The sample was then covered with aluminum foil and parafilm after which the sample was allowed to stand overnight at room temperature (about 22°C).

Two  $\mu$ l were withdrawn for injection to the G.L.C. for analysis from each sample.

### Apparatus

The Perkin-Elmer 990 Gas Chromatograph with a hydrogen flame detector (FID) was used. The packing materials of the column were 20 percent by weight diethylene glycol succinate (DEGS) on sixty to eighty mesh chromosorb W with a 12 ft x  $\frac{1}{4}$  inch circular shaped glass tube.

### Column Packing

Twenty gms of diethylene glycol succinate (DEGS) were dissolved

in approximately 80 ml of acetone and mixed with 80 gm of "chromosorb W." The acetone was removed by evaporation using nitrogen gas and an electric dryer. The last traces of solvent were removed with a fluidized bed apparatus which uses nitrogen and a heater.

The packing of the column with the above materials was done very slowly and carefully with the aid of an electric vibrator. The two ends of the packed columns were then covered with glass-wool to prevent disruption of the packed column. Approximately 2 inches of column above the inlet end was left empty to provide space for vaporization of sample.

Care in preparing a "superior" column was necessary for efficient separation of the esterified fatty acids. After mounting the newly prepared column, it was necessary to "cure condition" the column by heating it at 250°C overnight under a flow of nitrogen at 3 to 5 ml per minute. In the G.L.C., temperature of injection box (containing the column) was 190°C and the detector temperature was 250°. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min.

The column was then ready for injection of the mixture of fatty acid methyl esters.

#### Method of Calculation for Fatty Acids

After injecting the samples into the instrument, the recorder produces a chart with several peaks. For example, Figures 1 and 2 show typical profiles of methyl esters from a recorder that measured the response for each compound. Each peak represents a specific fatty acid. The recorder response is quantitative and the area of each peak was determined by triangulation (i.e. width at  $\frac{1}{2}$  height x

height = area of a triangle). Then, the total concentration of fatty acids is equal to the summation of the areas, as is shown in the following equation. The percentage of each fatty acid in the total amount of fatty acids was then calculated:

$$\% \text{ each fatty acid} = \frac{\text{the area of the acid}}{\text{the total areas of all acids}} \times 100$$

This is possible since the flame ionization detector responds to carbon mass of the compounds involved.

### Statistical Analysis

The main aim of this study is to develop a procedure to distinguish lard from other fats. Statistically this might be done by constructing confidence intervals for the percentage of each fatty acid in lard. This is because any fat is fully characterized by the contents of the different fatty acids in it. To construct the desired confidence intervals the values of the following parameters for each of the fatty acids in lard are needed.

- 1) Mean of the percentage of each fatty acid.
- 2)  $\hat{\sigma}_{Co}^2$  = variance between companies. Variance was introduced into the percentage of fatty acids due to variation in the manufacturing process and pork fat supply in the different companies.
- 3)  $\hat{\sigma}_s^2$  = variance between samples within companies which was introduced because of the variation due to factors of pork fat supply and day to day processing changes.

- 4)  $\hat{\sigma}_D^2$  = variance of determination. This variation is due to instrument determination from samples to samples.

Since these quantities are not known, they need to be estimated from the sample data. To estimate these components of variation the Analysis of Variance method is used. This necessitates the construction of Analysis of Variance for each percentage of fatty acids. According to the A.O.V. method the different mean squares in the A.O.V. are equated to their expected values, which are linear function of the variance components. These equations are then solved for the variance components to get the estimates of the variance components for each percentage of fatty acids.

In the present study the equations to be solved are:

1)  $\hat{\sigma}_D^2$  = Mean Square in A.O.V. due to determination in samples in companies.

2)  $\hat{\sigma}_D^2 + K_1 \hat{\sigma}_S^2$  = Mean Square in A.O.V. due to samples in companies.

3)  $\hat{\sigma}_D^2 + K_1 \hat{\sigma}_S^2 + K_2 \hat{\sigma}_C^2$  = Mean square in A.O.V. due to companies  
where  $K_1$  = number of samples in companies

$K_2$  = number of samples of all companies

$\hat{\sigma}^2$  = estimate of variance.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### Identification of the Major Fatty Acids in Lard

The chromatogram of methyl esters of lard, fatty acids separated according to chain length by gas chromatography, is shown in Figures 1 and 2. Seven peaks from the column were observed which indicated esters of 12, 14, 16, 16', 18, 18', and 18'' carbon acids which namely are as follows in Table I.

Results with Hornstein et al. (1961) and Joyce L. Beare (1962) agree with results presented in this study. Pork fat analyzed by using gas chromatography shows that major fatty acids composition is lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and trace amounts of linolenic.

#### Quantitative Fatty Acid Composition of Lard

Results of the analysis of 64 samples from four different companies are presented in Tables V-VIII shown in the Appendix.

The data indicated that the highest percentage of the fatty acids is oleic which was about 44 percent of the total fatty acid in these lard samples, followed by palmitic acid which was about 25 percent, and by linoleic and stearic both about 8.5-13.6 percent. The minority fatty acids found in the lard were lauric, myristic and palmitoleic and

Figure 1. Gas-Liquid Chromatographic Profile of Methyl Esters of Fatty Acids from Pork Fat from Two Different Columns

Column: DEGS 20% on Chromosorb W. 0.25" O.D. x 12' length. Used in Perkin-Elmer 990 GLC Instrument at 190°C temperature. Recorder speed, 1 cm = 1 min; Reduction of figure = .76.



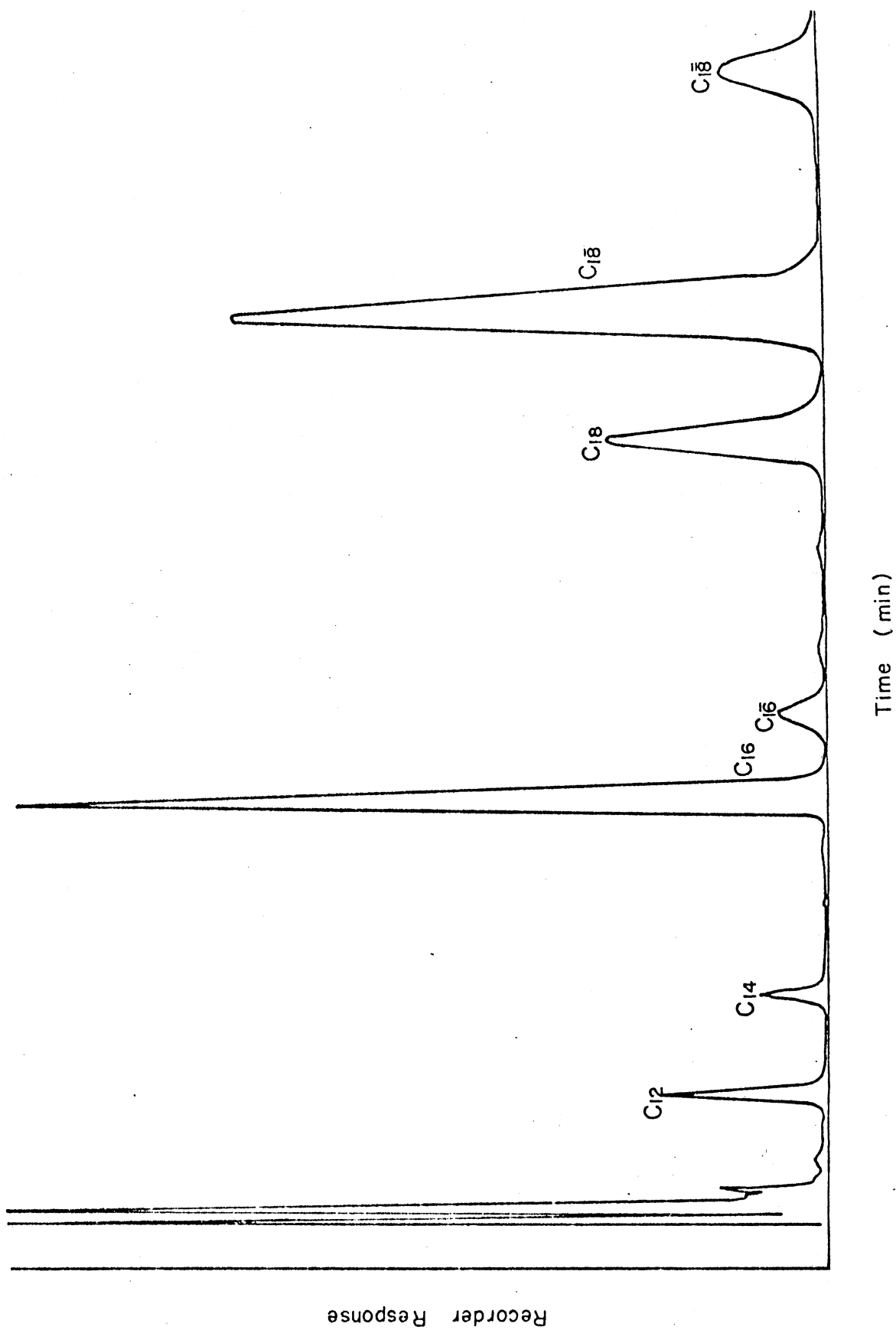


Figure 2. Gas-Liquid Chromatographic Profile of Methyl Esters of Fatty Acids from Pork Fat from Two Different Columns

Column: DEGS 20% on Chromosorb W. 0.25'' O.D. x 12' length used in Perkin-Elmer 990 GLC Instrument at 190°C temperature. Recorder speed, 1 cm = 1 min; Reduction of figure = .38.

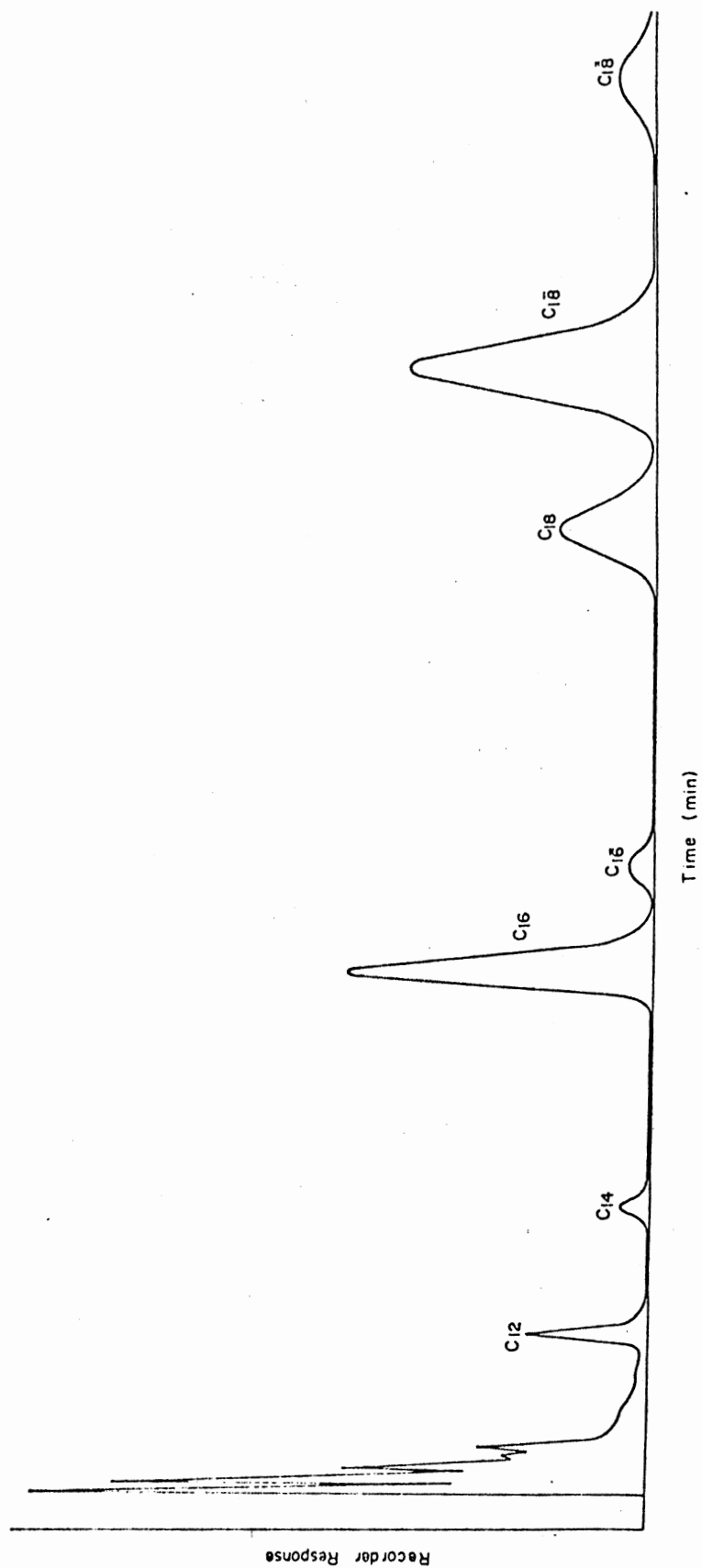


TABLE I  
FATTY ACIDS FOUND IN PORK

Number of Carbons	Number of Double Bonds	Common Name	Formula*
12	0	Lauric	$\text{CH}_3(\text{CH}_2)_{10}\text{COO}^-$
14	0	Myristic	$\text{CH}_3(\text{CH}_2)_{12}\text{COO}^-$
16	0	Palmitic	$\text{CH}_3(\text{CH}_2)_{14}\text{COO}^-$
16'	1	Palmitoleic	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}-(\text{CH}_2)_7\text{COO}^-$
18	0	Stearic	$\text{CH}_3(\text{CH}_2)_{16}\text{COO}^-$
18'	1	Oleic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}^-$
18''	2	Linoleic	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6\text{COO}^-$

\*Shown as salts of the acids; as esters methanol would be added to the carboxyl group.

comprise 3.4, 1.6 and 2.5 percent of the total fatty acids.

By comparing saturated and unsaturated fatty acids in lard samples it appears that unsaturated fatty acids make up approximately 55 percent of the total fatty acids present and the saturated fatty acids make up 45 percent.

Catchpale and Laurie (1972) studied porcine muscle from six different locations and reported that the major components of pig fat are oleic (40 percent), palmitic (25 percent), linoleic (10-20 percent) and stearic (10-20 percent) acids. These workers observed no difference in the fatty acid composition among muscle locations.

#### A Study of the Variation in Samples

Mean squares and estimated variance components of percentage of fatty acids of lard samples are presented in Table II. The variation between samples within companies were significant at 0.05 level of probability for all acids excepting palmitic ( $C_{16}$ ) and palmitoleic ( $C_{16}'$ ) acid.

The variation among companies turned out to be significant at 0.05 level of probability for all fatty acids with the exception of oleic acid ( $C_{18}'$ ).

The problem of developing a profile of fatty acids in lard is now considered. This is done on the basis of confidence intervals constructed for the mean percentages of the different fatty acids in lard.

For this purpose the distribution of the percentages of each fatty acid in lard is assumed to follow normal distribution. The basic statistical idea used is the following. If from a normal

TABLE II  
MEAN SQUARES AND ESTIMATED VARIANCE COMPONENTS OF PERCENTAGE FATTY ACIDS OF LARD SAMPLES

	D.F.	C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>16</sub> '	C <sub>18</sub>	C <sub>18</sub> '	C <sub>18</sub> ''
Between Company	3	4.640 <sup>a</sup>	1.560 <sup>a</sup>	10.3710 <sup>a</sup>	1.244 <sup>a</sup>	9.793 <sup>a</sup>	2.484	14.383 <sup>a</sup>
Sample Within Company	12	1.069 <sup>a</sup>	0.0343 <sup>a</sup>	0.8493	0.078 <sup>a</sup>	1.7115 <sup>a</sup>	3.674 <sup>a</sup>	1.925 <sup>a</sup>
Determination Sample	48	0.127	0.0168	0.4820	0.050	0.1613	0.5994	0.0971
$\hat{\sigma}_D^2$		0.127	0.0168	0.4820	0.050	0.1613	0.5994	0.0971
$\hat{\sigma}_S^2$		0.2355	0.0044	0.0968	0.007	0.3880	0.7700	0.4570
$\hat{\sigma}_{Co}^2$		0.2232	0.0954	0.5940	0.072	0.5100	0.0000	0.7790

<sup>a</sup>Significant (P < 0.05)

population with mean  $\mu$  and variance  $\sigma^2$ , at a random sample of size  $n$ , is drawn and  $\bar{x}$  is the sample mean, then it is a 0.997 confidence that  $\bar{x}$  will lie in the interval

$$\left( \mu - 3 \frac{\sigma}{\sqrt{n}}, \mu + 3 \frac{\sigma}{\sqrt{n}} \right).$$

This is due to a well-known property of normal distribution known as "3 $\sigma$  limits." The same can be looked at in a different manner. If a sample of size  $n$  is available and the mean  $\bar{x}$  of this sample does not lie in the above interval then it is almost certain that the sample does not belong to the reference population.

According to the above reasoning, if a can of lard is available and a number,  $n$ , of determinations is made on the percentage of each fatty acid in that sample of lard, then the mean percentage of that fatty acid should almost always lie between  $\mu \pm 3 \frac{\sigma}{\sqrt{n}}$  where  $\mu$  and  $\frac{\sigma}{\sqrt{n}}$  are the mean of the population and standard error of sample mean from that population for the percentage of that fatty acid. This is true for each of the fatty acids.

In the present study,

$\mu$  = population mean percentage of fatty acid

$\frac{\sigma}{\sqrt{n}}$  = standard deviation of the sample means of samples of size  $n$   
from the population for each fatty acid

$$= \sqrt{\frac{\hat{\sigma}_D^2 + \hat{\sigma}_S^2 + \hat{\sigma}_{Co}^2}{n}}$$

where  $\hat{\sigma}_D^2$  = estimate of variance of determination

$\hat{\sigma}_S^2$  = estimate of variance of samples within companies

$\hat{\sigma}_{Co}^2$  = estimate of variance between companies

Since these parameters are not known they need to be estimated using the sample data as explained in the section Statistical Methods.

Then the confidence intervals for the mean of each fatty acid based on a sample of  $n$  observations are given by

$$\bar{y} \pm 3 \sqrt{\frac{\hat{\sigma}_D^2}{n} + \hat{\sigma}_s^2 + \hat{\sigma}_{Co}^2}$$

- where: 1)  $\bar{y}$  = the estimate mean of each fatty acid  
 2)  $\hat{\sigma}_D^2$  = estimate of variance of determination  
 3)  $\hat{\sigma}_s^2$  = estimate of variance of samples within companies  
 4)  $\hat{\sigma}_{Co}^2$  = estimate of variance between companies  
 5)  $n$  = number of determinations of the samples.

The confidence ranges for each percentage of fatty acid are constructed in Table III. They are graphically represented in Figure 3. These confidence ranges could be used to determine whether any given fat sample was lard or not. First one should make " $n$ " determinations on each sample of fat and then find the mean of these " $n$ " determinations for each fatty acid. If the calculated mean percentage does not lie within the constructed confidence interval for any fatty acids then it may be concluded that the fat sample is not a lard sample. But it is to be noted that if the means of all fatty acids lie in the respective confidence intervals constructed, one cannot say that the sample is a lard sample. It could be lard or some other fat.

#### Comparing Fatty Acids of Pork Fat With the Beef and Lamb Fat

Results of the analysis of lamb and beef fat samples are presented

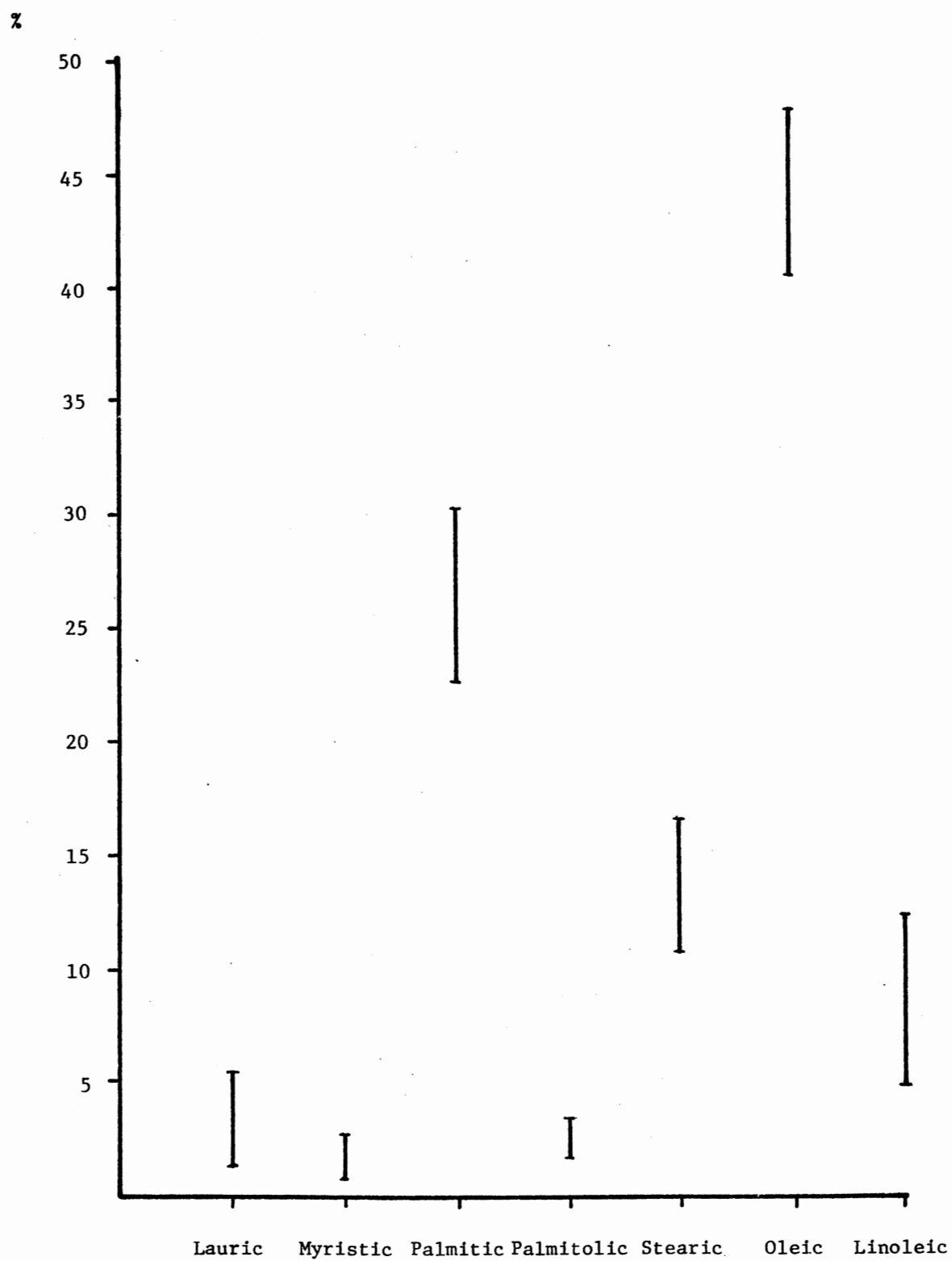


TABLE III

THE CONFIDENCE INTERVAL FOR EACH PERCENTAGE FATTY ACID IN LARD SAMPLES

	Fatty Acids						
	Lauric	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
Range of the confidence interval	1.3-5.5	0.5-2.7	22.3-30.1	1.6-3.5	10.7-16.5	40.8-47.8	4.5-12.4

Figure 3. Confidence intervals for percentage of fatty acids in lard



in Table IV.

From these results we can see that there is a difference between the fatty acid composition of these animal fats and that of lamb. Lamb fat contains higher amounts of stearic acid and lower amounts of oleic and linoleic acids over that observed in pork fat. In general, lamb fat contains lower amounts of unsaturated fatty acids than saturated fatty acids when compared to pork fat. Also by comparing beef fatty acids with pork we can see a difference. Beef fat contains decanoic acid which represented about 7.2 percent but was not found in the pork fat or in lamb fat. The linoleic acid also represented in trace amounts in beef while in lard about 8.5 percent and the amount of unsaturated to the saturated acid is lower than in lard. Hilditch and Williams (1964) reported that the fatty acids of lard are higher in oleic and linoleic acid than in beef and mutton tallow. Also, lard is higher in unsaturated fatty acids than beef and mutton tallow.

TABLE IV  
A COMPARISON OF PERCENTAGE FATTY ACIDS OF PORK, LAMB AND BEEF FATS

Animal Fat Samples	Fatty Acids							
	C <sub>10</sub>	C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>16</sub> <sup>'</sup>	C <sub>18</sub>	C <sub>18</sub> <sup>'</sup>	C <sub>18</sub> <sup>''</sup>
Pork* <sup>1</sup>	-	3.4	1.6	26.2	2.5	13.6	44.3	8.5
Lamb* <sup>2</sup>	-	5.9	4.3	24.5	2.4	21.9	34.9	2.7
Beef* <sup>3</sup>	7.2	3.6	2.2	28.2	7.6	8.1	41.9	trace

<sup>1</sup>Mean of 64 samples.

<sup>2</sup>Mean of 10 samples.

<sup>3</sup>Mean of 10 samples.

## CHAPTER V

### SUMMARY AND CONCLUSIONS

The fatty acid composition of pork fat was studied using gas-liquid chromatography. The objectives of this study were 1) to determine the major fatty acids in lard using a specific detecting column; 2) to estimate the quantitative fatty acid composition of lard; 3) to compare the fatty acids of lard with some lamb and beef fats; 4) to determine the relative variation in the fatty acids of lard in gas-liquid chromatography determination associated method. Samples had been obtained from four different companies and samples of lamb and beef fat obtained from the meat lab of the Oklahoma State University.

Fat samples were converted to methyl esters of fatty acids. Gas-liquid chromatography was employed to investigate the nature of the fatty acids present in the samples. Percentages of fatty acids in samples were calculated. Statistical analyses were carried out on all lard samples. The results indicate that the major fatty acids of lard are lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acid. The highest percentage of the fatty acids is oleic followed by palmitic, stearic and linoleic acid. Unsaturated fatty acids were higher in total amounts than saturated fatty acids.

The analyses of variance for the percentage of fatty acids showed that samples within companies were significant sources of variation

for all acids excepting palmitic and palmitoleic acids. Also companies were significant sources of variation for all fatty acids except oleic acid.

It was necessary to establish percentage of composition for each fatty acid studied in order to ultimately determine whether the source was indeed lard or whether the fatty acid came from another fat. A 0.997 confidence interval ranges for fatty acids were found to be:  $C_{12}$ , 1.3-5.5;  $C_{14}$ , 0.5-2.7;  $C_{16}$ , 22.3-30.1;  $C_{16}'$ , 1.6-3.6;  $C_{18}$ , 10.7-16.6;  $C_{18}'$ , 40.8-47.8;  $C_{18}''$ , 4.5-12.4 which could be used to detect any given fat sample as lard or not. Then, if the calculated mean percentage of each fatty acid from an unknown sample does not lie within the constructed confidence interval, it may be concluded that the fat sample is not a lard sample. And if it does lie in the interval, one can not say for sure that the sample is lard. It could be lard or some other fat. Comparing pork fat with lamb and beef fat showed that lamb and beef fat contained higher amounts of saturated fatty acid than pork. Lamb is higher in amounts of stearic acid and lower in amounts of oleic acid than pork. Beef contains decanoic acid and trace amounts of linoleic acid. Also, lower amounts of stearic acid were observed in beef than in pork fat.

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TABLE V  
PERCENTAGE FATTY ACIDS IN LARD

Sample Source		C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>16</sub> '	C <sub>18</sub>	C <sub>18</sub> '	C <sub>18</sub> ''	
Farmland Co.	1-	1	3.4	1.2	25	2.6	12	46.1	9.8
		2	3.8	1.2	27.4	2.4	11.5	44.1	9.6
		3	3.5	1.2	25.6	2.8	12.6	44.9	9.4
		4	3.6	1.4	27.2	2.7	11.2	45	9
	2-	1	3.6	1.3	26	2.5	13.2	43.7	9.8
		2	3.9	1.3	26.4	2.2	13.2	43.1	10
		3	3.7	1.4	26.3	2.5	13.2	43	9.9
		4	3.2	1.4	25.2	2.4	13.3	44.8	9.7
	3-	1	3	1.3	25.5	3.1	13.3	43.7	10.2
		2	2.6	1.4	26.2	2.7	13.9	43.8	9.4
		3	3.1	1.4	25.7	2.8	12.8	44	10.2
		4	3.7	1.4	25.2	2.4	12.1	44.9	10.2
	4-	1	3.6	1.6	25.5	2.6	12.9	44.2	9.7
		2	3.7	1.5	25.6	2.7	12.8	45	8.8
		3	3.8	1.6	25.4	2.5	12.2	45.8	9.9
		4	3.8	1.3	25	2.8	12.6	44.8	9.9
Mean		3.5	1.37	25.83	2.61	12.68	44.43	9.72	
Var.		0.12	0.015	0.484	0.044	0.486	0.73	0.154	
S. Dev.		0.345	0.121	0.696	0.211	0.697	0.85	0.392	

TABLE VI  
PERCENTAGE FATTY ACIDS IN LARD

Sample Source		C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>16</sub> '	C <sub>18</sub>	C <sub>18</sub> '	C <sub>18</sub> ''
Wilson's Co.	1	3.3	1.5	27.3	1.0	14.3	44.1	7.6
	2	3.6	1.4	26.5	2.2	14.9	44.3	7.2
	3	3.2	1.4	26.5	2.1	14.7	44.5	7.6
	4	3.2	1.4	26.4	2.1	14	45.1	7.8
	1	3	1.6	27.2	2.4	14.2	44.4	7.4
	2	2.7	1.4	26.3	2.2	15	45.1	7.3
	3	2.5	1.5	26.8	2.3	14.6	45.5	6.9
	4	2.5	1.5	28.6	2.3	14.7	43.1	7.3
	1	3.4	1.4	27.1	2.1	14.4	44.4	7.3
	2	3.3	1.3	26.8	2.3	14.8	44.1	7.5
	3	3.2	1.3	26.6	2.2	14.5	44.8	7.4
	4	3.1	1.4	26.4	2.1	14.3	45.2	7.5
	1	2.8	1.4	26.9	2	14.3	45.1	7.5
	2	2.9	1.5	27.1	2.3	14.3	44.7	7.3
	3	2.3	1.3	26.6	2.1	14.8	45	7.9
	4	1.9	1.5	28.4	2.2	14.6	44.1	7.3
Mean		2.93	1.43	26.963	2.175	14.525	44.594	7.43
Var.		0.1934	0.0069	0.432	0.0156	0.0744	0.332	0.053
S. Dev.		0.44	0.083	0.657	0.125	0.273	0.567	0.23

TABLE VII  
PERCENTAGE FATTY ACIDS IN LARD

Sample Source		C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>16</sub> '	C <sub>18</sub>	C <sub>18</sub> '	C <sub>18</sub> ''	
Wickham Co.	1-	1	4.6	2.2	26.4	2.8	13.4	41.7	9
		2	4.8	2.1	25.3	2.8	13.4	41.9	9.7
		3	5.2	2.1	26.7	2.8	13.2	40.4	9.7
		4	5.6	2	25.8	2.7	13.8	41.4	8.6
	2-	1	3.3	1.8	25.4	2.6	14.3	45.6	7.1
		2	3.4	1.8	24.4	2.8	14.8	46	6.9
		3	3.5	2.2	24.6	2.5	14.5	45	7.6
		4	3.4	2.3	24.7	2.7	15.1	43.8	8.1
	3-	1	4.4	2.2	24.7	2.7	12.8	44.3	9
		2	4	2.2	24.3	3.3	13.4	43.5	9.3
		3	4.4	1.7	24.7	2.8	13.4	44	9.1
		4	5.7	2.5	25.1	2.8	12.6	42.4	8.9
	4-	1	3.4	2	25	2.9	14	45.5	7.2
		2	3.8	1.9	25.3	2.2	14.9	44.3	7.6
		3	3.4	1.8	25.6	2.5	14.1	45.1	7.6
		4	3	1.9	26.6	2.9	14	44.5	7.2
Mean		4.12	2.044	25.29	2.74	13.86	43.713	8.29	
Var.		0.7	0.045	0.55	0.051	0.513	2.65	0.895	
S. Dev.		0.84	0.212	0.738	0.226	0.716	1.63	0.946	

TABLE VIII  
PERCENTAGE FATTY ACIDS IN LARD

Sample Source		C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>16</sub> '	C <sub>18</sub>	C <sub>18</sub> '	C <sub>18</sub> ''	
Schwab Co.	1-	1	1.8	1.5	27.3	2.5	14.6	44.7	7.7
		2	2.7	1.6	27.1	2.7	13.8	44.2	8
		3	3.3	1.5	26.6	2.9	13.7	44.2	7.9
		4	2.9	1.5	26.6	2.6	13.4	45.4	7.7
	2-	1	2.8	1.6	28.8	2.7	13.5	42.4	8.1
		2	2.8	1.7	26	2.4	14.1	44.9	8.1
		3	2.9	1.6	27.1	2.6	14.5	43.4	8
		4	3.1	1.5	26.5	2.7	14.1	44	8.3
	3-	1	3.3	1.5	26.1	2.7	12.4	44.3	9.8
		2	3.2	1.4	25	2.5	12	46.5	9.5
		3	3.8	1.3	27.1	2.5	12.2	43.6	9.6
		4	3.2	1.4	26.6	2.6	11.8	45.1	9.4
	4-	1	3.3	1.4	26.5	2.9	13.6	44.4	7.9
		2	3.3	1.4	27.6	2.6	13.3	44	7.8
		3	3.1	1.4	26.7	2.6	13.7	44.3	8.2
		4	3.2	1.5	27.5	2.7	13	45.5	7.7
Mean		3.044	1.49	26.82	2.64	13.4	44.43	8.4	
Var.		0.1712	0.0098	0.65	0.02	0.695	0.843	0.53	
S. Dev.		0.414	0.0992	0.8	0.132	0.834	0.92	0.727	

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