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Effect of Re-Use of Frying Fat From Selected Food Service Establishments on the Lipid Components of Fried Shrimp

Mary Ann Harvey
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To the Graduate Council:

I am submitting herewith a dissertation written by Mary Ann Harvey entitled "Effect of Re-Use of Frying Fat From Selected Food Service Establishments on the Lipid Components of Fried Shrimp." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Nutrition.

Ada Marie Campbell, Major Professor

We have read this dissertation and recommend its acceptance:

Jane R. Savage, Frances A. Schofield, Bernadine Meyer

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

THE UNIVERSITY OF TENNESSEE
THE GRADUATE SCHOOL

ABSTRACT OF EDUCATIONAL RESEARCH STUDY COMPLETED

Author of Study Mary Ann Harvey Date November 1, 1965

Title of Study "Effect of Re-Use of Frying Fat From Selected Food Service Establishments on the Lipid Components of Fried Shrimp."

Course Number _____

Under direction of what department Nutrition and Foods Degree Doctor of Philosophy

Abstract approved by _____ Quarter Graduating December 1965
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Note: The student should consult with his major professor and follow his advice concerning the general format of the abstract. Additional pages, if required, should be 8 1/2 x 11 inches and of quality equivalent to that required in the case of the thesis. (Master's thesis - submit one single-spaced typed copy; doctoral dissertation - submit two double-spaced typed copies.)

The effect of re-use of frying fat was studied in five types of food service establishments. Samples of shrimp were obtained in quantity and fried in each establishment during two complete uses of fat. Analyses of selected samples of frying fat and shrimp lipids included: viscosity of frying fats, silicic acid column fractionation of raw and fried shrimp lipids, silver-nitrate thin-layer chromatography of frying fats and shrimp glycerides, and gas-liquid chromatography of frying fats, shrimp glycerides, shrimp phospholipids, and thin-layer subfractions of frying fats and shrimp glycerides. An attempt was made to relate changes in lipids to frying practices in the food service establishments.

Changes occurring during re-use of fat were not pronounced. Lipid changes noted in frying fats were an increase in viscosity and some decrease in polyunsaturation. The degree of change in unsaturation varied with the initial concentration of unsaturates in the fat used. The shrimp glycerides also tended toward decreased polyunsaturation, probably reflecting the changed composition of the absorbed frying fats. None of the changes appeared sufficient to warrant any consumer concern regarding the use of commercially fried foods.

In contrast to lipid data available for land animals, the results of this study indicated that the shrimp glycerides were more unsaturated than the shrimp phospholipids.

October 30, 1965

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Accepted for the Council:

Dean of the Graduate School

EFFECT OF RE-USE OF FRYING FAT FROM SELECTED FOOD SERVICE
ESTABLISHMENTS ON THE LIPID COMPONENTS OF FRIED SHRIMP

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Mary Ann Harvey
December 1965

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TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.	1
II. REVIEW OF LITERATURE.	6
Early Methods of Analysis	7
More Recent Methods of Analysis	13
III. PROCEDURE	28
Information Obtained from Personnel in Food	
Service Establishments.	28
Sample Preparation and Collection	28
Viscosity Determinations.	30
Moisture Determinations	30
Extraction of Lipid	31
Fractionation of Lipids Extracted from Shrimp	32
Silver-Nitrate-Thin-Layer Chromatography.	34
Methylation of Total Lipids and Fractions	38
Gas-Liquid Chromatographic Analysis of Fatty Acid	
Methyl Esters	38
IV. RESULTS AND DISCUSSION.	41
Viscosity of the Frying Fats.	49
Lipid Content of Raw and Fried Shrimp	49
Fractions of Shrimp Lipids.	53
Fatty Acid Composition of Frying Fats	55

CHAPTER

PAGE

IV. (continued)

Fatty Acid Composition of Glyceride and Phospholipid

Fractions of Raw and Fried Shrimp 57

Subfractions of Frying Fat and Fried Shrimp Glycerides. . . 65

Fatty Acid Composition of Glyceride Subfractions. 70

V. SUMMARY 84

LITERATURE CITED. 86

APPENDIX. 94

LIST OF TABLES

TABLE		PAGE
I.	Frying Practices at Five Food Service Establishments	42
II.	Fatty Acid Composition of Unused Frying Fat from Five Food Service Establishments (Per Cent of Total Fatty Acids)	44
III.	Viscosity of Frying Fats Sampled Before, During, and After Use in Five Food Service Establishments (Centipoises at 45° C.)	50
IV.	Lipid Extracted from Raw Shrimp and from Shrimp Fried in Five Restaurants (Per Cent of Original Moist Weight). .	51
V.	Lipid Extracted from Raw Shrimp and from Shrimp Fried in Five Restaurants (Per Cent of Original Dry Weight). . .	52
VI.	Lipid Fractions from Fried Shrimp (Per Cent of Recovered Lipid)	54
VII.	Major Fatty Acids in Frying Fats from Five Food Service Establishments, Period I (Per Cent of Total Fatty Acids) .	56
VIII.	Major Fatty Acids in Frying Fats from Three Food Service Establishments, Period II (Per Cent of Total Fatty Acids). .	58
IX.	Fatty Acid Composition of the Glyceride and Phospholipid Fractions of Raw Shrimp (Per Cent of Total Fatty Acids). .	59
X.	Major Fatty Acids in the Glyceride Fraction of Fat Extracted from Fried Shrimp, Period I (Per Cent of Total Fatty Acids)	61

TABLE

PAGE

XI.	Major Fatty Acids in the Glyceride Fraction of Fat Extracted from Fried Shrimp, Period II (Per Cent of Total Fatty Acids).	62
XII.	Major Fatty Acids in the Phospholipid Fraction of Fat Extracted from Fried Shrimp, Period I (Per Cent of Total Fatty Acids)	63
XIII.	Major Fatty Acids in the Phospholipid Fraction of Fat Extracted from Fried Shrimp, Period II (Per Cent of Total Fatty Acids)	64
XIV.	Glyceride Subfractions of Frying Fats, Period I. (Per Cent of Recovered Glyceride)	67
XV.	Glyceride Subfractions of Fried Shrimp Lipids, Period I (Per Cent of Recovered Glyceride).	68
XVI.	Fatty Acid Composition of Glyceride Subfractions of Raw Shrimp Lipids, Period I (Per Cent of Total Fatty Acids).	71
XVII.	Fatty Acid Composition of Glyceride Subfractions of the Frying Fat Obtained from a Private Restaurant (R), Period I (Per Cent of Total Fatty Acids)	72
XVIII.	Fatty Acid Composition of Glyceride Subfractions of Fried Shrimp Lipids from a Private Restaurant (R), Period I (Per Cent of Total Fatty Acids).	73
XIX.	Fatty Acid Composition of Glyceride Subfractions of the Frying Fat Obtained from a Privately Owned Cafeteria (B), Period I (Per Cent of Total Fatty Acids)	74

TABLE

PAGE

XX.	Fatty Acid Composition of Glyceride Subfractions of Fried Shrimp Lipids from a Privately Owned Cafeteria (B), Period I (Per Cent of Total Fatty Acids)	75
XXI.	Fatty Acid Composition of Glyceride Subfractions of the Frying Fat Obtained from a Drive-in Restaurant (J), Period I (Per Cent of Total Fatty Acids)	76
XXII.	Fatty Acid Composition of Glyceride Subfractions of Fried Shrimp Lipids from a Drive-in Restaurant (J), Period I (Per Cent of Total Fatty Acids).	77
XXIII.	Linoleic Acid Concentrations in the Components of Glyceride Subfraction <u>Three</u> , Period I (Per Cent of Total Fatty Acids).	79
XXIV.	Concentrations of Fatty Acid Groups in the Most Unsaturated Component of Glyceride Subfraction <u>Three</u> , Period I (Per Cent of Total Fatty Acids).	82
XXV.	Moisture Content of Raw Shrimp Samples from Two Lots and of Shrimp from Each Lot Fried in Five Food Service Establishments (Per Cent of Original Weight).	95

CHAPTER I

INTRODUCTION

Although deep-fat fried foods are universally accepted today, a possible problem exists due to the susceptibility of frying fats to chemical and physical changes and the possibility of transfer of the degradation products to foods. The work reported prior to 1954 in relation to changes in fat during heating was reviewed by Bennion and Hanning (1956). Research up to that time was undertaken chiefly by commercial companies in an effort to lengthen the frying life of fat used for large scale production of potato chips, nuts, and doughnuts. These studies revealed that when fat was used repeatedly and held at frying temperatures for relatively long periods, three main changes occurred simultaneously: hydrolysis, oxidation, and polymerization.

In several more recent studies thermally treated fats were fed to experimental animals to determine whether or not heated fat causes any change in the over-all appearance or general health of such animals (Johnson et al., 1956, 1957; Kaunitz et al., 1955; Crampton, et al., 1951a, b, c, 1956; Firestone et al., 1961; and Poling et al., 1962). Thermally oxidized fats produced a growth depression, but the effect was not a permanent one; when animals were changed to a normal diet, they quickly recovered and grew to maturity (Johnson et al., 1956). In the studies of Johnson et al., the effect was found to be multiple in nature; in addition to the characteristic growth depression, diarrhea

was noted as well as a possible enzyme-splitting or vitamin destruction. It appeared that products formed during thermal treatment were related to unsaturated portions of the oil since animals fed a thermally treated saturated fat did not exhibit the characteristic effects. This seems to indicate that glycerides containing short chain fatty acids and a relatively small percentage of unsaturated fatty acids are more stable to thermal oxidation than are corn oil and hydrogenated fats. According to Johnson et al. (1956, 1957) a high degree of unsaturation is assumed to produce thermal polymers; and Firestone et al. (1961) found that such polymers are the agents in heated fats that are toxic to experimental animals.

It was pointed out by Melnick (1957b) and by Rice et al. (1957) that results from publications such as those mentioned above cannot be related directly to changes that occur during actual commercial frying operations. They believed that the practices employed in laboratory treatment--excessive heating for long periods of time and at very high temperatures, as well as constant aeration of fat by bubbling oxygen through it--were much more severe than any treatment such fat normally would receive in cooking operations. They could find no toxic effects in animals fed fat samples taken from commercial frying establishments. Melnick also pointed out that results of studies using highly unsaturated fats heated under severe laboratory conditions could not and should not be interpreted beyond the scope of such an arbitrary situation because in most commercial operations such polyunsaturated fats are not used and also because in commercial usage constant replenishing of the heated

fat with fresh fat greatly decreases the magnitude of changes.

A study reported in 1959 by Custot demonstrated that poorly controlled heating during cooking is likely to cause much more extensive chemical degradation of the fat than is prolonged use in commercial frying operations. Although it seems that prior development of off-flavors and odors should prevent humans from eating badly oxidized or polymerized fats, this might not always hold true if the odorous fission products are distilled off as they form, as happens to some extent in frying (Lea, 1962).

Today more and more busy Americans are rushing to restaurants and quick lunch counters for meals and snacks. Fried foods are very popular choices in such situations because they can be prepared and served quickly. Very little is known about the actual practices concerning the care of fat in commercial establishments and of the effect of these practices on the lipids in fried foods. A review of the literature reveals that most of the work on changes occurring with thermal treatment of fat has involved only analysis of either heated natural fat, purified synthetic triglycerides, or purified fatty acid methyl esters. There is very little information concerning the fat in the fried foods. Poling et al. (1962) stated that cooking of food in fat changes the physical and chemical conditions so greatly that direct extrapolation of the data obtained with cooking fat is not justified.

A study of the effect of re-using frying fat was carried out by the writer as the research for the M.S. degree (Harvey, 1962). Analysis was made of the kinds and amounts of fatty acids present in potatoes

deep-fat fried in cottonseed oil and hydrogenated cottonseed oil, Each fat was used repeatedly under controlled laboratory conditions. Because the role of phospholipids has become increasingly important in the deteriorative changes that occur in foods, the frying fat and the fat extracted from potatoes were fractionated into phospholipid and non-phosphorus fractions. The changes occurring in the fatty acid distribution of the two fats with prolonged use were mainly a small decrease in linoleate with increases first in oleate and later in palmitate. It was believed that the conditions used in treatment of the fats studied were not severe enough to show significant differences in fatty acid patterns, and it was hoped that further work could be done with fats subjected to more rigorous conditions such as those occurring during heating in commercial establishments.

In consideration of the above factors, the present research was undertaken. The purpose of this study was to obtain information concerning practices in the use of frying fats in selected food service establishments and the effects of those practices on the lipids in a fried food. French-fried shrimp, as well as the fat in which they were fried, were collected from five different food service establishments in Knoxville, Tennessee, including a university cafeteria, a hospital, a privately owned cafeteria, a privately owned restaurant, and a drive-in restaurant. Fat samples were obtained from each establishment immediately prior to use, during the period of use, and just prior to disposal of fat. Frozen raw breaded shrimp were obtained in quantity and fried at each establishment in order to assure uniformity in the

samples subjected to frying and in order to circumvent the possibility of shrimp not being fried in all establishments at the designated times of collection. Shrimp were cooked at the beginning and end of each period of use. The temperature of the frying fat was recorded at each collection. An attempt was made to obtain as much information as possible concerning frying practices without influencing those practices. Records were kept of the kinds and amounts of fats used, the time and temperature of heating, the details of care and storage, and the methods and frequency of replenishing the fat. The collections of samples were carried out during two complete uses of fat, each during a different season of the year.

Lipids were extracted from the shrimp and fractionated by means of a silicic acid column into glyceride and phospholipid fractions. Further study of selected fractions of the fat extracted from shrimp included gas-liquid chromatography of most of the fractions obtained from column separation, thin-layer fractionation of the glycerides into classes according to degree of saturation, and finally gas-liquid chromatography of each of these fractions to determine fatty acid changes occurring within the glyceride classes. The cooking fats obtained from each establishment were studied by gas-liquid chromatography and selected samples were analyzed further by thin-layer chromatography followed by gas-liquid chromatography. Viscosities of frying fat samples from each frying period were determined for estimating the variation in degree of polymerization.

CHAPTER II

REVIEW OF LITERATURE

Changes occurring during thermal treatment of fat have been under study for many years. As early as 1919, Woodruff and Blunt reported a study on changes in fats absorbed by fried foods. They found that lard and cottonseed oils were absorbed to the same extent when used under identical frying conditions. Changes in iodine number, acidity, and relative amounts of red substances in fats extracted from fried foods were similar. The changes were measurable but seldom great. Lard decomposed more than cottonseed oil and long cooking increased the decomposition more than did high temperatures. These workers believed that the fat absorbed by foods during frying underwent greater chemical changes than the fat in which they were fried. At about the same time Morgan and Cozens (1919) studied changes in physical and chemical constants of fats used for frying standard doughs. They found that fats used for frying a given quantity of dough showed consistent decreases in iodine number, lowering of melting point, and increases in acidity and refractive index. These workers believed that all changes pointed to partial hydrolysis and oxidation of fats.

Some of the results obtained today through the use of the early methods are similar to those of older studies. However, newer methods have been developed which help to clarify the changes which occur during

thermal treatment of fats and oils. For that reason, the studies to be reviewed will be subdivided into two sections: (1) early methods and (2) more recent methods. An effort will be made to review the studies within each section chronologically.

Early Methods of Analysis

Carlin and Lantz (1938) studied the rate of hydrolysis of fat held at a temperature of 380° F. in the presence of a constant flow of steam. Hydrolysis was found to be slow during the first 20 hr. but accelerated rapidly after free fatty acid levels of 0.5 to 1.0 per cent were reached. According to these workers the results coincide with actual production experiences of manufacturers of fried foods from which large volumes of water must be evaporated. Carlin and Lantz reported that more highly saturated fats developed free fatty acids at more rapid rates than did less saturated fats.

Work reported by Thiessen (1939) to determine the frying life of four different fats--lard, corn oil, cottonseed oil, and hydrogenated fat--when used under household conditions revealed consistent decreases in iodine number and smoke points of fats during heating. Evidence suggesting that free fatty acids developed in all of these fats on re-use for a total of 18 hr. was found. Lard and hydrogenated fat showed the greatest amounts of hydrolysis. Thiessen found that reheating and re-use of fat up to 12 hr. did not seem to affect the quality or flavor of the fried product.

Tendencies of various fats toward foam development when used for frying after preheating treatment were shown by Robinson et al. (1940). Thermal decomposition was evidenced by increased refractive indices, lowered active oxygen values, and increased viscosities.

Carlin and Lannerud (1941) found a rapid drop in oxidative stability of fats subjected to deep-fat frying temperatures and subsequent reduction of keeping quality of potato chips. They noted foaming tendencies in fat, a decreased iodine number, an increased saponification number, and an increased refractive index as fat was used.

Chemical characteristics of commercial fats and oils were studied by Vail and Hilton (1943). They hoped to determine some chemical or physical constants for fats that might be related to their suitability for use in deep-fat frying. Smoke points and per cent free fatty acids were determined for twenty-seven unused fats and oils. They found that the smoke points were in the range 190-245° C. for vegetable fats and oils and 174-234° C. for the animal fats. Free fatty acids increased with heating in all the samples tested. All fats produced a gum formation at the surface of the fat, close to the sides of the container, after being heated twelve or more hours. There was no evident increase in viscosity, but this was not determined experimentally. Color ranged from pale yellow to dark brown with a greenish cast as the fat was heated for 20 hr.

Arenson and Heyl (1943) found that the physical structure of doughnuts fried in various fats was modified by the addition of fatty acids and by the presence of substances thermally developed in the

normal process of frying. They concluded that the stability of shortening as measured by resistance to peroxide formation was without significance in the measurement of frying quality. They used frying conditions in which the free fatty acid content of the fats did not reach levels above 0.6 per cent.

A similar study was conducted by Fisher (1949) in which a correlation was shown between thermally developed free fatty acids of frying fat and physical characteristics of doughnuts. The fats used did not develop free fatty acids above the level of 0.4 per cent, but possible influence of other heat formed constituents in the frying fat was recognized.

A summary of the work done on commercially heated frying fats was compiled by Goodman and Black (1952). The changes which occurred were a decrease in iodine value, smoke point, and melting point; an increase in refractive index, free fatty acids, and acetyl value; the appearance of foaming in frying; and flavor deterioration. Fat absorption increased in foods fried in fats that were used repeatedly.

Carlin et al. (1954) studied factors affecting the decomposition of frying fats. Hydrolysis, polymerization, and oxidation were found to take place simultaneously at elevated temperatures. These workers concluded that oxidation of hot frying fat is a much more important aspect of the problem than recognized previously and hydrolysis is only of secondary importance. They believed that undue emphasis had been given to the hydrolysis reaction because of the ease of measuring free fatty acids.

Since changes taking place in frying fats and problems involved in deep-fat frying had been studied mostly on a commercial scale, Bennion and Hanning (1956) investigated changes in lard produced by frying potatoes or fritter batters under home conditions. They found very little change in free fatty acids. Peroxide values remained low at first but increased with further heating, reaching a higher value with potatoes than with the batter samples. Iodine and saponification numbers and the smoke point were only slightly affected. Development of a yellow color in fat in which potatoes were cooked occurred slowly. Fats darkened markedly with batter frying; the gross appearance of such fat was very undesirable.

Lowe et al. (1958) in an attempt to bring up to date the information on smoke points and free fatty acid content of fats as marketed, conducted a series of experiments with hydrogenated shortenings, oils, and lard. They found that the initial free fatty acid content was lowest in oils, slightly higher in shortenings, and highest in lards, although there was some variation. The free fatty acid content of all fats increased during heating, the final content in some cases being as much as fifteen times greater than the initial concentration. The free fatty acid content of lard did not increase as much as did that of oils. The smoke points of all fats were lowered with use.

The purpose of a study by Stern and Roth (1959) was to determine to what extent the state of deterioration of fat could be responsible for differences noted in fat absorption and to find a way to maintain fat so that absorption would remain constant. Using lard as the frying

medium, these workers cooked doughnuts and determined the fat absorption, viscosity, iodine number, free acidity, and specific gravity of fat. They tried to correlate the physical and chemical changes with the degree of fat absorption of doughnuts. Fat absorption did correlate with titratable acidity, specific gravity, and viscosity, and inversely with the iodine number of the fat. Stern and Roth stated that all these values could be related to the presence of either surface active materials or polymers. Because titratable acidity is associated with surface active agents present in fat, and density, viscosity, and iodine number are all related to polymers, these workers set out to determine which of these substances--surface active agents or polymers--were responsible for changes in fat absorption. Free fatty acids and monoglycerides, two well-known groups of surface active agents, were added to fresh fat in varying amounts. The same physical measurements were made as stated previously and the results showed that the addition of fatty acids to fresh fats in amounts normally present in commercial frying did not affect fat absorption. Monoglycerides at a similar level did affect fat absorption to a slight extent, but further increases in either free fatty acids or monoglycerides to levels present in excessively abused fat had no measurable effects. In attempting to relate fat absorption to chemical and physical properties, two approaches were used: First, fat was heated to a titratable acidity of more than 1 per cent, and fat absorption, per cent free fatty acids, iodine number, and viscosity were determined. This fat then was diluted to various concentrations with fresh fat and the

same properties were remeasured. Results were compared with those of similar determinations made on fresh fat and on fats obtained from normal frying procedures. In a second approach, eight different frying fats were brought to a free fatty acid level of 0.45 per cent. Fat absorption and viscosity were determined at both 212° and 294° F. The results of both experiments directly linked viscosity with fat absorption. For eight fats heated and tested at 294° F. the coefficient of correlation between viscosity and fat absorption was 0.84. These workers believed that viscosity itself rather than some unknown factor was responsible for changes in absorption of fat. It could be possible that the increased absorption resulted from an increase in the thickness of the fat film on food with increased viscosity of the fat. It was calculated that an increase in film thickness of 0.002 mm. on a doughnut leaving the fryer could cause a significant change in the fat absorption.

Stern and Roth (1959) made a further study to find the conditions necessary to maintain the fat so that it would not be a factor in excessive fat absorption. They found that with a high enough fat turnover so that free fatty acid content of fat remained at 0.40 to 0.45 per cent and the relative kinematic viscosity stayed within 1.06 to 1.08, the cooking fat did not change with respect to fat absorption.

Effects of ill-controlled heating of domestic fat were demonstrated by Custot (1959). He found that domestic cooking is likely to cause much more extensive chemical degradation of fat than are commercial frying operations when iodine value, per cent free fatty acids, peroxide value, and per cent oxidized acids were used as the criteria.

Rust and Harrison (1960) studied the effect of method of care on the frying life of fat. This work revealed that filtering and refrigerating fats and cleaning the fryer between frying periods did prolong the frying life of fat. Regardless of the method of care, there was a significant negative correlation between acid number of the fat and acceptability of French-fried potatoes.

A relationship was found between smoke point and free fatty acid content by Zabik (1962). As smoke point decreased, free fatty acid content increased. This was interpreted as suggesting that either of these analyses provides a valid means of determining progressive deterioration of fat subjected to repeated heating.

All of the above studies have been concerned with changes occurring during thermal treatment and re-use of fat and they all have employed essentially the same types of physical and chemical measurements. Information concerning changes in specific fatty acids is lacking, mainly because of inadequate methods of analysis. The quantities of some lipid components extracted from foods are too small for macro-analysis; yet modern technology has and is continuing to develop newer methods so that the study of these minute substances now is being carried out.

More Recent Methods of Analysis

With the advent of newer methods of analysis, changes occurring during thermal treatment of fats are being studied in more detail. The long used chemical and physical tests such as iodine number, acetyl

value, free fatty acid determinations, peroxide number, and others are still used, but only as supplements to newer methods such as gas-liquid chromatography, thin-layer chromatography, column chromatography, spectrophotometric analysis, densitometric determination, and infrared analysis.

Swift et al. (1948) studied the decomposition of methyl hydroperoxido oleate using spectrophotometry and identification methods commonly used in organic chemistry. The results indicated that one of the decomposition reactions of methyl hydroperoxido oleate is fission to produce α, β -unsaturated carbonyl compounds, one of which is 2-undecenal. These workers stated that aldehydes of the type represented by 2-undecenal readily undergo oxidation, reduction, and isomerization thus making it possible to account for many of the end products which have been identified in various types of fat-oxidizing systems.

Chang et al. (1952) studied fatty acid changes in commercial fats, (lard, butter, and vegetable oils) when used in baked products, hard candy, and as a medium for deep-fat frying. The changes occurring during deep-fat frying included an initial increase in peroxide number when fat was heated to 180° C. and kept at that temperature for 10 min., followed by a decrease in the peroxide number to a negligible value after 40 min. of additional heating. The changes occurring with prolonged heating at 180° C. indicated that peroxides were decomposed as fast as they formed.

Conjugated dienes increased significantly in the corn oil with

the additional heating period. Total unsaturation as measured by iodine number showed a gradual decrease with heating, resulting in an increase in the calculated value of saturated fatty acids. Changes in oleic acid were small, while linoleic definitely decreased and linolenate decreased slightly at first and then increased to a higher value than the original. According to Chang et al., the apparent increase in linolenate and decreased percentage of linoleate probably could be explained by slight oxidation of the large amount of linoleic acid present. The fatty acid determinations were made by saponification with potassium hydroxide followed by spectrophotometric analyses to determine linoleic, linolenic, arachidonic, and conjugated diene acids.

In 1952 James and Martin published the first practical details of the technique known as gas-liquid chromatography. Since the development of this apparatus, analysis of small quantities of mixtures of volatile liquids has been possible. The apparatus has been found useful in most aspects of research and chemical industry and recently has come into much use for analysis of changes occurring in food lipids.

Some of the early work in which gas chromatography was used in study of food lipids was that reported by Lee and Mattick (1961) and Mattick and Lee (1961) on vegetable lipids. These workers studied the influence of prior treatment upon alteration in vegetable lipids during freezer storage. After one year of storage decreases in several fatty acids in the phospholipid fraction from raw peas and spinach were large in comparison to changes in blanched samples. These workers thought that

changes in fatty acids in foods could be involved in the development of off-flavors in frozen foods during storage.

Johnson et al. (1956, 1957) studied the effect of heating on the nutritive value of edible oils. The changes in heated fat that were believed to produce a growth depression in experimental animals were found to be related to unsaturated portions of the oil. These workers also found a relationship between the linoleic acid content of fat and thermal oxidative damage as measured by comparative growth in rats.

Methyl esters of lauric, stearic, and oleic acids were oxidized thermally and their decomposition products and residual materials were analyzed by Ramanathan et al. (1959), who hoped to gain insight into the mechanism of oxidation. The loss in weight of each ester and amount of volatile non-condensable phase in terms of carbon dioxide, carbon monoxide, and hydrogen were measured. The condensable vapor phase was analyzed and found to contain a variety of carbonyl and carboxyl compounds, indicating a random oxygen attack of the ester molecule. The ester was analyzed for carboxyl, ester, carbonyl, and hydroxyl groups, and for unsaturation. Longer carbon chains were more susceptible to oxidation. These workers, using the analytical values and decomposition products that they had found, proposed a possible mechanism of thermal oxidation. Their suggestion as to sequence of steps was as follows: unsaturation; hydroperoxide formation; instantaneous decomposition of the hydroperoxide to form various hydroxy, carbonyl, and carboxyl compounds; decomposition of these compounds to release carbon dioxide, carbon monoxide, and water; and polymerization

through either carbon-carbon or ester linkages.

Nutritional and chemical changes in heated fats were reviewed by Perkins (1960). He recognized and defined three types of degradative changes which can occur in fats or oils: autoxidation, thermal polymerization, and thermal oxidation. Perkins believed that from results of available research it is not possible to predict whether unsaturated oils are damaged sufficiently during processing and commercial frying operations to be harmful. He did think, however, that data available were sufficient to justify the suspicion that the use of oils containing polymeric materials might not be desirable from a nutritional point of view. A need for more practical research in the area of commercially heated fat was emphasized.

Wurziger and Ostertag (1960) studied the relationship between chemical and physiological properties of heat-treated fats and determined the changes occurring in fats heated for 12 hr. at 180° C. Soybean oil, lard, hydrogenated soybean oil, and hydrogenated lard were used in the study. Results revealed mainly a decrease in unsaturation with heating regardless of whether heating occurred in the presence or absence of air. This was evidenced mainly by decreases in linoleic and linolenic acid content. Rats fed the various oxidized fats at the 10-20 per cent level were found to have carbonyl compounds from highly oxidized fats deposited in their carcass fat.

In an effort to secure more direct information on changes occurring in fats during commercial deep-fat frying, Poling et al. (1960) obtained thirty-four samples of "used" fat from a variety of

establishments. These fats along with laboratory heated samples were fed to rats for twelve weeks. The rats receiving the laboratory heated fat showed adverse symptoms while those receiving the commercially heated samples did not. These workers believed that the differences between laboratory heated samples and commercially used ones could have been due to any one of a number of factors. The conditions of commercial usage differ so widely from those of laboratory testing that extrapolation of the laboratory data to commercially used fats is of questionable significance. Under conditions of commercial usage, fats are exposed to varying quantities of water, to foodstuffs of various types, and to varying amounts of agitation; they may or may not be purified by decanting or filtration; and they often are supplemented with fresh fats to an extent which, in some applications, as the frying of potato chips, results in a rapid turnover of the frying fat. Any or all of these conditions could alter the effect of heat on fat and result in differences in the biological responses of rats fed laboratory heated and commercially heated fats.

In a similar study Rice et al. (1960) undertook the development of methods which would detect readily changes in fat quality. They used a restricted feeding technique to determine whether heat damaged oil had less available energy. They found that available energy was markedly reduced by severe heating or oxidation of fat. This technique was applied in a series of studies on factors which might damage fat.

1. Several samples of salad oil and shortenings were heated in the presence of oxygen and results indicated that decreases in available

energy were consistent with all fats. These findings were the same whether the fat was heated at 60° C. for long periods or at 182° C. for shorter lengths of time.

2. The influence of varying the percentage of oil exposed to air at any given temperature also was studied. Varying amounts of oil were heated in the same pan for one to five days; and in other tests constant amounts of oil were heated at different temperatures. Results indicated that a change in the amount of fat heated per unit of surface area had more influence than did a change in temperature. When oil was heated in small quantities, it became very viscous and after 6 hr. of heating had to be scraped from the pan. These workers stated that treatments in which changes in color, viscosity, and flavor were readily detectable were much more severe than conditions encountered in reasonable home or commercial cooking.

The conclusions drawn by Rice et al. (1960) were in agreement with those of Melnick's studies on oils from eighty-nine manufacturers (1957a,b). Both found insignificant changes in iodine values during processing and claimed on this basis that products had not been changed to any significant extent.

The studies which have shown significant biological effects of heated fats have been those where fats were subjected to excessive abuse under laboratory conditions (Kaunitz et al., 1955; Andrews et al., 1960; Crampton et al., 1951b,c; Perkins and Kummerow, 1959; Firestone et al., 1961; and Poling et al., 1962).

Crossley et al. (1962) used tricaprin and 2-oleo-dipalmitin in an

attempt to determine the mechanism of degradation of triglycerides at temperatures of 190-300° C. They heated the triglycerides with and without oxygen and studied the many degradation products formed. Even the pure fully saturated triglyceride was rendered organoleptically unacceptable due to breakdown in this temperature range, although the mechanism differed depending on the presence or absence of oxygen. The unsaturated glyceride was more readily degraded, with fission at or near the double bond being superimposed on the breakdown process established for saturated triglycerides. Crossley and associates found that the degraded triglycerides could be refined to an acceptable standard by conventional means, but the refined triglycerides did not remain stable as long as the original compounds.

The nutritional value of fats in cooked meats was studied by Warner et al. (1962). The purpose of the study was to demonstrate that fats in meats can withstand normal cooking conditions without objectionable changes in flavor, odor, or nutritive value. Several types of meat--beef rib roast, ground beef patties, skillet fried chicken, T-bone steak, bacon, short shank ham, pork chops, spareribs, leg of lamb, and turkey--were cooked in a research kitchen under conventional household conditions. For maximum exposure to heat, each cut was cooked at a temperature higher than usually recommended and to an over-done stage. Fats extracted from the meats were fed to weanling rats for seven days. The diets of these animals were limiting only in energy so that weight gains depended on the available energy in the test materials. The results indicated that the biologically

available energy of these fats was not changed during cooking. Also, none of these fats caused any noticeable biological defect in organ size of animals as had been shown previously when animals were fed laboratory heated fats (Rice et al., 1960). Warner et al. concluded that cooking does not alter the biological value of meat fats.

Florin et al. (1963) determined the fatty acid composition of various cooking fats and oils by the use of gas-liquid chromatography. The linoleic acid values reported as per cent of total were: sesame, 45.55; corn, 66.50; coconut, 0.57; cottonseed, 65.46; olive, 2.51; peanut, 39.40; safflower, 91.40; and soybean, 64.50. Analysis of oils which had been used to fry potatoes or chickens or of oils which had been heated under laboratory conditions (495° F.) indicated that the polyenoic acid content had been decreased significantly (in some cases as much as 30 per cent), with a concomitant apparent increase in the palmitic acid content.

Fats and fatty acids are polymerized by oxidative or thermal processes. Structures have been deduced by a number of chemical and physical techniques. Firestone (1963) reviewed the general methods applicable to the analysis of polymerized oils including determinations of acetone number, iodine value, molecular weight, dielectric constant, viscosity, and refractive index. Monomers, dimers, and trimers have been separated by molecular distillation. In addition urea fractionation and a number of chromatographic techniques including column, thin-layer, paper, and gas-liquid chromatography were suggested as useful for detection of monomers, dimers, and polymers.

Polymerization in frying fats and fats extracted from foods was studied by Sahasrabudhe and Bhalerao (1963). Isolation of polymerized products in frying fats was carried out by the use of urea fractionation methods. Corn oil and a hydrogenated vegetable oil were heated under laboratory conditions and for comparison oils were obtained from two private restaurants. Samples of commercial potato chips and frozen French-fried potatoes were fried in the fats and then analyzed for the amount of non-adduct forming fraction. The results revealed that the amount of urea non-inclusion fraction (NAF) was in direct relation to the duration of heating. Oils heated at 200° C. for 24 hr. yielded 15-18 per cent NAF having molecular weights of 500-550. Some of the fats extracted from fried foods yielded up to 2.5 per cent of the polymeric fatty acids.

A method was developed by Youngs and Subbaram (1964) for direct determination of the glyceride composition of natural fat, involving oxidation of the fat by permanganate-periodate, esterification of the oxidized glycerides, and subsequent gas-liquid chromatographic analysis. The composition of four vegetable oils--cottonseed, cocoa butter, olive, and soybean--was determined. Results were compared with those obtained by other methods.

Subbaram and Youngs (1964a) devised a method which gives the distribution of saturated and unsaturated fatty acids. It involves fractionation of triglycerides into groups on the basis of total unsaturation by employing chromatography on a silicic acid-silver nitrate column. The glyceride composition of each fraction then can

be determined by gas-liquid chromatography.

Combining the two methods described above, Subbaram and Youngs (1964b) determined the glyceride and fatty acid composition of fat from seven animals: human, dog, squirrel, chicken, pig, rabbit, and guinea pig; they also analyzed seven vegetable fats: palm oil, corn oil, olive oil, soybean oil, cottonseed oil, linseed oil, and cocoa butter.

Triglycerides of natural fats and oils also have been studied by thin-layer chromatography on silicic acid impregnated with silver ion. The method of thin-layer chromatography was given its real impetus through the work of Stahl, who in 1956 demonstrated the first practical method of applying thin layers of adsorbents to glass plates and applied the technique to the separation of a great number of substances. Litchfield et al. (1964) separated the triglycerides of Cuphea llavia seed fat according to the number of double bonds per molecule, using thin-layer chromatography. The recovered fractions were quantitated by the chromotropic acid technique. Each fraction was studied further by gas-liquid chromatography. This multiple chromatography procedure resolved C. llavia triglycerides into seventeen different components.

Many other workers have employed thin-layer chromatography with silver nitrate-silicic acid in the study of triglycerides. Reports by Privitt and co-workers (1961, 1962, 1963, and 1965) and by Blank et al. (1964, 1965) as well as those by many others have demonstrated the effectiveness of this method in determining characteristics and changes of micro amounts of substances. Reviews on practical uses and pitfalls

of thin-layer chromatography have been written by Mangold (1961), Fontell et al. (1960), and Pelick et al. (1965) and discussion of various aspects of the subject can be found in books by Truter (1963), Bobbitt (1963), and Randerath (1964).

The effects of heating fats in air and the effects of heating them in an inert atmosphere, as well as the relation of air to changes in the frying characteristics of fat, were studied by Rock and Roth (1964a). The frying characteristics of fat did not change to an extent that was commercially significant even after 48 hr. of heating at 375° F. in the absence of air; fats heated under identical conditions but in the presence of air changed radically. It was found, also, that fat which had changed appreciably in frying characteristics when heated in air did not continue to change significantly when heated further under nitrogen. Hence, the presence of oxygen seemed to be a necessary condition for deterioration of frying qualities of fat at frying temperatures. The rate of change in the frying characteristics was found to be directly proportional to the degree of exposure of fat surface to oxygen.

In a related study Rock and Roth (1964b) found that the rate of fat deterioration varied inversely with temperature of the heating element when a commercially prepared hydrogenated lard and heating methods simulating those commonly employed in commercial fryers were used. This finding was attributed to differences in the rate and duration of convection circulation, which are considered to be a measure of the amount of air-fat contact, and therefore, of thermal

oxidation. When the element temperature was increased from 900° to 2400° F. by the use of different heating elements and oil temperature was maintained thermostatically at 375° F., the rate of convection circulation increased while the duration of convection circulation decreased. These workers postulated that the over-all effect of an increasing element temperature was one of decreasing the total amount of fat in contact with the air. They also demonstrated that mechanical circulation of fat, as required in an externally heated fat system, significantly increased the rate of fat deterioration compared to that of fat heated by conventional direct gas heat if both were heated and maintained at 375° F. for identical periods of time.

Kilgore and Luker (1964) studied chemical changes which occur in a fat during thermal oxidation by frying two foods, chicken and potatoes, in cottonseed oil and in lard. These foods were chosen since it had been shown that factors affecting chemical changes in fat were: length of time fat is exposed to heat and the heating temperature (Johnson et al., 1957); mixed fatty acid composition and position of fatty acid in the triglyceride (Ramanathan et al., 1959); presence of metallic ions such as iron (Lundberg, 1954); presence or addition of hematin compounds (Maier and Tappel, 1959); presence of water vapor (Porter et al., 1932); amount of fat heated per unit surface area (Rice et al., 1960); presence of amino acids and carbohydrates (Lips, 1951, and Marcuse, 1962); and processing conditions used to refine, decolorize, and deodorize the fat (Wilding et al., 1963). The chicken represents a food with a relatively high fat, protein, and hematin content; and

potatoes have a high carbohydrate and low fat, protein, and hematin content. The frying fats as well as fats extracted from the chicken and potatoes were studied. The fats were used up to 10 hr. The fatty acid values for cottonseed oil, as obtained by gas chromatography, showed that the linoleic acid content decreased from 57 to 49 per cent. This decrease was observed regardless of whether chicken or potatoes were fried. There was no change in the linoleic acid content of lard when chicken was fried but a decrease from 11.3 to 5.8 per cent when potatoes were fried in fat heated 10 hr.

The fatty acid content of the fat extracted from potatoes and the fat used to fry them was the same at the end of 5 hr. of heating. However, at the end of 10 hr., fat extracted from potatoes had a lower linoleic acid content than did the fat used to fry them. Results were the same for lard and cottonseed oil. Fatty acid components of fat extracted from chicken reflected the fatty acid composition of the chicken as much as of the fat used for frying regardless of whether the fat was used for 5 or 10 hr.

Results of a study on the effect of heating corn oil in air at 299° C. were reported by Sahasrabudhe and Farn (1964). The heated oil was separated on a silicic acid column into eight fractions. The first four fractions, making up 62 per cent of the original oil, were found to be unchanged triglycerides. The remaining four fractions were composed of polymeric and degraded products of high molecular weights. Percentage losses from the different positions in the oleo- and linoleo-glyceride fractions suggested that fatty acids in the primary positions

are slightly more susceptible to heat than those in the 2-position. Assuming a 1,3-random 2-random distribution, triglyceride fractions in the heated oil contained 6.7 per cent triolein as compared to 17.7 per cent in the same fractions of fresh oil. Evidence was presented showing the presence of branching in short chain unsaturated acids and of hydroxy acids in the saponified polymeric fractions.

CHAPTER III

PROCEDURE

Information Obtained from Personnel in Food Service Establishments

Managers or owners of five different food service establishments were interviewed personally to determine whether they would be willing to cooperate in the study. If willingness was expressed, questions were asked concerning the type of fat used and some of their practices regarding use and care of fat. Information was obtained concerning capacity of fryer, foods fried, frying schedules, and method of replenishment. Schedules for collection of samples during two complete uses of fat were based on frying practices of the various establishments.

An attempt was made to obtain more specific information from kitchen personnel during the periods of collection. Observation at times of sample collections supplemented the information provided by personnel with respect to care of fat.

Sample Preparation and Collection

Sample collections were made during the summer (period I) and again during the winter (period II). In each period samples were collected from each establishment about midway between two changes of fat, as well as when the fat was fresh and when it was about to be replaced. Collection schedules differed with the establishments because the length of time that a given lot of fat was used varied from three days to two months. For period I a quantity of jumbo

frozen breaded shrimp sufficient for frying at all establishments was obtained for the sake of uniformity of samples subjected to frying. Portions consisting of six shrimp each were wrapped with heavy duty aluminum foil, flushed with nitrogen, and stored at -20° C. until fried. For period II the shrimp that were obtained were smaller. Again the shrimp were used in six-shrimp portions.

At the time of sample collection the temperature of the fat was recorded and the frozen shrimp were fried for 3-5 min. depending on the length of time required for them to rise to the top of the fat, which varied with the temperature. After the shrimp were removed from the fat, they were drained on six thicknesses of paper toweling for 3 min. and wrapped with foil.

A sample of frying fat was taken immediately prior to each frying of shrimp. Samples of fat and shrimp, which were collected from all five establishments before use of the fat, at the midpoint of the period of use, and just prior to disposal of the fat, will be referred to hereafter as samples 1, 2, and 3, respectively.

Immediately after collection the samples were taken to the laboratory. A portion of each fat sample was stored at -20° C. under nitrogen in a stoppered Erlenmeyer flask. The remainder of each fat sample was used for measurement of viscosity. The shrimp were cooled for 1 hr. and then minced in a Waring Blendor. Samples of the ground shrimp were used for the determination of moisture content. Lipids were extracted from the remainder of the ground shrimp. Moisture determination and lipid extraction were carried out also for samples

of the raw shrimp from the two periods of collection.

Viscosity Determinations

By means of a Brookfield Synchro-lectric Model LVF viscometer, viscosity measurements were made on all frying fat samples. The calibration of the instrument was checked with Standard Viscosity Oils L and M obtained from the National Bureau of Standards. Spindle 1 was used with the guard for all viscosity measurements and readings were recorded for each of three speeds--12, 30, and 60 r.p.m. A temperature of 45° C.* was used for all measurements because that temperature was found to give the most consistent readings for the standard oils at the three speeds. The values obtained at three spindle speeds were averaged.

Moisture Determinations

Samples weighing 3-5 g. each of the minced shrimp were weighed on an analytical balance into tared moisture pans. The pans containing the samples were placed uncovered in an air oven and dried for 18 hr. at 100° \pm 10° C. At the end of the drying period samples were covered, removed from the oven, and cooled in a desiccator for 1 hr. Weighing was repeated and the loss, representing moisture, was expressed as per cent of original weight.

*Maintained within 2° C. by means of a sand bath.

Extraction of Lipid

Lipid extraction was accomplished by a modification of the method of Ostrander and Dugan (1962). Approximately 100 g. of shrimp were extracted at a time. The fresh or fried shrimp were chopped finely in a Waring Blendor and weighed accurately into tared beakers. The samples then were transferred to a stainless steel blender jar with the aid of one-fourth piece of facial tissue. The beaker was rinsed with small portions of methanol. Methanol (130 ml. including that used for rinsing the beaker) was added to the sample along with a small piece of dry ice and approximately 0.5 g. hydroquinone. This mixture was blended for 5 min. with a rheostat setting of approximately 20. Sixty-five milliliters of chloroform were added and blending was continued for another 5 min. The sides of the blender jar were scraped with a polyethylene spatula and 65 ml. of chloroform were added. Blending then was continued for 30 sec. Addition of 65 ml. of distilled water containing 1.5 g. zinc acetate was followed by blending for 10 sec. At the end of this time the sample-solvent mixture was poured quantitatively into a No. 3 Büchner funnel which had been fitted with Whatman No. 1 filter paper. The sample was filtered with suction into a 1000-ml. suction flask. For protection of the sample during the filtration process, the Büchner funnel was placed under carbon dioxide vapors. The carbon dioxide, from a separatory funnel containing a piece of dry ice in water, was carried through rubber tubing from the top of the separatory funnel to the stem of a filter funnel inverted over the Büchner funnel.

After filtration the residue was transferred along with the filter paper back into the blender jar. The funnel was wiped with one-fourth piece of facial tissue and the sample again was blended with chloroform (100 ml.) for 2 1/2 min. The sample then was transferred to the funnel. When the residue on the filter appeared to be dry, the residue and funnel were rinsed with 50 ml. of chloroform. The residue was discarded and the filtrate was transferred to a 1000-ml. graduated cylinder with a small volume of chloroform. The cylinder was flushed with nitrogen, covered tightly with aluminum foil, and stored in the refrigerator for 4 hr. or longer until phase separation was clear-cut. At the end of the storage period, the volume of the bottom layer containing lipid in chloroform was recorded and the aqueous top layer was siphoned off and discarded. Two 10-ml. portions of the lipid extract were taken by pipette for determination of weight of extracted lipid. The last traces of water were removed by anhydrous sodium sulfate which was added to the cylinder and left in the lipid extract for at least 1 hr. The mixture then was filtered into a 1000-ml. boiling flask and the volume was reduced in a rotary evaporator. The lipid was transferred to a 25-ml. Erlenmeyer flask with a small amount of chloroform and stored under nitrogen at -20° C. Lipid concentration in shrimp was calculated on both moist and dry weight bases.

Fractionation of Lipids Extracted from Shrimp

Fractionation of the lipid extracted from shrimp was carried out by a modification of the method of Hornstein et al. (1961). A glass chromatographic tube with a teflon stopcock was used. Fifty grams of

silicic acid (100 mesh), which had been activated by heating in an air oven at $110^{\circ} \pm 10^{\circ}$ C. for 20 hr., was mixed with 100 ml. of 3:1 chloroform-methanol* (v/v) in a 250-ml. graduated cylinder and allowed to stand, covered, for 30 min. The fine suspension and liquid on top were poured off and the mixture was made back to 130 ml. with the same solvent system. The silicic acid slurry was stirred and poured into the chromatographic tube. The slurry was stirred with a long glass rod to remove air bubbles. The sides of the column were rinsed with the same solvent mixture and the silicic acid was allowed to settle. Settling was hastened by means of slight nitrogen pressure applied to the top of the tube. When the solvent level was approximately 2 in. above the silicic acid, granular anhydrous sodium sulfate was added, forming a layer approximately 2.5 cm. thick over the silicic acid. Washing of the column, begun when the solvent was just above the packing, consisted of a wash with 200 ml. of 20:1 chloroform-methanol followed by 300 ml. of chloroform. After the chloroform washing, the column was ready to be used.

Chloroform extract containing approximately 5 g. of shrimp lipid was applied to the column. For determination of the volume of extract to be applied, two 1-ml. portions of the lipid extract were removed and dried. The average weight of these subsamples was used also for calculating the weight of sample fractionated.

*Methanol used in fractionation was dried over anhydrous sodium sulfate and redistilled.

After the sample in chloroform was poured into the column, it was rinsed down with three small washings of 20:1 chloroform-methanol and eluted with 300 ml. of 20:1 chloroform-methanol, followed by 200 ml. of 1:1 chloroform-methanol, and finally 300 ml. of methanol. The neutral lipids were collected in a 1000-ml. boiling flask. The receiver was changed when the second solvent front (1:1 chloroform-methanol) reached a marked height (approximately 10 cm.) from the bottom of the column. The two eluates containing phospholipids were collected in a single 2000-ml. boiling flask. The drop rate was maintained at 2-4 ml./min. by means of slight nitrogen pressure. The lipids were protected during collection by a small stream of nitrogen directed into the boiling flasks. After the collection of each fraction, the extract was transferred quantitatively into a graduated cylinder and the volume was recorded. Two 5-ml. portions were removed from each extract for determination of weight of the lipid fraction. The remainder of each fraction then was concentrated in a rotary evaporator, rinsed with chloroform into a 25-ml. Erlenmeyer flask, and flushed with nitrogen. The flask was stoppered and stored at -20° C. The concentration of each fraction was calculated as percentage of total lipid recovered.

Silver-Nitrate-Thin-Layer Chromatography

A modification of the thin-layer chromatographic method described by Litchfield et al. (1964) was used for separation of the neutral lipids of shrimp. The procedure also was used for the study of selected samples of cooking fats.

Silica Gel G* (30 g.) was slurried with 60 ml. of a 12.5 per cent (w/v) aqueous solution of silver nitrate in a beaker. The slurry was applied quickly to 10-x 20-cm. glass plates in a 1.0-mm. layer. The plates, each containing 10-15 g. of Silica Gel G, were dried in air for 40 min. and then activated in an atmosphere of nitrogen at $110^{\circ} \pm 10^{\circ}$ C. for 45 min. The inert nitrogen prevented darkening of the adsorbent layer during heating. After activation, the plates were stored in a metal rack which excluded light and contained a desiccant.

Lipid in chloroform was applied as a narrow streak across the base of the plate by means of a 50- μ l. microsyringe. Streaking was continued until approximately 100 mg. of sample had been applied. Sample application required three to seven streaks, depending on lipid concentration. The plate then was placed in a stream of nitrogen for removal of solvent before development.

The plates were developed by the usual ascending technique with a mixture of 1 per cent ethanol in alcohol-free chloroform. The alcohol normally added to chloroform as a preservative was removed by treatment of the chloroform with activated alumina** just prior to use. Development was carried out in a metal cylinder covered with an asbestos pad that eliminated light. For complete saturation of the cylinder, the solvent was placed in the container at least 1 hr. before the plate was developed. Each plate was developed twice in the same

*Brinkmann Instruments, Inc.

**Brockman, Activity grade 1.

direction for satisfactory resolution. This double exposure required about 50 min. After development, plates were dried under nitrogen, sprayed with a 0.02 per cent solution of sodium fluorescein, and viewed under long-wave ultra-violet light. Seven distinct bands were observed and were eluted by the method of Van Handel (1964). Each band was scraped from the plate into a 25-ml. test tube. To each tube, 10 ml. of chloroform were added. The samples were mixed well and flushed with nitrogen. Tubes were stoppered and stored overnight in the refrigerator.

The amount of triglyceride in each thin-layer fraction was determined by a modification of the chromotropic acid color reaction of Van Handel and Zilversmit (1957). Values obtained from triplicate determinations for two thin-layer separations were averaged. The thin-layer fractions were brought to room temperature and each was filtered rapidly through coarse, chloroform-washed paper into a 25-ml. volumetric flask. The sodium fluorescein remained with the silica gel. The samples were made to volume with chloroform and three 1-ml. aliquots were pipetted quickly into separate 15-ml. conical tubes. The remaining chloroform extract was returned to the 25-ml. test tubes, flushed with nitrogen, and stored at -20° C. A solution of corn oil in chloroform, containing 0.05 mg./ml. was prepared for use as a standard. One milliliter of the standard solution was pipetted into a 15-ml. conical tube, and the solvent was evaporated from all of the tubes. After evaporation of the solvent, a 1-ml. portion of 0.07 N alcoholic potassium hydroxide was added to each

sample and standard tube and to an empty tube (reagent blank). The samples were saponified at 60-70° C. in a thermostatically controlled water bath for 30 min. The tubes were removed from the bath and cooled, and 1 ml. of 0.2 N sulfuric acid was added. The tubes were immersed to half their length in a boiling water bath for 15-20 min. until the odor of alcohol disappeared. After cooling of the tube contents, one drop of 0.025 M (0.5 per cent) sodium periodate was added, and after 10 min. at room temperature, one drop of 5.0 per cent sodium bisulfite was added. After another 10 min. at room temperature 10 ml. of chromotropic acid* were added to each tube, and the tubes were stirred well with separate glass rods. This proved to be a very important step because samples which were not mixed well gave inconsistent results. A marble was placed on each tube and the tubes were immersed to half their length in a 90° C. water bath and held for 30 min. The water bath containing the tubes was covered with aluminum foil which excluded excessive light. The tubes then were removed from the water bath and cooled for at least 1 hr. Optical density of the samples was determined at 570 mμ. in a "Spectronic 20" spectrophotometer (Bausch and Lomb). The instrument was zeroed with the reagent blank and the weight of glyceride in each band was calculated according to the equation:

$$\text{mg. triglyceride} = \frac{\text{O. D. unknown}}{\text{O. D. standard}} \times 0.05 \times 25$$

The amount of glyceride in each band then was expressed as a percentage

*4,5-dihydroxy-2,7,naphthalenedisulfonic acid, 0.2 per cent in 60 per cent sulfuric acid.

of the total glyceride recovered.

Methylation of Total Lipids and Fractions

Selected samples of frying fats and fractions obtained by column and thin-layer chromatography were methylated by a modification of the method of James (1960). Approximately 40 mg. of lipid were placed in a 15-ml. conical tube. To the sample were added 5 ml. of 5 per cent sulfuric acid in methanol (v/v). Each tube was covered with a marble and heated for 1 hr. in a thermostatically controlled water bath at 70° C. The sample was stirred frequently. At the end of the reflux period the sample was poured into a 30-ml. separatory funnel equipped with a teflon stopcock. To the funnel were added 6 ml. of redistilled petroleum ether and 2 ml. of distilled water. Separation into two phases occurred immediately, and the lower phase, containing the methanol, sulfuric acid, and water, was drawn off and discarded. The petroleum ether, containing the methyl esters, was washed once more with water; after disposal of the aqueous bottom layer, the petroleum ether-methyl ester layer was poured into a 15-ml. conical tube. Anhydrous sodium sulfate was added to remove the remaining traces of water and was allowed to remain at least 1 hr. The mixture then was poured into a clean 15-ml. conical tube and stored under nitrogen at -20° C. until chromatographed.

Gas-Liquid Chromatographic Analysis of Fatty Acid Methyl Esters

The petroleum ether was evaporated from the methyl esters under nitrogen. When all the solvent was removed, the esters remaining were

diluted with hexane* and injected into a Barber-Colman Model 61C gas chromatograph equipped with an argon ionization detector with radium source. A 7-ft. coiled aluminum column (1/4-in. inside diameter) packed with diethylene glycol succinate polyester (13.0 per cent by weight on 80-100 mesh Gas-Chrom P**) was used. The column, with argon flowing through, was preconditioned by heating for approximately 48 hr. at a temperature of 190° C. before it was connected to the detector.

The following conditions were used for gas-liquid chromatographic analyses:

Column Temperature	175° C.
Cell Temperature	215° C.
Flash Heater	220° C.
Split Heater	230° C.
Voltage	900
Argon Pressure	20 psi
Sensitivity	10

Peaks were identified by comparison of their retention times with those of the components of a standard mixture of fatty acid methyl esters*** (Horning et al., 1964).

The "area" under each peak was calculated as the product of peak height and width of peak at half-height. Fatty acid concentration

*Matheson Coleman and Bell, Chromatoquality.

**Applied Science Laboratories, State College, Pennsylvania.

***National Institutes of Health Standard Mixture D.

was calculated as per cent of total fatty acids:

$$\text{per cent fatty acid} = 100 \times \frac{\text{"area" of peak}}{\text{total "area" under all peaks}}$$

Two chromatograms were obtained for each methyl ester sample and the results were averaged.

CHAPTER IV

RESULTS AND DISCUSSION

All of the restaurant managers or owners expressed a willingness to assist with the study. They continued to be cooperative in their attitude but collection of samples according to plan was difficult; the chief problem was the investigator's dependence on restaurant employees who had neither sufficient time nor sufficient knowledge to be ideally cooperative.

A summarization of the frying practices in the five different establishments is presented in Table I. Each place will be discussed separately because the practices varied greatly.

In the privately owned restaurant, which will be designated throughout this paper as R, pure lard* was used as the principal component of the frying medium. Beef tallow, which had been trimmed from steaks and rendered, was added to the frying fat in undetermined and variable quantities. The predominating fatty acids of this mixed fat were oleic and palmitic acids (Table II). The difference in composition between periods I and II probably was due to the use of a lower proportion of beef fat in period I than in period II. A small amount of linoleic acid was detected in the mixture used in period I.

Three electric deep-fat fryers, each having a capacity of 50 lb.

*Armour or Morrel

TABLE I

FRYING PRACTICES AT FIVE FOOD
SERVICE ESTABLISHMENTS

Practices	Establishment				
	R	B	J	F	S
Fat used	Lard and beef fat	Modified soybean oil with and without cottonseed oil	Animal-vegetable fat mixture	Cottonseed oil	Modified soybean oil
Fryer capacity (lb. of fat)	50	35	29	45	29
Foods fried	Meats chicken fish shrimp Vegetables potatoes eggplant okra onions	Meats fish shrimp Vegetables potatoes eggplant Batters fritters doughnuts	Meats fish filets Vegetables potatoes	Meats fish shrimp cutlets chicken Vegetables potatoes okra eggplant others	Meats chicken fish chops cutlets croquettes
Temp. of fat (°C.)	172-206	161-164	196-222	162-178	187-212

TABLE I (continued)

Practices	Establishment				
	R	B	J	F	S
Approximate heating time per day (hr.)	16	8	24	5	6
Frequency of use	Constantly during hours	During three meals per day	Constantly	During meals; not at all meals	During lunch and dinner
Total hours of heating per period of use	48	480	72	45	24
Frequency of straining fat	Once daily	Twice daily	Once daily	Average once daily*	After each use
Storage between uses	Left in fryer	Left in fryer	Left in fryer for continuous use	Left in fryer	Refrigerated
Approximate amount of fresh fat added per day	1-25 gal.	1 gal.	8 gal.	1 gal.	1 gal.

*Depended on the cook and the foods fried.

TABLE II .

FATTY ACID COMPOSITION OF UNUSED FRYING FAT
FROM FIVE FOOD SERVICE ESTABLISHMENTS^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Establishment							
	R		B		J		F	S
	I	II	I	II	I	II	I	I
14	0.7	3.7	--- ^b	0.4	1.8	1.6	0.4	--- ^b
16	34.1	50.8	10.3	21.7	35.3	31.0	34.0	9.5
16:1	0.1	2.1	tr	tr	0.7	1.0	--- ^b	tr
18	11.1	10.7	tr	tr	20.6	6.6	1.2	tr
18:1	50.0	32.6	85.9	7.4	37.0	56.0	18.1	88.4
18:2	3.9	--- ^b	3.8	70.6	4.6	3.7	46.4	2.1
Saturates	45.9	65.2	10.3	22.1	57.7	39.2	35.6	9.5
Monoenes	50.1	34.7	85.9	7.4	37.7	57.0	18.1	88.4
Diene	3.9	--- ^b	3.8	70.6	4.6	3.7	46.4	2.1

^aEach value is an average calculated from two chromatograms.

^bNot detected.

of fat, were used for frying meats--chicken, fish, and shrimp--and vegetables--potatoes, eggplant, okra, and onion rings. The fat was rotated among the three fryers according to the foods fried. Fresh fat was placed in fryer one every night and used on the following day only for frying chicken; the fat in fryer one was transferred that night to fryer two and used the following day only for vegetables; the fat in fryer two was placed in fryer three at the end of the second day and used for frying fish and other seafood on the third day; at the end of the third day the fat was discarded.

The temperature used for frying at restaurant R varied with the food to be fried. The temperatures recorded ranged from 172° to 206° C. Fat was kept hot in all three fryers for at least 16 hr. per day. At the end of each day's use, the fat was strained and transferred to the next fryer in the sequence, where it remained, uncovered, until it was heated again. The amount of fresh fat required to keep the level in each fryer constant varied with the type and amount of food fried. When chicken and vegetables were fried about 1 gal. of fresh fat was added each day; when large quantities of fish were fried at a single meal, one-fourth to one-half of the fat had to be replaced. Lard was used for replenishment.

In the privately owned cafeteria, designated hereafter as B, a partially hydrogenated and fractionated soybean oil* was used in period I and a mixture of the soybean oil and a "thermostabilized" cottonseed oil**

*Crystal Oil

**Kraft Yellow Label Oil

was used in period II. The major fatty acid component of the soybean oil was oleate (Table II, page 44). The addition of cottonseed oil in period II changed the fatty acid composition of the frying medium drastically and linoleate was the major fatty acid in the frying fat in period II.

Two electric fryers, each with a capacity of 35 lb. of fat, were used at cafeteria B in the frying of meats, vegetables, and batters. The recorded temperature range of the fat used in frying was 161-164° C., a somewhat lower as well as more narrow range than those in other establishments. The fat was heated only during meal preparation for a total daily time of approximately 8 hr. The care of the fat included straining twice daily--once after breakfast and again after the evening meal. For maintenance of a constant level of fat in the fryer, approximately 1 gal. was added each day. The fat was used in this establishment for a considerably longer time than in the other places observed. During period I, the fat was used for 2 mo.; in period II samples were collected over a 2-mo. period, although the fat was still in use when the last sample was taken. The owner stated that his more recent practice was to continue use of the fat indefinitely with daily replenishment.

The drive-in restaurant, J, is a member of a local chain of restaurants. The establishment prepares and serves mainly sandwiches and French fries; therefore, the only foods fried were potatoes during period I and potatoes and breaded fish filets during period II. The fat used for frying was a commercial hydrogenated animal-vegetable

compound.* Oleic and palmitic acids were the major components of the fat before use. In period I there was more stearate present in this fat than in any other fat studied (Table II, page 44). The fat used in period II differed considerably in composition from that used in period I. Linoleate was present in small amounts in the fat used in both periods.

Restaurant J stays open 24 hr. each day and during this study the fat, held in the range 196-222° C. for three days of use, was not cooled except slightly during the once-daily straining. The strained hot fat was returned immediately to the fryer. The electric fryer had a capacity of 29 lb. of fat and approximately 8 gal. were added per day in maintenance of the fat level. Replenishment was not systematic.

In the hospital food service, designated here as F, cottonseed oil** was used for frying chicken, fish, veal and pork cutlets, pork chops, and vegetables. This oil was quite highly polyunsaturated (Table II, page 44); the major fatty acids were linoleic, palmitic, and oleic.

More frying was done in this hospital than would be expected for routine feeding of patients. The reason for the large amount of frying was that this kitchen provides food for an employees' cafeteria in addition to the food supplied for patients. Fried foods seemed to be preferred over other foods by employees.

*HIR

**Kraft Oil

The electric fryer used at F had a capacity of 45 lb. of fat. The temperature of the frying fat ranged from 162 to 178° C. and varied with the cook and type of food being prepared. The fat was used approximately 5 hr. each day, being heated only at mealtimes and even then not at every meal. Care of the fat presumably included straining once a day, but it was observed that this care depended on the foods fried and the cook on duty. Sometimes the fat was used as many as three times without straining. Approximately 1 gal. of fat was added each day for maintenance of a constant level. Each frying period covered a time span of about eight days.

In the university cafeteria, designated as S, partially hydrogenated fractionated soybean oil* was used as the frying medium for both meats and vegetables. The fatty acid composition of this oil was similar to that of the same brand of oil used during period I in the privately owned cafeteria, B (Table II, page 44). Two electric fryers were used: one was used only for vegetables and batters and the second fryer was used exclusively for meats. Only samples from the meat fryer were used in this study because the only food that was fried was shrimp. The capacity of the fryer was 29 lb. of fat. The temperatures employed for frying ranged from 187 to 212° C. The fat was used only during the luncheon and dinner meals with a total daily use of approximately 6 hr. Each frying period extended for about four days, depending on the food to be fried. The fat was strained at the end of each meal

* Crystal Oil

and stored in the refrigerator between uses. Approximately 1 gal. of oil was added to the fryer each day for maintenance of a constant level.

Viscosity of the Frying Fats

Viscosity of the frying fats showed some increase (Table III) with heating. Over-all means for samples 1, 2, and 3 from both periods of use were 32, 34, and 42 cp., respectively. Total change in viscosity of fats from the different establishments apparently bore little relationship to the total time of use (Table I, page 42) or to polyunsaturation (Table II, page 44) probably because of additions of fresh fat. Increased viscosity of fat during thermal treatment has been attributed to formation of polymers (Firestone, 1963; and Rock and Roth, 1964). Apparently some polymerization of the frying fats occurred in the present study, but no attempt was made to isolate polymers.

Lipid Content of Raw and Fried Shrimp

In Tables IV and V the values obtained for the lipid content of the shrimp are shown on moist and dry weight bases, respectively. The fat content of the shrimp increased considerably with frying; raw shrimp from the two lots contained about 1 per cent fat, while the fried samples contained from 9 to 14 per cent (moist weight basis). There was no consistent change in the fat content of shrimp as the extent of re-use of the frying fat increased.

Although viscosity of the frying fat increased with use, a relationship between viscosity of the fat and the apparent fat absorption by the food fried in it, as had been reported by Stern and

TABLE III
 VISCOSITY OF FRYING FATS SAMPLED BEFORE, DURING, AND
 AFTER USE IN FIVE FOOD SERVICE ESTABLISHMENTS
 (CENTIPOISES AT 45° C.)

	Establishment					Mean
	R	B	J	F	S	
Period I						
1	38	----- ^a	33	23	32	32
2	38	----- ^a	36	24	34	33
3	48	----- ^a	48	37	38	43
Period II						
1	35	28	36	30	35	33
2	42	40	40	31	35	36
3	48	37	44	32	39	40

^aThe samples collected were too small for viscosity determinations.

TABLE IV.
LIPID EXTRACTED FROM RAW SHRIMP AND FROM SHRIMP FRIED IN
FIVE RESTAURANTS^a (PER CENT OF ORIGINAL MOIST WEIGHT)

Shrimp sample	Raw	Establishment					Mean
		R	B	J	F	S	
Period I	1.0						
1		12.1	13.6	9.2	13.2	10.2	11.6
2		11.6	12.7	9.6	11.4	10.6	11.2
3		12.4	10.7	10.6	12.0	10.9	11.3
Period II	1.4						
1		11.7	12.3	12.2	11.6	11.9	11.9
2		10.6	13.8	11.8	11.1	12.3	11.9
3		10.8	13.2	11.6	11.3	13.0	12.0
Mean		11.5	12.7	10.8	11.8	11.5	

^aEach value is an average calculated from a triplicate determination for a single extraction.

TABLE V
LIPID EXTRACTED FROM RAW SHRIMP AND FROM SHRIMP FRIED IN
FIVE RESTAURANTS^a (PER CENT OF ORIGINAL DRY WEIGHT)

Shrimp sample	Raw	Establishment					Mean
		R	B	J	F	S	
Period I	2.0						
1		27.9	30.6	19.0	33.8	26.4	27.5
2		25.7	26.2	21.8	25.6	25.0	24.8
3		27.7	23.4	20.9	28.6	25.9	25.3
Period II	2.6						
1		30.9	35.9	32.9	28.2	30.2	31.6
2		26.2	34.9	32.6	29.0	32.9	31.1
3		26.1	34.0	29.9	30.5	35.3	31.1
Mean		27.4	30.8	26.2	29.3	29.3	

^aEach value is an average calculated from a triplicate determination for a single extraction.

Roth (1959) for doughnuts, was not found in this study. The lipid concentration of the fried shrimp apparently was influenced slightly by frying temperature; the highest and lowest average fat absorption occurred at establishments B and J, where the temperature range was lowest and highest, respectively.

Fractions of Shrimp Lipids

Average recovery of lipid from the silicic acid columns was 98.6 per cent. The fractionation data, reported as percentages of total lipid recovered, are presented in Table VI for the lipids extracted from fried shrimp. The glyceride content of the fried shrimp lipids ranged from 95 to 97 per cent of the total lipid, while the phospholipid made up only 3-5 per cent. The phospholipid concentrations of the raw shrimp lipids were 32 and 42 per cent for periods I and II, respectively. The great dilution of the shrimp lipid with frying fat, indicated by the fat content data presented previously (Table IV, page 51), also is reflected in the large differences in phospholipid concentration between the lipids of raw and of fried shrimp. When an attempt was made to fractionate the cooking fats, the amount of phospholipid in these samples was negligible. Variations in phospholipid concentration with continued use of fat were not consistent but rather appear to reflect variations in the extent of dilution of shrimp fat with frying fat. Phospholipid concentrations were slightly lower in period II than in period I, reflecting the slightly higher average fat absorption in period II.

TABLE VI
LIPID FRACTIONS FROM FRIED SHRIMP
(PER CENT OF RECOVERED LIPID)^a

Sample	Establishment					Mean
	R	B	J	F	S	
<u>Period I</u>						
Sample 1						
glyceride	95.9	96.9	96.4	96.2	94.6	96.0
phospholipid	4.1	3.1	3.6	3.8	5.4	4.0
Sample 3						
glyceride	96.2	95.4	96.6	95.4	95.7	95.8
phospholipid	3.8	4.6	3.4	4.6	4.3	4.2
<u>Period II</u>						
Sample 1						
glyceride	96.9	97.4	96.8	96.7	96.0	96.7
phospholipid	3.1	2.6	3.2	3.3	4.0	3.3
Sample 3						
glyceride	96.2	97.0	96.6	95.7	96.9	96.5
phospholipid	3.8	3.0	3.4	4.3	3.1	3.5

^aEach value is an average calculated from a duplicate determination for a single fractionation.

Fatty Acid Composition of Frying Fats

The major fatty acid components of frying fats for period I are shown in Table VII. A slight tendency toward increased saturation suggests that some autoxidation occurred. Decreases in concentration of linoleic acid due to autoxidation have been reported by Lundberg, 1954; Melnick et al., 1958; and, Kilgore and Luker, 1964. The decrease in linoleate was not pronounced except possibly in the oil used in establishment F, which had the highest concentration originally. The final value, 37.3 per cent linoleate, unfortunately represents a sample taken immediately after the addition of fresh oil. In fat obtained from R, B, and J, the decreases in linoleate were small but progressive. In the case of the frying fat from the university cafeteria, S, there appears to have been a slight increase in the percentage of linoleate, which cannot be explained on the basis of replenishment.

A tendency toward increased concentrations of saturated fatty acids with continued use of frying fats does not necessarily indicate change in absolute amounts of these components. Breakdown and loss of unsaturated fatty acids would result in increased percentages of saturated acids. The assumption has been made by other workers that saturated fatty acids remain at constant levels during thermal treatment (Kilgore and Luker, 1964).

Fatty acid data were obtained for frying fats from only three of the five places during period II: the privately owned restaurant (R), the privately owned cafeteria (B), and the drive-in restaurant (J). These establishments were selected because they used different types

TABLE VII

MAJOR FATTY ACIDS IN FRYING FATS FROM FIVE
FOOD SERVICE ESTABLISHMENTS, PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Fat sample	Establishment				
		R	B	J	F	S
16	1	34.1	10.3	35.3	34.0	9.5
	2	38.0	8.3	33.7	45.9	11.6
	3	40.4	21.0	28.9	41.6	13.3
18	1	11.1	tr	20.6	1.2	tr
	2	13.4	tr	12.0	1.8	tr
	3	11.6	5.1	10.2	1.0	tr
18:1	1	50.0	85.9	37.0	18.1	88.4
	2	46.3	89.3	51.1	21.1	83.9
	3	46.0	71.1	59.1	19.5	81.3
18:2	1	3.9	3.8	4.6	46.4	2.1
	2	1.3	2.4	1.5	30.8	4.5
	3	tr	2.1	1.0	37.3	5.5

^aEach value is an average calculated from two chromatograms.

of fat and had different frying practices. The data for the major fatty acids are presented in Table VIII for period II. Only in the fat from the privately owned cafeteria (B) was a decrease in polyunsaturation noted. This was the establishment which used the most polyunsaturated fat in period II and which used its fat the longest. Concentrations of saturated fatty acids did not show the expected increases. One of the peculiarities of the data can be explained on the basis of the information obtained. The fat from the private restaurant, R, showed a decrease in palmitic acid during period II, along with an increase primarily in oleic acid. During this period large quantities of fish were fried necessitating extensive replenishment of fat. The manager of the establishment stated that as much as 50 per cent of fresh lard was added to the fryer just prior to the second frying. Because lard is much lower in palmitic acid and higher in oleic acid than was the original mixture of lard and beef fat, this addition of lard could explain the decrease in palmitic acid and increase in oleate in the second fat sample. The changes in saturates and oleate between fryings 2 and 3 follow the expected trend of increase and decrease, respectively.

Fatty Acid Composition of Glyceride and Phospholipid Fractions of Raw and Fried Shrimp

The fatty acid compositions of the glycerides and phospholipids of raw shrimp are presented in Table IX. As would be expected, the fatty acid patterns differed more between lipid fractions than between the two lots of shrimp. The major fatty acid in the phospholipid fraction was

TABLE VIII

MAJOR FATTY ACIDS IN FRYING FATS FROM THREE FOOD
SERVICE ESTABLISHMENTS, PERIOD II^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Fat sample	Establishment		
		R	B	J
16	1	50.8	21.7	31.0
	2	32.9	32.4	41.6
	3	36.4	20.3	40.0
18	1	10.7	tr	6.6
	2	4.9	tr	6.2
	3	13.1	tr	2.9
18:1	1	32.6	7.4	56.0
	2	55.2	17.4	46.2
	3	46.3	39.0	51.0
18:2	1	--- ^b	70.6	3.7
	2	--- ^b	49.1	3.9
	3	1.9	40.6	4.4

^a Each value is an average calculated from two chromatograms.

^b Not detected.

TABLE IX
FATTY ACID COMPOSITION OF THE GLYCERIDE AND
PHOSPHOLIPID FRACTIONS OF RAW SHRIMP^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Phospholipids		Glycerides	
	Period		Period	
	I	II	I	II
10	----- ^b	0.7	----- ^b	----- ^b
12	----- ^b	0.8	----- ^b	----- ^b
14	3.2	1.0	0.5	0.8
14:1	----- ^b	2.9	----- ^b	tr
16	44.8	49.6	23.2	14.6
16:1	4.2	1.9	tr	tr
18	13.3	4.9	----- ^b	----- ^b
18:1	14.0	12.7	12.4	14.3
18:2	20.5	25.4	64.0	70.2
Total monoenes	18.2	14.6	12.4	14.3
Total saturates	61.3	60.3	23.6	15.5

^aEach value is an average calculated from two chromatograms.

^bNot detected.

palmitic acid while in the glyceride fraction linoleic acid was present in the greatest concentration. The high degree of polyunsaturation of the shrimp glycerides as compared with the phospholipids represents a reversal of the results reported for land animals (Hornstein et al., 1961; Taylor, 1964; Giam and Dugan, 1965; and Campbell and Turkki, in preparation).

Fatty acid compositions of the glyceride fraction of fat extracted from fried shrimp are shown in Tables X and XI for periods I and II, respectively. The samples are from shrimp fried in each lot of fat at the beginning and end of the period of use. Changes in glyceride fatty acids with continued re-use of frying fat were small, but tended toward slightly decreased polyunsaturation, reflecting changes in the frying fats. The one really large decrease in glyceride polyunsaturation was in period II for the shrimp fried at establishment B; this was the establishment that not only used the most highly polyunsaturated fat during that period but also used the fat for the longest time.

Concentrations of the major fatty acids in the phospholipid fraction of fat from fried shrimp are shown in Tables XII and XIII for periods I and II, respectively. The phospholipid fatty acids apparently were influenced little by the frying fats (Table II, page 44). The above findings are explained by the fact that the frying fats contained no appreciable amounts of phospholipid. The fact that the shrimp itself was the source of phospholipid also explains the smaller variation among the establishments than was seen with the glyceride fatty acids. Under such circumstances re-use of the frying fats would not be expected to

TABLE X
 MAJOR FATTY ACIDS IN THE GLYCERIDE FRACTION OF
 FAT EXTRACTED FROM FRIED SHRIMP, PERIOD I^a
 (PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Shrimp sample	Establishment				
		R	B	J	F	S
16	1	29.8	8.9	37.4	27.1	11.7
	3	44.6	14.9	32.2	28.8	10.7
18	1	13.4	1.9	8.3	0.3	--- ^b
	3	10.1	2.6	3.6	tr	--- ^b
18:1	1	51.4	81.1	40.4	7.2	79.6
	3	41.9	78.3	54.5	11.8	81.8
18:2	1	4.5	7.9	9.3	64.8	8.8
	3	0.8	3.7	7.8	58.6	7.5

^aEach value is an average calculated from two chromatograms.

^bNot detected.

TABLE XI

MAJOR FATTY ACIDS IN THE GLYCERIDE FRACTION OF
FAT EXTRACTED FROM FRIED SHRIMP, PERIOD II^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Shrimp sample	Establishment		
		R	B	J
16	1	32.8	22.2	31.2
	3	36.0	27.1	38.9
18	1	5.9	tr	3.0
	3	10.2	0.6	3.6
18:1	1	56.9	9.4	58.2
	3	47.2	65.2	52.3
18:2	1	--- ^b	67.2	7.5
	3	--- ^b	5.9	5.0

^aEach value is an average calculated from two chromatograms.

^bNot detected.

TABLE XII

MAJOR FATTY ACIDS IN THE PHOSPHOLIPID FRACTION OF FAT
EXTRACTED FROM FRIED SHRIMP, PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Shrimp sample	Establishment				
		R	B	J	F	S
16	1	41.1	44.6	43.2	35.5	39.4
	3	41.1	45.1	43.2	44.4	41.7
18	1	12.8	15.1	22.8	20.4	13.4
	3	13.9	11.4	9.6	18.9	10.6
18:1	1	17.1	18.4	17.7	20.4	19.8
	3	19.3	19.4	16.2	17.4	19.8
18:2	1	25.2	19.0	14.0	21.4	22.9
	3	23.0	21.3	26.8	16.7	24.2

^aEach value is an average calculated from two chromatograms.

TABLE XIII

MAJOR FATTY ACIDS IN THE PHOSPHOLIPID FRACTION OF FAT
EXTRACTED FROM FRIED SHRIMP, PERIOD II^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Shrimp sample	Establishment		
		R	B	J
16	1	50.2	48.7	46.0
	3	35.9	36.6	44.4
18	1	2.2	2.6	0.9
	3	4.5	2.4	3.7
18:1	1	12.6	8.3	6.5
	3	12.2	15.1	11.9
18:2	1	29.3	35.3	38.8
	3	42.0	40.8	32.6

^a Each value is an average calculated from two chromatograms.

affect phospholipid fatty acids and comparison of the values for samples 1 and 3 showed no consistent effect.

Subfractions of Frying Fat and of Fried Shrimp Glycerides

Thin-layer chromatography was used for further study of the glycerides. It was thought that changes not detected in study of the whole glyceride fraction might be found through study of glyceride subfractions. Frying fats from three of the establishments for period I and corresponding samples of fried shrimp glycerides were studied.

Separation of glycerides into subfractions on the basis of number of fatty acid double bonds per molecule resulted in seven distinct bands as indicated in Figure 1. The figure is a tracing of a typical thin-layer plate. The relative proportions of the various glyceride subfractions are shown in Tables XIV and XV for the frying fats and fried shrimp glycerides, respectively. Although the seven bands were eluted and analyzed separately, the values for fraction 3 represent a pooling of values for the four most unsaturated bands. Litchfield et al. (1964) actually analyzed these bands as a single fraction.

The amount of glyceride in each subfraction varied with the kind of fat that was used at the establishment. As should be expected, the lard from restaurant R and the hydrogenated compound from drive-in J had higher percentages of glyceride in the subfractions containing zero and one double bond per molecule than did the fat from establishment B. The oil used in B had only 10.3 per cent of saturated fatty acids originally as compared with 45.9 and 57.7 per cent for the fats of R

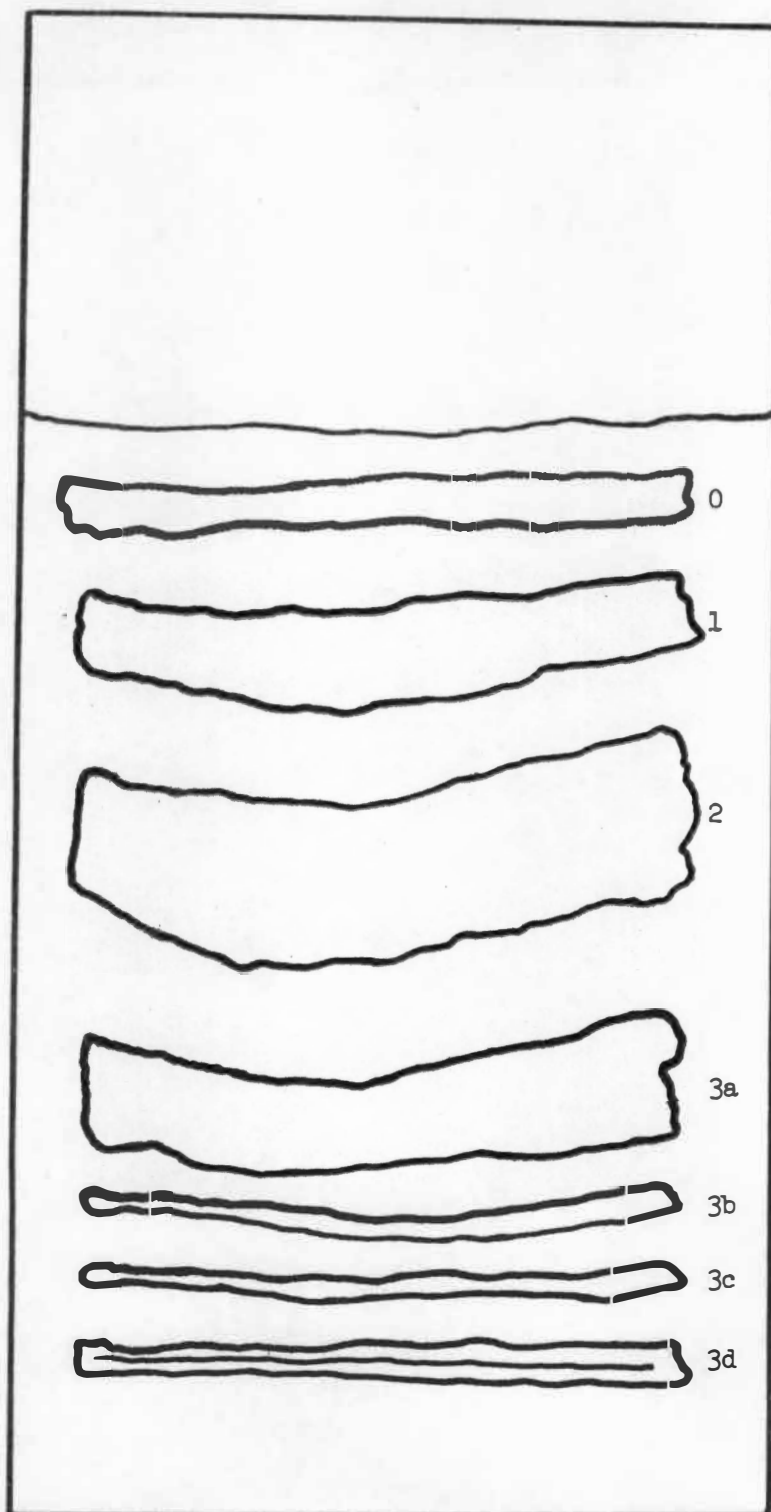


Figure 1. Glyceride subfractions, separated on basis of number of double bonds per molecule. (From top to bottom: 0, 1, 2, and 3 or more double bonds per glyceride molecule.)

TABLE XIV
GLYCERIDE SUBFRACTIONS OF FRYING FATS, PERIOD I^a
(PER CENT OF RECOVERED GLYCERIDE)

Subfraction ^b	Establishment		
	R	B	J
<u>0</u>			
Sample 1	14.4	3.2	15.7
Sample 3	13.9	4.3	12.8
<u>1</u>			
Sample 1	21.2	5.9	29.7
Sample 3	23.5	18.0	14.8
<u>2</u>			
Sample 1	35.2	14.2	30.7
Sample 3	30.9	18.1	34.6
<u>3</u>			
Sample 1	29.2	76.8	23.9
Sample 3	31.8	59.6	37.9

^aEach value is an average calculated from six determinations.

^bNumber of double bonds per molecule.

TABLE XV
GLYCERIDE SUBFRACTIONS OF FRIED SHRIMP LIPIDS, PERIOD I^a
(PER CENT OF RECOVERED GLYCERIDE)

Subfraction ^b	Establishment		
	R	B	J
<u>0</u>			
Sample 1	10.6	1.6	12.5
Sample 3	9.1	4.8	10.3
<u>1</u>			
Sample 1	28.2	1.4	8.7
Sample 3	38.4	1.6	6.8
<u>2</u>			
Sample 1	30.7	19.4	32.2
Sample 3	25.2	24.7	31.3
<u>3</u>			
Sample 1	30.6	77.5	46.5
Sample 3	27.3	68.9	51.6

^aEach value is an average calculated from six determinations.

^bNumber of double bonds per molecule.

and J, respectively (Table II, page 44). Glycerides of the fried shrimp similarly were lower in subfractions 0 and 1 for establishment B than for the other two restaurants.

The proportion of the glyceride sample falling in the most saturated subfraction was consistently somewhat higher for the frying fats (Table XIV, page 67) than for the fried shrimp glycerides (Table XV, page 68). This apparently reflects the fact that the shrimp glycerides were lower in saturated fatty acids and more polyunsaturated than the frying fats. The difference in saturation between shrimp glycerides and frying fats was great enough to show an effect on the subfractions even though the contribution of the shrimp lipid to the glyceride of the total extracted lipid was small.

A decrease in the proportion of the most highly unsaturated subfraction, 3, occurred in the frying fat used at establishment B between the first and last fryings (Table XIV, page 67). Although this was not a highly polyunsaturated oil, it was the most unsaturated fat used and was used the longest. The subfraction that showed the greatest simultaneous increase in concentration was that in which the glycerides theoretically had one double bond per molecule. If some of the oleate in the original fat were present as triolein, and if during use the α -fatty acids were oxidized, the net result would be a decrease in subfraction 3 and an increase in subfraction 1 as seen here.

The proportion of subfraction 3 in the frying fat and shrimp glycerides from establishment J increased with use of the fat,

particularly in the frying fat itself. This could be an apparent rather than a real effect if hydrolysis of the fat occurred during use. The free glycerol then would contribute to the values for the most unsaturated subfraction. Occurrence of such an effect at only the one establishment could be due to its having fried particularly large quantities of potatoes, which have a high moisture content. Establishment J also used higher frying temperatures than did the other restaurants. The difference in magnitude of effect between the frying fat and the fried shrimp glycerides again probably was due to the contribution of the shrimp glycerides themselves. Another possible contributor of polyunsaturated glycerides was fat from the partially cooked potatoes that were fried in huge quantities at establishment J. This restaurant used the institutional packs of pre-cooked frozen French-fries, which according to U.S.D.A. Handbook No. 8 (1963) contain approximately 6.5 per cent fat as compared with approximately 13.2 per cent in completely fried potatoes.

Fatty Acid Composition of Glyceride Subfractions.

The fatty acid compositions of the glyceride subfractions are shown in Tables XVI through XXII. The fatty acid values for the four components of subfraction 3, weighted by the relative proportions of the components, were combined into a single set of values representing the entire subfraction for each sample. The fatty acid values for the different subfractions indicate that the thin-layer chromatographic procedure yielded fractions according to theory. Each of the fractions

TABLE XVI
FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS
OF RAW SHRIMP LIPIDS, PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Fatty acid	Subfraction ^b			
	0	1	2	3
12	0.6	0.6	0.9	2.1
14	4.6	2.0	0.7	1.3
14:1	--- ^c	0.2	tr	tr
16	48.8	49.6	28.3	11.4
16:1	--- ^c	tr	0.8	2.6
18	46.0	15.9	3.9	1.0
18:1	--- ^c	31.7	65.3	60.1
18:2	--- ^c	--- ^c	--- ^c	23.0
18:3	--- ^c	--- ^c	--- ^c	0.2

^aEach value is an average calculated from two chromatograms.

^bNumber of double bonds per molecule.

^cNot detected.

TABLE XVII

FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS OF THE FRYING
FAT OBTAINED FROM A PRIVATE RESTAURANT (R), PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Subfraction ^b	Fatty acid						
	12	14	16	16:1	18	18:1	18:2
<u>0</u>							
Sample 1	--- ^c	3.7	47.5	--- ^c	48.8	--- ^c	--- ^c
Sample 3	1.4	6.5	56.6	--- ^c	35.5	--- ^c	--- ^c
<u>1</u>							
Sample 1	1.5	3.2	55.2	--- ^c	13.2	26.9	--- ^c
Sample 3	0.7	3.0	51.2	--- ^c	9.6	35.5	--- ^c
<u>2</u>							
Sample 1	0.7	1.8	31.6	tr	2.3	58.5	5.2
Sample 3	tr	3.8	31.3	tr	5.9	59.0	tr
<u>3</u>							
Sample 1	tr	2.1	21.7	0.9	0.2	54.7	20.5
Sample 3	3.0	8.2	23.8	1.1	6.5	49.5	7.9

^aEach value is an average calculated from six determinations.

^bNumber of double bonds per molecule.

^cNot detected.

TABLE XVIII.

FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS OF FRIED SHRIMP
LIPIDS FROM A PRIVATE RESTAURANT (R), PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Subfraction ^b	Fatty acid						
	12	14	16	16:1	18	18:1	18:2
<u>0</u>							
Sample 1	0.7	3.8	41.0	--- ^c	54.1	--- ^c	--- ^c
Sample 3	tr	10.0	58.4	--- ^c	31.5	--- ^c	--- ^c
<u>1</u>							
Sample 1	tr	5.2	48.9	0.1	16.3	29.5	--- ^c
Sample 3	tr	3.8	49.2	0.5	13.8	27.5	--- ^c
<u>2</u>							
Sample 1	tr	2.3	26.2	tr	tr	61.0	10.5
Sample 3	tr	8.6	21.4	tr	8.9	61.1	tr
<u>3</u>							
Sample 1	tr	3.7	27.0	2.4	2.0	50.9	14.0
Sample 3	--- ^c	6.8	22.3	3.8	2.2	59.4	5.3

^aEach value is an average calculated from two chromatograms.

^bNumber of double bonds per molecule.

^cNot detected.

TABLE XIX

FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS OF THE FRYING
FAT OBTAINED FROM A PRIVATELY OWNED CAFETERIA (B), PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Subfraction ^b	Fatty acid							
	12	14	15	16	16:1	18	18:1	18:2
<u>0</u>								
Sample 1	18.1	7.9	--- ^c	47.8	--- ^c	26.2	--- ^c	--- ^c
Sample 3	5.1	9.6	1.2	58.9	--- ^c	25.2	--- ^c	--- ^c
<u>1</u>								
Sample 1	0.8	2.2	--- ^c	51.8	tr	6.8	38.4	--- ^c
Sample 3	0.6	3.0	0.6	45.5	tr	7.4	43.0	--- ^c
<u>2</u>								
Sample 1	tr	4.1	--- ^c	25.0	tr	tr	70.8	--- ^c
Sample 3	0.4	0.8	tr	10.1	tr	1.6	87.1	--- ^c
<u>3</u>								
Sample 1	3.0	0.8	--- ^c	9.0	tr	tr	72.6	17.1
Sample 3	1.1	2.9	0.1	9.2	0.9	tr	72.9	14.7

^aEach value is an average calculated from two chromatograms.

^bNumber of double bonds per molecule.

^cNot detected.

TABLE XX

FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS OF FRIED SHRIMP
LIPIDS FROM A PRIVATELY OWNED CAFETERIA (B), PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Subfraction ^b	Fatty acid						
	12	14	16	16:1	18	18:1	18:2
<u>0</u>							
Sample 1	tr	7.3	47.6	--- ^c	45.1	--- ^c	--- ^c
Sample 3	--- ^c	4.3	68.5	--- ^c	27.9	--- ^c	--- ^c
<u>1</u>							
Sample 1	--- ^c	1.1	59.5	--- ^c	4.4	35.1	--- ^c
Sample 3	tr	3.0	62.0	--- ^c	8.0	27.0	--- ^c
<u>2</u>							
Sample 1	--- ^c	0.3	17.3	tr	1.4	81.0	--- ^c
Sample 3	tr	1.5	22.0	tr	3.0	73.5	--- ^c
<u>3</u>							
Sample 1	--- ^c	tr	6.9	--- ^c	--- ^c	74.3	17.6
Sample 3	--- ^c	0.7	9.2	0.7	0.1	82.7	6.9

^aEach value is an average calculated from two chromatograms.

^bNumber of double bonds per molecule.

^cNot detected.

TABLE XXI

FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS OF THE FRYING
FAT OBTAINED FROM A DRIVE-IN RESTAURANT (J), PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Subfraction ^b	Fatty acid							
	12	14	15	16	16:1	18	18:1	18:2
<u>0</u>								
Sample 1	0.4	6.0	1.1	42.9	--- ^c	49.6	--- ^c	--- ^c
Sample 3	0.2	6.9	tr	51.6	--- ^c	41.2	--- ^c	--- ^c
<u>1</u>								
Sample 1	0.5	5.6	1.2	47.0	--- ^c	21.2	24.5	--- ^c
Sample 3	0.3	3.5	--- ^c	48.2	tr	17.0	30.9	--- ^c
<u>2</u>								
Sample 1	0.4	1.2	0.4	22.9	0.7	8.0	65.4	--- ^c
Sample 3	0.2	1.7	0.1	23.2	0.5	6.0	68.2	--- ^c
<u>3</u>								
Sample 1	0.8	2.2	1.7	13.6	2.2	4.6	49.8	25.1
Sample 3	0.5	1.1	0.6	10.6	0.4	5.1	61.1	20.6

^aEach value is an average calculated from two chromatograms.

^bNumber of double bonds per molecule.

^cNot detected.

TABLE XXII

FATTY ACID COMPOSITION OF GLYCERIDE SUBFRACTIONS OF FRIED SHRIMP
LIPIDS FROM A DRIVE-IN RESTAURANT (J), PERIOD I^a.
(PER CENT OF TOTAL FATTY ACIDS)

Subfraction ^b	Fatty acid							
	12	14	15	16	16:1	18	18:1	18:2
<u>0</u>								
Sample 1	tr	9.4	--- ^c	55.3	--- ^c	35.3	--- ^c	--- ^c
Sample 3	1.6	8.6	--- ^c	39.0	--- ^c	50.7	--- ^c	--- ^c
<u>1</u>								
Sample 1	1.3	10.7	--- ^c	36.6	tr	20.4	30.0	--- ^c
Sample 3	--- ^c	10.2	--- ^c	38.9	--- ^c	17.8	33.1	--- ^c
<u>2</u>								
Sample 1	0.6	3.9	tr	39.1	0.7	14.6	37.9	3.2
Sample 3	1.9	5.1	0.4	40.7	0.2	10.4	40.4	1.0
<u>3</u>								
Sample 1	0.3	3.0	0.3	19.5	1.5	6.4	57.0	12.6
Sample 3	2.2	4.1	0.9	39.3	0.2	8.3	39.5	5.0

^aEach value is an average calculated from two chromatograms.

^bNumber of double bonds per molecule.

^cNot detected.

which theoretically contained no double bonds indeed appeared to have only saturated fatty acids. The subfraction that supposedly consisted of glycerides having one double bond per molecule contained approximately one-third oleic acid and two-thirds saturated acids. These proportions are correct according to theory, although the actual distribution among molecules is not known. The subfraction that presumably contained two double bonds per molecule had an average of 64 per cent oleic acid and a small amount of linoleate in addition to saturated fatty acids. These average proportions also are theoretically correct, though it must be pointed out that there was considerable variation among samples with respect to oleate concentration. Because oleic acid did appear in such large amounts in subfraction 2, it seems that some dioleo-triglyceride was present. The subfraction theoretically containing at least three double bonds per molecule apparently was very complex, as indicated by its having separated into four bands, containing progressively higher proportions of unsaturated fatty acids. Values for the linoleate concentrations in the components of subfraction 3 are shown in Table XXIII. Although the fatty acid values for the combined subfraction 3 did not account for three double bonds per molecule, the most polyunsaturated component of this subfraction did have a fatty acid distribution accounting for more than three double bonds per molecule in every sample except shrimp glycerides from establishment R.

Because the fatty acid composition of the frying fat as well as of the fried shrimp glycerides varied considerably among the three establishments, the fatty acid data will be discussed for each

TABLE XXIII
 LINOLEIC ACID CONCENTRATIONS IN THE COMPONENTS
 OF GLYCERIDE SUBFRACTION THREE, PERIOD I^a
 (PER CENT OF TOTAL FATTY ACIDS)

Components of subfraction <u>3</u>	Establishment					
	R		B		J	
	1	3	1	3	1	3
<u>Frying fat</u>						
a	6.4	tr	--- ^b	7.4	--- ^b	--- ^b
b	10.6	tr	17.5	18.0	--- ^b	13.9
c	26.9	23.9	34.8	15.8	14.9	19.5
d	63.7	11.3	65.3	45.9	49.7	34.7
<u>Shrimp glyceride</u>						
a	--- ^b	--- ^b	--- ^b	--- ^b	9.3	0.7
b	tr	--- ^b	--- ^b	0.8	tr	1.4
c	11.1	2.9	12.1	9.1	16.0	1.6
d	18.3	9.2	52.7	22.9	34.4	12.3

^a Each value is an average calculated from two chromatograms.

^b Not detected.

establishment separately.

The fatty acid data for fat used in restaurant R appear in Table XVII, page 72. Even though little change had occurred in the actual amount of glyceride in subfraction 3 with the use of fat (Table XIV, page 67), these results revealed that a major change occurred in the fatty acid composition in that subfraction. Linoleate decreased from 20.5 to 7.9 per cent as the fat was used. The same tendency was apparent in this subfraction of fried shrimp glycerides (Table XVIII, page 73) for establishment R. The decrease in linoleate was not as great in the shrimp as in the fat, which again suggests that the glycerides of raw shrimp did contribute to the total fried shrimp glyceride.

The fatty acid composition of frying fat from cafeteria B (Table XIX, page 74) tends to reinforce the subfractionation data (Table XIV, page 67) by showing large amounts of monoene in subfractions 2 and 3. In the frying fat there were no pronounced changes in the concentrations of unsaturated fatty acids as the fat was used. A large decrease in unsaturation was not expected in subfraction 3 because the proportion of frying fat glyceride in that subfraction decreased markedly with use of fat (Table XIV, page 67). In the case of the glyceride of fried shrimp from cafeteria B, there was a large decrease in linoleate in subfraction 3 (Table XX, page 75). Due to the fact that there was more of the original glyceride remaining in subfraction 3 of the shrimp glycerides than of the frying fat glycerides, more change in the unsaturated fatty acids was to be expected in this

subfraction. Again autoxidation might be an explanation for the apparent decrease in unsaturation that was observed.

Some decrease in linoleate occurred in subfraction 3 of the frying fat and glycerides from fried shrimp during re-use of fat in drive-in J (Table XXI, page 76 and Table XXII, page 77). Other changes were not clear-cut.

Of the four components of subfraction 3 the most polyunsaturated, d in Table XXIII, page 79, showed the greatest loss of 18:2 with use of the fat. This was the band that remained at the line of origin on the thin-layer plate (Figure 1, page 66). The concentrations of fatty acid groups in this d-band are shown in Table XXIV for the frying fats from the three establishments, period I, and for the corresponding shrimp glycerides. Losses in dienoate tended to vary with the original values. In any fat fraction containing more than 33 per cent diene prior to use of the fat some diene must be in the Δ -position. The greater losses in the fractions containing the higher amounts of 18:2 thus may have been due to larger proportions of diene in the vulnerable Δ -position. On this basis the decrease from 65.3 to 45.9 per cent in diene in fraction 3d of the frying fat of establishment B might be expected to be as great as that for the same fraction for establishment R; however, the oil used at B was that which is described by the manufacturer as "thermostabilized."

The over-all effect of re-use of fat was not pronounced. Although some rather large decreases in percentage of linoleate were observed in the most polyunsaturated fractions, these fractions

TABLE XXIV

CONCENTRATIONS OF FATTY ACID GROUPS IN THE MOST UNSATURATED
COMPONENT OF GLYCERIDE SUBFRACTION THREE, PERIOD I^a
(PER CENT OF TOTAL FATTY ACIDS)

Sample	Fatty acid group						Δ ,Diene
	Saturate		Monoene		Diene (18:2)		
	1	3	1	3	1	3	
<u>R</u>							
Frying fat	15.2	55.6	21.1	33.0	63.7	11.3	-52.4
Shrimp glyceride	37.9	35.1	41.9	55.7	18.3	9.2	- 9.1
<u>B</u>							
Frying fat	13.0	20.4	21.7	33.8	65.3	45.9	-19.4
Shrimp glyceride	10.2	12.4	37.1	64.7	52.7	22.9	-29.8
<u>J</u>							
Frying fat	15.6	18.0	34.6	47.2	49.7	34.7	-15.0
Shrimp glyceride	24.2	44.8	41.3	42.8	34.4	12.3	-22.1

^a Each value is an average calculated from two chromatograms.

represented relatively small portions of the entire fat sample. To the extent that changes did occur, they varied with the kind of fat used and to a limited extent with the frying practices. If an establishment frying foods in a highly polyunsaturated oil, such as was used at F in period I of this study, were to re-use that oil for as long a period as did B, the effects on fatty acids of the fried foods could be drastic. Probably the nature of the fat governs its use, however. There appears to be a trend toward commercial use of frying fats and oils of a low degree of polyunsaturation, probably because of their greater stability. Neither the slight decrease in unsaturation of fatty acids nor the slight increase in viscosity of fat found in this study seems sufficient to warrant consumer concern with respect to use of commercially fried foods. Perhaps it is in the home situation, where highly unsaturated oils are commonly used for frying, that caution particularly needs to be exercised in the re-use of frying fat.

CHAPTER V

SUMMARY

Information concerning frying practices was obtained from five food service establishments. Samples of frying fat were collected immediately prior to use, during the period of use, and just prior to disposal of fat. Frozen raw breaded shrimp were obtained in quantity and fried at each fat collection. Samples were collected during two complete uses of fat.

Lipids were extracted from the raw and cooked shrimp. Analyses included: viscosity of frying fats, silicic acid column fractionation of lipids extracted from shrimp; thin-layer chromatographic analysis of frying fats and of shrimp glycerides from three establishments during period I; gas-liquid chromatographic study of selected samples of frying fats, shrimp phospholipids, shrimp glycerides, and sub-fractions of frying fats and shrimp glycerides. An attempt was made to relate changes in lipids to frying practices in the food service establishments.

The viscosity of the frying fats increased slightly with use but not enough to affect the percentage of fat in shrimp and not enough to indicate a large amount of polymerization.

Although the phospholipid content of the raw shrimp was 32 and 42 per cent for periods I and II, respectively, the corresponding values for fried shrimp were 4 and 3 per cent, indicating the large

extent of dilution of shrimp lipids by frying fat. The glyceride fraction of shrimp lipid was much more polyunsaturated than the phospholipid fraction.

Changes occurring in frying fats with re-use appeared small but in general tended toward decreased unsaturation, particularly in the most polyunsaturated fats. The fatty acid compositions of the fried shrimp glycerides were similar to those of the frying fats and reflected changes in the fats. The changes were not as pronounced in the fried shrimp glycerides as in the frying fats probably because of the contribution of the natural shrimp glycerides. The fried shrimp phospholipids were quite different from the glycerides in fatty acid composition and were quite similar to the raw shrimp phospholipids. This fraction did not appear to be affected by the frying fats.

The amount of glyceride in each subfraction obtained by thin-layer chromatography varied with the kind of fat that was used at each establishment. Changes in fatty acids of the subfractions also varied with the kind of fat used. The subfractionation procedure permitted the detection of greater fatty acid changes than could be detected in unfractionated samples, but over-all changes were small. In general, decreases were observed in the percentage of the most polyunsaturated glyceride subfraction and/or in the linoleate concentration in that subfraction. Although some autoxidation apparently occurred, the magnitude of change under the conditions of this study would not seem to warrant consumer concern regarding the use of commercially fried foods.

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APPENDIX

TABLE XXV

MOISTURE CONTENT OF RAW SHRIMP SAMPLES FROM TWO LOTS AND OF SHRIMP
FROM EACH LOT FRIED IN FIVE FOOD SERVICE ESTABLISHMENTS^a
(PER CENT OF ORIGINAL WEIGHT)

Sample	Raw	Establishment				
		R	B	J	F	S
<u>Lot I, Period I</u>						
Raw shrimp	50.6					
Fried shrimp 1		43.3	44.5	48.3	39.1	44.0
Fried shrimp 2		45.1	48.4	44.1	44.5	44.9
Fried shrimp 3		44.8	45.8	50.8	42.5	43.7
<u>Lot II, Period II</u>						
Raw shrimp	53.9					
Fried shrimp 1		37.9	34.3	37.1	41.1	39.4
Fried shrimp 2		40.5	39.5	36.2	38.3	37.4
Fried shrimp 3		41.4	38.8	38.2	37.0	36.8

^aEach value is an average calculated from three determinations.