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Analysis of Fats and Fat-Soluble Components in Foods Using Supercritical Fluid Extraction and Chromatographic Techniques

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ANALYSIS OF FATS AND FAT-SOLUBLE COMPONENTS IN FOODS USING SUPERCRITICAL FLUID EXTRACTION AND CHROMATOGRAPHIC TECHNIQUES

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The purpose of this study was to develop experimental procedures for the extraction of fats from common foods using SFE and to determine by chromatographic techniques the fat-soluble components that are extracted with the fats. In this study various food samples were examined including french fries from Wendy's, McDonald's and Burger King, as well as potato chips, peanut butter and donuts. Soxtec extraction was used as the standard method for comparison of results (percentage of fat) obtained from SFE. Gas chromatography and gas chromatography/mass spectrometry were used for the determination of fat-soluble components (fatty acids), after extraction of fat from food products.

I INTRODUCTION

In 1990, the Nutrition Labeling and Education Act (NLEA) mandated, for the first time, labels containing information about nutritional content of nearly all processed foods. To accomplish this task, analytical data must be generated for foods. To address the analytical demands of NLEA, AOAC INTERNATIONAL formed the Task Force on Methods for Nutrient Labeling Analyses, which identified and documented the availability of methods for nutritional analysis. The AOAC manual supports and expands the efforts of the task force and provides guidance for the proper selection of methods for nutrient labeling.[1] Our study focused mainly on the extraction and measurement of fat and fat-soluble components (fatty acids).

NLEA requires that total fat be calculated as the sum of lipid fatty acids from all sources, expressed as triglycerides, and that classes of fat (e g , saturated fat) may be calculated and expressed as free fatty acids. The two criteria for an appropriate method for fatty acid measurements that satisfy NLEA requirements are complete extraction and recovery of fat/fatty acids and accurate measurements of individual fatty acids.[2]

Fats and oils provide the most concentrated source of energy in the diet, about 9 kcal/g. Animal tissue, dairy products, and oils extracted from certain seeds contribute most of the fat in the diet. A fat is distinguished from oil by its solid state at a particular temperature, usually room temperature.[3]

A Fats and Oils Definition

Fats and oils consist primarily of the three-carbon glycerol molecule with three straight-chain fatty acids attached as esters This large molecule is commonly called a triglyceride The declaration of "fat" as the sum of fatty acids expressed as trigylcerides takes into account all the possible sources of fatty acids in food They occur naturally or are derived through processing and storage of the fats The natural fats are made up mostly of mixed triglycerides with only trace amounts of mono- and diglycerides and little or no free fatty acids. In contrast, processed fats may contain up to 20% of mono- and diglycerides. However, minor components also present in natural fats are sterols, sterol esters, phosphotides, glycolipids, hydrocarbons, glycerol ethers, fat-soluble vitamins, trace metals, water, and oxidation products [3]

The major components of fats and oils are triglycerides (neutral lipids), which are formed by esterification of glycerol (a trihydroxy alcohol) and fatty acids The common constituent of all fats and oils is glycerol. Glycerol is a trihydric alcohol containing primary and secondary alcohol groups, the compound shows all the chemical reactions of alcohol For example, on oxidation some of the compounds formed are dihydroxyacetone, glyceride aldehyde, glyceric acid and tetronic acid.[6,7]

The term "lipid" is more appropriate than the term "fat," since lipid includes not only triglycerides but also additional chemical substances that are present in varying amounts depending on which food fat is being discussed. The term "total fat" is a classical term, which by definition means ether extract Ether extracts primarily the neutral lipids and does not quantitatively remove the more polar lipids such as cholesterol, cholesterol esters, and the phospholipids. Thus the term "total fat" must not be confused with the term "lipid."[3]

Fats are a subclass of lipids, but "fat" is often used interchangeably with lipid Lipids have been defined as a heterogeneous group of naturally occurring substances that are insoluble in water but soluble in organic solvents such as ether, chloroform, benzene, and acetone All lipids contain carbon, hydrogen, and oxygen, and some also contain phosphorus and nitrogen Most lipids are soft solids or liquids at room temperature and are difficult to crystallize.

Techniques of Lipidology includes in the definition of "lipid," those substances which "contain long chain hydrocarbon groups in their molecules and those present in or derived from living organisms." It also classifies two main groups of lipids which have significantly different natural functions and structures; the "neutral lipids" (including acylglycerides, fatty acids, alcohols, hydrocarbons, waxes) and "complex" or "polar" lipids (including phospholipids and glycolipids).[4]

The major classes of lipids can be summarized as follows: [5]

- Simple lipids (esters of fatty acids and alcohols)
- Fats and oils (esters of glycerol and alcohols)
- Waxes (esters of long-chain monohydroxy alcohols and fatty acids)
- Compound Lipids (simple lipids conjugated with nonlipid molecules)
- Phospholipids (esters containing phosphoric acid in place of one mole of fatty acid)
- Phosphoglycerides
- Sphingolipids
- Inositol phosphatides
- Glycolipids (compounds of carbohydrate, fatty acids, and sphingosinol)
- Lipoproteins (complexes of various lipids and proteins)

- Derived Lipids (products of hydrolysis of lipids)
- Fatty acids
- Alcohols (long chain or cyclic, including sterols)
- Hydrocarbons (carotenoids)
- Fat-soluble vitamins

The main (95%) component of dietary lipids are triglycerides, compounds composed of glycerol with three fatty acids esterified to glycerol The remaining 5% of lipids in the diet consist of mono- and diglycerides, phospholipids, glycolipids, sterols and others. The focus of the nutritional concern has been the triglycerides, because they constitute most of the fat

B. Fatty Acids

Fatty acids form homologous series of "straight" chain hydrocarbon compounds terminating with a methyl group at one end and a carboxylic acid at the other end may be fully saturated or contain one, two or more (up to six) double bonds (varying in lengths and degree of saturation or unsaturation).[4] They are readily obtained by the hydrolysis of lipids such as the glycerides and phosphoglycerides. Fatty acids that are used for energy metabolism are stored in triacylglycerol skeleton. A variety of saturated and unsaturated fatty acids are present in triacylglycerols.

The chain length of natural fatty acids varies typically from C_4 to C_{30}, with the majority lying between C_{10} and C_{22}. The C_4 through C_8 saturated acids are liquid at ordinary temperatures (20°C); C_{10} and higher fatty acids are solids Fatty acid chains are generally composed of an even number of carbon atoms (saturated fatty acids) by virtue of their biochemical synthesis, only small quantities of fatty acids containing an odd number of carbon atoms occurs.[4] Unsaturated fatty acids are more chemically reactive than saturated fatty

acids The addition of hydrogen atoms to the double bond(s), in the presence of a suitable catalyst, gives the corresponding saturated fatty acid. The hydrogenation process must be controlled in order to yield the type of product desired.[5] Hydrogenation of unsaturated fats and oils increases their melting point and hardness. Many of the fatty acids are better known by their trivial names rather than by the systematic nomenclature. The systematic nomenclature for fatty acids is based upon recommendations of the Geneva convention. The name of saturated fatty acids is derived from that of the related hydrocarbon with the same number of carbon atoms. For example, the acid with sixteen carbon atoms and structural formula $CH_3(CH_2)_{14}COOH$ is correctly termed hexadecanoic acid, although it also has a trivial name *palmitic acid*[6]

For indicating the position of unsaturation, the carbon atoms are numbered from the carboxyl carbon. Unsaturated fatty acids show two types of isomerism: geometric (cis, trans) and positional (difference in position of the double bonds in polyunsaturated acids). Because of the restricted rotation about the double bond in unsaturated fatty acids, geometric isomers can occur when two different atoms or groups are attached to the carbon atoms involved in the double bond. The terms "cis and trans" refer to the geometry of the groups (alkyl or others) attached to the carbons of a double bond. Since the carbons of the double bond are not free to rotate on their axis, a group attached to one of the carbons of the double bond can be on the same side of the double bond as a group on the other carbon; this arrangement is the "cis" form. When the groups are on opposite sides, they are "trans" to each other. Fatty acids that contain more than one double bond can exist in more than two geometrical isomeric forms.

Most naturally occurring unsaturated fatty acids exist in the "cis" form, however, ruminant fats and commercial hydrogenated fats contain considerable amounts of "trans" acids.[5] Fat analysis usually starts with time-consuming hydrolysis of the foodstuff and extraction of the lipids. The determination of fat content, fat composition and fat quality are some of the most important methods in food analysis.

The NLEA protocol for fat analysis consists of the following steps.

- An acid or base hydrolysis for producing free fatty acid from the lipid constituents and for releasing bound lipids from the food matrix.
- Solvent extraction of hydrolyzed fat.
- The preparation of fatty acid methyl esters (FAMEs) for analysis by gas chromatography (GC) or GC/MS.

The effectiveness of the above method relies on the complete extraction of fat and fatty acids, as well as accurate measurement of the individual fatty acids.[7] Lipid analysts have always been on the forefront of separation science, and development of chromatographic techniques has often been catalyzed by their research activities. For example, the first application of gas chromatography by James and Martin, the inventors of the technique, was the analysis of fatty acids. Today, the analysis of lipids and their derivatives is one of the most important and successful applications of modern chromatography. The quantitative measurement of fatty acids is done by gas-liquid chromatography after transesterification.[8]

C Transesterification

Before the fatty acid composition of a lipid can be determined by gas chromatography, it is necessary to prepare the comparatively volatile methyl ester derivatives of the fatty acid components. When fats and oils are heated in the presence of certain

catalysts, the fatty acids attached to glycerol rearrange in a process called transesterification In the literature, the term interesterification is often used interchangeably to describe reactions that involve the exchange of acyl radicals between an ester and an acid (acidolysis), an ester and an alcohol (alcoholysis), or an ester with ester (transesterification). Confusion too, exists in the use of the term transesterification because it has been used on numerous occasions to describe reactions that are in fact acidolytic.[9]

Rearrangement of the fatty acid positions of TG (triglyceride) molecules of fats and oils through transesterification processes can alter the initial physical properties of the oils and can lead to the formation of new products. Often tranesterification is carried out to counter problems associated with interchangeability or blending of fats and oils and to reduce the melting points of oil mixtures. Transesterification turns all fatty acids into the methyl esters, which are in form of mono-, di-, and triglycerides, wax and sterolesters, or emulgators. There are a large number of official methods for the transmethylation of fatty acids. Recommended temperature ranges are from ambient to reflux and the duration from minutes to several hours. These differences are partly the result of varying amounts of methoxide added, but probably also from a lack of optimization and the assumption that extra time would render the method more robust.[10]

Esterification reagents (referred to reagents that convert free fatty acids to ester) and transesterification reagents (those which convert acylglycerols to fatty acid esters), generally fall into two categories.

- Acid-catalyzed
- Base-catalyzed

Sheppard and Inversion surveyed the various reagents and methods and concluded that "there is no method of esterification that is ideal " They agreed, however, that most of these methods are acceptable if used properly.

1 Acid-catalyzed Reagents

Free fatty acids are esterified and O-acyl lipids transesterified by heating them with a large excess of an acidic catalyst Among the various acid-catalyzed reagents, such as methanolic hydrochloric acid, sulfuric acid in methano,l and acetyl chloride in methanol, boron trifluoride in methanol has found wide application as a good reagent to convert both acylglycerols and rapid esterifying reagent for converting free fatty acids into methyl esters The popularity of this reagent may be due, in part, to its early acceptance in the American Oil Chemists' Society (AOCS) method. The Association of Official Analytical Chemists (AOAC) procedure involves the saponification of the acylglycerols using sodium hydroxide in methanol followed by esterification with BF_3-methanol to give the FAMEs. It is the author's opinion that boron trifluoride in methanol has been greatly overrated as a transesterifying reagent, although it is undoubtedly of value for the rapid methylation of unesterified fatty acids This method has been stated as not useful for the fatty acids containing unusual functional groups such as epoxy, cyclopropenyl, cyclopropyl, and acetylenic or conjugated unsaturation. Although the first step is generally considered to be saponification, which involves the use of alcoholic sodium hydroxide, potassium hydroxide, etc.[11]

Christie stated that BF_3-methanol has been overrated as a transesterification reagent; it is known to form artifacts and is not particularly suitable for seed oils containing unusual fatty acids. For example, the reagent reacts with the plasmalogens to liberate the aldehyde, which is then converted into dimethyl acetals.

The reagent BF_3/MeOH is expensive and has a limited shelf life if not refrigerated Use of old or concentrated reagents has been shown to result in loss of polyunsaturated fatty acids (PUFA) In spite of these various disadvantages, BF_3-methanol is currently accepted as being of great value for the rapid methylation of fatty acids.[11]

Aluminum chloride in methanol has been used to transesterify lipids sample One of the disadvantages with the aluminum chloride reagent is that it does not esterify the FFAs. Hence it appears that in spite of being a Lewis acid, like borontrifluoride, it does not behave in a similar way.[11]

2 Base Catalyzed Reagents

O-Acyl lipids are transesterified very rapidly in anhydrous methanol in the presence of a base catalyst. Free fatty acids are not esterified. Sodium methoxide (0.5 M) in anhydrous methanol, prepared by dissolving fresh clean sodium in dry methanol, is generally accepted for the conversion of the acylglycerols to methyl esters. Potassium methoxide or hydroxide have also been used as catalysts. The reagent is stable for some months at room temperature, especially if oxygen-free methanol is used in its preparation. Nevertheless, this technique is not very useful when large amounts of free fatty acids (FFAs) are present in the sample. This transesterification technique is rapid and can be carried out at room temperature. Under such mild conditions the reagent does not cause any isomerization of double bonds nor does it liberate the aldehyde from the plasmalogens, which could complicate the chromatogram. The sphingolipids and cholesterol ester need more vigorous conditions for transesterification with sodium methoxide. A disadvantage with this base-catalyzed transesterification reagent is that it does not convert the FFAs into FAMEs The condition needs to be anhydrous as the presence of water causes saponification, thereby resulting in a

loss of fatty acids Negligent and prolonged use of this reagent may cause alteration in the fatty acid's composition Use of high temperature and high concentration of base could result in the formation of conjugated fatty acids Potassium hydroxide in methanol is another transesterification reagent commonly employed in the transesterification of oils.

Bennon and coworkers discussed the superiority of sodium methoxide over potassium hydroxide in methanol, taking into consideration that the latter could result in saponification. They found the methoxide-reflux method (which takes only 2 min) to be suitable for the determination of fatty acids in a variety of fats. However, this technique is not accurate for the determination of short-chain fatty acid esters such as methyl butyrate and caproate, which are present in considerable amounts in dairy fat. Sodium methoxide is a popular methylating agent in the oil industry, especially for refined oils, where the FFAs have already been removed during the refining stage.[11] More recently, guanidine and its alkylated derivatives in methanol have been used to catalyze the esterification of fats and oils. This basic reagent causes the complete methanolysis of oils, converting both the acylglycerols and the free fatty acids into methyl esters The reagent is inexpensive and mild, causing no iosmerization of double bonds. This derivatization reaction is rapid, requiring 2 min of heating in a boiling water-bath.[11] Based-catalyzed transesterification can occur in water containing foods, such as ice cream or milk, because it happens much faster than saponification. Diazomethane is a rapid esterification reagent, which esterifies FFAs to FAMEs in the presence of methanol. The reaction is almost instantaneous at room temperature, reacting rapidly with unesterified fatty acids forming methyl esters in the presence of a little methanol, which catalyzes the reaction The excess of reagent is easily eliminated by evaporation under nitrogen However, diazomethane is extremely toxic Moreover, diazomethane is known to form artifacts by

reacting with double bonds or carbonyl groups. It is recommended that diazomethane be used only if absolutely necessary.[11] Strongly basic quaternary salts of ammonia such as trifluoromethylphenyltrimethylammonium hydroxide (TFMPTAH) in methanol, trimethylphenylammonium hydroxide (TMPAH) and tetramethylammonium hydroxide (TMAH) have found applications as transesterification catalysts for acylglycerols. The FFAs, which may be present in the sample, are converted into quaternary ammonium salts, which under the pyrolytic condition of the gas chromatograph, give the corresponding methyl esters. The advantages of these methods include single-step transesterification, instead of the conventional saponification followed by esterification. These reagents do not require an extraction step, which is of particular concern when dealing with the short-chain fatty acids. Direct transesterification is also possible. This method circumvents the extraction and isolation of lipids, thus saving considerable amounts of time and chemicals. The direct esterification method is more efficient than the prevailing methods because of its simplicity, rapidity and higher accuracy.

Key elements of direct transesterifications are as follows.[10]

- Reaction in the homogenized food
- Fast transesterification: 1 min at ambient temperature
- Conditions providing robust optimum
- Stopping the reaction before relevant saponification occurs
- Internal standards verifying transesterification for each sample.

D Preparation of Esters Other than Methyl

Esters other than methyl can be prepared for a variety of reasons, for example, butyl ester for the analysis of short-chain fatty acids present in dairy fats The wide range of fatty acids (C_4-C_{26}) present in dairy fat and the volatility and solubility of the short-chain fatty acids require special attention in the preparation of FAMEs and GC analysis The best methods for transesterification of such fats would be those in which no heating or solvent evaporation (which could result in the loss of volatile fatty acids) or extraction with water (resulting in the loss of soluble short-chain fatty acids) is involved.[11] Usually methyl esters are prepared, because their analysis by GC is more accurate and separation more selective than that of the free fatty acids.

Methoxide transesterifies triglycerides and other fatty acid esters into methyl esters. However, in the presence of water, methoxide also forms hydroxide, which may saponify the triglycerides or the newly generated methyl esters. While transesterification is a reversible reaction, saponification is irreversible. Acidifying the mixture after complete transesterification can stop saponification.[10]

Most of the reagent discussed above would serve for esterfication and analysis, depending on what type of sample is to be analyzed However, care must be taken to follow the method exactly as specified because minor modification such as the use of cold instead of tepid water for extraction of the reagent, shaking manually instead of vortex mixing of solution, or heating for an extra few minutes could account for an inaccurate fatty acid composition. When introducing a new reagent it is not always practical to test its prolonged use on the GC column. The accuracy of the method should be demonstrated on primary standards, short- and long-chain and polyunsaturated fatty acids It should be carefully

assessed whether the method results in a preferential loss of certain fatty acids or causes the formation of conjugated or different geometric isomers [11] Mediator solvent and enclosed fat are important factors that need to be considered for esterification

1 Mediator solvent

A mediator solvent is used to create a one-phase system of the fat, solid fats must be dissolved in solvent prior to transesterification When a large proportion of the sample consists of water (e.g., milk, ice-cream), the mediator solvent also has to integrate the water into the one-phase system.[10] Solvents that can be used as mediator solvents include benzene, dichloromethane, toluene, hexane, dioxane, dimethylformamide (DMF), methyl-*tert* butyl ether (MTBE).

2. Enclosed fat

Fat transesterification is a drawback if time is needed for solubilizing enclosed fat in the reagent mixture. In the presence of water, the reaction time cannot be prolonged or temperature increased; i.e., solubilization must be achieved in a previous step.[10]

E. Derivatization Reaction

The derivatization of fatty acids, and acidic substances in general, has been a problem in analytical chemistry for many years. The classically used derivatives have been amides and esters. Derivatization can be performed pre-as well as postcolumn Postcolumn derivatization is most convenient for detection.[12] Derivatization reactions are used to decrease the polarity of polar analytes, which in turn increases their solubility in supercritical fluids, promotes their separation from aqueous and solid samples, improves their detectability and enables the coupling of supercritical fluid extraction with various chromatographic methods of separation and detection.[13] Derivatization is also used to derivatize active matrix sites to facilitate the

release of analytes A variety of derivatizing agent has been used to simultaneously derivatize and extract organic acids for example, triglycerides in oil seeds, triglycerides in edible fat, and microbial phospholipid fatty acids from whole cells.[14]

Three factors that need to be considered for derivatization reaction are volatility, reducing polarity and promoting separation, and detection.

1. Volatility

Many simple and complex lipids are either too polar or have too high a molecular weight to be subjected to some chromatographic procedures, so it is necessary to convert them to volatile and/or nonpolar derivatives for further analysis. Gas chromatography has the advantages of superior separation compared to liquid chromatography and a compatibility with a wide variety of detectors. However, volatility is the principal requirement for analyte analysis by gas chromatography. Polar organic compounds that contain active hydrogen (e.g., hydroxyl, amino and thiol groups) are typically of low volatility due to their tendency to self-associate or to associate with polar liquid or solid media through hydrogen bond formation In order to increase polar analyte volatility, the forming of alkyl, silyl and acyl derivatives typically masks active hydrogens, which effectively reduces the tendency of polar analytes to form hydrogen bonds. Derivatization can also be used to increase an analyte's thermal stability [13]

2. Reducing Polarity and Promoting Separation

Reducing the polarity of analytes through chemical derivatization makes polar analytes more amenable to extraction by either conventional means (e.g ,organic solvents) or by supercritical fluid extraction. Derivatization of active hydrogen decreases the reactivity of polar analytes with active sites of complex sample matrices and increases the solubility of

polar analytes in non-polar fluids (e g , organic solvents, supercritical fluids) Decreasing an analyte's polarity through derivatization reactions can be used to decrease the aqueous solubility of the compound from water using solid-phase extraction media, which can then be eluted with supercritical fluids.

3. Detection

Another important use of chemical derivatization reactions is to improve the detectability of compounds by detectors that are commonly used with gas chromatographs (e g., flame ionization, electron capture and nitrogen-phosphorus detectors as well as mass spectrometers). Halogenated derivatives are used typically to promote the sensitive detection of analytes by electron capture detectors and negative chemical ionization mass spectrometry. Derivatization can alter ion fragmentation patterns, which can be used to elucidate compound structure and functional group composition. Derivatization also can be used to enhance analyte detection using single wavelength ultraviolet, diode array, or fluorometric detectors, which are typically coupled with high performance liquid chromatography.[13]

F Lipid Oxidation

Oxygen is necessary for the oxidation of fats. At very low oxygen pressures, the rate of oxidation is approximately proportional to the pressure Therefore, removal of atmospheric oxygen from a fat or food product exerts a protective effect. Lipids become rancid as a result of oxidation, and this oxidative rancidity is a major cause of food deterioration. Oxidation of lipids and the resulting off-flavor and off-odors are of major concern to the food industry. These off-flavors and off-odors significantly affect the sensory quality of foods, their shelf-life, and nutritional quality. Lipid oxidation is influenced by factors such as the presence of oxygen, metal ions (trace metals, especially copper and iron,

act as pro-oxidants in fat), light (all forms of light radiation from the ultraviolet to the infrared region are conductive to fat oxidation), temperature, and enzymes. Ultraviolet light has a more pronounced effect than visible light due to the higher energy of ultraviolet. At ordinary temperatures, the effect of increasing temperature on the rate of autoxidation is slightly greater than for most chemical reactions because increasing temperatures accelerate both the chain propagation reactions and peroxide decompositions. Low temperature storage helps to minimize temperature-induced oxidation.[5]

These factors contribute to lipid oxidation, which generates hydroperoxides and eventually peroxide radicals. One of the factors that affects the autoxidation of common food fats is the total number of unsaturated linkages in the sample. Subsequent chain reactions take place leading to the formation of secondary products that contribute to off-flavors and off-odors.[15] The acceptability of a food product depends on the extent to which this deterioration has occurred. Whereas other deteriorative reactions such as microbial or enzyme attack can be largely controlled by lowering the temperature, this reaction is not particularly helpful in preventing oxidation since low thresholds are involved. From the standpoint of food oxidation, the important lipids are the unsaturated fatty acid moieties. The susceptibility and the rate of oxidation of these fatty acids increase in a somewhat geometric fashion in relation to their degree of unsaturation. Oxidation of unsaturated fatty acids has been well reported and unless mediated by other oxidants or enzyme systems proceeds through a free-radical chain mechanism involving initiation, propagation, and termination steps.

The mechanism of autooxidation involves attack by free radicals and is enhanced by high-energy photons (e g , ultraviolet light) and certain metal ions, once initiated, the reaction is autocatalytic

These can be formulated as

Initiation

$RH + O_2 \longrightarrow R\bullet + \bullet OH$

Propagation

$R\bullet + O_2 \longrightarrow ROO\bullet$

$ROO\bullet + RH \longrightarrow ROOH + R\bullet$

Termination

$R\bullet + R\bullet \longrightarrow RR$

$R\bullet + ROO\bullet \longrightarrow ROOR$

$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$

RH refers to any unsaturated fatty acid in which the H is labile by reason of being on a carbon atom adjacent to a double bond. R• refers to a free radical formed by removal of a labile hydrogen

Hydroperoxides (ROOH) are the major initial reaction products of fatty acids with oxygen [14] Hydroperoxides are the predominant, but not exclusive, primary products of autoxidation of unsaturated fats. ROOH are relatively unstable at or above 80°C; whereas, at room temperatures they are relatively stable. Therefore, different end products may be produced under different reaction temperatures.[5]

Oxidation is the most important cause of fat spoilage because all edible fats, as such or as components of foods, contain unsaturated triglycerides. Oxidative deterioration of fats

results in the destruction of vitamins (A, D, E, K,and C), destruction of essential fatty acids, and the development of a pungent and offensive off-flavor

Polyunsaturated fatty acids will autooxidize very rapidly if left unprotected in air. Although natural tissue antioxidants such as tocopherols may afford some protection, it is advisable to add an additional antioxidant such as BHT ("butylated hydroxytoluene" or 2,6-di-*tert*-butyl-*p*-cresol) at a level of 50-100 mg per liter to the solvents in an atmosphere of nitrogen. Pyrogallol can also be used as an antioxidant and protects fatty acids from oxidative degradation in the low-pH, high-heat hydrolysis environment, resulting in complete or near-complete recovery of fat. Degradation of unsaturates will lead to errors in determining individual fatty acids.[2]

The total amount of unsaturation may not be as important as the degree of unsaturation within a given molecule. Autoxidation of lipids can give rise to very real difficulties in chromatography separation as a function of unsaturation and the greater the degree of unsaturatation of the fatty acid components, the greater the risk Each additional double bond in the molecule increases this rate by a factor of at least two. For example, a fat high in linolenic acid (three double bonds) would be more susceptible to oxidation than one containing a similar amount of oleic acid (one double bond).[5] With fatty acid moieties containing two or more double bonds, one effect of autoxidation is that the double bonds move to form conjugated systems and exhibit distinctive ultraviolet spectra which can be used to estimate the extent of reaction.[6] An antioxidant reacts with free radicals to produce antioxidant-free radicals that will not react with further O_2. Antioxidants act primarily as hydrogen donors or free radical acceptors.

The reaction of an antioxidant (AH_2) may be represented as follows.

$$ROO\bullet + AH_2 \longrightarrow ROOH + AH\bullet$$

$$AH\bullet + AH \longrightarrow A + AH_2$$

The primary role of antioxidants is to break the chain reaction of autoxidation by reacting with hydroperoxy radicals. The antioxidants may be classed as natural and synthetic. Many fats and oils, particularly in the unrefined form, are quite stable to oxidative rancidity because they contain natural antioxidants. The tocopherols (α, β, and γ) are the most important of the natural antioxidants and are widespread in both plant and animal tissues. Vegetable oils contain much higher concentrations of tocopherols than animal fats.

Naturally occurring compounds BHT, BAH (butylated hydroxyanisole), ascorbic acid, α-tocopherol, and tert-butylhydroquinone (TBHQ) are present in most food samples (processed food) at the parts-per-million level, and are sufficient to protect the unsaturated acids from degradation under hydrolytic conditions.[2] Naturally occurring antioxidants exhibit relatively weak antioxidant properties. As a consequence, synthetic antioxidants have been developed for use in foods. These substances, to be allowed in foods, must have a low order of toxicity, should be effective in low concentrations in a wide variety of fats, should contribute no objectionable flavor, odor or color to the product, and should have approval of the Food and Drug Administration.[5]

Since antioxidants vary in their effectiveness to stabilize fats or fat products, and in their mode of action, combinations of antioxidants are often used to stabilize fatty foods

G Tests For Oxidative Rancidity

Several tests have been developed to indicate oxidative rancidity in fats Some of these tests are purely qualitative, whereas others give a quantitative indication of the degree of rancidity Three such tests are the Kreis Test, the Thiobarbituric Acid Test, and the Peroxide value.

The Keris test depends upon the formation of red color when an oxidized fat is treated with concentrated hydrochloric acid and a solution of phloroglucinol in ether. In the Thiobarbituric acid test, the oxidation of fats produces compounds that react with 2-thiobarbituric acid to give red-colored products. For the peroxide value, peroxides are the first compounds formed when a fat oxidizes, all oxidized fats give positive peroxide tests.[5]

H. Moisture Effect

Since water is a competing reagent, the efficiency of the tranesterification reaction should be highest when conducted under anhydrous conditions Although it is possible to remove moisture from samples by freeze drying, this method is somewhat expensive, time consuming, and may cause the loss of volatile analytes. If heat is used to dry the sample before extraction (oven drying), the heat may cause the loss of the more volatile lower chain fatty acids may cause air oxidation of unsaturated components, or may possibly cause the polymerization of oils [16]

A recent development has been the use of a drying agent. There are several advantages to use drying agents in that they are both sample dispersants and sorbent traps for water. A number of drying agents have been used with varying success, namely, Hydromatrix (pelletized diatomaceous earth), magnesium sulfate, sodium sulfate, calcium chloride, molecular sieves and silica. The drying agent can be used as 4:1 or 1:1 (w/w) drying agent-

to-sample ratio Ideally, the successful drying agents should be able to retain selectively the extracted water from real samples but not retain the extracted analyte of interest [17]

I Supercritical Fluid Extraction (SFE)

Various economic, environmental and social factors have motivated government agencies and industry to search for cheaper and safer extraction and separation technologies for food processing. A substance that is above its critical temperature and pressure is defined as a supercritical fluid. The cost of energy was initially the incentive to develop more flexible and energy-efficient extraction processes based on supercritical fluid technology. The most recent interest in supercritical fluid separations has resulted from government regulations regarding solvent residues and fugitive hydrocarbons in the environment, increased performance demands on the product, and increased food-safety awareness.

Analytical SFE is finding widespread application in the analysis of foodstuffs, agriculturally derived materials, and natural products The high efficiency that SFE demonstrates toward the removal of oils and fats from such matrices makes it a natural technique for the determination of their fat and oil content. Because the equipment required to perform supercritical extraction is somewhat sophisticated, the initial costs of SFE equipment is generally higher than that for other "standard" extraction methods such as soxhlet or soxtec extraction Although SFE equipment can be self-assembled relatively easily, most labs do not have the expertise or desire to fabricate such equipment and prefer to purchase SFE systems from a manufacturer. Fortunately a variety of commercial SFE units are available from several vendors [16]

The advantages of SFE, in terms of safety, expediency, and selectivity, together with the variety of instruments commercially available for its implementation, have promoted a

rapid growth that has relied on technical improvements and an expanding scope of applications in the past five years, as reflected in the exponential increase in the number of publications devoted recently to this technique. Liquid-extraction methods are being superseded by new SFE methods for food analysis that takes advantage of the rapidity and automation of this technique. Typically, conventional methods such as ether extraction can be replaced with SFE methods since supercritical CO_2 is suitable for dissolving lipids and minimizes toxicological risk to laboratory personnel and disposal problems of residual solvents.[18]

Supercritical fluid extraction of lipids is a well known technique for sample clean-up and preparative applications.[19] SFE has emerged as an alternative sample preparation technique that uniquely possesses the ability to incorporate carbon dioxide for the solubilization of target analytes from various sample matrices.[20]

In SFE, a supercritical fluid (CO_2) is used as the extraction solvent instead of the typical liquids encountered in the laboratory today (e.g., methylene chloride, hexane, and methanol). Carbon dioxide has certain characteristics that makes it suitable for use as a solvent. It is completely miscible with low molecular weight hydrocarbons and oxygenated organics and is therefore a good solvent for many organic. It has a very high volatility compared to virtually any organic extracted, thus facilitating its separation from extract solutions for product recovery and CO_2 recycle.[21]

CO_2 also has favorable transport properties, its viscosity (gas-like) is low, its diffusion coefficients are high (diffusivities that are intermediate between those of gases and liquids), and thermal transport properties are very good. In an extraction process, the liquid-like density enables the fluid to dissolve components from a matrix, subsequently, the gas-like

behavior allows easy separation of the solvent fluid from the resultant extracted material and sample matrix, often at mild thermal conditions

Carbon dioxide's critical temperature and pressure are readily accessible with well established process technology and equipment. Its heat of vaporization is low, especially near the critical point, leading to low energy requirements in many processes From a practical viewpoint, it is a nontoxic and nonflammable solvent, and is readily available at a low cost.[22] However, by means of introduction, this "hybrid" nature results in the overall simplification that supercritical fluids "*dissolve like liquids and behave handle like gases.*" [23]

J. Carbon Dioxide as Solvent

SFE has a good potential for selective extractions. In fact, with SFE temperature takes an even more important role since the fluid density is a strong function of temperature in the supercritical fluid region. The other parameter impacting density is the pressure. Supercritical fluids are highly compressible; significant pressure must be applied to them to achieve liquid-like densities. The high degree of compressibility leads to variable densities with the density directly dependent upon both temperature and pressure. At maximum densities, the supercritical fluid has maximum solvent power so usually everything that is soluble at the various discrete lower densities is soluble at the maximum density; i.e., there is no selectivity if just the highest density is used in the extraction scheme. With CO_2, the solvent power occurs at about 0.1 g/mL, this is the point at which the CO_2 makes a transition from ideal gas behavior (PVT equation) to critical-region behavior where the density is an even more sensitive function of pressure (compared to ideal gas behavior). The result is that liquid-like, but selective, solvation occurs for CO_2 over the density range 0.1 g/mL to 1 g/mL. Density vs pressure relationship for CO_2 is shown in Figure 1

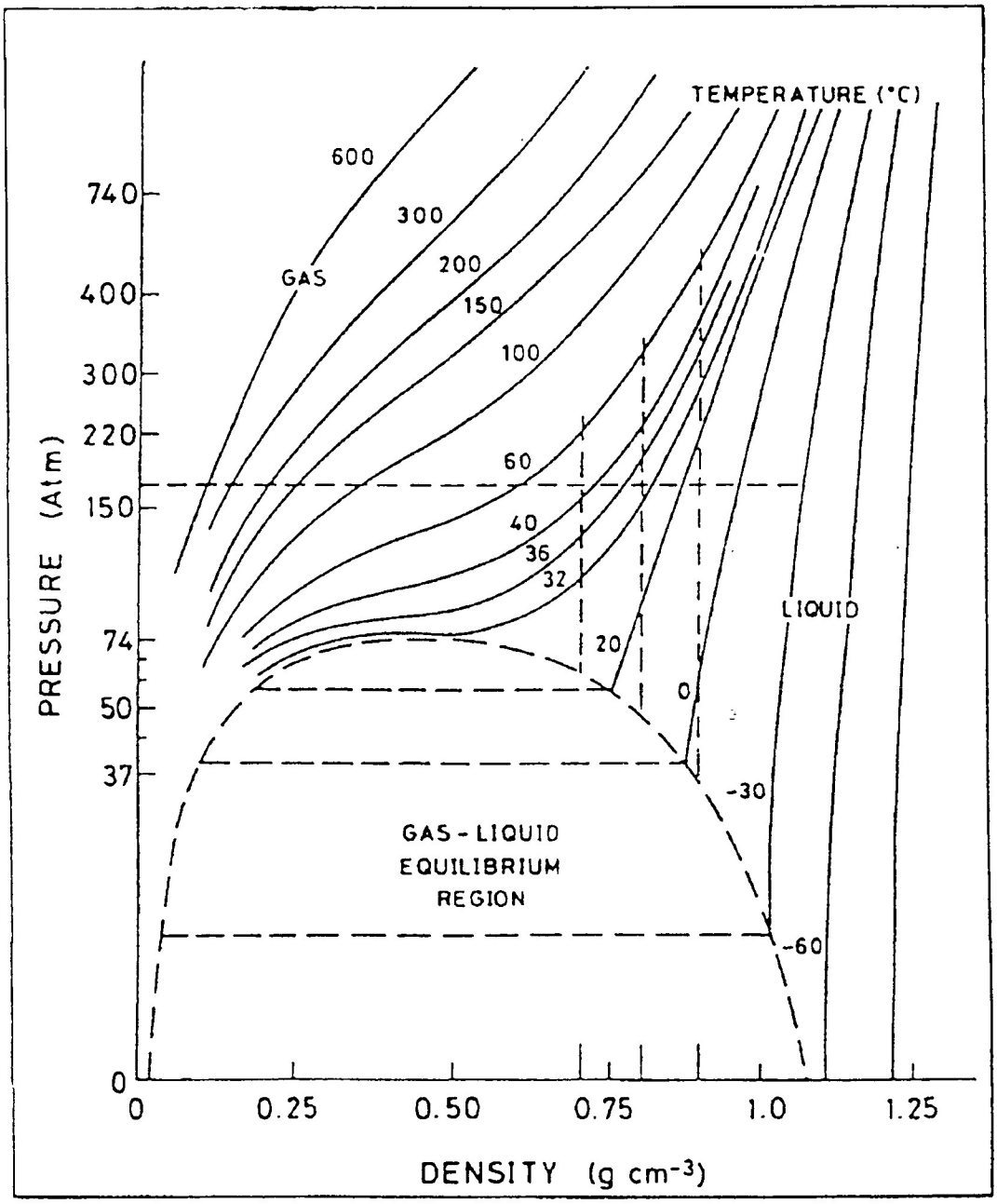


Figure 1. Density vs pressure relationship for CO_2 at various temperatures.

There are several steps one can take to increase efficiency of introduction by supercritical fluids (SFs), an increase in density of SFs is achieved by increasing the pressure or decreasing the temperature A higher density enhances the solvent strength of the SF's An increase in extraction temperature decreases the fluid's density but increases the vapor pressure of the solute It also enhances solute extraction (i.e., if the compound is below its melting point at the extraction temperature, the temperature should be increased because liquid solutes are generally more soluble than solids). Increasing the amount of modifier enhances analyte solubility. The polarity of the extraction fluid may change, and the sample matrix could swell. Increasing time of extraction , volume of extractant phase, or repetitive extraction per discrete sample allows equilibrium, kinetics, and mass-transfer effects to take place and finally grinding the sample size (0.01-0.1 mm) enhances the extraction kinetics.[24]

K Components of SFE systems

1. Fluid Delivery Module

When fluid coming from the cylinders is pressurized CO_2 is a liquid at ambient temperatures. In order to exploit the adjustable density of a supercritical fluid and to achieve optimal diffusivities and viscosity, the compressed liquid carbon dioxide must be turned into a supercritical fluid by adjusting both the temperature and pressure of the fluid before it contacts the sample to be extracted. The pressure exerted on the liquid CO_2 in the cylinder is a function of its ambient temperature There are a number of fluid delivery devices, including diaphragm compressors, gas or liquid booster pumps, reciprocating piston pumps, syringe pumps, and thermal pumps. Liquid pumps (such as used in liquid chromatographs) are more commonly used Pumping systems include pressure measuring transducers that provide an indication of the actual supply pressure or, in more sophisticated equipment,

provide a feedback signal for pressure (density) control. After the pump, the CO_2 (still in the liquid phase) passes through small diameter tubing until it approaches the extraction region where the sample is contained. The design of the preheat zone must accommodate transferring enough heat to the flowing stream of solvent at all possible operating values of pressure and flow rate so that the CO_2 is at the correct temperature as it first contacts the sample.

2. Source of Fluid

The purity of CO_2 used for extraction can be very important in avoiding the potential addition of interferences. Although CO_2 manufacturers do provide SFE grades (e.g., 1 ppb volatile hydrocarbon residue) of CO_2, these grades can be very expensive. The gravimetric-based SFE determinations do not require ultrapure CO_2.

3. Extraction Cell

All of the commercial extraction cells are designed to hold solid samples, although there are instances where the ability to extract a liquid sample would be useful. For quantitative extraction, large samples generally require longer extraction times. Therefore, smaller samples are preferred unless larger samples are required to insure sample homogeneity or to provide the required analytical sensitivity. In some cases the void volume should be filled with inert material such as glass wool, glass beads, or hydromatrix. Inert materials prevents channeling of the solvent through the cell and reduces the volume of the solvent required for the extraction.[25]

4. Separating the Extracted Components from the SF

After passing through the necessary capillary tubing, the moving phase of CO_2 (now containing analytes/solutes that were extracted from the sample in the extraction region) is

directed on to the "flow restrictor." Some devices provide automated flow control, some are heated, and some are simply pieces of capillary tubing Back pressure control can be accomplished using either restrictors or variable restrictors With the fixed restrictors, the flow rate and pressure cannot be independently selected and controlled The restrictor is the throttle point or the narrowing of the passageways It is the most restrictive flow region between the pump and ambient pressure conditions The restrictor is the point at which the major pressure drop occurs, i.e., where density of the extraction fluid changes from liquid-like to gas-like.

A simple outline of the key steps in the SFE process is straightforward. This involves bringing a fluid to the appropriate operating condition. The fluid is then introduced into a region containing a (finely divided or well-distributed) sample at precisely controlled conditions of temperature, density, and composition for some repeatable time of static extraction followed by a time dynamic extraction (having a controlled flow rate) to dissolve and remove components from the sample. The dissolved components are then removed from the supercritical solvent by drastically lowering the pressure of the solution. Finally, reconstitution of the extracted components in a solution that is appropriate for the subsequent analytical experiment.[26]

5. Static versus Dynamic Extraction

Not all commercial instruments offer both static (stopped flow) and dynamic (constant flow) extraction. Dynamic extraction requires a restrictor to maintain pressure, whereas static extraction can be done with or without a restrictor. In many cases, both processes will work to remove the analyte of interest [24]

6 Polarity and Solubility

Total fat content, cholesterol, fatty acid composition, triglycerides and phospholipids can be determined by using SFE methods With liquid extraction, nonpolar analytes are removed with nonpolar solvents and polar solutes are removed with polar solvents. CO_2 is nonpolar, so it has some limitation, particularly for the extraction of polar compounds In SFE, polarity is only one variable. The solvating power of the supercritical fluid can be altered simply by changing some physical conditions such as temperature and pressure or by using modified CO_2 Changes in the pressure and temperature of the SF, the use of modified CO_2, ion pair formation, esterification, the use of organometallics, or complex formation are the most common ways of increasing the extraction efficiency for polar and ionic analytes.

Polarity is adjusted by using a fluid other than the most commonly used (CO_2), by modifying CO_2 with a cosolvent or additive, or by changing the density. Varying temperature or pressure can change density At low densities (0.2 to 0.45 g/mL), the solubilizing power of CO_2 is similar to that of hexane. As the density increases, the polarity gradually changes so that 0.6 g/mL CO_2 behaves more like the moderately polar solvent methylene chloride. Large amounts of fat are extracted at 80°C and high densities; however, fat extraction is dramatically reduced below 0.6 g/mL. The extraction of total fat is usually carried out at high pressure (350-700 bar) and temperature between 60 and 80°C [18]

The extraction of polar compounds such as drugs, toxins, and polar pesticides requires high densities The polarity of CO_2 can be adjusted to enable it to dissolve very polar (water-soluble) molecules by adding small amounts of organic modifier such as methanol, ethanol, or isopropyl alcohol Other modifiers are water, acetonitrile, tetrahydrofuran (THF), and methylene chloride.[24] In general, the solubility of triglycerides increases with both extraction

temperature and pressure, although there is an inversion in the solubility-temperature relationship as a function of pressure. The modifier exerts its effect mainly in two basic ways: by interacting with the analyte/matrix complex to promote rapid desorption into the SF, and by enhancing the solubility properties of CO_2.[25] CO_2 is soluble in triglycerides and may be imbibed in the collected oil and fat after SFE. Hence, this dissolved CO_2 can give high gravimetric fat values if not removed (e.g., by rotary evaporation) from the oil before being weighed. Because the solubility of triglycerides increases greatly with pressure, supercritical fluid extractions of fat are most efficiently done at high pressure. Although some SFEs have high maximum operating pressure (e.g., 9500 - 10000 psi), others have much lower maximum pressure capabilities (e.g., 5500 or 6000 psi), limiting the achievement of the maximum solubility of triglycerides in CO_2. It should be noted that there can be some discrimination with respect to fatty acid chain length during SFE of fatty acids. It is important to determine the length of extraction required to quantitatively remove all of the target analyte, because in some food products the solubility of longer chain fatty acids are less than shorter fatty acids during extraction, which would lead to an incorrect determination of fatty acid profile. Using some of the highly automated commercial SFE septems, method optimization studies can be conducted very quickly and easily.[16]

The modifier can either be added to the sample in the extraction cell prior to SFE or be mixed with the CO_2. The latter is more effective since the modifier is continuously passed through the sample, whereas in the former the modifier is swept from the extraction cell when the SF starts to circulate through the sample.[27]

1. Matrix Effects Lipid Distribution/Association of Fat with Other Components

The total composition of the matrix is rarely known, although general characteristics may be available In SFE, as in most extraction procedures, the effects of the sample matrix are the least understood Variability of matrix type and the physical and chemical complexity of matrices can make extraction difficult, for example, oil or moisture contents, various constituents, or the presence of chlorophyll. Such matrix characteristics that affect classical liquid-liquid or liquid-solid extraction also influence the efficiency of SFE Polar solutes may be removed more easily from matrixes with higher moisture content. It is believed that analyte is solubilized in the entrained water and removed from the matrix with physical removal of water. Polar analytes benefit from the presence of water in the sample, water has been added as a mobile-phase modifier to enhance extraction recoveries. For the nonpolar analytes, the presence of moisture in the matrix can be detrimental.[28] For extraction of nonpolar analytes, drying agents are mixed with the matrix to adsorb moisture before extraction. Hydromatrix (Celite 566) has been used frequently. Sodium sulfate and calcium sulfates (drierite) are also used to dehydrate the matrix. Ratios of sample to drying agent of 1:1 up to1:5 have been reported. No rules of thumb exist for determining this ratio. Because matrix constituents are not always known, the extract contribution of a matrix constituent toward the success or failure of an extraction method cannot be determined. Matrices with salt concentrations causes problems in classical extractions Typically, subsequent concentration steps are difficult. In SFE, an aqueous modifier can remove the salt or it may actually aid in the extraction by salting out the analyte of interest.[24]

Although the distribution of lipids within foods is often ignored, it can have significant effects on the extraction of the lipids from the foods In low-fat foods, the lipids are generally

in a noncontinous lipid phase dispersed within polar components (e g , polysaccharides or proteins), similar to an oil-in-water emulsion. These polar compounds may form a barrier and may preclude the complete extraction of lipids, because the lipids must be transported across this layer before the solvent can remove them. However, in high-fat foods, the lipid phase is usually continuous with polar components dispersed within the fat. In these cases, the solvent has direct contact with the lipids, making their subsequent extraction easier [16] Removal of analyte from the matrix takes place in 2 steps, first, the analyte moves to the extractant phase, second, the extractant phase is removed from the extraction vessel Figure 2 shows the SFE flow chart.

If the first stage is rate-limiting, then a longer static extraction soak may enhance overall recovery by increasing the time the analyte has to reach equilibrium in the extraction phase. A lower dynamic flow rate yields the same result. The second stage is rate-limiting, then reducing the time it takes to completely remove the extractant phase from the extraction vessel should enhance overall recovery.[24]

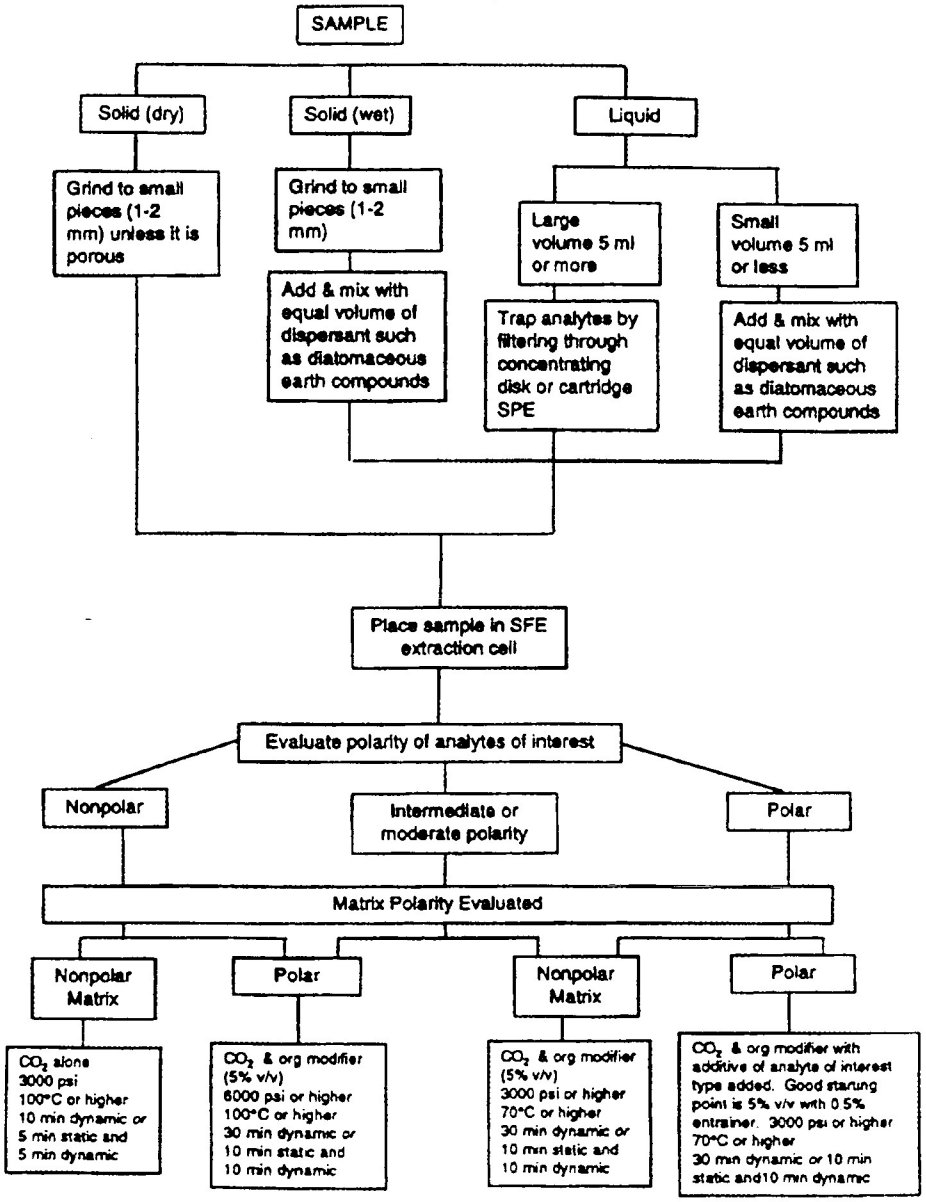


Figure 2. SFE strategy flow chart.

M. Gas chromatography (GC)

GC is a form of partition chromatography in which the compounds to be separated are volatilized and passed in a stream of inert gas (the mobile phase) through a column in which a high boiling point liquid (the stationary phase) is coated on a solid supporting material. The chromatographic process is based on passing the sample through three main parts of the instruments: an injector, a column, and a detector. The substances are separated according to their partition coefficients, which are dependent on their volatility and on their relative solubility in the liquid phases. They emerge from the column as peaks of concentration, these peaks are detected by some means which converts the concentration of the component in the gas phase into an electrical signal, which is amplified and passed to a continuous recorder so that a tracing is obtained with an individual peak for each component as it is eluted.

The most useful application of GC in lipid analysis is in the determination of the fatty acid composition of lipids and, by a judicious choice of liquid phases, a complete analysis of fatty acids separated both by chain-length and degree of unsaturation in a given sample can be achieved. Volatile derivatives of lipids must be prepared for GC analysis; for example, fatty acids are customarily converted to methyl esters and free hydroxyl groups of partial glycerides are acetylated. In addition to fatty acid analysis, GC can be used to determine cholesterol, glycerol, inositol, carbohydrates and many other compounds, released on hydrolysis of lipids in the form of volatile derivatives.

The heart of the GC is the column on which the substances are separated. There are two basic types of columns, packed columns, and wall-coated open-tubular (capillary or WCOT).

Packed columns are filled with inert solid support coated with the liquid phase The list of liquid phases available is almost endless In practice, certain polyester liquid phases have proved themselves most useful for fatty acid analysis and a few silicone elastomers have advantages for higher molecular weight components The solid supporting materials for the liquid phases are generally diatomaceous earth, graded so that the particles are of uniform size, and deactivated by acid washing to prolong the life of the liquid phases and by silylation to minimize any adsorptive effects on the solutes.[6]

The column may be an open-tubular or capillary column, the wall of which is coated with the liquid phase. Capillary injection techniques can in principle be divided into two groups: direct injection (on-column) and split-injection (split/splitless).

The on-column injection gives the highest recovery and reproducibility, while with split-injection technique recovery and reproducibility decreases The split injection technique is based on the sample splitting between the column and a split valve, in proportion to the "split ratio," which is regulated by the carrier gas flow rate A capillary column has advantages over packed columns: better separation efficiency, quantitative recovery and reproducibility of the retention data, shorter analysis time, longer life times of chemically bonded stationary phases, the possibility of multiple on-line column connection, and multidimensional analysis. They are particularly useful when coupled directly to the inlet of a mass spectrometer as each peak is likely to contain only a single component and more positive identifications can be made [6]

Various types of injection systems are available to ensure that the column is not overloaded and to minimize the amount of air and solvent injected onto the column with the sample Injectors introduce the sample onto the column in the form of an aerosol, under a

relatively high carrier gas pressure In many instruments, there are two identical columns and two detectors, which are balanced electrically against each other to minimize background variations The columns are held in an oven, the temperature of which is maintained accurately at a required point within 0 05-0.1^{0}C.

Temperature programming is a useful facility on many instruments If the sample to be analyzed contains components differing widely in volatility, it is advantageous to start the analysis with the column at a low temperature so that the more volatile components are separated as single coherent peaks and to raise the temperature at a fixed reproducible rate so that the less volatile components are eluted in a reasonable time

Neutral lipids are analyzed exclusively by programmed temperature because of the wide range of their boiling points; isothermal analysis is useful only for compounds with lower carbon numbers. High initial temperature causes zone broadening during initial sorption of the sample, while lower initial temperature result in incomplete mass transfer from the injector to the column. The sample emerges from the column into the detector, which is maintained by a separate temperature control, at a slightly higher temperature than that eventually reached by the column, ensuring that there is no change in the response of the detector during temperature programming.

N. Components of a Gas Chromatography

1. Detector

Two detector types are generally used in the GC analysis of lipids: flame ionization and mass spectrometric detectors. A comparison of various detectors with respect to their sensitivities, selectivity and operational ease indicates that the flame ionization detector (FID) comes closest to being the most sensitive The system of the relative response factors using

the effective carbon number concept supports the use of FID in the lipid analysis as the most suitable detector Eluted components are burned in a flame of hydrogen and air forming ions that are detected and measured by an electrical system. The FID detector does not deteriorate significantly with prolonged use [6]

2 Carrier gas

For analysis of lipids on capillary columns, hydrogen is used increasingly as the carrier gas with longer column length, because of considerably high linear velocities required for the elution of such compounds, (the use of hydrogen as a cerrier gas, however, requires precaution, as it constitutes hazard in a confined area). Helium as the carrier gas can also be operated effectively with shorter column length. The carrier gas flow rate affects both column efficiency and quantitative recovery, especially for triacylglycerols with higher carbon numbers and degree of unsaturation. The total gas flow rate through the detector is a contribution from the carrier gas and the fuel gas

O. Gas Chromatography-Mass Spectrometry (GC-MS)

The combination of the GC with mass spectrometry is recognized as the "gold standard" analytical method for the identification and quantitative analysis of organic compounds. Mass spectrometry involves four steps: important to consider and understand an ionization of molecules in a sample, separation of ions according to their masses, measurement of the amounts of the ions produced for each mass, storage, display, and interpretation of the results.

Ions can be separated according to their masses (mass-to-charge ratio) using electric or magnetic fields. Electron ionization (EI) continues to be the most common ionization technique for the lipid analysis. The MS serves as a detector Mass spectrometry is more

properly thought of as a chemical, and not as a classical spectroscopic technique because the process involves the ionization of molecules and subsequent chemical reaction (fragmentation) [25]

II EXPERIMENTAL

A. Materials

McDonald's French fries, Burger King French fries, Wendy's French fries Lays and Golden Flake potato chips, Dixie Cream and Great American donuts, and JIF Peanut Butter are the materials that were used for the extraction of fat.

Reagents used were boron trifluoride-methanol (140 g BF_3 per liter of methanol) stored at 4°C until used, SFC/SFE grade CO_2 (used to perform SFE), saturated sodium chloride, 0.5 N methanolic sodium hydroxide (dissolve 2 g of sodium hydroxide in 100 mL methanol) were ACS reagent grade. Organic solvents used were methanol, hexane, and petroleum ether. Anhydrous magnesium sulfate was used as a drying agent.

B. Supercritical Fluid Extractor

1. Extraction Condition

A LECO FA-100 SFE equipped with automated extraction capability and automated variable restrictors was used for the extraction of fat from different food products The SFE parameters were 9000 psi extraction pressure, 100°C temperature static extraction (5 min hold) at CO_2 flow rate 2 mL/min, and restrictor temperature 100°C. The extract collection was in 10 mL glass vials containing glass wool. The total extraction time was 12 min. Figure 3 is a picture of the LECO FA-100 SFE system.

2. Sample Preparation

Commercial food products including potato chips, French fries and donuts were chopped separately (mortar and pestle or micromill grinder) to make the sample size 0.1 mm

to 1 mm. Peanut butter was used directly from the jar. The procedure was to weigh 1 to 2 g of LECO DRY first into a beaker and then 1.5000 ±0.0010 g of chopped sample (peanut butter) was added. The mixture was thoroughly mixed with LECO DRY. The weight of the sample was registered directly in the analyzer. The glass vial with glass wool for the sample collection was weighed and the sample mixture was then added to the thimble containing a small disk of filter paper at the one end of the thimble such that the thimble cap was completely covered. Almost three scoops of LECO DRY was added to the thimble before the sample mixture was added to the thimble. The vials were inserted into the collection slot and thimbles were than loaded into the instrument (thimble chambers), which were in turn lowered into heated extraction cells that are pressurized by the addition of liquid CO_2. There are three thimble/collection vial chambers in the FA-100. After the extraction was complete, the glass vials were removed and placed in a tray to evaporate CO_2 that was collected in the vial during extraction. After 5 min the full collection vials were weighed, the FA-100 calculated the percent fat and printed the results.

3 Saponification of Extracted Fat from Samples for Fatty Acid Composition

The extracted fat (the vial) from the FA-100 SFE was dissolved by 5-10 mL of hexane. Then 25-50 mg of sample (fat) was taken out by medicine dropper and weighed into a 50 mL beaker. Three mL of 0.5 N methanolic sodium hydroxide was added to the beaker. The mixture was then heated over a water bath until a homogeneous solution was obtained. Then 5 mL of BF_3-methanol was added to the saponification reaction mixture which was boiled for 2 to 3 minutes under the hood. The solution was cooled and transferred to a separatory funnel containing 25 mL of hexane and 20 mL of saturated NaCl solution. The mixture was shaken well (gently) and the layers allowed to separate. The hexane layer

containing fatty acid methyl esters was dried with about 1 g of anhydrous $MgSO_4$ and filtered into a small vial. The solution (22-25 mL) was concentrated on the steam bath to a volume of about 0.5 mL under the hood. This solution of fatty acid methyl esters was capped to prevent air oxidation and stored in a refrigerator until GC and GC/MS analysis.

C. GC Analysis

A model SRI 8610C GC-FID (flame ionization detector) was used for the analysis of fatty acid methyl esters. A 3 m × 0.25 mm I.D. capillary column coated with an apolar stationary phase of 0.25 μm film thickness. A 0.5 μl portion of the sample was injected by the on-column technique. During injection the oven temperature was 60°C, which was held for 4 min, then programmed at 10°C/min to 250°C. Complete analysis of the sample took 30-33 min for each sample.

D. Gas Chromatograph-Mass Spectrometry (GC/MS)

Fatty acid methyl esters were also analyzed using a Shimadzu QP-5000 gas chromatograph/mass spectrometer. The system used an ionization potential of 70 ev and a scanning range of 35-450 atomic mass units. A 2 μl portion of sample was injected directly onto a 60 m × 0.32 mm RTX-5 capillary column and a film thickness of 1 μm in the splitless mode. The injection temperature was kept at 250°C. The column was held at an initial temperature of 100°C for 5 min and then programmed from 100°C to 300°C at 5°C/min. The transfer line temperature between the GC and the mass spectrometer was 230°C. The detector was an electron multiplier. The components identified were based on software matching with the NIST library.

E Soxtec (Soxhlet) Extractor

A model 1045 Soxtec HT2 System with a 1046 Soxtec Service Unit was used for fat extraction. The solvent was petroleum ether at a temperature of 100-105°C.

Each sample was ground in a micromill. A 6-7 g portion of fries (Burger King, Wendy's) was ground separately. Also a 6-7 g portion of Burger King fries was weighed and dried in a vaccum oven at room temperature. Then the dried sample was weighed, and transferred quantitatively into the thimble (attached adapter on the top) and covered with defatted cotton wool. The extraction cup was then weighed and 40-45 mL of petroleum ether was poured into the extraction cup.

The extraction knob was moved to the boiling position and thimbles were immersed in the solvent-containing cup. After 30 minutes in the extraction mode the control was moved to the rinsing position (closed the condenser valve) for 45 minutes. After 45 min all the remaining solvent in the cup was collected in the condenser. For the last traces of the solvents in the cup, the air button was pressed and air valve was opened for 5-8 min. Then the air valve was closed, the extraction cups were removed, and then the thimbles. The extraction cup containing extracted fat was dried in an oven at 50-55°C for 20 minutes and cooled to room temperature. The cup was then weighed and the fat percent calculated by using the following formula

$$\text{Percent Fat Recovery} = (W_3\text{-}W_2/W_1)\times 100$$

W_3 = Weight of extraction cup (after extraction).

W_2 = Weight of extraction cup (before extraction).

W_1 = Weight of sample

For all three kinds of French fries, Potato Chips and Donuts, the same procedure was used Peanut Butter was extracted without drying A picture of Soxtec HT2 System is shown in Figure 4

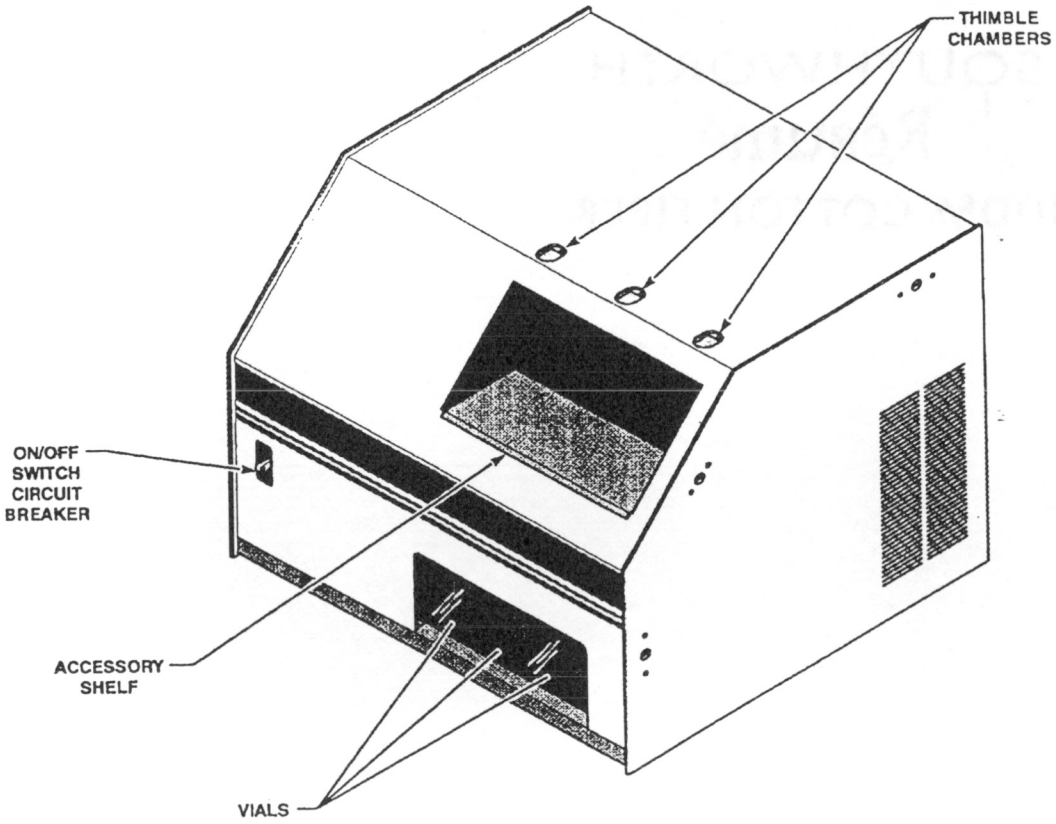


Figure 3. Diagram of LECO FA-100 SFE unit controls and functions.

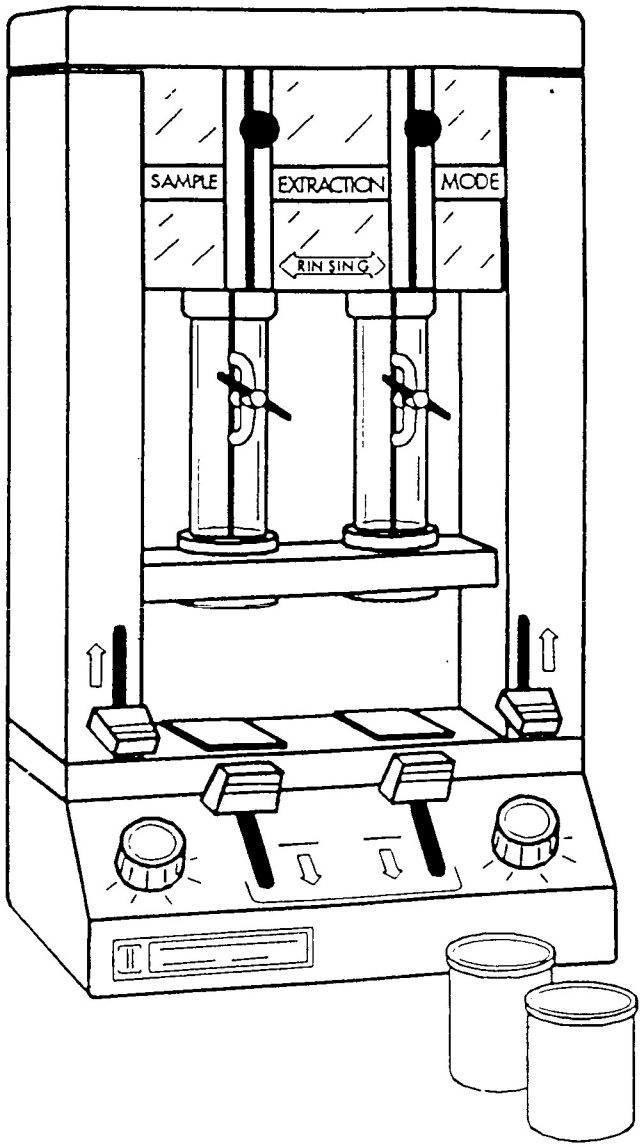


Figure 4. Soxtec extractor HT2 system.

III. RESULTS AND DISCUSSION

A. Parameters for Supercritical Fluid Extraction

The parameters for SFE-- chosen to be examined for their effects on the extraction of the fat content of the samples --were cell temperature, extraction time, static flow time, and the mass of the sample used in the extractions. The three cell temperatures used (the temperature at which the thimble and the sample are kept during the extraction period) were 70°C, 100°C, and 130°C. The amount of time the extractions were allowed to take place (the extraction times) were 5, 12, and 20 minutes. The three different static flows (amount of time that the samples set at a constant temperature and pressure before the extraction was begun) of 5,10, and 15 minutes were studied. The mass of the sample used in the extractions varied between 0.5000 and 1.5000 grams.

Using the three different values of the above four variables, an experimental matrix for our experimentwas designed. First, a three-by-three matrix was designed to take into account each of the three variables of extraction time and temperature. Then, each block of that matrix was broken down into a three-by-three matrix to take into consideration the three different static flows and masses of the sample. To reduce the number of trials that would be needed to complete the 81 possible trials of the matrix, we decided to run two diagonal cross-sections of the matrix. From these 17 trials, one should be able to accurately predict the operation of the LECO FA-100 over the entire matrix.

The three simultaneous trials of the same sample for each of the 17 squares of the matrix were run. The average percentage of fat was used in the generation of the tables and graphs.

The optimum extraction time for the extraction of fat from food samples was 12 minutes. However, when comparing the average % fat extracted between 12 and 20 minutes, one can see that there is an insignificant difference between these two values. Furthermore, the deviation between the values at 12 minutes is much smaller than the deviation between the values at 20 minutes, which would suggest a more accurate reading at the 12-minute mark. The data are shown in Table 1.

Table 1. Percent Fat Determined for Different Extraction Times.

Extraction Time, min	% Fat	Average % Fat	Std Deviation
5	31.2		
5	32		
5	32.3	30.6	1.17
5	30		
5	29.5		
12	31.2		
12	31.8		
12	32	32.0	0 52
12	32.4		
12	32 5		
20	30.3		
20	34		
20	31.4		
20	31 3	31.6	1 31
20	30.6		
20	31.8		

The optimum temperature for the extraction of fat from food samples is 100°C However, if one compares the values obtained at 100°C and 130°C, one would see that there is an insignificant difference in their average values The deciding mark then becomes how small or large a deviation there is in the values obtained at these two temperatures. Considering that 100°C values have a tight range (between 31% and 33%), whereas, the 130°C have a larger range (between 30% and 34%), it becomes evident that 100°C would help give the accurate reading The data are shown in Table 2.

Table 2 Percent Fat Determined for Different Temperatures.

Temperature	% Fat	Average % Fat	Std.Deviation
70	29.3		
70	30		
70	29.5	30.4	0.1
70	31 3		
70	30.6		
70	31.8		
100	31 2		
100	31.8		
100	32.0	32.0	0.5
100	32.4		
100	32.5		
130	31.2		
130	32		
130	32.3	31.9	1.25
130	30.3		
130	34.0		
130	31.4		

The optimum mass of the sample to use in the extraction of fat from samples is 1 5 grams When taking into consideration that 100°C is the optimum temperature, one can see that 1 5 g is definitely the optimum mass of the sample to use in the extraction process At 1 5 g of potato chip, the percent fat vs mass of sample data points at 100°C graph, show a significant increase in the percentage of fat extracted from the sample These data are shown in Table 3

Table 3. Percent Fat Determined for Different Masses of Sample

Mass of Sample, g	% Fat	Average % Fat	Std Deviation
0.5	32.3		
0.5	29.5		
0.5	32.4	31.7	1.13
0.5	32 5		
0.5	31.4		
0.5	31.8		
1	32		
1	30		
1	32	31.7	1.55
1	34		
1	30.6		
1.5	31.2		
1.5	29.3		
1.5	31.2	30.9	0.9
1.5	31.8		
1.5	30.3		
1.5	31.3		

An optimum static flow for the extraction of fat from food samples cannot be determined by the data obtained in this study From the data gathered the temperature of the cell, mass of the sample, and the duration of the extraction are the determining factors of the extraction process, with the static flow playing an insignificant role compared to these three factors There are almost no detectable changes in the average of the percent fat extracted at the three different static flows of 5, 10, and 15 minutes These data are shown in Table 4

Table 4. Percent Fat Determined for Different Static Flow Times.

Static Flow, Min	% Fat	Average % Fat	Std.Deviation
5	31.2		
5	31.4		
5	32 4	30.5	0.94
5	29.5		
5	31 3		
10	32		
10	34		
10	32	31.7	1.55
10	30		
10	30.6		
15	32.3		
15	30.3		
15	31.8		
15	32 5	31.6	1 26
15	29.3		
15	31.8		

Tables 1-4 show the data that are appropriate for the developed method SFE has been recognized as an important alternative to conventional liquid or soxhlet extraction Supercritical CO_2 is a natural solvent for the extraction of lipids because triglycerides and fatty acids are highly soluble in CO_2. After the procedure was developed fat was extracted from other samples, including French fries, peanut butter, and donuts Table 5 gives an example of a set of runs typical of the experimental extractions. In all cases LECO DRY was added to absorb the moisture

Table 5 Percent Fat Extraction from Various Foods

Trial	Weight of Sample	Fat Recovery (%)	Average % Fat
	Peanut Butter		
1	1.8573	48.1	
2	1.8255	48.3	48.4%
3.	1.8055	48.9	
	Wendy's French Fries		
1.	1.5008	16.6	
2.	1.5006	16.3	16.7%
3.	1.4991	17.1	
	McDonald's French Fries		
1.	1.5005	16.8	
2.	1.5007	16.3	16.3%
3.	1.4996	16.5	
	Burger King French Fries		
1.	1.5010	14.6	
2.	1.5004	14.3	14.2%
3.	1.5011	13.7	
	Dixie Cream Donut		
1.	1.5006	23.5	
2.	1.5008	22.8	22.8%
3.	1.5000	22.2	

Soxtec extraction was also done, which is the standard method for extractions. The principle of the soxtec extraction is the same as soxhlet extraction, but soxtec extraction requires much less time and smaller amounts of organic solvents compared to soxhlet extraction. Modifications of the soxhlet method to semi-automated equipment (Soxtec) is done to reduce the extraction time. Soxtec extraction can be completed within two hours, whereas soxhlet extraction can take almost 20 to 24 hours for the same kind of sample. Both methods require organic solvents for the extractions.

B. Soxtec (Soxhlet) Extraction

For the Soxtec extraction, the French fries and potato chip samples were used directly without any pretreatment. The extraction results are shown in Table 6. Petroleum ether was used for the extraction of neutral fat, which is a non-polar hydrocarbon solvent (mixture of pentane, hexane and heptane). Petroleum ether is the solvent of choice for the extraction of triglycerides, which is also non-polar and soluble in petroleum ether, based on the principle "like dissolve like." The ether extraction methods are suitable for analysis of fat in animal feed, raw cereal grains such as wheat, rye, oats, corn, buckwheat, rice, barley, soybeans, and their products, cereal adjuncts, meats and meat products, butter, nuts and spices. For nutrition labeling purpose, fat has been variously defined as triglyceride substances extracted with ether. The fat content of these foods was assayed by using 1.5-2 hrs, 45-50 mL petroleum ether extraction on a 3-3.5 g sample. The ether extract was dried completely, and the oil was weighed to determine the percentage of the fat. Soxtec extraction was done to compare the results of percent fat extracted to that of percent fat extracted by SFE. The % of fat extracted from Soxtec extraction was approximately 2-3% high compared to SFE

A number of specific factors hinder extraction of lipid or contribute to high fat values Particle size, product moisture, and formulation may all affect the extraction process. For example, the moisture content of a product and the extraction time influence the amount of lipid removed from the product or the sample Probably the moisture is the main factor that needs to be considered. The data in Table 6 for French fries and peanut butter were obtained without any sample pretreatment and are typical for procedure LECO DRY was added in equal amount of the weight of the sample.

Table 6 Results of Fat Extraction from Various Foods

Trial	Weight of Sampl	Fat Recovery (%)	Average % Fat
		Wendy's French Fries	
1	3.0049	17.67	
2	3.0010	18.15	17.7%
3.	3.0044	18.10	
		McDonald's French Fries	
1.	3.0020	18.25	
2.	3.0019	17.59	17.6%
3.	3.0090	17.23	
		Burger King French Fries	
1	3.0015	15.17	
2.	3.0002	15.98	15.4%
3.	3.0152	15.13	
		Peanut Butter	
1.	3.0015	45.34	
2.	3.0081	46.70	45.8%
3.	3.0012	45.20	

According to the method described in the AOAC, moisture is determined by drying the sample in a vacuum oven. For the moisture determination, 5-6 g of sample was ground and dried overnight in a vacuum oven. The next day, after 22-24 hours, the sample was reweighed. There was 1 5-2 g lost in the weight of the sample, equal to the moisture content of the sample. The extraction was then performed on the dry sample, using the petroleum ether. The SFE and Soxtec results were in good agreement for these samplea as shown in Table 7

Table 7 Results of Fat Extraction from Various Foods After Drying

Trial	Weight of Sample	Weight after Drying	Weight Loss	% Fat Recovery	Average
		Wendy's French Fries			
1	5 7970	3.9528	1 8442	16.73	
2.	6.1280	3.5341	2 5939	16.14	16 7%
3	4.9927	3 0888	1 8426	17 37	
		McDonald's French Fries			
1.	5.8255	3.9345	1.891	16.42	
2.	5.5300	3.7589	1.7711	16.61	16.4%
3.	5.0250	3.3249	1.7001	17.05	
		Burger King French Fries			
1.	5.8355	4.2718	1 5667	14.59	
2.	4.9150	3.4641	1.5091	14 11	14 3%
3.	4.6966	3.7730	1.6952	14.13	
		Potato Chips			
1.	5.2664	3 4252	1.8412	32 85	
2.	5.3901	3.5743	1.7912	33.22	33 2%
3.	5.1540	3.3525	1 8015	33.54	

The formula for calculating the fat recovery (%) is as follows:

Parameter Dry = {[Weight of extraction cup (after)-Weight of extraction cup (before)]/Weight of sample (after drying)}*100

= (39.6648-38.6947/3 9528)*100 = 24.54%

Fat Recovery (%) = Parameter Dry[100-{(loss in weight/weight of original sample)*100}/100]

= 24.54[100-{(1.8442/5.7970)*100}/100]

= 16.8%

All the calculations were done using the same formula for other samples

The effect of moisture is not very large. The peanut butter sample was used directly from the jar without any pretreatment. The percent fat extracted from peanut butter was similar to that determined by SFE. Figure 5 shows a graph comparing the SFE and soxhlet data. Figure 6 shows a graph comparing the SFE data with Nutritional Informational Tables.

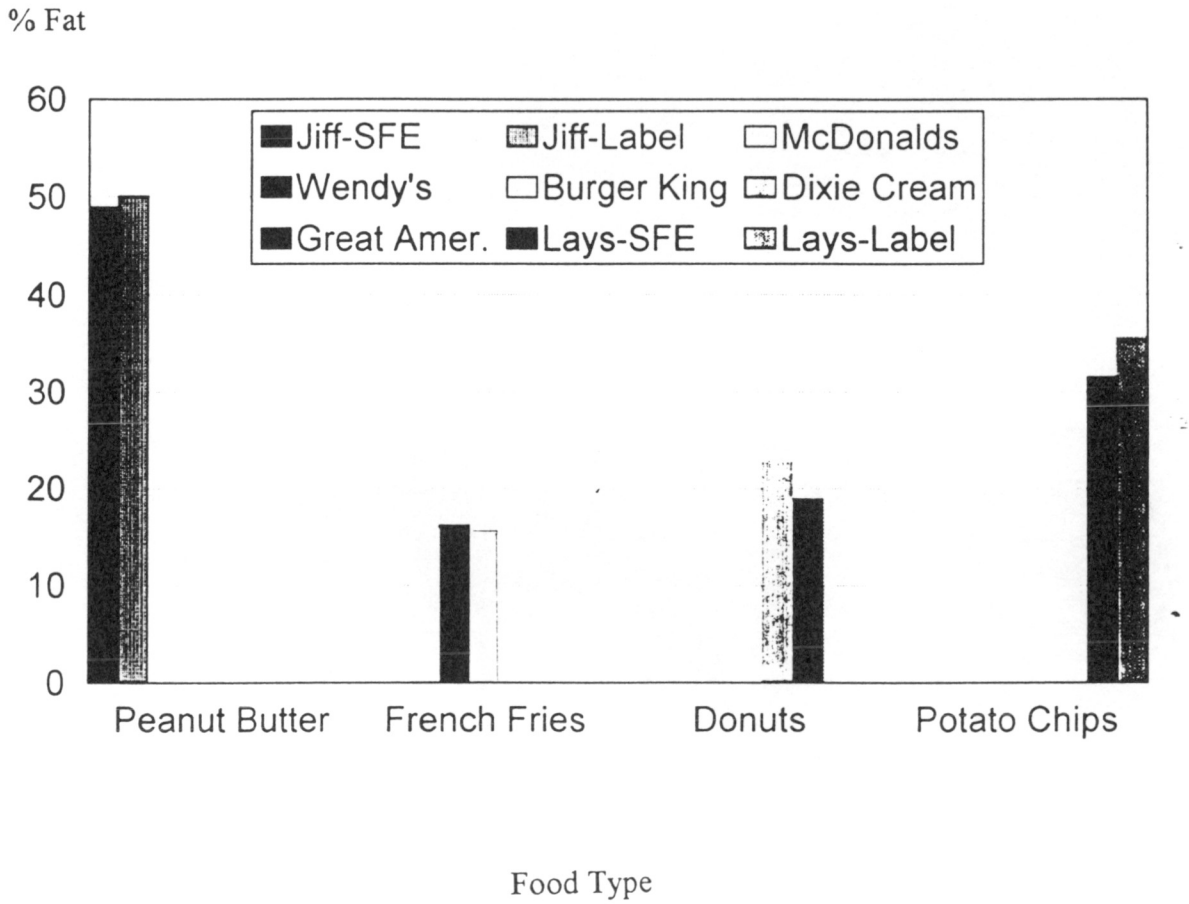


Figure 5. Comparison of percent fat of various foods extracted by SFE with Nutritional Information Label.

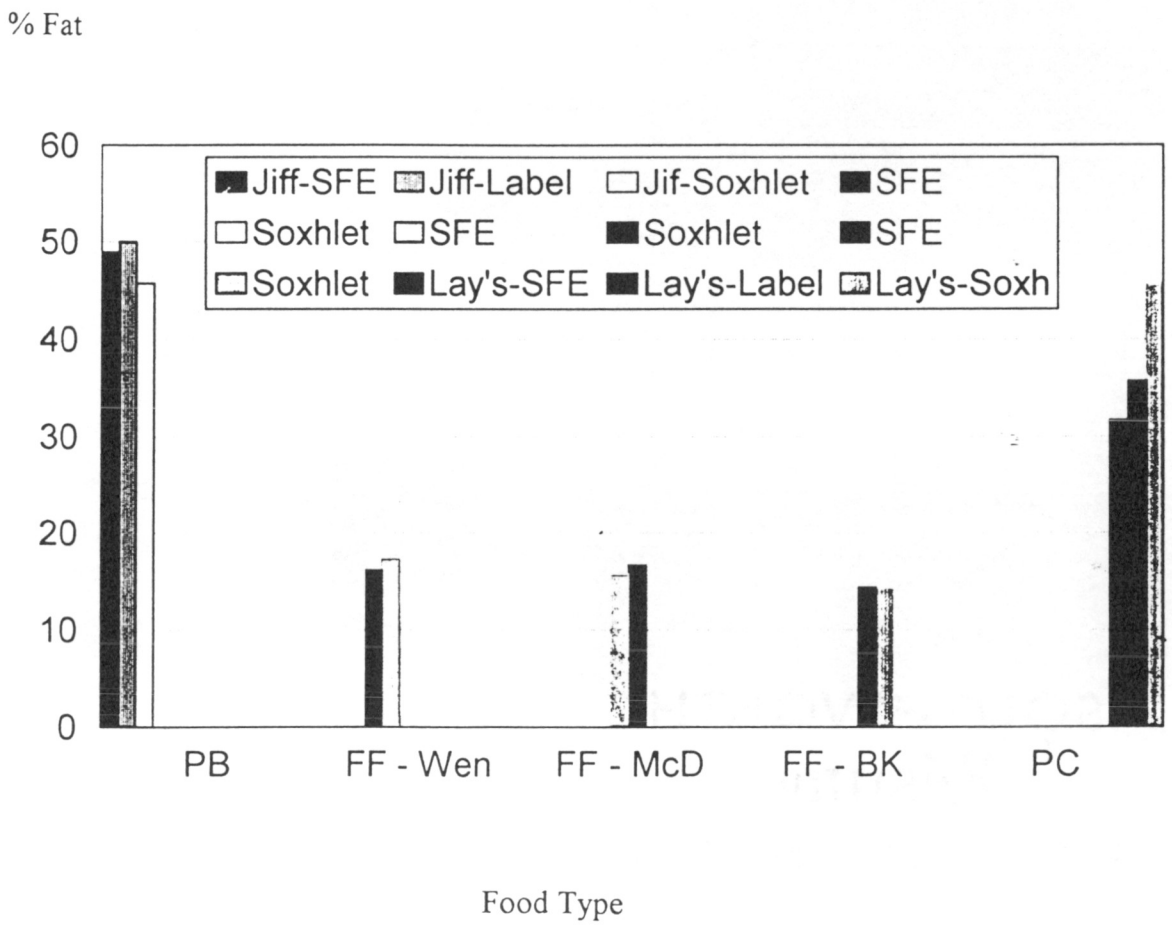


Figure 6. Comparison of percent fat of various foods extracted by SFE and soxhlet with Nutritional Information Label.

The data in Table 8 summarize the results of the SFE determination of fat in various foods. The averages and standard deviations were calculated from 15-20 runs on each type of food. The standard deviations are relatively high, but are calculated from data collected over a period of 3-4 months. During this time there were several problems with the instrument and extreme heat conditions in the building where the experiments were run.

Table 8. Average and Standard Deviation for SFE-Fat Analysis.

Food	Average	Std. Deviation
Dixie Cream Donut	23.20 %	1.99
Great American Donut	19.10 %	1.13
JIF Peanut Butter	48.88 %	0.85
Mc.Donalds French Fries	15.84 %	1.81
Wendy's French Fries	16.24 %	1.93
Burger King French Fries	13.96 %	1.22

C. Formation of Fatty Acid Methyl Esters

The formation of methyl esters is a function of time using catalysts. The BF_3/MeOH most commonly carries out the methylation of fat for the purpose of determining the fatty acid composition by GC. Methylation of short-chain esters is almost instantaneous, whereas the long-chain esters require slightly longer. Only those fatty acids which were positively identified and which were influenced by extraction conditions are reported. Also, only those extraction treatments that resulted in significant fractionation are reported. Fatty acid

contents of samples were determined using a modified fatty acid methyl ester method Fatty acid content of triacylglycerol is most easily determined by complete saponification with NaOH/MeOH followed by esterification of the released fatty acids We used 0 5 N NaOH in methanol Mangold indicated that 10 minutes saponification is sufficient for the base reagent as related to the sample size [27] Before injection, fatty acids were transesterified with 14% 5 mL BF_3/MeOH to form the methyl esters (FAMES) BF_3/MeOH reagent typically used is in the ratio of 1 mL of BF_3 per 4-16 mg lipid. The BF_3 bottle was stored in the refrigerator until used. BF_3 is highly corrosive. Before the reaction was started, the BF_3 bottle was removed from the refrigerator and warmed up to room temperature, and the bottle was opened under the fume hood After the 4-5 min reaction time period, the beaker containing the mixture was left in the fume hood to cool down to room temperature. Then the mixture was transferred into the separatory funnel containing 20 mL saturated NaCl and 25 mL hexane. The separatory funnel containing fatty acid methyl esters in the upper part was extracted very carefully, because the aqueous lower phase is very difficult to evaporate The upper hexane layer (FAMEs) was filtered through anhydrous magnesium sulfate to bind any residual water. Saturated NaCl was added to aid the extraction of esters. Pretreatment is recommended for various types of samples prior to transesterification. Samples with easily accessible fat are directly tranesterified. Solid fats are dissolved in 6-7 mL hexane prior to preparation of fatty acid methyl esters to create a one-phase system.

Fat transesterification is a drawback if time is needed for solubilizing enclosed fat in the reagent mixture The solubilization must be achieved in a previous step. It is concluded that BF_3/MeOH causes no greater losses of unsaturated esters than do the other reagents Losses of unsaturated esters are negligible when the treatment time with BF_3/MeOH is short,

as it is with most lipids BF_3/MeOH is the most electropolar of the boron halides and is extremely reactive toward many types of organic compounds When coordinated with methanol it becomes a useful reagent for the preparation of fatty acid methyl esters FAMEs are well resolved on both packed and capillary columns

D Analysis of FAMEs By GC and Gas Chromatography-Mass Spectrometry

The gas chromatography of fatty acid methyl esters with a flame ionization detector (FID) is the most often used method for the analysis. A less polar stationary phase and a somewhat longer column are needed for the separation of the saturates, monounsaturates, and polyunsaturates, as required for the declaration of nutritional values.[10] As a general rule, the use of the less polar phase provides satisfactory results.

The on-column injection is more reliable since it avoids discrimination against higher boiling point FAMEs by selective elution from the needle as well as problems related to sample evaporation and splitting inside the injector. With columns of some 30 m in length, inlet pressures are sufficiently high to enable injection at 55-60°C. Saturated fatty acids identified by GC/MS in the fractions included decanoic (capric) (C10:0), dodecanoic (C12:0), tridecanoic (C13:0), tetradecanoic (myristic) (C14:0), pentadecanoic (C15:0), hexadecanoic acid (palmitic) (C16:0), heptadecanoic (C17:0), octadecanoic (stearic) (C18:0), and ecosonoic (arachidic) (C20:0) acid. The saturates comprised the majority of the total fatty acids identified

Monounsaturated fatty acids identified in the fractions included tetradecenoic (C14:1), heptadecenoic (palmitoleic)(C16:1), heptadecenoic (C17:1), trans-9-octadecenoic (trans-9-C18:1), cis-9-octadecenoic (cis-9-C18 1), cis-11-octadecenoic (oleic) (cis-11-C18 1), and ecosenoic (C20:1) acids. Polyunsaturated fatty acids identified included, octadecadienoic

(C18.2), and octadecatrienoic (C18.3). These were due to the low concentration of polyunsaturated fatty acids in French fries and peanut butter m

Fatty acids in higher plants and animals usually contain an even number of carbon atoms. The hydrocarbon chain is almost invariably unbranched in fatty acids. The geometric configuration in the double bonds in most unsaturated fatty acids in nature is cis. Palmitic, oleic, and linoleic acids usually occur in high amounts, whereas the other acids are present in relatively small amounts. Unbranched, straight chain molecules with an even number of carbon atoms, particularly palmitic, stearic, and myristic acids are predominant among the saturated fatty acids. Conjugated linoleic acid is reported to be anticarcinogenic.[1]

The difference in the boiling point between adjacent members of the series is sufficient to permit efficient separation. Lauric acid is the most widely distributed naturally occurring of the saturated fatty acids, the others being palmitic and stearic acid. In most cases the double bonds have the cis configuration. Monounsaturated fatty acids contain two less hydrogens than the corresponding saturated fatty acids and have the empirical formula $C_nH_{2n-2}O_2$. Oleic (Cis-9-Octadecenoic acid) and palmitoleic acids are most widely distributed and are the most important. Diunsaturated fatty acids contain four less hydrogen atoms than the corresponding saturated fatty acids and have the empirical formula $C_nH_{2n-4}O_2$. The most important and widely distributed fatty acid is linoleic acid. It is one of the nutritionally essential fatty acids. It is the predominant naturally occurring octadecadionic acid.

The chromatograms obtained for McDonald's French fries and Wendy's French fries are different. The difference may be due to different sources of oils that had been used for frying, or different suppliers of materials. Both of these graphs show different amounts of fatty acids. Gas chromatography was also done on FAMEs. We did not have standards to

check the kind of FAMEs present The chromatograms of FAMEs of percent fat extracted from Wendy's and McDonald's French fries of GC and GC/MS are shown in Appendix A The selected mass spectra of both French fries are also shown in Appendix A

IV CONCLUSION

As stated in the introduction, the purpose of this paper is to provide information on fatty acid profile of different food products. The results of this study lead to the conclusion that SFE is the appropriate technique for the extraction of neutral non-polar lipid without addition of organic modifier in supercritical CO_2. SFE can reduce the time needed for quantitative extraction. Because SFs have solute diffusivities an order of magnitude higher and viscocities an order of magnitude lower than liquid solvents, they have better mass transfer characteristics. Soxhlet extraction requires a large amount of hazardous solvent and also the time needed for the extraction is very large compared to SFE.

Although, no single technique can solve the diversity of problems confronting the analytical chemist, SFE has a rightful place among other sample preparation methods. Method development and optimization can be performed rapidly and easily with automatic SFE. The decreased use of organic solvents associated with SC-CO_2 extraction can reduce the exposure of lab personnel to both the health and safety-related problems of organic solvents. In practice, the wide range of polarity of lipids due to their various structures make solvent selection quite difficult, and the choice of a solvent for fat and/or lipid extraction remains one of the most critical steps in the determination of fat in various matrixes. So SFE has many advantages over conventional liquid extraction.

The widespread use of capillary GC/MS is a direct result of its ability to provide quantitative and qualitative analysis at the same time, when combined with computerized data

identify the component FAMEs Extraction of fat from food is not always easy A total lipid extract is crucial to accurate total fat analysis The degree of difficulty in obtaining a total lipid extract will vary depending on the solvent system used and the nature of the sample

V BIBLIOGRAPHY

1 Darryl M Sullivan, Donald E. Carpenter *Methods of Analysis for Nutritional Labeling, J. Assoc. Off. Anal. Chem., 1,* **1993**.

2. House D Stephen, Larson A. Paul, Johnson R Rodney, DeVries W Jon, Martin L Diana *J. Assoc. Off. Anal. Chem.*, **1994**, *77 (4)*, 960-965.

3. Alan J. Sheppard, John L. Iverson, and John L. Weihrauch; *Handbook of Lipid Research,* Fatty acids and glycerides, **1978**, 1, 341-379.

4. Eugene W Hammond, *Chromatography for the Analysis of Lipids*, **1993**.

5. William. W. Christie, *Lipid Analysis*, 2nd edition, **1982**.

6. *Food Composition and Analysis*, **1987**, 178-231.

7 Janet M. Snyder , Jerry W. King, Michael A. Jackon. *J. Chromatogra.*, **1996**, *750*, 201-207

8. P Sandra and F. David. *Supercritical Fluid Technology in Oil and Lipid Chemistry,* 1996, 321-347

9 H. M. Ghazal, S Hamidah and Y. B CheMan. *J. Am. Chem. Soc.*, **1995**, *72 (6)*, 633-639

10 BeaSuter, Konard Grob, Bruno Pacciarelli *Z Lebensm Unters Forsch A,* **1997**, *204,* 252-258.

11. N. C. Shantha . *J. Chromatogra.*, **1992**, *624*, 37-51.

12. Jennifer A Field. *J. Chromaotgra.*, **1997**, *785*, 239-249

13. Ahmad A Gharaiben and Kent J. Voorhees. *Anal. Chem.*, **1996**, *68*, 2805-2810.

14 J I Gray *J Am.Chem.Soc.*, **1978**, *55*, 539-545

15. Chigizie Victor NWosu, Leon C. Boyd, and Brian Sheldon *J. Am. Chem. Soc.*, **1997**, *74 (3)*, 293-297

16 Fred J Eller and Jerry W. King *Seminar in Food Analysis*, **1996**, *1*, 145-162.

17 Mark D Burford, Steven B Hawthorne and David J Miller. *J. Chromatogra.*, **1993**, *657*, 413-427

18. M. Valc'arcel, M. T Tena. *Fersenius J. Anal. Chem.*, **1997,** *358*, 561-573

19. P Lembke, H. Engelhardt *Chromatographia*, **1993**, *35*, (9-12*)*, 509-516.

20. Joseph M. Levy, Robert M. Ravey, Victor G. Daniesol, Anita Cardamone, Kathy Holowczak, and Raymond K. Houck *LRA*, **1997**, *7*, 205-211.

21. P Maheshwari, Z. L. Nikolov, T M. White and Hartel. *J. Am.Chem. Soc.*, **1992**, *69*, (11), 1069-1076.

22. Richard P de Filippi. *Chemistry and Industry*, **1982**, *19*, 105-110.

23. Dennis R. Gere, Lenore G. Randall and Daniel Callahan. *Instrumental Methods in Food Analysis,* **1997**, *18*, 421-483.

24. Mary Ellen P McNally, *J. Assoc. Off. Anal. Chem.*, **1996**,79 *(2*), 380-387.

25. M D. Luque de Castro, M.T., Tena, *Trends in Analytical Chemistry*, **1996**, *6 (1*),32-37.

26. Jerry w. King, Maruin L. Hopper, *J. Assoc. Off. Anal. Chem.*, **1992**, *75, (3*)

27. Short Communication, *J. Assoc.Off. Anal.Chem.*, 1974,424-425

28. Lin Pan, Janusz Pawliszyn, *Anal. Chem.*, **1997**, *69*,196-205.

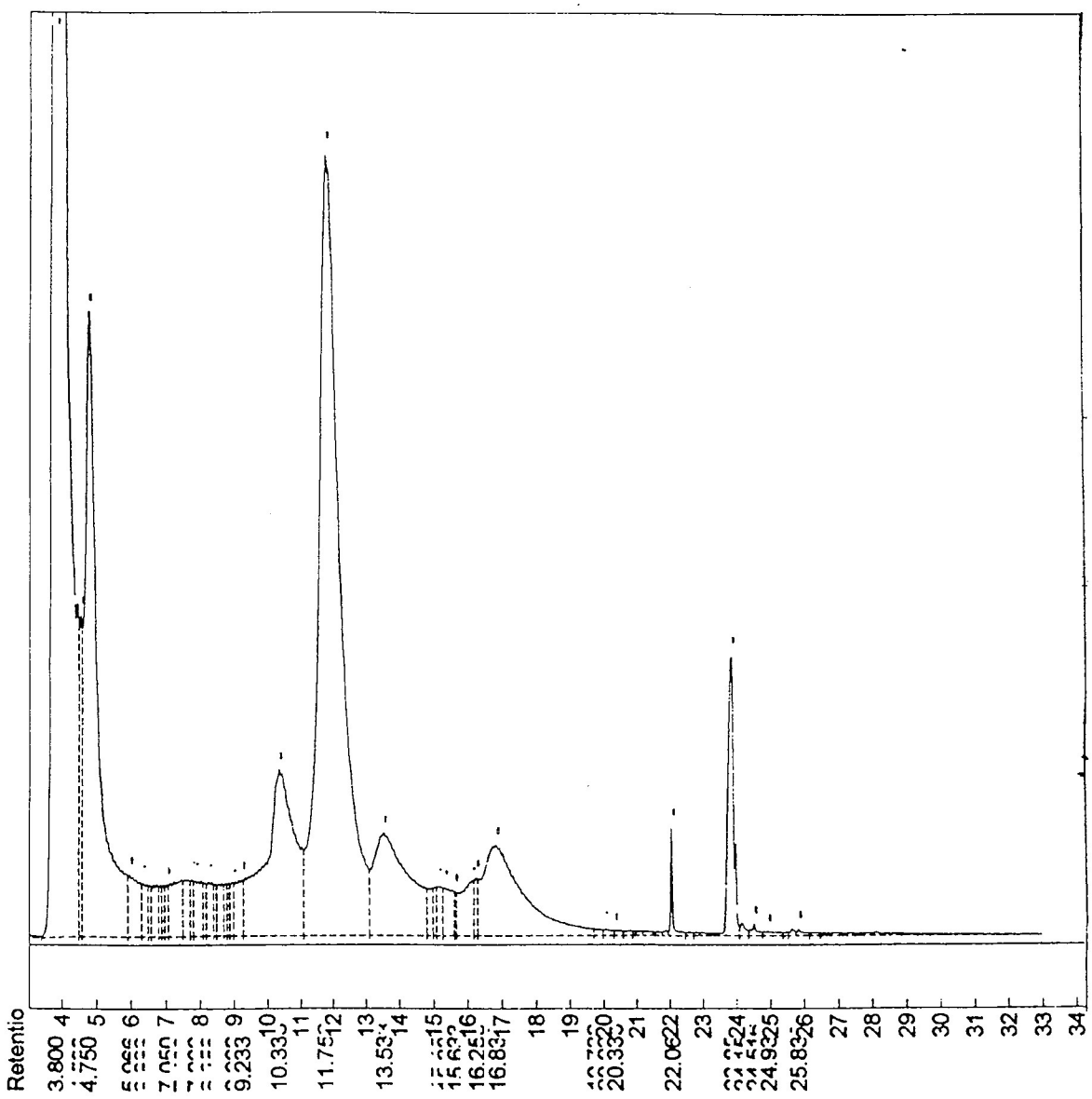
Appendix A

Representative

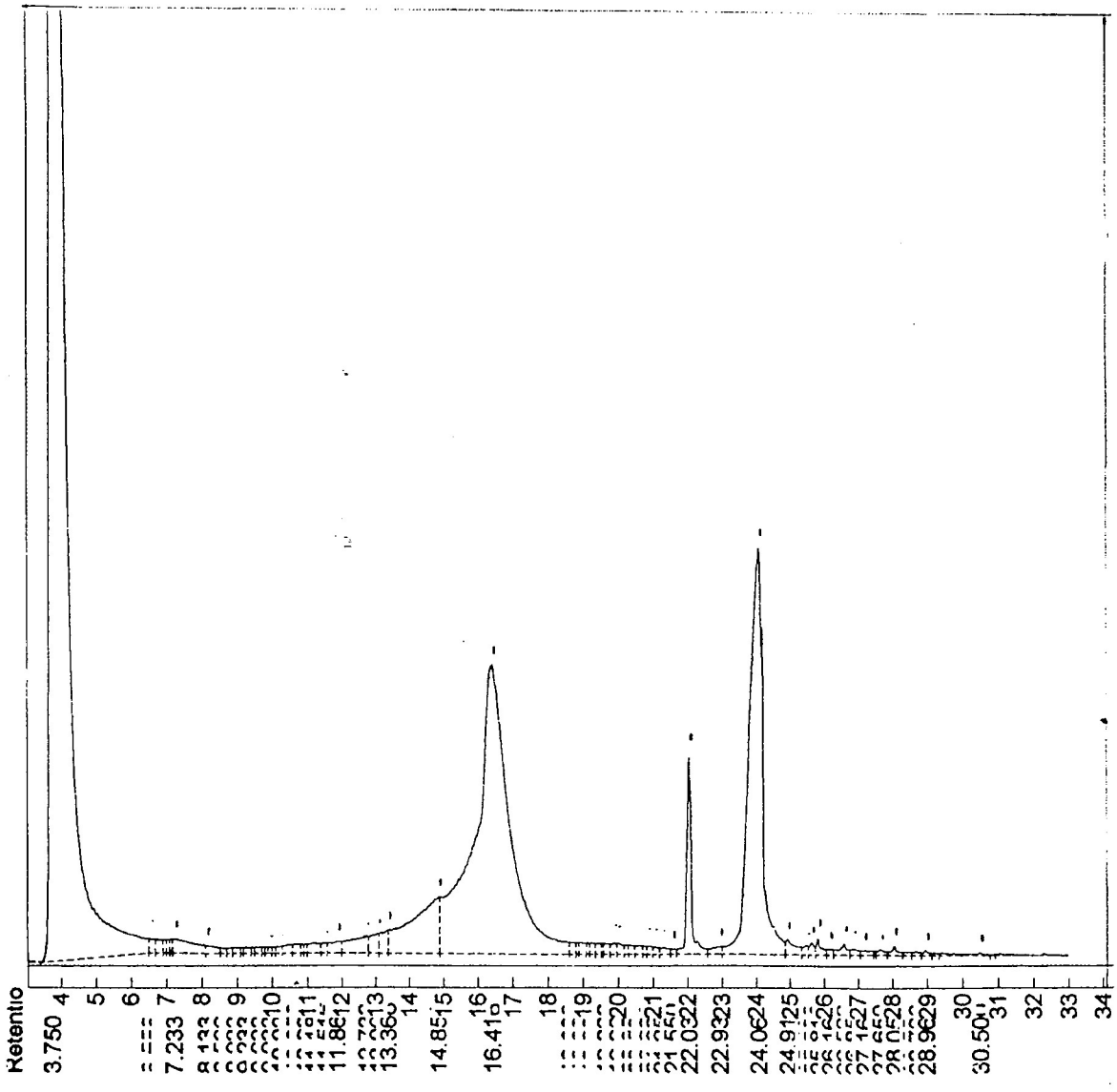
GC and Mass Spectra

From

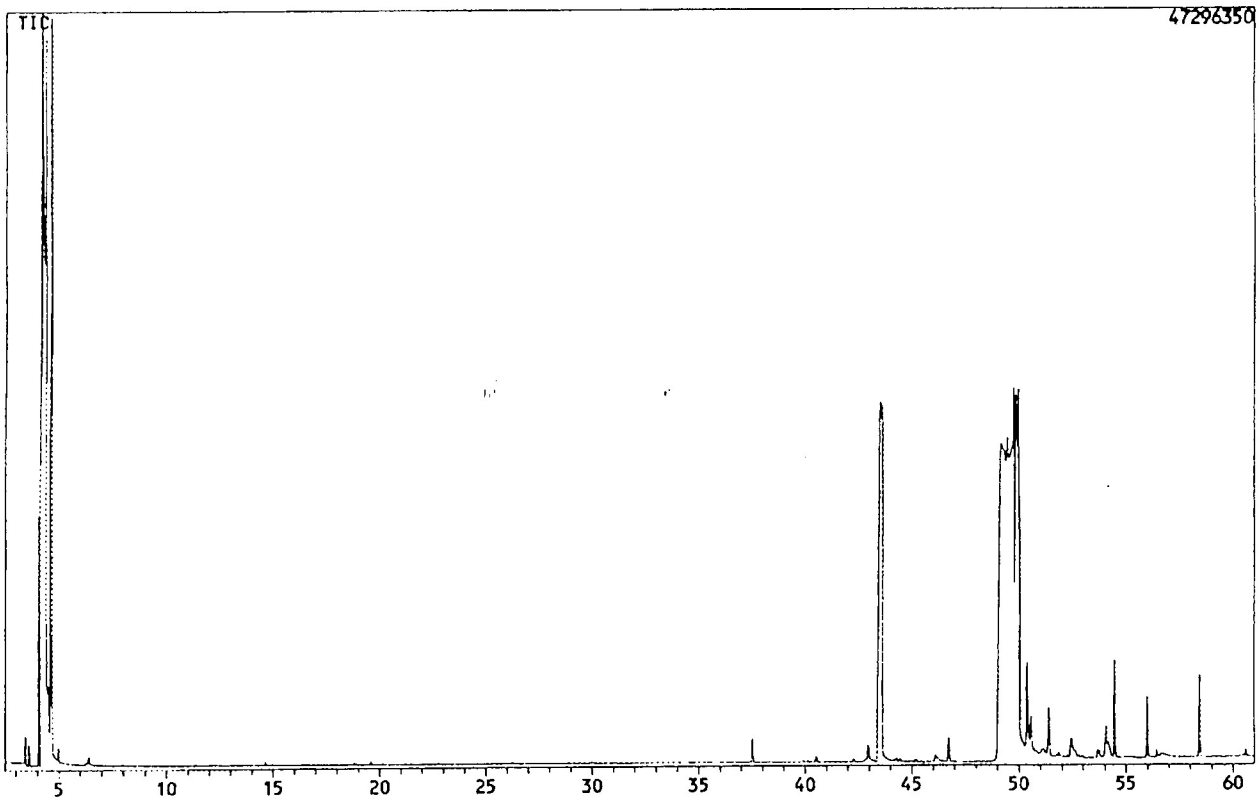
GC/GC-MS



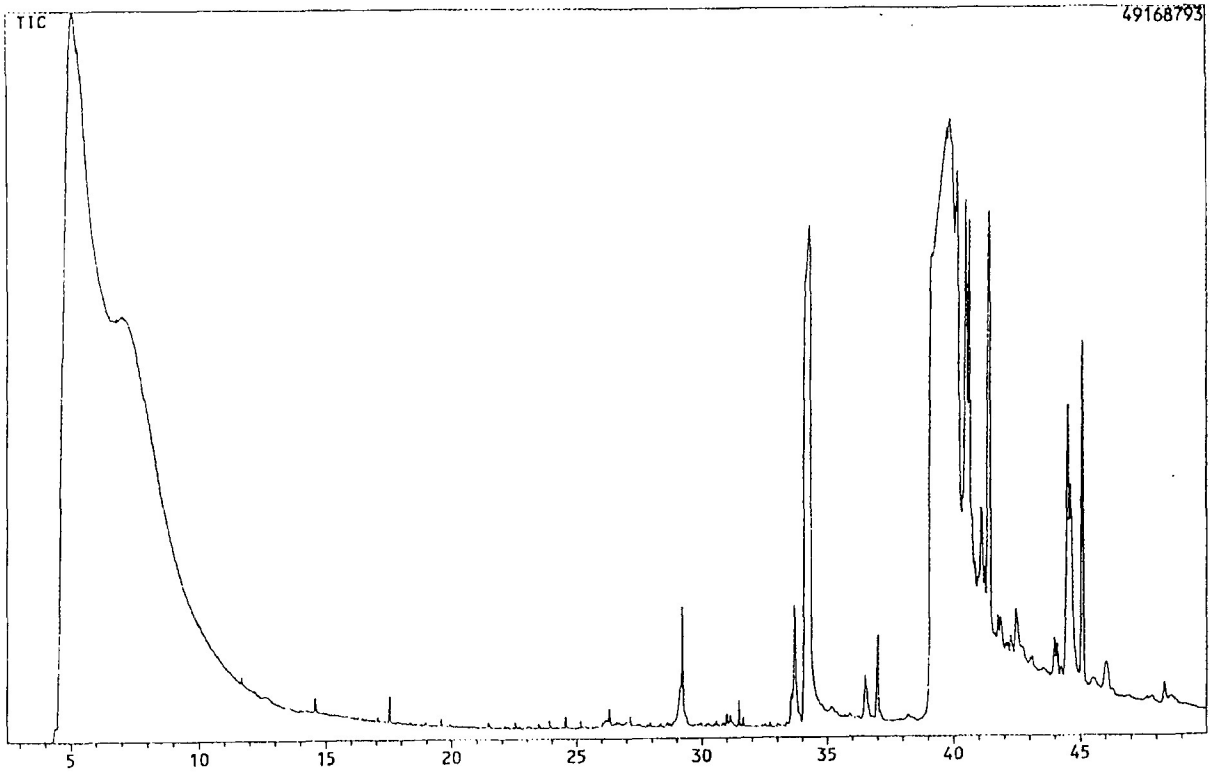
Gas chromatogram of FAME of 19.9 % fat. (Wendy's French fries)



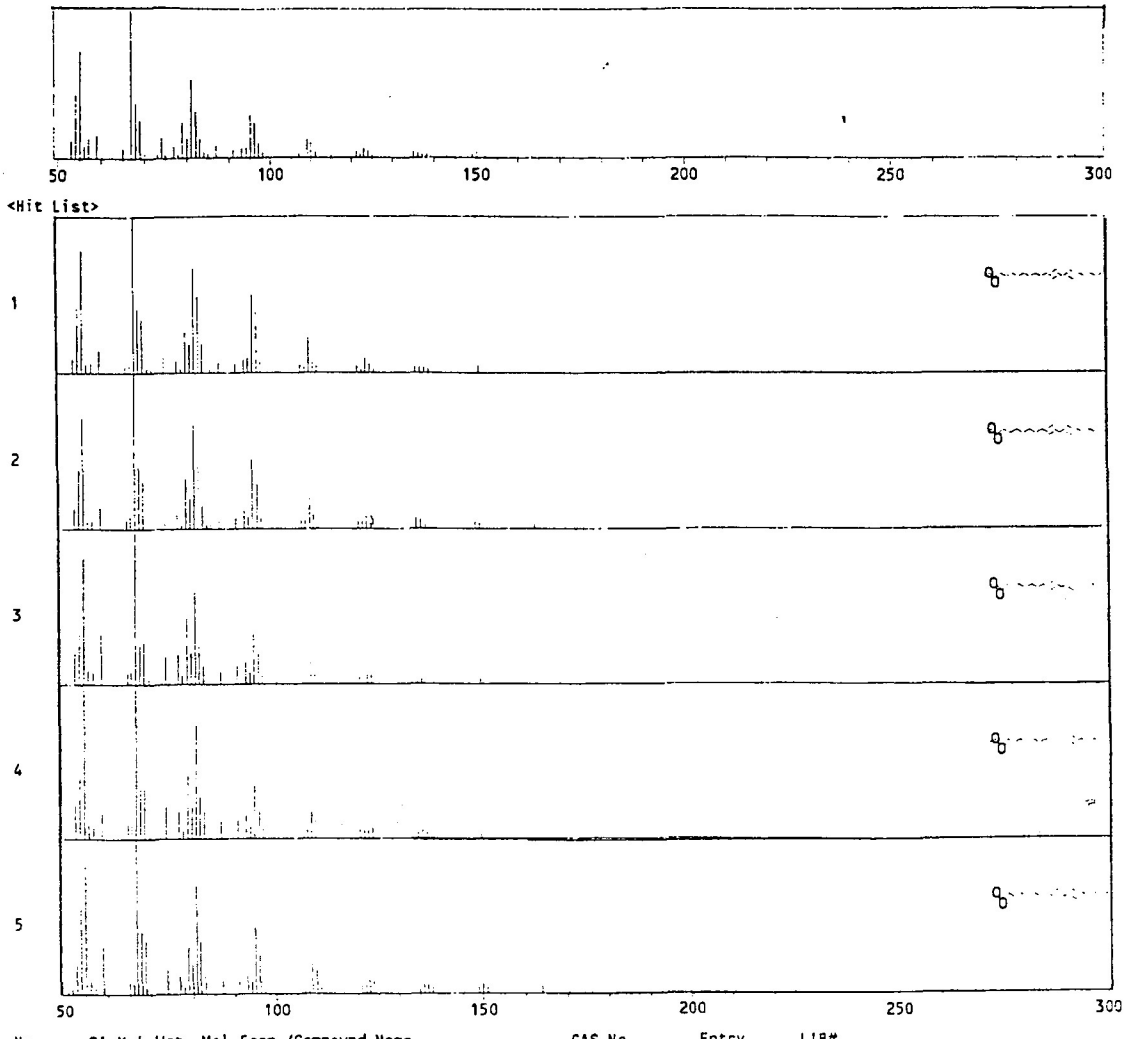
Gas chromatogram of FAME of 16.6 % fat. (McDonald French fries)



Chromatogram (GC/MS) of FAME of 16.6 % fat. (McDonald French fries)

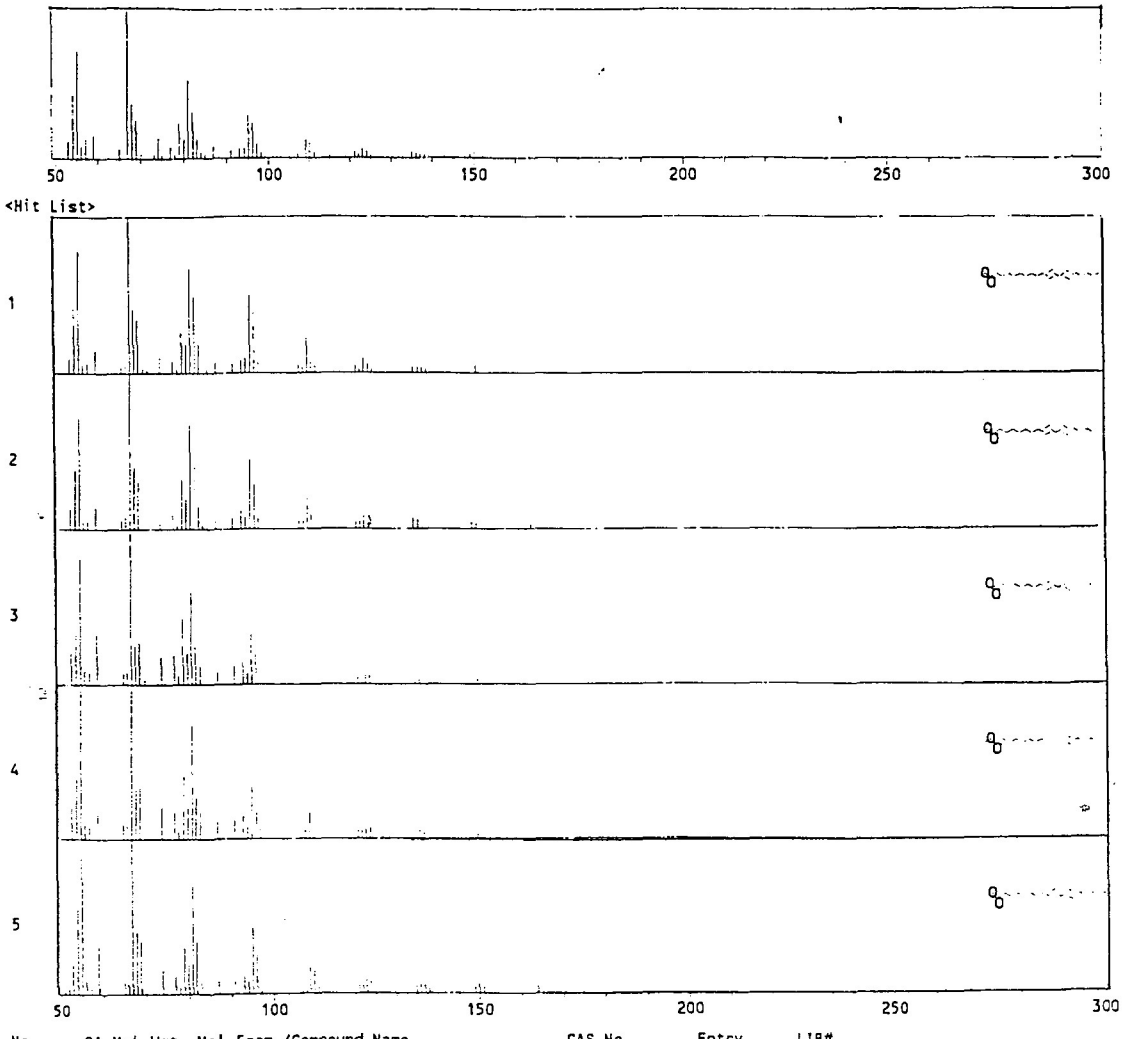


Chromatogram (GC/MS) of FAME of 19.9 % fat. (Wendy's French fries)



No	SI	Mol.Wgt.	Mol.Form./Compound Name	CAS No.	Entry	LIB#
1	92	322	$C_{21}H_{38}O_2$ 11,14-Eicosadienoic acid, methyl ester	2463-02-7	45900	2
2	92	294	$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid, methyl ester, (E,E)-	2566-97-4	10376	1
3	91	294	$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid, methyl ester, (E,E)- $$ Linolelaidic acid, methyl ester $$	2566-97-4	41833	2
4	90	294	$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	10385	1
5	90	294	$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	10384	1

Mass spectra of FAME of 16.6 % fat. (McDonald French fries)



No	SI	Mol.Wgt.	Mol.Form./Compound Name	CAS No.	Entry	LIB#
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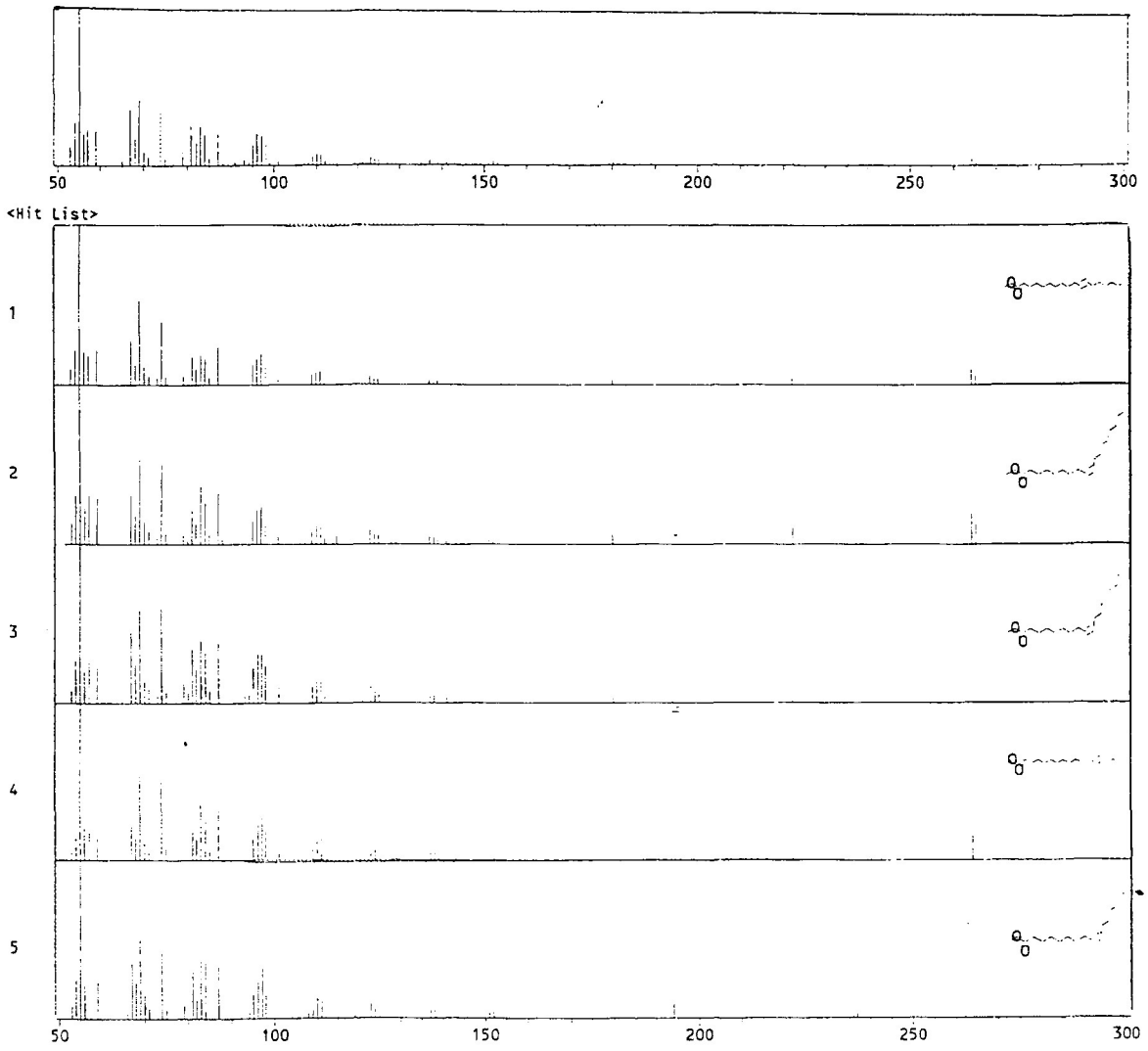
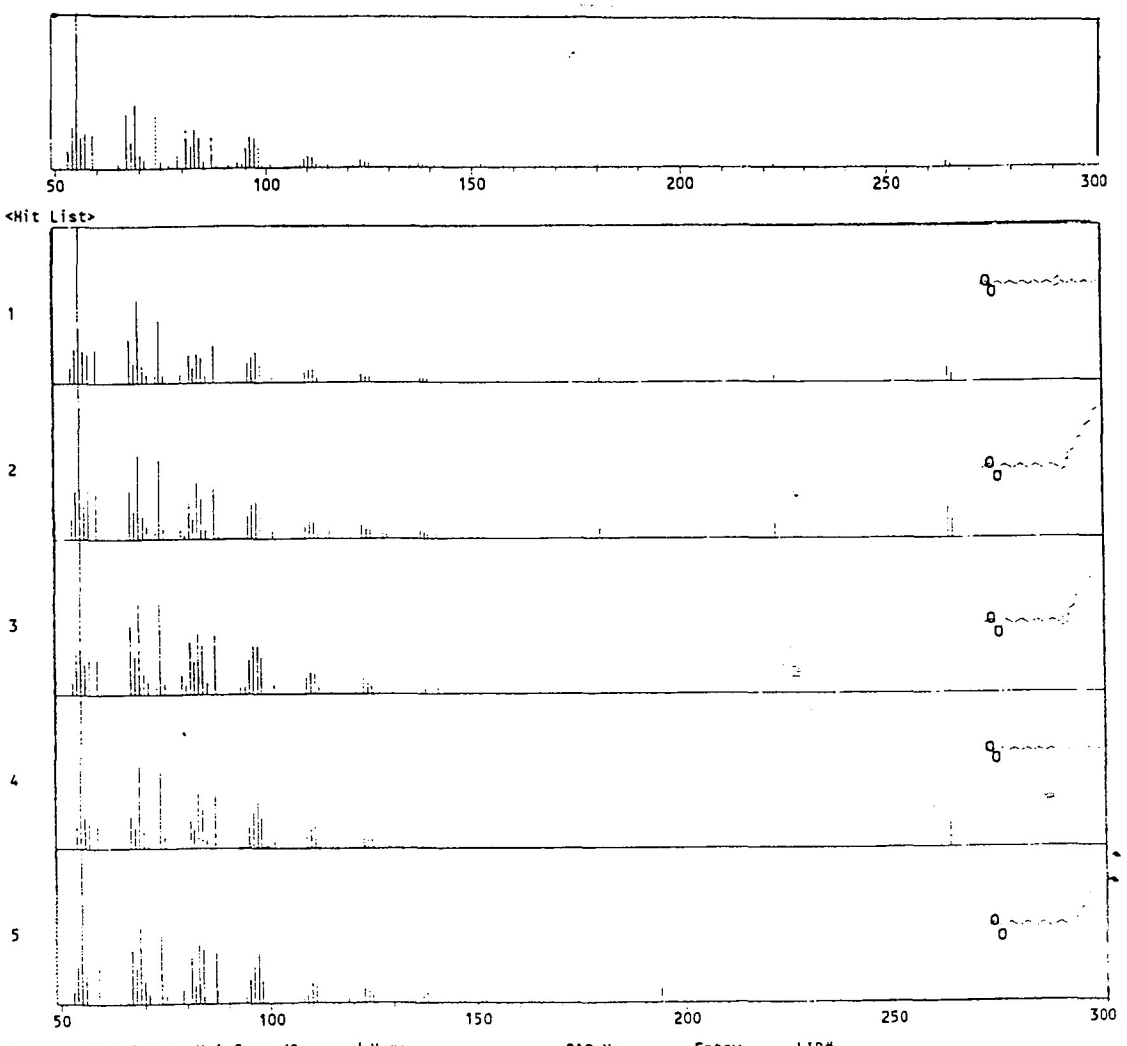
4	90	294	$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	10385	1
5	90	294	$C_{19}H_{34}O_2$ 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	10384	1

Mass spectra of FAME of 16.6 % fat. (McDonald French fries)



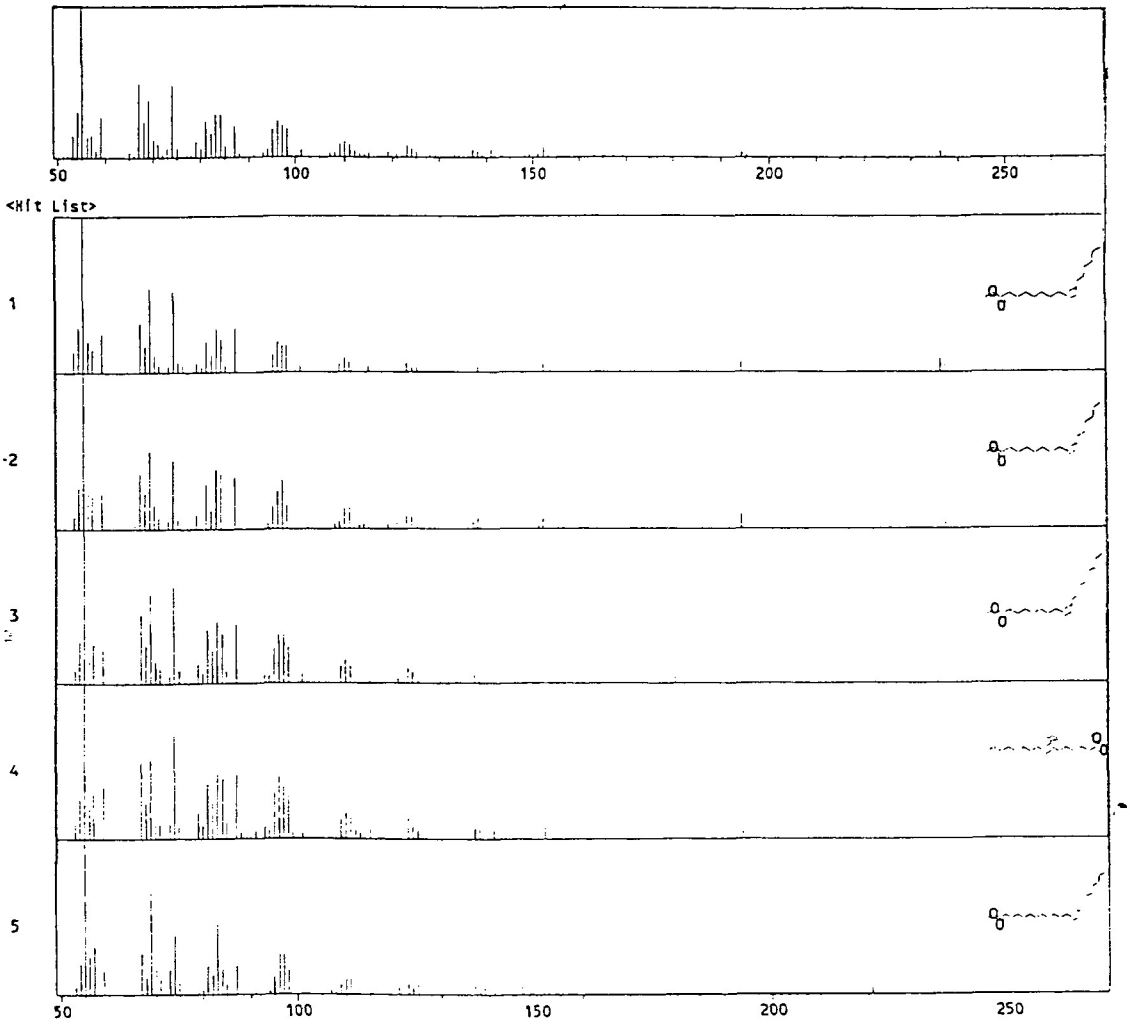
No	SI	Mol.Wgt.	Mol.Form./Compound Name	CAS No.	Entry	LIB#
1	93	296	$C_{19}H_{36}O_2$ 11-Octadecenoic acid, methyl ester $$ Methyl 11-octadecenoate	52380-33-3	42139	2
2	90	296	$C_{19}H_{36}O_2$ 9-Octadecenoic acid (Z)-, methyl ester	112-62-9	10436	1
3	90	296	$C_{19}H_{36}O_2$ 9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic aci	112-62-9	42154	2
4	89	296	$C_{19}H_{36}O_2$ 13-Octadecenoic acid, methyl ester	56554-47-3	42148	2
5	89	268	$C_{17}H_{32}O_2$ 9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	9700	1

Mass spectra of FAME of 16.6 % fat. (McDonald French fries)



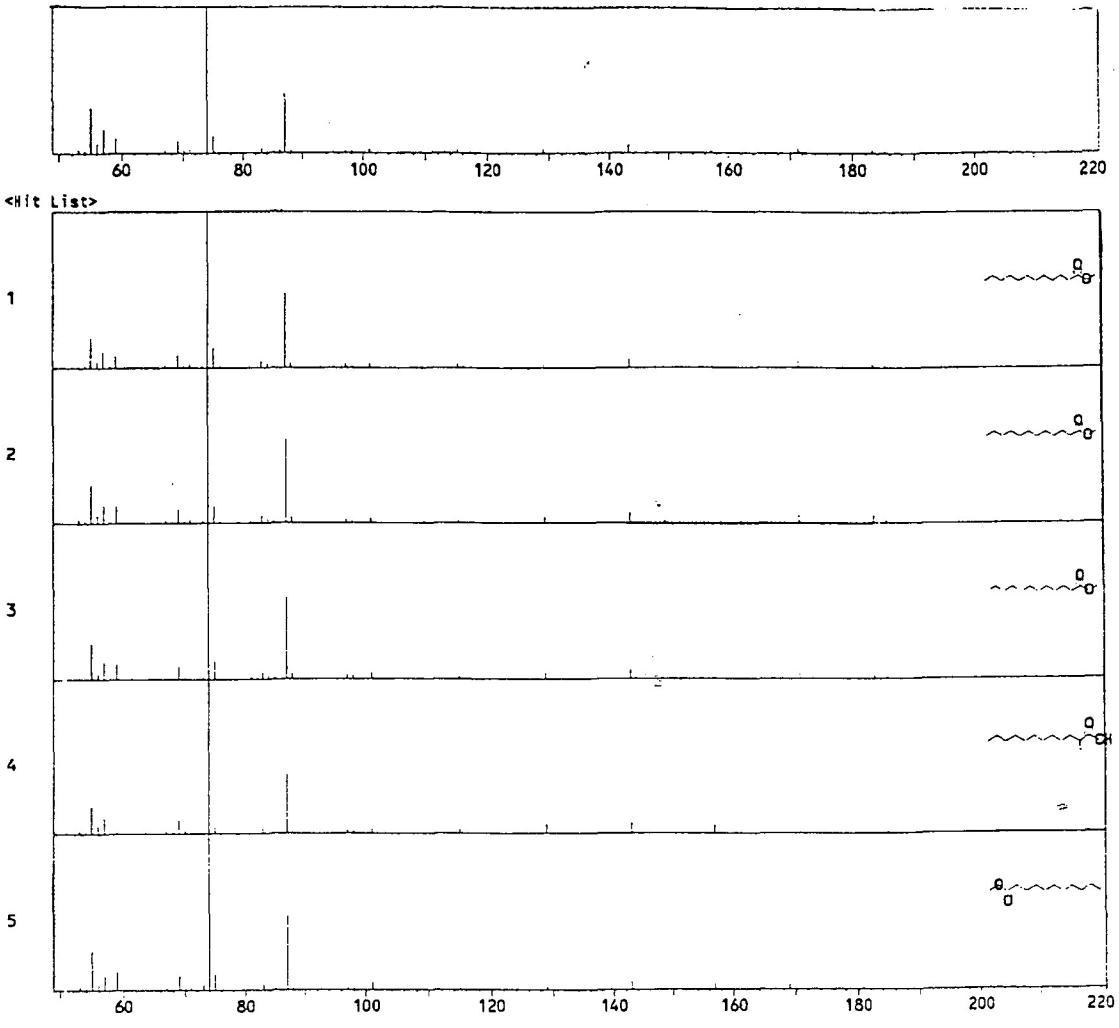
No	SI	Mol.Wgt.	Mol.Form./Compound Name	CAS No.	Entry	LIB#
1	93	296	$C_{19}H_{36}O_2$ 11-Octadecenoic acid, methyl ester $$ Methyl 11-octadecenoate	52380-33-3	42139	2
2	90	296	$C_{19}H_{36}O_2$ 9-Octadecenoic acid (Z)-, methyl ester	112-62-9	10436	1
3	90	296	$C_{19}H_{36}O_2$ 9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic aci	112-62-9	42154	2
4	89	296	$C_{19}H_{36}O_2$ 13-Octadecenoic acid, methyl ester	56554-47-3	42148	2
5	89	268	$C_{17}H_{32}O_2$ 9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	9700	1

Mass spectra of FAME of 19.9 % fat. (Wendy's French fries)



No	SI	Mol.Wgt.	Mol.Form./Compound Name	CAS No.	Entry	LIB#
1	91	268	$C_{17}H_{32}O_2$ 9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	9701	1
2	90	268	$C_{17}H_{32}O_2$ 9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	9700	1
3	90	296	$C_{19}H_{36}O_2$ 9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic aci	112-62-9	42154	2
4	90	268	$C_{17}H_{32}O_2$ 7-Hexadecenoic acid, methyl ester, (Z)-	56875-67-3	37409	2
5	87	352	$C_{23}H_{44}O_2$ 13-Docosenoic acid, methyl ester, (Z)- $$ Methyl (Z)-13 docosenoate $$ Methyl erucate	1120-34-9	49548	2

Mass spectra of FAME of 19.9 % fat. (Wendy's French fries)



No	SI	Mol.Wgt.	Mol.Form./Compound Name	CAS No.	Entry	LIB#
1	93	214	$C_{13}H_{26}O_2$ Dodecanoic acid, methyl ester $$ Lauric acid, methyl ester $$ Metholene 2296 $$ Methy	111-82-0	26365	2
2	93	214	$C_{13}H_{26}O_2$ Dodecanoic acid, methyl ester	111-82-0	8113	1
3	93	214	$C_{13}H_{26}O_2$ Dodecanoic acid, methyl ester	111-82-0	8114	1
4	92	214	$C_{13}H_{26}O_2$ Dodecanoic acid, 2-methyl- $$ 2-Methyldodecanoic acid	2874-74-0	26359	2
5	91	200	$C_{12}H_{24}O_2$ Undecanoic acid, methyl ester	1731-86-8	7479	1

Mass spectra of FAME of 19.9 % fat. (Wendy's French fries)