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Rapid Method for Determination of Iodine Numbers of Vegetable Oils

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by

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There is a great deal of interest by the public about the total unsaturation of fats and oils used in commercial products as an indication of nutritional value. Dealers in fats and oils are extremely interested in characterizing these products. The unsaturation is a guide to the expected stability of food additives, such as edible oils and foods in which they are used. Unsaturation is typically evaluated as an iodine number (IN), calculated for these additives based on their consumption of iodine through addition across the carbon-carbon double bonds. The classical titration method can typically take 45 minutes.

The investigation presented develops an alternative method for determining iodine numbers of chosen common oils using an infrared spectrophotometer. The quantitation technique employed involves a first derivative of infrared (IR) spectra. Results of the analytical data give iodine numbers with about a 5% relative standard deviation. The IN values from the newly developed IR method are compared with results from proton-NMR and with the AOCS standard techniques such as: gas chromatography and titration.

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INTRODUCTION

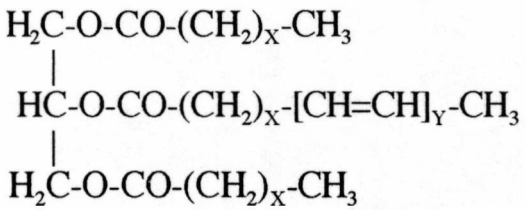
Characterization of Edible Oils

Lipids are one of the large groups of organic compounds which are of great importance in the food we eat because they are readily digested and utilized by the body. They are widely distributed and almost every natural food has considerable quantities of them.

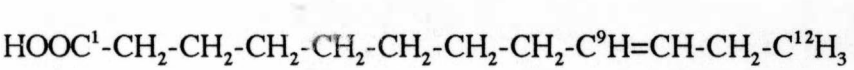
Fats are a subset of the lipid family. They are important in food and health science. Fats are the main component of fat cells in animals and plants, and act as an important storage medium of food reserves in the organism. These fats can be extracted from their native organisms to form the basis of many products which are used for food preparation. Fats and oils provide a concentrated source of food energy.

Chemically, fats and oils are esters of fatty acids derived from a single, trifunctional alcohol, glycerol. Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. The biological functions of the lipids are equally diverse. Fats and oils are the principal stored forms of energy in many organisms. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light absorbing pigments and etc.

The simplest lipids constructed from fatty acids are the triglycerides or commonly called fats or neutral fats. These compounds are composed of three fatty acids each in an ester linkage with a single glycerol.



Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons. In some fatty acids, this chain is fully saturated (contains no double bonds) and unbranched. Others may contain one or more double bonds. As shown in Table 1, the nomenclature of common fatty acids in vegetable oils specifies the chain length and number of double bonds. The 16-carbon saturated palmitic acid is abbreviated 16:0, and the 18-carbon oleic acid, with one double bond, is 18:1. The positions of any double bonds are specified by superscript numbers following Δ (delta). For example, a 20-carbon fatty acid with one double bond between C-9 and C-10, and another between C-12 and C-13, is designated 20:2 ($\Delta^{9,12}$). The Δ sign is used by chemists and describes the position of the double bond counted from carboxyl carbon. For example Δ^{9},



On the other hand, we may also see a number following omega to designate the position of a double bond in an unsaturated acid. This designation of double bonds in triglyceride molecules was established by nutritionists (1). For example ω-3,

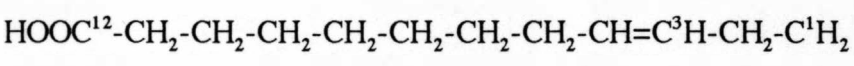


Table 1

The Nomenclature of Common Fatty Acids in Vegetable Oils (2)

Carbon Skeleton	Structure	Systematic Name	Common Name	Melting Point (^{0}C)	MW
Saturated					
4:0	C_3H_7COOH	n-Butanoic	Butyric	-7.9	88
6:0	$C_5H_{11}COOH$	n-Hexanoic	Caproic	-3.4	116
8:0	$C_7H_{15}COOH$	n-Octanoic	Caprylic	16.7	144
10:0	$C_9H_{19}COOH$	n-Decanoic	Capric	31.6	172
12:0	$C_{11}H_{23}COOH$	n-Dodecanoic	Lauric	44.2	200
14:0	$C_{13}H_{27}COOH$	n-Tetradecanoic	Myristic	54.4	228
16:0	$C_{15}H_{31}COOH$	n-Hexadecanoic	Palmitic	62.9	256
18:0	$C_{17}H_{35}COOH$	n-Octadecanoic	Stearic	69.6	284
20:0	$C_{19}H_{39}COOH$	n-Eicosenoic	Arachidic	75.3	312
22:0	$C_{21}H_{43}COOH$	n-Docosanoic	Behenic	79.9	340
24:0	$C_{23}H_{45}COOH$	n-Tetracosanoic	Lignoceric	84.1	368
Unsaturated					
16:1(Δ^9)	[1]	cis-9-hexadecenoic	Palmitoleic	0.5	254
18:1(Δ^9)	[2]	cis-9-octadecenoic	Oleic	16.3	282
18:1(Δ^9)	[2]	trans-9-octadecenoic	Elaidic	43.7	282
18:2($\Delta^{9,12}$)	[3]	cis-cis-9-12-octadecandienoic	Linoleic	-5.0	280
18:3($\Delta^{9,12,15}$)	[4]	cis-cis-cis-9-12-15-octadecatrienoic	Linolenic	-11.0	278
20:4($\Delta^{5,8,11,14}$)	[5]	cis-cis-cis-cis-5-8-11-14-eicosatetraenoic	Arachidonic	-49.5	304

[1] $CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
[2] $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
[3] $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
[4] $CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
[5] $CH_3(CH_2)CH{=}(CHCH_2CH)_3{=}CH(CH_2)_3COOH$

The most commonly occurring fatty acids have even numbers of carbon atoms in an unbranched chain of 12 to 24 carbons. The double bonds of polyunsaturated fatty acids are almost never conjugated, but separated by a methylene group.

The position of double bonds is also regular and in most monounsaturated fatty acids the double bonds are between C-9 and C-10 (Δ^9), and the other double bonds of polyunsaturated fatty acids are generally Δ^{12} and Δ^{15}, see Table 1.

The Technology of Edible Fats and Oils (1)

Three principal methods are used for the extraction of edible fats and oils from the animal or vegetable tissues in which they occur. These are rendering, pressing, and solvent extraction. Rendering is a process by which fat is removed from a tissue by heat. The process can be carried out either in the presence of water - "wet rendering" - or in its absence - "dry rendering". Pressing is the application of high pressures to the tissue to squeeze out the fat. In same cases, such as the pressing of olives, virgin oil is the first pressing of the fruit and is particularly bland in flavor. The fruit is then subjected to subsequent pressings to give other grades of oil. Solvent extraction uses solvents to extract oils from tissues. This method is practical in the removal of oil from tissues which have a relatively low percentage of oil. Processed and purified oils contain small amounts of compounds other than simple fats. The other compounds present are complex lipid-lecithins, cephalius, other phosphatides and hydrocarbons.

Adulteration

A great body of research has been built up around these foods because of efforts to differentiate one from the other so that an inexpensive oil is not sold as a more costly one. Each year there are incidents of olive oil that is labeled and sold as 100%

pure oil, but in fact, contains less-expensive vegetable oils. However, because of increased scrutiny by regulatory agencies both in the United States and in Europe, the number of incidents seem to be declining. This surveillance has increased both overseas and in the United States in part due to the Spanish Toxic Oil Syndrome incident where contaminated oil sickened and killed a number of people in Spain during 1981 (3).

Olive oil, due to its unique character, stability, and health benefits, has always been the subject of fraud or mixing. However, some of the analytical tests introduced during the past three to four years have minimized what can be done to adulterate extra virgin oil. Grades of olive oil from highest prized to the lowest are as follows: extra virgin olive oil, olive oil and olive pomace oil. One test determines the presence of *trans* fatty acid content (3). A second test measures steroidal hydrocarbons which may be degraded when olive oil is processed or other oils are refined (3).

In 1995 as a result of these analytical tests the U.S. Food and Drug Administration (FDA) was able to test 73 olive oils produced or distributed by companies based in the United States. Only one was found to be adulterated (3).

Nutrition and Health Aspects

In recent times, fat consumption has been carefully studied as a parameter relating to health issues. The dietary consumption of various fats and oils has been related to a variety of health statistics. Specifically, several studies have correlated various forms of fat consumption to heart related diseases. From a health standpoint, unsaturation has been identified as a desirable quality in fats. In recent years margarines, for example, have been produced with higher levels of dienoic and trienoic acids, and correspondingly lower levels of monoenoic and saturated acids. These oils, higher in polyunsaturates are manufactured to the desired semi-solid consistency by use

of oil solidifying agents as opposed to hydrogenation. Medical opinion is divided on the desirability of types of unsaturation. For example, it has been suggested that polyunsaturated fats are prone to production of a greater number of free radicals during metabolic processes. These free radicals are thought to then produce subsequent deleterious effects in the body (4).

A high intake of fat shows a positive correlation with blood cholesterol level. It has long been recognized that a high blood cholesterol level directly affects arteriosclerosis. Also there are conflicting reports that have indicated an increased consumption of trans fatty acids may lead to the development of coronary heart disease. General structure of cis and trans fatty acids is seen in Figure 1.

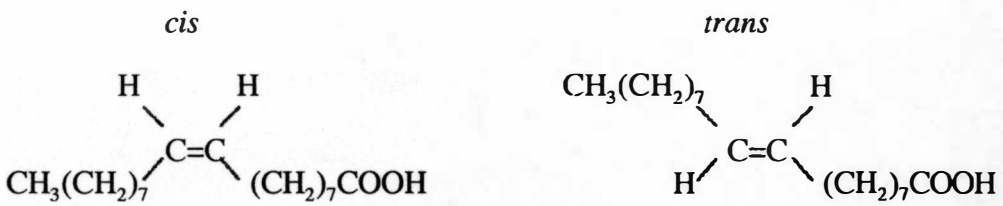


Figure 1. *Cis* and *Trans* Isomers of Oleic Acid.

Since *trans*- unsaturation in common fatty acid chains is typically a product of hydrogenation catalyst and not of natural synthesis, it has been argued that the human body is not accustomed to metabolizing these materials. Consequently, medical opinion also recommends reduction of consumption of *trans* - unsaturated materials. Interestingly, both saturated fats and *trans* - unsaturated fats have higher melting points than the equal chain length *cis* - unsaturated materials, due to packing inefficiencies in the *cis* - chain structures. While this higher melting point is at times a desirable commercial feature, its contribution to health is debatable.

Due to the above considerations it is desirable to qualify oils and fats on the basis of two parameters: the ratio of unsaturation to saturation in the acid chains and the ratio of *trans / cis* isomers in unsaturated materials. Fats also act as solvents for the fat - soluble vitamins that are naturally introduced into the diet in the fatty portion of the food. The fats and other lipids are, therefore, important in the diet for a number of reasons.

Chemical Properties

A number of chemical tests have evolved during years of studying of oils which are based on the partial determination of the chemical composition of the oil. These tests serve both to identify the oil and to detect the presence of adulteration as well as providing a measure of rancidity. All oils show a range of values and therefore sometimes more than one test is necessary. A few of the most commonly used tests are as follows: the Iodine Number is a measure of the true amount of unsaturation. The Saponification Number is related to the amount of potassium hydroxide required to saponify the fat and is inversely related to the average molecular weight of the fat or oil. The Reichert Meissl Number is a measure of the amount of water-soluble volatile fatty acids and the Polenske Number (5) measures the amount of volatile insoluble fatty acids. These tests can differentiate fats and oils on the basis of the chemical composition of the various triglycerides present in the mixture.

Proposed Methodology

There is a great deal of interest by the public and producers of oils to disclosure of total unsaturation in food additives, such as edible oils. The total unsaturation of an oil is an indication of the nutritional value, as well as a partial guide to its expected stability. Food technologists use the iodine number as an expression of the level of

unsaturation in a sample. The carbon-carbon double bonds of the fatty acids are the active reaction sites of the oil utilized for the determination of the iodine number. The iodine number is traditionally obtained from a titration technique called the Wijs or Hanus method. In these titrations excess iodine is added to a sample of an oil in chloroform where it may add across the carbon-carbon double bonds. The absorbed iodine is determined by back titration of the unreacted iodine with sodium thiosulfate. The iodine solutions for each method are somewhat different. The Wijs titration adds chlorine to increase the reactivity of the iodine solution, whereas the Hanus titration adds bromine. The typical reaction time of the oil and the iodine solution is 1 hour. Mercuric acetate can be added to the iodine solutions to speed the reaction time to 5-10 minutes. Total analysis time for an oil using these titration methods is at least 30 minutes. Other methods for determining iodine number of an oil are nuclear magnetic resonance (6, 7), and Raman spectroscopy (4). The classical titration method (8, 9, 10) and gas-liquid chromatography (11, 12) remain the standard methods used by the Association of Official Analytical Chemists (AOAC) and the American Oil Chemist's Society (AOCS).

More recently infrared spectroscopy has been used to obtain iodine numbers comparable to the titration methods. Lowry (13) applied the 1st and higher order (1st-4th) derivatives in infrared spectroscopy to resolve the weak carbon-carbon double bond band at 1651 cm^{-1} (shoulder or peak) from the carbonyl band at 1740 cm^{-1}. He developed a method for determination of iodine number based on the comparison of a ratio of derivative peak heights at 1740 cm^{-1} and 1651 cm^{-1} with standard oils. The major disadvantage of this technique was the stray light contribution to the carbonyl band. In order to minimize avoid this problem he had to prepare standards with low, middle and high iodine numbers and also use different dilutions for measuring each bond.

In our study we propose using the 1st derivative to resolve the overlapped peaks at 1740 cm^{-1} (carbonyl) and 1651 cm^{-1} (carbon - carbon double bond). However it is proposed that the carbonyl derivative absorption be measured at the overtone of the 1740 cm^{-1} band at 3460 cm^{-1}. In this case no significant contribution of stray light error is likely. Also it is proposed that this should produce an improvement in quantitation of the iodine number of an oil by infrared spectroscopy. The average molecular weight of oils, needed to calculate iodine numbers from infrared data, can be calculated from saponification numbers.

MATERIALS AND METHODS

Materials

Fourteen common oils were purchased from Spectrum and Sigma companies and subsequently studied. These vegetable oils with their literature iodine numbers are shown as following in Table 2. A range of iodine numbers for some of the oils in table are listed because oil types will vary in content of fatty acids from different locations and seasons. All samples are edible oils, except for linseed. Linseed oil is a highly unsaturated drying oil used in oil based paints. In Table 3 are listed fatty acids used as standards for determination of retention times with gas chromatography.

Table 2

Vegetable Oils Used

Name	Manufacture	IN_{lit}	Name	Manufacture	IN_{lit}
Almond	Sigma	95-102	Palm	Spectrum	51
Castor (Ricinus)	Spectrum	83-88	Peanut	Sigma	93
Coconut	Sigma	9	Safflower Seed	Sigma	145
Corn	Sigma	123	Sesame	Sigma	107
Cottonseed	Sigma	106	Soybean	Sigma	130
Linseed	Spectrum	190	Sunflower	Sigma	120
Olive	Sigma	81	Wheat Germ	Spectrum	115-132

Table 3

Standard Fatty Acids

Name	Purity [%]	Manufacture	Name	Purity [%]	Manufacture
Caproic	N / A	NBC[1]	Arachidic	≥ 99	Sigma
Caprylic	≥ 99	Fluka Chemika	Behenic	≥ 99	Sigma
Capric	≥ 98	Fluka Chemika	Lignoceric	≥ 98	Sigma
Lauric	98	J. T. Baker	Oleic	N / A	MC&B[2]
Myristic	≥ 99	Sigma	Linoleic	99	Sigma
Palmitic	99	Sigma	Linolenic	99	Sigma
Stearic	99	Lancaster			

[1] National Biochemical Corporation
[2] Matheson - Coleman & Bell
N/A = Not Available

Classical Titration Method

The iodine number is defined as the number of grams of iodine absorbed by 100 gams of fat. The double bonds present in the unsaturated fatty acids react readily with iodine. The iodine number (IN) is therefore a measure of the extent of unsaturation of the fatty acids present. While oleic acid contains one double bond in its 18 carbon chain, linolenic acid contains three double bonds in its 18 carbon chain. Thus a molecule of fat containing one oleic acid can react with only one third as much iodine as a molecule of fat containing one linolenic acid residue. The fatty acids present in natural fats are fairly characteristic of the fat. While there will be variation in each vegetable oil due to climate and soil factors, evaluating their iodine numbers are of great

value in identifying the oils. Standard methods have been developed for these analyses.

The classical standard method for the determination of IN involves a time intensive titration method. The method involves dissolving a weighed sample of fat (0.1 - 0.5g) in chloroform and adding an excess of halogen such as I_2 and/or Br_2. After standing in the dark for a controlled period of time, the excess unreacted iodine is measured by thiosulfate titration. The most recent revision of the standard method for the determination iodine number involves using a cyclohexane/acetic acid solvent. Further information on latest revisions of AOCS standard methods can be found on the world wide web (14).

Reagents

Potassium Triiodide Standard Solution, 0.1000 molar: KI_3 (HARLECO, concentrate).

Starch Solution, 1% w/v: dissolve 1.0 g of soluble starch (J.T. Baker Chemical Co., ACS grade) in 100 ml distilled water. Boil for 5 minutes and keep the clear supernatant liquid after it is cooled to room temperature.

Sodium Thiosulfate Standard Solution, 0.1 molar: dissolve about 25 g of $Na_2S_2O_3 \cdot 5\,H_2O$ (J.T. Baker Chemical Co., ACS grade) with 1 g of NaOH (Columbus Chemical Industries, Inc.) in 1.0 L of freshly boiled distilled water.

Standardization of Sodium Thiosulfate: dilute 25 ml mixture of 0.1000 molar standard solution of Iodine (KI_3) with approximately 25 ml distilled water and 5 ml of glacial acetic acid (Fisher Scientific Company). Titrate with $Na_2S_2O_3$ until the solution became light yellow. Several drops of starch solution may be added near the end point. Titrate until the solution became colorless.

$$I_3^- + 2S_2O_3^{2-} \rightleftarrows S_4O_6^{2-} + 3I^-$$

Potassium Iodide, 15% w/v: dissolve 45 g KI (Mallinckrodt Inc., U.S.P., granular) into 300 ml of boiled distilled water.

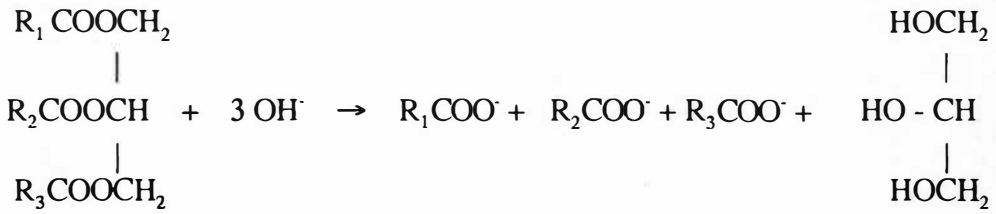
Kaufmann's Solution: 150 g NaBr anhydrous (Fisher Scientific, ACS grade) are added to 1.0 L methanol (Fisher Scientific, ACS grade). Filter the methanol solution into a 1.0 L amber bottle with a glass stopcock. Add 8.0 g liquid bromine (Columbus Chemical Industries) and mix.

Procedure

Each oil was titrated in triplicate as neat samples by the Hanus method. This method has been approved as a standard technique (8) by the AOCS. Experimentally determined iodine numbers (IN_{exp}) of the selected oils are listed as average values with their relative standard deviation (RSD) and compared with iodine numbers from literature in Table 4.

Saponification

The Saponification Number is defined as the number of milligrams of potassium hydroxide required to saponify 1 g of oil. This method is also a time consuming titration procedure. Potassium hydroxide reacts with a triglyceride consuming three moles of potassium hydroxide for each mole of oil. A measured excess of potassium hydroxide is added to a given weight of triglyceride. The mixture is allowed to react at elevated temperature for a prescribed time. Then the unreacted potassium hydroxide is titrated with a standard solution of hydrochloric acid. If the



triglyceride contains significant amounts of low molecular weight fatty acids, the number of molecules present in a 1 g sample of the oil will be greater than if the fatty acids have long carbon chains and high molecular weights. The oils with the low

Table 4

Iodine Numbers From Classical Titration

Name	IN_{class}	IN_{lit}	Name	IN_{class}	IN_{lit}
Almond	102.2 (±0.2)	102.5	Palm	43.3 (±1.4)	51.0
Castor	91.1 (±3.7)	83-88	Peanut	91.3 (±0.7)	93.4
Coconut	15.1 (±3.1)	9.0	Safflower	134.7 (±3.6)	145.0
Corn	123.5 (±2.3)	122.6	Sesame	110.4 (±1.6)	106.6
Cottonseed	109.4 (±1.4)	105.7	Soybean	128.7 (±0.9)	130.0
Linseed	180.0 (±8.3)	190.3	Sunflower	120.0 (±2.1)	120.5
Olive	81.3 (±0.6)	81.1	Wheat Germ	126.7 (±9.1)	115-132

(±) denotes relative standardard deviation

molecular weight fatty acids will consequently have a high saponification numbers. The average molecular weight is inversely proportional to the saponification number.

$SN = mg\ KOH / g\ sample = 10^{-3}\ g\ KOH / g\ sample \times mole / 56.1\ g\ KOH$

$\times\ 1\ mole \times sample / 3\ mole\ KOH = 1.68 \times 10^5 / Mw$ [1]

$SN = 1.68 \times 10^5 / Mw$ [2]

Average molecular weights were also determined for each vegetable oil by proton nuclear magnetic resonance measurements and compared with the values obtained from saponification data in Appendix A.

Reagents

Sodium Hydroxide Standard Solution, 0.5000 molar: NaOH (HARLECO, concentrate CO_2 free).

Hydrochloric Acid, 0.5 molar: HCl accurately standardized against standard 0.5000 M NaOH.

Phenolphthalein Indicator Solution, 1% w/v in 95% ethanol.

Alcoholic Potassium Hydroxide: Prepared according the reference from AOCS (15).

Procedure

Each oil was saponified with ethanolic sodium hydroxide and titrated with hydrochloric acid in triplicate. This method has been approved as a standard technique by both the AOCS (15) and AOAC (16). Experimentally determined saponification numbers of the selected oils are listed as average values with their RSD in Table 5 and compared with literature available values.

Gas Liquid Chromatography (GC)

This method for determining IN values is applicable to methyl esters of fatty acids having 8 - 24 carbon atoms and to vegetable oils after their conversion to their methyl esters. The method permits quantitative separation of mixtures containing saturated and unsaturated methyl esters. The method involves two steps: (1) preparation of the methyl esters, and (2) GC analysis. Although gas chromatography gives very good results, it still requires a significant amount of wet chemistry since oil samples are

Table 5

Saponification Numbers of Vegetable Oils

Name	SN_{exp}	SN_{lit}	Name	SN_{exp}	SN_{lit}
Almond	189.6(±0.1)	N/A	Palm	199.1(±0.4)	200.0
Castor	178.8(±0.7)	N/A	Peanut	189.6(±0.6)	192.1
Coconut	265.6(±0.4)	268	Safflower	192.0(±0.6)	192.0
Corn	190.1(±0.3)	192.0	Sesame	187.9(±0.5)	187.9
Cottonseed	193.0(±0.5)	194.3	Soybean	191.2(±0.8)	190.6
Linseed	191.2(±0.2)	190.3	Sunflower	186.7(±0.3)	188.7
Olive	187.5(±0.3)	189.7	Wheat Germ	189.6(±0.2)	189.7

(±) denotes relative standard deviations

not sufficiently volatile for the GC technique. The measurements are made on the assorted reaction products from the esterification reaction. It is necessary to determine retention times from a collection of methyl ester standards. While this method provides useful information about the oil it is time intensive.

Apparatus and Parameters

All chromatographic measurements were done with a Varian 3600 Star gas chromatograph, with flame ionization detector (FID) and a SP - 2380 (Supelco; 30 m, 0.25 mm ID, 0.20 μm film thickness) fused silica capillary column with a 90% (biscyanopropyl) and 10% (cyano-phenyl) siloxane bonded phase. The peak areas were measured by a Shimadzu CR601 Chromatopac electronic integrator with 0 attenuation. A 50 μL syringe (Hamilton), graduated to 0.05 μL was used.

Reagents

Boron Trifluoride reagent, w/v = 14 %: BF_3/methanol (Alltech).

Saturated Sodium Chloride Solution: NaCl (Fisher Scientific, A.C.S. grade).

Methanolic Sodium Hydroxide, 0.5 molar: 2 grams NaOH are dissolved in 100 ml methanol (Fisher Scientific) containing ≤ 0.5 % H_20.

Standard fatty acids: see Table 3, necessary to determine retention times.

Heptane: C_6H_{14} (Baxter Corporation, High Purity Grade).

Procedure

Fatty acid methyl ester preparation followed the AOCS and AOAC standard method (11, 12). Both vegetable oils and standard acids were analyzed according same procedure.

An example of a chromatogram of esterified coconut oil is shown in Appendix A-1. Iodine Numbers were calculated with an equation given in the AOCS Book of Methods as shown in Ce 1-62 (12). The equation is as follows:

$$IN = (\%\ 16{:}1 \times 0.95) + (\%18{:}1 \times 0.860) + (\%18{:}2 \times 1.732) + (\%18{:}3 \times 2.616) + + (\%20{:}1\ a \times 0.785) + (\%22{:}1 \times 0.723) \quad [3]$$

Experimental results are reported in Table 6.

Table 6

Determination of Iodine Number Values by Gas Chromatography

Name	IN_{GC}	IN_{lit}	Name	IN_{GC}	IN_{lit}
Almond	96.77(±5.79)	95-102	Palm	47.87(±1.42)	51.0
Castor	93.99(±10.91)	83-88	Peanut	98.88(±4.98)	93.4
Coconut	33.70(±9.66)	9.0	Safflower	138.70(±7.10)	145.0
Corn	133.8(±5.95)	122.6	Sesame	123.46(±6.44)	106.6
Cottonseed	131.58(±11.66)	105.7	Soybean	133.48(±7.30)	130.0
Linseed	185.40(±2.93)	190.3	Sunflower	110.82(±5.92)	120.5
Olive	81.29(±3.63)	81.1	Wheat Germ	142.53(±5.75)	115-132

(±) denotes relative standard deviation

Since a splitter was not available, it was necessary to vent the injector manually in order to approach the conditions of the standard method, e.g. 3 μL injection with a 100 to 1 split ratio. This limited day to day reproducibility. A typical chromatogram obtained under these conditions may be seen in Appendix A-2.

Proton Nuclear Magnetic Resonance (^{1}H-NMR)

Nuclear magnetic resonance spectroscopy makes it possible to determine the various kinds of hydrogen atoms in the triglyceride molecule. This is due to the fact

that hydrogen atoms in a strong magnetic field absorb radio frequency energy. The frequency of absorption depends on where the hydrogen atoms are located in the molecule. The ^{1}H-NMR technique has now become a standard method for determination of IN values of oil in oilseed in the AOCS Book of Methods (7).

The ^{1}H-NMR has multivariate application for oil chemist. First, the ^{1}H-NMR can be used to determine the average molecular weight of triglyceride molecule. Second, the iodine number for different natural oils can be determined from the number of hydrogen atoms directly attached to double bond carbon atoms (6). The relationship between the average number of double bonds per molecule and the iodine value is described for a general triglyceride molecule as is shown in Appendix A-3. The four hydrogen atoms marked Δ in the glycerol molecule give a multiplet at 4.2 ppm, and its integrated signal is proportional to the number of these hydrogen atoms in the sample. The two hydrogen atoms bonded to the olefinic carbons are marked with x and the methine proton of the glycerine moiety give a multiplet at 5.3 ppm. The integral for these resonances is proportional to the number of protons. Thus the ratio between the integral at 4.2 ppm and the integral at 5.3 ppm (corrected for the methine proton) depends on the number of double bonds present in the triglyceride. The last integrated area from 3.0 to 0.3 ppm represents remaining protons, designated as H_r, on triglyceride molecule. From the following sets of equations the final formulas for both, the average molecular weight of triglyceride molecule and the Iodine Number is obtained.

integration @ 4.2 ppm is: $H_{4.2} = 4\,k$ [4]

where k = proportionality constant for a single proton response (integration)

$\therefore\ k = H_{4.2}/4$ [5]

integration @ 5.3 ppm is: $H_{5.3} = k(1 + 2X)$ [6]

where X = average number of >C=C< per triglyceride molecule

$\therefore H_{5.3} = (H_{4.2}/4)(1 + 2X)$ [7]

rearranging $2X = 4(H_{5.3}/H_{4.2}) - 1$ [8]

or $X = 2(H_{5.3}/H_{4.2}) - 0.5$ [9]

the number of olefinic protons per triglyceride molecule is given by

$V = 2X = 4(H_{5.3}/H_{4.2}) - 1$ [10]

and the average molecular weight is as follows:

$MW = 173.1 + 45.1 + 13.02V + 14.03\{[H_r - 9(H_{4.2}/4)]/[2(H_{4.2}/4)]\}$ [11]

$IN = (126.91)(100)V/MW$ [12]

.Procedure

Measurements were made on an AC - 200 (Bruker) NMR spectrometer. Each sample was analyzed in triplicate as follows: 2-4 mg of sample was dissolved in about 1 ml $CDCl_3$, the tube was shaken, transfered to an NMR tube, and then placed in the spectrometer. Integration values for the resonances at about 5.3 ppm [$H_{5.3}$], 4.2 ppm [$H_{4.2}$], and from 3.0 to 0.4 ppm [H_r] were obtained. Computation of values was carried out using equations [4] through [12]. Experimental results from Proton-NMR method are reported in Table 7 with RSD values.

Infrared Spectroscopy and Its Derivative Spectra

Vibrational spectroscopies, including infrared and Raman, are extremely valuable tools for elucidating the structure and reactivity of many different types of samples. Infrared spectroscopy provides a powerful tool to investigate the molecular structure characterized by "polar" bonds and vibrations that change the molecular dipole. In other words, the chemical bonds between atoms of different

electronegativity, change the net charge during atomic movement. The determination of IN by infrared spectroscopy has been studied from several different perspectives.

Mainly, the focus of this study was aimed at two overlapped peaks e.g. carbonyl band (1740 cm^{-1}) and carbon - carbon double bond band at (1651 cm^{-1}). The principle reason that infrared methods have had only limited success for determining iodine numbers is due to the extreme difference in the strength of the carbonyl (>C=O) and the carbon-carbon double bond (>C=C<) bands. In order to satisfactorily measure the >C=C< band the concentration must be so high that the >C=O band is so large that only stray light is measured. Conversely, adjusting the concentration for measurable values of the >C=O band, causes the >C=C< band to be so weak as to negate its validity (see Appendix A). These problems are further complicated by strong degree of overlap of these two bands.

Table 7

Determination of Iodine Number Values From Proton - NMR

Name	IN_{PNMR}	IN_{lit}	Name	IN_{PNMR}	IN_{lit}
Almond	102.4(±5.6)	95-102	Palm	45.1(±2.5)	51.0
Castor	84.4(±3.7)	83-88	Peanut	90.5(±1.5)	93.4
Coconut	15.2(±6.2)	9.0	Safflower	139.8(±9.9)	145.0
Corn	126.1(±7.9)	122.6	Sesame	124.0(±14.9)	106.6
Cottonseed	105.8(±2.6)	105.7	Soybean	121.0(±9.8)	130.0
Linseed	180.1(±8.2)	190.3	Sunflower	140.3(±4.6)	120.5
Olive	86.2(±4.7)	81.1	Wheat Germ	132.1(±2.9)	115-132

(±) denotes relative standard deviation

Derivative Spectroscopy

Derivative spectroscopy is a relatively new analytical technique. Even though it was introduced more than twenty-five years ago, it has only gained limited acceptance. This has been due to the lack of reliable analog circuits and computational features of instrumentation. Today reliable derivatives can be taken computationally. The 1st derivative of a spectrum is a graphic representation of the mathematical differentiation of an absorbance spectrum, e.g. $dA/d\nu$ vs. ν, where A = absorbance and ν = wavenumber.

Derivative spectroscopy has been applied in UV-visible (17), flame emission (18), flame absorption (19), luminescence (18), fluorescence (20, 21), and also infrared (22) spectroscopic instruments. Most experimentation has been performed in the UV-visible region. The principal advantage of derivative measurements is the improvement in the detectability of minor spectral features. This spectral enhancement reduces the potential for measurement errors caused by overlapping and unresolved bands. Minor differences in the spectra can be so enhanced such that quantitation can become possible. The derivative is a measure of the rate of change in the slope of the spectrum.

The type of presentation obtained from the application of 1st to 4th derivative on a spectrum peak, may be seen in Figure 2. This illustrates idealized representations of derivatives applied to a single gaussian peak. In the first order derivative, the absorption maximum of the zero order curve appears as a zero crossing (Y) and the points of inflection appear as maxima and minima (X_1 and X_2). This representation shows the gradient increasing to point (X_1) and decreasing until gradient or rate of change of slope is zero, (Y), or zero crossing. The gradient increases again in the opposite direction until reaching (X_2) and finally decreases gradually again to zero. The

second order derivative can be thought of as the rate of change of gradient. In this mode, the absorption maximum appears as a minimum (Y) and the points of inflection, or positions on the zero order curve where the rate of change of gradient is greatest, appear as maxima (X_1/X_2). This presentation is perhaps more immediately familiar than the first derivative as a peak has now been resolved at the absorption maximum.

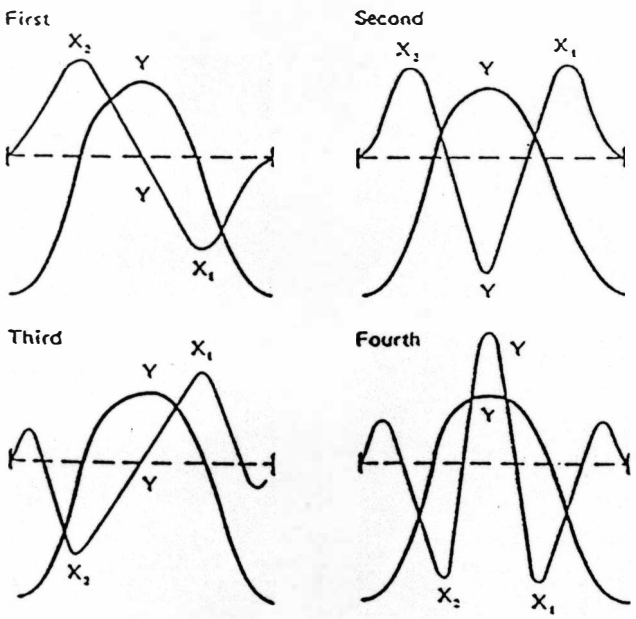


Figure 2. Idealized Representation of 1st/4th Derivative on Single Peak.

Also, it can be seen that the original peak has been significantly sharpened by the application of the second derivative. The representation of the third derivative shows an inverted sense of the first derivative when the peak of the zero order curve still being at the zero crossing. However, the fourth derivative is potentially much more interesting. The use of the fourth derivative produces a peak at the maximum absorbance and is extremely convenient for interpretation, because it is in the same sense as the original. It also exhibits a further sharpening of the peak band, thereby allowing a further improvement in apparent resolution. The use of even higher

derivative orders is possible, but would really be applicable only to extremely specialized applications and are generally limited by signal-to-noise considerations.

In Figure 3. we consider the case of two compounds A and B (maxima: A = 2 and B = 6) with overlapping peaks, a very common occurrence in IR spectra. Thus, the derivative can emphasize the slope change with respect to wavelength and can provide selectivity of B relative to A. The application of quantitative derivative spectroscopy is based on the measurement of the maxima and minima of the derivative spectra. Figure 4. depicts the corresponding 1st derivative spectra and their maxima. Occasionally a lesser maximum may be used in order to improve selectivity, e.g. selectivity for B, $S_3 > S_2 > S_1$. The limiting factor in derivative infrared spectroscopy is the inherent noise accompanying the signal. As the signal-to-noise ratio decreases within the spectrum, the error in the derivative measurements becomes more significant. Based on the previous discussion, the 1st derivative of each fundamental oil spectrum was processed as follows: measure the height in cm of the 1st derivative peak caused by >C=C< bond at 1651 cm^{-1} and the height in cm of 1st derivative of the harmonic band caused by >C=O at 3460 cm^{-1}.

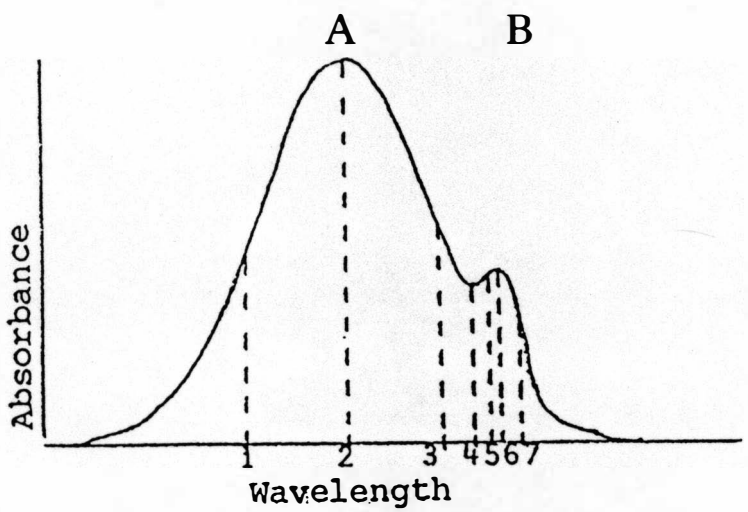


Figure 3. Unresolved Absorption Bands in Fundamental Spectra.

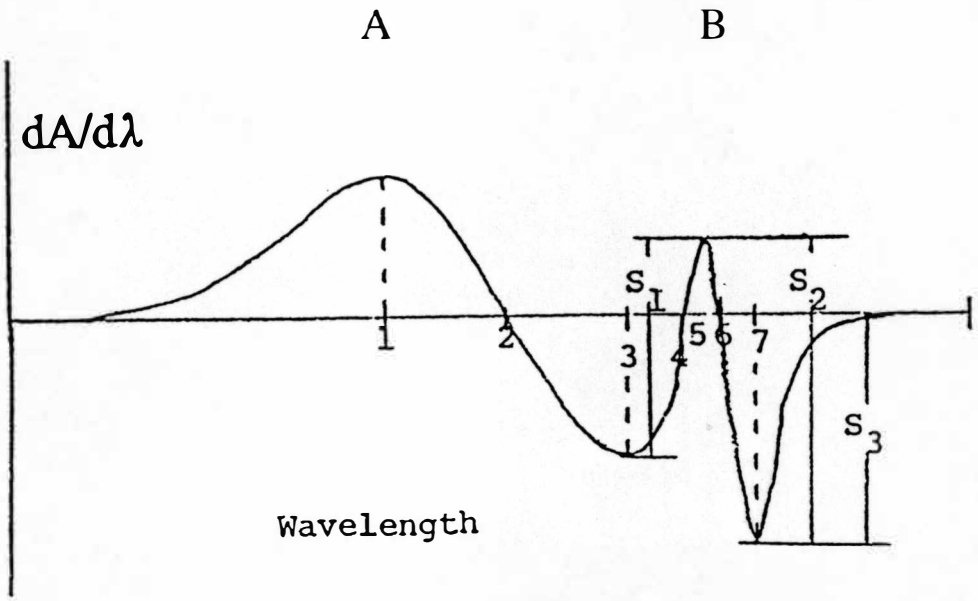


Figure 4. First Derivative of Absorption Bands.

Instrument and Parameters

All IR measurements of neat oils were done on a Nicolet 5DXC FTIR using 10 scans and a resolution of 4 cm^{-1}. A NaCl cavity cell with a path length of 0.05 mm was used in all measurements.

Reagents

The oil samples were used neat.

Carbon Tetrachloride solvent: CCl_4 (Fisher Scientific, ACS grade) was used to rinse the cell.

Procedure

Zero and first order spectra of some samples are shown in Appendix A-(4-7) which were obtained using the Nicolet spectrometer. In order to obtain 1st derivative

spectra the 0th order spectrum had to be obtained first. The wavenumber range of interest for the 0th order spectra was entered as 4,000 to 400 cm^{-1} and for the 1st derivative spectra 4,000 to 1,000 cm^{-1}. The ordinate (dA/dv) for 1st derivative spectra was entered as -0.333 to 0.277 units. The cell was polished before each day of analysis.

Each sample was scanned three times and coadded. Between sample analyses, a cell cleaning procedure was followed. First, the cell was rinsed three times with chloroform. It was then dried with a stream of air and rinsed several times with the next sample solution. Finally, the cell was filled with the sample and tightly capped. The outside of the cell was rinsed with chloroform and dried with soft paper tissue to remove all residual sample. Chloroform was used as a cleaning agent because it exhibits no absorbance bands in the wavenumber range of interest.

No sample preparation was needed. A small amount of each sample was simply placed in the clean cavity cell with Pasteur pipette. The cell was mounted in the instrument using the standard cell holder. The time required for each infrared spectrum was approximately two minutes to place the sample in a cavity cell and to position the cell in the holder. Approximately three minutes was required for data acquisition, processing and calculation.

Calculation of Iodine Numbers From Collected Data

In order to calculate iodine numbers from collected derivative peak heights of individual oils an attempt was made to derive an equation for this purpose. The derived equation for IN calculation is presented by equations [13]-[29]. The ratio of absorptivities was calculated from model compounds as shown in Table 8 and found to be $(a'_{C=O} / a'_{C=C}) = 0.28 (\pm 0.07)$. Peaks of interest were at 1651 cm^{-1} and 3460 cm^{-1}.

Table 8

Calculated Absorptivities for Model Compounds From Derivative Spectra

Name	% (w/w)	A'_{1651} [cm] >C = C<	A_{3460} [cm] >C = O	a'_{1651} 10^{-6} >C=C<	a'_{3460} 10^{-6} >C=O
cis3Hex1ol	13.40	5.61		0.60 (±0.02)	
cis5Oct1ol	13.40	3.70		0.53 (±0.04)	
cis4Dec1ol	10.80	2.50		0.49 (±0.01)	
mean $a'_{C=C}$				**0.56 (±0.06)**	
capMeEst	6.13		0.34		0.14
capMeEst	48.27		1.78		0.12
capMeEst	48.92		1.81		0.09
mean $(a'_{C=O})_1$					**0.12 (±0.025)**
capEtEst	6.10		0.35		0.15
capEtEst	31.42		1.30		0.13
capEtEst	48.92		1.40		0.10
mean $(a'_{C=O})_2$					**0.14 (±0.012)**
olMeEst	2.99		0.10		0.13
olMeEst	13.62		0.42		0.13
olMeEst	48.92		1.20		0.13
mean $(a'_{C=O})_3$					**0.13 (±0.000)**
triolein	neat	7.21	1.62	2.32	0.52
trilinolein	neat	11.38	1.70	1.80	0.54
mean $a'_{C=C}$				**2.06***	
mean $a'_{C=O}$					**0.53***

* Standard deviations could not be reported due to the fact of limited amount of sample.

From the data in Table 8 the ratio of absorptivities using isolated functional groups is seen to be:

$a'_{C=C} = 0.56(\pm 0.06)$

average $a'_{C=O} = \{(a')_1 + (a')_2 + (a')_3\} / 3 =$

$= [0.12(\pm 0.025) + 0.14(\pm 0.012) + 0.13(\pm 0.000)] / 3 = 0.13(\pm 0.012)$

$(a'_{C=O} / a'_{C=C})_{exp} = 0.28(\pm 0.07)$

using triolein and trilinolein

$(a'_{C=O}) / (a'_{C=C}) = 0.53/2.06 = 0.26$

Chromophore Study

The purpose of this study was to determine the theoretical absorptivity ratio, for carbonyl and >C=C< bond, that would be applicable to a derived equation for IN in equations[13]-[32]. This study utilized standard solutions containing the two chromophores of interest for the determination of absorptivites of both carbonyl (>C=O) and carbon - carbon double (>C=C<)bond as they are present in the triglyceride molecule. In order to calculate a theoretical absorptivity ratio the other variables, including IN and SN, have to be substituted into the derived equation.

Reagents

cis- 3-hexen-1-ol (Lancaster, 98% purity)

cis- 5-octene-1-ol (Lancaster, 98+% purity)

cis -4-decene-1-ol (Lancaster, 96% purity)

Caproic methyl ester (Sigma, 98% purity)

Caproic ethyl ester (Sigma, 99%)

Oleic methyl ester (Sigma, 98%)

Carbon Tetrachloride: CCl_4 (Fisher Scientific, ACS grade)

Triolein: (Sigma, 99%)

Trilinolein (Sigma, 99%)

Derived Equation for Iodine Number Calculation

The Beer-Lambert-Bouguer Law states:

$A = abc$ [13]

where

A = absorbance

b = cell path length

c = concentration

a = absorptivity

However if one only considers the volume in spectrophotometer cell (Figurre 5.) bdw in cm^3 of solution illuminated, one can write

$V = bdw$ in cm^3 or mL [14]

Now if c is expressed in molar concentration, then it can be stated that

$Vc = mm$ where mm is millimoles of absorbing species [15]

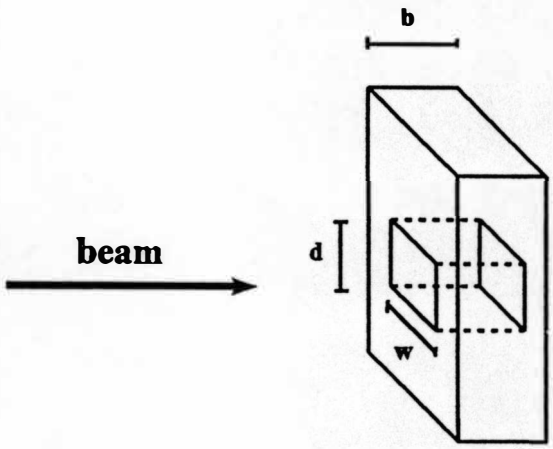


Figure 5. Spectrophotometer Cell.

under these conditions the absorptivity, a, for the absorbing species takes the form

$$a = A/mm \qquad [16]$$

While the Beer-Lambert-Bouguer law does not strictly apply to derivative spectra, practice has clearly shown that the derivative of absorbance, A', is proportional to concentration, c, over a limited concentration range. Thus equation [16] can be written in terms of derivative values, e.g.

$$a' = A'/mm \qquad [17]$$

Logically the IN must be proportional to the ratio of the number of moles of >C=C< to the number of moles >C=O. This ratio can be expressed as follows:

$$c_{C=C}/c_{C=O} = \{A'_{C=C}/a'_{C=C}\}/\{A'_{C=O}/a'_{C=O}\} \qquad [18]$$

It can be seen that the volume term cancels out. Rearranging one can write

$$c_{C=C}/c_{C=O} = \{A'_{C=C}/A'_{C=O}\}.\ \{a'_{C=O}/a'_{C=C}\} = \{A'_{C=C}/A'_{C=O}\}R$$

$$= \text{moles } >C=C</\text{moles } >C=O \qquad [19]$$

where $R = \{a'_{C=O}/a'_{C=C}\}$ = ratio of absorptivities [20]

The saponification number, SN, is defined by

$$SN = \text{mg KOH/g sample} \qquad [21]$$

It is well known that the average molecular weight of a fat or oil is inversely proportional to the saponification number. Experimentally it has been observed that the IN is proportional to the SN. Therefore applying equation [18] we can write

$$IN = k\ \{A'_{C=C}/a'_{C=C}\}/\{A'_{C=O}/a'_{C=O}\}\ .\ SN \qquad [22]$$

Now applying equation [19] the IN is given by the following relationship:

$$IN = k\ \{A'_{C=C}/A'_{C=O}\}\ .\ \{a'_{C=O}/a'_{C=C}\}\ .\ SN \qquad [23]$$

From equation [19] we see that

$$IN = k\ \{\text{moles } >C=C</\text{moles}>C=O\}\ .\ SN \qquad [24]$$

Therefore since IN is defined as the number of g I_2 per 100 g sample, it follows that

$$\frac{g\ I_2}{100\ g\ sample} =$$

$$= \left\{\frac{100}{(100^*)} \cdot \frac{254\ g\ I_2}{1\ mole\ I_2} \cdot \frac{1\ mole\ I_2}{1\ mole\ C{=}C} \cdot \frac{1\ mole\ C{=}O}{1\ mole\ KOH} \cdot \frac{1\ mole\ KOH}{56.1\ g\ KOH} \cdot \frac{10^{-3}\ g\ KOH}{1\ mg\ KOH}\right\}$$

$$\cdot \left\{\frac{mole\ >C{=}C<}{mole\ >C{=}O}\right\} \cdot \left\{\frac{mg\ KOH}{g\ sample}\right\} \qquad [25]$$

where (100*) is not a factor but a label. Thus the quantity in the first set of brackets is a proportionality constant k and the last term in brackets is the SN.

The proportionality constant is given by $k = 0.453$ [26]

Now applying equations [20] and [23] the expression for the IN can be written as

$$IN = 0.453\ \{A'_{C=C}/A'_{C=O}\} \cdot R \cdot SN \qquad [27]$$

Rearranging one can write

$$IN = K \cdot \{A'_{C=C}/A'_{C=O}\} \cdot SN \qquad [28]$$

where $K = k \cdot R = 0.453 \cdot 0.27 = 0.122$ [29]

Where R was determined using triolein and trilinolein as model componds. This value is consistent with the ratio found with studied model componds. This value could also be determined experimentally using a wide variety of oils. The value for K used to determine IN's in this study was 0.133 using equation [28] and calculated as the average of 14 different oils.

Instrument and Parameters

All IR measurements of neat oils were carried out on a FTIR - Nicolet 5DXC with 10 scans and resolution 4 cm^{-1}. The NaCl cavity cell, path length 0.05 mm was used throughout all measurements. A Mettler balance was used to determine the

concentration [w/w %] of diluted standard samples with carbon tetrachloride prepared in 10 mL volumetric flasks.

Procedure

The first set of standard compounds: *cis*- 3-hexen-1-ol, *cis*- 5-octene-1-ol, *cis* - 4-decene-1-ol, were used as model compounds to simulate the >C=C< double band present in many fatty acids which absorb at 1651 cm^{-1}. The second group of compounds: caproic methyl ester, caproic ethyl ester and oleic methyl ester were used to simulate the first harmonic of the carbonyl band of fatty acids present at 3460 cm^{-1}.

The concentration range for the first set of standards was adjusted so that the intensities of the standard bands simulated the >C=C< double band as in oil samples. This concentration was in the range between 10 and 13% w/w of standard in carbon tetrachloride. The concentration range for the second set of standards was adjusted so that the intensities of the bands produced simulated the carbonyl band intensities of oil samples. This ranged between 3.0 and 50.0% w/w of standard in carbon tetrachloride. The absorptivity values were then calculated using the modified form of Beer's Law, equation [17].

Each standard in the first set was measured three times and the average absorptivity value for the >C=C< double bond was calculated. The same procedure was done for the second set of carbonyl standards. The final absorptivity value for carbonyl bond was obtained as an average of the three standards. The results of the absorptivities for these standards are presented in Table 8. The experimental absorptivity ratio as determinated from model compounds was equal to 0.28 (±0.07).

Triolein and trilinolein (Sigma 99%) were also chosen as model compounds and their absorptivities were determined from sample weight, molecular weights, and

absorbance measurements. The average ratio of the absorptivities for these two compounds was found to be 0.26, compared to 0.28 for the model compounds in Table 8. Also using equation [27], theoretically calculated values for IN and SN of triolein and trilinolein, measured values for absorbances at 1651 and 3460 cm^{-1}, and the average value of their absorptivity ratios was found to be 0.27. This ratio too is in very good agreement with the previously determined ratios for the model compounds.

In Table 9 are listed Iodine Number values calculated by newly developed IR method. Each iodine value determined spectroscopically is the average from three separate measurments. As seen in Table 9, all of the IR determined iodine numbers (average values with their relative standard deviations) fall within the accepted range of literature values.

Table 9

The Iodine Number Values Calculated From IR Technique

Name	IN_{exp}	IN_{lit}	Name	IN_{exp}	IN_{lit}
Almond	108.4(±4.9)	95-102	Palm	43.7(±2.5)	51.0
Castor	N/A	83-88	Peanut	90.6(±4.8)	93.4
Corn	124.7(±6.4)	122.6	Safflower	134.6(±6.5)	145.0
Coconut	15.1(±4.8)	9.0	Sesame	118.3(±5.5)	106.6
Cottonseed	106.3(±5.3)	105.7	Soybean	121.0(±4.1)	130.0
Linseed	174.4(±8.3)	190.3	Sunflower	118.6(±5.4)	120.5
Olive	90.0(±5.9)	81.1	Wheat Germ	128.0(±4.9)	115-132

RESULTS AND DISCUSSION

The goal of this study was to determine Iodine Numbers of various selected vegetable oils by standard techniques and compare them with results from a newly developed IR technique. We can evaluate our results based on two different levels from the data as shown in Table 10. First, comparison of IN values from each individual technique vs. IN values cited in literature. Secondly, and perhaps more realistically, they can be compared to those obtained by the classical titration method.

The classical method provides highly accurate and precise results for each sample. For this reason the classical method was used in this research as the reference technique. Proton-NMR is also a known and established technique, but the results appears less accurate and precise relative to the classical titration method. This was caused primarily by difficulties in defining the areas for peak integration. Fair evaluation of the gas chromatographic method suffered from the lack of a split/splitless injector. This accounts, in large part, for the large value in standard deviations. The IR method is quite accurate and precise with respect to literature IN values and also the classical method. However, this method was not able to determine iodine number of castor oil and lecithin. The castor oil contains approximately 87 % ricinoleic acid. A strong band appearing around 3500 cm^{-1} is caused by OH stretch of hydroxyl group present in ricinoleic acid and overlaps with the harmonic band of carbonyl at 3460 cm^{-1} (23). Lecithin exhibits a similar problem.

$CH_3(CH_2)_5CH(OH)CH_2CH=CH(CH_2)_7COOH$

Ricinoleic acid (d-12-Hydroxy-cis-9-octadecenoic acid)

Table 10

Comparison of Iodine Number Results From Different Methods

Name	$IN_{classical}$	IN_{PNMR}	IN_{IR}	IN_{GC}	IN_{lit}
Almond	102.2(±0.3)	102.4(±5.6)	108.4(±4.5)	96.77(±5.79)	95-102
Castor	91.1(±2.9)	84.4(±3.7)	NA	93.99(±10.91)	83-88
Coconut	15.1(±18.6)	15.2(±6.1)	15.1(±6.6)	33.70(±9.66)	9.0
Corn	123.5(±1.8)	126.1(±7.9)	124.7(±6.4)	133.80(±5.95)	122.6
Cottonseed	109.4(±1.2)	105.8(±2.6)	106.3(±5.0)	131.58(±11.66)	105.7
Linseed	180.0(±4.6)	180.1(±4.6)	174.4(±4.5)	185.40(±2.93)	190.3
Olive	81.3(±0.8)	86.2(±4.7)	90.0(±6.6)	81.29(±3.63)	81.1
Palm	43.3(±3.1)	45.1(±2.5)	43.7(±5.7)	47.87(±1.42)	51.0
Peanut	91.3(±0.7)	90.5(±1.5)	90.6(±5.3)	98.88(±4.98)	93.4
Safflower	134.7(±2.7)	139.8(±14.9)	134.6(±4.8)	138.70(±7.10)	145.0
Sesame	110.4(±1.4)	124.0(±14.9)	118.3(±4.6)	123.46(±6.44)	106.6
Soybean	128.7(±0.7)	126.4(±9.8)	121.0(±3.4)	133.48(±7.30)	130.0
Sunflower	120.0(±1.8)	140.3(±4.6)	118.6(±4.6)	110.82(±5.92)	120.5
Wheat Germ	126.7(±7.2)	132.1(±2.9)	128.0(±3.8)	142.53(±5.75)	115-132

(±) denotes relative standard deviation

The hydroxyl group of phospholipids (Figure 6) tends to overlap the harmonic band of the carbonyl group (18). There were two types of saponification numbers applied in the

iodine number calculation which were obtained both experimentally and from the literature.

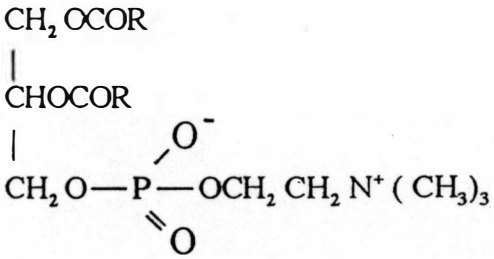


Figure 6. Lecithin Molecule.

From Table 11 it can be seen that calculated iodine numbers, IN_{lit} (calculated from literature SN values), and IN_{exp} (calculated from experimentally determined SN values) are almost identical. This is due to the fact saponification numbers from the literature and those experimentally determined are very similar. This implies that iodine numbers can be calculated from the derived equation [28] using literature values of the saponifacation numbers and thus avoiding the tedious titration necessary to obtain this variable.

In order to confirm the validity and reproducibility of the new IR technique three edible oils, avocado (Anglia Oils Ltd., Hull, Product of U.K.), canola (Spartan, Grand Rapids), and macadamia (Loriva Supreme Foods, Hauppauge) were purchased and analyzed. The data in Table 12. illustrates the agreement of iodine numbers obtained by titration and by infrared measurements. With the exception of macadamia oil, the values are in good agreement with the literature (24). The variation in measured iodine numbers from the literature might be interpreted as possibly being caused by additives introduced during the manufacturing process to enhance the quality of the cooking oil.

Table 11

Comparison of Calculated Iodine Numbers IN_{lit} and IN_{exp} Obtained From IR Method Base on Incorporated Values, SN_{lit} and SN_{exp}

NAME	SN_{lit}	SN_{exp}	IN_{lit}	IN_{exp}
ALMOND	188-197	189.6 (±0.2)	102.2 (±7.9)	108.4 (±4.9)
CASTOR	176-187	178.8 (±1.3)	N/A	N/A
COCONUT	268.0	265.6 (±1.2)	15.2 (±1.0)	15.1 (±1.0)
CORN	192.0	190.1 (±0.5)	126.0 (±6.4)	124.7 (±6.4)
COTTONSEED	194.3	193.0 (±0.9)	107.0 (±5.3)	106.3 (±5.3)
OLIVE	189.7	187.5 (±0.5)	91.0 (±6.0)	90.0 (±5.9)
PALM	199.1	200.0 (±0.8)	43.5 (±2.4)	43.7 (±2.5)
PEANUT	192.1	189.6 (±1.1)	91.8 (±4.8)	90.6 (±4.8)
SAFFLOWER	192.0	192.0 (±1.2)	137.9 (±6.4)	134.6 (±6.5)
SESAME	187.9	187.9 (±0.9)	118.3 (±5.5)	118.3 (±5.5)
SOYBEAN	190.6	191.2 (±1.5)	120.6 (±4.9)	121.0 (±4.1)
SUNFLOWER	188.7	186.7 (±0.6)	119.9 (±5.4)	118.6 (±5.4)
WHEAT GERM	179-189	189.6 (±0.4)	124.0 (±4.8)	128.0 (±4.9)

(±) denotes relative standard deviation

Table 12

Determined Iodine and Saponification Numbers by Classical and Newly Developed Method

Name	IN_{class}	IN_{lit}	IN_{IR}	SN_{exp}	SN_{lit}
Avocado	83.9(±4.8)	71-95	83.1(±2.2)	180.2(±1.3)	177-198
Canola	105.0(±1.5)	97-107	101.9(±1.7)	176.0(±1.6)	168-179
Macadamia	100.2(±2.9)	74-76	99.7(±1.8)	199.6(±1.8)	193-197

(±) denotes relative standard deviation

CONCLUSION

The derivative infrared spectroscopic technique provides a quantitative method that is very comparable to the standard methods. This method provides data with a relative standard deviation of about 5 percent. This technique permits quantitation in the infrared region at the same level of precision and accuracy as some of the more elaborate standard techniques. The method does not require any standards or sample preparation since spectra are measured on single neat samples. It does not require expensive instrumentation for analysis and takes only about 5 minutes to obtain an iodine number.

Appendix A

Gas Chromatograms, Proton - NMR and Infrared Spectra of Selected Vegetable Oils

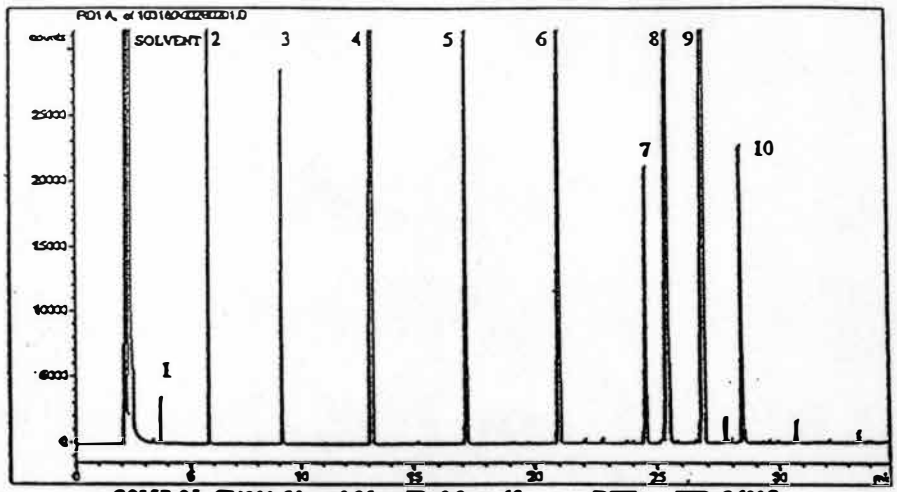
Certificate of Analysis

DESCRIPTION: COCONUT OIL

CATALOG NO.: 4-6949 LOT NO.: LA-53898 CAS NUMBER: 8001-31-8

PHYSICAL PROPERTIES	ASSAY
APPEARANCE	white semisolid with faint yellow cast
SAPONIFICATION VALUE	257
SOLUBILITY	clear colorless solution at 200 mg plus 4.0 ml of chloroform

CHROMATOGRAPHIC ASSAY OF COCONUT OIL AS METHYL ESTERS*



COLUMN: SP2380, 30m x 0.25mmID, 0.2μm df
CAT.NO.: 2-4110
OVEN: 80°C to 250°C @ 4°C/min
CARRIER: helium, 20 cm/sec @ 80°C
DET.: FID, 260°C
INJ.: 3μL
SPLIT: 100:1

ELUTION ORDER	RETENTION TIME (min.)	AREA PERCENT (%)
1. Caproic, C6:0	3.7	.24
2. Caprylic, C8:0	5.8	3.4
3. Capric, C10:0	9.2	2.9
4. Lauric, C12:0	13.3	24.3
5. Myristic, C14:0	17.4	10.5
6. Palmitic, C16:0	21.1	9.5
7. Stearic, C18:0	24.6	3.5
8. Oleic, C18:1	25.5	13.2
9. Linoleic, C18:2	26.9	19.6
10. Linolenic, C18:3	28.5	2.6

Kenneth J. Herwehe
Quality Control Supervisor

* Methyl Esters were prepared following procedures outlined in AOAC Method 969.33 or AOCS Method Ce 2-66.

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Figure A-1. Chromatogram of Coconut Oil Obtained from Supelco Company.

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Coconut oil

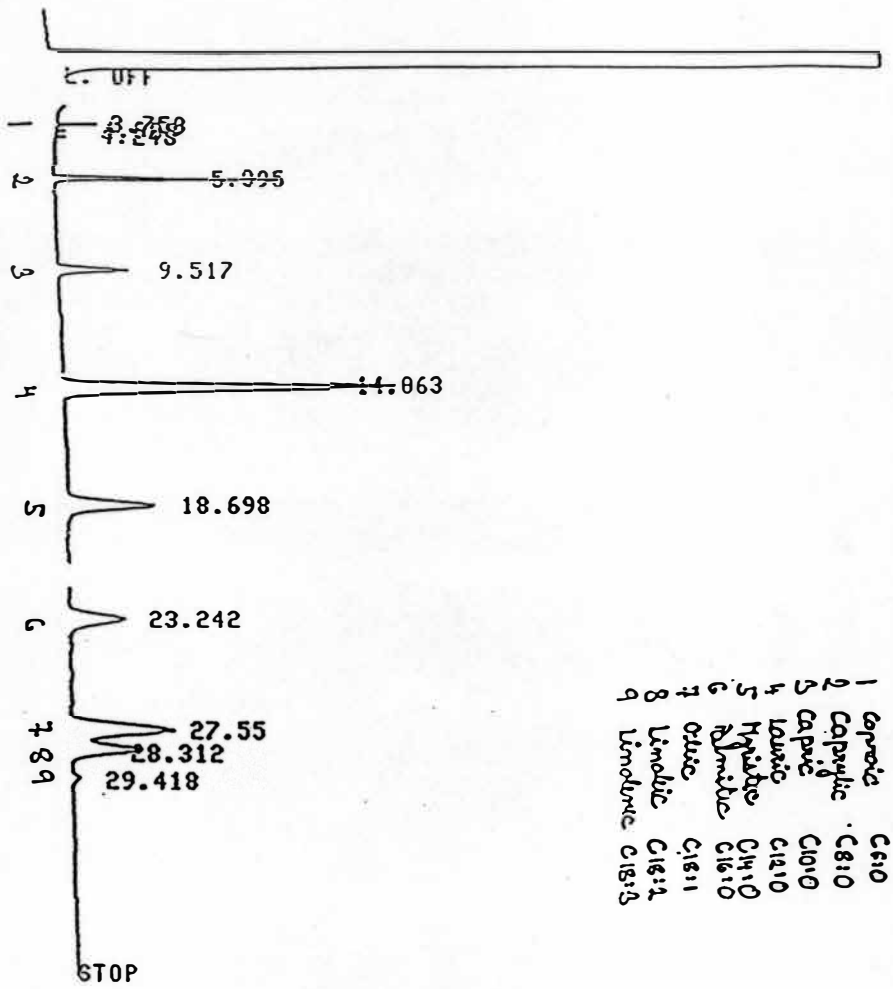

Figure A-2. Chromatogram of Coconut Oil.

CHROMATOPAC C-R3A FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 43

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.758	149			0.7763	
2	4.008	36	V		0.1865	
3	4.248	34			0.1791	
4	5.895	1569			8.193	
5	9.517	936			4.8873	
6	14.063	6811			35.566	
7	18.698	2295			11.9822	
8	23.242	1779			9.2895	
9	27.55	3413			17.82	
10	28.312	1972	V		10.2946	
11	29.418	158			0.8255	
	TOTAL	19152			100	

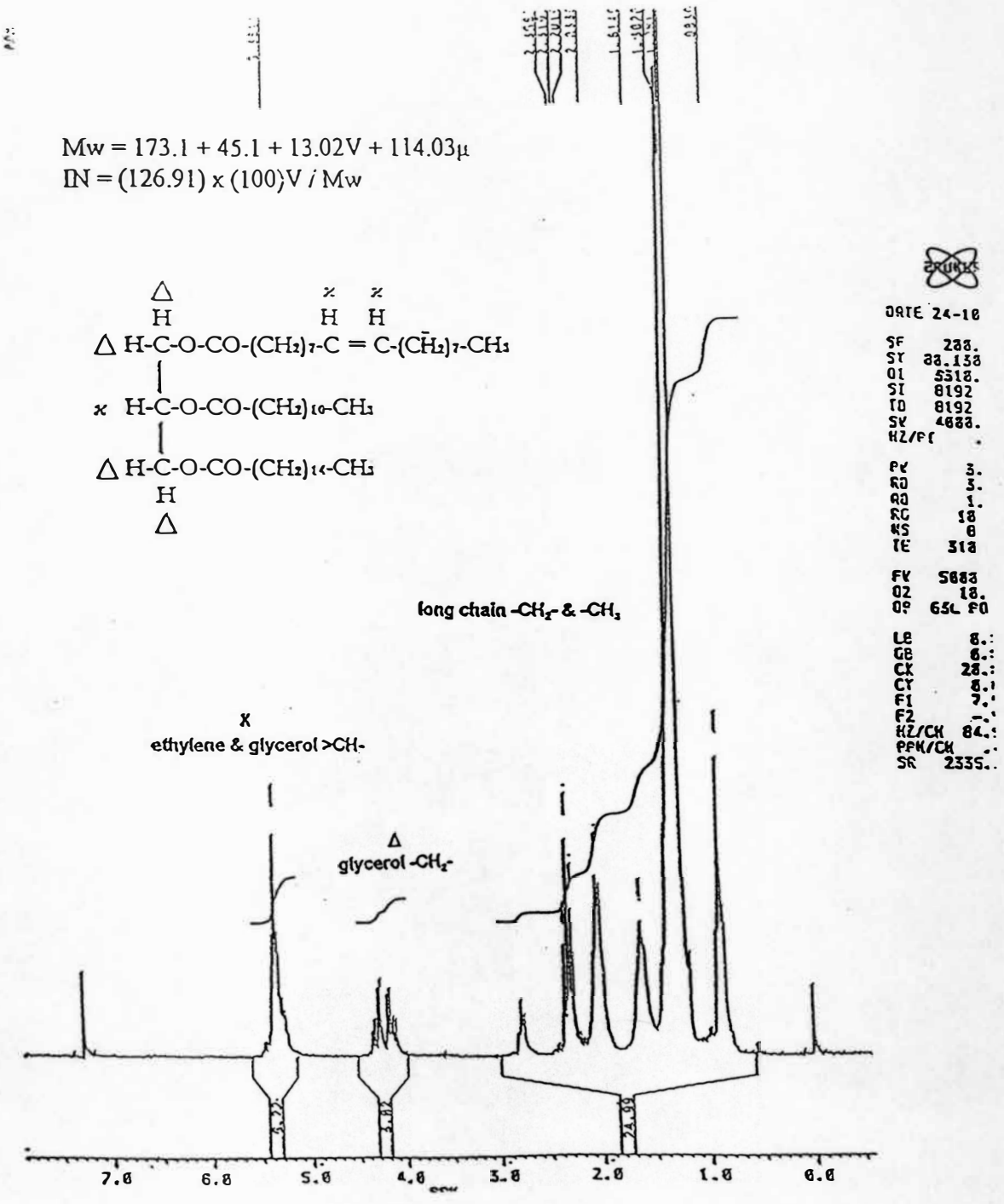

Figure A-3. Proton-NMR Spectrum of Peanut Oil.

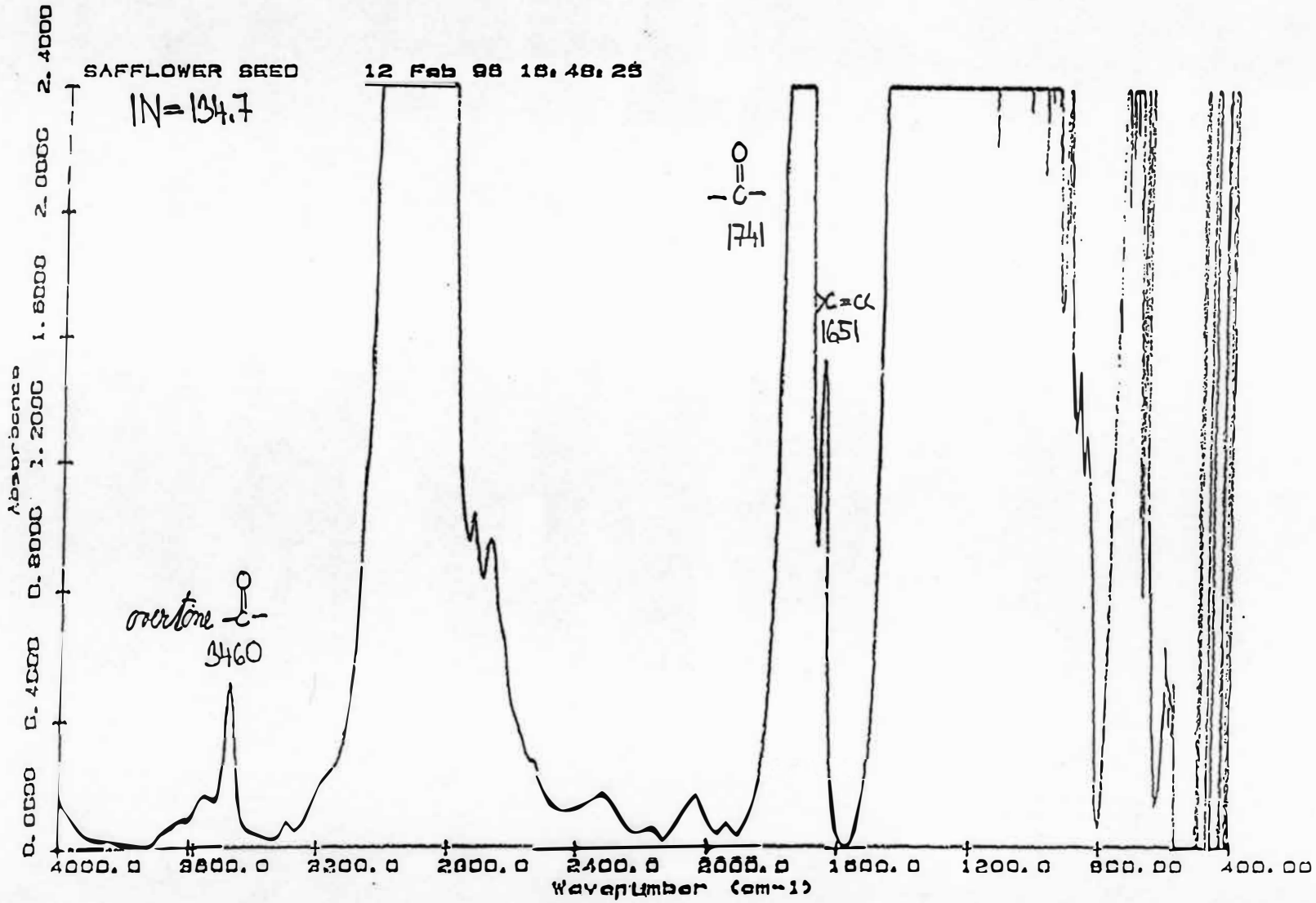

Figure A-4. IR 0th Order Derivative Spectrum of Safflower Oil.

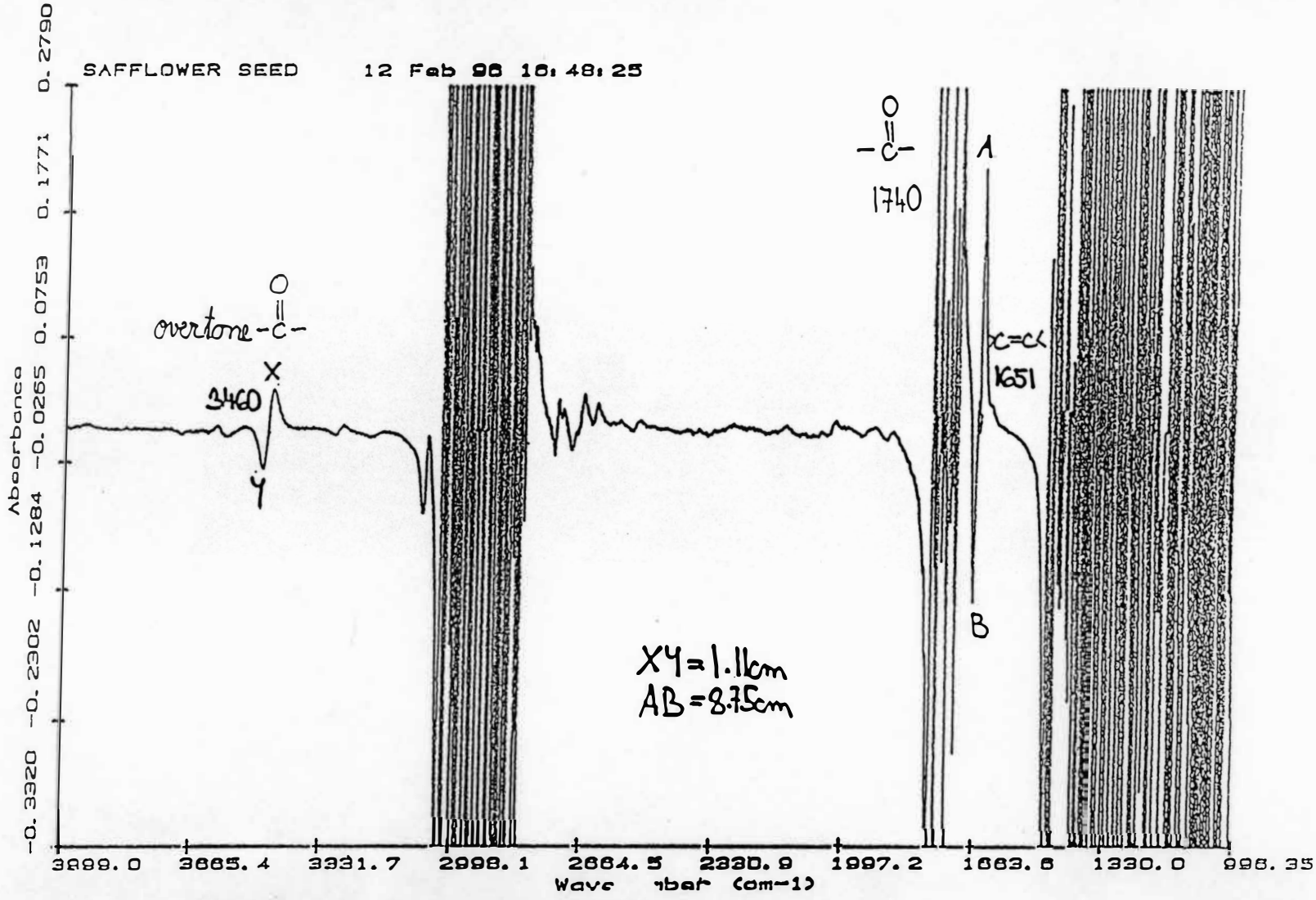

Figure A-5. IR 1st Order Derivative Spectrum of Safflower Oil.

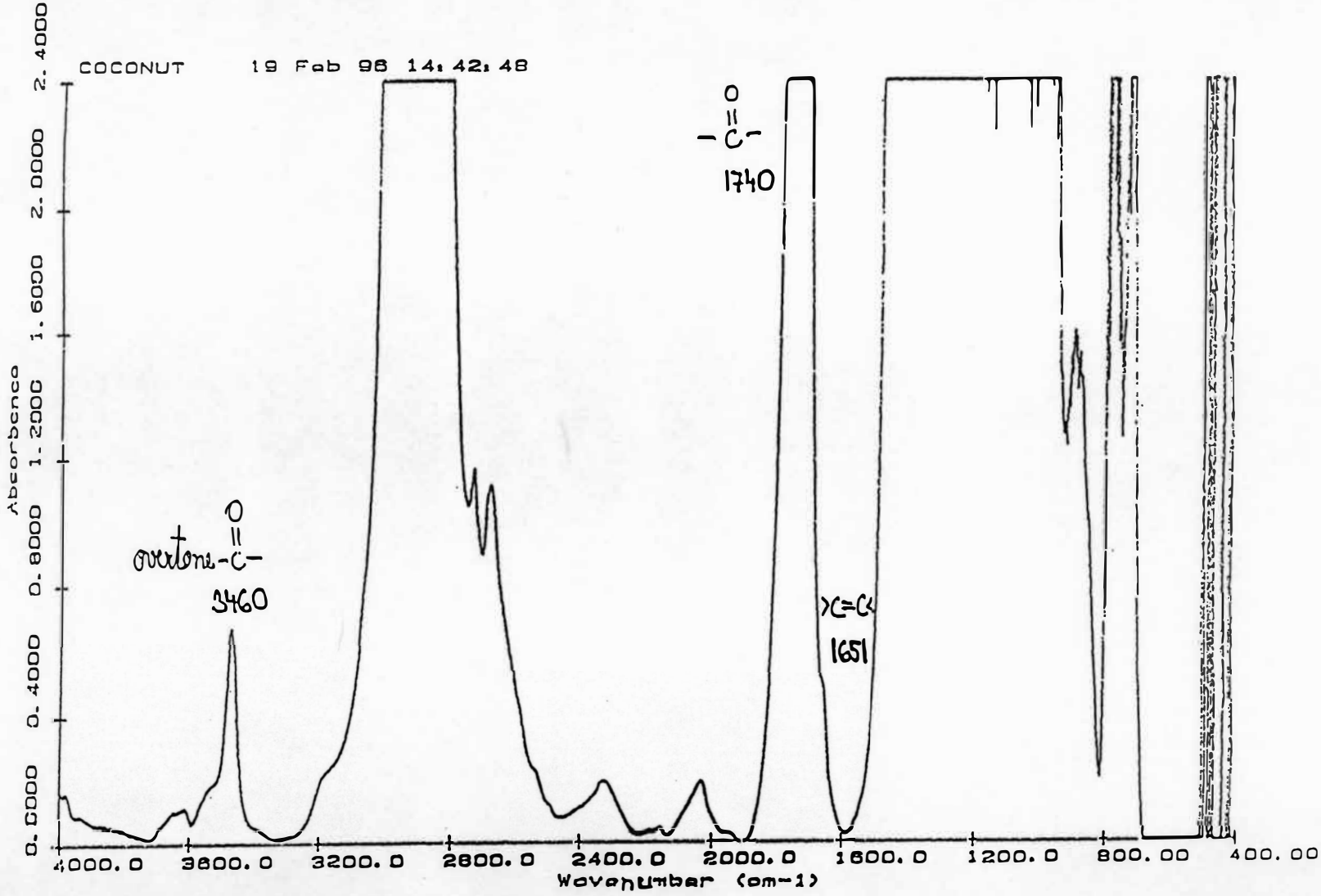

Figure A-6. IR 0th Order Derivative Spectrum of Coconut Oil.

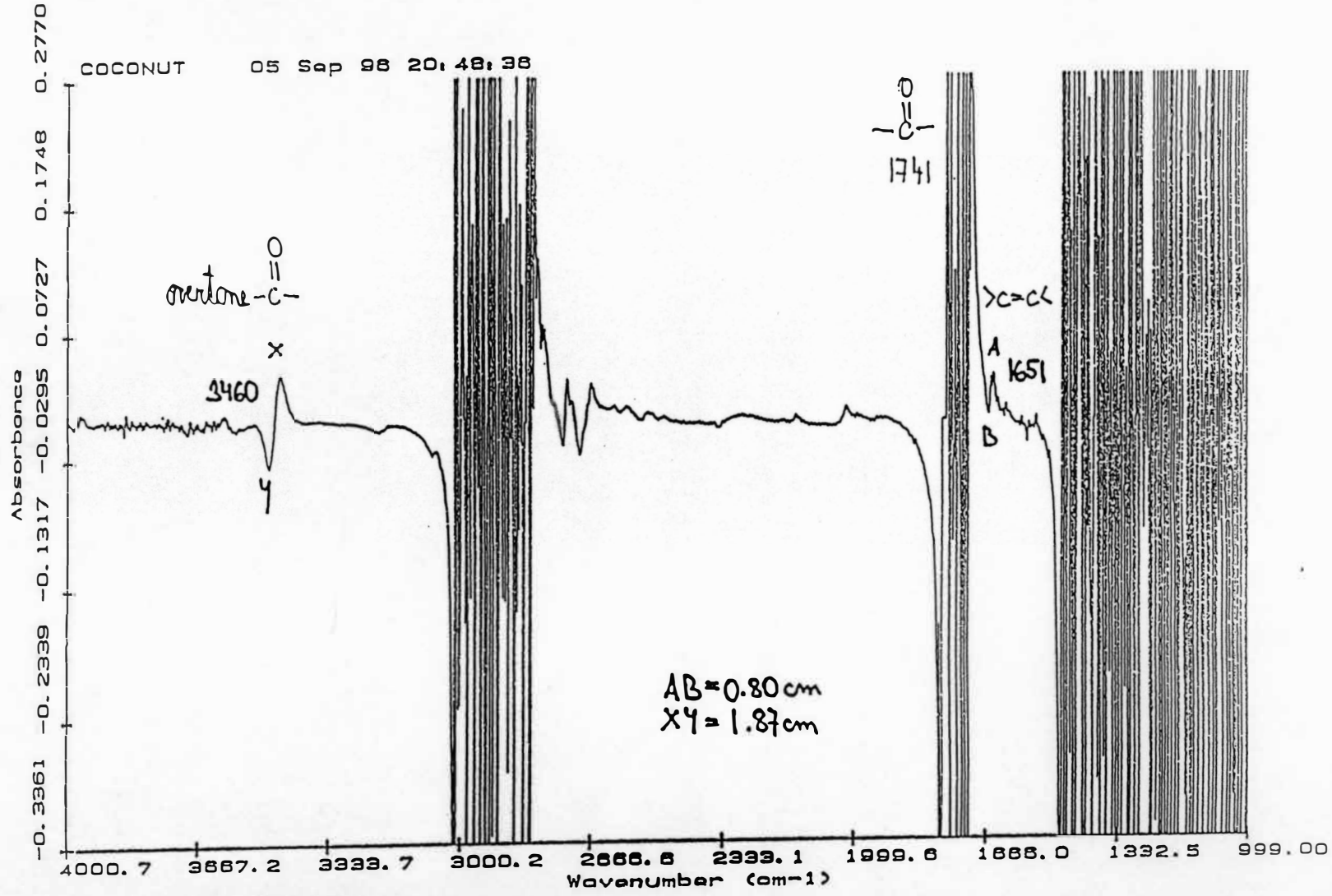

Figure A-7. IR 1st Order Derivative Spectrum of Coconut Oil.

Name	MW_{SN}	$MW_{P\text{-}NMR}$
Almond	886.1(±0.9)	872.3(±47.7)
Castor	939.6(±6.8)	948.6(±41.6)
Coconut	632.5(±2.8)	658.1(±268.4)
Corn	883.7(±2.3)	878.9(±55.1)
Cottonseed	870.5(±4.0)	862.0(±21.2)
Linseed	878.7(±1.4)	879.4(±40.0)
Olive	896.0(±2.4)	908.3(±49.2)
Palm	843.8(±3.4)	852.7(±47.3)
Peanut	886.1(±5.1)	862.8(±47.3)
Safflower	875.0(±5.5)	877.5(±62.1)
Sesame	894.1(±7.1)	912.9(±109.7)
Soybean	878.7(±6.9)	856.3(±69.4)
Sunflower	899.8(±2.9)	872.3(±28.6)
Wheat Germ	886.1(±1.9)	873.5(±19.2)

(±) denotes relative standard deviation

Figure A-8. Average Molecular Values from P-NMR and SN Measurements.

REFERENCES

1. Meyer, L.H. (1961). Food Chemistry, Reinhold Publishing Corporation, New York, p. 42-44.

2. ibid., pp. 22.

3. Haumann, F.B. (1996). Mediterranean Product Consumed Worldwide, Inform, 7: pp. 890-901.

4. Petty, Ch., Walser, F. (1992). An Introduction to FT - Raman (Near - Infrared) Spectroscopy, Nicolet FT -IR Spectral Lines, Introducing the Raman 91 Spectrometer, pp. 2- 3.

5. Melvin, J.A., Skelton, J.R. (1953). Organic Chemistry, Harper & Brothers Publishers, New York, p. 223.

6. Vinter, N.L. (1976). Studies on the Relationship Between Unsaturation and Iodine Value of Butterfat by High Resolution Nuclear Magnetic Resonance (NMR) Milchwissenshaft, 3 (10), pp. 598-602.

7. The American Oil Chemists' Society (1996, September 28). Oil in Oilseeds by Proton - NMR, Official Method, Internet, www.aocs.org.

8. Association of Official Analytical Chemistry (1980). 13th Ed., Iodine Value, Hanus Method, 28.018, Official Methods of Analysis.

9. Association of Official Analytical Chemistry (1980). 13th Ed., Iodine Value, Wijs Method, 28.020, Official Methods of Analysis.

10. The American Oil Chemists' Society (1983). 13th Ed., Iodine Value, Wijs Method, Vol. 1, Cd 1-25, Official Method.

11. Association of Official Analytical Chemistry (1980). 13th Ed., Preparation of Methyl Esters and Gas Chromatography, 28.052-28.65, Official Methods of Analysis.

12. The American Oil Chemists' Society (1983). 13th, Fatty Acid Composition by Gas Chromatography:, Vol. 1, Ce 1-62, Official Method.

13. Lowry, J.C. (September 26, 1984). The application of Multiderivative Infrared Spectroscopy to Iodine Number Determination, M.S. Thesis, WMU.

14. The American Oil Chemists' Society (September 28, 1996). IN Using Cyclohexane/Acetic Acid, Official Method, Internet, www. aocs.org.

15. The American Oil Chemists' Society (1983). 13th Ed., Saponification Number , Vol. 2, Tl 1a-64, Official Method.

16. Association of Official Analytical Chemistry (1980). 13th Ed., Saponification Number, 28.018, Official Methods of Analysis.

17. Kapoulas, M.V., Andrikopoulos, K.N. (1987). Detection of Virgin Olive Oil Adulteration with Refined Oils by Second-Derivative Spectrophotometry, Food Chemistry, 23: pp. 183-192.

18. Snellman, W. (1970)., Flame Emission Spectrometry with Repetitive Optical Scanning in the Derivative Mode, Anal. Chem. 42, p. 394.

19. Fowler, W.K. (1974). Double Modulation Atomic Fluorescence Flame Spectrometry, Anal.Chem., 46, p. 601.

20. Kolb, D.A. & Shearin, K.K. (1977). Fingerprinting Petroleum Oils with Low Temperature Derivative Fluorometry, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio.

21. O'Haver, T.C., Green, G.L. (1974). Derivative Luminescences Spectrometry, Anal. Chem., 46, p. 2191.

22. Overland, J,. Gilby, A.C., Russell, J.W., Brown, C.W., Beutes, J., Bjork, C.W., Paulet, H.G. (1967). A Littrow-McCubbin High Resolution Infrared Spectrometer, Appl. Opt., 6, p. 457.

23. Stadler Laboratories Inc. (1963). Ricinoleic Acid, Vol. 21, No. 21992, Stadler Standard Spectra, Grating Spectrum, Philadelphia, PA.

24. Eckey, E.W. (1954). Vegetable Fats and Oils, Reinhold Publishing Corporation, New York.