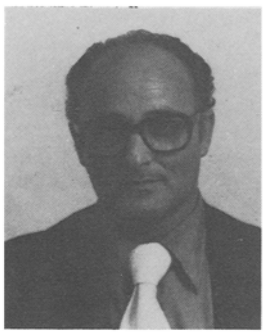

Methods for Analysis of Processed Soya Oil

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ABSTRACT

In a soybean oil processing plant, instrumental analysis plays an important role in monitoring the quality of oil produced. It is capable of providing analytical data with greater sensitivity, selectivity and efficiency than manual analysis. Some of the latest developments in instrumental methods are discussed with regard to their use in soybean oil analysis. Particular attention is paid to liquid chromatography, atomic absorption spectrophotometry, nuclear magnetic resonance and colorimetry. Relevant applications of each are given.

INTRODUCTION

A great demand exists for new and improved analytical methods for monitoring processed soybean oil quality. This demand originates from commercial and nutritional interest in quality of the finished products. Color, storage stability, odor and physical properties are quality attributes of commercial interest whereas unsaturation and fatty acid isomers (positional and geometric) are subjects of nutritional concern.

It is known that processing greatly affects the oil quality. Some changes that occur, such as elimination of some of the odoriferous components or their precursors and reduction of color, are deliberate and beneficial to the quality. Other changes, such as isomerization and polymerization, are coincidental and/or unwanted. Monitoring and assessing oil quality at any given processing step is of utmost importance. Proper process control allows more efficient production of oils of superior quality.

To be useful for quality and process control, analytical methods must fulfill three basic requirements: (a) selectively detect components of interest; (b) detect compounds at very low levels; (c) efficiently provide analytical data in a high through-put operation.

A vast number of analytical methods have been developed which serve quality control needs at almost all conceivable processing operations. A book containing an authoritative collection of such methods is published by the American Oil Chemists' Society (1). Although the methods included in that book are very useful and practical, there are many cases in which instrumental methods seem preferable. The intention of this paper is to show some instrumental methods which can supplement those contained in the AOCS method book.

Because the entire field of analytical and process control instrumentation is so large that its discussion would not fit into the scope of this presentation, only specific segments have been chosen for discussion. Particular attention is given to liquid chromatography (LC) because of its increasing importance in characterization and quantification of vegetable oil components. Other new trends such as nuclear magnetic resonance (NMR) and color measurements are reviewed, as are some of the advancements in atomic absorption spectrophotometry (AA) and some aspects of in-process instrumentation.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

For use in an atomic absorption spectrophotometer, a sample must be converted to atomic vapor. Each of the constituent elements (metals) in the vapor phase will absorb radiation at a specific wavelength. The flame AA achieves this by atomization of the sample solution in the nebulizer followed by atomic vaporization in the flame. The spectral absorption of the sample metal is measured by the spectrophotometer.

This instrument is included in AOCS methods for determination of Fe, Cu and Ni in vegetable oils. Although flame AA is capable of detecting trace amounts of metals, conditions exist in which a higher sensitivity is desirable. It has been established (2) that concentrations of less than 0.01 ppm of Cu, for instance, can adversely affect the oxidative stability of soybean oil. A recently developed carbon rod furnace modification for AA (also called "flameless AA") can accurately assess this level of metals in oils. The detection limit of metals by flameless AA is much below that of flame AA (Table I).

The breakthrough in this instrumentation is the substitution of a carbon rod furnace for the inefficient nebulizer and flame combination. The sample is directly deposited in the hollow of the carbon rod (Fig. 1) by injection or micropipetting. The carbon furnace is then heated in three stages. The first stage facilitates solvent evaporation. The second stage destroys the bulk of excipients (oil in this case) and the third stage heats the chamber to incandescence, atomizing the metals. The absorbance of the atomized metal is measured by the spectrophotometer.

The advantage of flameless AA is that the graphite chamber allows a higher concentration of atomized metal for the 3-4 sec of spectrophotometeric measurement. In conventional AA, the atomized metal is transient in the flame and requires continuous replenishment by new sample during the time of spectrophotometric measurement, which necessitates a large sample volume.

The consequences are: (a) the atomized sample in flameless AA is more efficiently used than in the flame, hence, higher sensitivity is obtained; (b) the sample is

TABLE I

Analytical Values for Atomic Absorption Spectrophotometer

Element	Wavelength (nm)	Lamp current (ma)	Flame		Graphite rod atomizer	
			Sensitivity[a] (ppm)	Detection limit (ppm)	Sensitivity (ppb)	Detection limit (ppb)
Copper	324.7	5	0.03	0.002	0.008	0.0013
Iron	248.3	8	0.04	0.005	0.003	0.0007
Nickel	232.0	10	0.06	0.008	0.02	0.015
Lead	217.0	5	0.1	0.002	0.8	0.07
Sodium	589.0	8	0.001	0.0004	0.004	0.004

[a]Sensitivity is concentration yielding 1% absorption.

stationary in flameless AA, whereas it is mobile in the flame and, therefore, the sample size requirement of flameless AA is a small fraction of that of flame AA; (c) sample extraction is unnecessary because of the second stage heating and subsequent charring of the vehicle (oil) associated with the sample metals.

The use of flameless AA for trace metal determination in soybean oil has been studied by Black (3). Data obtained by conventional flame AA were compared with flameless AA. Various methods of sample preparation are also described.

NUCLEAR MAGNETIC RESONANCE

Although it has been found that NMR is an excellent means of monitoring oil and water contents of seeds and the solid fat and moisture contents of liquid shortenings or oils, most of its applications have been research-oriented because of the intricacies associated with its use, and its high price. Some simplified, special-purpose instruments have been developed which are easy to operate and are reasonably priced. These instruments have made NMR more practical for use in quality control.

Two variations in NMR, wide-line and pulsed (4), were proposed for use in replacing dilatometry for determination of solid fat content (SFC). The measurement of SFC by both of the NMR techniques is based on the difference in nuclear spin relaxation time of hydrogen atoms in liquid oil and in solid fat. In essence, the ratio of hydrogen in liquid and solid phases is measured. In the U.S., pulsed NMR has gained more acceptance due to the experiments and evaluations of Madison and Hill (5). Their work demonstrated that the precision of solid fat content determination by pulsed NMR is nearly identical to that of dilatometry. Presently, AOCS is evaluating this instrumental method for inclusion into its official methods. The last report of J.H. Mellema, chairman of the NMR Subcommittee, was very encouraging in regard to the implementation of the method.

The pulsed NMR mode of SFC measurement has several advantages over dilatometry in that it is faster (20-30 analyses may be performed in a day) and simpler in operation, requires less sample preparation, and it uses less electrical energy.

LIQUID CHROMATOGRAPHY

The vegetable oil processor has an interest in chromatographic techniques because vegetable oil is a multicomponent system of constituents including triglycerides and fatty acids and an undetermined number of minor compounds. One of these chromatographic techniques, gas chromatography (GC), is well known and has been used in the laboratories of soybean processing establishments for

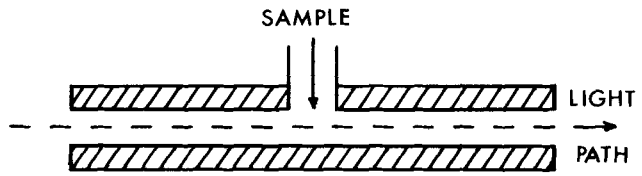

FIG. 1. Graphite furnace of a flameless atomic absorption spectrophotometer.

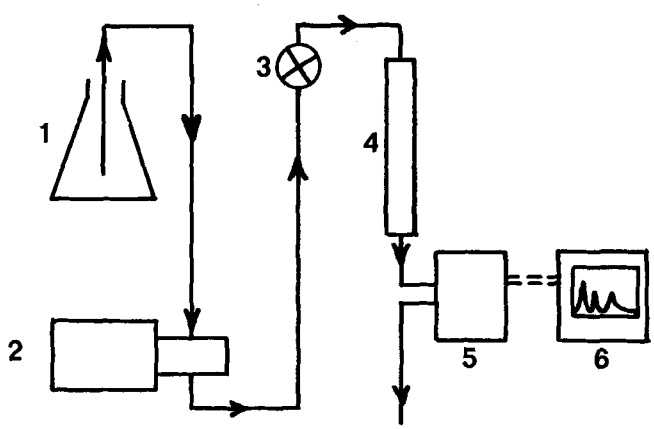

FIG. 2. Basic schematics of a liquid chromatographic system: (1) solvent reservoir; (2) pump; (3) sample introduction valve; (4) chromatographic column; (5) detector; (6) recorder.

many years. Analytical accomplishments using GC are numerous and well documented, yet GC is quite limited in application. It is useful only in separating compounds which are volatile and heat stable. Its appropriate counterpart is liquid chromatography (LC), which is only limited by the solubility of the compound mixture to be separated. Recently, the application of LC in the field of fats and oils analysis has significantly increased. Its importance may soon approach, if not surpass, that of GC for the soybean oil processing industry.

The renaissance of LC is mainly a result of the precision applied in the construction of the necessary hardware and the development of vastly improved, modified column packings. The basic schematic of an LC system is given in Figure 2. It consists of solvent reservoir, a solvent transfer pump, sample introduction valve, chromatographic column and detector. The reservoir is a simple device. The pump of choice is capable of operating at pressures of up to 6,000 psi while maintaining flow rates ranging from 0.1 to 10.0 ml/min. The columns are made of precision-bored stainless steel. A description of column packings is in the discussion of actual LC applications. The detector is closely connected to the effluent side of the column. Its heart is either a

minimum volume, flow-through cell (0.01-0.02 ml) in the case of spectrophotometric or refractometric detection, or a moving wire, in the flame ionization detection. As this list indicates, there are a variety of detectors available. The selection of the detector for chromatography depends on the solvent used and substances to be detected. For example, if the substances possess some specific optical absorption properties, spectrophotometric detection is the method of choice, but if nonvolatile organic compounds in a volatile solvent are to be measured, a moving-wire FID detector might be more appropriate. The most universally applicable detector is based on refractive index measurement. Finally, an auxiliary device is used to record the progress of chromatography and estimate the distribution of the separated components in a fashion analogous to GC. There is a great variety of equipment available to do this task, from simple graphic recorders with subsequent triangulation to sophisticated computers.

Liquid chromatography encompasses four distinctly different modes of separation: liquid-liquid, or partition chromatography; liquid-solid, or adsorption chromatography; exclusion, or gel permeation chromatography (GPC); and ion-exchange chromatography. The first three modes are very useful to the quality control laboratory of a soybean oil processing facility. Ion exchange chromatography has, at the moment, very little applicability. Each of the three important chromatographic methods will be discussed below with at least one application example.

PARTITION CHROMATOGRAPHY

Partition liquid chromatography is similar to GC. The essential parts of partition LC are, of course, the partitioning phase, which is fixed on a solid support and the mobile phase. For each solute, an equilibrium distribution develops between the stationary and mobile phases. Separation occurs because of different distributions of the various solutes in the sample between these phases.

Octadecylsilane is one of the many stationary phases which can be bonded to solid supports. This phase was selected by Plattner et al. (6) for the separation of triglycerides. The partition occurred between the bonded liquid phase and acetonitrile. Using this LC technique, a typical triglyceride distribution is obtained for soybean oil (Fig. 3). Triglycerides separate according to their polarity, the more polar compounds eluting first. For saturated triglycerides, the smaller the numbers of carbons in the three fatty acid chains ("carbon number"), the shorter the retention times. Unsaturated triglycerides elute more rapidly than expected on the basis of carbon numbers. For example, the carbon number of tripalmitin is $3 \times 16 = 48$ and for tristearin it is 54, but triolein has a carbon number of $(3 \times 18) - (3 \times 2) = 48$, which is equivalent to that of tripalmitin. Indeed, triolein coelutes with tripalmitin in a triglyceride separation experiment. There is a linear relationship between log retention volume and carbon number (Fig. 4). This relationship has great similarity to log retention time vs chain length of a homologous series of aliphatic compounds that exists with GC.

In Figure 5, a comparison is made between the triglyceride distributions of linseed, soybean and safflower oils. These chromatograms indicate that each oil has a characteristic distribution of triglycerides. With time, characterization of oils by LC may supplement or replace GC methods. The procedure for obtaining an LC chromatogram is faster and simpler than that for a GC chromatogram of fatty acids. For LC, there is less sample preparation.

ADSORPTION CHROMATOGRAPHY

Adsorption chromatography has been in use much longer

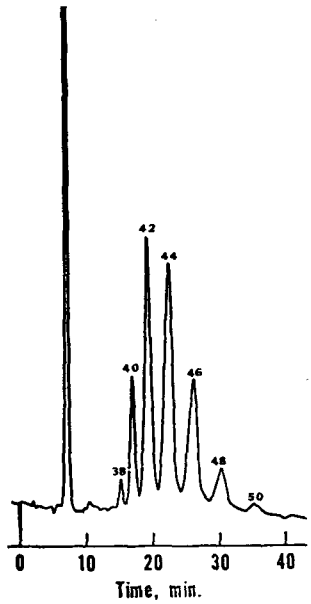

FIG. 3. Triglycerides distribution of soybean oil. Peaks are from carbon number 38 through carbon number 50 (6).

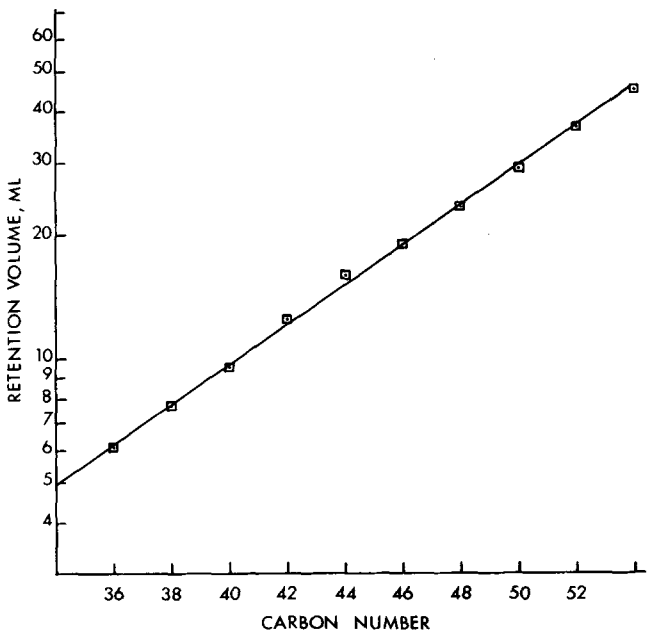

FIG. 4. A retention volume vs carbon number curve for triglycerides having carbon numbers 36-52.

than any of the other three types. For decades, column chromatography on silica or alumina columns was commonplace. Modern adsorption chromatography uses the same adsorbents and is based on the same theoretical principles as the "old" applications. The crucial differences, however, between older and modern adsorption LC are the changes in hardware and the quality of adsorbents. While "old" silica was irregular in shape and size, present-day LC silica is much smaller and uniform in size, spherical in shape and has a controlled adsorption surface. These properties make modern silica more efficient and predictable in its ability to separate complex samples. Furthermore, several modified surface silicas are on the market to serve special purposes.

The combined effects of precision hardware and highly developed adsorbents result in LC separations which were

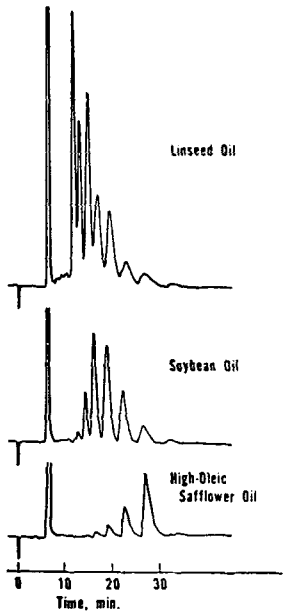

FIG. 5. Comparison of triglyceride composition of linseed, soybean and safflower oils (6).

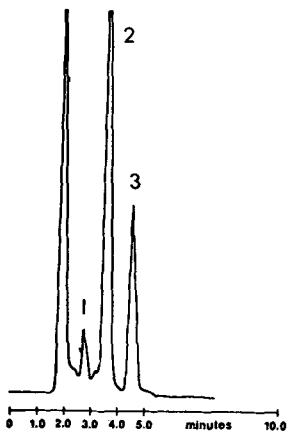

FIG. 6. HPLC separation of soybean oil tocopherols. (1) α-tocopherol; (2) γ-tocopherol; (c) δ-tocopherol. Chromatographic condition: 30 X 0.4 cm μ-Porasil column, solvent: 1.5% 2-propanol in hexane. Detector: spectrophotometer @ 295 nm.

formerly impossible. For instance, the separation of the tocopherols from vegetable oils has been reported by Carpenter (7), who found that separation was made without any sample preparation by using a 30 cm long, 0.4 cm id Porasil (a highly refined silica) column. The solvent was 1.5% isopropyl alcohol in hexane and a spectrophotometer was used for detection at 295 nm.

Quantitation was achieved by integration and use of a standard curve. A typical chromatogram (Fig. 6) shows ca. 40 ppm of α-, 700 ppm of γ-, and 300 ppm δ-tocopherol. The completion of this chromatogram takes less than 10 min, which is less time than that required to perform the nonspecific, colorimetric determination of total tocopherols. A similar determination by GC (through unsaponifiable analysis) may take as long as 2 hr.

GEL PERMEATION CHROMATOGRAPHY (GPC)

No other chromatographic technique is as suitable as GPC for the separation of mixtures of polymeric or high molecular weight compounds. Separation of a mixture on a GPC

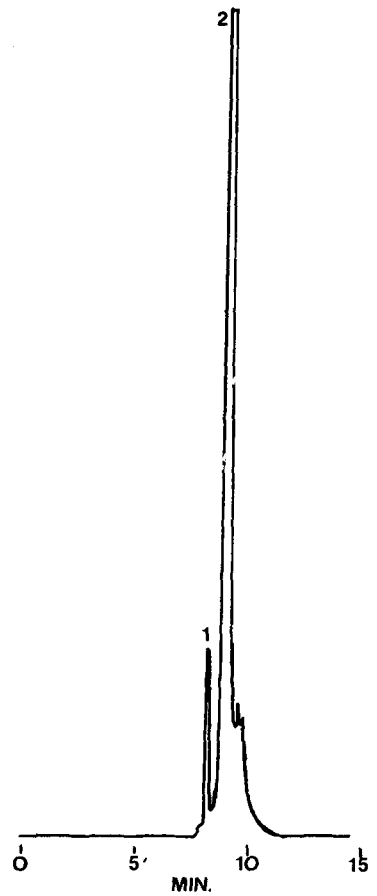

FIG. 7. Gel permeation chromatographic separation of polymers and triglycerides of soybean oil: (1) polymers; (2) triglycerides. Chromatographic condition: 25 X 0.62 cm Zorbax PSM-60S column; solvent: THF; flow rate: 1 ml/min; detector: spectrophotometer @ 233 nm.

column depends on molecular size, which is related to molecular weight (MW). The method of separation by GPC is based on the retention of solutes by the gel by means of incomplete penetration. Separation occurs as each solute is excluded from different fractions of the gel volume by steric effects. The penetration into the gel is smaller with increased solute size, which results in an earlier appearance in the effluent. Above a certain solute size no penetration occurs. This size is called the exclusion limit. The molecules which are completely excluded from the gel are washed down the column between the gel particles. These molecules elute from the column with the void volume.

GPC is useful in determining the extent of oil polymerization caused by heating (high temperature processing or frying) or oxidation. Polymer notation here includes all the compounds in vegetable oil of MW greater than that of triglycerides. Figure 7 illustrates a MW based separation of a deodorized soybean oil. Almost baseline separation was obtained between polymers (peak 1) and triglycerides (peak 2), even though the ratio between the two is 1:200. The shoulder peaks may be due to the presence of trace amounts of diglycerides. Ferren and Seery used this method for measuring dimerization of fatty acids in corn oil (8).

COLOR MEASUREMENT

With the advent of a new breed of colorimeters based on tristimulus color measurement, a definite drive has developed to replace the Lovibond color measurement (8). By

this proposed move, the subjective nature of color measurement will be eliminated, resulting in more reliable color detecting and resultant color data. Furthermore, the use of tristimulus colorimeters affords color data similar to that perceived by the human eye. The human eye has varied sensitivity to different wavelengths of visible light and sees all visible wavelengths of light (380-740 nm) at once, whether reflected or transmitted from a surface. Thus, the human eye integrates all wavelengths of light.

At the present, several manufacturers, such as Hunter Laboratory, IBM, Gardner Laboratories and Macbeth Corp., make excellent-quality tristimulus colorimeters. R.S. Hunter has proposed a Yellowness Index for vegetable oil colors. The simple conversion to Yellowness Index from Lovibond color (in case of 10/1 ratio of yellow to red) is shown in Figure 8.

IN-PROCESS INSTRUMENTATION

In-process instruments are installed at a specific site of processing, provide instantaneous data on a given quality attribute of that processing stream and perform unattended after initial calibration. Use of such instruments is beneficial in attaining uniform products and leads to increased automation.

There are two major installation arrangements for in-line instruments. One is the direct on-stream installation where the instrument sensor is imbedded in the process line and connects to the remote instrument. The other is the so-called loop arrangement, where the sensor is installed parallel to the process vessel or flow, and a small but representative portion of the oil is diverted to the loop. The direct installation is used when an on-stream sensor is attached to a remote instrument, e.g., an electrode wired to a pH meter. The loop is chosen when the conditions of the process stream are harmful to the sensor because of high temperature, pressure or flow. The parallel stream allows conditions to be changed to accommodate the needs of the sensor. Most in-process instruments are based on one of three operational principles, i.e., optics, electrochemistry or chromatography. Probably the most widely used are the first two because they are best suited for rapid, flow-through use.

The control of a hypothetical soybean oil hydrogenation plant will be used as an example of in-process instrumentation. Four existing process instruments are applicable in this particular situation. Three of them can monitor chemical changes, such as double-bond saturation and isomerization. The fourth instrument monitors the filtration efficiency after hydrogenation.

A continuously recording refractometer is one of the most appropriate means of monitoring hydrogenation. It follows the change of refractive index of an oil as hydrogenation proceeds. Hydrogenation of a soybean oil in a pilot plant has been continuously monitored by a refractometer while the IV of the oil was reduced from 132 to 100 (Fig. 9). The refractometer recorded a change of refractive index with time very similar to the change of IV.

A process control GC also can be installed. This allows the change of fatty acid composition with time during hydrogenation to be monitored. However, because of the nature of GC, the monitoring must be discontinuous in time and sequential. It cannot provide continuous and instantaneous data. Although process control GC can serve well in a vegetable oil processing plant, there is no information that such an installation exists. It is found, however, in chemical process and petroleum industries where it is used with great success.

The quantity of *trans* isomers in a hydrogenated soybean

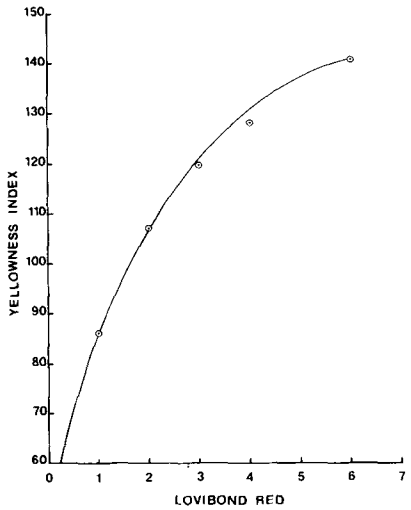

FIG. 8. Lovibond color conversion to Yellowness Index.

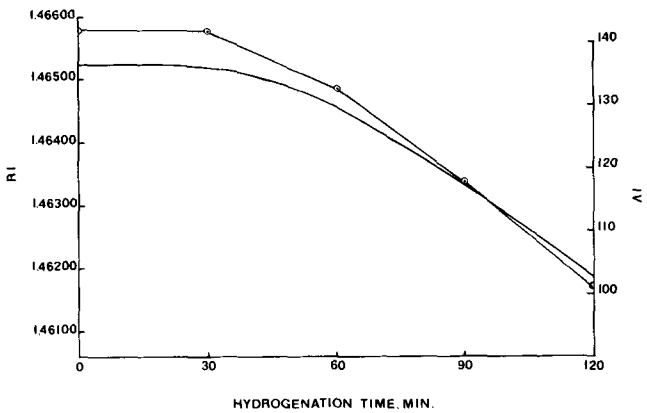

FIG. 9. Comparison of RI (—) and IV (-o-o-) measurements as monitors of hydrogenation. RI was determined continuously whereas IV was measured discontinuously.

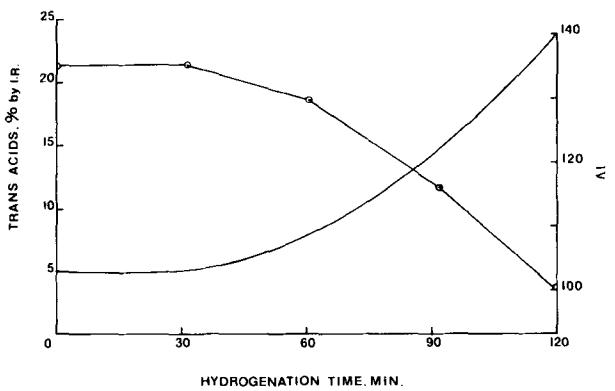

FIG. 10. Comparison of IR (—) and IV (-o-o-) measurements as monitors of hydrogenation. IR was determined continuously whereas IV was measured discontinuously.

oil or fat is a major concern of vegetable oil processors because *trans* isomers influence the melting and crystalline properties of the product and may affect its nutritional quality. Therefore, monitoring the formation of such isomers during hydrogenation is very beneficial. *Trans* isomers absorb infrared radiation around 10.3 μ. An on-line infrared spectrophotometer tuned to this wavelength can monitor the concentration of *trans* isomers as they are

formed in the oil. This spectrophotometer also can be used for indirect monitoring of hydrogenation. Figure 10 shows the application of this instrument for the previously mentioned pilot plant hydrogenation. Apparently, the progression of *trans* isomer formation is inversely related to the decrease of IV. Because the rate of *trans* isomer formation is greatly dependent on e.g., the catalyst and temperature used during hydrogenation, each set of hydrogenation conditions requires a special set of monitoring parameters.

Monitoring the clarity of a filtrate after hydrogenation or bleaching is also of interest to the oil processing industry. Another optically based process control instrument is on the market which can measure suspended particles in the process stream by means of nephelometry. Nephelometry measures the scattered light intensity at a right angle to the path of illumination caused by suspended particles. This instrument may be useful in monitoring filtration efficacy.

ACKNOWLEDGMENTS

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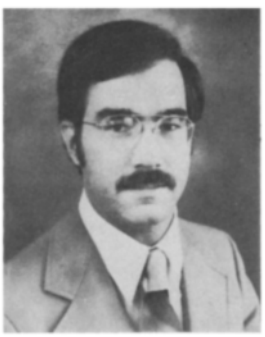

Analysis of Processed Soy Oil by Gas Chromatography

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ABSTRACT

Two basic gas chromatographic approaches are currently used for the analysis and characterization of processed soy oil and other lipid materials. One approach separates the intact glycerides after silylation whereas the complementary approach determines the fatty acid composition of the glycerides by the formation and subsequent separation of the corresponding fatty acid methyl esters. A brief history of the development of these gas chromatographic procedures and a more detailed discussion of current packed and capillary column technology used for the separation of both intact glycerides and fatty acid methyl esters is presented. Emphasis is placed on the advantages of the newly emerging capillary techniques over the more conventional packed column separations. High resolution chromatograms are shown for the separation of mixed glyceride isomers and also for the separation of unsaturated methyl esters with respect to the number, geometry and position of the double bonds. The separation of the methyl esters can provide information equivalent to classical *cis,cis*-lipoxygenase, iodine monochloride titration and infrared *trans* analyses. Finally, the use of short capillary columns to achieve rapid low cost separations (with resolution equivalent to packed column separations), ideal for quality control, also are discussed.

INTRODUCTION

Gas chromatography (GC) has become well established as a powerful tool in lipid research. The high resolving power, speed of analyses, sensitivity and adaptability to automation make GC a highly desirable technique where it is applicable. Two basic gas chromatographic approaches are currently used for the analysis and characterization of processed soy oil. One approach separates the intact glycerides after silylation to provide what will be defined as a carbon number profile (CNP) whereas the complementary approach determines the fatty acid composition (FAC) of the glycerides by the formation and subsequent separation of the corresponding methyl esters.

The fatty acid composition method, the older and more commonly used of these two methods, involves the transesterification of the triglycerides with sodium methoxide. The resulting methyl esters are suitable for chromatography. The quantitative data sometimes can be used to identify the source oil, provided that it has not been altered by hydrogenation or other processing beyond refining, bleaching and deodorization. The method is now well established and accepted in the lipid industry.

The separation of intact triglycerides by GC has been viewed as a valuable complement to GC-FAC. This has been recognized for quite some time, as investigations were started in 1959 (1). Since that time, Litchfield (2) and Kuksis (3) have worked independently to establish the quantitative aspects of triglyceride analysis by GC. More recently, Monseigny et al. (4) have used capillary column GC for the quantitation of triglycerides; however, the columns used in their work are not commercially available. The analysis of triglycerides is more difficult than the analysis of the fatty acid methyl esters, generally because of their higher boiling points. Because of this difficulty, there has been a significant time lag in the development of the triglyceride method. The advances in equipment and the state-of-the-art now make this method routine. The separation of intact lipid samples provides a characteristic profile or distribution of glycerides which often can be used to identify a specific fat or mixture of fats. The method aids in interpreting data where ambiguities exist in FAC. It is especially valuable in that the results are not affected