J Forensic Sci, Nov. 2003, Vol. 48, No. 6
Paper ID JFS2003122_486
Available online at: www.astm.org

TECHNICAL NOTE

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Analysis and Classification of Common Vegetable Oils*

ABSTRACT: The analysis of fatty acids from common vegetable oils was investigated for application to forensic casework. A base-catalyzed transesterification of the fatty acids to fatty acid methyl esters using tetramethylammonium hydroxide was simple, rapid, straightforward and inexpensive. Canola, corn, olive, peanut, safflower, soybean and sunflower oils were able to be classified based on their fatty acid methyl ester profiles. Using gas chromatography-mass spectrometry, the detection limits for canola, corn, olive, peanut and safflower oils were determined to be 0.4 mg/mL or less and 0.2 mg/mL or less for soybean and sunflower oils.

KEYWORDS: forensic science, fatty acids, fatty acid methyl esters, gas chromatography-mass spectrometry, transesterification, vegetable oils, classification of vegetable oils

Vegetable oils are very common in today's marketplace (1). They have replaced animal and petroleum products in a variety of applications. They are most commonly used as cooking oils, but are also found in a wide variety of foods, as well as in cosmetics and soaps. The ubiquitous nature of vegetable oils often results in their implication at crime scenes (2–5).

Vegetable oils are predominantly (95 to 98%) composed of triacylglycerols (i.e., triglycerides) with free long-chain carboxylic acids, mono- and diacylglycerols and other minor compounds (2 to 5%) also present (6). Triacylglycerols are esters derived from the reaction of a long-chain carboxylic acid with each of the alcohol functional groups of glycerol. The long-chain carboxylic acids are more commonly known as fatty acids. Biosynthesis of the fatty acids from smaller building blocks by site-specific enzymes produces characteristic carbon chain lengths, double bond positions and stereochemistry (7). The fatty acids generally have even-numbered carbon chain lengths and the introduction of a double bond into the structure normally occurs at carbon 9. If two or more double bonds are present in the fatty acid, they are generally isolated from each other by two carbon-carbon single bonds (e.g., 9,12-octadecadienoic acid). As well, the double bonds in unsaturated fatty acids are almost invariably *cis*- with trace amounts of the *trans*-isomers present naturally. More significant levels of *trans*-fatty acids can result from partial hydrogenation of an oil during the refining process (8).

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* Paper presented orally at the 2001 Canadian Society of Forensic Science meeting in Toronto, Ontario, Canada.
Received 5 April 2003; and in revised form 17 June 2003; accepted 18 June 2003; published 27 Aug. 2003.

The forensic significance of vegetable oils arises from a wide variance in their chemical compositions. Although the fatty acids are restricted to certain chain lengths, specific double bond positions and stereochemistry, there are still different amounts of ten to fifteen fatty acids present in vegetable oils. Small amounts of the less common fatty acids may be present in some oils but, in general, 90 to 99% of the fatty acid composition of most common vegetable oils is comprised of hexadecanoic, octadecanoic, 9-octadecenoic, 9,12-octadecadienoic and 9,12,15-octadecatrienoic acids.

This study will investigate a technique in which fatty acids from common vegetable oils are efficiently converted to their fatty acid methyl esters (FAMEs), separated by gas chromatography and analyzed by mass spectrometry. Classification of the vegetable oils, based on the distribution of the five main fatty acid methyl esters, will also be studied. Vegetable oils were targeted for investigation due to their purity, simplicity and the availability of information for these commercial products.

Experimental

Reagents

All of the reagents were of analytical reagent grade. Diethyl ether, tetramethylammonium hydroxide (TMAH), thymol blue (Alfa Aesar, Ward Hill, MA), hydrochloric acid (Anachemia Science, Montreal, Quebec, Canada) and methanol (Caledon Laboratories, Ltd., Georgetown, Ontario, Canada) were used in the analyses.

Vegetable Oils

The following commercial brand name vegetable oils were analyzed: Canola Harvest, Crisco, President's Choice, West (undetermined age) and Western Family canola oils, Rimini (undetermined age) canola and/or corn and/or soybean and/or sunflower oil, Mazola and Mazola (undetermined age) corn oils, Petrelli olive oil,

Planters peanut oil, President's Choice and Tosca safflower oils, Crisco soybean oil, Crisco canola and soybean oil blend and Canbra and Safflo (undetermined age) sunflower oils.

Twelve of the oils were purchased, at the time of this study, from local grocery stores while the date of purchase for four of the oils was unknown (i.e., of undetermined age). To the best of the authors' knowledge, the samples of undetermined age were purchased at least seven years prior to this study and were stored in capped bottles in a dark cabinet.

Sample Preparation (9)

Between 50 and 100 mg of vegetable oil and 3 mL of diethyl ether were mixed in a 12-mL screw cap vial. After the addition of 0.2 mL of 25% (w/w) TMAH in methanol, the solution was shaken for 1 min and the layers were allowed to separate for 2 min. The addition of one drop of 0.1% (w/v) thymol blue indicator in methanol was followed by a sufficient volume of 0.2 N hydrochloric acid in methanol to just change the indicator from blue to yellow (pH 7.5 to 8.0). A small amount of methanol (0.5 mL) was added and the solution was shaken to ensure a single phase resulted. An aliquot of the solution (0.1 μL) was taken for analysis by gas chromatography-mass spectrometry. Duplicate samples of each vegetable oil were prepared and analyzed.

Instrumentation

An HP Series 6890 gas chromatograph, an HP 5973 Mass Selective Detector, an HP G1513A autosampler and an HP G1701BA, Rev. B.01.00 software package (Hewlett-Packard Company, Palo Alto, CA) were utilized for the analyses. The gas chromatograph was equipped with a DB-Wax (J&W Scientific Inc., Folsom, CA) fused-silica capillary column (30 m X 0.32-mm inner diameter with an immobilized polyethylene glycol film thickness of 0.50 μm). The oven temperature was initially held at 100°C for 2 min, increased from 100 to 220°C at a ramp rate of 20°C/min and then held at 220°C for 12 min. The overall temperature program was 20 min in length. The injector temperature was 300°C with a 50:1 split ratio. Hydrogen at a constant flow rate of 1.1 mL/min was the carrier gas. Transfer line, quadrupole and source temperatures were 300°C, 106°C, and 230°C respectively. The mass spectrometer was operated in the electron impact mode at an ionization energy of 69.9 eV and scanned from m/z 40 to 400 at 3.99 scans/sec. The detector temperature was 300°C.

Detection Limits

A sample of Mazola corn oil, Petrelli olive oil or Canbra sunflower oil in the 10-mg range was prepared as outlined above but was brought to a final volume of 10 mL in a volumetric flask by the addition of methanol. An aliquot of the solution (0.1 μL) was taken for analysis by gas chromatography-mass spectrometry. Duplicate samples of each vegetable oil were prepared and analyzed.

Results and Discussion

Using TMAH, in a simple base-catalyzed transesterification reaction, for the preparation of fatty acid methyl esters from vegetable oil samples offered a number of advantages over the more traditional sample preparation procedures. An extra step in the sample preparation procedure was eliminated by combining the cleavage of the acylglycerols with the saponification and esterification reactions. An extraction step, which could be troublesome in obtaining a representative sample, was also avoided since a single phase resulted for final analysis. The reagents, including TMAH, were inexpensive. Little or no equipment besides glassware was necessary for sample preparation and there was no heating or centrifuging required. As a result, a sample could be fully prepared for analysis in as little as 5 min. If necessary, the procedure would also permit the determination of acylglycerols separate from the free fatty acids. Furthermore, the procedure was also reported to be suitable for samples (e.g., butters and certain soaps) containing a broad range of fatty acids (9).

Several parameters were evaluated in order to optimize the resolution, sensitivity and analysis time for the vegetable oil samples. In the analysis scheme, TMAH salts (formed from the free fatty acids) were pyrolyzed in the injector and re-arranged to methyl esters. As reported by Robb and Westbrook (10), the yield of methyl esters from TMAH salts increased rapidly between injector temperatures of 220 and 370°C. Investigation of this effect was studied by raising the injector temperature in steps, from 250 to 350°C. Upon increasing the injector temperature from 250 to 300°C, a corresponding increase in the signal intensity was noted. However, when the temperature was further increased to 350°C, the signal intensity decreased with small peaks appearing in the lower carbon chain length number region of the chromatogram. As a result of this experiment, it was determined that 300°C was the preferred choice for the injector temperature since it would maximize the signal intensity while maintaining the integrity of the sample. Initial column temperatures between 50 and 100°C and ramp rates ranging from 4 to 25°C/min were investigated. An initial temperature of 100°C and a ramp rate of 20°C/min did not significantly affect the chromatographic analysis but minimized, to a large extent, the analysis time. A final temperature of 220°C, a limitation imposed by the polyethylene glycol stationary phase, was also incorporated into the optimal parameter set. Constant carrier gas flow rates of 1.1 mL/min, 1.6 mL/min and 2.1 mL/min were investigated. An optimum signal intensity resulted from a flow rate of 1.1 mL/min. Due to better peak shapes being obtained, a scan rate of 3.99 scans/sec was chosen rather than a faster scan rate. Using the optimal parameter set, blanks analyzed subsequent to vegetable oil samples demonstrated no carryover effect.

The fatty acid methyl ester profiles for the brand name vegetable oils in this study appear in Table 1. Based on the distribution of the five main fatty acid methyl esters–methyl hexadecanoate (C16:0), methyl octadecanoate (C18:0), methyl 9-octadecenoate (C18:1), methyl 9,12-octadecadienoate (C18:2) and methyl 9,12,15-octadecatrienoate (C18:3)—classification schemes were developed for these oils. Four questions formed the framework for the classification schemes outlined in Figs. 1 and 2. First, the dominant fatty acid methyl ester was identified. Common vegetable oils had fatty acid methyl ester compositions dominated by either C18:1 or C18:2. The classification was narrowed further by looking for the presence of C18:3. Only canola oil, soybean oil and vegetable oil blends, containing canola and/or soybean oils, had C18:3 peak areas greater than one percent of the total fatty acid methyl esters detected. The C18:1 and C18:2 peak area ratio and the percentage of the total fatty acid methyl esters that were saturated were also useful for the classification of an oil. If these questions were applied in succession, it was possible to classify each type of vegetable oil in this study. For the Rimini vegetable oil, the only available information indicated the oil contained canola and/or corn and/or soybean and/or sunflower oil. Utilizing the classification schemes listed above, the observed fatty acid methyl ester profile was found to correspond to a canola oil-based product.

Total ion chromatograms representative of the chromatographic resolution and sensitivity attained for the various vegetable oils in this study appear in Fig. 3. Two peaks in the President's Choice canola oil profile, adjacent to the C18:3 peak and possibly geometric and/or positional isomers of C18:3, had intensities greater than in any of the other oils. Examination of Table 1 revealed further

TABLE 1—*Average FAME composition, reported as a percentage of the total FAMEs detected, and [percent relative standard deviation] for various brand name vegetable oils.*

FAMEs	Canola Oil Canola Harvest	Canola Oil Crisco	Canola Oil President's Choice	Canola Oil West Undetermined Age	Canola Oil Western Family	Vegetable Oil Rimini Undetermined Age	Corn Oil Mazola	Corn Oil Mazola Undetermined Age
C16:0	4.2 [2.5]	4.0 [0.9]	5.6 [2.8]	3.4 [0.8]	4.2 [0.8]	3.6 [1.2]	11.3 [0.8]	11.3 [0.9]
C16:1		0.2 [31.4]	0.2 [3.3]		0.2 [30.0]	0.2 [28.3]		
C18:0	1.8 [2.0]	1.7 [2.0]	1.6 [0.9]	1.5 [1.4]	1.7 [3.7]	1.4 [1.5]	1.5 [2.8]	1.6 [5.2]
C18:1	68.5 [0.5]	66.5 [0.7]	67.0 [0.7]	66.8 [0.2]	66.7 [0.2]	65.9 [0.2]	28.9 [0.4]	29.3 [0.3]
C18:2	18.6 [0.3]	18.7 [1.0]	20.2 [1.3]	19.6 [0.2]	19.2 [0.5]	20.0 [1.5]	57.5 [0.2]	56.8 [0.6]
C18:3	5.6 [1.6]	7.5 [5.6]	4.0 [2.6]	7.3 [0.7]	6.6 [2.2]	7.4 [1.0]	0.5 [13.3]	0.5 [2.6]
C20:0	0.4 [31.8]	0.4 [13.5]	0.5 [2.9]	0.4 [13.8]	0.4 [5.4]	0.3 [2.1]	0.3 [33.7]	0.3 [22.3]
C20:1	0.9 [2.2]	1.0 [2.9]	0.9 [7.0]	1.0 [15.7]	0.9 [5.3]	1.1 [15.0]		0.2 [3.6]

FAMEs	Canola Oil Canola Harvest	Canola Oil Crisco	Canola Oil President's Choice	Canola Oil West Undetermined Age	Canola Oil Western Family	Vegetable Oil Rimini Undetermined Age	Corn Oil Mazola	Corn Oil Mazola Undetermined Age
C16:0	13.1 [1.0]	11.0 [2.2]	4.6 [0.3]	6.9 [4.2]	11.4 [0.2]	6.8 [0.1]	6.1 [0.2]	6.1 [1.2]
C16:1	0.9 [0.8]							
C18:0	2.1 [4.3]	2.4 [1.8]	1.9 [0.0]	2.4 [2.4]	4.9 [0.4]	2.4 [1.5]	4.4 [2.8]	4.5 [0.0]
C18:1	76.3 [0.0]	54.0 [0.5]	78.7 [0.2]	15.9 [2.2]	24.9 [0.3]	51.6 [0.2]	17.2 [2.2]	14.0 [1.1]
C18:2	6.6 [4.2]	27.6 [0.4]	14.2 [0.6]	74.7 [0.8]	53.7 [0.3]	31.8 [0.3]	71.6 [0.3]	74.8 [0.3]
C18:3	0.4 [1.7]	0.2 [62.8]			4.8 [2.9]	6.5 [0.3]	0.2 [3.3]	0.2 [81.4]
C20:0	0.3 [33.0]	1.1 [0.7]	0.3 [6.7]	0.2 [55.7]	0.2 [6.7]	0.3 [20.9]	0.2 [32.6]	0.1 [84.8]
C20:1	0.3 [33.9]	0.9 [0.8]	0.2 [19.3]			0.6 [2.6]		
C22:0		2.3 [5.6]			0.1 [90.0]		0.3 [72.0]	0.4 [29.9]
C24:0		0.6 [3.4]						

characteristics useful as points of comparison among the vegetable oils. Greater amounts of the fatty acid methyl ester, tentatively identified as methyl 11-eicosenoate (C20:1), were detected in canola and peanut oils. Olive oil contained quantities of methyl 9-hexadecenoate (C16:1) and peanut oil contained quantities of methyl eicosanoate (C20:0), methyl docosanoate (C22:0) and methyl tetracosanoate (C24:0) exceeding those of the other oils. The President's Choice safflower oil was a high C18:1 safflower oil as opposed to the high C18:2 Tosca safflower oil. In direct contrast to all of the other vegetable oils, no C18:3 was detected for either of the safflower oils. As expected, the fatty acid methyl ester profile for the Crisco canola and soybean oil blend was intermediate between the Crisco canola oil and Crisco soybean oil profiles. For the vegetable oils of undetermined age, no olfactory evidence of rancidity was noted. In addition, there was close agreement between the fatty acid methyl ester profiles for the vegetable oils of undetermined age and the corresponding vegetable oils of the same classification purchased at the time of this study. Finally, the observed fatty acid methyl ester profiles for each oil were in close agreement with both the nutritional information, listed on the labels of the containers, and literature values (7,11–13).

The detection limit of an analytical technique is very important to determine its utility and to evaluate its performance for an application. To determine detection limits, the vegetable oils were subdivided into two different groups, soybean/sunflower and the rest of the oils, based on the amount of C18:0 present. Attention was focused on C18:0 because it was the first of the five main fatty acid methyl esters to disappear upon dilution of the vegetable oil samples. Its disappearance was important because the presence of the five main fatty acid methyl esters was considered essential for the classification schemes developed in this investigation. Soybean and sunflower oils were treated separately because they had a higher amount of C18:0 than the other oils. Corn, olive and sunflower oils were chosen as representative oils. The detection limit was defined, according to the International Union of Pure and Applied Chemistry approach (14), as the C18:0 concentration giving a chromatographic signal intensity equal to the average background signal intensity plus three standard deviations of the back-

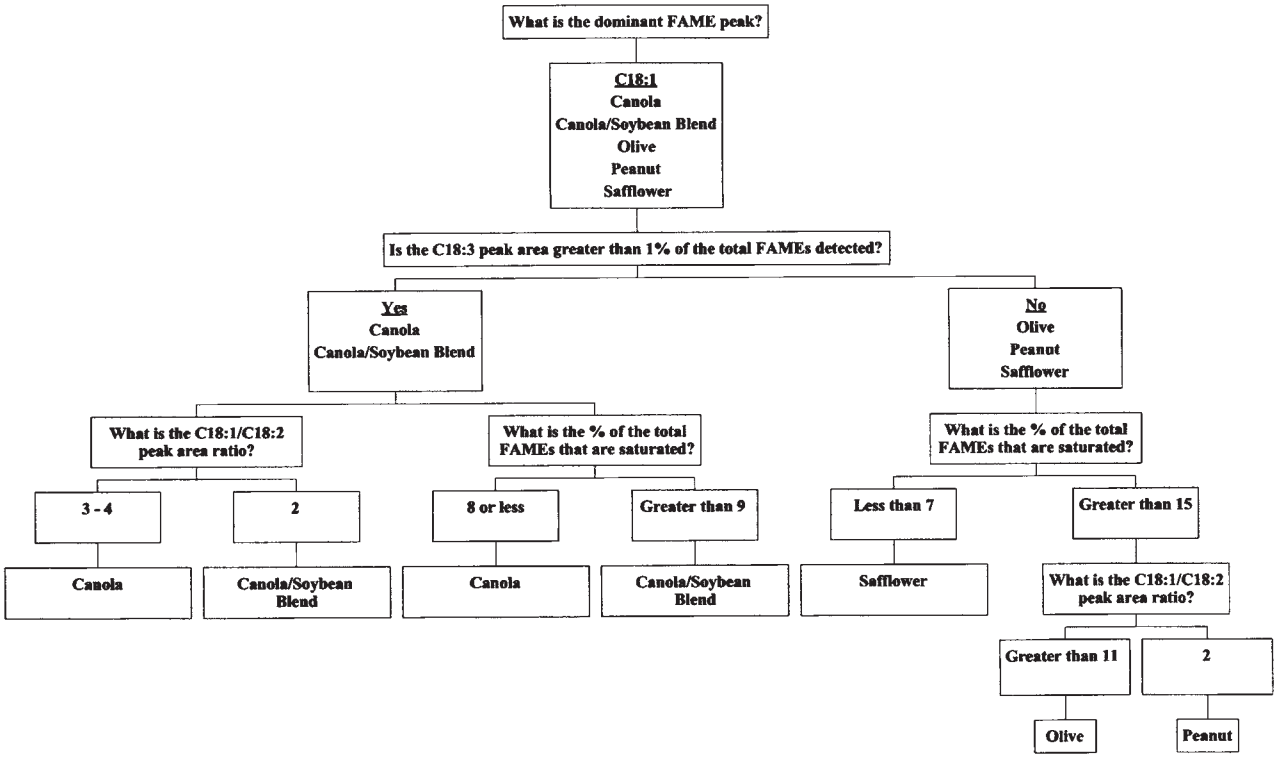

FIG. 1—*Classification of C18:1 dominated vegetable oils using FAMEs.*

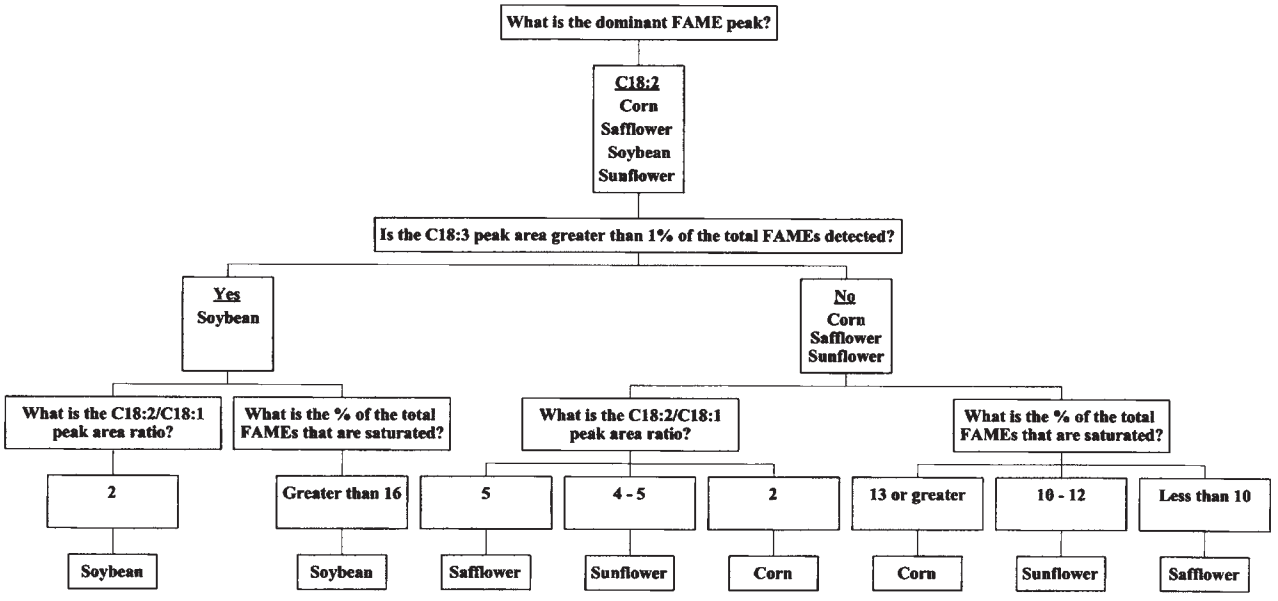

FIG. 2—*Classification of C18:2 dominated vegetable oils using FAMEs.*

ground signal intensity. The average and standard deviation of the background signal intensity were determined by taking 48 data points from a 0.20-min region between the glycerol (7.94 min) and C18:0 (8.42 min) peaks. The detection limits for Mazola corn oil, Petrelli olive oil and Canbra sunflower oil, expressed as the concentration of the oil in solution before an aliquot was taken for analysis by gas chromatography-mass spectrometry, were determined to be 0.4 mg/mL. 0.3 mg/mL and 0.2 mg/mL respectively.

Conclusions

For this investigation, the preparation of fatty acid methyl esters from common vegetable oils was simple, rapid and straightforward. The reagents required for the analyses were inexpensive while the equipment and instrumentation were readily available. Using a DB-Wax column, separation of the fatty acid methyl esters proceeded according to carbon chain length number and the degree

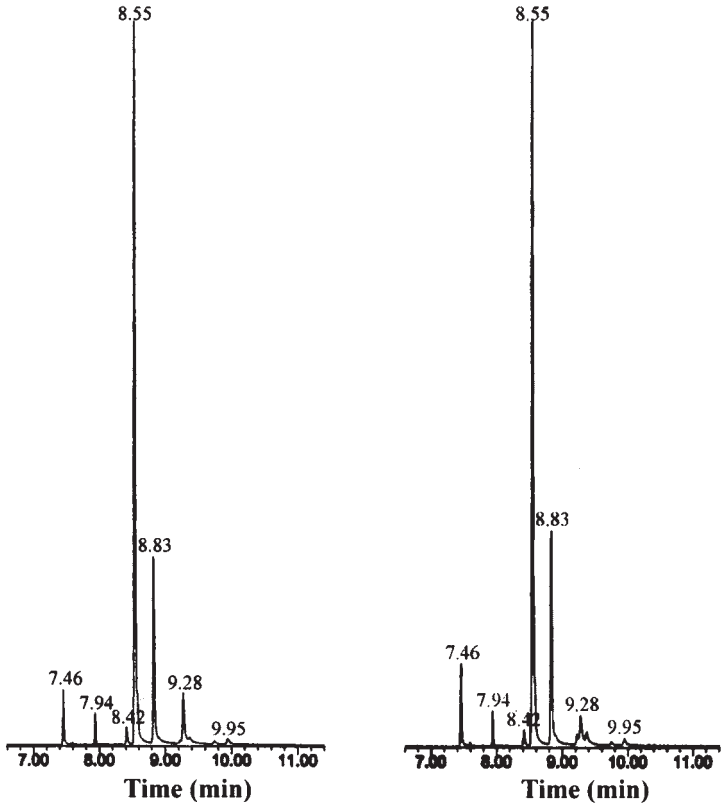

FIG. 3—*Total ion chromatograms of Canola Harvest (left) and President's Choice (right) canola oils. Peaks appearing in the chromatograms are C16:0 (7.46 min), glycerol (7.94 min), C18:0 (8.42 min), C18:1 (8.55 min), C18:2 (8.83 min), C18:3 (9.28 min), C20:0 (9.75 min) and a FAME tentatively identified as C20:1 (9.95 min).*

of unsaturation. Overall, the analytical technique was sensitive and it permitted the classification and, to a limited extent, discrimination of various types of vegetable oils.

Further studies are required to determine the utility of the analytical technique for other areas of forensic casework. The incomplete combustion products of vegetable oils should be examined. The chromatographic profiles of rancid oils may also be of future interest. In addition, it is unknown at this time whether additional components found in foods (e.g., butters, margarines and salad dressings), cosmetics (e.g., lipsticks, powders, creams and lotions) and soaps may complicate the sample preparation and analysis procedures. There is certainly more work to be done but it is clear that the technique is promising for forensic casework involving vegetable oil-based products.

Acknowledgments

The authors thank the following individuals: Dr. Gary R. Stringam of the Department of Agricultural, Food and Nutritional Science at the University of Alberta, Edmonton, Alberta, Canada for providing some of the references appearing in this manuscript and Dr. Margaret-Anne Vaughan for helpful suggestions in editing the manuscript.

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J Forensic Sci, Sept. 2004, Vol. 49, No. 5
Paper ID JFS12446
Available online at: www.astm.org

Erratum

Erratum/Correction of Pitts SJ and Thomson CI. Analysis and Classification of Common Vegetable Oils. J Forensic Sci 2003 Nov;48(6):1293–1297.

It has come to the attention of the Journal that the second set of headings for Table 1 was misprinted. Below is the correct listing for the second set of headings:

FAMEs	Olive Oil Petrelli	Peanut Oil Planters	Safflower Oil President's Choice	Safflower Oil Tosca	Soybean Oil Crisco	Canola/Soybean Oil Blend Crisco	Sunflower Oil Canbra	Sunflower Oil Safflo Undetermined Age

The Journal regrets this error. Note: Any and all future citations of the above-referenced paper should read: Pitts SJ and Thomson CI. Analysis and Classification of Common Vegetable Oils. [Published erratum appears in J Forensic Sci 2004 Sept;49(5)] J Forensic Sci 2003;48(6):1293–1297.