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TECHNICAL NOTE

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Extraction, Derivatization, and Analysis of Vegetable Oils from Fire Debris

ABSTRACT: Vegetable oils have the ability to spontaneously heat under certain conditions, which may lead to spontaneous ignition. While the oils are not often encountered in forensic casework, they may be suspected in some fire cases. As these oils are not effectively analyzed using traditional fire debris analysis methods, a protocol must be established for extracting vegetable oils from fire debris. In this study, a protocol was developed for the extraction, derivatization, and analysis of vegetable oils from fire debris. Three derivatization methods were compared to establish an optimal derivatization procedure to convert the fatty acids found in vegetable oils to the fatty acid methyl esters (FAMEs) used in analysis. Three different gas chromatograph columns and programs were examined to determine which was best suited for the separation and analysis of FAMEs. The procedure was tested and refined using a variety of neat and burned vegetable oils, in addition to extractions from oils burned on commonly encountered fire debris materials. The findings of this research will serve as a starting point for further understanding and research of vegetable oils in fire debris.

KEYWORDS: forensic science, fatty acid methyl ester, fire debris, vegetable oils

Vegetable oils and animal fats are composed of ~95% triglycerides, which contain three fatty acids (FAs) attached to a glycerol backbone (1,2). Oils and fats are differentiated from each other by their physical state at room temperature or from the FA composition of the material. Substances that are liquids at room temperature are "oils" and are mainly comprised of unsaturated FAs. These characteristics are generally found in the oils produced from plants (3). Materials that are solid at room temperature are "fats," comprised mostly of saturated FAs. Fats are generally animal derived (3). However, some exceptions exist. For example, palm and coconut oils are plant derived, yet are solids at room temperature. Another exception is found with fish oils, which are produced from an animal but are liquids at room temperature.

Vegetable oils are traditionally produced by pressing or crushing an oil source, such as plant stalks, leaves, or seeds, to extract the fats by direct compression followed by filtration. A more modern method of producing vegetable oils is solvent extraction, which uses a solvent, such as hexane or heptane, to extract the fats. This extraction method has improved efficiency and yield of vegetable oil production (1).

Vegetable oil FAs are generally straight chains composed of an even number of carbon atoms, although a small amount of odd numbered chains also exist. The chains are predominantly 16 or 18 carbons in length, with a few exceptions, such as coconut oil and animal fats, which have a higher proportion of shorter chain FAs (1). These FAs may be saturated, with no double bonds, or unsaturated, with double bonds. Any number of double bonds may exist, though the first double bond is usually found at the carbon 9 (C_9) position with each double bond isolated from other double bonds (4).

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FAs may be named in a number of ways, such as by their formal International Union of Pure and Applied Chemistry name, a common name, or a shorthand designation. The shorthand designation for a FA is the same for the corresponding fatty acid methyl ester (FAME). The shorthand designation is based on two characteristics of the molecule: the number of total carbons and the number of double bonds. Each shorthand designation begins with a "C" followed by the number of total carbons, a colon, and then the number of double bonds. For example, octadecanoic acid methyl ester, which has 18 total carbons and no double bonds, is designated as C18:0. Octadecatrienoic acid methyl ester, which also has 18 total carbons, but three double bonds, is designated as C18:3. This system for naming compounds is easily understood and will be used throughout this paper.

FAs are derivatized by transesterification of the triglycerides or by esterification of free FAs. The transesterification technique is a one-step derivatization which is much faster than the two-step technique that first forms free FAs, and then esterifies these free FAs. Derivatization of vegetable oils to FAMEs is useful as FAMEs are less reactive and more volatile than triglycerides or free FAs. FAMEs can easily be analyzed by gas chromatography (GC), whereas FAs are difficult to detect via normal laboratory instrumentation. While the FAs may be derivatized to other compounds, methyl esters are the simplest esters formed, offering a low molecular weight and elution from a chromatographic column at lower temperatures (5).

Vegetable oils are not often encountered in fire debris, but may be used as an "accelerant" to start or spread an intentional fire. However, vegetable oils differ greatly from more commonly encountered petroleum-base ignitable liquids. The triglycerides that form at least 95% of vegetable oils are not volatile and are not easily ignited. Some vegetable oils are prone to spontaneous heating, but petroleum products are not (6). As vegetable oils are composed primarily of unsaturated FAs, the molecules are not extremely stable. Polyunsaturated fatty acids (PUFAs), which have two or more double bonds, are chemically the least stable, causing them to have

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the highest propensity for self-heating. The more double bonds present in a FA, the more chemically unstable it will be (1). As a result, vegetable oils have a wide range of propensities for spontaneous heating from low (e.g., castor oil, coconut oil, and peanut oil) to moderate (e.g., corn oil, olive oil, and Tung oil) to high (e.g., linseed oil, fish oil, and cod liver oil) (7).

Spontaneous heating is the result of a slow exothermic oxidation process occurring within a combustible material. With vegetable oils, spontaneous heating occurs due to the exothermic autooxidation of the double bonds present in unsaturated FAs. If the heat generated from this reaction is not able to escape or be adequately dissipated by the surrounding material, spontaneous heating may occur. However, other conditions must still be met for spontaneous ignition to take place. These include the presence of enough air to sustain a fire, but not enough to deplete the heat being generated, and a material that is sufficiently insulated to hold the heat until it reaches its autoignition temperature. The spontaneous heating process may occur naturally or be initiated by the presence of additional heat, as preheating the material or oil increases the rate of oxidation (6). Spontaneous combustion, or spontaneous ignition, occurs when these conditions are met and the spontaneous heating has progressed to a runaway rise in temperature, resulting in reaching the ignition temperature of the surrounding material (8). While spontaneous heating and spontaneous ignition may occur at any location, most published accounts are of fires caused after laundered materials containing vegetable oils have spontaneously ignited (9,10). Laundered materials may be a source of spontaneous heating and ignition as laundering does not always remove all of the oil present in a material. With the addition of heat from drying and storage in a poorly ventilated area, all conditions necessary for spontaneous heating and ignition may be met.

Current fire debris analysis methods generally utilize passive or dynamic headspace concentration, which relies on the volatility of ignitable liquids. The volatile components of ignitable liquids are adsorbed onto charcoal for later elution and analysis by GC–mass spectrometry (MS). As vegetable oils are not volatile, passive and dynamic headspace concentration methods are not suited for their analysis. The components of vegetable oils will not be adequately present in the headspace and will therefore not be adsorbed onto the surface of the charcoal.

Due to the lack of volatility of vegetable oil, a solvent extraction is necessary. As triglyceride molecules are very large, heavy, and nonvolatile, it can be difficult to analyze them without derivatization. By derivatizing triglycerides into FAMEs, a sample can be analyzed with better peak shape and separation, and with shorter GC–MS program times. In addition, the difference in chemical properties between FAMEs and other ignitable liquid compounds may affect the ability of certain GC columns to separate the molecules.

There has been minimal published research to date regarding the analysis of vegetable oils in forensic evidence. There are even fewer published papers examining the extraction and analysis of vegetable oils in fire debris. Current articles on vegetable oils recognize a strong need for further study in this area (5).

This research sought to develop a protocol for the extraction, derivatization, and analysis of vegetable oils in fire debris. Three derivatization reagents were compared to determine the derivatization method best suited for vegetable oils in debris samples. In addition, multiple GC–MS programs were tested to establish an optimum temperature program. Neat liquids of reference vegetable oils were analyzed using the best derivatization and analysis methods. Various fire debris samples were spiked with a neat vegetable oil and analyzed using the extraction, derivatization, and analysis methods of the proposed protocol.

Materials and Methods

Reference Standard

A FAME reference standard was prepared in methanol (Burdick & Jackson, Muskegon, MI) using NuChek Prep #17AA (NuChek Prep, Inc., Elysian, MN). The standard contained C8:0, C10:0, C12:0, C14:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, C20:0, C22:0, C22:1, and C24:0 (Table 1). The entire ampule (100 mg) was dissolved in 200 mL methanol and heated until the ampule contents went into solution. The combination of FAMEs in this standard was chosen to cover a wide range of FAMEs that may be encountered in vegetable oil analysis.

GC–MS

Samples were analyzed using at least one of three GC–MS instruments, each with different methods presented in Table 2. Instruments included a PerkinElmer (PerkinElmer, Inc., Wellesley, MA) Clarus 500 GC–MS, a Hewlett-Packard (Agilent, Palo Alto, CA) HP 6890 GC with an HP 5973 MS, and a Hewlett-Packard HP 6890 GC with an HP 5972 MS. The PerkinElmer Clarus 500 GC–MS was equipped with a SGE (SGE, Inc., Austin, TX) HT-5

TABLE 1—*Designations and characteristics of FAMEs present in reference standard.*

Designation	Common name	IUPAC name	Formula	MW (amu)	Percentage (by weight)
C8:0	Methyl octanoate	Methyl octanoate	$C_7H_{15}COOCH_3$	158	2.0
C10:0	Methyl decanoate	Methyl decanoate	$C_9H_{19}COOCH_3$	186	3.0
C12:0	Methyl laurate	Methyl dodecanoate	$C_{11}H_{23}COOCH_3$	214	4.0
C14:0	Methyl myristate	Methyl tetradecanoate	$C_{13}H_{27}COOCH_3$	242	4.0
C16:0	Methyl palmitate	Methyl hexadecanoate	$C_{15}H_{31}COOCH_3$	270	10.0
C16:1	Methyl palmitoleate	Methyl hexadecenoate	$C_{15}H_{29}COOCH_3$	268	1.0
C17:0	Methyl heptadecanoate	Methyl heptadecanoate	$C_{16}H_{33}COOCH_3$	284	1.0
C18:0	Methyl stearate	Methyl octadecanoate	$C_{17}H_{35}COOCH_3$	298	8.0
C18:1	Methyl oleate	Methyl octadecenoate	$C_{17}H_{33}COOCH_3$	296	10.0
C18:2	Methyl linoleate	Methyl octadecadienoate	$C_{17}H_{31}COOCH_3$	294	10.0
C18:3	Methyl linolenate	Methyl octadecatrienoate	$C_{17}H_{29}COOCH_3$	292	10.0
C20:0	Methyl arachidate	Methyl eicosanoate	$C_{19}H_{39}COOCH_3$	326	5.0
C22:0	Methyl behenate	Methyl docosanoate	$C_{21}H_{43}COOCH_3$	344	10.0
C22:1	Methyl erucate	Methyl docosenoate	$C_{21}H_{41}COOCH_3$	342	10.0
C24:0	Methyl lignocerate	Methyl tetrasanoate	$C_{23}H_{47}COOCH_3$	372	12.0

FAME, fatty acid methyl ester; IUPAC, International Union of Pure and Applied Chemistry.

TABLE 2—*GC–MS conditions.*

PerkinElmer Clarus 500 GC–MS		
Column	Type	HT-5 (5% phenyl equivalent polycarborane-siloxane, aluminum clad)
	Dimensions	25 m × 0.22 mm × 0.1 μm
Mobile Phase	Carrier gas	Hydrogen
	Flow rate	1.0 mL/min (electronically controlled)
Injection	Type	Liquid/autosampler
	Volume	1 μL
	Split ratio	30 : 1
Temperatures	Injector	480°C for 2 min, 400°C for 3 min
	Column	60°C for 1 min 5°C/min to 170°C for 0 min 1°C/min to 180°C for 0 min 20°C/min to 250°C for 3 min Total run 39.5 min
	Transfer line	280°C
	Quadrupole	n/a
	Source	230°C
Mass Spectrometer	Scanning range	50–550 amu
	Solvent delay	2.00 min
	Sampling	n/a
Hewlett-Packard HP 6890/HP 5973 GC–MS		
Column	Type	Supelco® SP-2380 (95% Cyanopropyl, 5% Phenyl Polysiloxane)
	Dimensions	30 m × 0.25 mm × 0.20 μm
Mobile Phase	Carrier gas	Helium
	Flow rate	1.0 mL/min
Injection	Type	Liquid/autosampler
	Volume	1 μL
	Split ratio	20 : 1
Temperatures	Injector	250°C
	Column	105°C for 0 min 4°C/min to 200°C for 0 min 20°C/min to 260°C for 0 min Total run 26.75 min
	Transfer line	265°C
	Quadrupole	150°C
	Source	230°C
Mass Spectrometer	Scanning range	50–550 amu
	Solvent delay	1.90 min
	Sampling	2.94 scans/sec
Hewlett-Packard HP 6890/HP 5972 GC–MS		
Column	Type	J&W Scientific DB-1MS (dimethylpolysiloxane)
	Dimensions	30 m × 0.25 mm × 0.25 μm
Mobile Phase	Carrier gas	Helium
	Flow rate	0.5 mL/min
Injection	Type	Liquid/autosampler
	Volume	1 μL
	Split ratio	20 : 1
Temperatures	Injector	250°C
	Column	60°C for 3 min 5°C/min to 120°C for 0 min 12°C/min to 300°C for 5 min Total run 35 min
	Transfer line	280°C
	Quadrupole	150°C
	Source	230°C
Mass Spectrometer	Scanning range	33–300 amu
	Solvent delay	2.80 min
	Sampling	2.80 scans/sec

GC–MS, gas chromatography–mass spectrometry.

capillary column, and the HP 6890/HP 5972 GC–MS was equipped with a J&W Scientific (Folsom, CA) DB-1MS capillary column. The HP 6890/HP 5973 GC–MS was equipped with a Supelco (Bellafonte, PA) SP-2380 capillary column, which was chosen specifically for use in separating FAMEs found in the derivatized vegetable oil extracted samples.

Derivatizations

Tri-Sil® reagent (Pierce Chemical Company, Rockford, IL), composed of hexamethyldisilazane, chlorotrimethylsilane, and high-purity pyridine, was tested using canola oil, following a procedure derived from information accompanying the reagent package (11). One drop of oil was placed in a 15 mL glass vial, followed by 1 mL of dichloromethane (DCM) (Burdick & Jackson), then 1 mL (one ampule) of Tri-Sil® reagent. The sealed vial was heated in a 60°C oven for ~15 min, and then allowed to cool to room temperature. The contents of the vial were diluted with DCM until the vial was ~¾ full. Samples using DCM as the solvent appeared milky and cloudy, which did not improve after filtration with a Whatman AUTOVIAL® (Whatman, Florham Park, NJ). The Whatman filters have a 0.45 μm polytetrafluoroethylene membrane with glass microfiber prefilter and polypropylene housing. The procedure was repeated using pentane (Fisher Scientific, Fair Lawn, NJ) as the solvent, which produced a colorless sample with no milky or cloudy appearance. The sample was then analyzed using GC–MS.

Derivatization using Methyl-8® reagent (Pierce Chemical Company), which contained 2 mEq/mL *N,N*-dimethylformamide (DMF) dimethyl acetal in pyridine, followed a procedure derived from reagent instructions (12). One drop of vegetable oil was placed in a 15 mL glass vial, followed by 1 mL methanol and 1 mL Methyl-8® reagent. The sealed vial was heated in a 60°C oven for ~15 min, allowed to cool to room temperature, then diluted with methanol until the vial was ~¾ full. The sample was then filtered and analyzed using GC–MS.

A third derivatization procedure was tested using a base-catalyzed esterification of FAs to yield FAMEs. The method was based on previously published material utilizing potassium hydroxide (KOH) in methanol as the derivatizing reagent (13). The procedure was modified for use in this research. A 2.0 N KOH (Fisher Scientific) solution was prepared in methanol for use in derivatizations. For neat liquid analysis, one drop of oil was placed in a 15 mL glass vial, followed by 10 mL pentane and 0.5 mL KOH solution. The vial was sealed and shaken by hand for ~10–15 sec and the layers were allowed to separate. The top layer was removed and filtered, and then this extract was analyzed on GC–MS.

Reference Oil Analysis

Various oils and fats, listed in Table 3, were derivatized using KOH and analyzed using the HP 6890/HP 5973 GC–MS with the SP-2380 capillary column. All oils were derivatized and analyzed in triplicate. The animal fats were tested to compare the results of vegetable oils versus animal fats and the presence of differing FAMEs. In addition, the essential oils and mineral oil were tested using the same procedures as for vegetable oils to verify differing responses between these types of oils.

Extractions

Extractions from all spiked materials were performed in a new, clean plastic beaker. Samples were extracted using 50 mL pentane and allowed to soak for ~10 min. The sample was removed from the pentane extract and squeezed to remove any excess pentane from it. Two milliliters of extract were initially removed and placed in a 4 mL glass vial for derivatization, filtration, and analysis by GC–MS. If the sample was determined to be too dilute, the remaining extract was concentrated using a nitrogen gas stream, and then derivatized, filtered, and analyzed using GC–MS.

TABLE 3—*Various oils used as references.*

Number	Oil Type	Brand (City, State)
1	Canola	Super G (Landover, MD)
2	Corn	Mazola (Bestfoods, Englewood Cliffs, NJ)
3	Linseed, Boiled	E.E. Zimmerman Company (Pittsburgh, PA)
4	Linseed, Raw	E.E. Zimmerman Company
5	Tung	H. Behlen and Brothers (Hudson, NC)
6	Olive, Extra Virgin	Pompeian (Baltimore, MD)
7	Sesame, Extra Virgin	Loriva (San Leandro, CA)
8	Sunflower	Loriva
9	Vegetable	Wesson (CA)
10	Peanut	Super G
11	Blend, Vegetable and Canola	Wesson
12	Olive	Safeway Select Verdi (Pleasanton, CA)
13	Olive, Extra Light	Safeway Select Verdi
14	Almond Extract	McCormick (Sparks, MD)
15	Butter, Sweet Cream Salted	Land O'Lakes (St. Paul, MN)
16	Shortening, Butter Flavor	Crisco (Orrville, OH)
17	Cottonseed	Unknown
18	Canola	Richfood (Richmond, VA)
19	Sesame	Unknown
20	Canola	Giant (Landover, MD)
21	Vegetable	Giant
22	Olive, Extra Virgin	Giant
23	Oil of Pine Needles	LorAnn Oils (Lansing, MI)
24	Cedar Wood Oil	LorAnn Oils
25	Coconut	Unknown
26	Palm	LorAnn Oils
27	Castor	CVS Pharmacy (Woonsocket, RI)
28	Sweet Almond	Aarhus United UK Ltd. (Saddle Brook, NJ)
29	Vegetable	Giant
30	Canola	Richfood
31	Olive, Extra Virgin	Bertolli (Unilever, Englewood Cliffs, NJ)
32	Olive, Extra Virgin	Oliovita (San Juan, Argentina)
33	Light Buttery Spread	Smart Balance (Paramus, NJ)
34	Buttery Spray	Smart Balance
35	Buttery Spray	I Can't Believe It's Not Butter (Unilever)

For debris containing water, a slight modification was made. The debris and water contained in the quart can was placed in a new clean plastic beaker, with 50 mL pentane added for extraction. The pentane was mixed with the water and the sample. The sample was removed from the liquid and allowed to drip before any excess liquid was squeezed from the material and the two layers were allowed to separate. The upper pentane layer was decanted from the beaker into a second new clean plastic beaker. The pentane layer was dried using anhydrous sodium sulfate (Mallinckrodt Chemical, Paris, KY). Two milliliters of the dried pentane extract were removed and placed in a 4 mL glass vial for derivatization, filtration, and analysis by GC–MS. If the sample was determined to be too dilute, the remaining extract was concentrated using a nitrogen gas stream, and then derivatized, filtered, and analyzed using GC–MS.

Debris Analysis

Materials representative of those that are commonly found as fire debris, such as wood, carpet, carpet padding, and kitchen towels, were spiked, burned, and extracted in triplicate to test the extraction and derivatization procedures using debris examples. Each material was cut into pieces $\sim 1\frac{1}{2} \times 1\frac{1}{2}$ inches. An initial set of unburned and unspiked materials was extracted and derivatized to establish a background for substances that naturally occurred in the products. Then, a set of unburned materials was spiked with 1 mL raw linseed oil and allowed to soak into the material for $\sim$1 h. The spiked, unburned samples were then extracted, derivatized, and analyzed using GC–MS.

Thirteen samples each of wood, carpet, carpet padding, and kitchen towel material were burned using a propane blowtorch until thoroughly charred but still recognizable. Seven of these 13 samples were spiked after burning, and six were spiked prior to burning. All samples were burned until they were able to sustain a flame, and then allowed to burn for a number of seconds with the self-sustained flame. Burned samples were extinguished with or without the use of water. If extinguished with water, the burning sample was dropped into its unlined quart can and a portion of tap water was poured on top to extinguish the flame and sealed for future extraction and analysis. If extinguished without water, the burning sample was dropped into its unlined quart can and the lid was held over the top of the can, but not sealed to prevent the can from collapsing, until the flames were extinguished. After the flames were extinguished, the lid was sealed on the can.

An unspiked, burned blank for each of the sample substrate materials was also produced, without the use of water to extinguish flames. The unspiked samples were burned, returned to their respective cans, and cooled to room temperature. The cooled samples were removed from the cans, extracted, derivatized, and analyzed.

Of the seven samples that were spiked after being burned, three were extinguished using water and four were extinguished without the use of water. These samples were burned, and then placed into the cans and sealed until the contents cooled to room temperature. After cooling, the samples were spiked with 1 mL raw linseed oil, and then removed from the can, extracted, derivatized, and analyzed.

Of the six samples that were spiked prior to being burned, three were extinguished using water and three were extinguished without the use of water. The samples were spiked with 5 mL raw linseed oil and then allowed to sit for 2 h so that the oil could soak into the sample material. The samples were burned as described previously, placed into their respective cans, and sealed until the contents cooled to room temperature. After cooling, the samples were removed from the can, extracted, derivatized, and analyzed. All spiked samples used a large enough volume of raw linseed oil such that the extractions did not need to be concentrated and to ensure that enough oil would remain on the sample after burning for extraction, derivatization, and analysis.

Results and Discussion

GC–MS

Initially, different GC–MS columns and programs were tested to determine the most suitable analytical method. The PerkinElmer Clarus 500 GC–MS with a HT-5 column is a GC–MS typically used in the high temperature analysis of oils, waxes, and plasticizers, although it was not used in that capacity for this research (14). The Hewlett-Packard HP 6890/HP 5972 GC–MS with DB-1MS column is used for general fire debris analysis. However, as shown in Figs. 1 and 2, the DB-1MS and HT-5 columns are not able to separate the FAMEs, especially the C18:1 and C18:3 peaks, to an acceptable level. The separation of C18:1 and C18:3 using the

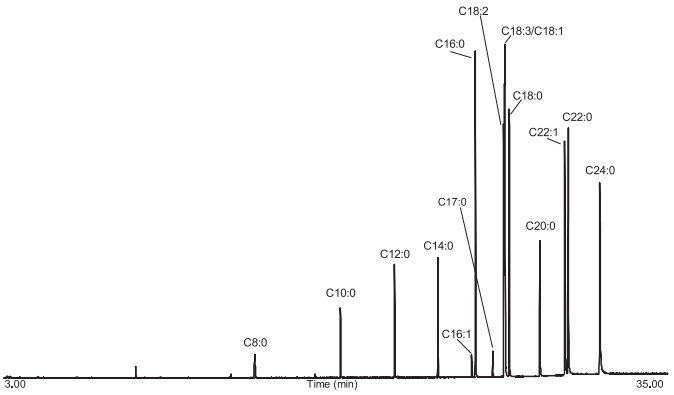

FIG. 1—*Total ion chromatogram (TIC) of the FAME reference standard from the HP 6890/HP 5972 GC–MS with DB-1MS capillary column using a standard fire debris program.*

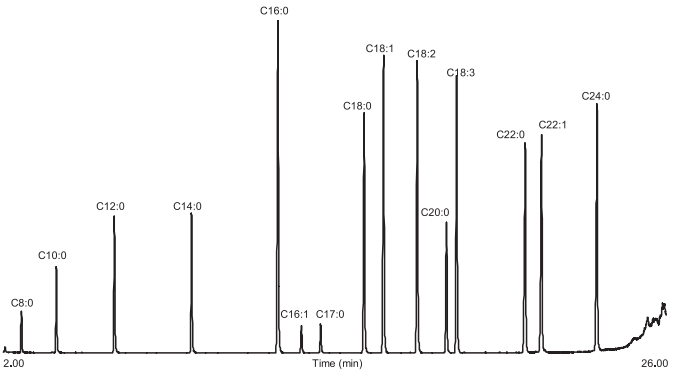

FIG. 3—*TIC of the FAME reference standard from the HP 6890/HP 5973 GC–MS with SP-2380 capillary column using the optimized vegetable oil program.*

HT-5 column was maximized at half-way to the baseline (inset for Fig. 2). The SP-2380 column used in the Hewlett-Packard/HP 5973 is a more polar column and is able to separate all FAME peaks with baseline resolution (Fig. 3).

All FAME peaks, with the exception of the C18:1 and C18:3 peaks, were separated and baseline resolved using each of the capillary columns; however, only the SP-2380 column separated the C18:1 and C18:3 peaks. In addition, the quality of the mass spectra produced with the HP 6890/HP 5973 was better, with a larger abundance of the molecular ion present when compared with the other two instruments. Representative mass spectra of the major C18 peaks have been published by Stauffer (5). The total program lengths ranged from 26.75 min for the HP 6890/HP 5973 GC–MS with the SP-2380 column to 39.50 min for the PerkinElmer Clarus 500 with the HT-5 column.

The HP 6890/HP 5973 GC–MS with the SP-2380 column was chosen for all vegetable oil analyses as it produced the best quality mass spectra, best resolution and separation in the chromatograms, and had the shortest program length. Samples analyzed were of widely varied concentrations. Many extracted samples were extremely concentrated, yet very few samples had any carryover to the following solvent blank. It appeared that the program was successful in allowing all of the FAMEs and other compounds to be eluted from the column before the next sample run began. Although the typical fire debris column is not ideal, it may be suitable for a preliminary examination of the evidence and will provide an indication of the presence of FAMEs.

Derivatizations

Tri-Sil® Reagent and Methyl-8® Reagent were chosen because of their ease of use and documentation supporting their use as a FA derivatizing agent. Currently, there are no published reports of previous use of Methyl-8® in forensic science. Derivatization using Tri-Sil® reagent yielded no FAME peaks. Tri-Sil® reagent and other tetramethylsilyl (TMS) derivatizing agents are most often used in controlled substances and toxicology analyses. TMS is used to produce TMS derivatives of polar compounds for GC or biochemical synthesis. It is used for the derivatization of sugars, alcohols, phenols, steroids, sterols, some amines and organic acids, as well as for the optimal conversion of organic hydroxyl and

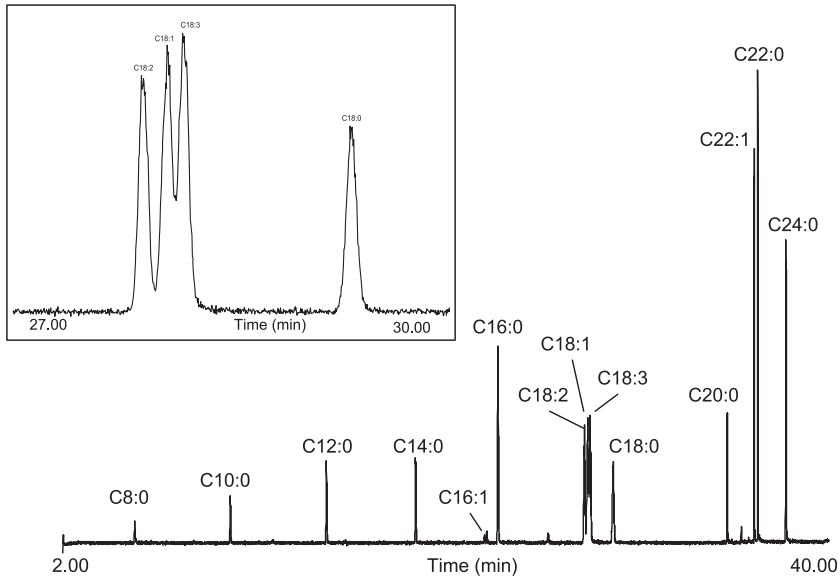

FIG. 2—*TIC of the FAME reference standard from the Clarus 500 GC–MS with HT-5 capillary column using the optimized program for vegetable oils. Inset: A magnified view of the separation for C18:1, C18:2, and C18:3.*

polyhydroxyl compounds into TMS esters (11). A different type of TMS agent may have worked, but was not investigated further due to the positive results of the KOH procedure.

Methyl-8® Reagent was successful in derivatizing the vegetable oil FAs to FAMEs, as seen in Fig. 4; however, the large pyridine solvent peak produced a tail which nearly overlapped the C8:0 peak. In addition, a peak identified as DMF was observed on the chromatograms nearly coeluting with the C10:0 peak. While this may not be a problem, depending on the sample, it was not optimal and was not used further in this study.

The base-catalyzed transesterification using KOH successfully derivatized the FAs in vegetable oils to FAMEs, as shown in Fig. 5*A–E*. The solvent, pentane, was completely eluted well before the solvent delay of 1.90 min, and no additional peaks due to the derivatization reagent were present. The original, published procedure used a volume of 2.0 mL 2 N KOH in methanol, as well as a larger volume of oil or extract; however, it was shown that the results were equivalent when using 0.5 mL 2 N KOH in methanol.

As this was intended as a qualitative technique, quantitative limits of detection were not determined. Preliminary testing indicated that as little as 0.5 μL of vegetable oil spiked onto an inert substrate could be effectively extracted, derivatized, and identified by this method.

Reference Oil Analysis

Vegetable oils are distinguished from each other by differing ratios of FAs, which can be seen in previously published research (4,5). Various oils were analyzed in this study using the optimum derivatization technique and GC–MS program to compare the FAs present and to investigate the possibility of characterization. Distinct differences were observed between the oils tested. Depending on the oil, the most abundant peak was either C16:0, C18:1, C18:2, or C18:3. No oils tested had C18:0 as the most abundant peak. While the major peaks seen in samples were C16:0, C18:0, C18:1, C18:2, and C18:3, many other FAMEs ranging from C8:0 to C26:0 were also observed, usually in very small concentrations. Only linseed oil, raw and boiled, had C18:3 as its tallest peak (Fig. 5*B*). Raw and boiled linseed oil differ in the refining process of the oil. Raw linseed oil is unrefined, while boiled linseed oil is refined for a quicker drying time. Currently, most boiled linseed oil is not actually boiled, but is raw linseed oil with the addition of chemical additives to achieve the same goal of a quicker drying time (15). Linseed oil was originally boiled to rearrange the double bonds to a conjugated configuration, which quickened the drying

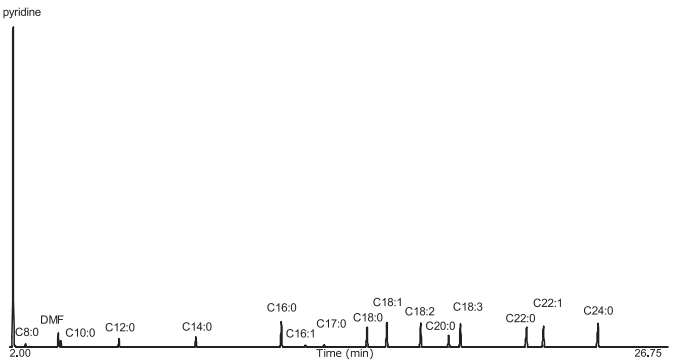

FIG. 4—*TIC of Methyl-8® Reagent with the FAME reference standard using the HP 6890/HP 5973 GC–MS with the SP-2380 column.*

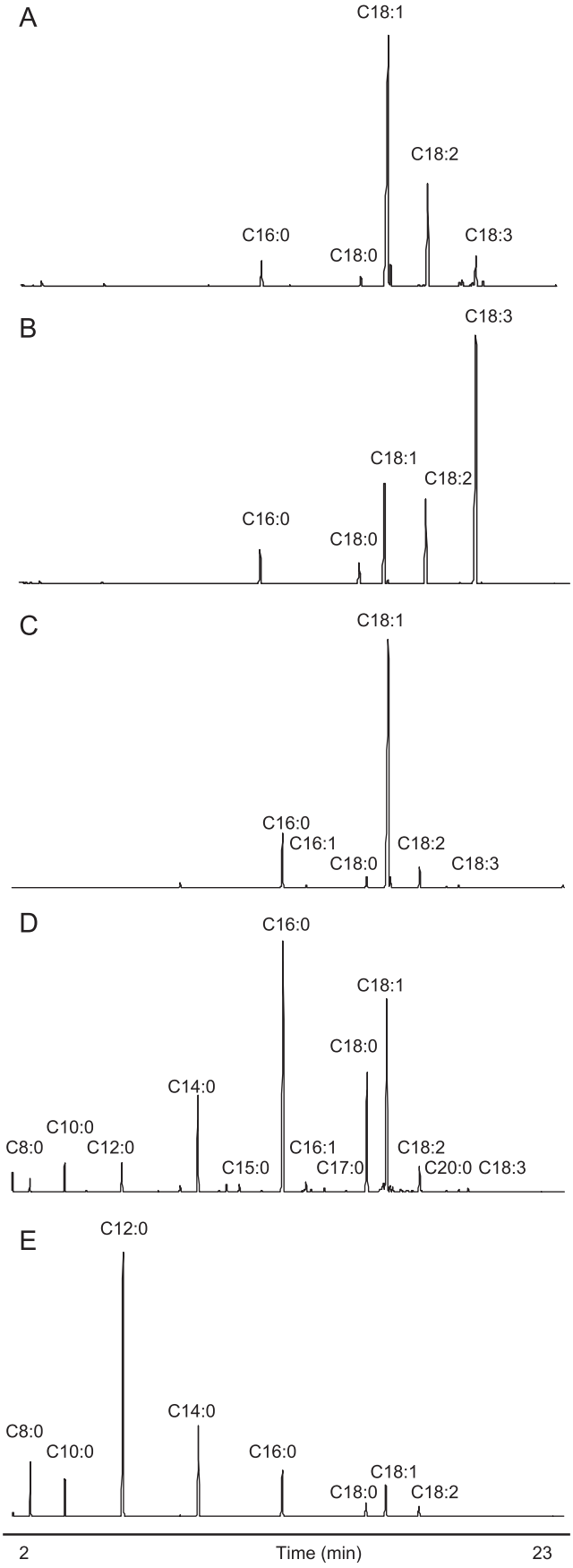

FIG. 5—*TIC of KOH derivatized (A) canola oil, (B) raw linseed oil, (C) extra virgin olive oil, (D) salted, sweet cream butter, and (E) coconut oil using the HP 6890/HP 5973 GC–MS with the SP-2380 column.*

time, but also made the oil more reactive and prone to autooxidation.

Vegetable oils, such as linseed oil, have a higher proportion of PUFAs, especially C18:3, and are characterized by having a high propensity for self-heating. Oils with much lower amounts of PUFAs and higher concentrations of monounsaturated or saturated FAs, such as olive oil (Fig. 5*C*), have a lower propensity for self-heating. The butter-flavored vegetable oil spreads and sprays, as well as the shortening, produced FAME peaks that were consistent with vegetable oils.

The essential oils derivatized and tested did not exhibit any FAMEs. While essential oils are derived from plants and plant material, they are produced in a different manner than vegetable oils, possibly accounting for the lack of FAMEs derivatized from FAs or triglycerides. Essential oils are most often produced through steam distillation, while vegetable oils are typically extracted from seeds or nuts through pressing or by solvent extraction. Essential oils are more volatile than vegetable oils, which accounts for why they are able to be produced using steam distillation. As triglycerides and FAs are not volatile, they are not removed from the plant material during steam distillation (16,17). No FAMEs were observed for almond extract.

The animal fat tested, salted sweet cream butter, exhibited many additional saturated FAMEs in larger abundances than the vegetable oils. The chromatogram shown in Fig. 5*D* exhibited peaks for C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, and C20:0. The solid vegetable shortening tested was similar to the vegetable oils, with the primary peaks being C18:2, C18:1, C18:0, and C16:0, smaller C18:3 and C20:0 peaks, and additionally, small C14:0 and C22:0 peaks.

While palm and coconut oils are technically vegetable oils, they are each nearly solid at room temperature. The physical characteristics of these oils are more consistent with animal fats even though they are vegetable oils. C12:0 was the tallest peak in the coconut oil (Fig. 5*E*), followed by C14:0, C8:0, C16:0 C10:0, C18:1, C18:0, and finally C18:2 (in order of decreasing peak heights). The palm oil samples produced a chromatogram similar to the other vegetable oils, but with a wider range of FAME peaks. FAMEs present ranged from C8:0 to C20:0, with C16:0 and C18:1 as the tallest peaks.

Debris Analysis

Extractions from unburned and burned debris were derivatized to test the method using samples that may be encountered at a fire scene. Extractions, derivatizations, and analyses of debris samples tested for interference from the material with the FAMEs from linseed oil. The debris analysis also tested if burned materials extinguished with water responded any differently than materials extinguished without water.

Extractions from unspiked carpet padding and kitchen towels were nearly free of FAMEs. The unspiked wood sample extraction exhibited a small C18:1 peak; however, when concentrated, small C16:0, C18:0, C18:1, and C18:2 peaks were observed. The initial extraction from the unspiked carpet sample did not show any FAME peaks, but revealed numerous FAMEs including C8:0, C9:0, C10:0, C12:0, C14:0, C16:0, and C18:0, when concentrated. As this method will derivatize all FAs, not only those in vegetable oils, determining the source of the FAs would be difficult. These FAMEs may have been introduced by handling the materials without gloves, as skin contains various FAs that would be derivatized to FAMEs. The unspiked, burned samples yielded the same results as the unspiked, unburned samples that were not concentrated.

Derivatized neat linseed oil, seen in Fig. 5*B*, exhibited peaks for C16:0, C18:0, C18:1, C18:2, and C18:3, at significant levels, with smaller peaks for C14:0, C16:1, C17:0, C20:0, C22:0, and C24:0. Higher concentrations of linseed oil caused the C18:0 peak to develop a shoulder at the front of the peak, though the mass spectrum remained identifiable to C18:0. Higher concentrations also caused the C18:1 peak to become shorter relative to the C18:2 peak, yet the C18:1 peak continued to have a larger peak area as the peak was wider than the C18:2 peak. At much higher concentrations, the C20:0 peak was often not separated from the large C18:3 peak. While C20:0 did not appear as a separate peak in these cases, the mass spectrum for the front of the large C18:3 peak still exhibited a pattern and molecular ion matching that of C20:0. Many small C18 peaks eluted between C18:3 and C22:0 at very high concentrations, but as there were no other targeted FAMEs in this area, the small peaks did not interfere.

All burned, then spiked samples yielded the same FAME peaks from the linseed oil with approximately the same peak characteristics (Fig. 6). In the samples that were extinguished without the use of water, the wood had the lowest abundance with the other three sample materials producing approximately equal responses. The burned, then spiked samples extinguished with water demonstrated a greater range of instrument responses, but the mass spectra and relative peak intensities remained consistent (Fig. 7).

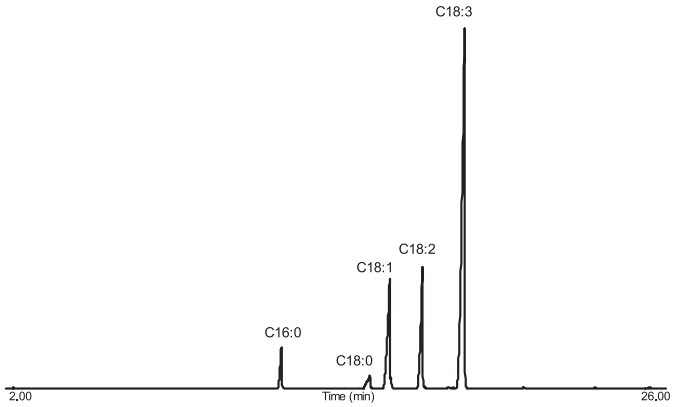

FIG. 6—*TIC of burned, then spiked carpet padding, extinguished without water. The padding was spiked with raw linseed oil, and the sample was analyzed on the HP 6890/HP 5973 GC–MS with the SP-2380 column.*

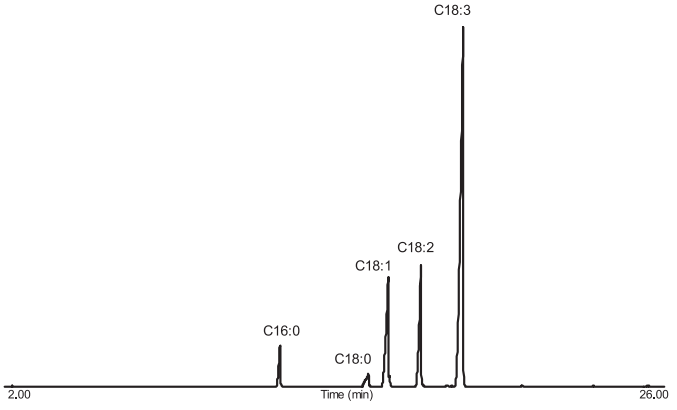

FIG. 7—*TIC of spiked, then burned carpet padding, extinguished with water. The padding was spiked with raw linseed oil, and the sample was analyzed on the HP 6890/HP 5973 GC–MS with the SP-2380 column.*

Samples that were spiked and then burned were spiked with a greater volume of oil to ensure oil would remain on the sample to be extracted after burning. The larger volume of oil resulted in a higher abundance on the chromatogram, but did not cause the mass spectra to be adversely affected. With the higher concentration of linseed oil, the shoulder on the front of the C18:0 peak was larger and the height of the C18:2 peak was taller than that of the C18:1 peak. A selection of samples that were extinguished with water was derivatized and analyzed at the initial concentration and diluted. The diluted samples exhibited much lower abundances on the chromatograms, and the only FAMEs observed were C16:0, C18:0, C18:1, C18:2, C18:3, and C20:0. In the diluted samples, the C18:2 peak is shorter than the C18:1 peak, all peaks are sharper, and the shoulder is no longer visible on the front of the C18:0 peak.

While the materials may have introduced other compounds to the extraction and analysis, the overall peak and chromatogram characteristics were basically unchanged between the neat oil analysis and the debris extractions. The debris material did not appear to affect the results of the derivatization or analysis. No significant differences were seen between samples spiked before or after burning, except for differences in concentration or abundance. The results of the samples extinguished without water and those extinguished with water demonstrated no noticeable differences.

Additional burned carpet padding debris samples were spiked with lower volumes of raw linseed oil. Padding samples that were burned first were spiked with either 50 or 100 μL of raw linseed oil. Samples that were spiked prior to burning were spiked with 500 μL or 1 mL of raw linseed oil. All samples were extracted as previously described, with initial derivatization and analysis completed prior to concentrating any samples. FAMEs were easily detected after the initial derivatization and analysis without concentration necessary for any sample. The total ion chromatograms (TICs) produced from the initial derivatization had clear mass spectra with strong molecular ions.

Additional carpet padding spiked with 1 mL raw linseed oil then burned and extinguished with water was extracted a second time. The debris and the water used to extinguish the debris were re-extracted separately. Each sample was extracted in a new, clean plastic beaker using 50 mL of pentane. The pentane extraction from the water was decanted from the water into a separate new, clean plastic beaker and dried using anhydrous sodium sulfate. Two milliliters of the extract were removed to a 4 mL glass vial for an initial derivatization and GC–MS analysis. While the abundance was lower for the second extraction than the primary extraction, the initial analysis produced TICs with good response for both the debris samples and the water from the debris. The peaks in the TICs had a strong molecular ion with identifiable mass spectra.

Conclusions

FAME peaks were clearly separated using a more polar GC column. The use of a nonpolar column caused certain FAME peaks to overlap. The selected GC–MS program resulted in peaks with a good response at consistent retention times and high quality mass spectra. Base-catalyzed transesterification of vegetable oil triglycerides to FAMEs for GC–MS analysis is a robust technique with good reproducibility and consistent results. The FAME reference standard was used to evaluate GC–MS programs and to identify instrumental characteristics of the FAMEs most commonly found in vegetable oils and animal fats. Sample fire debris materials, burned and spiked with linseed oil, were successfully extracted, derivatized, and analyzed to detect and identify FAMEs. Neither the debris material interfered with the derivatization or analysis of the FAMEs. Analysis of both neat liquids and burned debris demonstrated that brand and type identifications are difficult if not impossible. Identifying FAMEs through the derivatization and GC–MS analysis procedure discussed in this paper will indicate to the chemist that FAs, which are components of vegetable oils, were present.

There are numerous areas in which future research is needed to gain a greater understanding of vegetable oil extractions from fire debris. Additional research should be carried out on oils that have been exposed to heat, such as what may be encountered during a prolonged fire, or on oils that have undergone spontaneous heating and ignition. Research should also investigate how the passive or dynamic headspace concentration procedure may affect the extraction and analysis of vegetable oils from fire debris. Additional materials should also be tested, especially material such as linoleum, which is present in many buildings and is primarily composed of a mixture of three FAs (C18:1, C18:2, and C18:3).

The results presented in this paper provided a basis for developing a protocol for the analysis of vegetable oils from fire debris, but more work is necessary to establish a true understanding of vegetable oil characteristics as related to fire investigations.

Acknowledgments

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