TECHNICAL NOTE

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A Method for Forensic Identification of Vegetable Oil Stains—Rapid Analysis of Carboxylic Acids with Methyl Esterification Using Purge-and-Trap Gas Chromatography/Mass Spectrometry

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ABSTRACT: A simple method using purge-and-trap gas chromatography/mass spectrometry (P&T-GC/MS) for forensic examination of oil stains was studied. Carboxylic acids, chosen as target components for discrimination of oil samples, were extracted from stains with ether, methyl esterified by tetramethylammonium hydroxide, and analyzed by P&T-GC/MS. Vegetable oils were discriminated according to their carboxylic acid compositions. Carboxylic acid composition was independent of the substrate material of the stain. Although the carboxylic acid composition of the oil changed on exposure to sunlight, identification of oil was possible for oil stains that had been in the shade, if analysis was made within 20 days.

KEYWORDS: forensic science, vegetable oil, purge-and-trap gas chromatography, oil stain, methyl esterification, carboxylic acid, substrate material, exposure, steroid analysis

Vegetable oil stains on paper or clothes are encountered in forensic science laboratories as important evidence in criminal investigations. The most frequent type of oil stain examination involves comparison between an oil stain from a suspect or a victim and an oil sample collected at a crime scene. When a control sample cannot be found, identification of the kind of oil or even the identification of the plant species from which the oil was produced is required.

Techniques that have traditionally been used for oil examination are analysis of carboxylic acids in a saponified fraction of a sample and analysis of steroids in an unsponifiable fraction. When gas chromatography or mass spectrometry is used for carboxylic acid analysis, esterification or transesterification is required, making the whole examination procedure complicated and time-consuming.

Purge-and-trap gas chromatography (P&T-GC), which does not require complicated pretreatment such as saponification, is a useful technique for analysis of volatile organic compounds. P&T-GC is as a convenient method for many purposes (1–6) and is especially effective for trace analysis, where a simple procedure is inevitable because of a limited amount of sample (7–9). Because substances with low boiling points can be easily lost during exposure to air, it is desirable to analyze more volatile resistant components in examining physical evidence. In this study, we chose carboxylic acids present in vegetable oil as target components for discrimination of vegetable oils. We used P&T-GC/MS to analyze the carboxylic acid compositions of samples first extracted with ether and then esterified with tetramethylammonium hydroxide (TMAH) (10). We discuss the discrimination of vegetable oils on the basis of their carboxylic acid compositions and the effect of substrates, such as paper or cloth, on the recovery of carboxylic acids from stains. Furthermore, we describe changes of the carboxylic acid compositions of the samples on exposure to sunlight.

Additionally, after the carboxylic acid analysis, we attempted a gas chromatographic steroid analysis of the residues to further discriminate the vegetable oils.

Materials and Methods

Samples

TMAH (12% w/w in methanol), methanol, N,N-dimethylformamide, and methyl iodide were analytical grade (Wako Pure Chemicals, Tokyo, Japan). Dodecanoic acid, tetradecanoic acid, hexadecanoic acid, 9-octadecenoic acid, octadecanoic acid, and methyl esters correspond to these carboxylic acids and were analytical grade (Tokyo Kasei Co., Ltd., Tokyo, Japan). Olive oil, sesame oil, corn oil, and sunflower oil were obtained from The Honen Oil Mills, Ltd. (Tokyo, Japan), Ajinomoto Co., Ltd. (Tokyo, Japan), Showa Sangyo Co., Ltd. (Tokyo, Japan), and Asahi Oil and Fat Chem. Co., Ltd. (Osaka, Japan), respectively. Soybean oil, canola oil, corn oil, sunflower oil, and safflower oil were obtained from The Nishin Oil Mills, Ltd. (Tokyo, Japan). Vegetable oils

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used in this study were all fresh samples. Substrates used for preparing oil stains were newspaper, toilet paper, filter paper (No. 5A), cotton, silk, wool, polyester, nylon, rayon, acrylic, and acetate.

Apparatus and Analytical Conditions

The P&T system used for carboxylic acid analysis consisted of a Model JHS-100 Curie-point headspace sampler (Japan Analytical Industry, Tokyo, Japan), directly attached to a Model G1800A GCD gas chromatograph/mass spectrometer (Hewlett-Packard, Palo Alto, CA). The G1800A GCD was equipped with a high-resolution fused-silica capillary column (0.25-mm i.d., 30-m length) coated with an immobilized polyethylene glycol layer of 0.25-μm thickness (HP Innowax, Hewlett-Packard). Substances purged from extracts or standard solution after esterification treatment were trapped on an adsorbent (Tenax TA), desorbed by Curie-point heating with a Pyrofoil, and introduced into the GC column. P&T-GC/MS analytical conditions were as follows: Purging, 150°C for 10 min under a flow of 50 mL of He/min; trap temperature, −10°C; desorption, 358°C for 20 s; column temperature, 100 to 240°C, programmed to increase by 10°C/min (initially hold at 100°C for 5 min); injection temperature, 230°C; transfer line, 250°C; column flow rate, 1.0 mL of He/min; split ratio, 40:1. The MS was operated in the electron ionization mode at 70-eV electron energy, and with a scan range of m/z 45–425.

Detection Limits

Detection limits of the carboxylic acids were established by dissolving their methyl esters in methanol over the range of 1 to 500 μmol/L and analyzing the mixture by P&T-GC/MS. The volume of the mixture introduced into the P&T apparatus was always 2 μL.

Analysis of Carboxylic Acids

Methyl esterification (11,12) was applied to both the carboxylic acid solutions (10 mmlL/L of each carboxylic acid in methanol) and the vegetable oil samples. The carboxylic acid solutions were treated by first adding 0.5 mL of 12% TMAH to the carboxylic acid solution, and heating mixture in a water bath for 10 min. N,N-dimethylformamide (2 mL) was then added, with shaking, followed by addition of 0.5 mL of methyl iodide and shaking. After precipitation of tetramethylammonium iodide, 2 μL of the supernatant was subjected to P&T-GC/MS analysis. Vegetable oil samples (10 mg) were similarly treated, using 50 μL of 12% TMAH, 200 μL of N,N-dimethylformamide, and 50 μL of methyl iodide.

Analysis of Oil Stains

Oil stains were prepared by dropping 5 mg of oil samples onto the substrates (2 × 2 cm). The stain specimen was placed in a glass tube and extracted with 300 μL of ether. The ether extract was

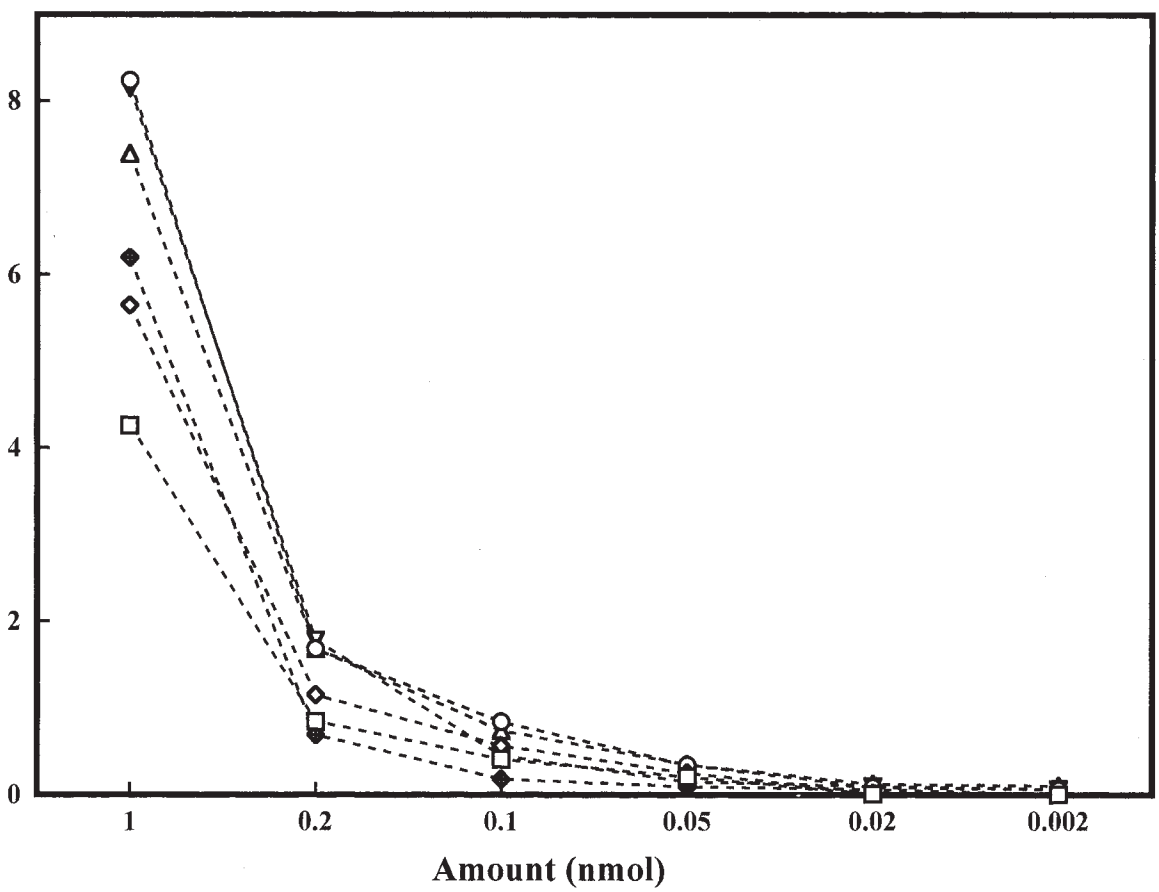

FIG. 1—*Relation between peak area and amount of carboxylic acids introduced into the P&T apparatus. (□) Dodecanoic acid; (◊) Tetradecanoic acid; (○) Hexadecanoic acid; (△) 9-octadecenoic acid; (▼) 9,12-octadecadieoic acid; (◆) Octadecanoic acid. The corresponding methyl esters were analyzed.*

treated by the procedure described for the vegetable oil samples (cf. previous paragraph).

Effect of Exposure to Sunlight

Oil stains on a quartz glass filter paper were placed in the shade as well as in a sunny place for up to 37 days. After exposures of 3,7,15,27, and 37 days, the samples were extracted with 300 μL of ether, the ether extracts were then treated by the procedure described for the vegetable oil samples.

Results and Discussion

Detection Limits of Carboxylic Acids

GC was carried out on a methanol mixture of the methyl esters corresponding to the carboxylic acids (Fig. 1). Measurement was made on 2 μL of the mixture, with methyl ester concentrations ranging from 1 to 500 μmol/L (total amount of methyl esters, 0.002 to 1 nmol). According to the results and the S/N ratios on the chromatograms, the detection limits of the carboxylic acids were estimated to be 0.1 nmol as the amount injected into the P&T apparatus.

Carboxylic Acid Compositions of Vegetable Oils

Total ion chromatograms of vegetable oil samples are shown in Fig. 2 and carboxylic acid compositions of the samples are shown in Fig. 3. All vegetable oil samples analyzed in this study could be successfully discriminated on the basis of carboxylic acid composition. The presence of 9,12,15-octadecatrienoic acid and the hexadecanoic acid/octadecanoic acid ratio are especially important in discriminating vegetable oil samples.

Recovery of Carboxylic Acids from Oil Stains

Soybean oil was used as a reference to examine the recovery of carboxylic acids from oil stains on various substrates (Fig. 4). Although hexadecanoic acid showed a slight tendency to vary from substrate to substrate, the variation did not prevent clear identification the original oil by carboxylic acid composition from any substrate materials.

Recoveries of carboxylic acids from soybean oil stains on various substrates are summarized in Table 1. The recoveries varied from 73.0 to 103.6%, but for given substrates, recoveries were similar. The differences in the recoveries are probably due to the characteristics of the fibers. Even though there is variation of recoveries with different substrates, no significant difference was observed in the carboxylic acid composition (Fig. 4).

Effect of Exposure to Sunlight

Fats and oils gradually deteriorate during exposure to the sunlight (13,14). In this study, olive oil, sesame oil, and soybean oil were used to examine changes in carboxylic acid composition for various exposure conditions. When these oil samples were exposed to sunlight, production of dicarboxylic acids and a decrease of unsaturated carboxylic acids were clearly observed. The chromatograms in Fig. 5 show the change in carboxylic acids composition when soybean oil stains were exposed to direct sunlight for 3, 7, 15, 27, and 37 days from the 9th August to the 15th September, the hottest, most humid season in Japan. Nonanedioic acid appeared after just three days of exposure, followed by the appearance of octanedioic acid (15 days), heptanedioic acid (27

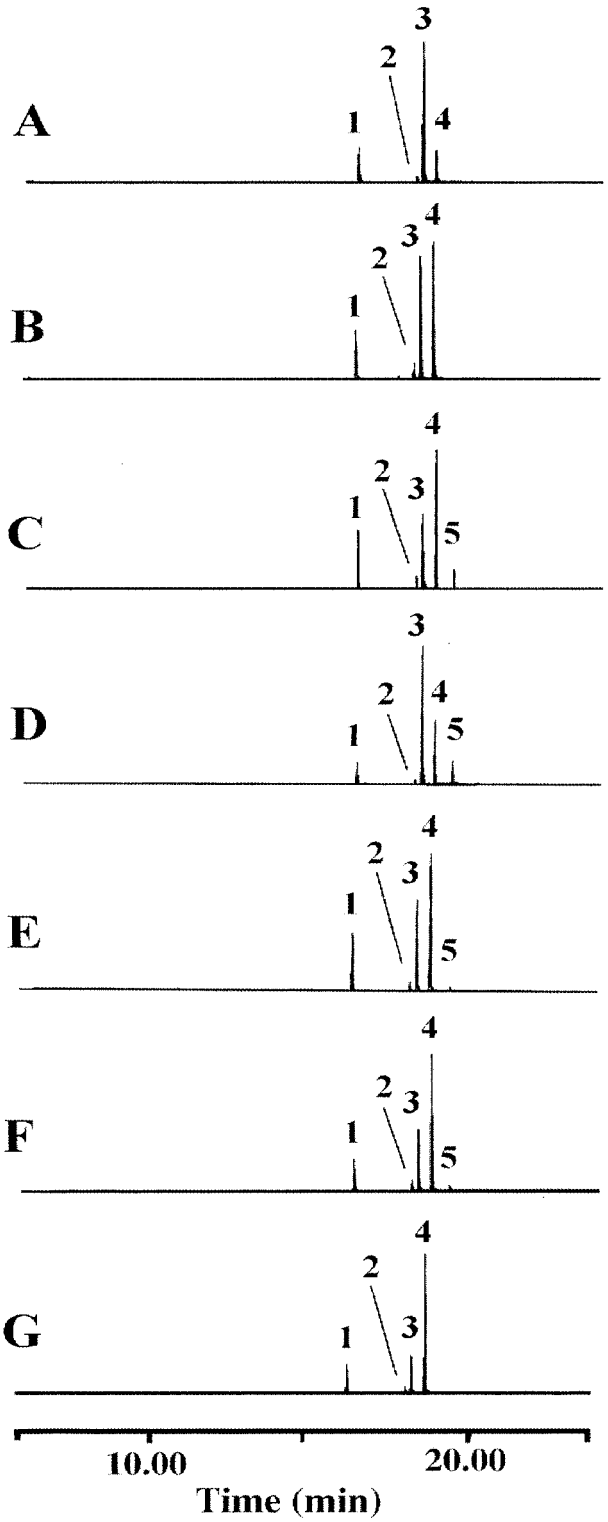

FIG. 2—*P&T gas chromatograms of vegetable oils. (1) Hexadecanoic acid; (2) Stearic acid; (3) 9-octadecenoic acid; (4) 9,12-octadecadienoic acid; (5) 9,12,15-octadecatrienoic acid; (A) Olive oil; (B) Sesame oil; (C) Soybean oil; (D) Canola oil; (E) Corn oil; (F) Sunflower oil; (G) Safflower oil.*

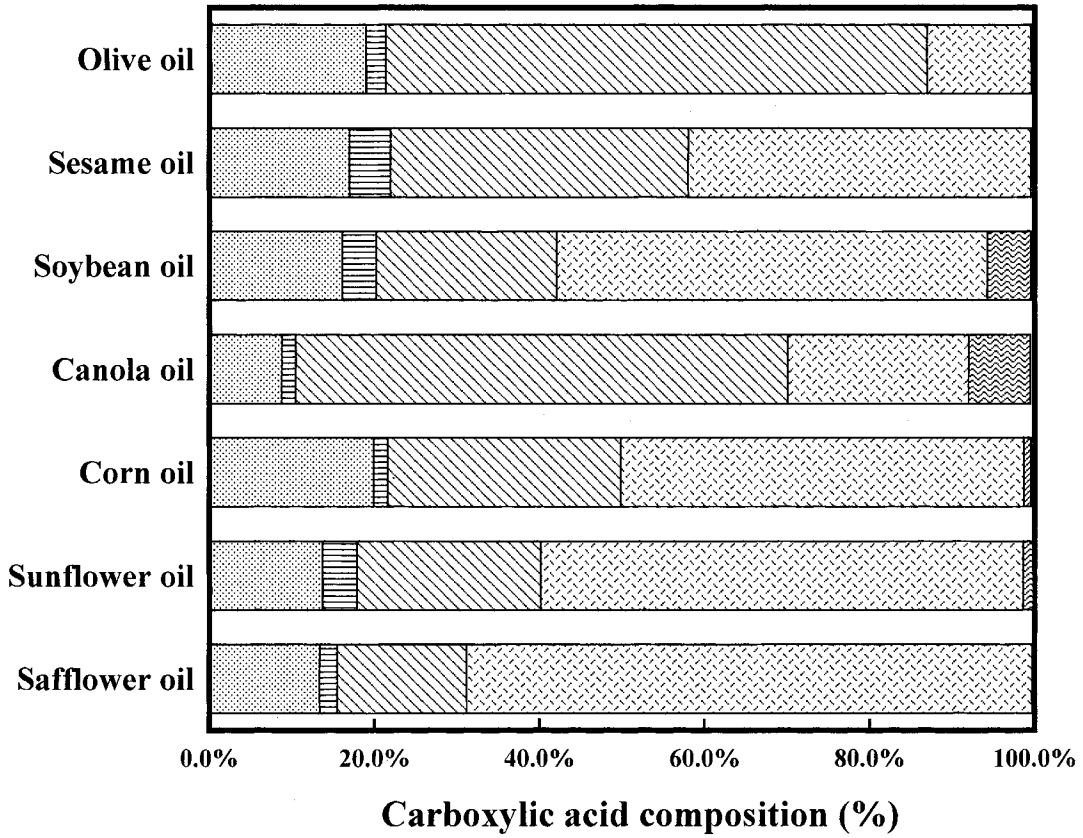

FIG. 3—*Carboxylic acid compositions of vegetable oils. (▨) Hexadecanoic acid; (▤) Octadecanoic acid; (▧) 9-octadecenoic acid; (▨) 9,12-octadecadieoic acid; (▨) 9,12,15-octadecatrieoic acid.*

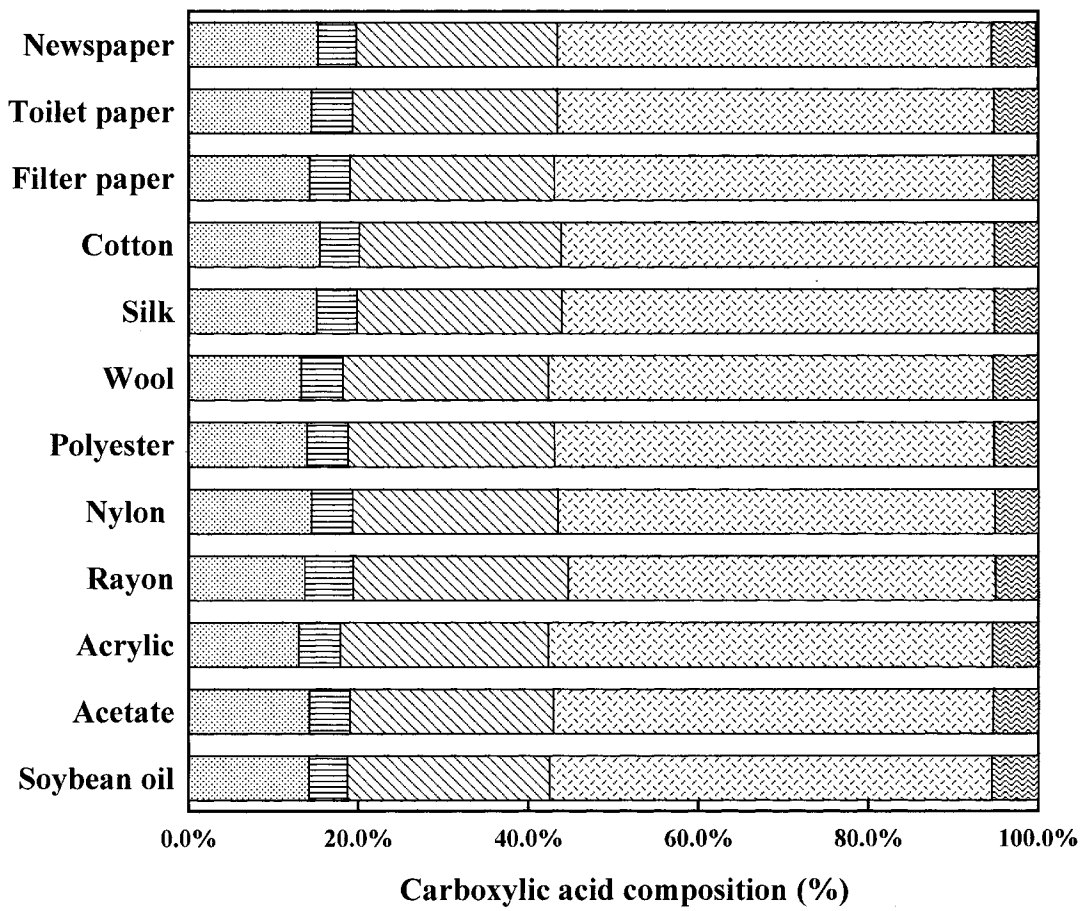

FIG. 4—*Carboxylic acid compositions measured from soybean oil stains on various substrate materials. (▨) Hexadecanoic acid; (▤) Octadecanoic acid; (▧) 9-octadecenoic acid; (▨) 9,12-octadecadieoic acid; (▨) 9,12,15-octadecatrieoic acid.*

TABLE 1—*Recoveries of carboxylic acids from oil stains with soybean oil.*

Carboxylic acid \ Substrate	Recovery, %										
	News Paper	Toilet Paper	Filter Paper	Cotton	Silk	Wool	Polyester	Nylon	Rayon	Acrylic	Acetate
Hexadecanoic Acid	83.8	82	78.4	73	96.4	93.6	91.5	95.1	88.2	71.6	96.9
Octadecanoic Acid	87.5	86.4	81.6	78.8	98.3	96.6	96.4	103.6	91.6	73.6	91.6
9-Octadecenoic Acid	81.2	82.5	76.7	72.3	92.2	90	89.2	91.4	84.7	70.2	89.5
9,12-Octadecadienoic Acid	85.2	87.8	81.7	75.2	98	93.6	92.2	91.1	88	74.4	94.8
9,12,15-Octadecatrienoic Acid	86.7	89	81.6	73	97.7	97.4	95.5	91.1	85.8	76.3	103.4

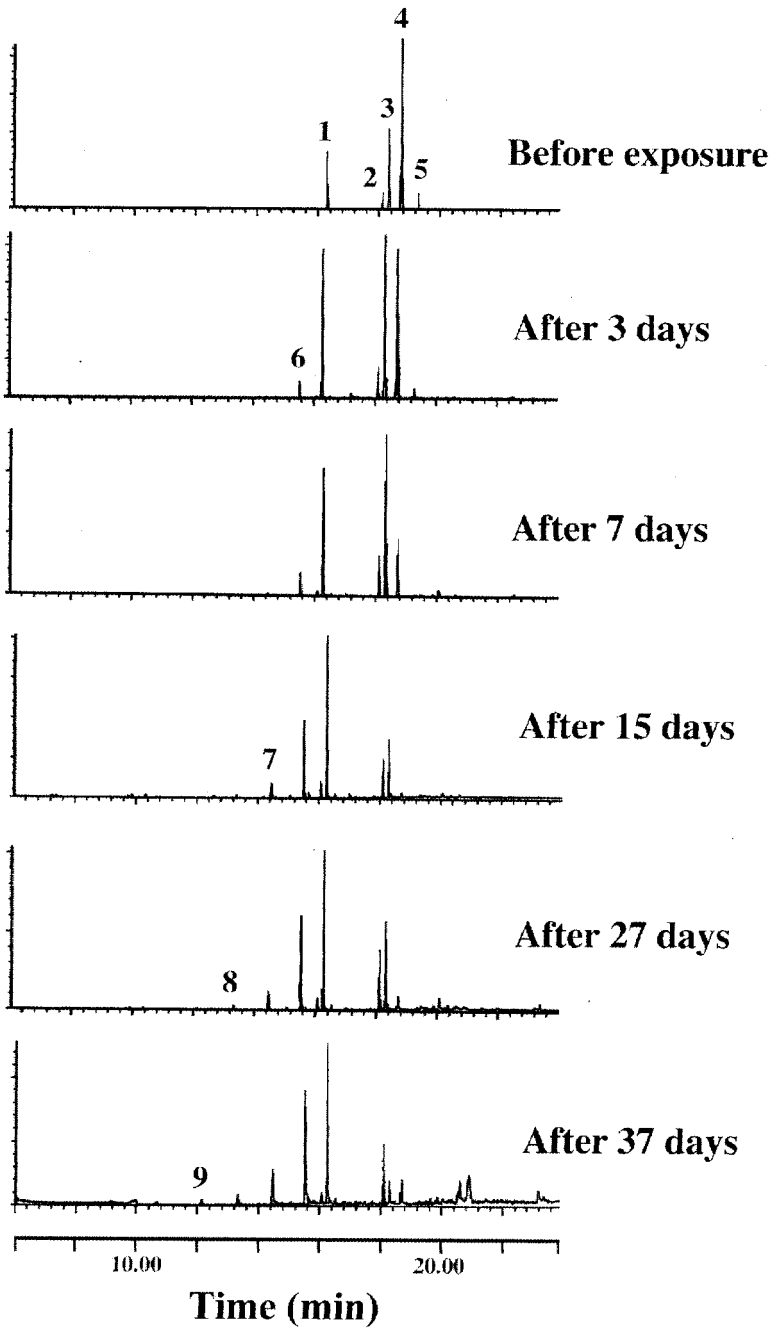

FIG. 5—*Changes of chromatogram of carboxylic acids on exposure of soybean oil stains to sunlight. (1) Hexadecanoic acid; (2) Stearic acid; (3) 9-octadecenoic acid; (4) 9,12-octadecadienoic acid; (5) 9,12,15-octadecatrienoic acid; (6) Nonanedioic acid; (7) Octanedioic acid; (8) Heptanedioic acid; (9) Hexanedioic acid.*

days), and hexanedioic acid (37 days). On the other hand, 9-octadecenoic acid, 9,12-octadecadienoic acid, and 9,12,15-octadecatrienoic acid were markably decreased on exposure to sunlight. The same experiment was carried out in a rainy season (May to June) and in winter (December to January). The similar results were observed, but changes of carboxylic acids were slower than those in Fig. 5.

These data show that it becomes impossible to identify the oil of a stain when the stain is exposed to sunlight for more than a week. When the oil samples were kept in the shade, however, production of dicarboxylic acids was so slow that nonanedioic acid barely appeared after 15 days. Figure 6 compares nonanedioic acid/hexadecanoic acid peak area ratios for samples exposed, and not exposed to sunlight. Since the change of the carboxylic acid composition is slow in the shade (Fig. 6), we conclude that it is possible to identify oil stains if they are analyzed within 20 days of exposure to the atmosphere. However, identification of oil stains that had been in a sunny place, even for only a week, was difficult.

Steroid Analysis

Increase of discriminating power can be expected if steroid analysis is successively applied to the residue after analysis of carboxylic acids. In this study, steroid analysis was additionally attempted.

The gas chromatograph used for steroid analysis was a Model 6890 series (Hewlett-Packard) equipped with a high-resolution fused-silica capillary column (0.53 mm i.d., 15 m length) coated with an immobilized phenyl-methyl-siloxane layer of 0.15-μm thickness (HP-1, Hewlett Packard). Analytical conditions were as follows: column temperature, 200 to 300°C, programmed to increase by 5°C/min; injection temperature, 250°C; detector temperature, 300°C; column flow rate, 3.5 mL of He/min; split ratio, 10:1.

Fifty micro liters of remaining supernatant, which was previously used for P&T-GC/MS analysis of carboxylic acids, were heated at 150°C for 10 min to evaporate volatile compounds. The resulting brown residue was dissolved in 10 μL of hexane-ether mixture (1:1), and the solution was subjected to GC.

Seven vegetable oils were analyzed for steroids by GC/MS (Fig. 7). Sitosterol, kampesterol, stigmasterol, brassicasterol, and cholesterol were clearly detected in the chromatograms of all seven oils. Cholesterol and brassicasterol were distinctive steroids of sesame oil and canola oil, respectively. Although corn oil, sunflower oil, and safflower oil could not be differentiated by the chromatograms, other oil samples gave all different chromatograms. Use of steroid analysis in combination with carboxylic acid analysis can give more certainly to identification of vegetable oils. The detail of steroid analysis from vegetable oil stains should be studied in future.

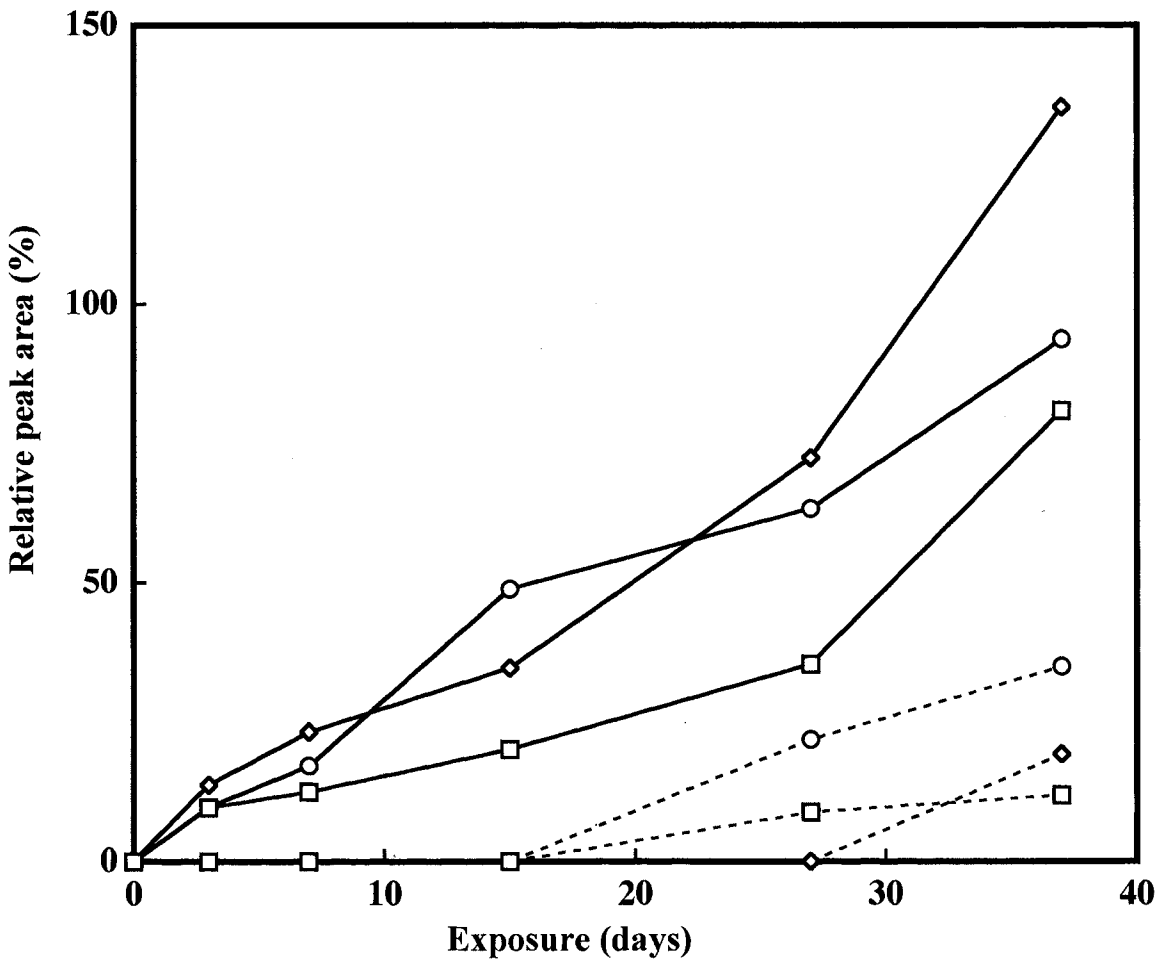

FIG. 6—*Changes of nonanedioic acid/hexadecanoic acid peak area ratio on exposure of oil samples to air. (□) Olive oil; (◊) Sesame oil; (○) Soybean oil; (—) Sunlight; (---) Shade.*

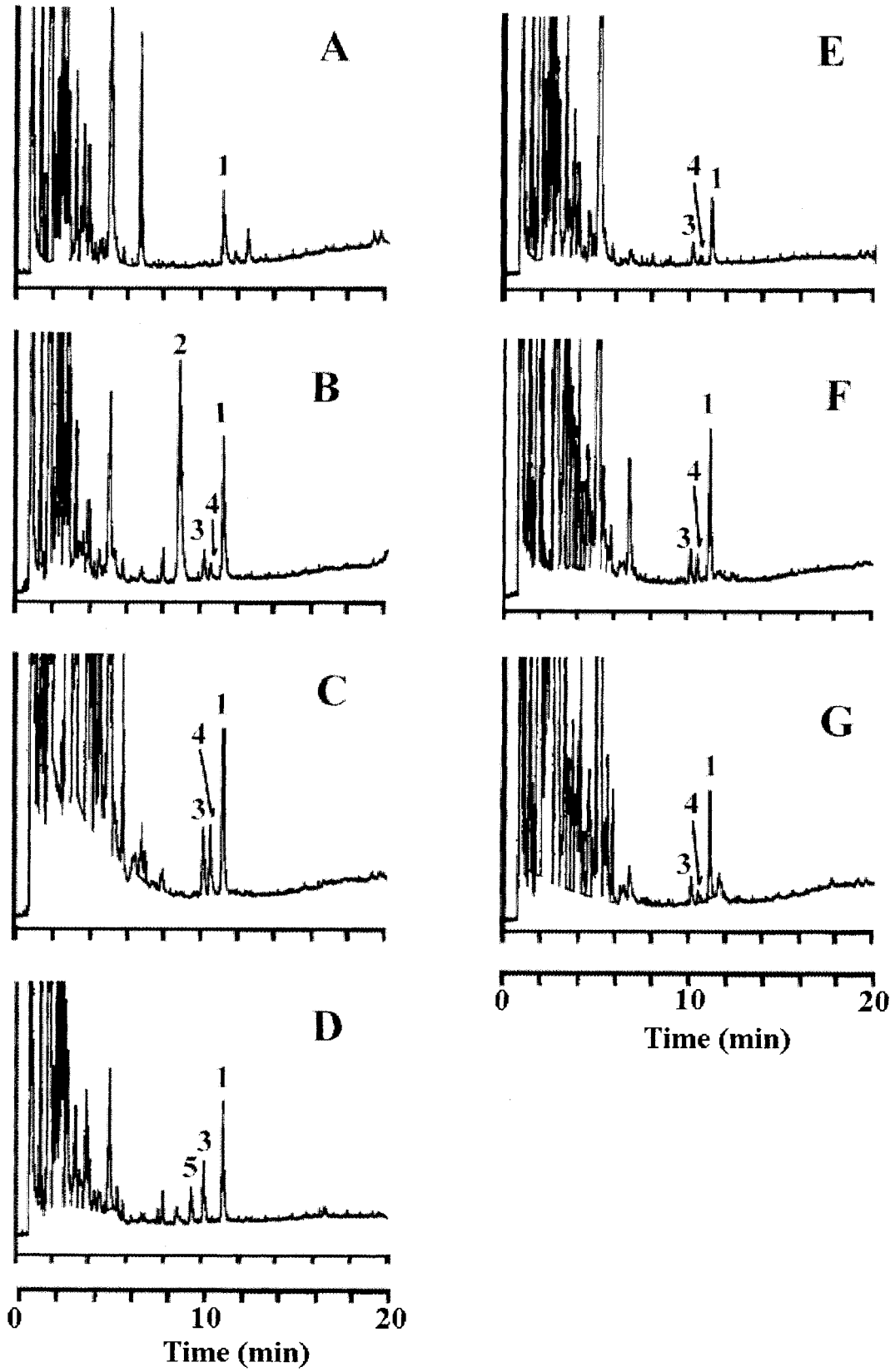

FIG. 7—*Gas chromatograms of steroids from P&T-GC/MS analysis of the residue after carboxylic acid analysis. (1) Sitosterol; (2) Cholesterol; (3) Kampesterol; (4) Stigmasterol; (5) Brassicasterol. (A) Olive oil; (B) Sesame oil; (C) Soybean oil; (D) Canola oil; (E) Corn oil; (F) Sunflower oil; (G) Safflower oil.*

Conclusions

A simple and rapid method for the forensic examination of oil stains was proposed. Carboxylic acids extracted with ether from oil stains were methyl esterified by TMAH and analyzed by P&T-GC/MS. Identification and determination of carboxylic acids were carried out using methylesters for the corresponding carboxylic acids. Vegetable oils were successfully discriminated by their carboxylic acid compositions. Identification of oil was possible for oil stains kept in the shade if analysis was performed within 20 days of exposure to the atmosphere, even though the carboxylic acid composition changed gradually due to deterioration. However, for oil stains exposed to sunlight, the carboxylic acid composition rapidly changed, making oil identification difficult, even for exposures of only a week. Steroid analysis additionally attempted. The results indicated that steroid analysis can help in identifying vegetable oils.

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References

1. Ojanperä I, Hyppölä R, Vuori E. Identification of organic compounds in blood by purge and trap PLOT-capillary gas chromatography coupled with Fourier transform infrared spectroscopy. Forensic Sci Int 1996;80:201–9.
2. Ashley DL, Bonin MA, Cardinali FL, McCraw JM, Holler JS, Needham LL, et al. Determining volatile organic compounds in human blood from a large sample population by using purge and trap gas chromatography/mass spectrometry. Anal Chem 1992;64:1021–9.
3. Schumacher BA, Ward SE. Quantitation reference compounds and recoveries from soils by purge-and-trap GC/MS. Environ Sci Technol 1997;31:2287–91.
4. Philippaerts J, Vanhoof C, Vansant EF. Waste water analysis by purge and trap capillary GC-FTIR spectrometry. Talanta 1992;39(6):681–5.
5. Oguri N, Onishi A, Hanai T. Development of a curie-point headspace sampler for capillary gas chromatography. J High Resolut Chromatogr 1991;14:79–82.
6. Oguri N, Onishi A, Uchino S, Nakanishi K, Jin X. Analysis of polyester by a combination of curie-point heating techniques and preparative liquid chromatography. Anal Sci 992;8:57–61.
7. Ehara Y, Oguri N, Saito S, Marumo Y. Purge and trap/GC for the forensic analysis of lipstick. Bunseki Kagaku 1997;46:733–6.
8. Ehara Y, Marumo Y. Identification of lipstick smears by fluorescence observation and purge-and-trap gas chromatography. Proceedings of The International Workshop on the Forensic Examination of Trace Evidence; 1998 Jan 22–23; Tokyo: 91.
9. Ehara Y, Marumo Y. Identification of lipstick smears by fluorescence observation and purge-and-trap gas chromatography. Forensic Sci Int 1998;96:1–10.
10. Ehara Y, Sakamoto K. Purge-and-trap gas chromatography/mass spectrometry for analysis of carboxylic acids by esterification. Anal Sci 2000;16:283–6.
11. West JC. Rapid preparation of methyl esters from lipids, alkyd paint resins, polyester resins, and ester plasticizers. Anal Chem 1975;47:1708–9.
12. Greeley RH. Rapid esterification for gas chromatography. J Chromatogr 1974;88:229–33.
13. Fujimoto K. Studies on oxidative deterioration of fats and fat-containing foods deterioration assessment. J Jpn Oil Chem Soc 1997;46(3):249–59.
14. Totani Y. Antioxidative effect of nitrogen-containing phospholipids on autoxidation of polyunsaturated oil. J Jpn Oil Chem Soc 1997;46(1):3–15.

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