Reversed-Phase High-Performance Liquid Chromatographic Analysis of Triacylglycerol Autoxidation Products with Ultraviolet and Evaporative Light-Scattering Detectors

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The oxidation products of triacylglycerol standards (trilinolenin, trilinolein and triolein) and a natural mixture of rapeseed oil triacylglycerols were analyzed with a reversed-phase high-performance liquid chromatographic method. The oxidation products were detected with ultraviolet (UV) and evaporative light-scattering detectors (ELSD). The chromatographic profiles obtained with these two detectors were similar for all samples except triolein. ELSD is a mass detector and can detect the oxidation products of triolein and other compounds that do not have conjugated dienes in their structure. The two detectors can be used in series. The sensitivity of ELSD approached that of the UV detector used. ELSD seems to be a good universal detector type for monitoring autoxidation products of edible oils.

KEY WORDS: Autoxidation, ELSD, evaporative light-scattering detector, RP-HPLC, triacylglycerols.

Reversed-phase high-performance liquid chromatography (RP-HPLC) has been used to analyze the oxidation products of triacylglycerols (TAG) in edible oils (1-5). The detection is often based on monitoring the conjugated dienes with an ultraviolet (UV) detector (234-235 nm). However, the UV detector provides no information about oxidation products without a conjugated diene structure, e.g., products of oleic acid. Information about these compounds is important when oils with a high oleic acid content are studied. The most common universal detector types—refractive index and flame-ionization detectors—are not sensitive enough to detect small amounts of oxidation products.

This paper describes an evaporative light-scattering detector (ELSD) that can be used to detect autoxidation products of TAG standards and of a natural mixture of rapeseed oil (RSO) TAGs.

EXPERIMENTAL PROCEDURES

The sample materials used in the work were TAG standards trilinolenin (TLn), trilinolein (TL) and triolein (TO) (Nu-Chek-Prep. Inc., Elysian, MN) and a natural mixture of rapeseed oil triacylglycerols (RSO-TAG) (6). The samples were oxidized at 40°C in the dark in open 10-mL test tubes. Sample aliquots of 500 mg were taken for analyses at regular intervals. Peroxide values (PVs) were measured by the colorimetric ferric thiocyanate method (7).

Autoxidized samples were separated into polar and nonpolar components through solid-phase extraction (NH_2 stationary phase, Bond Elut 500 mg; Analytichem International, Harbor City, CA) (8). The sample size for solid-phase extraction was 100-150 mg. After separation of the polar and nonpolar components, the polar fractions were concentrated into 1 mL dichloromethane and analyzed by RP-HPLC with a Nova-Pak C18 cartridge column (3.9 × 150 mm, 60 Å, 4 μm, Waters, Milford, MA). The effectiveness of the solid-phase extraction was checked by thin-layer chromatography on plates coated with Kieselgel 60 (Merck, Darmstadt, Germany) (8).

The RP-HPLC method was modified from the method described by Neff and co-workers (2-5). The HPLC system consisted of two Waters 501 pumps equipped with a Waters 700 WISP autosampler. The mobile phase was acetonitrile/dichloromethane/methanol (80:10:10, vol/vol/vol) and the flow rate was 1.5 mL/min. Between analyses, the column was cleaned with 100% dichloromethane. All of the solvents used were HPLC-grade (Rathburn Chemicals Limited, Walkerburn, Scotland). The injection volume was 10-100 μL, representing 1-10 mg of original sample. Autoxidation products were detected with a Cunow DDL21 (Cunow Department DMS; Gergy, St. Christophe, France) ELSD and a Waters 486 Tunable Absorbance Detector (UV, 235 nm). The gas used in ELSD was compressed air filtered before the detector through a 0.45 μm filter. The gas pressure was 1.0 bar, giving a flow rate of 7 L/min. Make-up gas was off. The detector temperature was 87°C, and the photomultiplier sensitivity was 650 mV (gain area 400-800 mV).

RESULTS AND DISCUSSION

The RP-HPLC chromatograms of the autoxidized TLn, TL, TO and RSO-TAG obtained from the UV and ELS detectors are shown in Figures 1-4.

Comparison of the two different types of detector, UV and ELSD, showed that the chromatographic profiles of the autoxidized samples are similar except for TO (Fig. 3). Compounds without conjugated dienes in their structure cannot be detected with a UV detector at 235 nm; thus, the autoxidation products of TO are not detected. The detection limit of ELSD was 150 ng, quantified by 1,3-diolein and 2-monolinolein, and its sensitivity in detecting autoxidation products of TLn and TL approached that of the UV detector used in the study. The two detectors could therefore be used in series. The sensitivity of ELSD used in this study is higher than that of other universal detector types reported previously (9,10). The peroxide values of TLn, TL, TO and RSO-TAG samples in these example chromatograms are 236.4, 198.2, 149.7 and 393.8 meq/kg, respectively. By using the analytical procedure presented in this study, it was possible to detect autoxidation products of the samples at PVs as low as 2.1 meq/kg (TLn) and 2.6 meq/kg (TL), indicating that the ELSD can also be used when samples of low oxidation level are to be studied.

Each of the standard TAG chromatograms (Figs. 1-3; ELSD) exhibits one main peak, which grows during the autoxidation process of the samples. The retention times of these main peaks in the chromatograms are: TLn, 3.6 min; TL, 7.5 min; and TO, 18.6 min; and the major autoxidation products of these TAG standards are thus

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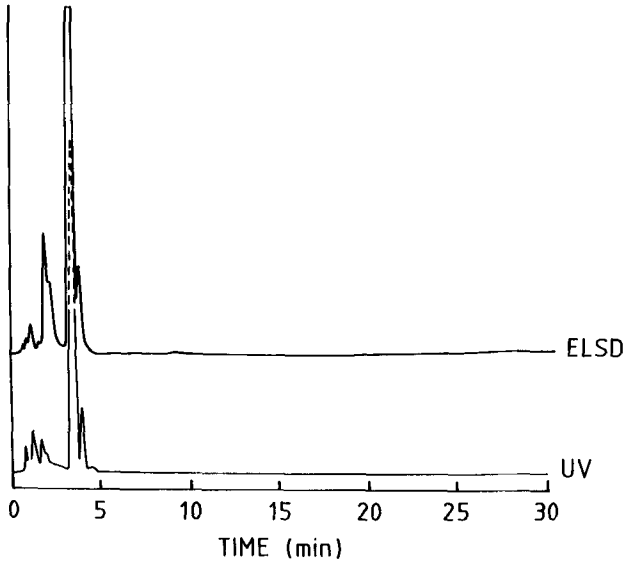

FIG. 1. Reversed-phase high-performance liquid chromatography of autoxidized trilinolenin (peroxide value = 236.4 meq/kg). Nova-Pak C18 cartridge column (Waters, Milford, MA) (3.9 × 150 mm, 60 Å, 4 μm), mobile-phase acetonitrile/dichloromethane/methanol (80:10:10). Ultraviolet (UV) detector (235 nm) and evaporative light-scattering detector (ELSD). Primary oxidation products, double peak at 3.6 min; secondary oxidation products elute before primary oxidation products. For details see Results and Discussion section.

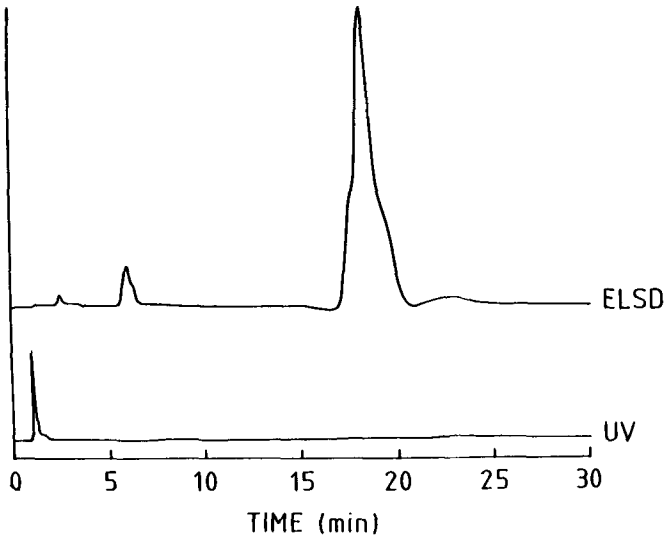

FIG. 3. Reversed-phase high-performance liquid chromatography of autoxidized triolein (peroxide value = 149.7 meq/kg). See Figure 1 for abbreviations and chromatographic conditions. Primary oxidation products, peak at 18.6 min; secondary oxidation products elute before primary oxidation products. For details see Results and Discussion section.

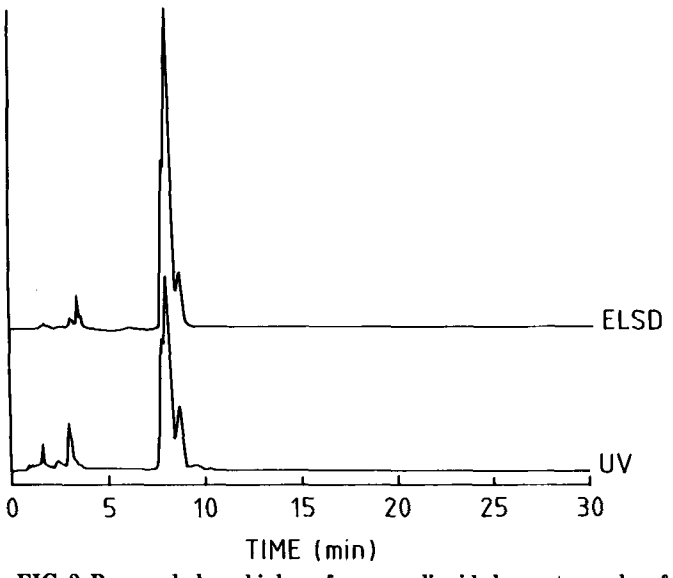

FIG. 2. Reversed-phase high-performance liquid chromatography of autoxidized trilinolein (peroxide value = 198.2 meq/kg). See Figure 1 for chromatographic conditions and abbreviations. Primary oxidation products, peaks at 7.4–9.1 min, secondary oxidation products elute before primary oxidation products. For details see Results and Discussion section.

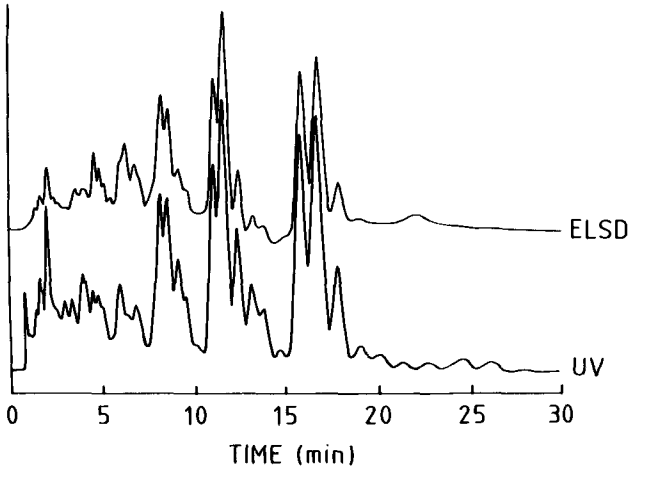

FIG. 4. Reversed-phase high-performance liquid chromatography of autoxidized rapeseed oil triacylglycerols (peroxide value = 393.8 meq/kg). See Figure 1 for abbreviations and chromatographic conditions. Peaks correspond to both primary and secondary oxidation products of rapeseed oil triacylglycerols.

separated. These main peaks do not characterize one pure compound because shoulders can be seen in them. Present knowledge about the oxidation of TAGs and the capabilities of RP-HPLC methods to separate different compounds suggest that these main peaks are mixtures of different geometric isomers of monohydroperoxides (1,11,12). The PVs of the samples in our example chromatograms are 149.7–393.8 meq/kg, which indicates that oxidation of the samples has proceeded rather far. Therefore, the peaks in the chromatograms most likely represent not only primary but also secondary oxidation products of TAGs. According to Neff and co-workers (2–5), the minor peaks eluting before the main peaks of standard compounds (Figs. 1–3; ELSD) illustrate secondary oxidation products, such as hydroperoxy epidioxides or *bis*- or *tris*-hydroperoxides.

Corresponding to standard chromatograms, the main peaks in the RSO-TAG chromatograms (Fig. 4) appear between the elution volumes of the main autoxidation products of TL and TO at retention times of 5–20 min. The peaks of the RSO-TAG chromatograms are not totally separated. Linolenic, linoleic, oleic and palmitic acids are the dominant fatty acids in RSO-TAG (13). The peaks of the autoxidized RSO-TAG chromatograms thus represent autoxidized heteroacylglycerols containing these fatty acids. Earlier, Neff *et al.* (5) studied autoxidation of soybean oil TAGs by similar chromatography. The authors reported that peaks of RP-HPLC chromatograms of autoxidized soybean oil represented monohydroperoxides and hydroperoxy epidioxides of soybean oil heteroacylglycerols, which eluted out of the C18-column in order of their polarity.

The RP-HPLC method used here permits separation of the main autoxidation products of TAG standards. The formation of autoxidation products in RSO-TAG and the nature of these autoxidation products also can be studied. The accurate identification of oxidation products, though, requires further study. Unlike the UV detector, which is often used in autoxidation studies to detect conjugated dienes at wavelengths of 233–235 nm, the ELSD is a mass detector and can be used to detect all high-molecular weight autoxidation products. Thus, it can also be employed to detect TO oxidation products and is a useful instrument for studying the oxidation of monounsaturated compounds at different stages of oxidation. As the consumption of high oleic acid oils is increasing, it is very important to use methods that detect any autoxidation of oleic acid. The results of our study suggest that ELSD promises to be a good universal detector type for monitoring autoxidation products of edible oils.

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