Detection of Pressed Hazelnut Oil in Admixtures with Virgin Olive Oil by Analysis of Polar Components

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ABSTRACT: Analysis of the polar fraction from virgin olive oil and pressed hazelnut oil by high-performance liquid chromatography showed marked differences in the chromatograms of the polar components in the two oils. Six commercial samples of pressed hazelnut oil and 12 samples of virgin olive oil (or blended olive oil including virgin olive oil) were analyzed. The phenolic content of the pressed hazelnut oil samples was 161 ± 6 mg·kg^{-1}. Inspection of the chromatograms showed that the pressed hazelnut oil extracts contained a component that eluted in a region of the chromatogram that was clear in the olive oil samples, and consequently this component could be used to detect adulteration of virgin olive oil by pressed hazelnut oil. The component had a relative retention time of 0.9 relative to 4-hydroxybenzoic acid added to the oil as an internal standard. The ultraviolet spectrum of the component showed a maximum at 293.8 nm, but the component could not be identified. Analysis of blends of oils showed that adulteration of virgin olive oil by commercial pressed hazelnut oil could be detected at a level of about 2.5%.

Paper no. J9756 in *JAOCS 78*, 621–624 (June 2001).

KEY WORDS: Adulteration, hazelnut oil, olive oil, phenolics.

Adulteration of olive oil with hazelnut oil is a practice that benefits unscrupulous oil producers because of the difference in price between the two oils. The problem is most serious for adulteration of virgin olive oil because of the high economic value of this oil. Detection of hazelnut (*Corylus avellana* L.) oil in olive (*Olea europaea*) oil is a difficult problem because the common components used to identify oils, e.g., fatty acids, triacylglycerols, sterols, or tocopherols, are not suitable because of the similarities between the two oils or variability in the components in different cultivars. One promising approach is to detect pressed hazelnut oil by the analysis of (*E*)-5-methylhept-2-en-4-one (1–3). The polar fraction of hazelnut oil represents a fraction that has been little studied. However, virgin olive oil has been extensively studied with the polar components comprising phenols and related aromatic substances at levels of 50–200 mg·kg^{-1} (4). Recent analysis of six virgin olive oil samples found 62–114 mg·kg^{-1}, when determined by the Folin-Ciocalteu method, and 47–566 mg·kg^{-1}, when determined by a more specific enzymatic assay (5). The polar fraction of the virgin oil includes esters and ether derivatives of polyphenols. Ester and ether derivatives of phenols and *o*-diphenols are present in greater quantities than the simple phenols and *o*-diphenols, which include tyrosol and hydroxytyrosol (6). High-performance liquid chromatography (HPLC) analysis of the polar fraction has been most useful in identification and quantification of olive oil polar components (7,8).

Much less is known about the polar components in pressed hazelnut oil. Polar lipids isolated from hazelnut oil were identified as glycolipids (1.4%), with phospholipids present at <1% (9). Minor aromatic compounds were not investigated in this study, which was not designed to detect components present at mg·kg^{-1} levels. The presence of aromatic compounds has not been reported in hazelnut oil, but flavonol glycosides occur in the winter buds and leaves of the plant (10). However, no further information is available about the composition of the polar components in hazelnut oil.

EXPERIMENTAL PROCEDURES

Materials. Folin-Ciocalteu reagent, caffeic acid, and chrysin were purchased from Sigma-Aldrich Company Ltd. (Gillingham, United Kingdom). Myricetin was purchased from Extrasynthese (Lyons-Nord, France). Olive oil samples were purchased from retail outlets. Hazelnut oil samples were purchased from retail outlets or supplied by Anglia Oils Ltd. (Kingston-upon-Hull, United Kingdom) or Leon Frenkel Ltd. (Belvedere, United Kingdom).

Extraction of polar fraction from oils. Pressed hazelnut oil or virgin olive oil (500 g) dissolved in hexane (500 mL) was extracted with a 60:40 methanol/water mixture (4 × 250 mL). The aqueous phase was separated, and the repeat extracts were combined and extracted with 150 mL of hexane to remove any remaining lipid. The methanol was removed under vacuum to leave an aqueous solution, which was extracted with ethyl acetate (3 × 100 mL). The organic solution was dried over anhydrous sodium sulfate, and the solvent was removed under vacuum to leave a residue.

HPLC–diode array ultraviolet (uv) analysis. The analysis of the extracts was carried out on a Hewlett-Packard (model 1050 Series II, Stockport, United Kingdom) liquid chromatograph with a quaternary solvent delivery system and auto-injector, coupled to a uv-visible (UV-Vis) diode-array detector. The Hewlett- Packard ChemStation 3D software package was used for data acquisition and analysis.

The chromatographic separation of the polar fractions from virgin olive oil and hazelnut oil was achieved on a Kro-

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masil 100-5C18, 5 µm column (25 cm × 4 mm i.d.) connected to a 100 RP-C18, 5 µm guard column (Phenomenex UK Ltd., Macclesfield, United Kingdom) at ambient temperature. Gradient elution was employed using a two-solvent system: solvent 1 was 78% water, 20% methanol, and 2% concentrated hydrochloric acid, and solvent 2 was acetonitrile. The flow rate was set at 0.8 mL·min^{-1}. The gradient employed was as follows: 95% solvent 1/5% solvent 2 (0–10 min) changing to 50% solvent 1/50% solvent 2 (maintained 35–50 min) changing to 95% solvent 1/5% solvent 2 by 60 min.

Colorimetric determination of total phenols. The total phenolic content of the oils was estimated by the Folin-Ciocalteu method according to Gutfinger (10). A suitable aliquot of the extract in methanol (1 mL) was diluted with water to 5 mL in a 10-mL volumetric flask. Then 0.5 mL of Folin-Ciocalteu reagent was added and left for 3 min. Sodium carbonate solution (35%, 1 mL) was added. The contents were mixed well and then diluted to volume with deionized water. The absorbance of the solution was measured after 1 h at 725 nm in a Pyrex cell against the reagent blank. The procedure was repeated with caffeic acid as a standard for the preparation of a calibration curve with concentrations in the range 20–120 µg/mL.

RESULTS AND DISCUSSION

Extraction of polar components from six commercial pressed hazelnut oil samples by the method described above showed that the level of polar components determined by mass was 321 ± 20 mg·kg^{-1}, and the phenolic content was 161 ± 6 mg·kg^{-1}. The use of ethyl acetate in the final extraction step leaves very polar components in the water phase, but this solvent was chosen because flavonols and compounds of similar polarity are readily soluble in it. Initial investigation of the HPLC-diode array (UV) chromatograms of virgin olive oil (Fig. 1) and pressed hazelnut oil (Fig. 2) showed that there were clear differences in the components present and the relative amounts of these components. The use of external standards allowed identification of myricetin and chrysin as minor components in the polar extract of pressed hazelnut oil.

Comparison of the chromatograms of the extracts from pressed hazelnut oils and virgin olive oils shows that components present in pressed hazelnut oil would co-elute with components from virgin olive oil over quite a wide range of the chromatogram. However, a component eluting at about 10.8 min in the chromatogram of the pressed hazelnut oil appeared to elute in a region of the chromatogram that was clear for the polar fraction of virgin olive oil. This component had a UV spectrum with a wavelength of maximal absorbance at 293.8 nm (Fig. 3). The component could not be identified despite the use of 35 flavonoids, phenols, and phenolic acids as external standards. Washing the oil three times with water prior to extraction removed a small proportion of this component, but most of it remained in the oil. However, this component was found to be absent in three commercial refined hazelnut oil samples and was also removed during a laboratory refining procedure employing neutralization and deodorization. From the UV spectrum, it appears probable that the component is a phenol or phenolic acid derivative, and hence it is likely to be removed during the neutralization step. 4-Hydroxybenzoic acid was selected as an internal standard, since it absorbs at 293 nm and elutes just after the hazelnut

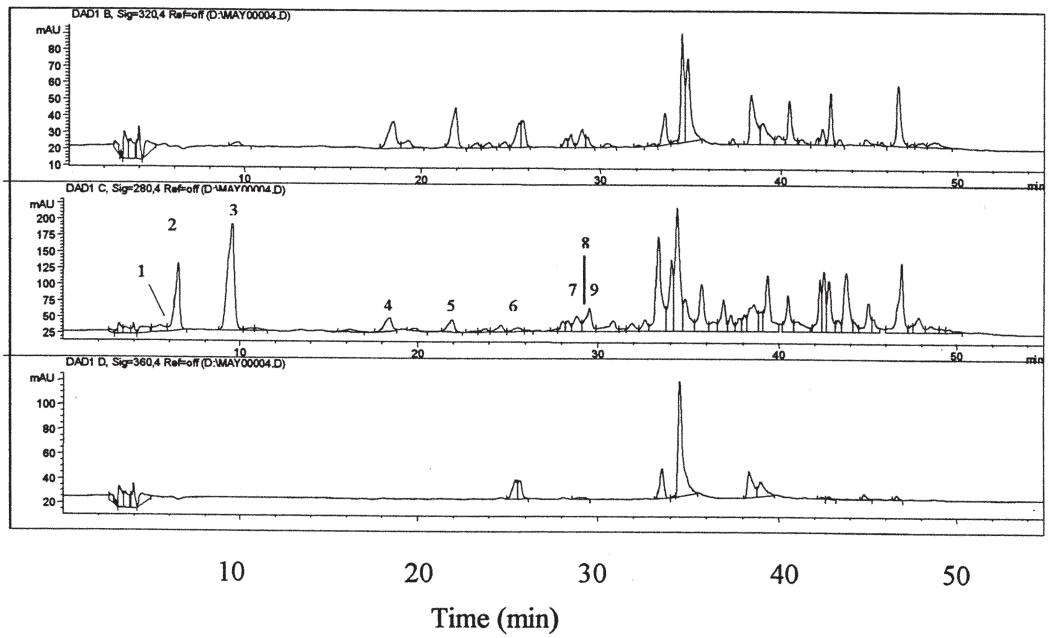

FIG. 1. Chromatogram of extract from extra virgin olive oil (detection at: top 320 nm, middle 280 nm, bottom 360 nm) (1 = gallic acid, 2 = hydroxytyrosol; 3 = tyrosol; 4 = vanillic acid; 5 = *p*-coumaric acid; 6 = ferulic acid; 7 = *p*-hydroxyphenyl acetic acid; 8 = *o*-coumaric acid; 9 = oleuropein).

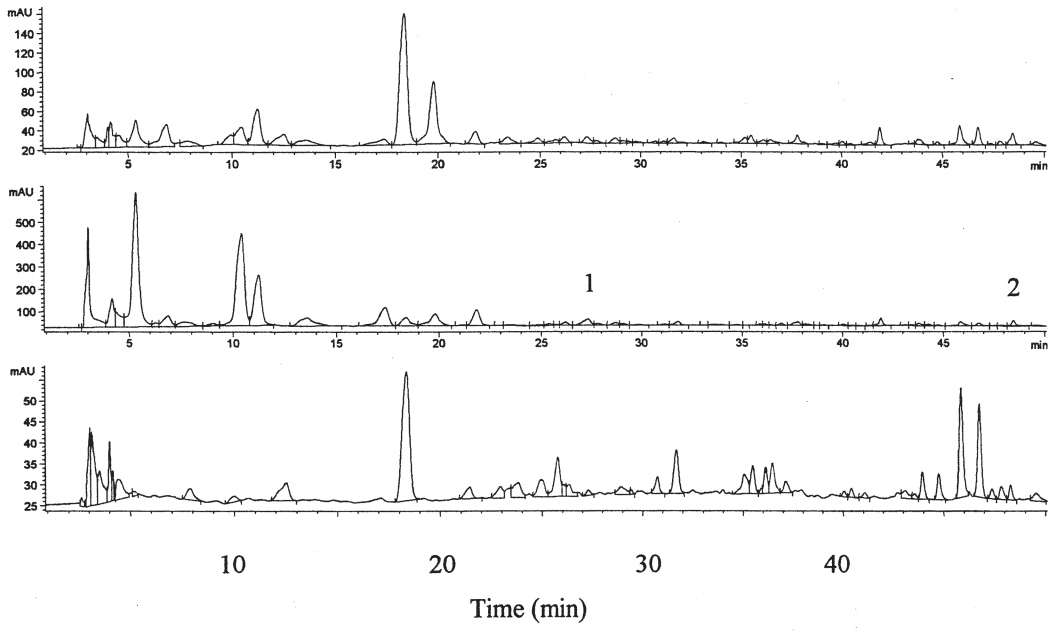

FIG. 2. Chromatogram of extract from pressed hazelnut oil (detection at: top 320 nm; middle 280 nm, bottom 360 nm) (1 = myricetin; 2 = chrysin).

oil component. The component eluting at about 10.8 min in the chromatograms of pressed hazelnut oil eluted with a relative retention time (RRT) of 0.9 relative to the 4-hydroxybenzoic acid. Use of an internal standard facilitated identification of the hazelnut oil component, despite variations in the retention times, and also allowed a check on the efficiency of the extraction. Recovery of the internal standard was 92.4 ± 5.5% for 18 samples of oils analyzed with 40 mg·kg^{-1} added to the oil prior to extraction.

Variability of pressed hazelnut oil phenolic composition. The concentration of the polar extract of commercial pressed hazelnut oil samples was 321 ± 20 mg·kg^{-1} (based on mass extracted), and the phenolic content was 161 ± 6 mg·kg^{-1} (six samples). There was quite a wide variation in the composition of the pressed hazelnut oil samples determined by HPLC, but the component at RRT 0.9 was present in all the samples examined. Based on the HPLC peak areas of the extracts from the six oils, the coefficient of variation for this component was 43.6%.

Variability in composition of olive oil. To determine whether the presence of a component eluting at RRT 0.9 could be used to detect pressed hazelnut oil, it was necessary to inspect a wide range of olive oil samples to ensure that olive oil is always free from this component. The polar fractions from eight virgin and extra virgin olive oils and four blended oils prepared from mixtures of virgin and refined olive oil were analyzed. The total phenolic content for the oils was in the range of 204–342 mg·kg^{-1}. In general, the chromatograms of the extracts from virgin and extra virgin olive oil samples showed many differences, but the chromatograms appeared to be free of any component that would interfere with the pressed hazelnut oil component at RRT 0.9.

Establishment of limits for detection of unrefined hazelnut oil in virgin olive oil by analysis of polar components. In

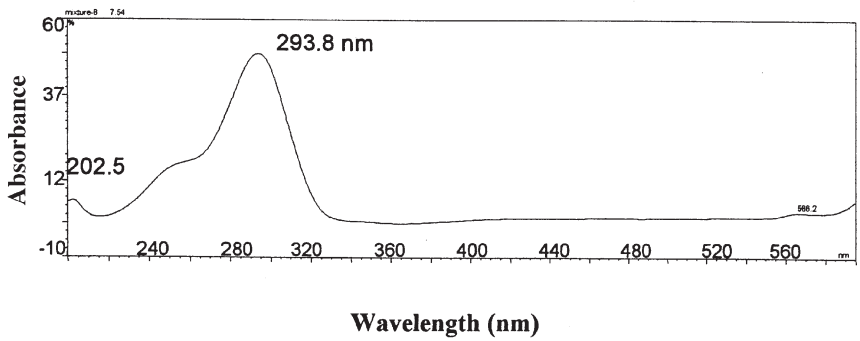

FIG. 3. Ultraviolet spectrum of component in extract from pressed hazelnut oil eluting at relative retention time 0.9.

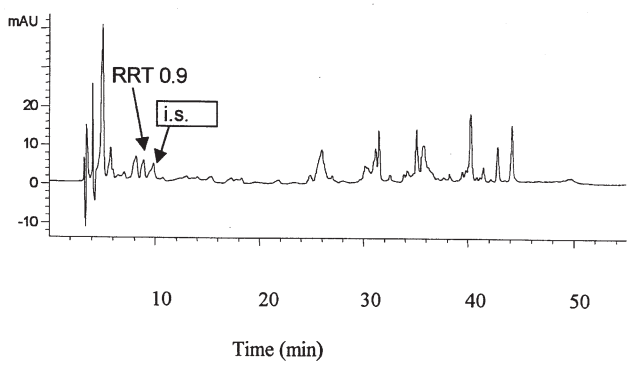

FIG. 4. Chromatogram of extract from 90:10 mixture of virgin olive oil and unrefined hazelnut oil with 4-hydroxybenzoic acid added as an internal standard (i.s.). Detection at 293 nm.

order to establish a limit for the detection of pressed hazelnut oil in virgin olive oil, a range of mixtures of virgin olive oil and unrefined hazelnut oil were examined, with the hazelnut oil present in varying proportions from 50 to 1%. A typical chromatogram for an olive oil–hazelnut oil mixture is shown in Figure 4. Even at the 1% dilution level, a clean peak, albeit small, was observed at RRT 0.9, and at a 2.5 % dilution level a clear UV spectrum could be extracted from the photodiode array data for the component and was confirmed to match that of the component eluting at RRT 0.9 in unrefined hazelnut oil polar extract. Hence, it appears that this component can be used to act as a marker peak for the detection of adulteration of olive oil by pressed hazelnut oil to a level of 2.5%. This method of detecting pressed hazelnut oil in virgin olive oil is an alternative to that proposed by Blanch *et al*. (1), which involves gas chromatographic analysis of (*E*)-5-methylhept-2-en-4-one.

ACKNOWLEDGMENTS

This work was supported by grants from the United Kingdom Ministry of Agriculture, Fisheries and Food, and the Food Standards Agency.

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[Received September 5, 2000; accepted March 15, 2001]