Rapid Recognition of Olive Oil Adulterated with Hazelnut Oil by Direct Analysis of the Enantiomeric Composition of Filbertone

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On-line coupling of reversed-phase liquid chromatography to gas chromatography (RPLC-GC) using a programmable temperature vaporizer (PTV) as an interface is used for detecting adulterations of hazelnut oil in olive oil on the basis of the determination of the enantiomeric composition of (*E*)-5-methylhept-2-en-4-one (filbertone). Different variables (i.e., packing material used for trapping the LC−GC transferred solutes, desorption temperature, and eluent composition of the mobile phase during the LC preseparation) are investigated in terms of their influence on the sensitivity achievable in the RPLC-GC analysis. The method does not require any type of sample pretreatment, thus allowing the direct and rapid analysis (under 50 min) of sample oils. Working under the experimental conditions proposed, adulterations of olive oils with hazelnut oil percentages lower than 5% are detectable.

Keywords: *Olive oil; hazelnut oil; adulteration; on-line coupling RPLC-GC*

INTRODUCTION

In the past few years, the development of both selective chiral stationary phases (König, 1992; Juvancz and Petersson, 1996; Armstrong, 1997) and new separation strategies for chromatographic techniques has been beneficial to a wide variety of areas. Specifically, the food industry is becoming increasingly concerned with enantiomeric separations and analyses because they can be used in different studies including nutritional aspects, characterization of flavor and fragrance components, more exact control and monitoring of fermentation processes and products, identification of adulterated foods, evaluation of treatment and storage effects, etc. (Armstrong et al., 1990; Stalcup et al., 1993; Ekborg-Ott and Armstrong, 1997).

However, further development of new and more useful approaches for the analysis of enantiomers is still required for some applications, as it is clear that the complexity of food matrices makes it difficult or even impossible to separate enantiomers specially when the compounds of interest occur at low concentrations. Overlap is an additional problem because its possibility imposes constraints on the certainty of peak identification as well as lowering quantitative analysis reliability when the area of the peak supposedly corresponding to the analyte is augmented by the presence of one or more coeluted compounds. In these cases, multidimensional techniques usually provide a means for enhancing the resolution of the components of complex mixtures and consequently its use is the most convenient way to determine the enantiomeric composition (Werkhoff et al., 1991; Full et al., 1993).

On-line coupled liquid chromatography−gas chromatography (LC−GC) has already proven its usefulness for the analysis of minor compounds in complex matrices, as the technique combines the effectiveness of LC as a sample pretreatment procedure (thus replacing time-consuming and unreliable conventional methods) with the high efficacies achieved in capillary GC (Grob, 1991; Vreuls et al., 1994; Grob, 1995; Mondello et al., 1996). Moreover, the possibility of using reversed-phase in the LC step has recently enlarged the application field of on-line coupled LC−GC thus allowing its use for the analysis of different fractions in a variety of samples (Señoráns et al., 1995, 1998; Blanch et al., 1998a).

On the other hand, the detection of adulterated olive oils, at least on some occasions, is satisfactorily performed by analysis of minor components (e.g., the sterol fraction, including sterol dehydration products) (Grob and Romann, 1993; Grob et al., 1994a,b; Li-Chan, 1994; Paganuzzi, 1997). In other cases, however, recognition of some frauds is especially difficult, e.g., when the cheaper oil is added to olive oils at a level lower than a certain percentage. Therefore, the reduction of the current levels of fraud in olive oils demands the constant development of new analytical techniques for the detection of possible adulterations. In this regard, an interesting approach is the study of compositional differences among olive oils and those edible vegetable oils which could be used for fraud (e.g., because of their low cost). Evidently, a critical point concerning this aspect is the sensitivity achievable with the analysis inasmuch as it establishes the smallest detectable additions. An interesting example in this respect is the fact that the widely applied adulteration of olive oil with hazelnut oil has only been detectable at high concentrations (approximately 30−40% hazelnut oil in olive oil) using conventional methods.

Recent work has demonstrated the adequate selectivity of on-line LC−GC analysis to determine compositional differences between olive oils and hazelnut oils. Specifically, the use of (*E*)-methylhept-2-en-4-one (filbertone) was proposed as a marker to distinguish between hazelnut oil and olive oil (Blanch et al., 1998b),

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10.1021/jf9807014 CCC: $15.00
Published on Web 11/19/1998

although the lack of sensitivity of the method did not allow the use of direct RP-LC−GC analysis for the recognition of adulterated oils at the concentrations of interest. Actually, filbertone had been previously identified as the flavor-impact component of hazelnut (Emberger et al., 1985), and its occurrence in raw and roasted hazelnuts and in hazelnut cream had already been reported (Jauch et al., 1989; Schurig et al., 1990a; Güntert et al., 1991), although its usefulness for the rapid and direct recognition of low percentages (e.g., under 5%) of hazelnut oil has not been demonstrated yet.

The aim of this work was to develop a rapid, sensitive, and reliable procedure to detect adulterations of olive oils with hazelnut oils on the basis of the determination of the enantiomeric composition of filbertone and the use of on-line coupling RP-LC−GC.

EXPERIMENTAL PROCEDURES

Samples and Materials. (*E*)-5-Methylhept-2-en-4-one (filbertone) used for identification purposes was obtained from Haarmann & Reimer (Holzminden, Germany) while olive oil and hazelnut oil were purchased in the commercial market. Methanol (HPLC grade) was obtained from Lab Scan (Dublin, Ireland) and the water used was collected from a Milli-Q water purification system (Millipore, Milford, MA). Before its analysis by on-line coupling LC−GC, sample oils were simply filtered through a 0.22-μm Pro-X filter (Teknokroma, Madrid, Spain), no further pretreatment being required.

Different adsorbents were used, namely Tenax TA (80−100 mesh), Tenax GR (35−60 mesh), and Gaschrom and Thermotrap (60−80 mesh). In all cases, a plug length of approximately 15 mg was used in the glass liner (80 mm × 1 mm i.d. × 2 mm o.d.) of the PTV injector (Perkin-Elmer, Norwalk, CT) between two plugs of glass wool to keep it in place. Tenax TA and Thermotrap were purchased from Chrompack (Middelburg, The Netherlands), while Tenax GR and Gaschrom were obtained from Alltech (Deerfield, IL). Prior to their use all materials were conditioned for 2 h under a helium stream at either 350 °C (Tenax TA and Tenax GR), 275 °C (Gaschrom), or 400 °C (Thermotrap).

Under the experimental conditions investigated, the use of Thermotrap is not recommended as a large number of peaks appears during thermal desorption. In contrast, use of Tenax TA, Tenax GR, and Gaschrom allowed the analysis of filbertone to be performed, although the yield of the technique is clearly lower when using Tenax. Use of Gaschrom enabled us to obtain filbertone recoveries approximately 12−15 times higher than those obtained for Tenax TA and Tenax GR, respectively.

LC Preseparation. The HPLC system consisted of a Hewlett-Packard Model 1050 (Wilmington, DE) chromatograph involving a manual injection valve (Model 7125, Rheodyne, Cotati, CA) having a 20-μL sample loop and an ultraviolet (UV) detector. All separations were performed using a slurry packed with 10-μm homemade column (50- × 4.6-mm i.d., Vydac 214 TPB).

Different compositions of the eluent (methanol/water) were tested to optimize the preseparation of the fraction of interest, namely 80:20, 70:30, 65:35, 60:40, and 50:50.

Selection of water and methanol percentages was carried out to reduce LC column activity and also to establish the modifier concentration adequate to minimize the solubility of the analyte in the mobile phase (i.e., to avoid losses due to wash-off from the packing material). According to results obtained, the lower water percentages were, the more symmetric the peaks were. Unfortunately, however, lower recoveries were achieved in these cases. Methanol/water (65:35) was finally selected, as adequate shaped peaks were then obtained also yielding higher filbertone recovery than that for eluent composition 70:30.

All throughout the experimentation, the column temperature was kept at 45 °C and the detector was operated at 205 nm, 2000 μL/min being both the flow rate during LC preseparation and the speed of sample transfer from LC into GC. Acquisition of data from the UV detector was performed with a HPChem Station (Hewlett-Packard).

LC−GC Transfer. Transfer of the selected fraction was performed by using a multiport valve placed immediately after the UV detector of the LC system, the PTV of the GC acted as an interface of the on-line coupled LC-GC equipment, as described elsewhere (Señoráns et al., 1995). Upon elution of the beginning of the fraction to be transferred, the multiport valve was switched from the waste position to the transfer position (the PTV being maintained at 21 °C) and was switched back to waste after the transfer time. Solvent elimination during transfer was achieved through both the split exit and the injector bottom (by withdrawing the column end). Moreover, solvent elimination was also promoted by sweeping the injector with a helium flow (1500 mL/min) which was maintained during an additional purge time (6 min), established once the transfer is finished. Subsequently, the column end was again connected and thermal desorption of the trapped material was achieved by increasing the PTV temperature (at 14 °C/s) to different values (i.e., 150, 200, 225, and 275 °C). The lowest value did not allow the desorption of the retained filbertone and low recoveries were obtained when performing the experimentation at 200 °C. Finally, 225 °C was judged to be high enough to achieve acceptable recoveries while giving satisfactory blanks between consecutive runs.

GC Analysis. A Perkin-Elmer Model 8500 gas chromatograph fitted with a PTV injector and a flame ionization detector (FID) was used. GC separations were carried out on a homemade chiral column (a 25-m × 0.25-mm i.d. fused silica column coated with a 0.25-μm layer of Chirasil-β-Dex) (Schurig et al., 1990b) with helium as the carrier gas. In all analyses the FID temperature was 320 °C. Chromatography software from Perkin-Elmer Nelson Systems (Model 2600-rev 5.0) was used for data acquisition from the FID.

RESULTS AND DISCUSSION

Taking into account both the complexity of the sample to be analyzed and the additional difficulty generally involved in the detection of adulterated olive oils, a reliable analysis of relevant components is considered essential. In this respect, it is clear that the use of an enantioselective fingerprint may allow a more exact evaluation of specific compounds in comparison to that resulting from achiral stationary phases. Consequently, the information resulting from a chiral column is more definitive and much more difficult to duplicate in an adulterated food.

As previously mentioned, data obtained by performing the experimentation under different conditions were evaluated in terms of its suitability for the direct RP-LC−GC analysis of the enantiomeric composition of filbertone in sample oils. As a result, the following values were established for the investigated variables: packing material in the PTV, Gaschrom; desorption temperature, 225 °C; and eluent composition in the LC step, methanol/water (65:35). Under these conditions, the recoveries of (*R*)- and (*S*)-filbertone were 36% and 41% respectively, using as reference a 2-μL splitless injection of filbertone in GC.

Figures 1 and 2 show the chromatograms obtained by LC−GC transfer of a 3000-μL fraction resulting from the preseparation performed in LC of a hazelnut oil and an olive oil, respectively. Filbertone was identified in the hazelnut oil by matching retention times of both *R* and *S* enantiomers with those measured when standards were analyzed under identical conditions. More-

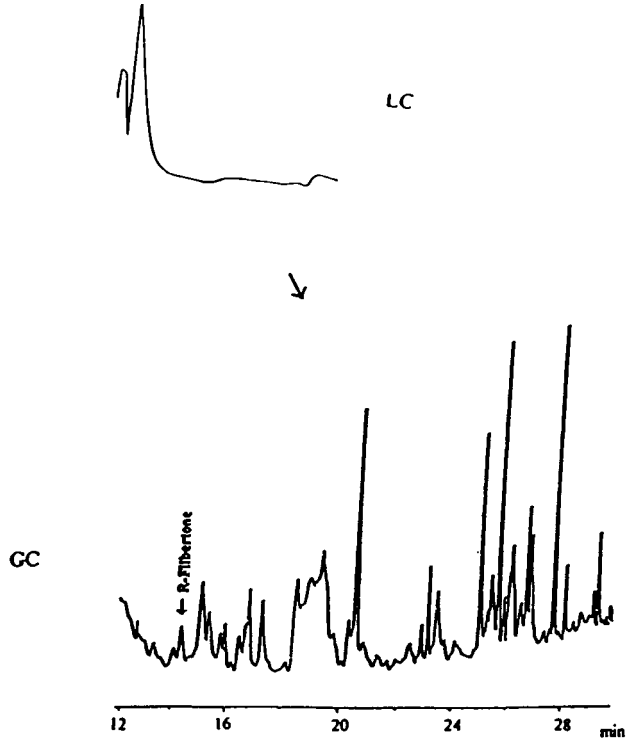

Figure 1. LC preseparation obtained from the direct analysis of a hazelnut oil and GC separation resulting from online LC−GC transfer of a 3000-μL fraction of methanol/water eluent (65:35). Conditions: fused silica capillary column, 25 m × 0.25 mm i.d., coated with a 0.25-μm layer of Chirasil-β-Dex; oven temperature, 45 °C (10 min) and then programmed at 5 °C/min to 180 °C; speed of sample transfer, 2000 μL/min.

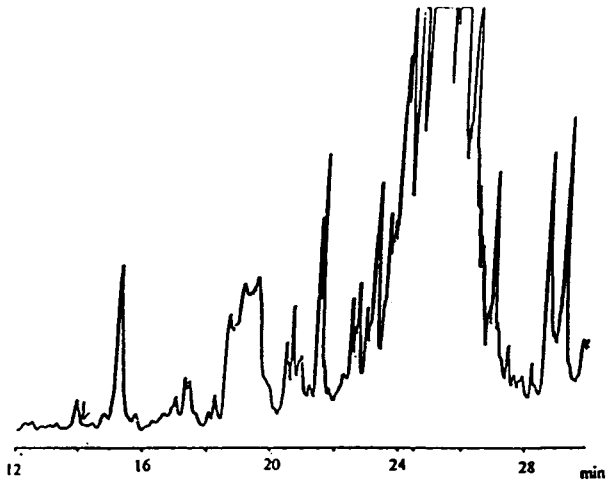

Figure 2. Gas chromatogram obtained after transfer of a 3000-μL fraction of mehanol/water eluent (65:35), resulting from the direct LC−GC analysis of an olive oil. Capillary column, oven temperature, and speed of sample transfer are as in Figure 1.

over, in all cases, the presence or absence of filbertone was confirmed by adding the reference compound to the sample. Although enantiomer *S* coeluted with other unidentified compounds, the presence of filbertone in hazelnut oil could be established from detection of enantiomer *R* while from Figure 2 it is clear the absence of filbertone in the olive oil. Concentration of filbertone in hazelnut oil (approximately 10 mg/L) was established from peak areas resulting from analysis, performed

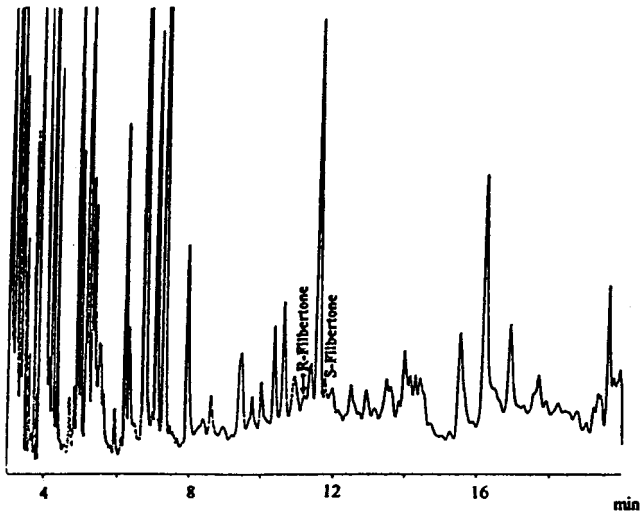

Figure 3. Gas chromatogram obtained after transfer of a 3000-μL fraction of methanol/water eluent (65:35), resulting from the direct LC−GC analysis of an olive oil mixed with hazelnut oil (96:4). The sample was cold trapped on the column and the GC oven was programmed at 2 °C/min from 45 °C to 180 °C. Capillary column and speed of sample transfer are as in Figure 1.

under identical conditions, of the original sample and of a spiked sample.

As far as the recognition of adulterated olive oils is concerned, detection of filbertone in mixed oils can be easily performed under the experimental conditions proposed providing that hazelnut oil percentage is higher than 10%. Analysis of lower percentages is also possible, although less accurate. In this respect, it is clear that a more reliable detection of adulterated olive oils would be performed on the basis of the determination of both (*R*)- and (*S*)-filbertone enantiomers. To this aim, the improvement of the chromatographic resolution was attempted by narrowing the band beginning the chromatographic process, i.e., by performing an on-column focusing of the sample by cooling a trapping section. As a result, Figure 3 depicts the chromatogram obtained after transfer of a 3000-μL fraction resulting from the LC preseparation of an olive oil mixed with hazelnut oil (96:4), which shows the separation achieved for both enantiomers. The enantiomeric excess calculated from *R* and *S* concentrations resulted in being 73%. The relative standard deviations (RSD, calculated from three replicate analyses) were 11% (for enantiomer *R*) and 4% (for enantiomer *S*), 0.02 and 0.03 ppm being the corresponding detection limits (estimated from a signal equal to 5 times the baseline noise).

Summarizing, filbertone can be used as a chiral marker for the rapid recognition of olive oils adulterated with hazelnut oils by direct RP-LC−GC analysis. Since preconcentration is not needed, danger of contamination and loss of samples is minimized, and the time required for the performance of the overall procedure (i.e., including oil filtration, LC preseparation, LC−GC transfer, and GC analysis) is 50 min.

ACKNOWLEDGMENT

We thank Professor V. Schurig (University of Tübingen, Germany) for the gift of the chiral column used in this study.

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Received for review June 29, 1998. Revised manuscript received October 2, 1998. Accepted October 6, 1998. This work was made possible by financial support from the CICYT (Project OLI96-2145-C03-01). M. L. Ruiz del Castillo and M. del M. Caja thank the Ministerio de Educación y Cultura and CSIC, respectively, for their grants.

JF9807014