Study of the Enantiomeric Composition of Chiral Constituents in Edible Oils by Simultaneous Distillation-Extraction. Detection of Adulterated Olive Oils

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ABSTRACT: A method is proposed for detecting adulterations of olive oil with hazelnut oil at percentages as low as 5%. The method is based on the identification of *R*- and *S*-enantiomers of *E*-5-methylhept-2-en-4-one (filbertone) and involves the use of simultaneous distillation–solvent extraction in the sample preparation step and subsequent gas chromatographic analysis with a chiral stationary phase. Relative standard deviation values obtained from three replicates of adulterated mixtures of olive oil and hazelnut oil (95:5) were less than 9% for both *R*- and *S*-filbertone.

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KEY WORDS: Adulteration, enantiomeric composition, filbertone, hazelnut oil, olive oil.

Reliable identification of adulterated edible oils can be very difficult from an analytical point of view, especially when frauds involve the use of low percentages of cheaper oils (1–3). Moreover, specific adulterations (i.e., olive oil with relatively high percentages of hazelnut oil) are difficult to detect even with conventional parameters usually considered for oil control. In those cases, the development of new methods providing high sensitivity and selectivity is clearly necessary.

The possibility of establishing the enantiomeric composition of chiral compounds is of great interest either to evaluate the authenticity of a consumer product or to detect its adulteration (4–6). Such studies, however, are not common in foods because the complexity of the matrix may cause a number of problems. Specifically, when the enantiomeric composition has to be established, the eventual overlapping of some groups of compounds may be accentuated because of the difficulty involved in separating enantiomers from one another (7–9). In any case, it is clear that the knowledge of the enantiomeric composition may result in a more reliable evaluation of some products.

On the other hand, the sample preparation step is a critical aspect in the analysis of complex mixtures as it can alter the composition of the sample to be analyzed. Specifically, eventual losses of volatile compounds or chemical changes of some components can be observed during isolation and concentration, and there is no universal sample preparation procedure suitable for all samples under all conditions. Consequently, selection of the isolation and concentration techniques best suited to the characteristics of each specific sample is always advisable.

In this respect, the simultaneous steam distillation–solvent extraction technique (SDE) (10,11) has proved its usefulness for enriching different types of samples (12–16). Our previous research demonstrated the potential of SDE for distinguishing between different edible oils on the basis of the study of compositional differences, although detection of adulterations of olive oil with hazelnut oil below 15% was not achieved (17).

The object of this work was to develop a reliable method, based on the enantiomeric composition of a suitable chiral marker, for detecting adulterations of olive oil with low percentages of hazelnut oil (e.g., 5%). For this purpose, we used SDE in the sample concentration step and the subsequent gas chromatographic (GC) analysis, and the chosen chiral marker was *E*-5-methylhept-2-en-4-one, a compound previously identified as the flavor-impact component of hazelnuts (18–21).

EXPERIMENTAL PROCEDURES

Materials. *E*-5-Methylhept-2-en-4-one (filbertone), used for identification purposes, was obtained from Haarman & Reimer (Holzminden, Germany). Dichloromethane was purchased from SDS (Peypin, France) and glass wool from Phase Separation (Deeside, United Kingdom). Olive and hazelnut oils were obtained from either the commercial market or an oil mill.

SDE. Sample was concentrated using a Chrompack (Middelburg, The Netherlands) microdistillation–extraction device, as proposed by Godefroot *et al.* (10). The SDE device was used in the high-density solvent configuration (i.e., its design allows the use of extraction solvents denser than the sample solvent). In all instances, the concentration procedure was started by heating the sample by applying a silicone bath at 135–140°C. The sample consisted of a 75-mL volume of the oil (either pure or adulterated) and a 25-mL volume of

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water purified in a Milli-Q system (Millipore, Milford, MA) which was added to the oil and homogenized for 15 min in an ultrasonic bath. Simultaneously, a 2-mL volume of the extractive solvent (dichloromethane) was distilled by heating it with a water bath at 60–65°C. Vapors of both solvent and sample were condensed with a cold finger maintained at –3°C. The design of the apparatus is such that both vapor and liquid-phase extraction can be achieved while a continuous reflux of water and solvent is maintained during the extraction time (2 h). Finally, the steam-distillable material present in the sample is collected in *ca*. 2 mL of dichloromethane, thus achieving a concentration factor near 50. After finishing the SDE operation, the obtained extracts were analyzed by GC. Between consecutive runs, the SDE apparatus was rinsed with acetone and with purified water.

GC analysis. For the GC system, a gas chromatograph (Perkin Elmer, Norwalk, CT) model 8500 fitted with a PTV (programmed temperature vaporizer) as the injection system and a flame-ionization detection system (FID) was used. The glass liner of the PTV-injector was packed with a 2-cm plug of glass wool. All analyses were performed on a 25 m × 0.25 mm i.d. fused-silica column coated with a 0.25-μm layer of Chirasil-β-Dex (22) by sampling a 2-μL volume of the solvent extract after having added 0.2 μL of *n*-nonane (Schuchardt, Munich, Germany) as internal standard. The PTV was operated in the splitless mode and maintained at 45°C upon sample introduction. Thereafter, the temperature of the injector was increased (approximately at 14ºC/s) to the temperature required (350ºC) for transferring the solutes to the capillary column. The ending PTV temperature was kept at 350°C for 11 min. The temperature of the GC oven was programmed from 40 to 80ºC at 5ºC/min and maintained there for 20 min. In all cases, helium was used as the carrier gas.

Throughout the experimentation, the FID was operated at 300ºC and the chromatography software from an HPChem Station (Hewlett-Packard) was used for data acquisition from the detector.

RESULTS AND DISCUSSION

According to our previous research, the presence or absence of filbertone was a convenient marker used to distinguish between pure hazelnut oil and pure olive oil, respectively. However, some difficulties, mainly concerning the selectivity, sensitivity, and reliability of the method, arose when analyzing adulterated oils.

In this work, the quantitative estimation of filbertone in hazelnut oil was performed from the straight line (Fig. 1) determined from the absolute peak areas obtained in the GC analysis of various standard solutions containing *n*-nonane, as an internal standard, and different amounts of filbertone, namely 6, 10, 16, 20, 30, 40, 50, and 60 mg/L in dichloromethane. The analysis is linear in the considered interval and the relation between the peak area (y) obtained for the sum of *R*- and *S*-filbertone in each run and the corresponding concentration (x) is given by equation $y = 2225.3x + 608.0$ (correlation coefficient equals 0.999). According to this equation, the average amount of filbertone estimated from three SDE extracts of the same hazelnut oil analyzed under identical conditions was to be 40 mg/L.

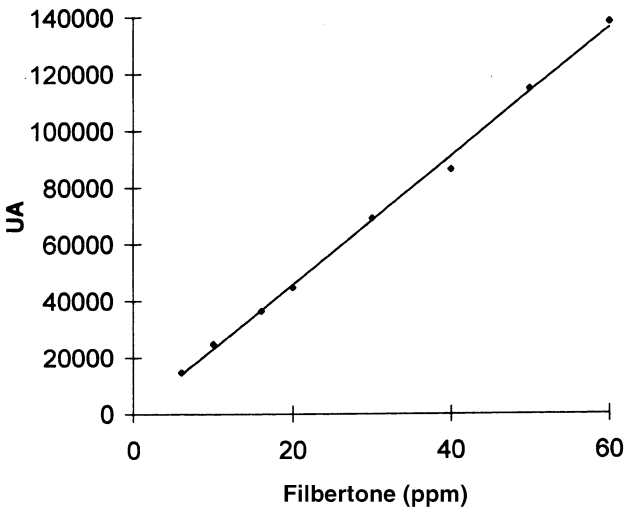

FIG. 1. Absolute peak areas (in units of area, UA) obtained from different standard solutions of filbertone (*E*-5-methylhept-2-en-4-one).

Under the conditions given in the Experimental Procedures section, the absence of filbertone in pure olive oil was confirmed and its presence was easily detected in pure hazelnut oil, but the sensitivity of the method was not sufficient for detecting adulterations of olive oil with hazelnut oil. However, solvent elimination (approximately up to a 0.2-mL volume) from the SDE-extract prior to its injection into the gas chromatograph enabled us to achieve enough sensitivity for the analysis.

Figure 2 shows the chromatogram of the SDE extract resulting from a mixture of the olive and hazelnut oils mentioned above and demonstrates the potential of the method for detecting adulterations of high-quality oils with low percentages of cheaper oils (even at concentrations as low as 5%). Identification of filbertone was performed by matching the retention times of both enantiomers with that obtained for the authentic reference compound. The identification was confirmed by adding the standard to the oil sample and performing a new analysis under identical conditions as shown in trace A (Fig. 2).

Enantioselective fingerprints for detecting adulterations are useful even in cases when an enantiomer is chromatographically overlapped by other component(s) of little or no importance regarding authentification. Indeed, data obtained from a second enantiomer can be sufficient to perform a reliable analysis.

With respect to precision, the relative standard deviations (n = 3) obtained from relative areas (ratio of filbertone peak area to that for the internal standard) were 5.2 and 3.5% for enantiomers *R* and *S*, respectively, when considering the chromatograms of the SDE extracts obtained from standard solutions of filbertone. In performing the total analysis (i.e., including SDE and GC) of three different samples of the same olive oil adulterated with 5% of the same hazelnut oil, the relative standard deviation values were 8.7 and 5.9% for enantiomers *R* and *S*, respectively.

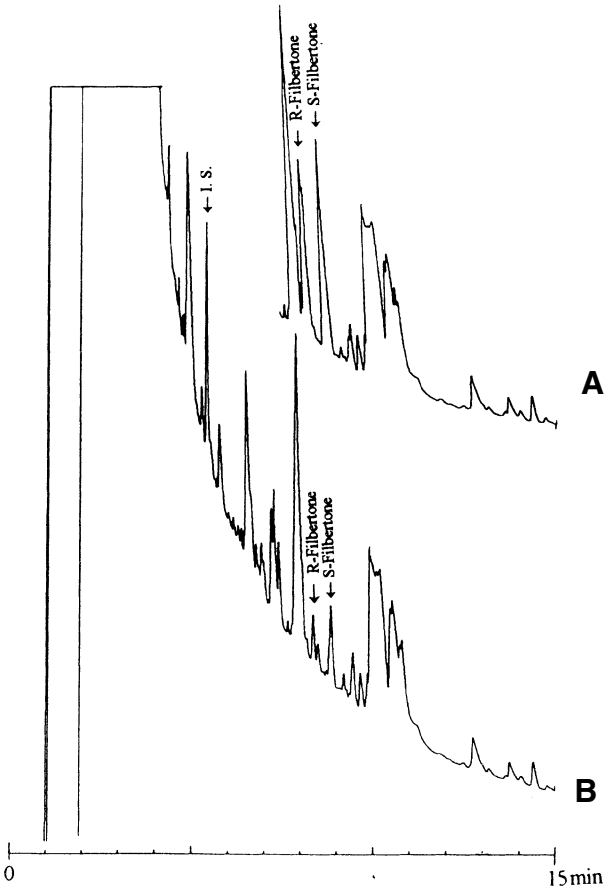

FIG. 2. Chromatogram (trace B) of a 5-µL injection of the simultaneous distillation–extraction (SDE) extract obtained from an olive oil mixed with a hazelnut oil (95:5) and subsequent concentration under a stream of inert gas. Trace A corresponds to the chromatogram resulting from addition of the standard solution of filbertone to the extract giving trace b. Fused-silica capillary column: 25 m × 0.25 mm i.d., coated with a 0.25-µm layer of Chirasil-β-Dex. The oven temperature was programmed at 5°C/min from 45 to 80°C and the final temperature was kept for 20 min. Extraction time: 2 h. Injection mode: splitless. Chromatograms a and b were recorded at the same full range. I.S. internal standard; for other abbreviations see Figure 1.

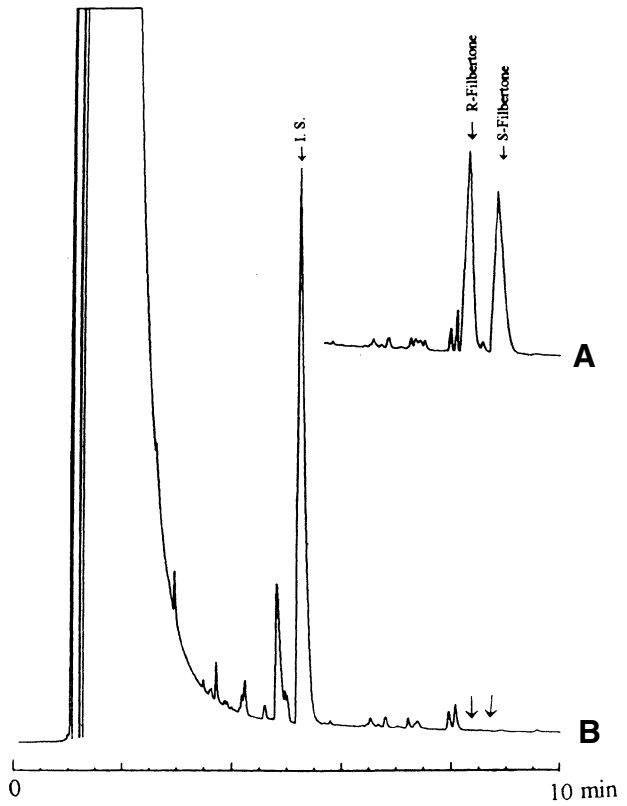

FIG. 3. Chromatogram (trace B) of a 2-µL injection of the SDE-extract obtained from a sunflower oil. Trace A corresponds to the chromatogram resulting from addition of the standard solution of filbertone to the extract giving trace B). Chromatographic column and experimental conditions as in Figure 2. Chromatograms a and b were recorded at the same full range. For abbreviations see Figures 1 and 2.

The proposed method allows one to obtain recoveries as high as 99.5 and 97.1%, for enantiomers *R* and *S*, respectively. These values were calculated using as a reference the average peak area resulting from sampling into GC three replicates of a standard solution.

Peak areas measured from *R*- and *S*-filbertone enabled us to obtain the enantiomeric excess (ee) equal to 45%. This value agrees with that previously obtained on roasted hazelnuts (21), although it must be considered that higher values have also been occasionally reported (19–21). In this respect, partial racemization of filbertone owing to heat may occur during the sample concentration procedure proposed in this work, as heating at 140°C is necessary. On the other hand, the enantiomeric distribution of filbertone may not be influenced by extraction and isolation conditions and, moreover, filbertone does not show a tendency for partial racemization in response to heat. In any case, the temperature used in this work during SDE operation is slightly higher than those reported in the literature (140 vs. 100–120°C) for racemization studies of filbertone (23). Consequently, racemization due to heat should not be excluded although the low enantiomeric excess obtained is more likely due to the shift of the enantiomeric excess to a higher (*R*)-content that may be caused by the presence of a unknown precursor in the hazelnut matrix which forms racemic filbertone by a chemical mechanism as previously suggested (23).

Keeping in mind that filbertone has not been reported so far as a component of virgin olive oil, whereas its presence has been detected in hazelnut oil, it is clear that the lack of filbertone does not necessarily imply the authenticity of an olive oil but its detection seems to suggest the presence of hazelnut oil. In this respect, however, further investigation about compositional differences between other vegetable oils is required. To illustrate this aspect, Figure 3 gives the chromatogram of a 2-µL injection of the SDE extract obtained from a sunflower oil, and Figure 4 shows the chromatogram obtained from a rapeseed oil. In both cases, the absence of filbertone was confirmed by adding a standard solution of filbertone to the corresponding SDE extracts and performing new runs under identical conditions (see Fig. 3A and 4A).

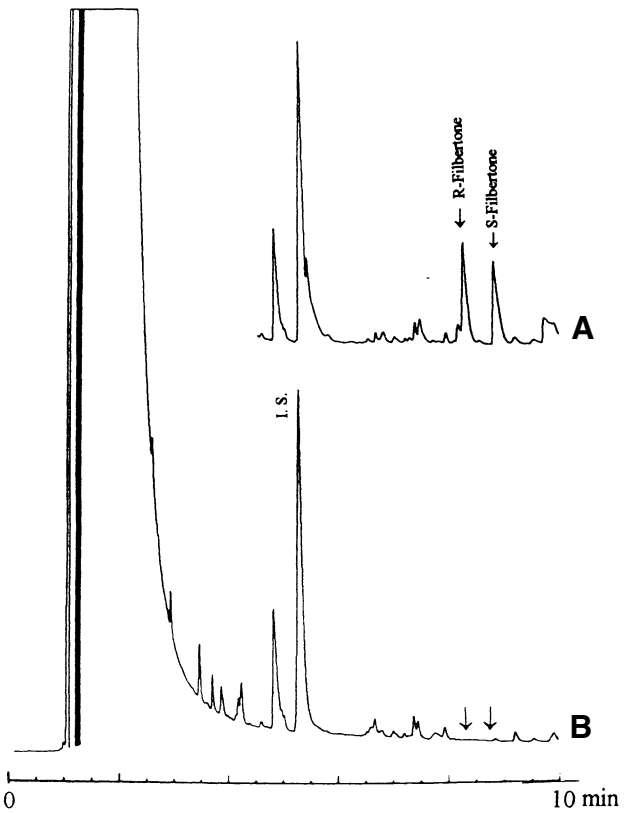

FIG. 4. Chromatogram (trace b) of a 2-μL injection of the SDE-extract obtained from a rapeseed oil. Trace (a) corresponds to the chromatogram resulting from addition of the standard solution of filbertone to the extract giving trace b). Chromatographic column and experimental conditions as in Figure 2. Chromatograms a and b were recorded at the same full range. For abbreviations see Figures 1 and 2.

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