Comparison of Different Methods for the Evaluation of the Authenticity of Olive Oil and Hazelnut Oil

Gracia P. Blanch, María del Mar Caja, María Luisa Ruiz del Castillo, and Marta Herraiz*

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

Different methods for the analysis of hazelnut oil and olive oil are compared in terms of the potential of each procedure in detecting compositional differences for evaluation of authenticity. These methods involve the use of different sample preparation techniques, namely simultaneous distillation−extraction (SDE) and supercritical fluid extraction (SFE), and the subsequent analysis by capillary gas chromatography using a programmable temperature vaporizer (PTV). On-line coupling of reversed-phase liquid chromatography to gas chromatography (RPLC−GC) was also considered for the analysis of the samples investigated. Working under the experimental conditions proposed, identification of olive oil, hazelnut oil, and mixtures of both oils (85:15) may be possible on the basis of the determination of the presence or absence of (*E*)-5-methylhept-2-en-4-one (filbertone).

Keywords: *Olive oil; hazelnut oil; filbertone; adulteration*

INTRODUCTION

The ability of an analytical method to establish compositional differences in food samples may be of great interest for the characterization of commercial products or to reveal falsifications and adulterations. To this aim, an interesting approach may be the identification of those compounds that are expected to be of significance for the character of the product or the intensity of its aroma. This is, however, a difficult task if the sample to be analyzed contains many species with wide ranges of polarities, concentrations, and molecular sizes. Specifically, control and evaluation of the authenticity of edible oils usually requires the isolation and analysis of minor compounds of the unsaponifiable matter by means of different procedures that are laborious, time-consuming, and, occasionally, a source of significant errors and not sufficiently sensitive for specific applications.

Obviously, separation, identification, and quantitation of such complex mixtures demand not only the previous enrichment of the compounds but also the high separation efficiency and selectivity achievable in capillary gas chromatographic analysis. When only applying standard GC instrumentation, however, various difficulties may arise, as the sample preparation step and the separation efficiency achievable may not be sufficient. For that reason, fraud cannot be shown in many cases with conventional control methods and development of new analytical procedures is strongly required. In this respect, different sample preparation techniques and multidimensional approaches should be considered in developing new methods suitable to analyze minor compounds in edible oils.

Several isolation and concentration methods have been previously tried for the analysis of complex mixtures. The simultaneous steam distillation−solvent extraction technique (Godefroot et al., 1981, 1982) has proved its usefulness for achieving an effective enrichment using small amounts of extractive solvents without requiring a further concentration step (Núñez et al., 1984; de Frutos et al., 1988; Blanch et al., 1993a,b).

On the other hand, the advantages of supercritical fluids (i.e., high diffusivities, low viscosities, wide density range available, etc.) with respect to traditional liquid solvent extraction procedures have been widely reported (Lee and Markides, 1990; Wenclawiak, 1992; Rizvi, 1994). Also, the potential of different multidimensional approaches based on SFE (e.g., off-line and on-line supercritical fluid extraction and gas chromatography) in food composition studies has already been investigated (Blanch et al., 1994, 1995).

On-line coupling of liquid chromatography and gas chromatography (LC−GC) is also of great interest for the analysis of complex mixtures, as it combines the effectiveness of sample preparation achievable in the LC step with the performance of the capillary gas chromatographic separation (Grob, 1991, 1995; Vreuls et al., 1994; Mondello et al., 1996). Specifically, a number of practical applications in food analyses have been reported including the use of both normal phase (NP) in the LC preseparation (Grob et al., 1990; Biedermann et al., 1996) and reversed phase (RP) (Señoráns et al., 1995a,b, 1996).

To date, most of the work that has been performed to detect adulteration of expensive edible oils, such as extra virgin olive oil, with oils of lower quality was based on the study of the unsaponifiable matter and mainly refers to the sterol composition (Grob and Romann, 1993; Grob et al., 1994a,b; Li-Chan, 1994; Paganuzzi, 1997). In this respect, however, the development of new analytical techniques for the detection of adulterated olive oils is strongly required. Specifically, no methods have proved to be reliable on detecting the adulterations of olive oil with hazelnut oil, although it is certainly a widely applied adulteration which so far has not been able to be detected at the concentrations of interest (i.e., around 5−20% of hazelnut oil in olive oil).

On the other hand, (*E*)-5-methylhept-2-en-4-one (filbertone) has been previously identified as the flavor-

* Fax (341) 564 48 53; e-mail mherraiz@fresno.csic.es.

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impact component of hazelnuts (Emberger et al., 1985), and its occurrence in raw and roasted fruits as well as in commercially available hazelnut cream has been reported (Jauch et al., 1989; Schurig et al., 1990; Güntert et al., 1991). To our knowledge, however, the presence of filbertone in edible oils has not been investigated yet.

The objective of this work was to evaluate the potential of various analytical techniques to detect the adulterations of olive oils with lower valuable oils, such as hazelnut oil, on the basis of compositional differences occurring in the mentioned oils. Specifically, our investigation was based on the ability to detect the presence or absence of filbertone in the sample oil.

EXPERIMENTAL PROCEDURES

Materials. For identification purposes, a test solution containing (*E*)-5-methylhept-2-en-4-one (Haarmann & Reimer, Holzminden, Germany) was used. Olive oil and hazelnut oil were purchased in the commercial market.

Tenax TA (80−100 mesh; Chrompack, Middelburg, The Netherlands) was used as packing material in the glass liner (80 mm × 1 mm i.d. × 2 mm o.d) of the PTV injector (Perkin-Elmer, Norwalk, CT). Prior to its use, it was conditioned under a stream of helium at 350 °C for 120 min.

Different extraction procedures (i.e., SDE and SFE) and separation techniques (capillary GC and RPLC−GC) were used under the following experimental conditions.

Steam Distillation−Solvent Extraction (SDE). Sample concentration was performed by means of the high-density solvent configuration of the commercial version (Chrompack) of the microdistillation−extraction device proposed by Godefroot et al. (1981). The SDE extracts were achieved starting from a 75-mL volume of the corresponding oil. In all cases, a 25-mL volume of water purified in a Milli-Q system (Millipore, Milford, MA) was added to the oil and homogenized in an ultrasonic bath and a 2-mL volume of distilled dichloromethane (SDS, Peypin, France) was employed as the extraction solvent. The sample and the solvent were heated by using a silicone bath at 140 °C and a water bath at 60 °C, respectively. In all instances, the coldfinger was maintained between −3 and −4 °C and the extraction time was established to be 2 h. Subsequently, the solvent extract was analyzed by capillary gas chromatography. The SDE apparatus was cleaned between successive extractions with acetone and Milli-Q purified water.

Supercritical Fluid Extraction (SFE). SFE experimentation was carried out in two different modes: (a) off-line supercritical fluid extraction using the original commercial configuration detailed below and (b) supercritical fluid extraction−capillary gas chromatographic analysis using the glass liner of the programmed temperature vaporizer (PTV), which served as the injector of the gas chromatograph, for trapping the extracted solutes.

All throughout the experimentation, a Hewlett-Packard 7680 extraction module (Wilmington, DE) provided with a nozzle/trap assembly (variable restrictor) was used. The mentioned extractor allowed the instant depressurization of the supercritical fluid as well as the decoupling of flow and pressure. The extracted analytes were deposited either on an internal trap (held at 20 °C) made of octadecylsilane (ODS) material where the supercritical fluid evaporates and leaves the system (approach a) or on the glass liner of the PTV located in place of the analyte trap of the commercial configuration (approach b) as described elsewhere (Blanch et al., 1994; Ibáñez et al., 1997). In the latter case, the chromatographic analysis was subsequently performed just by placing the glass liner in the PTV body of the gas chromatograph and achieving the thermal desorption of the analytes retained by increasing the PTV temperature, while in approach a, dichloromethane/methanol (50/50) was pumped through the trap once the extraction step had been completed. The SFE extract was then collected in a 1-mL vial, and subsequently, its RPLC−GC analysis was performed as described below.

Other experimental variables affecting the SFE step were established as follows:

Approach a. Extraction cell temperature: 60 °C. Trap temperature: 20 °C. Supercritical CO_2 flow: 4.0 mL/min. Sample weight in the cartridge: 1 mL. Extraction time: 5 min. CO_2 density: 0.80 g/mL (carbon dioxide conditions, T = 60 °C; P = 260 atm).

Approach b. Extraction cell temperature: 60 °C. Trap temperature: −25 °C. Supercritical CO_2 flow: 4.0 mL/min. Sample weight in the cartridge: 1 mL. Extraction time: 5 min. CO_2 density: 0.80 g/mL (carbon dioxide conditions, T = 60 °C; P = 260 atm).

Gas Chromatographic Analysis. In all cases (i.e., GC analysis of SDE extracts and thermal desorption of the analytes retained in the packing material of the glass liner after either SFE or LC−GC transfer), a 25-m × 0.25-mm-i.d. fused silica column coated with a 0.25-μm layer of SE-54 (5%/95% diphenyl/dimethylpolysiloxane) was used with helium as the carrier gas. Filbertone was identified by gas chromatographic−mass spectrometric analysis using an ITD-50 ion-trap detector (EI 70 eV) linked to the gas chromatograph. The typical fragment ions of the mass spectra and their relative intensities (%) were m/z 41 ($C_3H_5^+$, 34), m/z 69 (MW − C_4H_9, 100), m/z 98 (MW − C_2H_4, 14), and m/z 111 (MW − CH_3, 12).

On-Line Coupling Liquid Chromatography−Gas Chromatography (LC−GC). LC preseparation was performed using a Hewlett-Packard Model 1050 chromatograph (Wilmington, DE) equipped with a manual injection valve (Model 7125, Rheodyne, Cotati, CA) having a 20-μL sample loop and an ultraviolet (UV) detector (operated at 205 nm). Analyses were carried out on a 50- × 4.6-mm-i.d. column slurry packed with 10-μm silica phase modified with a *n*-butyl chain structure (C4, Vydac 214 TPB) which was maintained at 45 °C, by using a mixture of eluent A (methanol) and eluent B (water) as the mobile phase, at a flow rate of 2.0 mL/min. The initial conditions (70:30 eluent A:B) were followed from the third minute on by a linear gradient up to 22% water within 3 min, 78:22 eluent A:B was kept for 4 min, and a subsequent linear gradient was applied within 2 min up to 14% eluent B. Then, 86:14 eluent A:B was kept for 3 min, and finally, 100:0 eluent A/B was established within 4 min and maintained during the analysis. Methanol (HPLC grade) was purchased from Lab-Scan (Dublin, Ireland). A HPChem Station (Hewlett-Packard) was used for data acquisition from the UV detector.

For the GC system, a gas chromatograph (Perkin-Elmer, Model 8500, Norwalk, CT) provided with a flame ionization detector (FID) operated at 320 °C was used. Acquisition of data from the FID was performed with chromatography software from Perkin-Elmer Nelson Systems (Model 2600−rev 5.0).

LC−GC Transfer. Transfer into the GC of the volume fraction previously selected from the LC preseparation step was carried out using a multiport valve placed after the LC detector, a transfer line (a 80-cm × 0.32-mm-i.d. fused silica tube), and the PTV injector of the GC system acting as the interface, as described elsewhere (Señoráns et al., 1995a,b).

According to our previous experience regarding large sample introduction into capillary GC and on-line LC−GC transfer (Villén et al., 1992; Señoráns et al., 1993, 1995a,b, 1996), the helium flow rate applied during transfer was 1000 mL/min and the speed of sample transfer (equal to the flow rate during the LC preseparation step) could be established as high as 2000 μL/min. The silylated glass insert from the PTV injector was packed with a 5-cm plug length of Tenax TA, and the PTV initial temperature was held at 21 °C. Solvent elimination was achieved both in the evaporative mode (through the split line) and in the nonevaporative mode (through the injector bottom). To facilitate the elimination of the solvent which passes through the injector as a liquid, the GC column end from the injector body was removed before starting the transfer procedure and it was again connected after the purge time (6 min) established for each analysis to promote elimination of the remaining solvent from the glass liner (held at a fixed

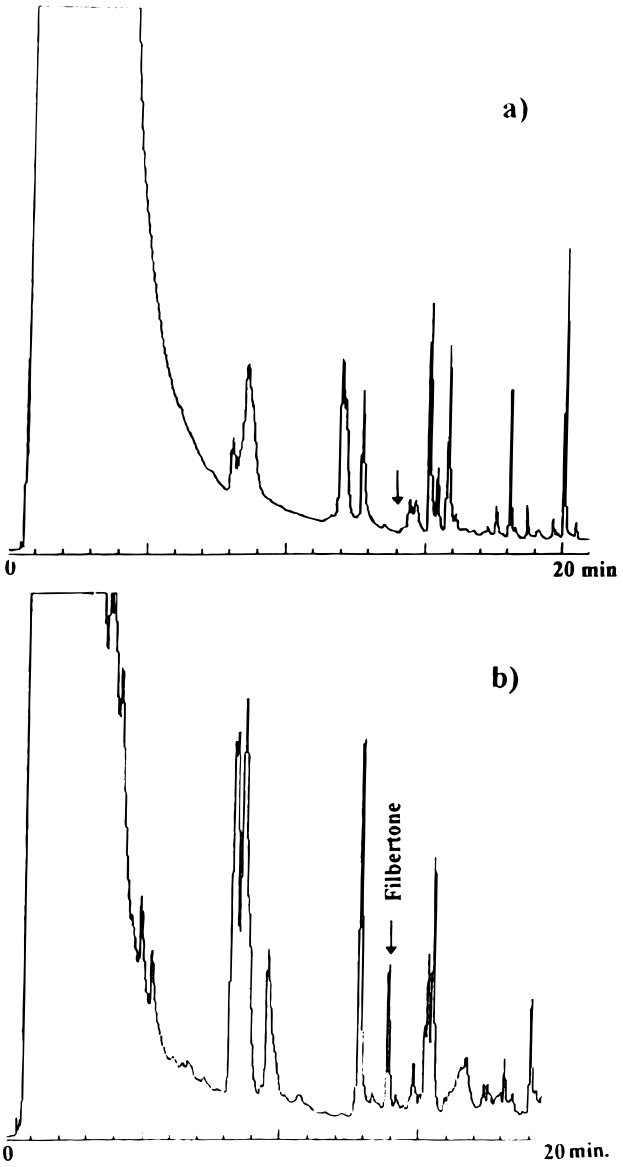

Figure 1. Chromatograms of a 1-μL injection of the SDE extracts obtained from an olive oil (a) and a hazelnut oil (b). Fused silica capillary column, 25 m × 0.25 mm i.d., coated with a 0.25-μm layer of SE−54. Initially the GC oven was maintained at 45 °C for 10 min and then programmed to 290 °C at 5 °C/min. The final temperature was kept for 30 min (chromatograms a and b were recorded at the same full range).

temperature under a helium flow). Once the solvent elimination step was complete, the transfer line was taken out from the PTV injector and its temperature was increased at 14 °C/s to 350 °C and maintained there for 11 min to achieve the thermal desorption of the retained solutes and its subsequent transfer to the capillary column. Between successive runs, the PTV injector was also heated to 350 °C to obtain acceptable blanks.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms resulting from the analysis of extracts obtained under identical experimental conditions by simultaneous distillation−extraction (without further concentration) of an olive oil (Figure 1a) and a hazelnut oil (Figure 1b). As can be seen, filbertone was detected in hazelnut oil (around 25−30 mg/L) while no peak was eluted at the corresponding retention time when analyzing an olive oil. Identification of filbertone in hazelnut oil was established by matching its retention time with that obtained for the authentic reference compound analyzed under identical conditions, and it was confirmed by gas chromatographic−mass spectrometric analysis. Moreover, the identification of filbertone was also confirmed by adding the standard to the oil sample and performing a new run.

When using SFE in such a way that the extracted analytes were trapped in octadecylsilane (approach a) and subsequently analyzed by capillary GC, filbertone could be detected neither from olive oil nor from hazelnut oil, most probably because the required dilution of the analytes to remove them from the trap, as explained in Experimental Procedures, decreased the overall sensitivity achievable with the analysis.

However, use of supercritical fluid extraction under the experimental conditions defining approach b resulted in an interesting alternative for filbertone detection purposes, as the ability to quantitatively transfer the extracted analytes to the chromatographic column without requiring the use of liquid extraction solvents yielded high sensitivity for the overall analysis. As a result, the occurrence of filbertone in the hazelnut oil (Figure 2b) and its absence in the olive oil (Figure 2a) was also confirmed by means of SFE.

As far as the use of on-line coupling of reversed-phase chromatography and capillary gas chromatography is concerned, parts a and b of Figure 3 depict the gas chromatograms obtained from LC−GC transfer of a 3000-μL volume of methanol:water eluent resulting from LC preseparation of the SFE extracts of an olive oil and a hazelnut oil, respectively. From the chromatogram given in Figure 3b, the occurrence of filbertone in the hazelnut oil can also be confirmed, while the analysis performed under identical experimental conditions did not reveal its presence in olive oil (Figure 3a). It is interesting to mention that although, under the experimental conditions used, the volume of the whole fraction of interest was as high as 3000 μL, its transfer only took a relatively short period of time (i.e., 90 s).

Concerning the repeatability achievable with the different analytical methods proposed, it should be noted that the relative standard deviation values (RSD, calculated from a minimum of three replicates) obtained for filbertone were 4.7%, 25.5%, and 9.1% when SDE, SFE (approach b), and RPLC−GC analysis, respectively, were performed. The high value obtained for the SFE method (approach b) is most likely due to rearrangement of the packing material which is repetitively maintained at high pressure and then depressurized.

As far as the time required for each analysis is concerned, the overall procedure (i.e., including sample preparation if necessary and chromatographic analysis itself) demands approximately 2.5 h if SDE is performed, 45 min when using SFE (approach a and approach b as well), and less than 85 min if RPLC−GC analysis is carried out.

From Figures 1−3, it can be concluded that under the investigated experimental conditions, the SFE method is not sufficiently selective and the more selective LC−GC is not sufficiently sensitive, while the SDE method may allow sensitive and selective analyses. To illustrate the practical possibilites of the latter mentioned method to detect adulterations, Figure 4 gives the chromatogram of the SDE-extract resulting from a mixture of the same olive and hazelnut oils (85:15) given in Figure 1. As can be seen, the presence of filbertone is easily

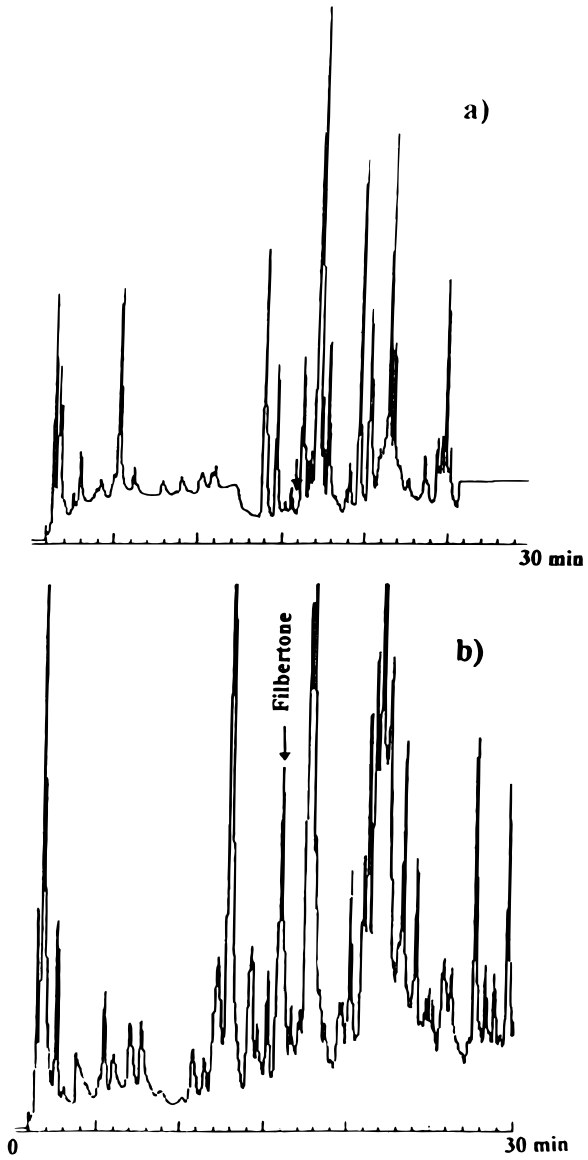

Figure 2. Chromatograms resulting from the thermal desorption of the trapped material retained in the glass liner of the PTV located in place of the analyte trap in the commercial equipment after SFE of an olive oil (a) and a hazelnut oil (b). Chromatographic column and oven temperature as in Figure 1 (chromatograms a and b were recorded at the same full range).

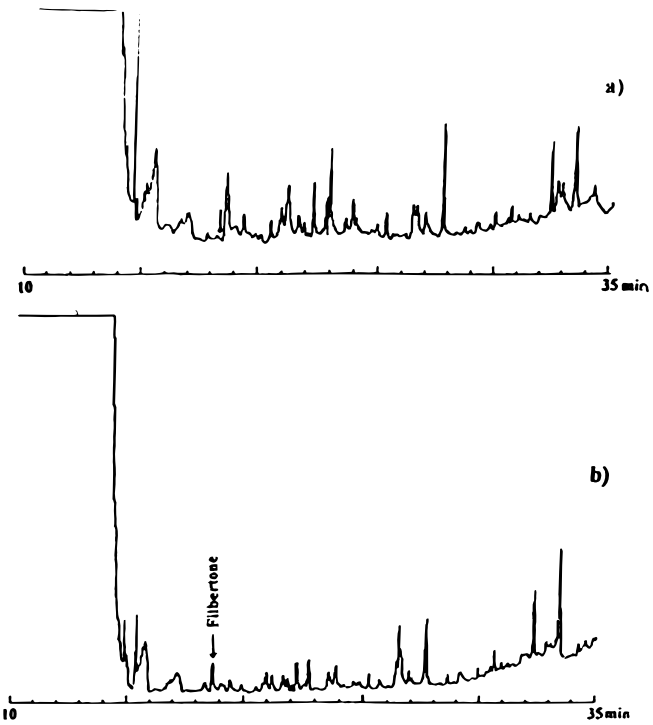

Figure 3. Chromatograms obtained from LC−GC transfer of a 3000-μL fraction of mehanol:water eluent (flow rate, 2000 μL/min) resulting from the SFE extracts obtained from an olive oil (a) and a hazelnut oil (b). Chromatographic column and oven temperature as in Figure 1 (chromatograms a and b were recorded at the same full range).

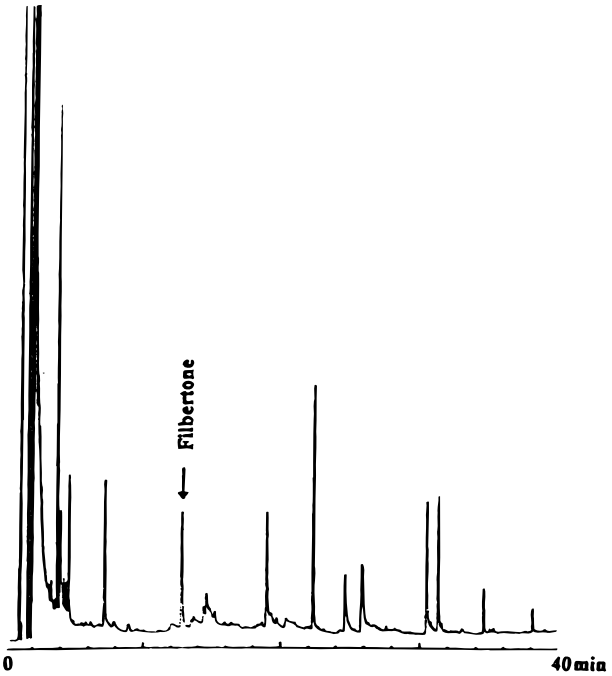

Figure 4. Chromatogram of a 1-μL injection of the SDE extract obtained from an olive oil mixed with a hazelnut oil (85:15). Chromatographic column and oven temperature as in Figure 1.

detected simply by concentrating under a stream of inert gas the SDE-extract obtained.

An important aspect concerns the question if the filbertone may be easily removed upon gentle deodorization of the oil. However, adulteration of virgin olive oil by deodorized oil does not seem to be very common, as it would lead to an increase of trans fatty acids so that its content in such adulterated oils could exceed the legal tolerances for cold pressed oils, and moreover, such adulteration would be easily detectable (Brühl, 1996). Therefore, it can be assumed that, at least in some cases, virgin olive oil will be adulterated with unrefined (i.e., nondeodorized) hazelnut oil, thus allowing the detection of filbertone as proposed here.

Summarizing, from the obtained results, it can then be concluded that the ability to use various analytical procedures suitable to detect compositional differences in olive oil and hazelnut oil may be of interest for their characterization and evaluation of their authenticity on the basis of establishing the presence or absence of filbertone. Satisfactory precision is achieved in the determination of filbertone by using both SDE and RPLC−GC analysis, but a rather high RSD value is obtained if SFE (approach b) is used, although, as a counterpart, higher sensitivities may then be observed. However, the low sensitivity achievable with the LC−

GC method should not be taken to conclude its lack of suitability for the detection of adulterated olive oils on the basis of the determination of filbertone. On the contrary, the selectivity of the technique suggests its potential in this respect, although further optimization is obviously required.

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