Ultrasonically Assisted Solid-Phase Extraction and GC Analysis of Filbertone in Hazelnut Oil

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ABSTRACT: Ultrasound was used to assist solid-phase extraction (SPE) of filbertone (*E*-5-methylhept-2-en-4-one) from hazelnut oil. Interferences from TG were reduced for effective separation and detection during chromatographic analysis. The enantiomeric distribution of filbertone was determined. Different sorbent materials, sample volumes, and eluents were tested, and the effect on filbertone recovery by ultrasound during the elution step was evaluated. Experimentation performed on a 2-mL volume of oil diluted with a 2-mL volume of *n*-hexane, using a silica modified with either a cyano or phenyl group during the extraction, and ultrasound-assisted elution with a 1-mL volume of chloroform allowed filbertone recoveries of up to 94.4% (relative SD 6.5%) vs. 11% obtained when ultrasound was not applied. This improvement is most likely due to a mechanism of cavitation. Ultrasonically assisted SPE is proposed as an accessible and simple alternative to multidimensional chromatographic techniques to accomplish the reliable determination of the enantiomeric excess of chiral compounds in complex matrices, such as hazelnut oil, since experimental conditions that can bring about racemization are not required.

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KEY WORDS: Chirality, filbertone, hazelnut oil, SPE, ultrasound-assisted extraction.

Filbertone (*E*-5-methylhept-2-en-4-one) has been described as a major flavor component of hazelnut (1,2). The absence or presence of filbertone also has been proposed to differentiate olive oil from hazelnut oil (3) and, thus, to detect adulterated samples (4). The interest in analyzing both *R*- and *S*-enantiomers of filbertone in a hazelnut oil has been underlined as a means to improve the reliability of filbertone detection as well as to investigate the natural compositional variability of filbertone in hazelnut oils (5).

The importance of the enantiomeric composition of chiral compounds in foods recently has been highlighted (6–8). Stereochemical analysis has been useful in differentiating naturally occurring compounds from synthetic ones (9) and monitoring fermentations, adulteration, and storage (8,10). However, the fact that foods are very complex matrices may make enantiomer separation difficult, especially at low concentrations, because of the lack of peak resolution during chromatographic enantiomeric separations. Therefore, identification of enantiomers in foodstuffs has been largely ignored. Isolation of minor chiral compounds in oils may be difficult as it requires a sample handling procedure adequate to preclude racemization conditions as well as complete elimination of TG to avoid undesirable interferences.

Multidimensional chromatographic techniques (11,12) may be used to overcome these problems by allowing improved peak resolution of complex mixtures. We have already proposed on-line coupling of reversed-phase LC and GC (RPLC-GC) for the detection of filbertone in hazelnut oil (4,13,14).

Multidimensional techniques are not currently available in most laboratories as they are complex and tedious to operate and must be optimized before the analysis can be done. Therefore, alternative simpler separation strategies to isolate and concentrate analytes from complex matrices using simple and rapid techniques are needed. Solid-phase extraction (SPE) is an efficient alternative for sample preparation prior to chromatographic analysis (15). In the last few years, SPE has been used to separate a variety of edible oil components, including tocopherols and sterols (16,17), phenolic compounds (18,19), and DG (20,21). In the same way, some authors have also demonstrated the efficiency of ultrasound in extraction procedures (22,23) and, in particular, its use in the isolation of oil components (24,25). However, the applicability of SPE for the analysis of filbertone in oils as well as the use of ultrasound to improve the recovery of the compound of interest from the sorbent during the SPE procedure, to our knowledge, has not been demonstrated before.

The objective of this research was to evaluate the potential of ultrasonically assisted SPE and GC for the chiral analysis of filbertone in hazelnut oil.

EXPERIMENTAL PROCEDURES

Samples and materials. (*E*)-5-Methylhept-2-en-4-one (filbertone) was obtained from Haarmann & Reimer (Holzminden, Germany), and hazelnut oil was purchased from the commercial market. Chloroform and *n*-hexane (HPLC grade) were acquired from Lab Scan (Dublin, Ireland). Methanol (HPLC grade) was purchased from Scharlau Chemie, S.A. (Barcelona, Spain), and the water used was obtained from a Milli-Q water purification system (Millipore, Milford, MA).

Ultrasonically assisted SPE (UASPE). A low-frequency ultrasonic bath (Selecta, Madrid, Spain) was used to perform

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the UASPE procedure. Ultrasonic radiation (40 kHz, 150 W) was applied to each sample for 2 min at approximately 22°C. The UASPE was performed by immersing a vessel containing the SPE cartridge in an ultrasonic bath during the elution step.

SPE. Packed conventional 3-mL SPE cartridges with a 500-mg weight of different sorbents (i.e., silica chemically modified by groups of different polarity such as cyano, phenyl; and some hydrocarbon chains, namely, C_2, C_8, and C_{18}) were obtained from International Sorbent Technology (IST, Hengoed, Mid Glamorgan, United Kingdom). Methanol, methanol/water, and chloroform were tested as elution solvents. SPE operation mode included the following steps: (i) sample pretreatment, which involved dilution of the oil in a solvent; (ii) SPE tube packing conditioning, which was accomplished by rinsing the tube with a 1-mL volume of both the matrix and the elution solvents; (iii) sample application, which was carried out by flowing slowly through the SPE tube; and (iv) analyte elution with an appropriate solvent at a flow rate of approximately 1 mL/min.

Sorbent materials and elution solvents were evaluated relative to recovery obtained for filbertone (defined as the ratio between the amounts extracted vs. that in the sample before SPE). The choice of the solvent for filbertone release was made on the basis of several factors: (i) tested sorbent material; (ii) strength necessary to elute the filbertone with the lowest possible volume; (iii) enough volatility to guarantee compatibility with the subsequent GC analysis; and (iv) ease of elimination if further concentration was required.

To establish the so-called "safe sampling volume" (i.e., the sample volume that can be applied without loss of analyte), the SPE step was carried out starting with different sample volumes (i.e., 1, 2, 5, and 8 mL). The bed weight (500 mg) and the size (3 mL) of the SPE cartridge were selected on the basis of both the sample volume and the mass of the compound to be extracted. The results showed that percolation through the packing material of more than 2-mL volumes yielded significant decreases of filbertone recoveries. Most probably, this is due to exceeding the analyte breakthrough (maximum analyte mass that can be retained by the SPE packing) in the experimental conditions proposed. Consequently, the use of a 2-mL volume was considered to be preferable to 1 mL because of the higher filbertone content obtained in the final extract with no apparent decrease in its recovery. In all cases, the elution was performed in a single step with a 1-mL volume, as the use of two aliquots (2×1 mL) did not result in higher recoveries. Once the SPE step had been completed, the sensitivity required for the GC analysis was achieved by concentration under a nitrogen stream.

GC analysis. A PerkinElmer (Norwalk, CT) Model 8500 gas chromatograph fitted with a programmed temperature vaporizer (PTV) injector and an FID operated at 250°C was used. Samples were placed on a 25-m × 0.25-mm i.d fused-silica column coated with a 0.25-μm layer of Chirasil-β-Dex (Chrompack, Middelburg, The Netherlands), and using helium as the carrier gas. Initially, the column was maintained 5 min at 45°C and then raised (3°C/min) to 90°C and then to 180°C (at 5°C/min). A 0.2-μL volume of the concentrated extract was injected into the chromatographic column using the PTV injector in the splitless mode (at 250°C). Data acquisition from the FID was performed by using an HP ChemStation (Hewlett-Packard, Wilmington, DE).

The use of Chirasil-β-Dex as the GC column allowed the determination of both *R*- and *S*-enantiomers of filbertone by using the term enantiomeric excess, ee, expressing the excess of one enantiomer over the other (26). Identification of filbertone was carried out from both *R*- and *S*-enantiomers by matching their retention times with those measured when standards were analyzed under identical experimental conditions. Moreover, the presence of both enantiomers of filbertone was confirmed by adding a solution of the standard to a corresponding extract similarly analyzed.

RESULTS AND DISCUSSION

Experimental conditions were evaluated to achieve both the elimination of TG from the hazelnut oil and the isolation of filbertone. *n*-Hexane was chosen as the most suitable solvent to reduce oil viscosity, thus ensuring its free flow through the sorbent.

Despite the low volatility of the eluent methanol/water (65:35, vol/vol), it was included in the present study because of its proven ability to elute filbertone from alkyl-modified silica used as the stationary phase when reversed-phase LC (RPLC) was employed as the preseparation technique (14). However, the use of the mentioned eluent was finally discarded due to the difficulty in eliminating it in the preconcentration step prior to GC analysis. Other combinations, namely, C_2-methanol, cyano-methanol/water (65:35), and C_{18}-methanol, were ruled out as well because of poor separation of the matrix components or extremely low recoveries, which made the identification of filbertone impossible.

Initially, the use of silica modified with a phenyl (or cyano) group as the sorbent together with chloroform as the elution solvent for SPE seemed to be the best option to isolate filbertone from a hazelnut oil. However, under these conditions low filbertone recoveries of 11–16% were achieved. SPE performance was improved by establishing experimental conditions using ultrasound to enhance filbertone release from the sorbent of the cartridge. The UASPE was conducted by performing the elution step with the SPE cartridge in a vessel immersed in an ultrasonic bath.

The application of ultrasonication greatly improved extraction of filbertone. Specifically, filbertone recoveries (calculated for *R*- + *S*-enantiomers from three replicates) increased from 12.1 to 80.0% (when the sorbent silica was modified with a phenyl group) and from 11.0 to 94.4% (when silica was modified with a cyano group) (Fig. 1). As the temperature is kept constant (22–23°C) throughout UASPE, it seems evident that the mechanism of filbertone release must be that of cavitation. This phenomenon, which is based on the formation and activity of bubbles in a liquid due to local negative pressures, is frequent in ultrasonic procedures and results in a variety of chemical effects (27,28).

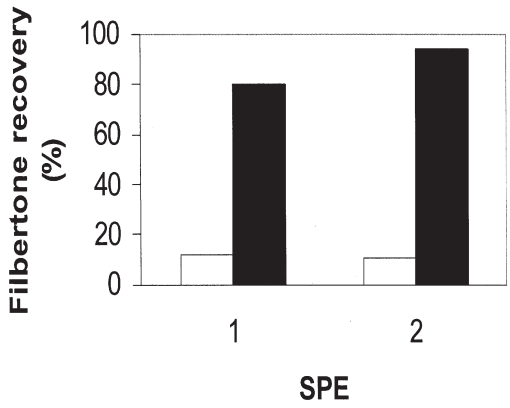

FIG. 1. Effect of the use of ultrasonically assisted solid-phase extraction (SPE) on filbertone recovery from hazelnut oil. Differences in series 1 and 2 refer to the use of phenylsilica or cyanosilica, respectively, as the packing material during the SPE step. In both cases, chloroform was used as the elution solvent. (□) Without ultrasonication; (■) with ultrasonication.

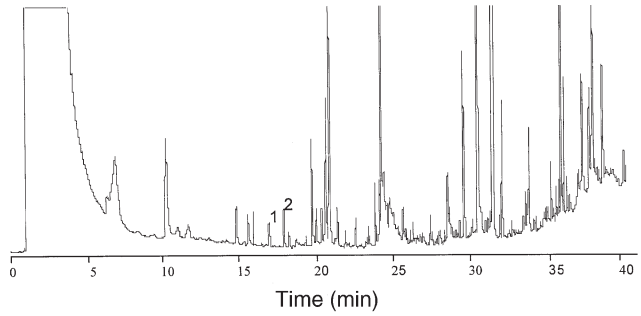

FIG. 2. Gas chromatogram obtained from a hazelnut oil using ultrasonically assisted SPE with endcapped cyanopropyl silica as the sorbent material and chloroform as the eluent. Conditions: fused-silica capillary column: 25 m × 0.25 mm i.d., coated with a 0.25-µm layer of Chirasil-β-Dex. Sample volume: 0.2 µL. Identification: peak number 1, *R*-filbertone; peak number 2, *S*-filbertone. See text for further experimental details.

To verify the suitability of the developed method, it was applied to a conventional sample of hazelnut oil. Figure 2 shows the chromatogram of an SPE-GC analysis of hazelnut oil under the experimental conditions presented above. The high resolution achieved for *R*- and *S*-filbertone allowed detection and quantification of the compound. In this respect, it is clear that a more reliable detection of filbertone could be performed on the basis of determining both *R*- and *S*-filbertone enantiomers (4).

Figure 2 also shows that other constituents of hazelnut oil were extracted and eluted together with filbertone. This is probably due to omission of a washing step, which is usually recommended immediately after adding the sample and prior to the analyte elution, to remove undesired compounds from the SPE column. This step was found to be unnecessary for filbertone separation, and its omission minimized losses of the compound.

The relative SD value (RSD, calculated from three replicates) was 6.5% (for *R* + *S* forms); the corresponding detection limits (estimated from a signal equal to five times the baseline noise) were 0.14 and 0.11 mg/L for *R*- and *S*-enantiomers, respectively. Likewise, filbertone exhibited an ee of 20.2% (26), the *S*-enantiomer being the predominant isomer. The ee value was calculated from peak areas by considering the excess of the predominant enantiomer, expressed as a percentage, i.e., [(predominant enantiomer − minor enantiomer)/(predominant enantiomer + minor enantiomer)] × 100. The concentration of filbertone in the analyzed oil was found to be 1.18 mg/L; 75 min was the time of the overall analytical procedure.

Considerably higher concentrations of filbertone in hazelnut oils previously had been detected in our laboratory by RPLC-GC [e.g., 17.4 mg/L in the hazelnut oil used in this study during the SPE optimization step vs. 1.18 mg/L in the hazelnut oil analyzed with the optimized procedure (14)]. Moreover, the comparison of the filbertone enantiomeric composition estimated from the latter hazelnut oil (i.e., about 20%) and from compositions in other oils (about 73%) (5) suggests that these differences may depend on whether the oil is obtained from roasted or unroasted hazelnuts. Also, it is evident that the exposure of the sample to some kind of heating (i.e., deodorization) could have promoted the loss of the analyzed compound and/or the modification of its enantiomeric distribution.

The method proposed here (i.e., the dilution of a 2-mL volume of oil with a 2-mL volume of *n*-hexane, the SPE step using a silica modified with either cyano or phenyl groups, the ultrasound-assisted elution with a 1-mL volume of chloroform, and the subsequent GC analysis) may be a valuable alternative for the identification of filbertone in hazelnut oil. The UASPE method is proposed as an interesting and versatile option. Besides its simplicity, accessibility, and low cost, further advantages of UASPE are the requirement of low volumes of organic solvents and the fact that it allows a reliable determination of the enantiomeric composition of a chiral marker since the sample is not exposed to conditions (e.g., heat) that could cause racemization. Moreover, the results obtained suggest the great potential of ultrasonication to enhance analyte recoveries in the elution step and, therefore, to enlarge the field of application of SPE by allowing the development of more sensitive and rapid methods based on ultrasonically assisted elution.

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REFERENCES

1. Jauch, J., D. Schmalzing, V. Schurig, R. Emberger, R. Hopp, M. Köpsel, W. Silberzahn, and P. Werkhoff, Isolation, Synthesis, and Absolute Configuration of Filbertone—The Principal Flavor Component of the Hazelnut, *Angew. Chem. Int. Ed. Engl. 28*:1022–1023 (1989).

2. Güntert, M., R. Emberger, R. Hopp, M. Köpsel, W. Silberzahn, and P. Werkhoff, Chirospecific Analysis in Flavor and Essential Oil Chemistry. Part A. Filbertone—The Character Impact Compound of Hazelnuts, *Z. Lebensm. Unters. Forsch. 192*:108–110 (1991).
3. Blanch, G.P., M.M. Caja, M.L. Ruiz del Castillo, and M. Herraiz, Comparison of Different Methods for the Evaluation of the Authenticity of Olive Oil and Hazelnut Oil, *J. Agric. Food Chem. 46*:3153–3157 (1998).
4. Blanch, G.P., M.M. Caja, M.L. Ruiz del Castillo, and M. Herraiz, A Contribution to the Study of the Enantiomeric Composition of a Chiral Constituent in Hazelnut Oil Used in the Detection of Adulterated Olive Oil, *Eur. Food Res. Technol. 210*:139–143 (1999).
5. Ruiz del Castillo, M.L., E. Gómez Caballero, G.P. Blanch, and M. Herraiz, Enantiomeric Composition of Filbertone in Hazelnuts and Hazelnut Oils from Different Geographical Origins, *J. Am. Oil Chem. Soc. 79*:589–592 (2002).
6. Armstrong, D.W., C.-D. Chang, and W.Y. Li, Relevance of Enantiomeric Separations in Food and Beverage Analyses, *J. Agric. Food Chem. 38*:1674–1677 (1990).
7. Stalcup, A.M., K.H. Ekborg, M.P. Gasper, and D.W. Armstrong, Enantiomeric Separation of Chiral Components Reported to Be in Coffee, Tea, or Cocoa, *Ibid. 41*:1684–1689 (1993).
8. Ekborg-Ott, K.H., and D.W. Armstrong, Stereochemical Analysis of Food Components, in *Chiral Separations, Applications and Technology*, edited by S. Ahuja, American Chemical Society, Washington, DC, 1997, pp. 201–270.
9. Blanch, G.P., M.L. Ruiz del Castillo, and M. Herraiz, Enantiomer Analysis of Chiral Lactones in Foods by On-line Coupled Reversed-Phase Liquid Chromatography–Gas Chromatography, *J. Chromatogr. Sci. 36*:589–594 (1998).
10. Marchelli, R., A. Dossena, and G. Palla, The Potential of Enantioselective Analysis as a Quality Control Tool, *Trends Food Sci. Technol. 7*:113–119 (1996).
11. Bertsch, W., Two-Dimensional Gas Chromatography. Concepts, Instrumentation, and Applications—Part 1: Fundamentals, Conventional Two-Dimensional Gas Chromatography, Selected Applications, *J. High Resolut. Chromatogr. 22*:647–665 (1999).
12. Liu, Z., and M. Lee, Comprehensive Two-Dimensional Separations Using Microcolumns, *J. Microcolumn Sep. 12*:241–254 (2000).
13. Caja, M.M., M.L. Ruiz del Castillo, R. Martínez-Alvarez, M. Herraiz, and G.P. Blanch, Analysis of Volatile Compounds in Edible Oils Using Simultaneous Distillation–Solvent Extraction and Direct Coupling of Liquid Chromatography with Gas Chromatography, *Eur. Food Res. Technol. 211*:45–51 (2000).
14. Ruiz del Castillo, M.L., M.M. Caja, M. Herraiz, and G.P. Blanch, Rapid Recognition of Olive Oil Adulterated with Hazelnut Oil by Direct Analysis of the Enantiomeric Composition of Filbertone, *J. Agric. Food Chem. 46*:5128–5131 (1998).
15. Hennion, M.C., Solid-Phase Extraction: Method Development, Sorbents, and Coupling with Liquid Chromatography, *J. Chromatogr. A 856*:3–54 (1999).
16. Toivo, J., V. Piironen, P. Kalo, and P. Varo, Gas Chromatographic Determination of Major Sterols in Edible Oils and Fats Using Solid-Phase in Sample Preparation, *Chromatographia 48*:745–750 (1998).
17. Lechner, M., B. Reiter, and E. Lorbeer, Determination of Tocopherols and Sterols in Vegetable Oils by Solid-Phase Extraction and Subsequent Capillary Gas Chromatographic Analysis, *J. Chromatogr. A 857*:231–238 (1999).
18. Litridou, M., J. Linssen, H. Schols, M. Bergmans, M. Posthumus, M. Tsidimou, and D. Boskou, Phenolic Compounds in Virgin Olive Oils: Fractionation by Solid Phase Extraction and Antioxidant Activity Assessment, *J. Sci. Food Agric. 74*:169–174 (1997).
19. Pirisi, F.M., P. Cabras, C.F. Cao, M. Migliorini, and M. Muggelli, Phenolic Compounds in Virgin Olive Oil. 2. Reappraisal of the Extraction, HPLC Separation and Quantification Procedures, *J. Agric. Food Chem. 48*:1191–1196 (2000).
20. Marquez-Ruiz, G., N. Jorge, M. Martin-Polvillo, and M.C. Dobarganes, Rapid, Quantitative Determination of Polar Compounds in Fats and Oils by Solid Phase Extraction and Size Exclusion Chromatography Using Monostearin as Internal Standard, *J. Chromatogr. A 749*:55–60 (1996).
21. Conte, L.S., O. Koprivnjak, S. Fiorasi, and L. Pizzale, Solid-Phase Extraction Applied to Diacylglycerol Determination in Foods, *Riv. Ital. Sostanze Grasse 74*:411–414 (1997).
22. De Lucas, A., A. Durán, M. Carmona, and M. Lapuerta, Characterization of Soluble Organic Fraction in DPM: Optimization of the Extraction Method, *Soc. Automot. Eng. 1477*:225–230 (1999).
23. Romdhane, M., and C. Gourdon, Investigation in Solid–Liquid Extraction: Influence of Ultrasound, *Chem. Eng. J. 87*:11–19 (2002).
24. Bruni, R., A. Medici, A. Guerrini, S. Scalia, F. Poli, M. Muzzoli, and G. Sacchetti, Wild *Amaranthus caudatus* Seed Oil, a Nutraceutical Resource from Ecuadorian Flora, *J. Agric. Food Chem. 49*:5455–5460 (2001).
25. Arino, A., I. Arberas, G. Renobales, and J.B. Domínguez, Influence of Extraction Method and Storage Conditions on the Volatile Oil of Wormwood (*Artemisia absinthium*), *Z. Lebensm. Unters. Forsch. A 209*:126–129 (1999).
26. Schurig, V., Terms for the Quantitation of a Mixture of Stereoisomers, *Enantiomer 1*:139–143 (1996).
27. Maisonhaute, E., P.C. White, and R.G. Compton, Surface Acoustic Cavitation Understood *via* Nanosecond Electrochemistry, *J. Phys. Chem. B 105*:12087–12091 (2001).
28. Laborde, J.L., G. Servant, A. Hita, J.P. Caltagirone, and A. Gerard, Cavitation Bubble Field at Low Frequency: Simulations and Experiments, *FED 253*:345–349 (2000).

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