Enantiomeric Composition of Filbertone in Hazelnuts and Hazelnut Oils from Different Geographical Origins

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ABSTRACT: The potential of enantiomeric analysis of (*E*)-5-methyl-hept-2-en-4-one (filbertone) for authenticity control of oils is evaluated for a selection of both hazelnuts and hazelnut oils from different geographical origins. The analytical method proposed involves the enantioselective GC analysis of the fraction resulting from the preseparation with HPLC of either a hazelnut extract or a hazelnut oil. The obtained results demonstrate that the proposed procedure avoids the partial or total racemization of filbertone and thus allows the reliable determination of its enantiomeric composition. Moreover, the enantiomeric ratio of filbertone is not affected by the cold pressing process of hazelnut oil production and was found to be nearly constant for oils obtained from unroasted hazelnuts of different geographical origins.

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KEY WORDS: Enantiomeric ratio, filbertone, hazelnut, hazelnut oil.

Over the last few decades, advances in analytical techniques have brought about a considerable increase in the knowledge of safety, authenticity, and quality issues of foods. In this respect, it is clear that recent improvements in chromatographic methods and the development of hyphenated and coupled techniques have contributed significantly to the progress of analytical food chemistry.

Specifically, the convenience of screening the optical purity of food components has been stressed during the past few years owing to the fact that enantiomers may have not only different sensory properties but also different nutritional values and biological activities (1–4). In fact, the potential of enantioselective capillary GC with different chiral stationary phases (e.g., amino acid derivatives or modified cyclodextrins) for authenticity control has already been demonstrated for different types of samples (5–9), but the complexity of food matrices still demands the development of new, rapid, and efficient methods for stereochemical studies.

Although some of the chiral food components are exclusively present as enantiomerically pure compounds, many chiral constituents occur as enantiomeric mixtures with specific concentrations. In these cases, the ratio in which both enantiomers are present can be taken as a parameter for quality assessment in food studies as it can be useful for the control of technological processes, determination of the geographical origin, identification of adulterations, evaluation of aging and shelf life, and so on.

It is necessary to establish whether the enantiomeric ratio for a specific chiral component is expected to be constant for a certain matrix or, on the contrary, whether it may vary depending on different factors (e.g., geographical origin and climatic conditions among others). Consequently, whether the variability of natural products can make the enantiomeric ratio insufficient for authenticity control must be investigated (10). In such a situation, it is of utmost importance to ensure the reliability of the enantiomeric composition of the chiral constituent of interest by applying a suitable analytical procedure that avoids its total or partial racemization during analysis.

In previous work, *(E)*-5-methyl-hept-2-en-4-one (filbertone) has been identified as the principal flavor component of hazelnuts (11–13). Its occurrence in raw and roasted fruits as well as in commercially available hazelnut cream has been reported (14,15). Moreover, previous studies on the presence or absence of filbertone in edible oils support the assumption that it is an adequate marker to distinguish between hazelnut oil and olive oil and, thus, to detect adulterated samples (16–18). However, data reported so far have not ascertained the possibility of using the enantiomeric composition of filbertone as a criterion to detect the adulteration of olive oil with hazelnut oil.

The objective of the present work was to evaluate the reliability of the determination of the enantiomeric composition of filbertone and to study the way in which the enantiomeric ratio of filbertone in hazelnut oil depends on its provenance or can be affected by the production procedure.

EXPERIMENTAL PROCEDURES

Materials. A standard of (*E)*-5-methyl-hept-2-en-4-one, used for identification purposes, was provided by Haarman & Reimer (Holzminden, Germany). The hazelnuts were either supplied by farmers or purchased in the commercial market, and the hazelnut oils were obtained either from the commercial market (three samples) or by cold pressing (two samples) the same hazelnuts previously analyzed. 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). Gaschrom (Alltech, Deerfield, IL) was used as the packing material to retain the solutes transferred from LC into GC by placing a 5-cm-length plug in the silylated glass liner (75 mm × 1 mm i.d. × 2 mm o.d.) of the GC injector. Prior to its use,

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Gaschrom was conditioned under a helium stream at 50ºC for 10 min, and the temperature was increased successively to 100, 150, 200, and 250ºC for 10 min each.

Sample preparation. Hazelnuts were ground, and a 1-g sample weight was transferred into a 10-mL flask. After adding a 2-mL volume of dichloromethane (SDS, Peypin, France), the flask was immersed in an ultrasonic bath and maintained for 1 min. Subsequently, the organic solvent was removed by filtration and the solid was again extracted with dichloromethane as described. The overall process was repeated three times, and the resulting extracts were combined and subsequently filtered through a 0.22-µm filter prior to their sampling in the gas chromatograph. Hazelnut oil extracts were prepared following the same procedure, although methanol was used as the solvent instead of dichloromethane.

Hazelnuts and hazelnut oils were analyzed by both GC and on-line LC coupled with GC. GC analyses were performed for both hazelnut and hazelnut oil extracts, and LC–GC was used for either the hazelnut extracts or the direct analysis of hazelnut oils.

GC analysis of both hazelnut and hazelnut oil extracts. A 1-µL volume of the extracts obtained as described was sampled through a programmed temperature vaporizer (PTV) operated in the splitless mode. A 2-cm plug length of glass wool was placed in the silylated glass liner of the PTV body. The injector temperature was kept at 225ºC upon sample introduction and maintained there for 11 min, whereas the FID system was operated at 250ºC. A 25 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.25-µm layer of Chirasil-β-Dex (Chrompack, Middelburg, The Netherlands) was used, and the GC oven was maintained for 10 min at 40ºC and then programmed to 180ºC at 5ºC/min.

On-line LC coupled with GC: Analysis of hazelnut extracts and direct analysis of hazelnut oils. The coupled LC–GC equipment used consisted of a Hewlett-Packard Model 1050 (Wilmington, DE) liquid chromatograph linked to a Perkin-Elmer Model 8500 (Norwalk, CT) gas chromatograph fitted with a PTV that acted as the system interface.

LC preseparation. Hazelnut oils analyzed directly by LC–GC as well as the extracts obtained from hazelnuts as detailed in the sample preparation section (above) were sampled through a manual injection valve (Model 7125; Rheodyne, Cotati, CA) having a 20-µL sample loop. In all analyses a UV detector operated at 205 nm was used for detection. Methanol/water (65:35 vol/vol) was the HPLC eluent at 2.0 mL/min. Acquisition of data from both the UV and FID detectors was performed using an HP ChemStation (Hewlett-Packard). When analyzing hazelnut oils, the only pretreatment of the samples was their filtration through a 0.22-µm filter. The analyses were performed using a 5 cm × 4.6 mm i.d. Kromasil column (100-10C_4; Symta, Madrid, Spain) maintained at 45ºC.

LC–GC transfer. Upon elution of the beginning of the fraction of interest (i.e., that containing filbertone), a 2.0-mL volume of the eluent was transferred to the GC. The transfer was performed using a multiport valve (Rheodyne, Model 7060) positioned after the UV detector in the LC system and a transfer line (a 80 cm × 0.32 mm i.d. fused-silica tube) previously inserted into the septum of the PTV (19). Thermal desorption of the material retained in the interface was achieved by increasing (at 14ºC/s) the PTV temperature up to 225ºC for 11 min.

GC analysis of the fractions transferred from LC. The fractions transferred from LC into GC were analyzed using the same column (Chirasil-β-Dex) detailed in this paper. The initial GC temperature (40ºC) was maintained for 5 min and then programmed at 3ºC/min to 85ºC, then to 180ºC at 5ºC min.

RESULTS AND DISCUSSION

As the sample pretreatment usually required to determine the enantiomeric composition of a chiral compound in a complex mixture may bring about the partial or total racemization of the compound of interest, we considered as a priority of our investigation the use of methods precluding such a risk. Therefore, we evaluated the possibility of performing the thermal desorption of a sample weight of hazelnut introduced directly (i.e., without previous isolation or concentration) in the glass liner of a PTV. However, the complexity of the chromatogram obtained did not allow a reliable determination of the peak areas of both enantiomers of filbertone.

Taking into account that the variation of the enantiomeric composition of the total content of a chiral compound can be caused under different experimental conditions (15,20,21), we considered it essential to minimize or even eliminate the sample preparation step and avoid heating the sample. Finally, we selected the two approaches described in the Experimental Procedures section, which involved either the cold extraction of the sample (hazelnut or hazelnut oil) with organic solvents and its subsequent analysis by GC, or the analysis by on-line coupled LC–GC. The first procedure is simple and rapid but may involve the introduction of large amounts of fat into the capillary column, which will eventually affect its performance. As an alternative, we proposed the use of the second procedure for either the analysis of the extract obtained from hazelnuts or the direct analysis of the oil. In both cases, the HPLC preseparation step is used to select the fraction containing filbertone, which is subsequently transferred to GC, thereby avoiding the introduction of the TG fraction into the GC column.

Figures 1 and 2 show the chromatograms obtained from hazelnut extracts of two different origins (1A and 2A) and from the oils obtained by cold pressing the same hazelnuts (1B and 2B) by using GC (chromatograms given in Fig. 1) or on-line LC coupled with GC (chromatograms given in Fig. 2). In all cases, satisfactory resolutions were achieved for both R- and S-enantiomers of filbertone, yielding relative SD values (calculated from a minimum of three replicates) lower than 7 and 12%, respectively.

It is interesting to note that the use of different temperatures (i.e., 150, 225, and 300ºC) during the desorption step, when transferring the selected fraction from LC into GC, did not result in significant differences between the enantiomeric

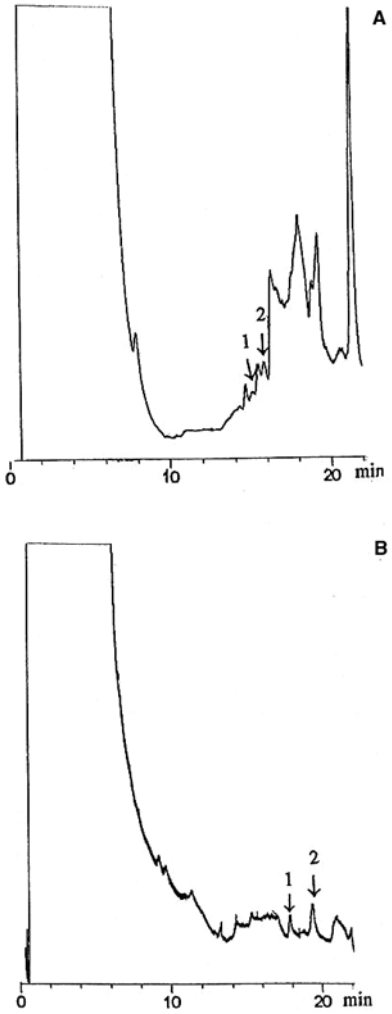

FIG. 1. Determination by GC of the enantiomeric composition of filbertone of (A) a hazelnut extract and (B) the extract of the hazelnut oil obtained by cold pressing the same hazelnuts (Spain-2) analyzed in (A). Fused-silica capillary column: 25 m × 0.25 mm i.d., coated with a 0.25-µm layer of Chirasil-β-Dex (Chrompack, Middelburg, The Netherlands). Peak identification numbers: 1, R-filbertone; 2, S-filbertone. See text for further details.

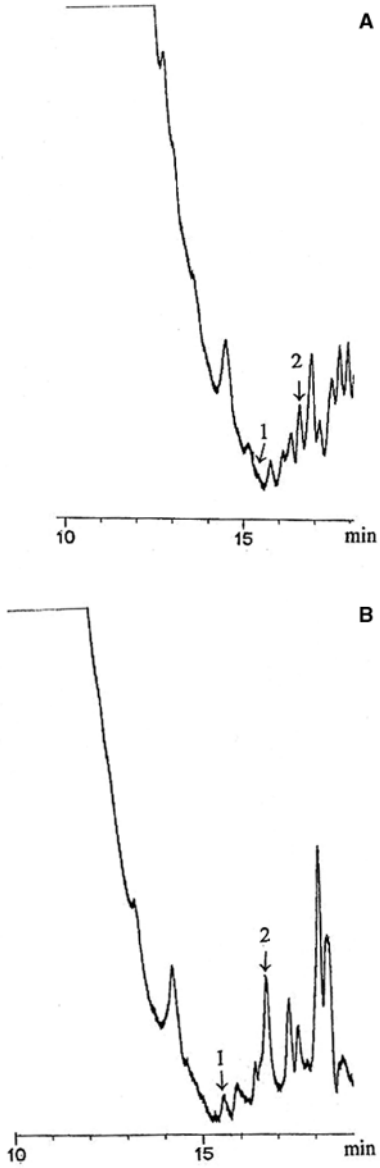

FIG. 2. Determination by LC–GC of the enantiomeric composition of filbertone of (A) a hazelnut extract and (B) the hazelnut oil obtained by cold pressing the same hazelnuts (Unknown) analyzed in (A). Both chromatograms were obtained after transfer from LC into GC of a 2.0-mL fraction of methanol/water eluent (flow rate: 2.0 mL/min). For identification of peak numbers and experimental conditions, see Figure 1.

excesses determined for filbertone. However, the use of the intermediate temperature (i.e., 225ºC) seemed to be more convenient to ensure the complete desorption of filbertone from the adsorbent material used to retain the solutes transferred.

The enantiomeric compositions (ee%) of filbertone from hazelnuts of different geographic origins were as follows: Turkey, 70.9; California, 70.0; Spain-2, 68.1; Unknown, 73.2. The compositions (ee%) of filbertone from hazelnut oils were as follows: France-1, 73.0; France-2, 43.0; Spain-1, 85.0; Spain-2, 72.9; Unknown, 73.5. In both cases, excess of the predominant enantiomer is expressed as a percentage, i.e., (predominant enantiomer – minor enantiomer)/(predominant enantiomer + minor enantiomer) × 100. It should be noted that when analyzing the same sample with either of the two analytical procedures tested (i.e., extraction with organic solvents and subsequent GC analysis or on-line LC coupled with GC), very close values for the enantiomeric ratios of filbertone were obtained. These results suggest that racemization during both the sample preparation step and the analysis itself was avoided. Consequently, both approaches are useful for the reliable determination of the enantiomeric composition of the chiral constituent being considered.

From enantiomeric excess (ee) values given, it is clear that the enantiomeric composition of filbertone in hazelnuts from different origins is relatively stable whereas values obtained for hazelnut oils ranged from ee = 73% to ee = 85% in four samples. However, the ee value dropped to 43% in one of the commercial oils analyzed, most likely because roasted hazelnuts must have been used for its production, even though the oil was not labeled as such. This observation corresponds not

only with the sensory evaluation of this oil but also with the apparent effect of the roasting on the ee of filbertone. Other authors (15,21) have studied the influence of the roasting process on the filbertone content in hazelnuts and have established its stable enantiomeric composition in both raw (with values of about ee = 67%) and roasted fruits (with ee close to 44%). They have also suggested that this significant difference may be due to the formation of racemic filbertone from an unknown precursor under the influence of heat. Although, to our knowledge, no data have previously been reported concerning the enantiomeric composition of filbertone in oils obtained from both raw and roasted hazelnuts of different origins, it seems clear that the results obtained in the present work are consistent with the hypothesis mentioned by the preceding authors.

Another interesting question concerns the influence of the processing conditions of the oil production on the enantiomeric composition of filbertone. Data collected reveal that ee values of 72.9 and 73.5% were obtained from the hazelnut oils obtained by cold pressing the hazelnuts, whose ee values were 68.1 and 73.2%, respectively. Considering the experimental error of the determination of the areas of both enantiomers, it is clear that no significant differences can be attributed to the oil production process.

It is also interesting to emphasize that the significant modification of the enantiomeric ratio of filbertone, which involved the addition of low amounts of the synthetic racemate of filbertone, can be useful for authenticity control of oils. Thus, the enantiomeric excess of filbertone in a hazelnut oil dropped from values of about ee = 73% to values of about ee = 23% or ee = 10% when 2 or 4 ppm, respectively, of the synthetic racemate of filbertone was added.

On the basis of the results given in this work, ee for filbertone can be considered constant in the context of the natural variability of oils. Moreover, hazelnut oil production by cold pressing does not markedly change the ee value found in the hazelnuts from which the oil was obtained.

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