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Solid-Phase Microextraction for Studies on the Enantiomeric Composition of Filbertone in Hazelnut Oils

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The enantiomeric distribution of filbertone was determined in unroasted and roasted hazelnut oils of different geographical origins by using solid-phase microextraction (SPME) and capillary gas chromatography. An optimization procedure including SPME fiber, extraction time, exposure temperature, and sample volume enabled the best conditions to be selected. Under the optimized conditions, detection limits were in the micrograms per liter level for both enantiomers of filbertone with relative standard deviation values of 7.1 and 4.9% for *R*-filbertone and *S*-filbertone, respectively. The proposed approach allowed the rapid determination of the enantiomeric composition of filbertone and demonstrated that its variability is an inherent property of the natural compound. Analysis of two batches of hazelnut oils obtained from either unroasted or roasted hazelnuts showed, in general, significantly higher amounts of filbertone in roasted hazelnut oils.

KEYWORDS: SPME; filbertone; enantiomeric analysis; hazelnut oil

INTRODUCTION

It is well-known that the different biological activities of some pairs of enantiomers result from different stereospecific interactions with receptors, enzymes, and transport systems. Therefore, the study of modifications of stereospecific enzymatic pathways in food processing relies upon the application of efficient chiral separation techniques adequate to establish the relationship between stereochemistry and metabolic transformations involved in the formation of a certain compound (*1*, *2*). For that reason, suggestions of possible biosynthetic pathways for a relevant component can be made from the reliable determination of the enantiomeric distribution of the compound of interest. In this respect, the development of new chromatographic methods specifically designed to overcome the difficulties involved in the separation of enantiomers is continually being sought. Despite the tremendous effort made during the past few years concerning chiral discrimination, some critical aspects must be still considered. In particular, the need to develop highly enantioselective methods while simultaneously avoiding those conditions giving rise to racemization of the compounds of interest must be emphasized.

(*E*)-5-Methylhept-2-en-4-one (filbertone) has been previously isolated from hazelnut fruits and identified as the principal flavor component of hazelnut (*3*). On the basis of former enantiomeric composition studies of filbertone in hazelnut fruits, the low enantiomeric purity of natural filbertone as an inherent property of the natural compound has already been reported (*4*). Other authors have found that filbertone enantiomeric composition seemed to be constant whatever the origin of the hazelnuts (*5*, *6*), although the limited number of samples investigated did not allow general statements to be made. Concerning the reliability of the determination of the enantiomeric composition of filbertone, the significant influence of the sample extraction procedure has also been previously underscored (*7*).

On the other hand, over the past few years solid-phase microextraction (SPME) has proved its usefulness for the rapid analysis of a wide variety of compounds (*8*−*11*) because of its numerous benefits. The main advantages of SPME include simplicity, speed, effectiveness, low cost, and compatibility with analytical systems. This way, different authors have successfully applied SPME methodology to the extraction of organic compounds from different matrices (*12*, *13*), but data in the literature on the use of SPME to determine the volatile profile of vegetable oils are rare (*14*). In this respect, the usefulness of SPME for enantiomeric composition studies in edible oils of different origins and, in particular, changes in the filbertone enantiomeric composition in hazelnut oils obtained from either unroasted or roasted hazelnuts have not been so far studied.

Our work was aimed to offer additional and new results about filbertone using SPME to establish its natural variability in hazelnut oils from different geographical origins. The objective was also to apply the SPME procedure to study the influence of the roasting process on the enantiomeric distribution of filbertone in hazelnut oils.

MATERIALS AND METHODS

Material. For identification purposes, (*E*)-5-methylhept-2-en-4-one (filbertone) was supplied by Haarman and Reimer (Holzminden, Germany). The samples analyzed were as follows: (i) 21 hazelnut oils obtained from unroasted hazelnuts of different origins (Spain, Italy, Turkey, France, and Croatia); (ii) 21 hazelnut oils obtained after roasting of the same hazelnuts described above.

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Procedure. *SPME.* An SPME holder (Supelco, Bellefonte, PA) was utilized to perform the experimentation. A fused silica fiber coated with a 65-μm layer of poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) was employed to retain the compound of interest. Before the SPME fiber was used, it was conditioned in the injector of the gas chromatograph at 260 °C for 30 min. Oil samples were not subjected to any kind of pretreatment prior to the extraction. A 0.5-mL volume of the oil was placed in a 5.0-mL vial that was sealed with plastic film. Experimentation was performed by exposing the fiber to the headspace of the sample for 5 min at 70 °C. Constant sample stirring was necessary throughout the experimentation to facilitate the mass transfer and the release of filbertone into the headspace. Once the extraction was finished, the target compound was thermally desorbed into the GC injector at 250 °C for 5 min and finally analyzed by gas chromatography (GC) by using an enantioselective column as explained below.

GC Analysis. A Hewlett-Packard model 5890 gas chromatograph fitted with a split/splitless injector and a flame ionization detector (FID) was used. GC separations were performed on a chiral column (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). After the initial temperature had been held at 45 °C for 5 min, the column was, first, programmed at 3 °C/min to 90 °C and then at 5 °C/min to 150 °C. In all analyses, helium was used as the carrier gas at an initial flow rate of 1 mL/min, the FID temperature was set at 250 °C, and sample introduction was carried out in the splitless mode. Data acquisition from the FID was performed by using an appropriate software (TotalChrom, Perkin-Elmer, Madrid, Spain).

Identification of the investigated compounds was performed by matching their retention times to those of (+)- and (−)-enantiomer standards analyzed under identical conditions. Additionally, SPME-GC analyses of spiked samples were accomplished to verify the identification in all cases and, when in doubt, typical fragmentation of filbertone and the resulting relative intensities were also positively confirmed by mass spectrometry. All analyses were made in triplicate.

RESULTS AND DISCUSSION

An optimization procedure was accomplished to select the experimental conditions most suitable for the determination of filbertone in hazelnut oil. To this aim, several temperatures (30, 40, 50, and 70 °C), extraction times (5, 15, and 30 min), SPME fibers [100-μm layer of poly(dimethylsiloxane), 65-μm layer of PDMS/DVB, 85-μm layer of carboxen/poly(dimethylsiloxane)], and hazelnut oil volumes (0.5, 1.0, and 2.0 mL) were tested. This optimization was performed as follows. Initially, experimental conditions, based on the literature (*14*) (extraction time of 30 min and exposure temperature of 50 °C) and on our own experience (sample volume of 1.0 mL) were used to optimize the SPME fiber. From this experimentation, a 65-μm layer of PDMS/DVB was finally selected as the best option because a 100-μm layer of poly(dimethylsiloxane) did not provide enough sensitivity to detect the enantiomers of filbertone in the samples considered and an 85-μm layer of carboxen/poly(dimethylsiloxane) resulted in unacceptable repeatability [the relative standard deviation (RSD), calculated for at least three replicates, was >20%]. Once selected, the fiber, sample volume, exposure time, and extraction temperature were optimized successively by carrying out extractions under conditions in which the chosen parameter was varied and the other parameters were kept constant. For all parameters, the optimal values were selected on the basis of which provided the highest peak areas by SPME-GC.

As a result of this optimization, 0.5 mL, 5 min, and 70 °C were selected as the optimal values of sample volume, extraction time, and exposure temperature, respectively. With regard to the sample volume, interestingly neither 1.0 nor 2.0 mL resulted in higher peak areas. It is convenient to clarify that in the case of 2.0 mL, the use of a 10-mL vial, instead of 5.0 mL, was required to perform the experimentation. This fact could explain the minor peak areas obtained as, obviously, the use of a greater vial size results in greater sample headspace and, thereby, lower filbertone amounts are finally retained on the fiber. With respect to extraction temperature and time, generally speaking, higher temperatures resulted in higher area peaks when short exposure times were used. On the contrary, at low temperatures, longer extraction times appeared to be more effective. As a consequence, 70 °C for 5 min seemed to be the conditions that provided the highest filbertone peak areas, although it is interesting to point out that a similar (although slightly lower) response (i.e., the second best response) was obtained when conditions were 30 °C for 30 min. This is, most likely, because the use of extraction temperatures as high as 70 °C results in a quick release of filbertone from the hazelnut oil so that 5 min corresponds to the extraction time adequate to retain the compound on the fiber with no subsequent losses. To the contrary, filbertone was given off far more slowly at 30 °C; however, an extraction time as long as 30 min compensates for the low extraction temperature, and a similar result is eventually obtained.

On the other hand, the use of 30 °C for 30 min, theoretically, involves lower risk of filbertone racemization than the use of 70 °C for 5 min. For that reason, we decided to evaluate comparatively enantiomeric excesses found for filbertone under the two different sets of experimental conditions above-mentioned.

For this purpose, a hazelnut oil sample was used for which the concentration and enantiomeric excess for filbertone had been previously estimated in our laboratory using on-line LC-GC (*6*). When the SPME-GC analysis of the same hazelnut oil was performed, the enantiomeric composition found for filbertone was 97.4 and 99.3% depending on whether 30 °C for 30 min or 70 °C for 5 min, respectively, was used during the experimentation. It is clear that extraction temperatures of up to 70 °C were found not to affect the resulting enantiomeric distribution of filbertone from SPME analysis, at least when extraction times as short as 5 min were established. Consequently, either of the two sets of conditions above-mentioned appears to be adequate for enantiomeric composition studies. Evidently, the experimentation could have been performed at 30 °C for 30 min, but the application of 70 °C for 5 min enabled the overall time to be considerably shortened as well as slightly higher peak areas to be obtained. Therefore, conditions of 70 °C for 5 min were eventually selected as the most favorable.

The repeatability of the proposed procedure was estimated by measuring the RSD from three replicates of the hazelnut oil when SPME was accomplished under the optimized experimental conditions. The values obtained for the *R*- and *S*-enantiomers of the target compound were 7.1 and 4.9%, respectively, when the same fiber was used. Equally, a study of fiber-to-fiber variation provided RSD values of <10%. Detection limits (calculated as the concentration of product giving a signal equal to 5 times the background noise) were 26 μg/L (*R*-filbertone) and 25 μg/L (*S*-filbertone), whereas the concentrations of both enantiomers in the hazelnut oil analyzed by SPME-GC were estimated to be within the same range as those previously reported using other analytical techniques (*16*, *17*). It is interesting to emphasize that the proposed procedure does not demand any kind of pretreatment of the oil, 30 min being the time necessary to perform the overall analysis. Under the optimized conditions, the SPME-GC analysis of filbertone proved to be linear, at least, over 2 orders of magnitude of concentration for each enantiomer (i.e., in the range of 0.4−40

Table 1. Enantiomeric Excesses (ee)[a] Obtained from SPME-GC Analysis of Filbertone from Unroasted and Roasted Hazelnut Oils of Different Origins

	ee (%)	
sample/origin	unroasted	roasted
1/Croatia	70.2	28.4
2/Cataloniaa, Spain	98.0	27.3
3/Catalonia, Spain	nd[b]	20.0
4/Catalonia, Spain	nd	36.9
5/Asturias, Spain	nd	14.8
6/Asturias, Spain	61.5[c]	29.6
7/Piedmont, Italy	nd	42.1
8/Piedmont, Italy	83.0	20.3
9/Isere, France	44.0[d]	14.2
10/Istanbul, Turkey	89.8	33.0
11/Istanbul, Turkey	48.9[c]	25.4
12/Istanbul, Turkey	nd	27.9
13/Istanbul, Turkey	77.2	41.8
14/Istanbul, Turkey	74.7	32.7
15/Istanbul, Turkey	93.0	38.0
16/Cancon, France	76.4	17.7
17/Cancon, France	73.1	19.5
18/Cancon, France	71.9	30.0
19/Cancon, France	88.8	37.8
20/Cancon, France	nd	34.7
21/Petralia, Italy	75.8	42.6

[a] Excess of predominant enantiomer expressed as percent, i.e., [(predominant enantiomer − minor enantiomer)/(predominant enantiomer + minor enantiomer)] × 100. [b] Not detectable. [c] Not reliable because of overlapping. [d] Not reliable. Areas are too small to be accurately quantified.

mg/L). The linear range was calculated by adding increasing volumes of a standard solution to a vegetable oil in which the absence of filbertone had been previously verified (*18*). In all samples, concentrations of both enantiomers of filbertone were found to be within this range.

Table 1 gives the enantiomeric excesses (ee) resulting from SPME-GC analysis of the hazelnut oils processed from either unroasted or roasted hazelnuts in the selected conditions. In all cases, the enantiomeric distribution was calculated from peak areas and excess of predominant enantiomer was expressed as a percent:

$$[(\text{predominant enantiomer} - \text{minor enantiomer})/(\text{predominant enantiomer} + \text{minor enantiomer})] \times 100$$

An important aspect to consider is that under the sorption temperature and exposure time used throughout SPME experimentation, as expected from the study mentioned above, no evidence of filbertone racemization was observed.

With respect to the enantiomeric distribution of filbertone in hazelnut oils of different geographical origins, data obtained in the present work demonstrated the wide range that can be found in nature. Specifically, enantiomeric excesses calculated for filbertone in unroasted hazelnut oils varied from 44 to 98%, although values between 70 and 90% were observed in most cases. In this regard, it should be kept in mind that previous work on the enantiomeric distribution of filbertone in nature, which has almost exclusively focused on hazelnut fruits, showed that ee values in hazelnuts of different origins seemed to be constant (*5*), although only four different varieties were investigated. As far as hazelnut oil is concerned, the enantiomeric composition found for filbertone in oil samples processed, most likely, from unroasted hazelnuts ranged from 73 to 85%, although data previously reported refer to only four hazelnut oils analyzed by using techniques other than SPME (*6*).

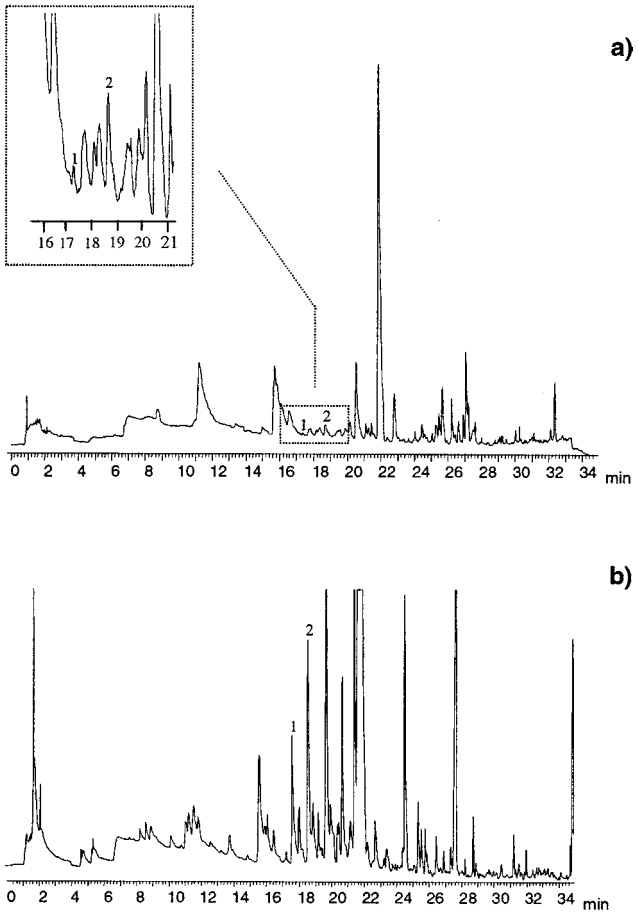

Figure 1. SPME-GC analysis resulting from the hazelnut oils obtained from unroasted hazelnuts (a) and from the same hazelnuts after roasting (b) (sample 16 in **Table 1**). Fused silica capillary column: 25 m × 0.25 mm i.d., coated with a 0.25-μm layer of Chirasil-β-Dex. Peaks: 1, *R*-filbertone; 2, *S*-filbertone. Chromatograms a and b were recorded at the same full range. See text for further details.

The influence of the roasting process on the enantiomeric distribution of filbertone is also clear from **Table 1**. As can be seen, there was a substantial difference between oils obtained from unroasted and roasted hazelnuts. In fact, in all cases the enantiomeric excess dropped significantly when roasted hazelnuts were used to obtain the hazelnut oils. Observation of the lower values obtained for the enantiomeric excesses in the roasted samples might seem to indicate that filbertone racemization is caused by heat. However, some authors have discarded this possibility as they attributed the increase of the *R*-enantiomer during the roasting process to the presence of a precursor from which filbertone is released under the influence of heat (*5*, *7*).

Figure 1 shows the chromatograms resulting from SPME-GC analysis of two hazelnut oils (sample 16, **Table 1**) obtained from unroasted hazelnuts (a) and from the same hazelnuts after roasting (b). Concerning the enantiomeric composition found for filbertone, this sample exhibited an enantiomeric excess equal to 76.4% when the oil was obtained from unroasted hazelnuts, the *S*-form being predominant. However, filbertone was made up of only 17.7% of the *S*-enantiomer when the same hazelnuts were roasted. Likewise, taking into account that the two chromatograms given in **Figure 1** were recorded at the same full range, it is clear the considerable increase in volatile compound concentrations results from roasting. In particular, as far as filbertone is concerned, concentrations in oils obtained

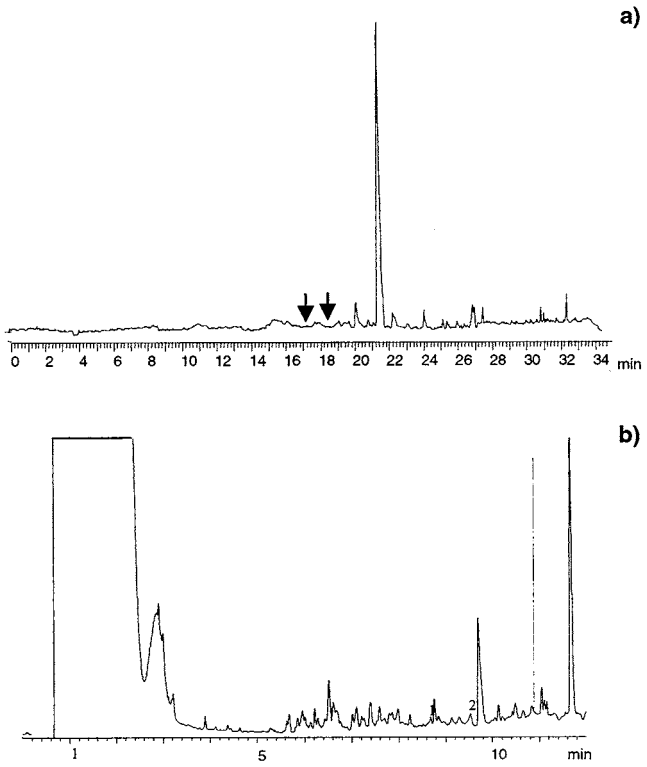

Figure 2. SPME-GC analysis (a) and on-line LC-GC analysis (b) resulting from the same hazelnut oil obtained from unroasted hazelnuts (sample 5 in **Table 1**). Capillary column and peak identifications are as in **Figure 1**. Column temperature program: 45 °C (at 5 °C/min) to 150 °C (10 min) (b). See text for further details.

from roasted hazelnuts were up to 35 times higher than those found in unroasted hazelnut oils. Specifically, filbertone concentrations ranged from 1 to 5 mg/L in unroasted hazelnut oils and from 32 to 36 mg/L in roasted samples. These results are in agreement with those previously reported by some authors (*15*) who described the effect of thermal treatments on filbertone generation.

In short, this approach can be considered as a valuable alternative to determine filbertone content in hazelnut oils. However, as indicated in **Table 1**, either the lack of sensitivity or the presence of overlapped peaks made the determination of the enantiomeric distribution of filbertone occasionally impossible. As an example, **Figure 2** gives the chromatograms obtained when SPME-GC was applied to a hazelnut oil (sample 5, **Table 1**), which results from unroasted hazelnuts, showing the region in which both *R*- and *S*-enantiomers of filbertone should elute in the unroasted hazelnut oil (**Figure 2a**). Obviously, filbertone could not be reliably identified in this sample. Consequently, the use of an alternative analytical approach suitable to obtain the sensitivity or selectivity required might be necessary in certain cases. In this regard, various techniques have already been tested in our laboratory for the determination of filbertone in hazelnut oils (*19*), the on-line coupling of liquid chromatography and gas chromatography (LC-GC) being the most advantageous. To illustrate this aspect, **Figure 2b** gives the chromatograms obtained from the same sample when using LC-GC as described elsewhere (*16*, *20*). Using this coupled system, the hazelnut oil analysis, which is performed directly (i.e., without sample preparation), includes the preseparation of the oil in the LC step, the transfer from LC into GC of the fraction in which filbertone is eluted, and, finally, the enantiomeric analysis of filbertone in the GC step. From **Figure 2b** it is clear that both enantiomers of filbertone were identified in the unroasted hazelnut oil (sample 5), 63.8% being the enantiomeric excess estimated. Interestingly, the enantiomeric excess value obtained by SPME-GC when the same roasted hazelnut oil (sample 5 in **Table 1**) was analyzed was confirmed by using LC-GC (18.6 vs 14.8% from LC-GC and SPME-GC, respectively).

Similarly, LC-GC was also applied to all samples in which overlapping with other matrix components made the identification of filbertone in unroasted oils through SPME-GC uncertain (i.e., samples 6 and 9 in **Table 1**). In these cases, filbertone enantiomeric excesses of 57.7 and 53.1% were obtained by LC-GC for samples 6 and 9, respectively. Because the enantiomeric ratios resulting initially from SPME-GC analysis of the same samples were 61.5 and 44.0%, respectively, the use of LC-GC appeared to be equally useful to verify unreliable data.

In short, the advantages of SPME were its simplicity, speed, and low cost, whereas LC-GC showed higher selectivity and sensitivity for the determination of the enantiomeric composition of filbertone. Both methods, however, displayed equally good repeatability (*16*).

CONCLUSIONS

Considering the obtained results, we can conclude that both SPME-GC and LC-GC are suitable for the enantiomeric analysis of filbertone in hazelnut oil samples, as neither of them brought about the racemization of filbertone and both of them yielded similar ee values. On the other hand, the relatively wide range of enantiomeric excesses found for filbertone in hazelnut oils according to their geographical origin reflects the variability of this compound in nature. Likewise, the results obtained from the roasted hazelnut oils suggest that the roasting process alters the enantiomeric composition and concentration of filbertone as substantially lower enantiomeric excesses and a far higher amount of filbertone were found in all roasted samples.

In addition, the results obtained in the present work might confirm previous observations by other authors concerning the existence of two possible biogenetic pathways of filbertone (*5*). According to their hypothesis, one of these pathways would lead to a greater extent to pure *S*-enantiomer and the other to a lesser extent to a racemate. Although in contrast to the results obtained by these authors from their filbertone enantiomeric composition studies, we have found a wider variability in nature, and the mentioned hypothesis can still be accepted to explain the low enantiomeric purity determined for filbertone in natural products.

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