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Detection of the adulteration of olive oils by solid phase microextraction and multidimensional gas chromatography

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Abstract

The presence or absence of filbertone in 21 admixtures of olive oil with virgin and refined hazelnut oils obtained using various processing techniques from different varieties and geographical origins was evaluated by solid phase microextraction and multidimensional gas chromatography (SPME–MDGC). The obtained results showed that the sensitivity achievable with the proposed procedure was enough to detect filbertone and, hence, to establish the adulteration of olive oil of different varieties with virgin hazelnut oils in percentages of up to 7%. The very low concentrations in which filbertone occurs in some refined hazelnut oils made difficult its detection in specific admixtures. In any case, the minimum adulteration level to be detected depends on the oil varieties present in the adulterated samples. In the present study, the presence of *R*- and *S*-enantiomers of filbertone could be occasionally detected in olive oils adulterated with 10–20% of refined hazelnut oil.

Keywords: Enantiomers; Multidimensional gas chromatography; Olive oil adulteration; Refined hazelnut oil; Solid-phase microextraction

1. Introduction

The adulteration of olive oil with other edible oils of lower commercial value is relatively frequent and, therefore, the development of new analytical techniques capable of detecting such adulterations is currently highly demanded. In this respect, we have previously proposed the use of (*E*)-5-methyl-hept-2-en-4-one (filbertone), the characteristic flavour compound in hazelnuts, as a suitable indicator of the presence of virgin hazelnut oil in olive oil (Blanch, Caja, Ruiz del Castillo, & Herraiz, 1998; Ruiz del Castillo, Caja, Herraiz, & Blanch, 1998). So far, however, the usefulness of this compound to detect such an adulteration when refined hazelnut oil is involved has not been studied.

Another interesting aspect is the possibility of performing rapid and simple analysis for screening procedures and confirmatory processes. In these cases, the advantages of using Solid-Phase Microextraction (SPME) have already been reported (Arthur & Pawliszyn, 1998; Pawliszyn, 1995; Zhang & Pawliszyn, 1993; Zhang, Yang, & Pawliszyn, 1994). Specifically, the potential of SPME for the determination of volatile constituents in vegetable oils has been occasionally described in the literature by different authors who have insisted on the need to carefully optimize the experimental variables involved in the extraction procedure (Bentivenga, D'Auria, De Luca, De Bona, & Mauriello, 2001; Vichi et al., 2003; Vichi, Pizzale, Conte, Buxaderas, & López-Tamames, 2003). Particularly, other authors (Jelén, Obuchowska, Zawirska-Wojtasiak, & Wasowicz, 2000) have underlined the importance of performing the extraction at relatively low temperatures as otherwise oil properties can be easily altered. These authors point out the fact that although unsaturated fatty acids are relatively stable to oxidation at low temperatures, they can oxidize when working at high temperatures

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thus promoting changes in the volatile profile through the formation of monohydroperoxides which eventually break down into volatile products.

We have recently proposed the use of SPME for the determination of filbertone in virgin edible oils as this technique allows the efficient enrichment of the target compound also avoiding the isolation of major components of the matrix, which would bring about interferences during the subsequent analytical step (Ruiz del Castillo, Flores, Herraiz, & Blanch, 2003). Moreover, in our earlier work we confirmed the convenience of working either at low temperatures over long extraction times or at high temperatures (up to 70 °C) over short extraction times.

Because of the complexity of the obtained extracts, the analytical separation is usually difficult as it requires the detection of a minor compound in a mixture in which chromatographically overlapped analytes of similar characteristics may strongly affect its detection when applying standard GC instrumentation consisting of a single column. In this respect, the use of Multidimensional Gas Chromatography (MDGC) may offer the separation efficiency, selectivity and retention factors required to achieve the chromatographic resolution of those target analytes occurring at extremely low concentrations that are not satisfactorily resolved when one-dimensional gas chromatography is used (Deans, 1981; Schomburg, 1995).

On the other hand, when the adulteration of olive oil with hazelnut oil is intended to be detected, the consideration of refined oils always implies an additional difficulty as the high temperatures reached under oil deodorization conditions may theoretically lead to partial or even total removal of highly volatile compounds, such as filbertone. As a consequence, it is likely that analytical methods developed to determine this chiral marker in virgin oils are not sensitive enough to detect adulterations accomplished with refined oils.

The objective of this work was to evaluate the suitability of SPME–MDGC as well as of SDE–MDGC and SDE–GC–MS to determine filbertone. A further purpose was to study the adequacy of this compound as a chiral marker to detect adulterations performed with refined hazelnut oils by applying the methods proposed.

2. Materials and methods

2.1. Materials

The 21 samples included in this study were obtained from different suppliers and geographical areas. These samples were prepared by mixing at different percentages (established by a computer program) oils produced by the current processing techniques from diverse olive and hazelnut varieties of different geographical origins. Hazelnut oils used for preparing the adulterated samples were obtained from roasted (sample 17 in Table 1) and unroasted nuts (samples 1, 2, 4, 6, 8, 12, 15, and 19 in Table 1). According to the information available, samples 3, 5, 10, 11, 13, 18, 20, and 21 in Table 1 were obtained from probably unroasted hazelnuts. (*E*)-5-methyl-hept-2-en-4-one (filbertone) used for identification purposes was acquired from Haarman and Reimer (Holzminden, Germany).

2.2. Solid-phase microextraction

An SPME holder (Supelco, Bellefonte, PA, USA) was used to carry out the experimentation as previously detailed (Ruiz del Castillo et al., 2003). A fused silica fiber coated with a 65-μm layer of poly (dimethylsiloxane)/divinylbenzene (PDMS/DVB) was employed to retain filbertone. Before using the SPME fiber, it was conditioned in the injector of the gas chromatograph at 260 °C for 30 min. A 1.0-ml volume of the oil was placed into 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. To facilitate the release of filbertone into the headspace and its transfer to the fiber, constant sample stirring was applied throughout the experimentation. Upon completion of the extraction step, the target compound was thermally desorbed into the GC injector at 250 °C for 5 min and finally analyzed by multidimensional gas chromatography (MDGC) by using two coupled columns, namely a precolumn and a main column with an enantioselective stationary phase, as explained below.

2.3. MDGC analysis of extracts obtained by SPME

The MDGC system consisted of two independent gas chromatographs (Varian model CP-3800), connected together through a transfer line, in which two columns of different characteristics were placed. The two columns (i.e., precolumn and main column) were serially coupled through a Deans based switching system (Deans, 1981) and the transfer line, which was maintained at 280 °C throughout the experimentation. A programmed temperature vaporizer (PTV) injector operated at 250 °C in the splitless mode was used for sampling introduction. In all analyses, a FID kept at 250 °C was used and nitrogen served as the carrier gas at an approximate speed of 1 ml/min.

The gas chromatographic analysis of the SPME extracts was performed using a 30-m × 0.25-mm i.d. capillary fused silica column coated with a 0.25-μm film of DB-5 (J&W Scientific, Folsom, CA) as the precolumn. The oven was temperature programmed from 45 °C (5 min) to 80 °C at 10 °C/min and then to 180 °C at

Table 1
Identification of adulterated olive oil samples through the determination of filbertone by SPME–MDGC

Samples	Composition[a]		SPME–MDGC analysis	Conclusion
	Olive oil	Hazelnut oil		
1	86% RTurTa 5% LTurTa	9% RTur2Haz		
2	93% LTurCe	7% VTur5Haz	*R*-filbertone, *S*-filbertone	Adulterated
3	70% RTurRi 15% RTurTa	10% RTur4Haz 5% RTur3Haz		
4	89% RTurBa	11% RTur5Haz		
5	90% VTurMe	10% VTur3Haz	*R*-filbertone, *S*-filbertone	Adulterated
6	40% RSpPi 40% RSpHo	20% DSpHaz	*R*-filbertone, *S*-filbertone	Adulterated
7	100%TVTurYa			
8	89% RTurBa	11% RTur5Haz		
9	100% LTurTa			
10	80% VTurMe	20% VTur3Haz	*R*-filbertone, *S*-filbertone	Adulterated
11	88% RTurCe	12% RTur4Haz		
12	93% LTurCe	7% VTur5Haz	*R*-filbertone, *S*-filbertone	Adulterated
13	92% VTurBu	8% VTur3Haz	*R*-filbertone, *S*-filbertone	Adulterated
14	100%LTurTa			
15	85% VGrKo	15% VItHaz	*R*-filbertone, *S*-filbertone	Adulterated
16	100% VTurAy			
17	90% LMoPm	10% VFrHaz	*R*-filbertone, *S*-filbertone	Adulterated
18	91% VTurBu	9% VTur3Haz	*R*-filbertone, *S*-filbertone	Adulterated
19	75% VSpVe	25% DSpHaz	*R*-filbertone, *S*-filbertone	Adulterated
20	89% VTurEg	11% VTur3Haz	*R*-filbertone, *S*-filbertone	Adulterated
21	10% LItCo 40% RSpPi 30% RTurMe 5% RTuCh	15% RTur4Haz		

[a] *Codes:* V, virgin; L, lampante virgin olive oil; R, refined; D, deodorized; Haz, hazelnut. *Geographical origin:* Fr, France; Gr, Greece; It, Italy; Mo, Morocco; Sp, Spain; Tu, Tunisia; Tur, Turkey. *Variety:* Ay, Ayvalik; Ba, Unknown; Bu, Büyük Topak Ulak; Cb, Cima di Bitonto; Ce, Çelebi and Ayvalik; Co, Coratina; Ch, Chemlali; Eg, Egrin Burun; Ko, Koroneiki; Ho, Hojiblanca; Me, Memecik; Mix, Blend; Pm, Picholine Moroccan; Pi, Picual; Re, Refined 2nd centrifugation; Ri, Type Riviera; Ta, Blend; Ve, Verdial; Ya, Yag Celebi.

5 °C/min, the final temperature being maintained for 15 min.

As the main column, a capillary (25 m × 0.25 mm i.d.) fused silica tube coated with a 0.25-μm layer of permethyl-β-cyclodextrin (Chirasil-β-Dex; Varian, Middelburg, The Netherlands) was used. The column temperature was increased from 45 °C (5 min) to 80 °C (3 °C/min) and then raised once more at 5 °C/min up to 150 °C.

Identification of the investigated compounds was performed by matching their retention times to those of (+) and (−) enantiomer standards of filbertone analyzed under identical conditions. In all cases, SPME–MDGC analyses of spiked samples were additionally accomplished to verify the identification. All analyses were made, at least, in duplicate.

2.4. Steam distillation-solvent extraction

In those cases in which enough sample volume was available, the presence or absence of filbertone was also confirmed by using SDE (Blanch, Tabera, Herraiz, & Reglero, 1993; Godefroot, Sandra, & Verzele, 1981). Sample concentration was performed by using the high-density solvent configuration of the commercial version (Chrompack, Middelburg, The Netherlands) of the microdistillation-extraction device. SDE extracts were obtained starting from an ultrasonically homogenized sample consisting of a 75-ml volume of the corresponding sample oil and a 25-ml volume of water. A 2-ml volume of distilled dichloromethane (SDS, Peypin, France) was used as the extraction solvent. The sample was heated in a silicone bath at 140 °C whereas dichloromethane was distilled by heating with a water bath at 60 °C. A cold finger at −3 °C (±1 °C) was employed to condense the vapors of both sample and solvent and the continuous reflux was maintained over the extraction time (2 h), the distillable material being finally collected in the dichloromethane. Between consecutive runs the SDE apparatus was cleaned with chromosulfuric acid, acetone, and water collected from a Milli-Q water purification system (Millipore, Milford, MA). Once the extraction was finished, a further concentration step under a nitrogen stream up to a 0.5 ml-volume was required to achieve the sensitivity demanded for identification purposes. The SDE-extracts were then analyzed by using MDGC as well as GC–MS. The MDGC analyses were carried out by sampling a 0.2-μl

volume of the extracts obtained by SDE in the splitless mode using the chromatographic system above described for SPME-extracts while GC–MS analyses were performed by sampling a 0.2-μl volume of the SDE-extracts as detailed below.

2.5. GC–MS analysis of extracts obtained by SDE

The presence or absence of filbertone in the analyzed oils was also confirmed by gas chromatographic–mass spectrometric analysis using a Hewlett–Packard model 6890 gas chromatograph coupled to an Agilent 5989A quadrupole instrument (Palo Alto, CA) in the selective ion monitoring mode (SIM), 70 eV being the electron energy. The source and the quadrupole temperatures were 230 and 100 °C, respectively. Filbertone identification was accomplished by recording four typical fragment ions (i.e., *m/z* 41, 69, 98, and 111). The GC separation was performed on a 25-m × 0.25-mm i.d. fused silica column coated with a 0.25-μm layer of Chirasil-β-Dex (Chrompack); helium was used as the carrier gas at an initial flow rate of 1 ml/min and the splitless mode was used in all cases. The injector was kept at 250 °C throughout the experimentation and the GC-column was temperature-programmed at 3 °C/min from 45 °C to 150 °C (20 min). Data acquisition from the MS was performed using the HP G1701BA ChemStation (revision B.01.00) that allows the control of both the GC and the MS systems.

3. Results and discussion

By using the experimental conditions described in Section 2, the application of SPME–MDGC resulted in relative standard deviation (RSD, $n = 3$) values of 7.1% and 4.9% for *R*- and *S*-filbertone, respectively. Likewise, detection limits (signal/noise = 5) of 0.025 mg/l were obtained for each enantiomer. However, from SDE–MDGC and SDE–GC analyses, RSD ($n = 3$) values of 8.4% and 5.9% were achieved, whereas detection limits (signal/noise = 5) were 4.7 and 6.6 μg/l for *R*- and *S*-filbertone, respectively.

Table 1 gives the results obtained from the SPME–MDGC analysis of the 21 samples included in this study as well as the specific composition of each sample. As can be seen, the method proposed enabled us to establish the genuineness of olive oils on the basis of the absence of filbertone (samples 7, 9, 14 and 16 in Table 1) as well as the occurrence of hazelnut oil in olive oil in some of the analyzed samples by means of the identification of filbertone. To improve the reliability of the analysis, the adulteration of a sample was only established when both *R*- and *S*-enantiomers of filbertone were unambiguously detected. Thus, samples 2, 5, 6, 10, 12, 13, 15, 17, 18, 19 and 20 were successfully identified as olive oils adulterated with hazelnut oils. Furthermore, the presence of the *S*-enantiomer of filbertone in sample 1 was also detected, although its low concentration did not allow its reliable identification as adulterated oil. Similarly, overlapping of both enantiomers of filbertone with other matrix components made difficult the recognition of sample 3 as adulterated.

Generally speaking, it can be stated that those adulterations of olive oil performed with virgin hazelnut oil mostly obtained from unroasted nuts could be easily detected through the identification of the proposed chiral marker. This is an interesting point as filbertone levels in oils obtained from unroasted hazelnuts have been reported to be significantly lower than those found in roasted hazelnut oils (Ruiz del Castillo et al., 2003). Specifically, adulteration levels of 20%, 11%, 10%, 9% and 8% of the same hazelnut oil (VTur3Haz) obtained from probably unroasted nuts were successfully detected in different olive oils on the basis of the presence of both filbertone enantiomers (see Table 1, samples 10, 20, 5, 18, and 13, respectively). Similarly, adulterations performed with other virgin hazelnut oils from different geographical origins (see samples 2, 12, 15, and 17) could be demonstrated even at percentages as low as 7 % (sample 2 in Table 1).

Concerning the adulteration of olive oils with refined hazelnut oils, it is interesting to note that filbertone can also be occasionally a suitable chiral marker, as percentages close to 20–25% of deodorized hazelnut oils could be detected (samples 6 and 19 in Table 1). However, the identification of both enantiomers was unreliable when percentages of around 10–15% of refined oils were involved in the adulteration (as in samples 1 and 3 in Table 1) or even impossible (samples 4, 8, 11, and 21 in Table 1). Consequently, the detection limit achievable with the proposed method was not sufficient to ensure unambiguous identification of *R*- and *S*-filbertone in the last six mentioned samples so that their adulteration could not be reliably established. In any case, it is clear that the adulteration level to be finally detected depends strongly on the type of refined oil used in the adulteration.

To confirm the results obtained by SPME–MDGC analysis, the SDE technique was also applied in those cases in which enough sample volumes were available, namely samples 2, 9, 12, 13, 14, 16, 18, 19, and 21. In all cases, the obtained extracts were subsequently analyzed by MDGC as well as GC–MS, as above detailed. As a result, the presence of *R*- and *S*-filbertone was confirmed in all those samples in which both enantiomers had also been previously identified by SPME (i.e., samples 2, 12, 13, 18, and 19). Actually, the higher initial volume of the sample used when performing the SDE procedure with respect to that of SPME (i.e., 75 ml vs. 1 ml) led to a higher enrichment factor which finally resulted in higher sensitivities and, consequently, in more

reliable identifications in those samples with the lowest levels of filbertone. In this way, the absence of both enantiomers of filbertone was also successfully confirmed in samples 9, 14, and 16, which should be then considered as genuine olive oils.

It is worth mentioning that none of the three considered techniques (i.e., SPME–MDGC, SDE–GC–MS and SDE–MDGC) allowed us to confirm adulteration in sample 21. Most likely that was due to the extremely low levels of filbertone in the hazelnut oil labelled as

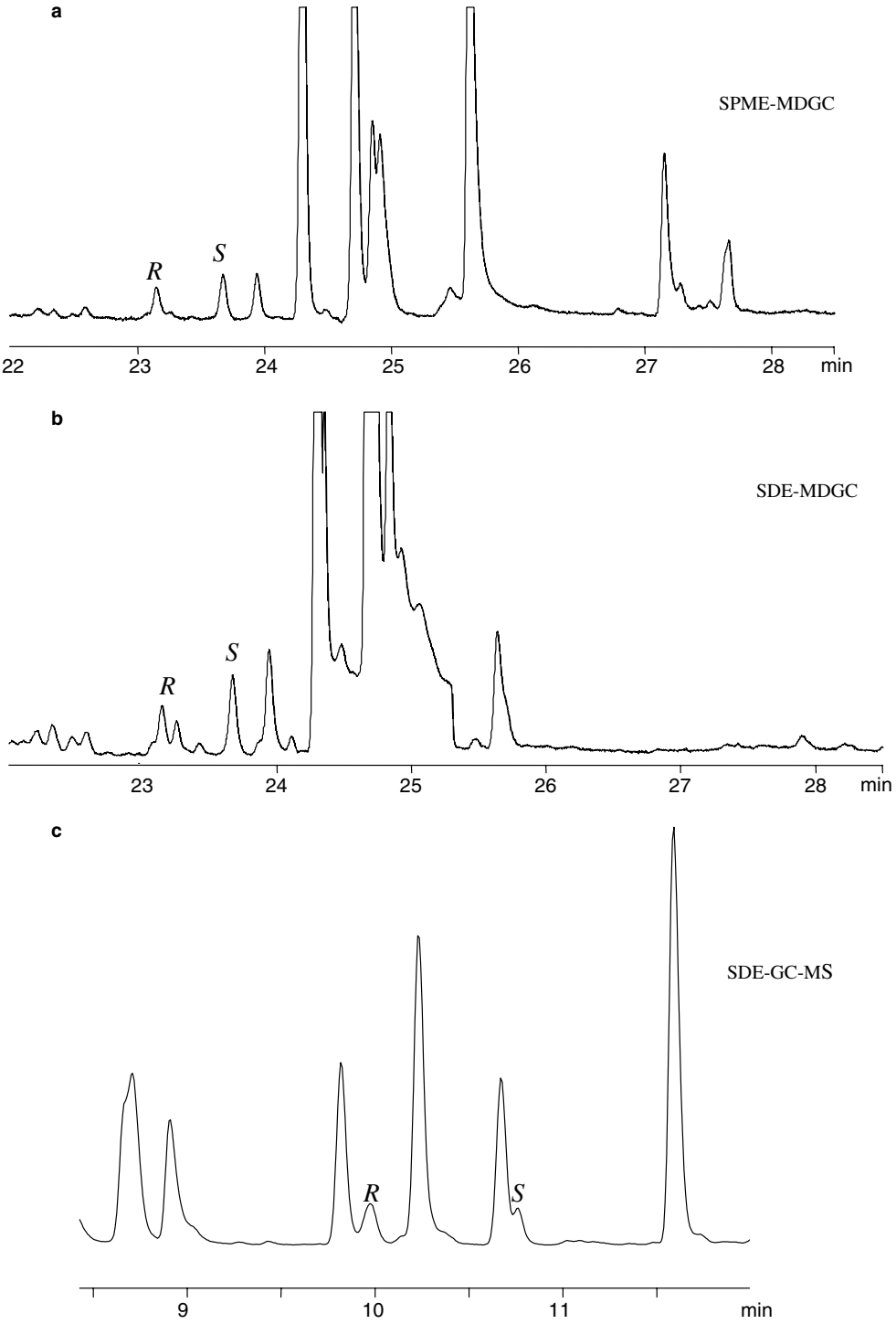

Fig. 1. Chromatograms resulting from an admixture of a lampante virgin olive oil (93%) with a crude hazelnut oil (7%) obtained from unroasted hazelnuts (sample 2 in Table 1). The analyses were performed by SPME–MDGC (a), SDE–MDGC (b) and SDE–GC–MS (c). Peak identification: *R*, *R*-filbertone; *S*, *S*-filbertone. See text for further details.

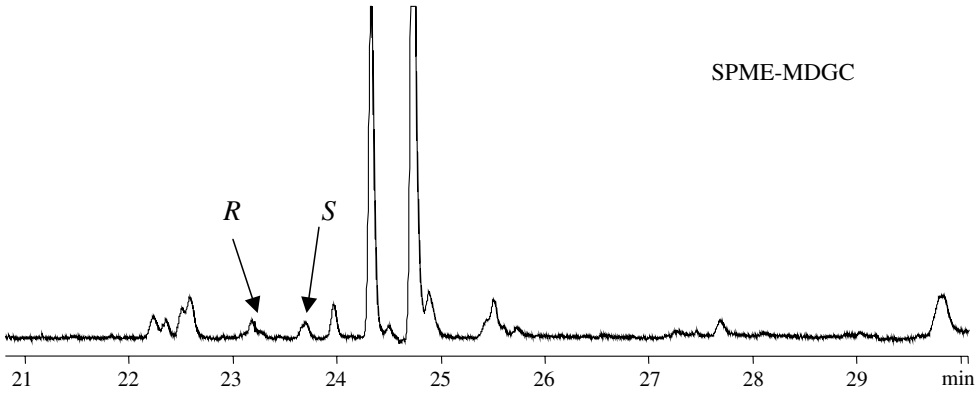

Fig. 2. Chromatogram resulting from SPME–MDGC analysis of an admixture of two refined olive oils (40% + 40%) and a deodorized hazelnut oil (20%) obtained from unroasted hazelnuts (sample 6 in Table 1). Peak identification as in Fig. 1. See text for further details.

RTur4Haz, which according to our information was obtained from probably unroasted nuts from Turkey. Further data concerning producer, variety and refining conditions were unknown to the supplier.

To illustrate the performance achievable with the analytical procedures proposed, Fig. 1 shows the chromatograms obtained by SPME–MDGC, SDE–MDGC and SDE–GC–MS of the same admixture (sample 2) resulting from the adulteration of a lampante virgin olive oil (93%) with a crude hazelnut oil (7%) obtained from unroasted hazelnuts. In this case, the use of MDGC enabled the transfer of the unresolved fraction containing filbertone from the precolumn to the main column thus avoiding peak co-elutions and, hence, making possible a more reliable identification of both enantiomers.

Fig. 2 shows the chromatogram resulting from SPME–MDGC analysis of the admixture of two refined olive oils with 20% of a deodorized hazelnut oil obtained from unroasted hazelnuts (sample 6 in Table 1). In spite of the fact that deodorization conditions (i.e., 250 °C for 4 h under a stream of nitrogen, 1 mmHg being the pressure of the system) might have promoted the loss of filbertone, its presence could be detected by the proposed method. Actually, the qualitative effect on filbertone levels of a deodorization process simulated in our laboratory had previously been considered (Blanch, Caja, León, & Herraiz, 2000), although so far the suitability of filbertone as a chiral marker to detect adulteration of commercial samples in which refined hazelnut oils are involved has not been studied.

In any case, it is clear that the use of different complementary concentration techniques, as SPME and SDE, combined with different separation techniques, such as one-dimensional chromatography or multidimensional gas chromatography, are useful to establish or confirm the adulteration of olive oil with hazelnut oil.

All in all, the simplicity, effectiveness, speed, low cost and compatibility with different analytical systems of SPME suggest the convenience of using this technique for rapid screening procedures and/or confirmatory tests for adulterated olive oils.

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