

Enantiomeric Composition of Filbertone in Hazelnuts in Relation to Extraction Conditions. Multidimensional Gas Chromatography and Gas Chromatography/Mass Spectrometry in the Single Ion Monitoring Mode of a Natural Sample

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The influence of the matrix and the isolation and concentration conditions on the enantiomeric excess of filbertone [(*E*)-5-methyl-2-hepten-4-one] in hazelnuts is studied. In hazelnuts, under mild conditions the filbertone obtained has a higher enantiomeric excess than that one extracted under forced conditions. Enantiomers were separated by inclusion gas chromatography on Chirasil- β -Dex using multidimensional gas chromatography with flame ionization detection and gas chromatography/mass spectrometry in the selected ion monitoring mode.

Keywords: *Filbertone; enantiomeric excess; multidimensional gas chromatography*

INTRODUCTION

Hazelnuts (*Corylus avellana*) are used for the production of sweets and cakes due to their unique and delicate flavor. The main aroma compound of hazelnuts was identified by Emberger et al. (1987) as (*E*)-5-methyl-2-hepten-4-one (filbertone). Recently, Jauch et al. (1989) and Schurig et al. (1990) synthesized (*E,S*)-5-methyl-2-hepten-4-one and applied complexation as well as inclusion gas chromatography (GC) to determine the enantiomeric composition of filbertone in hazelnuts and to study racemization in the presence of heat, silica, and basic alumina. Güntert et al. (1991) determined the optical rotation of synthesized (*E,S*)-5-methyl-2-hepten-4-one and studied the taste quality of the two filbertone *E,R* and *E,S* isomers, showing that the *E,S* isomer has a stronger impact than the (*E,R*)-filbertone. The differences between the enantiomers with respect to odor intensity represent an example of the importance of chirality in olfaction.

Techniques such as multidimensional enantioselective gas chromatography (MDGC) (Bertsch, 1978), progress in the development of the derivated cyclodextrins as chiral selector phases (König, 1992; Schurig and Nowotny, 1988; Weber et al., 1995), and combination of GC and mass spectrometry in the selected ion monitoring [GC/MS(SIM)] represent reliable and powerful methods to avoid peak overlapping and to establish the enantiomeric composition and absolute configurations (Schurig, 1987; Glausch et al., 1995; Blanch et al., 1996).

The most important step prior to the analytical separation is the acquisition of a representative concentrate. The past few years have seen the development of isolation and concentration techniques that use mild

conditions to avoid degradation reactions during sample preparation. In this respect, steam distillation combined with extraction (SDE) described in 1964 by Likens and Nickerson has received increasing attention and an SDE microversion has been previously developed (Godfroot et al., 1981, 1982). Recently, an improved SDE apparatus using a more efficient cooling surface and allowing operation with solvents of both higher and lower density than water with only one configuration was presented (Blanch et al., 1993b). This device has proved to be very versatile as it allows operation at normal pressure (SDE-NP), at reduced pressure (SDE-RP), and in the operation mode, which involves the concentration of the dynamic headspace from the sample (SDE-HS) (Blanch et al., 1993a,b).

Here we report our results concerning the influence of the hazelnut matrix on the racemization of filbertone during extraction, using an MDGC/FID and an off-line MDGC with GC/MS(SIM).

EXPERIMENTAL PROCEDURES

Samples. Hazelnuts and hazelnut ice cream were purchased from the local market. The hazelnuts were milled and homogenized using a laboratory mill and were kept refrigerated until extraction. (*E,S*)-5-Methyl-2-hepten-4-one (filbertone) as reference compound was synthesized as described by Jauch et al. (1989).

Forty grams of hazelnuts and 300 mL of distilled water or 100 g of ice cream and 200 mL of distilled water were placed in a 500 mL flask of the above-mentioned SDE apparatus. Extractions were carried out according to our previous papers using dichloromethane (2 mL; Sigma-Aldrich, Steinheim, Germany) (Blanch et al., 1993a,b) at normal pressure (SDE-NP) under the following conditions: sample heating bath temperature, 149 °C; solvent heating bath temperature, 67 °C; coolant temperature, 3 °C; and extraction time, 60 min. When the extraction was performed under mild conditions (SDE dynamic headspace extraction), that is, purging the sample with an inert gas (SDE-HS), the experiments were carried out under the following conditions: sample heating bath temper-

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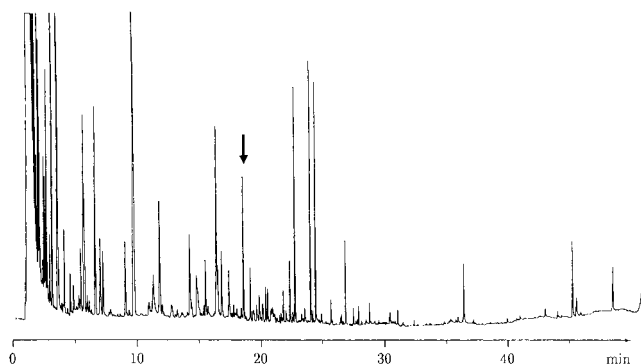


Figure 1. Gas chromatogram of volatile constituents of hazelnuts obtained from SDE-NP. (For chromatographic conditions see Experimental Procedures.)

ature, 40 °C; solvent heating bath temperature, 52 °C; coolant temperature, -5 °C; extraction time, 60 min; and inert gas flow, 100 mL/min (N₂). This process was repeated three times, and the resulting extracts were combined and concentrated to the final volume of 0.5 mL.

Instrumentation. GC was performed on a Siemens Sichromat 2 MDGC (Mannheim, Germany) with two separated ovens, on-column injector, and two FIDs. Column switching was achieved with a pneumatically controlled six-port valve (Valco, Schenck, Switzerland). Peak broadening was minimized by cooling the first 10 cm of the chiral column with air precooled with liquid nitrogen, thus focusing the cut fraction.

The achiral column was a 30 m × 0.25 mm i.d. glass capillary column coated with a 0.1 μm film of poly(5% diphenyl-95% dimethylsiloxane). Hydrogen was used as carrier gas at 1 bar. The column was held at 35 °C for the first 10 min, then programmed at 20 °C/min to 180 °C, and held at this upper temperature for further 20 min.

The chiral column was a 25 m × 0.25 mm i.d. glass capillary column coated with Chirasil-β-Dex (permethyl-β-cyclodextrin) (df = 0.25 μm). Hydrogen was used as carrier gas at 0.8 bar. The column was held at 40 °C and then programmed at 5 °C/min to 80 °C for 20 min and subsequently at 20 °C/min to 180 °C.

The MDGC-GC/MS(SIM) was accomplished using a modified MDGC instrument coupled off-line (Blanch et al., 1996; Blanch and Nicholson, 1998) with a second GC (Carlo Erba Fractovap 2900, Milano, Italy) coupled to a VarianMAT 112S mass spectrometer (Bremen, Germany) in SIM mode: ions with *m/z* 126 were detected. Electron energy was 80 eV.

The cut fraction containing the filbertone was trapped in a short uncoated fused silica capillary tube (80 cm × 0.25 mm i.d.) cooled with liquid nitrogen. The capillary tube was then installed in the GC/MS(SIM) instrument, connected to the Chirasil-β-Dex analytical column, and heated to achieve the thermal desorption of the retained solutes.

Racemization of *S*-(-)-Filbertone. (*E,S*)-Filbertone (2 μL) [enantiomeric excess (ee) 91%] and 300 mL of distilled water were placed in an autoclave. The sample was heated at 100 °C during 0, 24, 51, or 96 h.

To study the influence of the hazelnut matrix on the racemization of (*E,S*)-filbertone, 40 g of hazelnuts and 300 mL of distilled water were placed in an autoclave and 2 μL of (*E,S*)-filbertone (ee = 91%) was added. The sample was heated at 100 °C during 0, 6, 12, 24, 46, and 95 h. The same experiment was repeated at 140 °C. An aliquot of each SDE extract was injected on-column. The chromatographic conditions were as described above.

RESULTS AND DISCUSSION

The gas chromatogram of the hazelnut extract obtained at normal pressure is shown in Figure 1. The SDE method required no further purification due to the high quality of the extract obtained. The filbertone

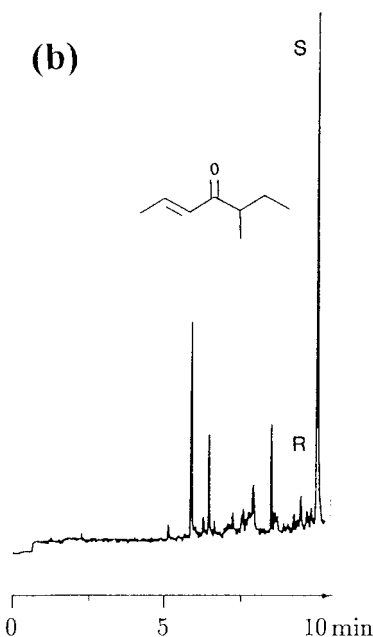
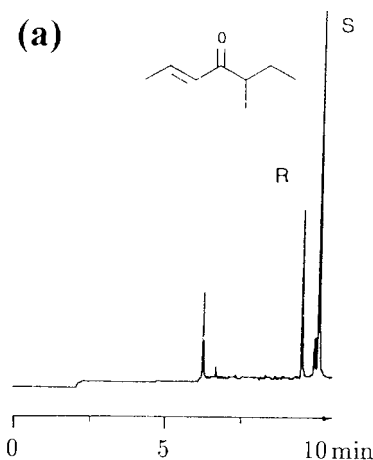


Figure 2. Chromatograms showing the enantiomeric composition of filbertone extracted from hazelnuts using SDE-NP (a) and SDE-HS (b) analyzed by MDGC-GC/MS(SIM). (For chromatographic conditions see Experimental Procedures.)

recoveries obtained were 83 and 60% using SDE-NP and SDE-HS, respectively.

Enantiomeric excess was determined by MDGC-GC/MS(SIM) (Figures 2 and 3), as described under Experimental Procedures. This represents an easy way to combine MDGC with MS and also offers the possibility to concentrate the substances of interest by repeated injections in the MDGC mode. Enantiomeric excesses in both hazelnuts and hazelnut ice cream are summarized in Table 1. Comparing the ee of filbertone obtained by SDE-NP and SDE-HS implies that racemization of filbertone seems to occur when the extraction of hazelnuts and hazelnut ice cream is performed at atmospheric pressure at 100 °C.

To confirm these results, we carried out some racemization experiments using synthesized (*E,S*)-filbertone. When the standard (*E,S*)-filbertone and distilled water are heated in an autoclave at 100 °C during 0, 24, 51, and 96 h, no racemization was observed (Figure 4, A). When the standard (*E,S*)-filbertone together with hazelnuts is heated at 100 °C for different times, the enantiomeric excess decreased (Figure 4B) and the rate

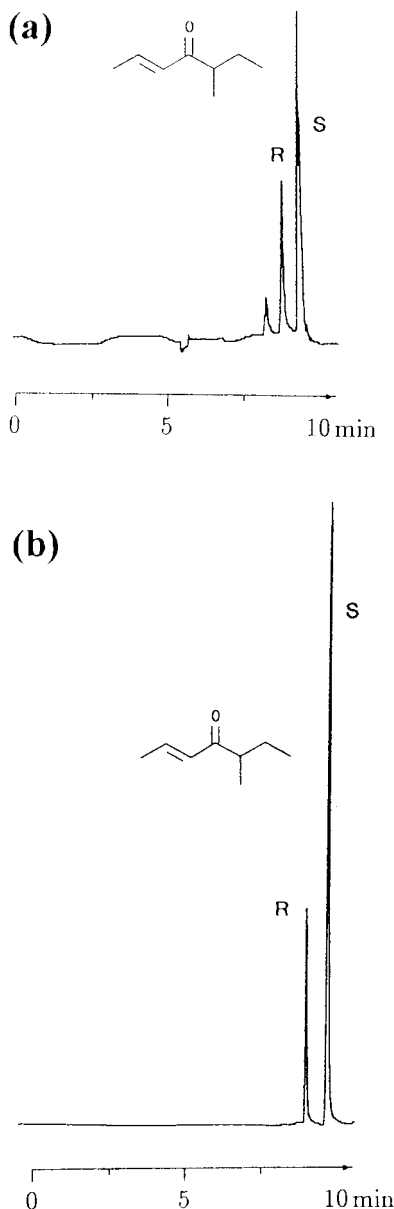


Figure 3. Chromatograms showing the enantiomeric composition of filbertone extracted from hazelnut ice cream using SDE-NP (a) and SDE-HS (b) analyzed by MDGC-GC/MS-(SIM). (For chromatographic conditions see Experimental Procedures.)

Table 1. Enantiomeric Excess (Percent) of Filbertone Obtained by MDGC-GC/MS(SIM)

| | SDE-NP | SDE-HS |
|--------------------|--------|--------|
| hazelnuts | 56 | 87 |
| hazelnut ice cream | 36 | 50 |

increased with temperature (Figure 4C). For example, when (*E,S*)-filbertone and hazelnuts were heated at 100 °C during 46 h, the enantiomeric excess dropped from 91.3 to 50.7%, and at 140 °C the enantiomeric excess dropped with the first 24 h, from 91.3 to 41.7%. As it has been previously suggested that filbertone shows no tendency for partial racemization due to heat (Jauch et al., 1989; Güntert et al., 1991), the results obtained in this work could indicate that a hitherto unknown precursor in hazelnut matrix forms racemic filbertone by a chemical mechanism, thus shifting the enantiomeric excess to a higher (*R*)-filbertone, and can explain the differences obtained in Table 1.

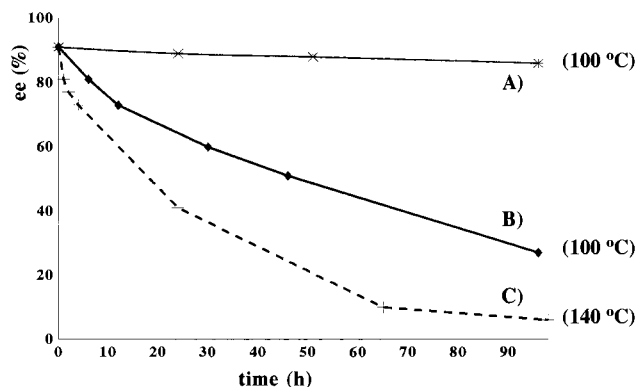


Figure 4. Evolution of the enantiomeric excess with the time of (A) (*E,S*)-filbertone and distilled water placed in an autoclave at 100 °C, (B) (*E,S*)-filbertone and hazelnuts placed in an autoclave at 100 °C, (C) (*E,S*)-filbertone and hazelnuts placed in an autoclave at 140 °C.

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