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Concentration of 4-methoxy-2-methyl-2-butanethiol in Spanish virgin olive oils

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Abstract

The sensitivity of a stable isotope dilution assay for 4-methoxy-2-methyl-2-butanethiol (MMB) causing the blackcurrant-like odour in olive oils was improved. After spiking the oil sample with labelled MMB, the thiols were separated from the volatile fraction with an immobilized reagent for thiols. MMB and its standard were purified by multi-dimensional gas chromatography of the thiol fraction and determined by mass spectrometry. The new procedure was applied to three Spanish oils differing in the intensity of their blackcurrant-like odour note. The concentrations of MMB lay between 0.4 μg kg^{-1} and 4.3 μg kg^{-1}. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Recently, 4-methoxy-2-methyl-2-butanethiol (MMB), which smells like blackcurrant in dilute solution, was detected in oils originating from Spain (Guth and Grosch, 1991, 1993). The two oil samples contained only traces (1.8 μg kg^{-1}) of MMB. However, because of its very low odour threshold (0.045 μg kg^{-1}) MMB was responsible for the intense blackcurrant-like note that was perceived in the aroma profile of these oil samples (Guth and Grosch, 1993).

The MMB concentration was determined in oil samples using 4-[$^2H_3$]methoxy-2-methyl-2-butanethiol (d-MMB) as internal standard (Guth and Grosch, 1993). Losses of the analyte and the standard do not affect the results, because the physical and chemical properties of the two isotopomers are identical apart from small isotope effects, which are discounted (Grosch, 1993). The reproducibility of this procedure, known as a stable isotope dilution assay, varies from 5 to 10%, assuming that the analyte and its standard are so pure that their peaks in the mass chromatograms are not overlapped by signals from other substances.

In the previous study (Guth and Grosch, 1993) MMB and its standard were enriched by high-performance liquid chromatography (HPLC). The aim of the present study was to improve the sensitivity of the analytical procedure by trapping the thiols using a specific reagent and multi-dimensional gas chromatography (MDGC) for the purification of MMB and d-MMB. The new procedure was applied to three Spanish oils differing in intensity of their blackcurrant-like odour note.

2. Materials and methods

2.1. Materials

The Spanish virgin olive oil S1 was purchased from the oil mill of Erfurt, Germany, the virgin olive oils S2 and S3 were gifts from the Instituto de la Grasa y sus Derivados, Sevilla, Spain. MMB and d-MMB were synthesized as reported previously (Guth and Grosch, 1991, 1993). Dithiothreitol and 2-methyl-1-pentanol were purchased from Aldrich (Steinheim, Germany). Affi-Gel 501 (organomercurial cross-linked agarose) was from Bio-Rad (München, Germany).

2.2. Concentration of d-MMB

The concentration of the labelled compound d-MMB was determined without correction factor by high resolution gas chromatography (HRGC) on capillary DB-FFAP (cf. below) with 2-methyl-1-pentanol as internal standard.

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2.3. Sensory evaluation of the olive oil samples

Sensory analysis was performed under the conditions described by Guth and Grosch (1993). The panel consisted of nine experienced assessors, seven males and two females, aged 25 to 35 years. A solution of MMB in refined sunflower oil (concentration 0.2 μg kg^{-1}) served as a reference for the blackcurrant-like odour quality. The panelists were asked to score the intensity of the blackcurrant-like odour in the olive oil samples nasally and retronasally on a category scale of 0 to 3.0 in 0.5 intervals. The samples were evaluated in two sessions and the results were averaged.

2.4. Quantification of MMB in oil samples

The sample (1 kg) was spiked with the internal standard d-MMB. Its amount varied between the 0.5- and 2-fold concentrations of the analyte MMB. The sample was then diluted with diethyl ether (1 litre) and the volatile fraction was stripped off together with the solvent under high vacuum (6 mPa, 50°C) using the apparatus described by Guth and Grosch (1989). The ethereal solution of the oil was dropped into the distillation flask within 6 h. Afterwards, distillation of the volatiles was continued for 1 h. The extract was concentrated to a volume of 50 ml by distillation. MMB and d-MMB were enriched by reversible covalent chromatography using Affi-Gel 501 (Full and Schreier, 1994; Semmelroch and Grosch, 1996). The resulting thiol fraction was separated from the displacing reagent dithiothreitol under high vacuum (6 mPa, 22°C) using the apparatus depicted by Sen et al. (1991). The traps were cooled with liquid nitrogen. The sublimate was concentrated by distillation, microdistillation (Bemelmans, 1979) and by the procedure of Dünges (Maarse and Grosch, 1996) to final volumes of 200 μl (S1) and 20 μl for S2 and S3, respectively.

The thiol fraction was analysed by MDGC using the moving capillary stream switching (MCSS) system (Fisons Instruments, Mainz-Kastel, Germany) installed into a gas chromatograph of the HRGC MEGA 2 series (Fisons Instruments) and controlled via a personal computer. The MCSS outlet had a flame ionization detector, and the main column outlet was connected to the HRGC/MS system reported earlier (Semmelroch et al., 1995) with an ion-trap detector (ITD 800, Finnigan, Bremen, Germany). DB-FFAP and DB-5 fused silica capillaries (30 m×0.32 mm, 0.25 μm film thickness) supplied from J&W Scientific (Folsom, CA, USA) were used as pre-column and main column, respectively. The carrier gas was helium at 2 ml min^{-1} for both the pre- and the main column. The samples (0.5 μl) were applied by the on-column injection technique at 35°C. The initial temperature was held for 2 min, then raised at a rate of 40°C min^{-1} to 60°C, held isothermally for 1 min and finally raised by 6°C min^{-1} to 230°C. The HRGC effluent of the pre-column eluting between 5 and 7 min was cut out, cryofocused with liquid nitrogen and then transferred onto the main column. After the start, the temperature of 40°C was held for 1 min, raised by 40°C min^{-1} to 50°C and then raised at a rate of 6°C min^{-1} to 230°C.

Mass chromatograms of MMB and d-MMB were recorded in the chemical ionization (CI) mode with methanol as the reagent gas (Guth and Grosch, 1993). The abundance of the selected ions m/z 101 (M^+-SH) and m/z 104 (d_3-M^+-SH) was evaluated; the calibration factor was 0.99 (Guth and Grosch, 1993).

3. Results and discussion

The intensity of the blackcurrant-like note was different in the three oil samples (Table 1). It was strong in

Table 1
Sensory evaluation of the blackcurrant-like odour of three Spanish virgin olive oil samples

	Intensity of the blackcurrant-like odour[a]		
	S1	S2	S3
Determined nasally	2.8	1.0	0.5
Determined retronasally	2.7	1.6	1.0

[a] Rating scale of the odour intensity: 0, odour quality is lacking; 1, bland; 2, moderate; 3, strong.

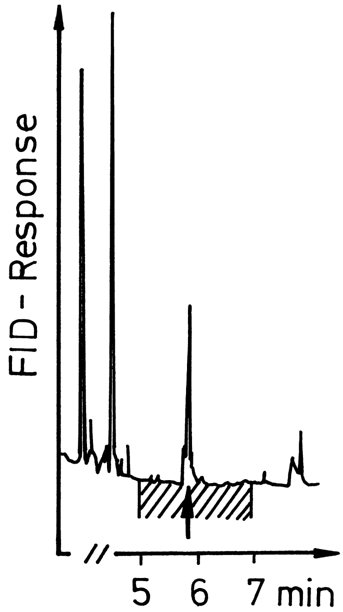

Fig. 1. Gas chromatogram (pre-column) of the thiol fraction obtained from S1. The effluent between the 5th and 7th minutes (hatched area) was transferred onto the main column.

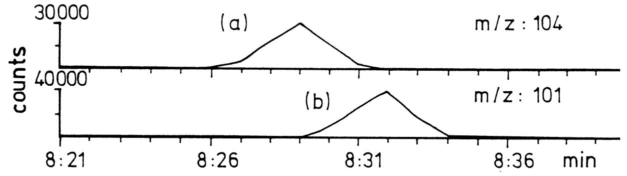

Fig. 2. Mass chromatograms of (a) d-MMB and (b) MMB after GC separation of the effluent (cut out from the pre-column) on the main column.

Table 2
Concentration of MBB in three Spanish virgin olive oil samples

	Concentration (μg kg^{-1})[a]		
	S1	S2	S3
MMB	4.3	0.9	0.4

[a] The results are mean values of duplicates differing by not more than 8.5%.

S1, medium in S2, when this sample was retronasally evaluated, and only weak in S3.

In the analytical procedure, MMB and d-MMB were enriched with other thiols by covalent chromatography according to Full and Schreier (1994). The analyte and its standard were purified by MDGC. Fig. 1 shows the gas chromatogram of the thiol fraction of S1 on the pre-column. MMB and its standard eluted between the 5th and 7th minutes. The effluent was cut out and transferred onto the main column. After GC separation mass chromatograms (Fig. 2) were recorded to differentiate between the unlabelled odorant (originating from S1) and the deuterated standard. The concentration of MMB in S1 (4.3 μg kg^{-1}) was calculated based on the areas of the two peaks (MMB and d-MMB in Fig. 2). In comparison to preseparation of the volatile fraction by HPLC (Guth and Grosch, 1993), the modified analytical procedure provides a higher degree of enrichment of MMB and its standard because of the application of a selective thiol reagent and the higher separation efficiency of the MCSS system.

The concentration of MMB was much lower in S2 and S3 than in S1 (Table 2). This difference correlated with the weaker blackcurrant-like odour note of these oil samples. The concentations of MMB in the three oils were in the same low range as found earlier (Guth and Grosch, 1993). The conclusion that these low levels of MMB causes the blackcurrant-like odour of olive oils agreed with the results of Rigaud et al. (1986) that the aroma of blackcurrant liquor is enhanced by the addition of 0.2 μg kg^{-1} of MMB.

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