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Journal of Chromatography A, 983 (2003) 283–288
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Simultaneous determination of long-chain aliphatic aldehydes and waxes in olive oils

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Received 6 May 2002; received in revised form 2 August 2002; accepted 2 August 2002

Abstract

A procedure for the simultaneous determination of long-chain aliphatic aldehydes, and aliphatic and triterpenic waxes in virgin olive oils is described. A fraction containing these compounds was isolated from the oil using solid-phase extraction on silica-gel cartridges. The fraction was analyzed by capillary GC on 35%-dimethyl–65%-diphenylpolysiloxane phase using on-column injection. In extra virgin olive oils, the long-chain aliphatic aldehydes with even carbon atom numbers from C_{22} to C_{30} were identified by comparison of retention times and mass spectra with those of synthesized standards. The concentration of total aldehydes ranged from 20.2 to 108.0 mg/kg—*n*-hexacosanal being the most abundant aldehyde. The determination of aliphatic waxes was achieved with similar or better precision than that of the EU official methods.

Keywords: Waxes; Olive oil; Food analysis; Aldehydes

1. Introduction

Wax esters occurring in vegetable oils are a group of compounds formed by esterification of high-molecular-mass alcohols with fatty acids. The length and structure of the alcoholic group is variable; thus, if the alcoholic groups are long chain aliphatic alcohols they result in aliphatic waxes of 34–46 carbon atoms. If the alcoholic group are sterols [1], triterpenic alcohols or methylsterols [2], the compounds are generally named terpenic waxes. In olive oils, the sum of C_{40}, C_{42}, C_{44} and C_{46} aliphatic waxes is a parameter used to detect olive-residue oil in olive oil [3]. In addition, a high content of waxes in vegetable oils, particularly sunflower oil could produce turbidity after long term storage [4]. Therefore, the determination of waxes in vegetable oils is of great interest in order to assess the quality.

Recently, with the aim of reducing time and also simplifying official methodologies [3], several authors have described procedures to quantify wax esters in olive oils [5–8]. In particular, Nota et al. [7] developed a method to isolate the wax fraction using solid-phase extraction (SPE) on silica cartridges from a small amount of sample (20 mg) and analyzing the fraction by gas chromatography on a polar phase. Nevertheless, carbon tetrachloride is used for eluting the wax fraction from the cartridge, and the use of nonchlorinated solvents would be advisable.

On the other hand, in distillates obtained during refining of soybean oil, long-chain aliphatic alde-

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)01608-4

hydes (LCAAs) have been detected [9]. Besides, aldehydes with large number of carbon atoms (C_{22}–C_{30}) have been found in various parts of different plants [10–13], including the waxy material covering the surface of the olive fruit [11,14,15]. It has been suggested that they are intermediates in the synthesis of alkanes and secondary alcohols [16]. Likewise, Kubo et al. [17] demonstrated the activity of medium-chain aldehydes (C_6–C_{10}) against microorganism and proved that increasing the chain length of the carbon tail seen to result in increased activity. Nonetheless, neither results nor references on LCAAs presence in olive oils have been previously reported. In this work a new method for the simultaneous determination of LCAAs, and aliphatic and triterpenic waxes is described. The methodology includes isolation of the corresponding fraction by SPE and quantifying by gas chromatography on a polar stationary phase using on-column injection.

2. Experimental

2.1. Samples

Genuine extra virgin olive oils, from several origin and varieties were used.

2.2. Material and reagents

All reagents were of analytical-reagent grade otherwise specified. Pyridium chlorochromate, 1-triacontanol, 1-docosanol, 1-hexacosanol, arachidyl laurate, cholesteryl stearate, and, dye Sudan I, were purchased from Aldrich (Steinheim, Germany). The SPE cartridge (6 ml) packed with silica (1000 mg) was purchased from Varian (Middelburg, The Netherlands).

A solution of dye Sudan I (1 mg/ml) in hexane–ethyl ether (99:1, v/v) was prepared.

2.3. Synthesis of standard long-chain aldehydes

Long chain aldehydes were prepared by oxidation of the corresponding alcohols using pyridinium chlorochromate, as described by Nass et al. [13]. A molar ratio of 0.1 m*M* alcohol (33 mg C_{22}-ol, 38.3 mg C_{26}-ol, 44 mg C_{30}-ol), to 0.9 m*M* pyridium chlorochromate in dichloromethane (195 mg/100 ml) was stirred for 1.5 h. The reaction mixture was passed through a short column (6 cm×2 cm I.D.), packed with silica-gel 60. The reaction products were eluted from the column using dichloromethane (50 ml), and the solutions evaporated to dryness. The GC analysis of the residues showed only the aldehyde peaks.

2.4. Preparation of stock solutions and glyceridic matrix

Oil samples (500 mg) were fractioned on a silica-gel (15 g) column chromatography prepared as indicated in EU regulations [3]. The oil in hexane (2 ml) was placed onto the column and eluted with 70 ml of hexane. This fraction containing hydrocarbons was discarded. Then the column was eluted with 70 ml of hexane–ether (98.5:1.5, v/v) and the fraction evaporated until dryness. The residue containing aldehydes and waxes was dissolved in heptane—these were the stock solutions. A third fraction was eluted with 150 ml of hexane–ether (87:13, v/v) and evaporated in a rotary evaporator at room temperature under vacuum. This oily residue, containing the triglycerides, was used as lipidic matrix.

2.5. Preparation of standard solutions

A solution of 0.2 mg/ml of arachidyl laurate (C_{32}-W) and 0.5 mg/ml of cholesteryl stearate, in hexane, was used as internal standard for determining aldehydes and aliphatic and terpenic waxes.

2.6. Solid-phase extraction–gas chromatography

2.6.1. Solid-phase extraction

A silica SPE cartridge (1000-mg) was placed in a vacuum elution apparatus, and conditioned by passing through 6 ml of hexane. The oil sample (≤50 mg) containing the standards C_{32}-W and cholesteryl stearate (50 μl) and two drops of the dye Sudan I solution was applied to the column and the solvent was pulled through, remaining the sample, the standard and the stain on the top of the column. The sample container was washed with two 0.5-ml portions of hexane that were run out the cartridge. The column was eluted with 7 ml of hexane–toluene

(85:15) in order to eliminate the hydrocarbon fraction including the majority of squalene. Then, the column was eluted with approximately 10 ml of the same mixture until the stain reached the bottom of the cartridge. This second eluate was evaporated in a rotary evaporator at room temperature under vacuum until dryness. The residue was redissolved in 500 μl of heptane and an aliquot (0.5 μl) was injected onto the gas chromatograph.

2.6.2. Gas chromatographic analysis

Chromatographic analysis was performed using a Chrompack (Middelburg, The Netherlands) CP9000 gas chromatograph fitted with a flame ionization detection system and a cold on-column injection system. Separations were carried out on an Rtx-65TG capillary column (30 m×0.25 mm I.D.) coated with 35% dimethyl–65% diphenylpolysiloxane (Restek, Bellefonte, PA, USA). The operating conditions were the following: oven temperature 90 °C for 0.2 min and then increased at 20 °C/min up to 160 °C; then increased at 4 °C/min up to 350 °C. Injector and detector temperatures were 90 and 365 °C, respectively. Hydrogen was used as carrier gas at flow-rate of 3.5 ml/min. Data acquisition and processing were carried out using a Chrom-Card Data System (Fisons, Altrincham, UK).

2.7. GC–MS identification

A mass spectrometer MAT 95-S (Finnigan, Manchester, UK) coupled directly to a gas chromatograph HP-5890 (Hewlett-Packard, Wilmington, DE, USA) fitted with a Rtx-65TG fused-silica capillary column (30 m×0.25 mm I.D.) was used. Sample injection was made in splitless mode, helium at a flow-rate of 1.6 ml/min was used as carrier gas. Electron impact ionization at 70 eV and resolution 2500 (10% valley definition) were used. The temperature programming was isothermal at 140 °C for 5 min, then increasing at 4 °C/min up to 350 °C.

2.8. Repeatability and recovery

For the determination of repeatability, two stock solutions containing different concentrations of aldehydes and waxes were obtained from two olive oils using the method described in Section 2.4. Lipidic matrices were spiked with mixtures of both stock solutions in different proportions, and then the oily samples processed according to the analytical procedure described in Section 2.6. Each sample was analyzed five times.

For the determination of recovery, the oily samples were processed without the addition of the standard solution and the extracts were obtained. An analogous series of extracts containing the same amounts of aldehydes and waxes were prepared in heptane. Both series of solutions were spiked with the standard solution (containing arachidyl laurate and cholesteryl estearate), evaporated and redissolved in 500 μl of heptane. A volume (1 μl) of solution was injected onto the gas chromatograph using the on-column injection system. The results of the oily matrices were compared with those obtained by direct injection of the heptane solutions.

3. Results and discussion

Compared with the EU official method, the procedure proposed by Nota et al. [7] is a simple and quick method to quantify wax esters using carbon tetrachloride as eluting solvent. However, since chloride solvents should not be used, because they are a source of pollution it was advisable to search for other solvents and hexane–toluene (85:15) was found to be the the most suitable. Sudan I, a dye that elutes with triacylglycerols, allows the visualization of the elution guaranteeing the complete elution of the waxes and remaining triacylglycerols in the cartridge. The first fraction (7 ml) was rejected because it contains hydrocarbons, mainly squalene, which is present in large amounts in olive oils and overlaps with the C_{26}-aldehyde peak. Subsequent elution with an additional amount of hexane–toluene (~10 ml) until the stain reaches the bottom of the cartridge yield a fraction containing aldehydes, waxes and a residual amount of squalene that does not interfere with the aldehyde determination.

Fig. 1 shows a gas chromatogram of the second fraction, where a group of five unknown compounds eluted just before the aliphatic waxes. Their mass fragmentation spectra exhibited the presence of an uniform difference of 28 mass unit and absence of $[M-Me]^+$ ion, suggesting a long straight-chain

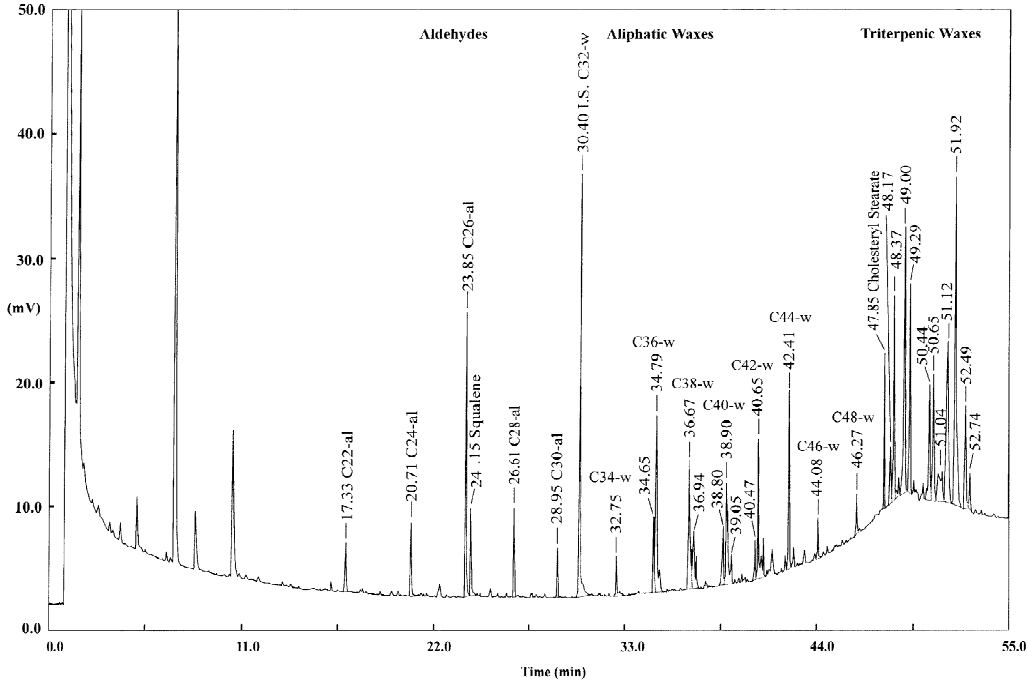

Fig. 1. Gas chromatogram of the second fraction containing long chain aldehydes, and aliphatic and terpenic waxes isolated by SPE from virgin olive oil. GC conditions as described in Section 2.6.2.

compound. Their molecular ion $[M]^+$ peaks at m/z, 324, 352, 380, 408 and 436, although small were recognizable, and the presence of peaks corresponding to $[M-18]^+$, $[M-44]^+$ and $[M-46]^+$ suggested the existence of an aldehydic function in all the unknown compounds [18]. Fig. 2 shows the mass spectrum of C_{26}-aldehyde isolated from olive oil.

On the basis of these results, these compounds were identified tentatively as long chain aliphatic alcohols with an even number of carbon atoms from C_{22} to C_{30}. In order to confirm the presence of these compounds in olive oil, C_{22}, C_{26} and C_{30} aldehydes were synthesized from the corresponding long chain alcohols, and then analyzed under the same conditions. All the synthesized aldehydes eluted in their expected place, confirming the structure and the presence of the formulated compounds.

For quantitative determination of LCAAs and aliphatic waxes, arachidyl laurate (C_{32}-W) was used as internal standard, because its retention time is close to those of the aldehydes, and it does not interfere with other compounds. The response factor of aldehydes was calculated for the C_{22}-al. The results ranged from 0.95 to 1.03, the mean being 0.99. Therefore, a response factor of 1 was adopted. For quantitative determination of terpenic waxes, cholesteryl stearate was used.

In Table 1, reproducibility and recovery of the determination of LCAAs and waxes are shown. Triterpenic and sterolic waxes are calculated adding up the areas of peaks eluting after the cholesteryl stearate. In the same way, each aliphatic wax is calculated by adding together the areas of neighbouring peaks corresponding to waxes with the same number of carbon atoms.

Extra virgin olive oils coming from olives of several varieties showed GC profiles of LCAAs similar to that depicted in Fig. 1. Aldehydes with even number of carbon atoms from C_{22} to C_{30} were

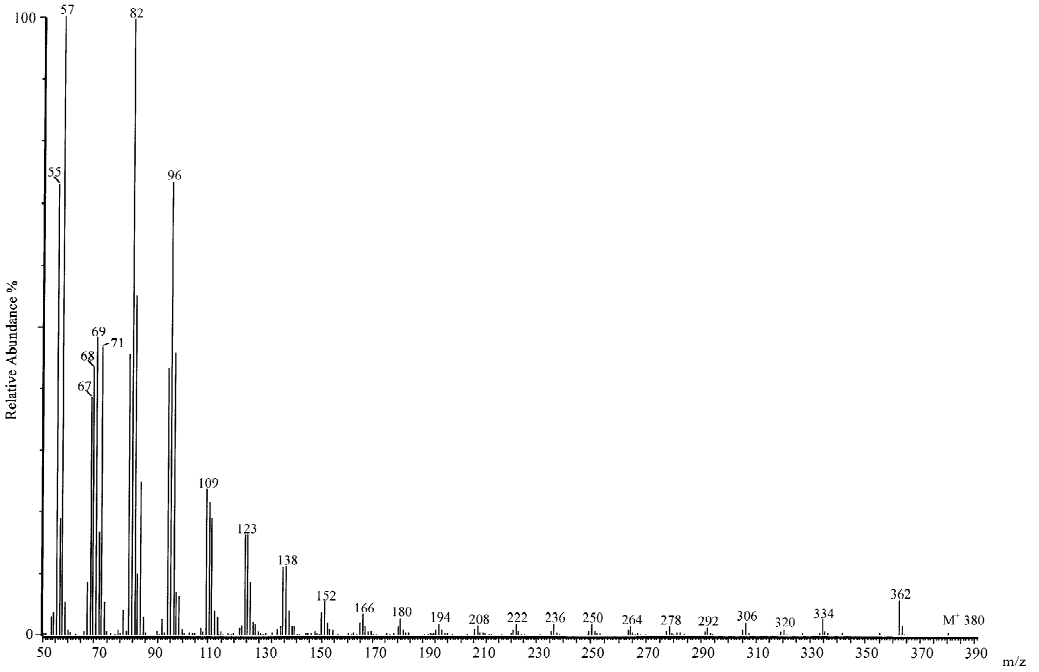

Fig. 2. Mass spectrum of the C_{26} aldehyde isolated from olive oil. MS conditions as described in Section 2.7.

detected, the C_{26}-aldehyde being the most abundant. The fact that the major hydrocarbon present in olive oil is the C_{25} [19] supports the hypothesis of different authors [20,21] that LCAAs are the precursors of the corresponding alkanes by decarboxylation. The concentrations of total LCAAs ranged from 20.2 to 108.0 mg/kg.

The determination of aliphatic waxes C_{40} to C_{46} was carried out with similar or better repeatability than the EU official methodology (RSD repeatability values of 6.4–10.5% for concentrations about 120 mg/kg) [22].

Acknowledgements

We are grateful to Mrs. R. González-Cordones and Mr. M. Rodriguez-Aguilar for technical assistance,

Table 1
Recovery and precision data of aldehydes and waxes analysis by SPE–GC

Sample	Aldehydes (ΣC_{22}–C_{30})			Waxes ($\Sigma C_{36}+C_{38}$)			Waxes (ΣC_{40}–$C4_{46}$)			Triterpenic+sterolic waxes		
	Mean (mg/kg)	RSD_r (%)	Recovery (%)	Mean (mg/kg)	RSD_r (%)	Recovery (%)	Mean (mg/kg)	RSD_r (%)	Recovery (%)	Mean (mg/kg)	RSD_r (%)	Recovery (%)
1	107	3.2	93	434	5.2	96	91	5.6	94	407	6.2	94
2	69	3.7	94	164	5.1	94	45	5.2	92	635	5.9	96
3	40	3.7	92	23	5.2	93	185	4.9	95	600	6.1	95
4	29	3.8	93	140	5.0	93	27	5.5	90	36	6.5	97

RSD_r, relative standard deviation of repeatability, $n=5$.

to project ALI-97-0644 for financial support and to Mr. J.J. Rios for the GC–MS analysis.

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