High-Resolution Gas Chromatographic Determination of Alkanols in Oils Extracted from Olives

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Polar columns with stationary phases characterized by high thermal stability have attracted a great deal of interest in the study of lipid compounds that have, until recently, been studied with nonpolar columns. Such polar columns have also proved useful in the study of the entire unsaponifiable matter of a lipid and in determining the alkanols of extra-virgin olive oil, husk oil and their relative mixtures. The proposed procedure permits the study of alkanols without having to isolate them from the other classes of compounds present in the unsaponifiable matter by means of thin-layer chromatography.

KEY WORDS: Alkanols, gas chromatographic analysis, olive oil, oil quality, unsaponifiable.

The main characteristics required of an analytical technique to evaluate food products in general, and fatty substances in particular, can be summarized as follows: precise results, repeatability and reproducibility, uncomplicated rapid execution and low cost. Unfortunately, in an analytical method all these objectives cannot always be reached because the matrices are often complex or because the compounds to be analyzed are present only in trace amounts. In such cases it becomes essential to manipulate the sample and/or to use complex analytical techniques, which causes longer times of execution as well as higher costs.

The aim of the present work was to quantitate the alkanols directly in the unsaponifiable fraction of olives through high-resolution gas chromatography (HRGC) with polar capillary columns that are stable at high temperature (1,2). This procedure permits the study of alkanols without having to isolate them by means of thin-layer chromatography (TLC) from other classes of compounds present in the unsaponifiable matter. This technique also avoids losses occurring during recovery from chromatographic plates. In fact, as has been shown by Mariani (3), in the TLC separation of unsaponifiable matter, the aliphatic alcohols with 22–28 carbon atoms do not move as a distinct band but show a "spray-out" effect on the plate due to the different number of carbon atoms in the aliphatic chain.

EXPERIMENTAL PROCEDURES

Five grams of each oil sample (extra-virgin olive oil, husk oil and their relative mixtures), after the addition of squalane ($C_{30}H_{62}$, internal standard IS_1) in benzene and 500 μL of 0.1% eicosanol in chloroform (internal standard IS_2), were saponified according to the procedures detailed in *Norme Grassi e Derivati* (4). After treatment with diazomethane (CH_2N_2) (5) to transform any free fatty acid present to methyl ester, 10% benzene solution of unsaponifiable matter was prepared. Fifty μL of this solution, after evaporation and treatment with silanizing mixture to transform the hydroxyl groups into trimethylsilyl derivatives according to Sweeley *et al.* (6), were analyzed by gas chromatography with a Carlo Erba HRGC 5160 Mega gas chromatograph (Carlo Erba, Rodano, Milano, Italy) interfaced with a Mega 2 computing integrator and equipped with a fused-silica capillary column (25 m × 0.32 mm i.d.) with a 0.1 μm film thickness of 50% phenyl/50% methylpolysiloxane (TAP, Chrompack, Middleburg, The Netherlands) stationary phase. Carrier gas was He at 0.8 mL/min column flow rate and 1:80 split ratio, with an inlet pressure of 100 KPa. Injector and detector temperatures were 330°C; the oven temperature was programmed from 200 to 300°C with a rate of 3°C/min.

Identification of the different components was carried out by comparing retention times with those of pure substances provided by Sigma Chemical Co. (St. Louis, MO) and Supelco Inc. (Bellefonte, PA), by comparing the results obtained with SE 52 nonpolar type column, and by comparing the results with those already published in the literature (1,2).

RESULTS AND DISCUSSION

By analyzing the entire unsaponifiable matter, it is not only possible to obtain an immediate view of the proportion of components in the same or in different classes of substances, but also to determine the alkanols directly because they are distinctly separated. In addition, this kind of analysis could be useful to test the quality of the raw material. The graph of the unsaponifiable matter of husk oil (Fig. 1) shows clear separation of the different components of the diverse classes of unsaponifiable compounds. Peaks 2, 3, 4 and 6 correspond to alcohols with 22, 24, 26 and 28 carbon atoms, respectively. The same figure also shows two gas chromatograms of the unsaponifiable matter of two different lampante olive oils (cold-pressed olive oils with more than 3.3% free fatty acids and/or unpleasant flavor). The peaks indicated as OS correspond to the oxidized products of squalene ($C_{30}H_{50}$, triterpenic hydrocarbon).

Figure 2 shows the graphs obtained from the unsaponifiable fraction of extra-virgin olive oil (Fig. 2A) and two different husk oils (Fig. 2B and 2C). Among the other components present in extra-virgin olive oil, graphs B and C of the same figure show the alkanols with 22–28 carbon atoms. This method allows estimation of the amount of alkanols corresponding to the total sum of alcohols with 22–24 carbon atoms.

In addition, the graphs indicate the presence of other components useful for the classification of oils extracted from olives: peak 15 (erythrodiol), peak 16 (uvaol) and peak 18 (oleanolic acid) are quantitative markers to ascertain whether the oils were obtained by pressing or by extraction. A further element to be noticed, as a confirmation

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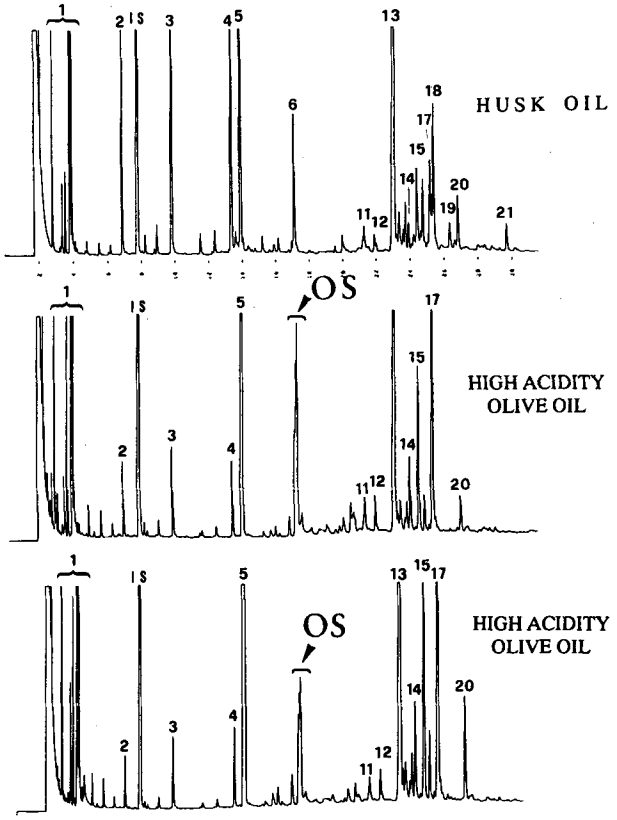

FIG. 1. Gas chromatograms of unsaponifiables of husk oil and two different lampante oils. 1, Free fatty acids; 2, docosanol; IS, internal standard (squalane); 3, tetracosanol; 4, hexacosanol; 5, squalene; OS, oxidation by-products of squalene; 6, octacosanol; 11, campesterol; 12, stigmasterol; 13, β-sitosterol; 14, Δ^5-avenasterol; 15, cycloartenol; 17, 24-methylenecycloartanol; 18, erythrodiol; 19, uvaol; 20, citrostadienol; and 21, oleanolic acid.

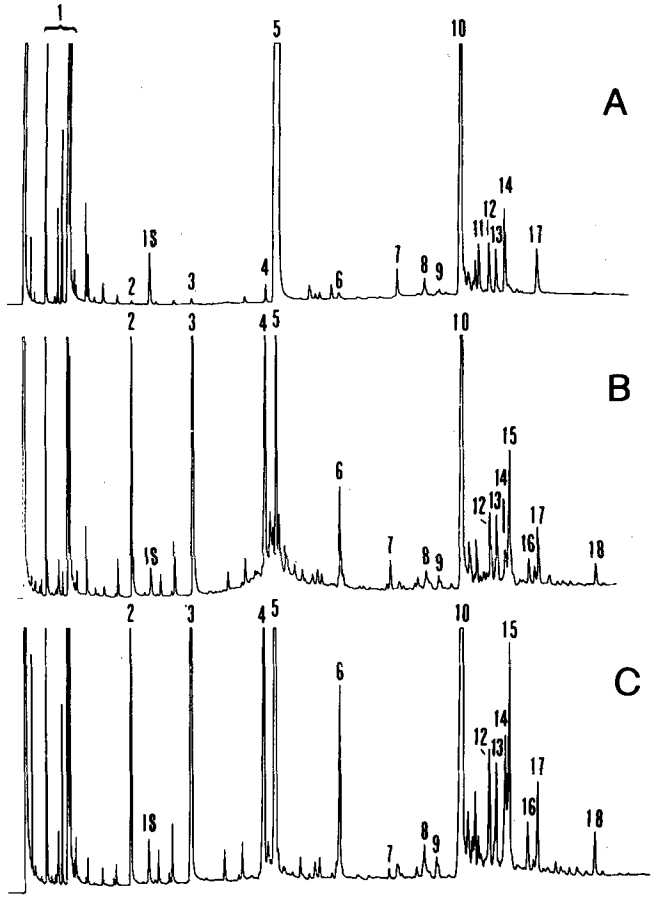

FIG. 2. Gas chromatograms of the unsaponifiable fraction of: A, extra-virgin olive oil; B and C, husk oils. 1, Free fatty acids; 2, docosanol; IS, internal standard (squalane); 3, tetracosanol; 4, hexacosanol; 5, squalene; OS, oxidation by-products of squalene; 6, octacosanol; 7, α-tocopherol; 8, campesterol; 9, stigmasterol; 10, β-sitosterol; 11, Δ^5-avenasterol; 12, cycloartenol; 13, unknown; 14, 24-methylenecycloartanol; 15, erythrodiol; 16, uvaol; 17, citrostadienol; and 18, oleanolic acid.

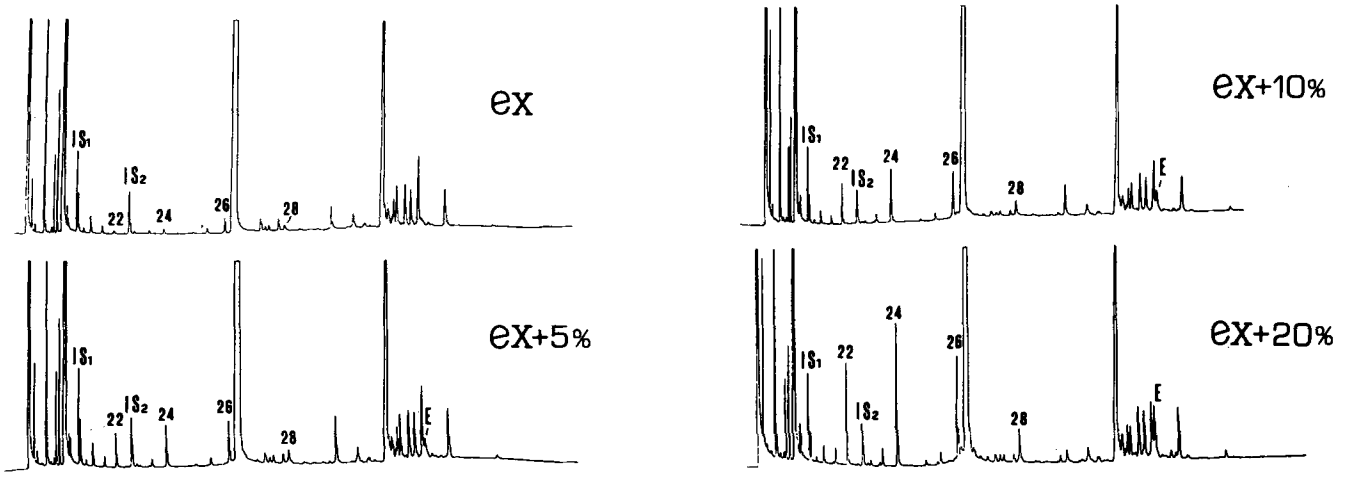

FIG. 3. Gas chromatograms of unsaponifiable matter of: ex, extra-virgin olive oil; ex + 5%, ex + 10% and ex + 20% = extra-virgin olive oil with 5, 10 and 20% of husk oil. 22, Docosanol; IS_1, internal standard (eicosanol); IS_2, internal standard (squalane); 24, tetracosanol; 26, hexacosanol; 28, octacosanol and E, erythrodiol.

of the classification, is the relative scarcity of squalene in extracted oils (graphs B and C in Fig. 2).

Figure 3 shows a graph of the unsaponifiable matter of a sample of extra-virgin olive oil to which small amounts of husk oil were gradually added. With the aid of internal standards (IS_1, IS_2) containing the same amount of additives so as to correspond, in most cases, to the quantitative evaluation of alkanols present in husk oil (10 mg/100 g, each standard), it is possible to recognize as little as 5% husk oil mixed with extra-virgin olive oil.

In conclusion, by direct gas chromatographic determination, the method proposed gives the following information after only 30 min: (i) a fingerprint of the unsaponifiable matter, which can be used to distinguish between different classes of components (1); (ii) an assessment of the quality and quantity of the alkanols, thus avoiding errors due to handling of the samples prior to the final chromatographic determination; (iii) feedback on the presence of marker components (erythrodiol, uvaol, oleanolic acid) that indicate the method of oil extraction; and (iv) evidence of the oxidation by-products of squalene, indicating the quality of the raw material.

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