Determination of the Enantiomeric Composition of γ-Lactones in Edible Oils by On-Line Coupled High Performance Liquid Chromatography and Gas Chromatography

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A new method is proposed for the determination of the enantiomeric composition of γ-lactones in different vegetable edible oils (i.e., olive oil, almond oil, hazelnut oil, peanut oil, and walnut oil), and its potential for authenticity control is underlined for a limited number of samples. The method is based on the direct injection (i.e., without requiring a sample pretreatment step) in on-line coupled reversed phase liquid chromatography to gas chromatography (RPLC-GC) using a chiral stationary phase in the GC-step. Different experimental values for both speed of sample introduction into GC and volume of the transferred fraction are considered to improve the recoveries obtained. Relative standard deviations lower than 10% and detection limits ranging from 0.06 to 0.22 mg/L were achieved for the investigated γ-lactones.

Keywords: *Enantiomeric composition; lactones; edible oils; on-line RPLC-GC*

INTRODUCTION

The evaluation of authenticity of edible oils is often difficult due to the complexity of the matrix. Specifically, the evaluation of oil quality and genuine character requires sensitive analytical methods suitable to determine specific compounds. In this respect, identification of compositional differences existing in the main vegetable edible oils is interesting and, among these oils, in those which for their low cost are used for frauds or are potential adulterants of olive oil.

The analysis of minor compounds of the unsaponifiable matter of edible oils is usually recommended for both their characterization and the detection of adulterated oils (Grob and Romann, 1993; Grob et al., 1994a,b; Li-Chan, 1994; Firestone, 1995; Paganuzzi, 1997). In some cases, however, the reliability of the analysis is not enough to establish the genuine character or the adulteration of a specific oil. For that reason, the performance of rapid and accurate analysis of suspect samples often demands the development of new analytical methods which ideally should not require a sample pretreatment step as it may be a source of unreliability.

An aspect of great interest concerning identification of adulterated consumer products is the enantiomeric analysis of suitable chiral markers. Actually, several studies have already been published regarding the usefulness of performing the stereochemical analysis of food components (Armstrong et al., 1990; Stalcup et al., 1993; Ekborg-Ott and Armstrong, 1997) and the availability of different stationary phases providing the enantioselectivity required for a number of applications has also been reported (Allenmark, 1991; König, 1992; Schurig, 1994; Juvancz and Petersson, 1996; Beesley and Scott, 1998). Specifically, the possibility of establishing the enantiomeric excess of chiral compounds is interesting for more reliable studies concerning the authenticity of a consumer product or the detection of its adulteration. However, the enantiomeric analysis of foods and beverages is not considered in most cases probably due to the lack of suitable methods for the efficient resolution of enantiomers in complex matrixes. Actually, the use of multidimensional systems or coupled techniques may be in some cases the alternative adequate as it allows to enhance the resolution of the components and to remove overlapped compounds by transfer cuts of limited broadness (Werkhoff et al., 1991; Full et al., 1993; Mondello et al., 1998).

In this respect, on-line coupling LC-GC (Grob, 1991, 1995; Vreuls et al., 1994; Mondello et al., 1996) is advantageous as it allows not only an effective sample fractionation in the LC step, even when reversed-phase eluents are required, but also an efficient and rapid separation by high-resolution capillary gas chromatography (Blanch et al., 1998a,b,c; Señoráns et al., 1998).

In previous work we have proposed the use of (*E*)-5-methylhept-2-en-4-one (filbertone) as a chiral marker to detect adulterated olive oil with hazelnut oil (Ruiz del Castillo et al., 1998), but further research is still needed to establish new chiral markers potentially useful to improve the reliability of the declaration of an oil as genuine or adulterated.

On the other hand, several studies regarding the chemical composition of different foods have already demonstrated the presence of γ-lactones and have emphasized their role in the sensory attributes that can be detected by consumers. In this respect, the use of coupled techniques has been previously recommended to improve the efficiency achieved in the separation of these compounds (Schmarr et al., 1990; Blanch et al., 1998b). However, the presence of γ-lactones in edible oils and their potential as chiral markers has not yet been sufficiently studied. Actually, γ-octa- and γ-nonalactone have been tentatively identified in peanut oil (Chung et al., 1993), but as far as we know the possibility of using the enantiomeric composition of

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γ-lactones in different oils as a reference to establish its authenticity has not been investigated.

The objective of this work was to develop a new method to determine the enantiomeric composition of γ-lactones in different vegetable edible oils by using on-line coupled reversed phase liquid chromatography with gas chromatography (RPLC-GC) analysis without any kind of sample pretreatment. A further aim of our work was to evaluate the possibility of using the determination of the enantiomeric composition of γ-lactones to establish the authenticity of an oil.

EXPERIMENTAL PROCEDURES

Samples and Materials. A standard solution containing 50 mg/L of different γ- lactones (i.e., C_8, C_9, C_{10}, and C_{11}) was used for identification purposes. Lactones were obtained from Aldrich (Milwaukee, WI), methanol (HPLC grade) was purchased from Scharlau Chemie, S.A. (Barcelona, Spain), and the water used was obtained from a Milli-Q water purification system (Millipore, Milford, MA). Tenax TA (80−100 mesh; Chrompack, Middelburg, The Netherlands) was placed in the silylated glass liner (75 mm × 1 mm i.d. × 2 mm o.d.) of the GC-injector as detailed below. Tenax TA was conditioned under a stream of helium for 120 min at 350 °C. Two olive oils (virgin and blend), one almond oil (unknown type), two hazelnut oils (virgin and unknown type), one peanut oil (virgin), and one walnut oil (unknown type) were analyzed. All oils were purchased in the commercial market except one of the two hazelnut oils which was obtained from an oil mill. In all cases, oils were filtered through a 0.22-μm filter prior to its RPLC-GC analysis.

On-Line Coupling Liquid Chromatography−Gas Chromatography (LC-GC). The analyses were performed using an on-line coupled LC-GC equipment. LC preseparation was accomplished with a Hewlett-Packard model 1050 (Wilmington, DE) chromatograph equipped with a manual injection valve (model 7125, Rheodyne, Cotati, CA) having a 20-μL sample loop and an ultraviolet (UV) detector operated at 205 nm. For the GC step, a Perkin-Elmer model 8500 (Norwalk, CT) fitted with a programmed temperature vaporizer (PTV) was used. The flame ionization detector (FID) was operated at 320 °C. Acquisition of data from both UV- and FID-detector was performed using a HPChem Station (Hewlett-Packard).

LC Analysis. The LC column was a 50 × 4.6 mm i.d. Kromasil 100−10C_4 (Symta, Madrid, Spain), and methanol/water was used as mobile phase at different flow rates (see below). All analyses were performed at 45 °C. The eluent composition (methanol/water, 35:65, v/v) was initially maintained for 18 s, and a subsequent linear gradient was applied within 3 s up to 90% methanol and maintained during the analysis.

LC-GC Transfer. Transfer into the gas chromatograph of the volume fraction previously selected from the LC preseparation step was carried out using the PTV injector of the gas chromatograph as interface for the LC-GC system. The operation mode also demands the use of a multiport valve (Rheodyne, model 7060) positioned after the UV detector of the LC-system and a transfer line. Upon elution of the beginning of the fraction of interest, a specific volume (see below) was transferred to the GC (by switching the mentioned valve from the waste position to the transfer position) through the transfer line (a 80 cm × 0.32 mm i.d. fused silica tube) previously inserted into the septum of the PTV body. Transfer solutes are then retained in the packing material (a 5-cm length of Tenax TA plugged at both ends with silanized glass-wool).

Effective solvent elimination during LC-GC transfer is achieved in both the evaporative mode (through the split line) and the nonevaporative mode. As discussed earlier (Blanch et al., 1998a), solvent elimination as a liquid is allowed by removing the GC column end from the injector body before starting the transfer procedure. Once finished the purge time established for each analysis (see below), the column is reconnected and the retained solutes are transferred to the GC column by increasing the PTV temperature at 14 °C/s to 350 °C (kept for 11 min). Between consecutive runs, the PTV injector was heated to 350 °C for 11 min and subsequently satisfactory blanks (70−180 °C, 5 °C/min) were obtained. During transfer, the PTV was kept at a fix temperature (10 °C) under a helium flow that is maintained a time (the so-called purge time) to promote elimination of the remaining solvent from the glass liner.

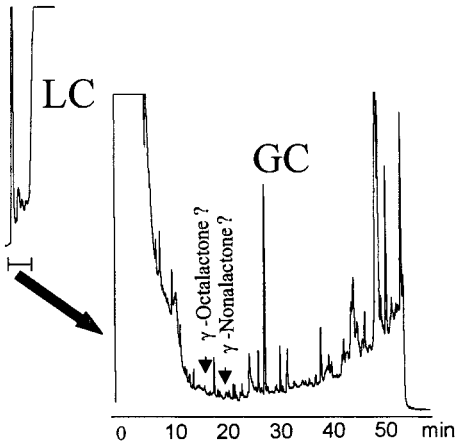

Figure 1. LC preseparation and gas chromatogram obtained after transfer of a 2550-μL fraction of methanol/water eluent (flow rate: 1700 μL/min) resulting from the direct LC-GC analysis of an olive oil. Fused silica capillary column: 25 m × 0.25 mm i.d., coated with a 0.25-μm layer of Chirasil-β-Dex. The GC oven was programmed at 2 °C/min from 70 to 130 °C. Split ratio, 5:1.

Experimentation was performed by considering different values for some of the variables involved in the LC-GC transfer step, namely speed of sample transfer and the transferred fraction volume. For the speed of sample transfer (equal to flow rate during the LC preseparation step) three values were considered (1400 μL/min; 1700 μL/min; 2000 μL/min) whereas different cuts (ranging from 0.2 to 1.5 min) were selected in each case.

Gas Chromatographic Analysis. GC analysis of the transferred fractions from the LC preseparation were carried out on a 25-m × 0.25-mm i.d. fused silica column of Chirasil-β-Dex (film thickness: 0.25 μm) (Schurig et al., 1990) using helium as the carrier gas. The oven temperature was increased from 70 °C up to 130 °C, at 2 °C/min.

Gas Chromatographic−Mass Spectrometric Analysis. The presence or absence of γ-lactones in the analyzed oils was also confirmed by gas chromatographic-mass spectrometric analysis using a gas chromatograph (Hewlett-Packard, model HP-5890) equipped with a mass spectrometer detector model 5971A (EI, 70 eV). Analyses were performed from the injection of a 1-μL volume of the concentrated fraction collected from the LC preseparation. Injection was performed in the splitless mode, 250 °C and 280 °C being the injector and detector temperatures, respectively. The presence or absence of γ-lactones was established by comparison of the corresponding spectra with those contained in the NIST library. The typical fragment ions of the mass spectra were as follows: *m/z* 85, *m/z* 57, *m/z* 56, and *m/z* 41.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram resulting from LC transfer into GC of a 2550-μL fraction of methanol/water eluent obtained from direct injection of an olive oil. Although initial experimental conditions were fixed as previously established in our laboratory for the analysis of γ-lactones in fruits and commercially available fruit-containing products (Blanch et al., 1998b), a satisfactory

analysis was not obtained when starting from an oil sample. As can be seen, reliable identification of γ-lactones is not easy as coelution of different compounds disturbed the chromatographic separation. Actually, γ-lactones were not only partially unresolved from other compounds, but also the obtained recoveries were extremely low. To overcome these difficulties, several modifications in experimental conditions were investigated. First, it was considered that application of a high helium flow rate during transfer may strongly accelerate passage of the liquid through the packing material placed in the PTV and, hence, a recovery decrease can be finally observed. Consequently, a helium flow rate equal to 800 mL/min was established and a purge time equal to 4 min was initially considered sufficient to promote elimination from the glass liner of the remainder solvent.

The influence of both the speed of sample transfer and the transferred volume fraction was estimated from the peak areas obtained under different experimental conditions for γ-octa- and γ-nonalactone. For the sake of simplicity, data for γ-deca- and γ-undecalactone are not considered here as the two latter compounds were not detected in the investigated oils although their analysis can be satisfactorily performed from the standard solution under the selected experimental conditions.

Working at a speed of sample transfer equal to 1700 μL/min, transfer of different cuts resulting from the LC preseparation (i.e., 0.2, 0.3, 0.4, 0.5, and 1.5 min) showed that the sensitivity was markedly increased when smaller fractions were transferred. In fact, the best recoveries for γ-octa- and γ-nonalactone were obtained from transfer of a 0.2- or 0.3-min fraction, and, consequently the optimum volume fraction seems to be close to 400 μL. Subsequently, the effect of higher (2000 μL/min) and lower (1400 μL/min) speeds of sample transfer was considered regarding the transfer of volume fractions close to 400 μL. As a result, a speed of sample transfer and a volume fraction equal to 1400 μL/min and 420 μL, respectively, were established as the best option to improve the recovery finally achievable for γ-lactones. Under these conditions, the sensitivity was increased as much as 30 times (for γ-octalactone) and 57 times (for γ-nonalactone) with respect to that resulting from working under the initial conditions.

Taking into account the experimental value applied for the transferred fraction in previous work (i.e., 2520 μL) (Blanch et al., 1998b), it is clear that solvent elimination must be easier in the present work, as a definitely lower volume is involved in the LC-GC transfer. Obviously, this situation is always preferable regarding the overall analysis, but it should be emphasized that it is only feasible when transfer of smaller fractions does not involve the loss of some of the solutes of interest. Precisely, that is the case of the analysis of γ-lactones in the investigated oils as only γ-octa- and γ-nonalactone were detected whereas γ-deca- and γ-undecalactone, if present, must be under the detection limit achievable with the proposed method.

Therefore, all oil samples were analyzed under the experimental conditions selected for the speed of sample transfer (1400 μL/min) and for the transferred volume fraction (420 μL). Under these conditions, a purge time as low as 0.4 min was sufficient to achieve satisfactory solvent elimination. As an example, Figure 2 shows the LC preseparation and the part of the gas chromatograms corresponding to elution times of γ-lactone enantiomers obtained after direct LC-GC analysis of an olive oil and a hazelnut oil. As can be seen, *R*- and *S*-enantiomers of γ-octalactone are detected in both oils whereas γ-nonalactone content in olive oil, if any, must be under the detection limit.

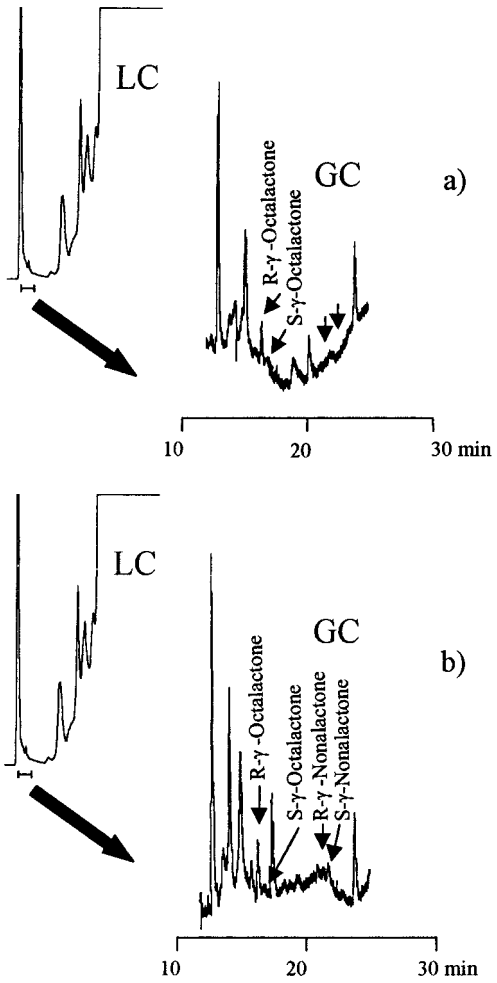

Figure 2. LC preseparations and GC separations resulting from on-line LC-GC transfer of a 420-μL volume of methanol/water eluent (flow rate, 1400 μL/min) from an olive oil (a) and a hazelnut oil (b). Fused silica capillary column, split ratio and temperature program as in Figure 1. Split ratio, 5:1. Chromatograms a and b were recorded at the same full range.

Throughout the experimentation, identification of γ-lactones was achieved by matching retention times with those obtained for the reference compounds analyzed under identical conditions, and it was confirmed from the chromatogram resulting by adding the reference compounds to the oil sample considered in each case and performing a new run. Moreover, as detailed in Experimental Procedures, the presence of γ-lactones was also confirmed by gas chromatographic−mass spectrometric analysis.

Under the experimental conditions proposed, the repeatability, detection limits, and recoveries obtained for γ-lactones were established as follows. Relative standard deviations (RSD) values obtained for absolute peak areas from three hazelnut oil replicates were 4.0% and 6.9% for *R*- and *S*-γ-octalactone and 10.0% and 2.4% for *R*-γ- and *S*-γ-nonalactone, respectively. Detection limits were calculated from the peaks giving a signal equal to five times the detector baseline noise (established from the width of the baseline over a certain period of time). Values obtained were almost identical

Table 1. Average Values[a] of *R*- and *S*-Enantiomers of γ-Lactones and Enantiomeric Excesses (ee)[b] of γ-Lactones in Edible Oils

	γ-octa-lactone			γ-nona-lactone			γ-deca-lactone		γ-undeca-lactone	
oil	*R*	*S*	ee	*R*	*S*	ee	*R*	*S*	*R*	*S*
olive	0.76	0.22	55	–	–	–	–	–	–	–
almond	0.50	0.28	28	0.06	0.18	50	–	–	–	–
hazelnut	2.94	0.73	60	0.20	0.42	35	–	–	–	–
peanut	0.37	0.23	23	0.09	0.02	64	–	–	–	–
walnut	–[c]	–	–	–	–	–	–	–	–	–

[a] mg/L obtained from three replicates. [b] Excess of predominant enantiomer expressed as percent, that is: [(predominant enantiomer − minor enantiomer)/(predominant enantiomer + minor enantiomer)] × 100. [c] − not detected.

for all oils. Concretely, detection limits for peanut oil varied from 0.06 mg/L (for *R*-γ-nonalactone) to 0.22 mg/L (for *S*-γ-octalactone). Recoveries of γ-lactones (measured from the absolute peak areas obtained from the spiked oils for the *R*- and *S*- enantiomers) were similar for all oils analyzed. As an example, values estimated from peanut oil were 19.0% and 18.9% (for *R*-γ-octa- and *S*-γ-octalactone, respectively) and 54.7% (for both *R*- and *S*-γ-nonalactone). This estimation was performed using as a reference peak areas obtained from splitless injection into GC of the standard solution.

Table 1 shows the average values obtained (from three replicates) when the on-line LC-GC analysis of γ-lactones in the investigated oils is carried out. Quantification was performed from peak areas obtained for the compounds of interest from both the original sample and the same sample spiked with a known amount of each analyte. Table 1 also includes the enantiomeric excesses calculated for the identified compounds. Identical enantiomeric excesses were obtained from the two olive oils (ee = 55% for γ-octalactone) and the two hazelnut oils (ee = 60% and 35% for γ-octalactone and γ-nonalactone, respectively). However, the limited number of samples did not allow concluding about the variations in enantiomeric excesses within same oils from various sources.

It should be emphasized that under experimental conditions proposed (i.e., direct LC-GC analysis of the oil sample without pretreatment) no significant racemization should be produced and, consequently, the method may allow a reliable determination of the enantiomeric excess of each sample oil. In this respect, another interesting question concerns the different enantiomeric excesses obtained for γ-lactones from direct RPLC-GC analysis of the different oils investigated (Table 1), as these values may illustrate the potential of the use of γ-lactones as chiral markers to detect adulterated olive oils.

Laboratory adulteration experiments showed that the enantiomeric excesses obtained for a mixture of olive oil and hazelnut oil (90:10) was 55% (γ-octalactone) and 36% (γ-nonalactone) whereas analysis of a mixture of the same olive oil with peanut oil (90:10) yielded an enantiomeric excess equal to 35% and 64% for γ-octa- and γ-nonalactone, respectively. According to Table 1, the presence of γ-nonalactone in an olive oil could be initially considered as a possible sign of adulteration. Moreover, the enantiomeric excess obtained for γ-nonalactone may indicate the adulterant oil used in each case, and this suspect may be confirmed comparing values obtained for the enantiomeric excesses of γ-octalactone in both pure olive oil and suspect oil.

In summary, we consider this preliminary study very promising, as it may support the declaration of an olive oil as authentic or adulterated. Obviously, the study is far from comprehensive, and more experimental work should be performed especially concerning the analysis of a high number of oil samples to establish the influence of different factors (e.g., olive cultivars, olive ripeness, oil extraction system, refination mode, and ageing) on the γ-lactone enantiomeric composition. In cases of naturally varying values, it is clear that the proposed method may prove insufficient as proof for authenticity of any olive oil, although it might be useful, providing that the pure olive oil is available.

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