A Rapid Argentation TLC Method for Detection of Reesterified Oils in Olive and Olive-Residue Oils

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ABSTRACT

A rapid, simple and reproducible argentation thin layer chromatographic method for the detection of reesterified oils in olive and olive-residue oil is described. The sensitivity of the method is comparable to the sensitivity to the IUPAC method 2.210 for the determination of fatty acids in the 2-position in the triglycerides of oils and fats.

INTRODUCTION

Reesterified oils are produced by direct reesterification of olive or olive-residue oils with high percentages of free fatty acids, or by esterification of glycerol and distilled fatty acids, usually derived from the same oils. Olive or olive-residue oil and reesterified oils can be characterized by their triglyceride structure (1). The IUPAC method 2.210 (2) for the determination of fatty acids in the 2-position in the triglycerides of oils and fats used for the detection of reesterified oils in olive or olive-residue oil is time-consuming, among other disadvantages described in our previous work (1). Essentially, it is a semiquantitative method, and its application needs two working days. The method proposed by Kaufmann and Wessels (3) and Mani and Wessels (4) has greater sensitivity than the IUPAC method (10%, compared to 15%) but is a tedious operation, requiring three working days. Our comparative studies on olive oil and reesterified oils by differential scanning calorimetry (5), which was reported as a suitable technique for the differentiation of these oils (6), showed that this rapid and reproducible technique could not solve the problem. Thus, it was considered useful to investigate the possibility of developing a rapid method for this purpose, suitable for routine work.

MATERIALS AND METHODS

Solvents of analytical grade were used without further purification. Samples of genuine virgin olive oil and refined olive-residue oil (chemically neutralized) were collected from the main oil-producing areas of Greece. Reesterified oil was prepared by esterification of glycerol and olive oil fatty acids in the presence of $SnCl_2 \cdot 2H_2O$ according to a published method (7). Triglyceride standards were products of Supelco Inc. (Bellefonte, PA).

For the triglyceride fractionation, 20 × 20 cm Merck type 5721 silica gel precoated thin layer chromatographic (TLC) plates were impregnated with silver nitrate (ethanol/20% silver nitrate in water [1:1, w/v]) and dried at ca. 70 C for 15-20 min (8). The samples were applied at 5% solutions in chloroform and the chromatograms developed in toluene/diethyl ether (96:4). After removal from the tank, the plate was allowed to dry for 5-10 min, sprayed by 0.2% ethanolic solution of 2′,7′-dichlorofluoroescein and observed under ultraviolet (UV) light. Otherwise, the chromatogram was detected by a two-stage dipping process: the plate is plunged into a solution of ethanol/15% ammonium hydrogen sulfate in water (1:1), removed after 0.5 min and excess liquid wiped off. After drying at ca. 150 C for 15-20 min, the cool plate is plunged into a solution of water/20% phosphomolybdic acid in ethanol (1:1), wiped and dried at 70 C for 15-20 min. Finally, the plate is heated at 175 C for 1 hr (purple-brown bands on a greenish yellow background) (8).

Fatty acid composition and *trans* oleic acid were determined according to AOCS methods (9, 10) and fatty acids in the 2-position after selective deacylation by pancreatic lipase (1). Gas liquid chromatography was performed with Tractor 550 or Sigma 2 with flame ionization detectors. Column chromatography was carried out with EGGS 10% on Gas Chrom Q, 100-120 mesh, 1.80 m × 3 mm id of stainless steel. Infrared spectra were obtained with a Perkin Elmer Model 337 spectrometer.

RESULTS AND DISCUSSION

Kaufmann and Wessels (3), by applying the 1,3-random-2-random distribution hypothesis to olive oil and reesterified oils, comparatively, showed that differentiation of the two oils could be based on the *trans* isomeric triglycerides which were not present in virgin olive oil and which were calculated as 4.1% dioleo-elaidin, 1.8% palmito-oleo-elaidin and 1.2% oleo-linoleo-elaidin. However, *trans* isomers do not only characterize reesterified oils because they may be formed in oils by other processes as well. Thus, the differences in positional isomers are more characteristic. For the monoenoic triglyceride dipalmito-olein (2-2.5% of the triglycerides), the ratio of 1-oleo-2,3-dipalmitin (100) to 1,3-dipalmito-2-olein (010) is 8:92 for olive oil and 55:45 for the reesterified oil. The results for the main dienoic triglyceride palmito-diolein, which comprises ca. 12% of the triglycerides, are similar. The ratio of the positional isomers 1,3-dioleo-2-palmitin (101) to 1-palmito-2,3-diolein (011) is 7:93 for olive oil and 55:45 for the reesterified oil. Finally, for the dienoic triglyceride dioleo-stearin (2.8% of the triglycerides), the ratio of the isomers 1,3-dioleo-2-stearin (101) to 1-stearo-2,3-diolein (011) is 16:84 for olive oil and 46:54 for the reesterified oil.

Triglycerides may be fractionated in groups by silver ion chromatography, mainly according to the number of their double bonds. However, other factors may influence the fractionation. The strength of the complex formed depends not only on the position of a double bond in the fatty acid chain or the position of the fatty acid in the triglyceride, but also on the type of unsaturation *cis* or *trans*, etc. (11). Thus, the above differences between olive oil and reesterified oils could be demonstrated by separation of their triglycerides on argentation TLC plates. The problem actually consists of finding a developing solvent to fractionate the

geometrical as well as the positional isomers of the triglycerides on the same precoated TLC 20 × 20 cm plate which is commercially available. By trying different solvent systems we found that with the toluene/ether (96:4) system, the results were quite satisfactory.

In Figure 1 are presented the chromatograms obtained with olive oil, olive-residue oil, reesterified oil and several triglyceride standards. The fatty acid composition and the percentage of fatty acids in the 2- and 1,3-positions of the samples of olive oil and reesterified oil analyzed are given in Table I. With olive and olive-residue oil beginning from the top of the plate (Fig. 1), the saturated 000, monoenoic 010, dienoic 011, trienoic 111, etc., fractions of triglycerides are visible. With the reesterified oil, the monoenoic triglyceride fraction 010 is followed by the monoenoic isomeric esterified fraction 100 which is characteristic of reesterified oils. Next appears the *trans* dienoic fraction preceding the *cis* dienoic fraction 011 (12). After the *cis* dienoic fraction 011, which also appears in olive and olive-residue oil, follows the symmetric dienoic fraction of triglycerides 101 which is characteristic of reesterified oils.

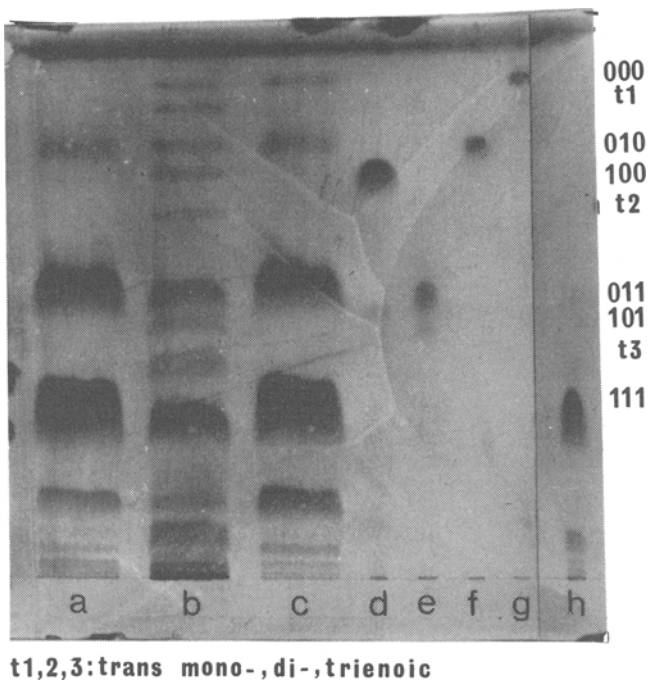

FIG. 1. Argentation TLC of (a) olive oil, (b) reesterified oil, (c) olive-residue oil, (d) 1-oleo-2,3-dipalmitin, (e) 1-palmito-2,3-diolein, (f) 1,3-dipalmito-2-olein, (g) tripalmitin, (h) triolein.

It was reported previously (13) that the symmetric isomers generally have the highest Rf value in each case. The results in Figure 1 suggest that this general conclusion is not correct: the symmetric isomer does not always form the weakest complex, the strength of the complex depending mainly on the unsaturation of the fatty acids in the 1,3-positions of glycerol. Thus, in the case of the monoenoic triglyceride fraction, the symmetric isomers 010 have the highest Rf value in comparison to the unsymmetrical triglycerides 100. On the contrary, the symmetric isomers 101, because of the presence of the unsaturated fatty acids in the 1,3-positions, form a stronger complex in comparison to the unsymmetrical triglycerides 011. Finally, the *trans* trienoic triglyceride fraction 111 appears above its *cis* isomer.

To investigate the sensitivity of the method, mixtures of 15, 20 and 25% of reesterified oil in olive oil were analyzed. As shown in Figure 2, detection of reesterified oils is safe at a level of ca. 15%. Consequently, the method which we have described here has comparable sensitivity to the IUPAC method 2.210, but is rapid, it needs 2 hr of work as opposed to two working days which are needed for the IUPAC method, and is relatively inexpensive. The method is also simple and reproducible, and with care it is possible to obtain chromatograms which can be compared in a semi-quantitative way.

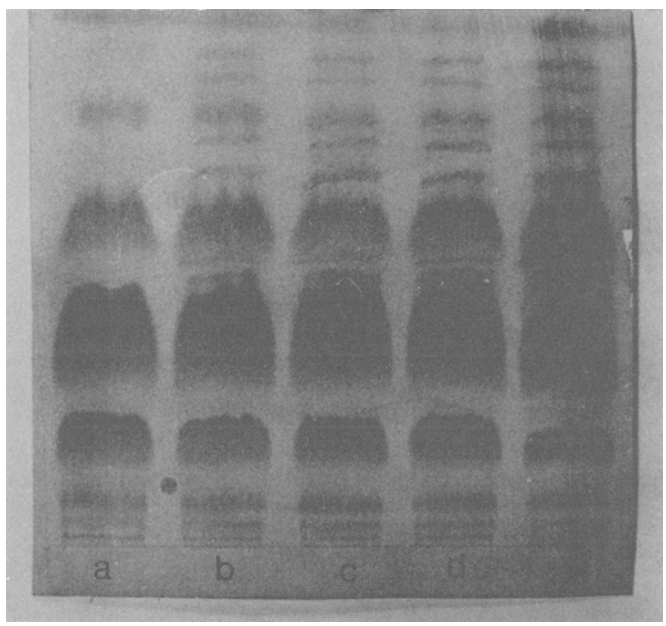

FIG. 2. Argentation TLC of (a) olive oil, (b) (c) (d) 15%, 20%, 25% reesterified oil in olive oil, respectively, and (e) reesterified oil.

TABLE I

Fatty Acid Composition of Olive Oil and Reesterified Oil

	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$ (*cis*)	$C_{18:1}$ (*trans*)	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
				Olive oil				
Total fatty acids (%)	11.1	0.3	1.8	79.1	–	6.7	0.7	0.3
Fatty acids in 2-position (%)	1.4	0.1	0.6	87.8	–	10.1	–	–
Fatty acids in 1,3-positions (%)	16.0	0.4	2.4	74.8	–	5.0	1.0	0.4
				Reesterified oil				
Total fatty acids (%)	12.1	0.6	2.8	70.5	4.0	9.5	0.5	–
Fatty acids in 2-position (%)	13.5	0.5	2.5	68.7	4.0	10.8	–	–
Fatty acids in 1,3-positions (%)	11.4	0.6	3.0	71.4	4.0	8.9	0.7	–

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Simultaneous Detection of Aflatoxin B_1 and Ochratoxin A in Olive Oil

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ABSTRACT

A screening method has been developed for simultaneous determination of aflatoxin B_1 and ochratoxin A in olive oil. The technique includes extraction of both mycotoxins with aqueous methanol, clean-up using lead acetate, partitioning in chloroform, and thin layer chromatography. The detection limits achieved are, respectively: 4 μg aflatoxin B_1 and 40 μg ochratoxin A/kg. 60 samples of crude farm olive oil were analyzed using this method: three of them contained traces of ochratoxin A while none was contaminated with aflatoxin B_1.

INTRODUCTION

The natural occurrence of aflatoxins in a wide range of fatty products has often been reported, but to our knowledge, comparatively few references dealing with mycotoxin contamination of olive oil can be found in the literature.

However, in 1962, Gracian et al. (1) identified *Aspergillus flavus* in olive fatty cakes; and in 1973, San Perez et al. (2) obtained aflatoxin on black olives artificially contaminated with either *A. flavus* or *A. parasiticus*.

More recently, olive oil samples originating from Greece (3) and Spain (4) have been reported to contain aflatoxins.

For our part, mycological surveys carried out in our laboratories on Moroccan olives, while not resulting in the isolation of *Aspergillus* from the *flavus* group, did result in the identification of *A. ochraceus* in many samples (5).

The toxigenesis of these strains was tested after cultivation on whole wheat, using two methods (6, 7) for extraction and analysis. None of the strains secreted measurable quantities of ochratoxin.

Nevertheless, we thought it interesting simultaneously to determine contamination levels for Moroccan crude farm olive oil with both ochratoxin A and aflatoxin B_1, since farmers sometimes store their olives for several weeks in conditions that promote the growth of molds. Furthermore it must be emphasized that refining which would remove aflatoxins from contaminated crude oil (8) is not applied to so-called "virgin" olive oil.

Therefore, this paper comprises two distinct parts: (a) the description of a method for simultaneous detection of aflatoxin B_1 and ochratoxin A in olive oil; and (b) an investigation of these two mycotoxins in crude farm olive oil samples collected from producers in the area of Beni-Mellal (250 km southeast of Rabat).

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EXPERIMENTAL PROCEDURES

Materials

Glass plates 20 × 20 cm; Quickfit applicator; dessicator; spotting template; microsyringe 10 μl; Desaga developing tank; longwave ultraviolet (UV) lamp (366 nm); Pleuger, chromatolux 2L.

All reagents are analytical grade chemicals. Solvents: acetonitrile, benzene, chloroform, ethyl acetate; formic acid 98-100%; hexane, toluene. Extraction solvent: methanol/water solution (6:4, v/v) containing 4% NaCl. 4% NaCl aqueous solution. Clean-up solution: 20% lead acetate aqueous solution, prepared from Pb $(CH_3COO)_2$, $3H_2O$. Drying agent: anhydrous sodium sulfate. Silica Gel for TLC: HR 60, Merck.

Mycotoxin standard solutions: aflatoxin B_1: determine concentration and purity, following basic methodology described by Rodricks and Stoloff (9); using a Pye Unicam UV-visible SP 1800 spectrophotometer (correction factor, CF = 0.997). Ochratoxin A: follow same method, using information given by Stoloff et al. (10).

Developing solvents: benzene/hexane (3+1) (11), toluene/ethyl acetate/formic acid (6+3+1) (11), ethyl acetate/formic acid (99+1), benzene-methanol/acetic acid (18+1+1) (7), chloroform/methanol (92+8).

Methods

Extraction and clean-up. Extraction procedure follows method of Toussaint et al. (3) with slight changes.

Pour a 50 g sample with 100 mL hexane into a 500 mL separating funnel. Extract first with three 100 mL portions of methanol/water extraction solvent (6:4; v/v, containing 4% NaCl), next with two 50 mL portions of aqueous 4% NaCl solution. Shake for 2 min each time.

Combine aqueous and aqueous/methanolic extracts in a separating funnel and wash twice with 50 mL hexane each time. Transfer aqueous/methanolic phase to a beaker, add 20 mL clean-up solution (20% acetate aqueous solution), and stir well. Filter the solution on Buchner funnel and wash the precipitate with 50 mL distilled water. Transfer the