Fast Quantitative Analysis of Soybean Oil in Olive Oil by High-Temperature Capillary Gas Chromatography

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Analysis of glycerides has always been of great interest, especially to the food industry. Several methods have been developed, and attempts to improve analytical conditions are prevalent. Among these methods, high-temperature capillary gas chromatography has received particular consideration because it rapidly provides a glyceride profile. In this paper, we discuss a method to identify and quantitate mixtures of olive and soybean oils with the intention of verifying the latter as an adulterant of olive oils. The contamination of olive oil with soybean oil was detected by the presence of the triglyceride trilinolein, which does not exist in pure olive oil, although it is abundant in soybean oil. A calibration curve was constructed with several levels of contamination, and the lowest amount of detectable soybean oil was determined to be 4%.

KEY WORDS: Adulteration, capillary gas chromatography, HRGC, HT-CGC, olive oil, soybean oil, triglycerides.

Due to economic factors, intentional adulteration of olive oils by addition of soybean oil has become widespread around the world. With the intention of detecting and quantifying such adulteration, many simple methods have been developed and applied in routine laboratory analyses. Among these are the chemical and physical tests (refractive index, iodine value determination, etc.) and chromatographic analyses of fatty acid methyl esters, generated by hydrolysis with further derivatization of the free fatty acids (1).

Although these methods are quite simple, they do not provide a high degree of accuracy because it is difficult to determine which oils are present in the sample. With the advent of high-temperature stationary phases (2,3), it became possible to inject the oil directly into the gas chromatograph, thus avoiding the hydrolysis and derivatization steps. Because high-temperature capillary gas chromatography (HT-CGC) analysis produces a triglyceridic profile of each vegetable oil, adulteration can be detected by profile comparison. In this case, the presence of soybean oil is verified by the presence of the triglyceride trilinolein (LLL) in the olive oil sample.

The analyses were carried out on a fused-silica capillary column with a polarizable phenyl methyl polysiloxane stationary phase. In these phases, the triglycerides are separated according to the total length of the carbon chain, the carbon number (carbon number separation). There is further separation according to the degree of unsaturation in the fatty acids. High-selectivity separations can be made in a relatively short time (10–20 min).

Triglyceride identification was made by comparison with the results of Sandra and Geeraert (4), who named the triglycerides by using abbreviations relating to the fatty acids. The nomenclature used to identify each fatty acid is as follows: P, palmitic acid, hexadecanoic acid, C16:0; Po, palmitoleic acid, *cis*-9-hexadecenoic acid, C16:1; S, stearic acid, octadecanoic acid, C18:0; O, oleic acid, *cis*-9-octadecenoic acid, C18:1; L, linoleic acid, *cis,cis*-9,12-octadecadienoic acid, C18:2; Ln, linolenic acid, *cis,cis,cis*-9,12,15-octadecatrienoic acid, C18:3; A, arachidic acid, eicosanoic acid, C20:0; Ga, gadoleic acid, *cis*-9-eicosenoic acid, C20:1.

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EXPERIMENTAL PROCEDURES

Chromatographic analyses were done with a fused-silica capillary column, 25 m × 0.25 mm × 0.10 μm OV-17 (50% phenyl/50% methyl polysiloxane) in an HP 5890 Series II (Hewlett-Packard, Palo, Alto, CA) gas chromatograph, equipped with a flame-ionization detector, split injector and HP 7673A autoampler.

Heated zones were maintained at 350°C (injector) and 360°C (detector). Oven temperature was held at 330°C for 1 min, then heated to 355°C at 1°C/min. The final temperature was held for 4 min. Hydrogen was used as carrier gas, with the column pressure being 100 KPa. The split ratio selected was 1:10. Five samples of representative olive oils were obtained from local supermarkets: two Brazilian oil, one Argentine, one Portuguese and one Spanish. Standard mixtures and pure oils were prepared in hexane solution at 1% vol/vol. Standard concentrations were 2, 4, 6, 8, 10, 20, 30 and 40% vol/vol soybean oil in olive oil.

The injection volume was 2 μL, and peak integration was carried out by an integrator (HP 3396A) without column compensation. Although it does not have the same accuracy and reproducibility obtained with on-column injection (5), the split injection can easily be automated by means of an autosampler.

RESULTS AND DISCUSSION

Chromatographic analysis of pure soybean oil, pure olive oil, 2% standard mixture and 40% standard mixture are shown in Figures 1 to 4, respectively. A calibration curve for this analytical method was constructed from the mean peak areas for LLL for all standard solutions, which were analyzed in triplicate. The calibration curve is shown in Figure 5.

The calibration curve shows that the point corresponding to the 2% standard mixture deviates from the linearity obtained for the other points. It was assumed that the lower limit of quantitation for this method was 4% of soybean oil in olive oil by the integration mode selected. The deviation is more visible in Figure 6, which depicts only the points ranging from 0 to 10%. This line was used as the standard curve for the quantitation of soybean oil adulteration in olive oil.

The results from analyzing the five commercial olive oils are shown in Table 1, and Figure 7 shows the chromatographic profile for the olive oil named Brazilian 1. The data in Table 1 show that only the Brazilian olive oils have been adulterated by soybean oil addition. The Brazilian 2 oil, although not electronically integrated, also shows the peak corresponding to LLL.

The proposed analytical method has shown confident quantitative data for adulterating concentrations above 4%, while below this value the points deviate from linearity.

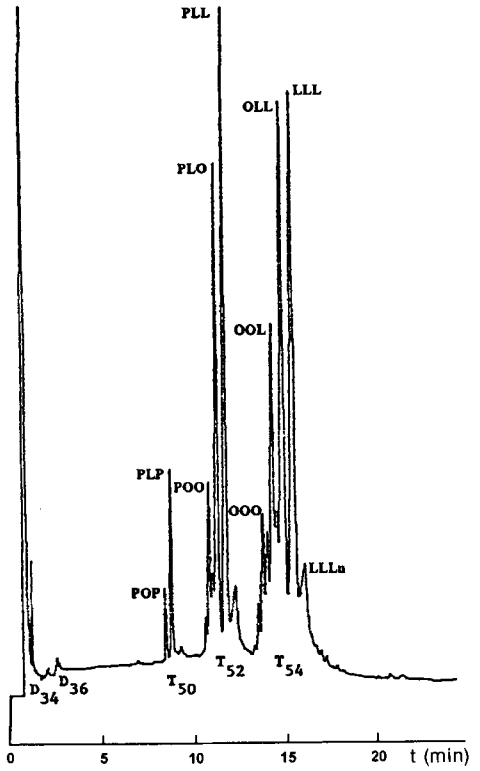

FIG. 1. High-temperature capillary gas chromatogram of pure soybean oil. Chromatographic conditions: capillary column 25 m × 0.25 mm × 0.10 μm OV-17; temperature program: 330°C (1 min) up to 355°C (4 min) at 1°C/min; carrier: hydrogen (100 KPa). P, palmitic acid, L, linoleic acid; O, oleic acid; Ln, linolenic acid.

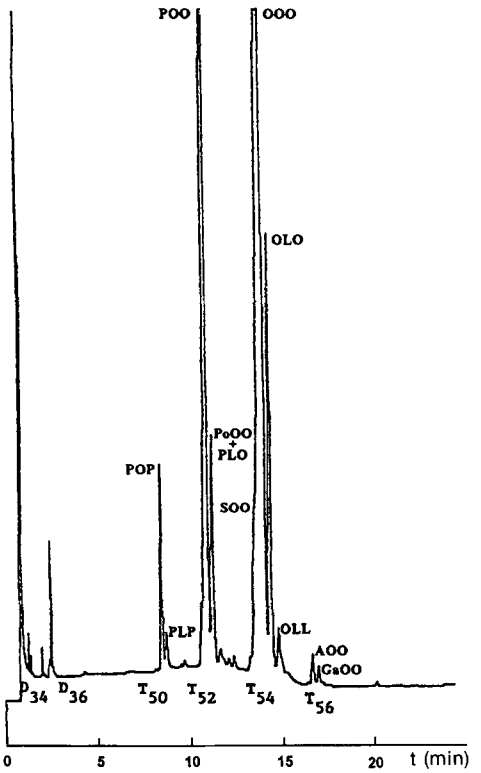

FIG. 2. High-temperature capillary gas chromatogram of pure olive oil. Same conditions and abbreviations as in Figure 1. Po, palmitoleic acid.

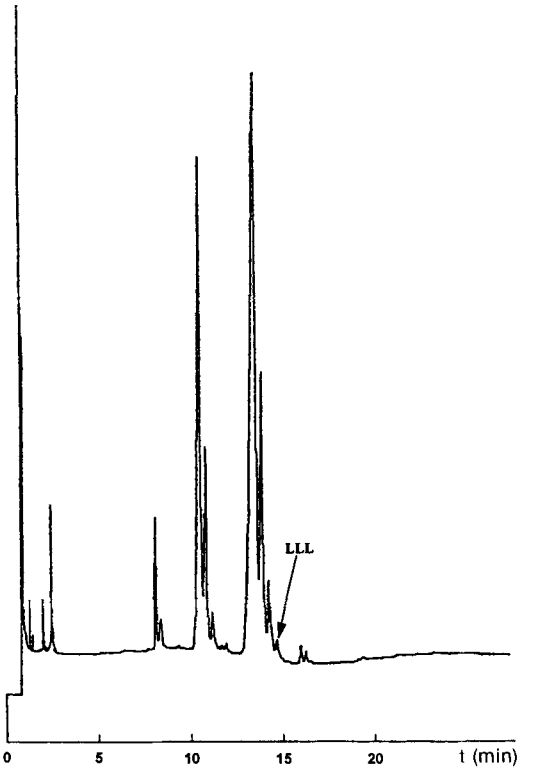

FIG. 3. High-temperature capillary gas chromatogram of 2% standard mixture (2% soybean oil in olive oil, vol/vol). Same conditions as in Figure 1. LLL, trilinolein.

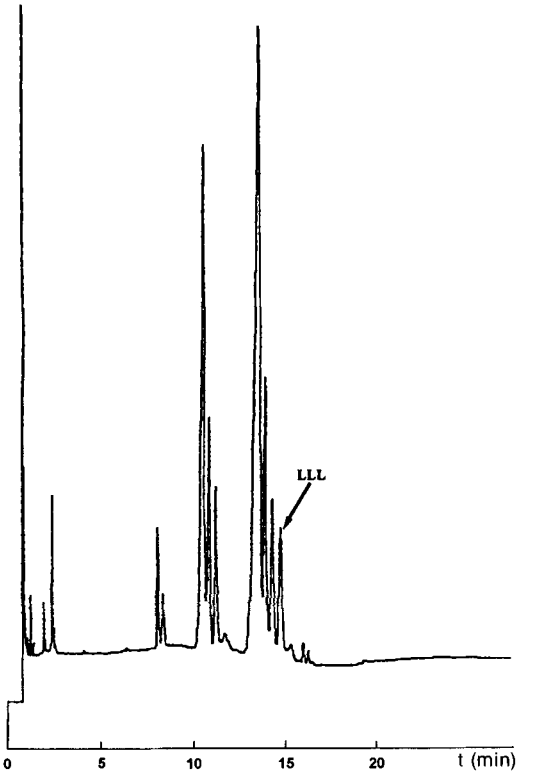

FIG. 4. High-temperature capillary gas chromatogram of 40% standard mixture (40% soybean oil in olive oil, vol/vol). Same conditions as in Figure 1. LLL, trilinolein.

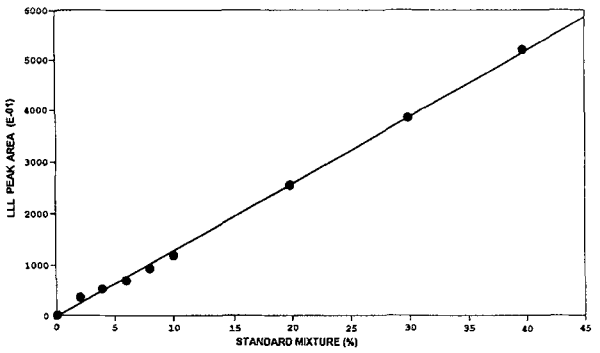

FIG. 5. Calibration curve of soybean oil in olive oil as a function of the trilinolein (LLL) peak area. Same conditions as in Figure 1.

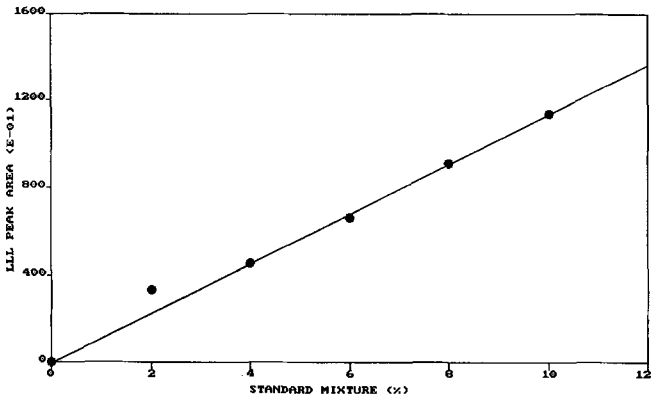

FIG. 6. Calibration curve for standard mixtures up to 10% of soybean oil in olive oil. Same conditions as in Figure 1.

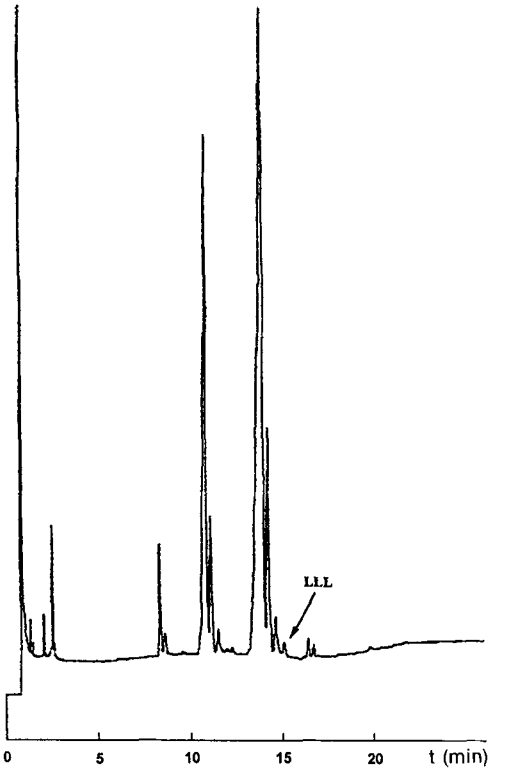

FIG. 7. Adulteration detection in olive Brazilian 1 oil. Same conditions as in Figure 1. LLL, trilinolein.

TABLE 1

Results Obtained for Commercial Olive Oils by High-Temperature Capillary Gas Chromatograph Analyses

Olive oil sample	Adulteration percentage based on trilinolein peak
Brazilian 1	5.3%
Brazilian 2	N.I.[a]
Argentine	N.D.[b]
Portuguese	N.D.
Spanish	N.D.

[a]N.I., not integrated.
[b]N.D., not detected.

One factor affecting this quantitation at low concentrations is the LLL peak form, which is difficult to integrate electronically. Better results can be achieved by interfacing the gas chromatograph to a data system that can provide manual integration, to choose the beginning and end of the peak.

Use of an autosampler has made the analysis easier as a routine process, but the proximity of the syringe needle to the hot injector (350°C) produces bubble formation in the sample within the syringe body. To solve this problem, the syringe was washed several times with hexane and acetone to cool it prior to use. Quantitation of soybean oil adulteration in olive oil at lower concentrations is being further investigated in our laboratory.

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[Received October 16, 1992; accepted July 27, 1993]