Detection of Adulteration of Almond Oil with Apricot Oil through Determination of Tocopherols

A procedure was devised for detection of adulteration of almond oil with apricot oil. γ-Tocopherol was found to be the main component in apricot oil, while it was present in almond oil in a limited amount. The developed method calls for a preliminary separation of the tocopherols from the oils' unsaponifiable matter by thin-layer chromatography. Subsequently, the α- and γ-tocopherols are determined either by gas–liquid chromatography or by colorimetry. It was shown that admixtures of as little as 5% apricot oil can be detected by the proposed procedure. γ-Tocopherol, present in apricot oil, was responsible for the color which developed during the Bieber test.

Almond oil is used in the cosmetic and pharmaceutical industries. The oils of the Rosacea family, such as those extracted from apricot, peach, cherry, and plum kernels, can be used for the same general purpose as that of almond oil (Ben-Gera and Kramer, 1969; Vaughan, 1970).

The oils of the Rosacea group are remarkably similar in their physical characteristics (Pearson, 1970) and fatty acid composition (Hilditch and Williams, 1964; Gutfinger *et al.*, 1972). Consequently, the relatively expensive almond oil is often substituted by or adulterated with oils extracted from kernels of fruits of the same family. One of the common adulterants is apricot oil (Cruess, 1958; Williams, 1966; Winton and Winton, 1950).

The detection of apricot oil is rather difficult. About 70 years ago, Lewkowitsch (1904) found that the Bieber test can be useful for distinguishing between apricot and almond oils (Pearson, 1970; Williams, 1966).

Pure almond oil does not change color in the Bieber test, whereas apricot oil assumes a deep red coloration. In a mixture with almond oil, additions of at least 30% apricot oil may be detected by the Bieber test, but the test is uncertain for smaller amounts of the admixed apricot oil. In general, the test seems to be unsatisfactory.

For lack of a better method the Bieber test is still recommended in several publications for detection of almond oil adulterations (Pearson, 1970; Williams, 1966).

Lately, attention has been drawn to a number of reports in which the detection was discussed of adulteration of some oils through the analysis of their minor constituents (Fedeli *et al.*, 1966; Firestone, 1968; LaCroix, 1969; Thorpe, 1970; Thorpe *et al.*, 1969). Individual oils can be characterized by gas chromatographic analysis of sterols, triterpene alcohols, and also other unsaponifiable components.

In a previous paper (Gutfinger *et al.*, 1972) it was shown that apricot and almond oils are similar not only in their fatty acid composition, but also in their sterol composition. There was, however, a difference in their tocopherol pattern. That difference is also supported by evidence from the literature. Slover *et al.* (1969) reported that α-tocopherol was the principal tocopherol in almond oil (total tocopherol content was 334 μg/g of oil), while Täufel and Serzisko (1963) found that γ-tocopherol was the principal tocopherol in apricot oil (total tocopherol content was 711 μg/g of oil).

The purpose of the present study is to establish whether or not a method of tocopherol analysis could be satisfactory for detection of an admixture of apricot oil to almond oil, even at low levels of the former. The authors wish also to identify the substances responsible for the color reaction in the Bieber test (Williams, 1966).

EXPERIMENTAL SECTION

Materials. The investigated oils were extracted from the kernels of almond (*Prunus amygdalus*, var. Victoria) and apricot (*Prunus armeniaca*, var. Canino (Raanana)). Extraction of the oils from the almond and apricot kernels was described in an earlier publication (Gutfinger *et al.*, 1972). Samples were prepared of the pure oils and also of 5:95 and 10:90 (w/w) mixtures of apricot and almond oils.

All solvents and chemicals were of analytical grade. α-Tocopherol was purchased from Fluka A.G. γ- and δ-tocopherols and tocopherol dimer [5-(γ-tocopheryloxy)-γ-tocopherol] were obtained by tlc separation from the unsaponifiables of soybean oil.

The genuineness of α- and γ-tocopherols was confirmed by both thin-layer and gas–liquid chromatographic analysis. Their R_f values and retention times were compared with those reported in the literature (Bunnel, 1967). The identity of γ-tocopherol dimer was established by reaction gas chromatography (Gutfinger and Letan, 1972).

Preparation of the Unsaponifiable Matter. Five grams of an oil sample were saponified and the unsaponifiables were extracted according to Ames (1971). The solvent was evaporated and the unsaponifiables were redissolved in 1 ml of chloroform (in a volumetric flask) and then separated by thin-layer chromatography (tlc).

Thin-Layer Chromatography. Analytical and preparative tlc separations were carried out on 20 × 20 cm plates coated with 0.5 mm of silica gel (Merck, catalog no. 7731), which contained sodium fluorescein (Stahl, 1969).

Three different methods were used to analyze the unsaponifiable matter: the Bieber test; the colorimetric (Emmerie-Engel) determination; and gas chromatographic determination of the tocopherols (previously separated by tlc). These methods are described in detail below. The chromatograms obtained in the tests were developed with two different solvent systems: (1) chloroform (contained 0.7% ethanol as stabilizer); and (2) petroleum ether (60–80°)–ethyl ether–acetic acid (50:50:1).

In all of the tlc separations, the solvent was allowed to advance 17 cm. The tocopherols were identified by cochromatography (with standards); they resolved as spots which were dark purple under ultraviolet light. Emmerie-Engel spray (Stahl, 1969) and freshly prepared Bieber reagent (a mixture of equal volumes of water, sulfuric acid, and fuming nitric acid) (Lewkowitsch, 1904) were used for detection of the studied compounds.

Bieber Test. The following experiment was performed for identification of the components which react in the Bieber test. A tlc plate was divided in two parts, and in each part was spotted 10 μl of the investigated chloroform solution of unsaponifiables. The amount of unsaponifiables in the mentioned volume corresponded to that in 0.05 g of oil. The unsaponifiables were separated in chloroform as described above. The plate was then dried and one part of it was covered with glass. The exposed part was sprayed with the Emmerie-Engel spray. After visualization of the unsprayed part under ultraviolet light, the exact location of the tocopherols and tocopherol dimers was marked. The Bieber reagent was dripped from a pipette along the strips of the separated components of the unsaponifiables. The positively reacting components were purple red colored. (It is shown below that only γ-tocoph-

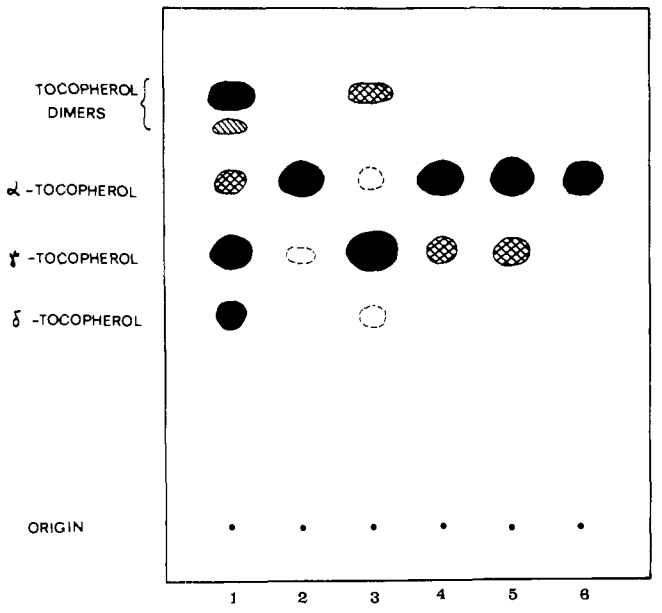

Figure 1. Thin-layer chromatogram of tocopherols from the oils of almond and apricot and their mixtures. Solvent, chloroform; detecting reagent, Emmerie–Engel spray. 1, Unsaponifiables from soybean oil; 2 and 3, unsaponifiables from almond and apricot oils, respectively; 4 and 5, unsaponifiables from 5:95 and 10:90 (w/w) mixtures from apricot and almond oils, respectively; 6, α-tocopherol.

erol and γ-tocopherol dimer reacted positively in the Bieber test; see Results Section.)

Isolation of Tocopherols for Colorimetric Analysis. The investigated solution of unsaponifiables in chloroform (30 μl, corresponding to 0.15 g of oil) was applied on the tlc plate with a Hamilton syringe as a strip of 2 cm. After development with chloroform, the plate was viewed under ultraviolet light and the location of α- and γ-tocopherols was marked. Silica gel with the examined tocopherols was scraped, and a similar area of silica gel was wetted with the developing solvent (for blank determination). The scraped silica gel was placed in a 15-ml centrifuge tube which contained 3 ml of absolute ethanol. The tube was then shaken for 20 sec in a vibrator (Vortex-Genie, Scientific Industries Inc., Springfield, Mass.). After centrifugation (10 min at 5000 rpm) and decantation, the ethanolic extract was subjected to colorimetric measurements.

Colorimetric Analysis (Emmerie–Engel Test). A Beckman DB spectrophotometer was used for the colorimetric measurements. The Emmerie–Engel reaction was carried out inside the cuvette to avoid exposure to light.

The examined ethanolic extract (1.5 ml) was transferred to the cuvette, which contained 1.4 ml of 0.5% ethanolic solution of α,α'-bipyridyl. Subsequently 0.1 ml of a 0.2% ethanolic solution of ferric chloride was added to the cuvette. The solution was mixed with a Teflon rod and, after exactly 2 min, the extinction was read at 520 nm.

The concentration of tocopherol was calculated as

$$\mu\text{g of tocopherol/g of oil} = (E_S - E_B) \times 200 \times f/0.041 \times W$$

where E_S and E_B = extinctions of sample and blank, respectively, 0.041 = extinction of 1 μg of α-tocopherol in 1 ml of assay solution (see above), 200 = dilution factor, W = weight of oil (g), and f = the corresponding factors for α- and γ-tocopherols—1.0 and 0.92, respectively. Those factors were used by the Analytical Methods Committee (1959) and by Newton and Pennock (1971).

Isolation of the Tocopherols for Glc Analysis. The investigated solution of unsaponifiables in chloroform (200 μl, corresponding to 1 g of oil) was applied as a strip of 12 cm. The plate was developed with petroleum ether (60–80°)–ethyl ether–acetic acid (50:50:1). The separated strips of the tocopherols were distinguished under ultraviolet light and marked. They were subsequently scraped and extracted with three 6-ml portions of absolute ethanol. The ethanol was evaporated at 60° under a stream of nitrogen. The residue was dissolved in 100 μl of chloroform and subjected to gas chromatographic determinations. (As good results were obtained in those determinations, no attempt was made to derivatize the tocopherols prior to the glc analysis.)

Table I. Amounts of Tocopherols in Almond and Apricot Oils

	γ-Tocopherol, μg/g of oil	α-Tocopherol,[a] μg/g of oil
Almond oil	266	19
Apricot oil	24	560
95:5 mixture of almond and apricot oil	247	50

[a] γ-Tocopherol and the unresolved β-tocopherol (when present) were calculated as γ-tocopherol.

Gas–Liquid Chromatography. The apparatus used was a Packard gas chromatograph, model 7821, equipped with a hydrogen flame ionization detector. Gas chromatographic determinations were carried out at 240° on a 6 ft × ¼ in. o.d. coiled glass column, packed with 3% SE-30 on Gas Chrom Q (Applied Science Laboratories). The temperatures of the inlet, outlet, and detector were 280°.

The carrier gas was argon at a flow rate of 30 ml/min. The peaks of tocopherols were identified by comparing their retention times with those of the standards. The relative amounts of the examined components were determined from the peak areas.

RESULTS AND DISCUSSION

Two different solvent systems were used in this investigation (see Experimental Section, thin-layer chromatography). In the first system (chloroform) the tocopherols resolved as individual spots. The R_f values for α- and γ-tocopherol were 0.66 and 0.53, respectively. This tlc separation made it possible to subsequently determine each tocopherol by the Emmerie–Engel test. In the second solvent system (petroleum ether–ethyl ether–acetic acid) there was no complete resolution of the tocopherols and they appeared on the plate as overlapping strips (R_f 0.56–0.72); a mixture of those tocopherols (separated from the other unsaponifiables) was then analyzed by glc.

β- and γ-tocopherol could not be resolved by our methods of thin-layer and gas–liquid chromatographies; these tocopherols were therefore calculated as γ-tocopherol.

Typical tlc separations of the unsaponifiable matter of almond and apricot oils are shown in Figure 1 (separations 2 and 3). There was a substantial difference of tocopherol patterns between the two oils. Both oils contained α- and γ-tocopherols, but apricot oils also contained very small amounts of δ-tocopherol and a tocopherol dimer [5-(γ-tocopheryloxy)-γ-tocopherol (Gutfinger and Letan, 1972)]. The tocopherol fraction of almond oil consisted mainly of α-tocopherol, while γ-tocopherol was the major tocopherol in apricot oil.

The total amounts of α- and γ-tocopherols in the investigated oils were determined by colorimetric analysis using the Emmerie–Engel reagent (Table I).

The combined content of α- and γ-tocopherols was higher in apricot oil than in almond oil (Table I). The amount of γ-tocopherol in almond oil was negligible in comparison with that present in apricot oil. The results obtained in a previous study (Gutfinger *et al.*, 1972) for the contents of tocopherols and for the α- to γ-tocopherol ratio in almond and apricot oils were similar to those found in the present investigation. The previous work was performed with an oil which had been extracted from a different variety of almonds (*Ne plus ultra*); α-tocopherol

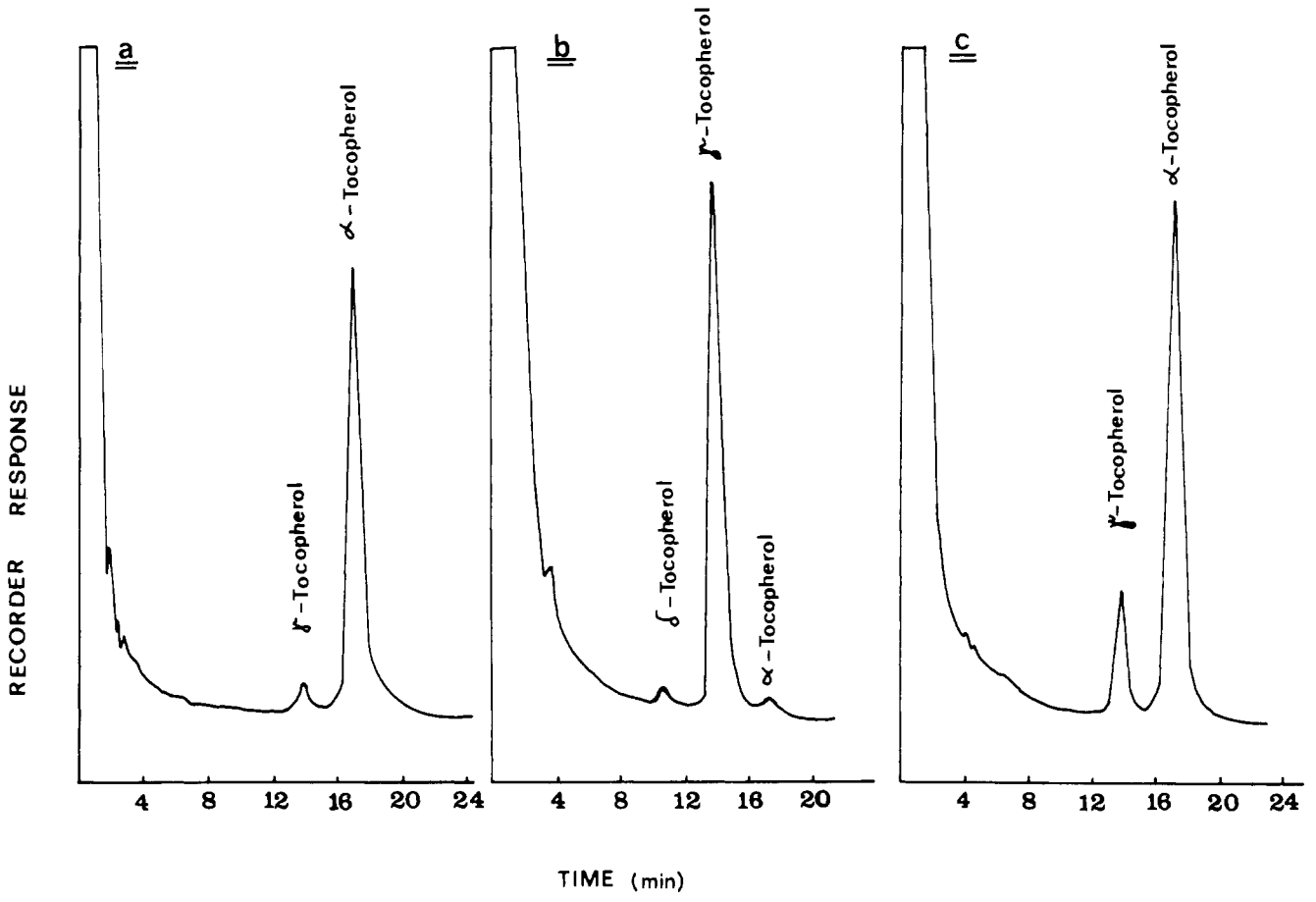

Figure 2. Gas chromatograms of tocopherols from almond and apricot oils and their 95:5 (w/w) mixture. Conditions of the gas chromatographic separations given in Experimental Section, gas–liquid chromatography. Tocopherols were isolated by tlc. a, Tocopherols from almond oil; b, tocopherols from apricot oil; and c, tocopherols from a 5:95 (w/w) mixture of apricot and almond oils.

content in that oil was 420 μg/g of oil. Apricot oil was extracted from the kernels of the Canino variety (also used in this work but the fruits came from a different crop); the γ-tocopherol content in that oil was 600 μg/g.

The data of Slover *et al.* (1969) for almond oil (329 μg of α-tocopherol/g of oil) and those of Taüfel and Serzisko (1963) for apricot oil (630 μg of γ-tocopherol/g of oil) support the results of the present investigation.

Presumably, the difference in tocopherol pattern from almond and apricot oils may explain the different results obtained for these oils in the Bieber test. The spots of the unsaponifiable components from almond oil (separated by tlc) gave no coloration in the Bieber test (see Experimental Section). As for apricot oil, the spots of R_f values which corresponded to γ-tocopherol and to the tocopherol dimer [5-(γ-tocopheryloxy)-γ-tocopherol] gave instant coloration in the Bieber test. The color of the tocopherol dimer was pale purple red, while that of γ-tocopherol was intense purple red.

It should be added that although δ-tocopherol was detected in apricot oil, it did not give coloration in the Bieber test. It is presumed that the lack of coloration was due to the small concentration of δ-tocopherol in that oil (Figure 2a).

Further and Meyer (1939) had shown that treatment of tocopherols with nitric acid resulted in red oxidation products. α-Tocopherol, however, failed to react with the Bieber reagent (which includes also fuming nitric acid), although the tocopherol concentration in almond oil was quite high (266 μg/g of oil). Nilsson *et al.* (1968) reported that oxidative dimerization of γ- and δ-tocopherols with *p*-benzoquinone proceeded rapidly (30 min) in contrast to the very slow formation of dimers from α-tocopherol (12–14 hr). Both the results of our experiments and of the data from the literature show that the respective reactivities in the Bieber test of α- and γ-tocopherols were different. A spot of α-tocopherol, which contained a higher amount of that component than that which was present in the spot obtained from almond oil, gave coloration with the Bieber reagent. It was concluded that the lack of color formation in almond oil was due to an insufficient concentration of α-tocopherol.

It is apparent that the Bieber test, which is usually carried out with oils as such, is not sensitive enough to detect γ-tocopherol in mixtures of almond and apricot oils which contain less than 30% of the latter. However, a direct colorimetric determination (by the Emmerie–Engel method) of γ-tocopherol will make it possible to detect adulteration of almond oil even with a low (5%) amount of apricot oil. Typical tlc separations of the unsaponifiables from mixtures of 95:5 and 90:10 of almond oil with apricot oil are shown in Figure 1 (separations 4 and 5). The γ-tocopherol spots from the 90:10 and 95:5 mixtures of almond and apricot oils were definitely larger than the corresponding spot obtained from the pure almond oil (compare separations 4 and 5 with separation 2, Figure 1).

In Table I are given the contents of α- and γ-tocopherol in a sample of almond oil which was adulterated with 5% apricot oil. The presence of apricot oil in almond oil more than doubled the amount of γ-tocopherol in the mixture (the ratios of α-tocopherol:γ-tocopherol in the pure almond oil and in the almond oil which contained 5% of admixed apricot oil were 93:7 and 83:17, respectively). A good correlation was found between the amount of γ-tocopherol in the oil mixtures and in the original oils. It seems, therefore, that through a determination of the relative amount of γ-tocopherol, the approximate degree of adulteration of almond oil with apricot oil can be estimated.

Quantitative glc determinations were carried out to confirm the results obtained by tlc separations. Typical

gas chromatograms of tocopherols from almond and apricot oil are shown in Figure 2, a and b. Also here, as in the previous tlc separations (see above), the pattern of the tocopherols was entirely different for the two investigated oils. The gas chromatograms of the tocopherols from pure almond and apricot oils reveal that the tocopherol fractions did not include interfering components from unsaponifiables with the same retention time as either α- or γ-tocopherol. In the gas chromatogram of tocopherols from a 95:5 mixture of almond and apricot oils (Figure 2c) the peak height of γ-tocopherol increased distinctly as compared with the corresponding peak in the gas chromatogram of almond oil (Figure 2a). The ratio of α- to γ-tocopherol in pure almond oil was 94:6 (Figure 2a), while for the adulterated oil it was decreased to 83:17. It was concluded also that gas chromatographic analysis may be used as a means for detection of adulteration of almond oil with apricot oil.

To summarize, three different techniques suitable for detecting adulteration of almond oil with apricot oil were described. The first one (tlc of the unsaponifiables) may be used in a primary investigation of a possibility of adulteration. The other two methods (whose results were in good agreement between themselves) were combinations of tlc of the unsaponifiables, followed by either colorimetric or gas chromatographic determinations of tocopherols. Both of these methods were found sensitive and are recommended for evaluation of the extent of the adulterations.

A survey of the level of γ-tocopherol in different varieties and crops of the investigated almond and apricot oils may help in establishing the usefulness of the γ-tocopherol content as an indication of the purity of almond oil.

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Received for review November 24, 1972. Accepted July 18, 1973.

A Method for Obtaining Reproducible Quantitative Gas Chromatograms of Volatiles Isolated from Foods

Statistical interpretation of the gas chromatographic data obtained from volatile flavor compounds isolated from foods requires reproducible gas chromatograms. Injection of the volatiles isolated from frying oils into a high sensitivity gas chromatograph yielded similar but quantitatively different gas chromatograms in consecutive injections. It was found that after the gas chromatographic column has been saturated with the volatiles by repeated injections, qualitatively as well as quantitatively reproducible gas chromatograms can be obtained.

Recent publications in the field of volatile flavor analysis by gas chromatography employ serious efforts in statistical interpretation of data (Dravnieks *et al.*, 1973; Pattee and Singleton, 1972). Since the volatiles isolated from the food samples are usually minute in quantity, high sensitivity gas chromatography is generally used to produce profile chromatograms. The most significant prerequisite for the statistical interpretation of such chromatograms is therefore the assurance of reproducibility of quantitation of each peak in the chromatograms.

McGugan and Howsam (1972) reported that the analysis of volatiles by gas chromatography is not always quantitative. It is generally thought that thermal degradation of labile compounds and "irreversible" sorption of compounds account for the nonquantitative passage of samples through the gas chromatograph.

In our recent study of the volatile flavor components in deep fat fried foods, we simulated commercial and restaurant practices, according to the method of Krishnamurthy *et al.* (1965), by frying moist cotton balls containing 75% by weight of water in six different oils, namely, cottonseed oil, corn oil, peanut oil, and soybean oil hydrogenated to iodine values of 70, 89, and 115, respectively. The volatile flavor compounds were then isolated from each of the fried oils by subjecting them to 90° under 0.05 mm for 6 hr.

A sample of the isolated volatiles from cottonseed oil was chromatographed with a Beckman GC-55 gas chromatograph using a hydrogen flame ionization detector set at high sensitivity. Two profile chromatograms, obtained by injecting the same sample into the instrument twice under exactly identical conditions, were qualitatively similar, but quantitatively different (Figure 1). Similar results were obtained with 1.8-mm i.d. stainless steel columns packed with either 10% methyl silicone SE-30 on 60–70 mesh Anakrom ABS, Porapak Q, 60–80 mesh, or 5% OV-101 on 80–100 mesh Chromosorb W-HP (AW-DMCS).

Since the injected volatile flavor compounds were from the same sample, any losses of heat-sensitive compounds should have been the same and should not have affected