Rapid Method for the Quantitative Determination of Individual Tocopherols in Oils and Fats

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ABSTRACT

Oils and fats are frozen two times from acetone solution at -80 C under protection by ascorbyl palmitate. Tocopherols (T) and tocotrienols (T_3) present in the filtered extract are separated into their homologues by one-dimensional thin layer chromatography on precoated silica gel plates in the n-hexane/ethyl acetate system 92.5:7.5. Complete separation of the positional isomers β-T and γ-T is accomplished as well, whereas β-T_3 and γ-T are assumed to form identical bands. Suitable spray- and detection systems including new found coloring ones are described for the qualitative estimation of the chromatograms. The content of individual tocopherols (T and T_3) of 24 commercial vegetable oils is quantitatively determined using the Emmerie-Engel procedure.

INTRODUCTION

During the past 15 years, tocopherols, as constituents primarily of vegetable oils and fats, have been to an increasing extent the object of qualitative and quantitative analytical methods of determination. The elaboration of such methods has been decisively influenced by the papers of Pennock et al. (1). In their "Reassessment of Tocopherol Chemistry," they confirmed the existence of eight natural individual tocopherols, four saturated and four unsaturated counterparts. At the same time, they standardized the nomenclature of these tocopherols. Up to now, no new tocopherol homologue compounds have been found in nature, so that, when investigating vegetable oils and fats, analysts now have to deal with the well-defined and already known homologues: the α-. β-, γ-, δ- tocopherols (T) and related tocotrienols (T_3). The latter were originally denominated by Bunyan (2). The term "tocopherol homologue" seems more appropriate here than the still frequently used designation "tocopherol isomer," which is strictly speaking valid only for the positional isomers β-T / γ-T and β-T_3 / γ-T_3.

Where as previously attention was focused chiefly on determination of α-T, during the past 15 years the most important methods of determination aim at the qualitative and quantitative estimation of all individual tocopherols. To this end they make use of paper chromatography (PC), thin layer chromatography (TLC), column chromatography (CC), gas liquid chromatography (GLC), gas liquid chromatography/mass spectrometry (GLC/MS), and recently also of high resolution liquid chromatography (HRLC) also known as high pressure liquid chromatography (HPLC) (1-20), separation techniques, all of which are also sometimes used in combination.

Only a few authors have hitherto succeeded in separating all eight homologue compounds along side one another simultaneously in a satisfactory manner (1,17,20). A survey of the literature shows that the majority of analysts have to be content with the determination of α-T alone, the determination of α-T and of β + γ-T, sometimes also of δ-T, the tocotrienols either not being estimated at all or being estimated together with the corresponding tocopherols. Determinations of total tocopherols, which are made on unsaponified oil, are mostly rapid methods used for specific purposes in which separation into individual tocopherols is not attempted (11,21). Our efforts were directed at finding a method of determining quantitatively the individual tocopherols without great expenditure for equipment. This has led to a method that is presented here and which we feel represents progress in the assay of tocopherols. The technique is so simple that it can be undertaken by any commercial laboratory in the food industry. We have tested the method on 24 commonly marketed vegetable oils and found it to be suitable. It can also be used for oils and fats extracted from foodstuffs.

The principle of the method is as follows: After twice freezing out the oils and fats from an acetone solution at ca. -80 C under the protection of ascorbyl palmitate [modified from Chow et al. (13)], the T and T_3 present in the filtered extract (uncrystallizable fraction) are separated into their homologues by means of one-dimensional TLC on silica gel after application by streaking. The qualitative evaluation is made on the chromatoplate with the aid of suitable detecting reagents, the quantitative estimation by eluting the scrapings of each T and T_3 band followed by the colorimetric Emmerie-Engel procedure (22), the procedure modified as described under Procedure.

Characteristics are: (a) The total tocopherols are separated from the oil by freezing; the Chow et al. method (13) is improved by repeating the freezing process and by adding ascorbyl palmitate as an antioxidant. The omission of the saponification step avoids losses of the tocopherol homologues by alkaline treatment.

(b) The total tocopherols are separated into their homologues by means of TLC on precoated silica gel plates. After testing ca. 75 developing solvents, we found one system with the aid of which seven of the eight homologues can be cleanly separated from one another using one-dimensional TLC with three runs. This is the solvent mixture n-hexane/ethyl acetate 92.5:7.5, which has already been used by Polesello and Vistarini (23) in 1965 and by Livingston et al. (24) in 1968 for the separation of α-T from interfering reducing substances and for the purification of α-T in alfalfa meal extract, respectively. Only β-T_3, which was not available to us, presumably has a band identical with that of γ-T. The unsatisfactory resolution of these two homologues is also described in (1) and (13).

(c) The notably difficult separation of β-T from γ-T (positional isomers), which was successfully achieved for the first time in 1963 by Stowe (5) with one-dimensional TLC and spot application, using a five-component solvent, was then achieved in 1964 in a two-dimensional thin layer chromatogram by Pennock et al. (1), using the data given by Stowe. Only in 1973 did Lovelady (18) once again succeed in obtaining this separation with a four-component solvent, one-dimensional, streak application (2.5-3 cm long). However, in a model experiment these authors' one-dimensional procedures failed to separate the tocotrienols. In practice, for the last 11 years the two-dimensional TLC separation technique has therefore remained a component of many methods of determining T(T_3), since a satisfactory separation of the positional isomers β- and γ-T has not yet been obtained, not even with the aid of CC and GLC. The one-dimensional procedure described in this paper, on the other hand, allows them to be completely separated with a two-component solvent. In this manner, we have been able to establish the presence of β-T in most oils.

(d) With our method, a larger amount of extract can be streaked (12-13 cm long) onto the TLC plate, so that sufficient quantities are available for qualitative and quantitative determination of the homologues, and the sensitivity of detection is therefore increased. In our investigations of oils, the microgram amounts of tocopherol homologues were between 0.2 and 335 mcg/band.

(e) Suitably sprayed thin layer chromatograms permit localization and definite identification of each homologue; the TLC "pictures" show one-colored to individually-colored T and T_3 bands according to choice.

(f) Naturally occurring tocopherol esters have hitherto been found only as fatty acid esters of the trienols in the latex lipids of *Hevea brasiliensis* (7,13). The search for esters of saturated tocopherol homologues in plant oils remained without result (10,11,13,14). We also searched for the presence of these esters after complete extraction of the free T and T_3. Only by alkaline saponification of the remaining crystallized fractions of 24 plant oils with safflower oil did we succeed in obtaining α-T in any considerable amount (70 mg/kg). By comparing TLC and GLC it was shown that the responsible, still unsaponified compound was not identical with α-T palmitate or stearate. It is assumed to be a saponifiable dimer of α-T. Generally, naturally saturated tocopherol fatty acid esters do not seem to be present in plant oils. Supplemental α-T acetate is, if present, completely coextracted with the free tocopherols and is therefore absent in the crystallized fraction.

(g) This method produces results rapidly, needs only the simplest apparatus, saves time and expenditure, requires a sample of a maximum of 500 mg of oil, and nevertheless enables detection of the slightest presence of T and T_3 homologues, since there is no saponification.

The four tocopherols required for the development of this method were available to us in the form of the Mixed Tocopherols Concentrate of the Distillation Products Industries (Rochester, NY); individual isolation was unnecessary. As to tocotrienols, we had at our disposal synthetic α-T_3 and natural α-, γ- and δ-T_3, isolated from latex, according to data in the literature (6).

EXPERIMENTAL PROCEDURES

Chemicals

1. Acetone, dist., enriched with 0.001% ascorbyl palmitate (ASCP); for dry ice-acetone bath without ASCP.
2. Methylene chloride, pure.
3. Chloroform, analytical-reagent grade.
4. Petroleum ether, bp 50-75 C, pure.
5. Cyclohexane, analytical-reagent grade.
6. n-Hexane, analytical-reagent grade.
7. Ethyl acetate, analytical-reagent grade.
8. Ethanol, abs. dist. from $KOH/KMnO_4$.
9. Mixed Tocopherols Concentrate N.F., Type 4-50, Distillation Products Industries, Rochester, NY; tocopherol distribution: 25% α-T, 15% γ-T, 5% β-T, 5% δ-T.
10. Reference solution of total tocopherols (for thin layer reference chromatogram): 0.6% w/v solution of Mixed Tocopherols Concentrate in petroleum ether (bp 50-75 C).
11. D,L-α- tocopherol, for biochemical use, Merck (D,L-α-T).
12. D,L-α- tocopherol standard solution: 25 mg D,L-α- tocopherol of known content, weighed exactly to 0.01 mg, calculated as 100%, are dissolved in cyclohexane, transferred quantitatively to a 100 ml volumetric flask, and made to volume with the same solvent. This D,L-α-T stock solution will keep for one month if stored in a refrigerator and if protected by CO_2 and from light. Diluting 5 ml to 50 ml in a volumetric flask with cyclohexane gives the D,L-α- tocopherol standard solution.
13. L (+)-ascorbyl palmitate (ASCP), Roche or Merck.
14. Dry ice, CO_2, solid.
15. Nitrogen.
16. ortho-Phosphoric acid 85%, analytical-reagent grade.
17. Iron (III) chloride hexahydrate solution 0.2% w/v in ethanol abs. dist.; store in refrigerator.
18. 2,2′-bipyridine solution 0.5% w/v in ethanol abs. dist.; store in refrigerator.
19. TLC spraying and detection reagents:
    - I. Mixture 1:1 from iron (III) chloride solution and 2,2′-bipyridine solution (see above) (Emmerie-Engel reagent)
    - II. Phosphormolybdic acid solution 10% w/v in ethanol abs. dist. (MPS)
    - III. α,α′-Diphenyl-β-picrylhydrazyl solution 0.03% w/v in ethanol abs. dist. (DPPH)
    - IV. Ethanolic sulfuric acid ca. 10% v/v, from sulfuric acid 95-97% (1,84) analytical-reagent grade and ethanol abs. dist.
    - V. Mixture of 0.5 g ceric sulfate, 20 ml dist. water, 1.0 g trichloroacetic acid; simmer; add sulfuric acid 95-97% (1,84) analytical-reagent grade until the solution becomes clear [reagent according to Sonnenschein; modified (25)]
    - VI. Iodine vapour (crystals in TLC chromatotank, tank saturation)
    - VII. Trifluoroacetic acid vapor (acid in TLC chromatotank, tank saturation)

Apparatus

1. Intensive cooling filter apparatus (own construction): A one liter Jena G 20 reagent bottle with ground joint is cut off at the height of ca. 15 cm (calculated from ground joint). A G_3 funnel with sintered filter disc, diameter 7 cm, with lengthened discharge outlet, is then inserted into this flask, the ground joint being sealed with a well-greased (stopcock grease "Glisseal") pierced rubber stopper. The free space between funnel and bottle side serves as a dry ice-acetone jacket. External isolation is obtained with cellulose wadding and aluminum foil. A suction ring is drawn over the discharge outlet; a 500 ml round bottom flask with a ground joint serves as a receiver.
2. Funnel with sintered filter disc G_3, diameter 4 cm.
3. Filter apparatus, Witt's, inside diameter 7 cm, height 11 cm.
4. Dewar vessel, 2.5 liter, as cooling bath (acetone; dry ice).
5. Rotary evaporator, e.g. Rotavapor Büchi.
6. TLC plates silica gel 60 F 254, precoated, layer thickness 0.25 mm, 20 x 20 cm, Merck.
7. TLC applicator, e.g. applicator ICN (e.g. with Hamilton syringe).
8. Usual glass laboratory apparatus and TLC equipment.

Procedure

Extraction of the tocopherols (T) and tocotrienols (T_3) by freezing process: Approximately 500 mg oil are accurately weighed to 0.1 mg, put into a 100 ml Erlenmeyer flask, and dissolved in 25 ml acetone, containing 0.001% ASCP. With oils, which are in some cases more easily dissolved in acetone at -80 C, the weighed sample and corresponding amount of acetone should be reduced (e.g. 200 mg oil in 10 ml acetone), since the oil may otherwise flood the TLC plate (see Separation of the Individual Tocopherols T and T_3 by TLC). The solution is left in a cooling bath at ca. -80 C (Dewar vessel) for 10 min and occasionally shaken thoroughly. The oil rapidly freezes into a fine crystallate. At the same time, five portions each of 15 ml acetone or less (containing ASCP) are subjected to intensive cooling in reagent test tubes.

Using apparatus 1, the contents of the Erlenmeyer flask

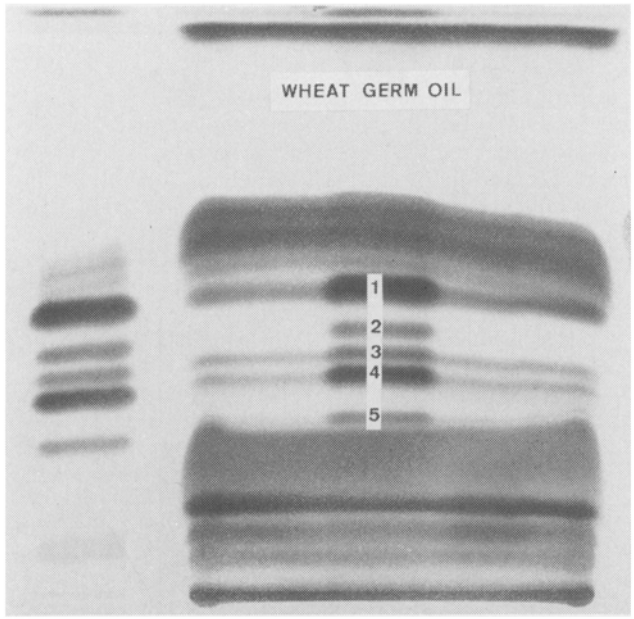

FIG. 1. Separation of individual tocopherols of 100 mg wheat germ oil on 0.25 mm TLC plate, silica gel 60 F 254, precoated, 20 x 20 cm. Solvent system–n-hexane/ethyl acetate 92.5:7.5 (v/v); 3 runs. Reference solution 0.1 ml on the left-hand edge and in the middle of the plate, containing 0.6% natural Mixed Tocopherols Concentrate + 0.04% synthetic α-T_3. Indicator: Emmerie-Engel reagent I, followed by respraying with Phosphormolybdic acid reagent II; heated 10 min 90 C. 1 = α-T, 2 = α-T_3, 3 = β-T, 4 = γ-T, 5 = δ-T. The oil does not contain α-T_3.; γ-T includes also β-T_3. At the start: ASCP.

are then sucked through the precooled G_3 funnel with sintered filter disc into a 500 ml round bottom flask with ground joint and washed in portions with the precooled acetone, the suction process being interrupted each time. The receiver is exchanged for a 100 ml round bottom flask with ground joint and the frozen oil redissolved under suction at room temperature in 25 ml acetone (containing ASCP), the solvent being added in small portions.

If more acetone is required, this must be concentrated again afterwards under vacuum to 25 ml in a rotary evaporator. After repeating the freezing process once again, drawing off by suction and rewashing–as described above–the second extract is combined with the first one. The remaining cyrstallized tocopherol-free fraction of the oil is discarded; the G_3 funnel must be thoroughly cleaned (order: methylene chloride, ethanol, acetone) and, after being used several times, annealed. The acetonic extract contains–in so far as they are present–four tocopherols (T) and four tocotrienols (T_3) (total tocopherols).

Obtaining the total tocopherol concentrate: After evaporation of the extract in vacuum to dryness in a rotary evaporator, the residue is quantitatively transferred by petroleum ether (bp 50-75 C) into a 100 ml round bottom flask with ground joint, concentrated again, and taken up in a little petroleum ether (total tocopherol concentrate). The vacuum should always be released under nitrogen. The transfer from acetonic into petroleum ether solution is made in order to obtain narrow streaks in the TLC.

Separation of the individual tocopherols T and T_3 by TLC and qualitative evaluation:

(A) TLC. By means of an applicator the total tocopherol concentrate is quantitatively and evenly streaked (length 12-13 cm) on two precoated and prewashed (developing solvent) silica gel 60 F254 TLC plates; the flask is rinsed out with petroleum ether.

0.1 ml of a reference solution of total tocopherols is streaked separately 3 cm long on the left hand edge of the plate and in the middle of the applied sample. During application nitrogen is used as protective gas.

One-dimensional development is performed three times successively by the ascending technique at room temperature, the tank being saturated with the solvent mixture n-hexane/ethyl acetate 92.5:7.5; solvent front to the edge of the plate; migration time ca. 3 x 40 min. The four tocopherols (T) and four tocotrienols (T_3) are thereby separated in the following order: α-T/α-T_3/β-T/β-T_3/γ-T/γ-T_3/δ-T/δ-T_3 with decreasing Rf values: 0.50/0.43/0.38/?/0.34/0.27/0.26/0.20. The corresponding Rf value for β-T_3 could not be determined because the pure homologue was not available, but we assume it to be nearly identical with that of γ-T. The T and T_3 references in the middle of the plate–influenced by the applied sample–often migrate somewhat higher than those streaked on the left; the added polar antioxidant ASCP remains at the start; tocopherol dimers, if present, migrate to the proximity of the solvent front (see Figure 1).

(B) Evaluation (qualitative). For location and visualization of the individual tocopherols T and T_3, several detection systems are available that produce both one-colored and color-differentiated chromatograms. We recommend using several systems conjointly, which is also possible on the same plate (two plates are available in each case). The following detection systems have proved to be effective in our investigations of oils:

(a) Intensive spraying with I: very sensitive, all T and T_3 bands are of a uniform color, reddish to red.
(b) Same as (a); followed by respraying with II: intensification of the T or T_3 band–coloration and change of color from reddish to violet; extremely sensitive; bands of foreign substances also become visible. Can be stored for months.
(c) Spray lightly with III: very sensitive; all T and T_3 bands as yellowish-white negative "picture" against blue background. Sharply contoured depiction of the entire width of the bands! Chromatogram sometimes fades gradually.
(d) Same as (c); followed by respraying with IV and heating for 5 min/120 C: individual change of color of the single T and T_3 bands and visualization of foreign bands. Typical T and T_3 band colors (intensity and shade depend on the concentration of T(T_3) in each band): q-T greenish-yellow; α-T_3 grey-blue; β-T yellow-orange; β-T_3 ?; γ-T bright orange-brown; γ-T_3 violet; δ-T orange-brown; δ-T_3 grey-blue to violet.
(e) Spray lightly with V and heat for 5 min/120 C: very sensitive; rich in colors, chromatogram includes all bands of foreign substances. Typical T and T_3 band colors: α-T brownish-yellow; α-T_3 brown to violet; β-T brownish-yellow; β-T_3 ?; γ-T rust colors; γ-T_3 violet; δ-T yellowish-grey to violet; δ-T_3 yellowish-grey to violet.
(f) Spray lightly with IV and heat for 5 min/120 C:T and T_3 bands individually colored as with (d); bands of foreign substances do not show up so clearly. Simplest detection method giving differentiated colors.
(g) 3-5 min under influence of VI; followed by spraying with IV and heating for 5 min/120 C: excellent color differentiation; color chromatogram of all T and T_3 bands and those of foreign substances.
(h) Leave under influence of VI until color penetration of all T and T_3 bands has taken place–5-10 min (brown coloration); afterwards leave under influence of VII for 2 hr without previously exposing the plates to the air. Then leave lying open to air and light. Clear chromatogram giving an extremely good overall view, as bands of foreign substances fade

while T and T_3 bands, on the other hand, show up in typical colors and with particularly sharp contours (no spraying; only action of iodine and acid vapours!). Colors can be kept for months.

The detection systems (a) to (g) respond on both Merck precoated plates and on self-coated silica gel plates; system (h) is suitable only for Merck precoated plates. The band colors obtained can be kept in the light. For documentation purposes, systems (b) and (h) are particularly appropriate. Systems (b), (d), (f) and (h) are the result of our own work and, as far as we know, have not yet been described.

The spraying reagents diazotized o-dianisidine, ceric sulfate and antimony pentachloride, known from the literature, proved to be unsuitable in our case. The last named produces color-differentiated but not always adequately reproducible chromatograms. With 2,6-dichloroquinone-chlorimide (0.5% in ethanol) all T and T_3 bands are visible, and after heating, numerous bands of foreign substances are also visualized, but a grey plate background remains beneath the strongly shaded, generally grey-blue band colors.

The width of the bands and intensity of their colors allow an estimation to be made at the same time of the type and quantity of the T and T_3 homologues present in the oil. On the basis of chromatograms made in this manner, oils and oil mixtures can be largely characterized.

Separation of the individual tocopherols T and T_3 by TLC and quantitative estimation:

(A) TLC. The same procedure is followed as in the previous TLC section except that the additional application of reference solution in the middle of the streaked sample is omitted. After separation of the T and T_3 bands, these are marked—using the available qualitatively evaluated chromatograms as a guide—with the aid of the reference strip: the bands of the reference strip can already be clearly recognized by flourescence quenching (254 nm) and can easily be made visible by separate spraying (spraying reagents I or III).

The T and T_3 bands of the two plates are scraped off individually with a suitable spatula, identical scrapings being collected together in each case in a 100 ml Erlenmeyer flask prepared with 15 ml chloroform. After the scrapings are heated gently, this solution is sucked off over a funnel with sintered filter disc G 3 (diameter 4 cm) and eluted with a total of 50 ml chloroform.

(B) Estimation (quantitative).

Colorimetry (modified Emmerie-Engel procedure). After evaporation of the filtrate in vacuo to dryness, the residue is taken up in 5.0 ml absolute ethanol and mixed with 1.0 ml of a freshly prepared mixture 1:1 of iron (III) chloride solution and 2,2′-bipyridine solution (Emmerie-Engel reagent I). With high T and T_3 content the residue should, however, be taken up in more ethanol (10, 15, 20 ml), 5.0 ml being used for colorimetry.

After the solution stands for 2 min (δ-T and δ-T_3 10 min!) protected from light, 0.5 ml ortho-phosphoric acid 85% is added and reading is taken against pure absolute ethanol at 525 nm in a 1 cm cell. The measured value of absorbance includes the blanks for reagents and plate-material.

Calculation. The standard absorbance of a standard solution of D,L-α-T serves as a basis for calculation in determining the T and T_3 homologues. The absorbance values found for the T(T_3) homologues should in all cases be corrected with the conversion factors given by the AMC (Analytical Methods Committee) (22) in 1959, since the individual tocopherols, despite one reactive OH group, do not give the same absorbances on either a mass or a molar basis, on the premise that the reaction of color development is finished (with δ-T, according to our measurements, an exception; 10 min are necessary for this). The plate blank value—estimated for a free area of silica gel 1 cm x 13 cm of a precoated or self-coated plate 0.25 mm thick—corresponds to 2 mcg D,L-α-T. This, like the reagent blank values, should in all cases be deducted before using the conversion factors. Recovery determinations for α-T have shown that ca. 80% is recovered using the method described, both in the range of 60-70 mg α-T and that of 600-630 mg α-T/kg (avocado and sunflower oil).

If recovery-corrected results are desired, we recommend that the recovery rate be determined separately for each oil. In practice, the uncorrected base values will be adequate.

The following is an example for recovery determination: sunflower oil; uncorrected base value found 621 mg α-T/kg, corresponding to 310.5 mcg α-T/500 mg oil; the assay is repeated after addition of 12 ml D,L-α-T standard solution (=314.0 mcg α-T) to the weighed sample of 500 mg and evaporation of the solvent cyclohexane; only half of the tocopherol concentrate (see Obtaining the total tocopherol concentrate) is applied to the two TLC plates followed by the usual procedure. Calculation: added α-T = 314.0 mcg corresponding to 628 mg α-T/kg. Total α-T found: 1120 mg/kg; added α-T recovered: 1120-621 = 499 mg = 79.4%. Recovery-corrected value: 782 mg α-T/kg.

The mg T or T_3/kg oil (base value) is calculated by means of the following equation:

$$\text{mg T or } T_3\text{/kg oil} = \frac{\alpha\text{-}T_{ST} \times (Abs_S - Abs_R) \times F_D}{Abs_{ST} - Abs_R} - 4 \times \frac{F_C}{W_S \times 100}$$

where $\alpha\text{-}T_{ST}$ = mcg D,L-α-T 100%/2 ml D,L-α-T standard solution; Abs_S = absorbance of the T or T_3 solution in ethanol (5.0 ml); Abs_R = absorbance of the reagent blank value; Abs_{ST} = standard absorbance of 2 ml D,L-α-T standard solution (after evaporation and taking up in 5.0 ml ethanol); F_D = dilution factor for ethanolic solution (colorimetry with 5.0 ml); 4 = plate blank value, calculated as 4 mcg α-T (constant, for 2 plates), ditto valid for all T and T_3 homologues; F_C = conversion factor of the AMC (22), valid for 2 min reaction time and 520 (sic!) nm : α-T 98, β-T 96, γ-T 90, δ-T 75, α-T_3 94, β-T_3 96, γ-T_3 88, δ-T_3 unknown [according to Whittle and Pennock (9) same reaction time as with δ-T (10 min)]; W_S = wt of sample in g.

The base value may be corrected, if desired, by the recovery percentage for α-T, a simplification assumed to be valid also for all other individual tocopherols.

QUANTITATIVE DETERMINATION OF T AND T_3 IN 24 COMMERCIAL VEGETABLE OILS

The amount of individual tocopherols present was quantitatively determined in 24 vegetable oils by the method described. The results are given in Table I as uncorrected base values in mg/kg oil as well as in percentage of the individual tocopherols of total tocopherols. The values for γ-T include those for β-T_3 if present [e.g., wheat germ oil, known as a source for β-T_3 [12,16,17,26)]. For pure oils the percentage distribution is to a certain extent a characteristic of the type of oil. The T_3 content of the edible oils C and I (Table I) seems to indicate mixed oils (with palm oil).

The accuracy of the rapid method was checked for each oil by comparing the results for α-T with those obtained after saponification of the sample and isolation of α-T by TLC (27)]. The results of both methods (recovery-corrected in each case) were in good agreement.

The precision was statistically evaluated [159 measurements (N); 47 groups (g) representing averages ($\bar{x}$) of similar levels of mg T(T_3)/kg; one laboratory; two analysts; during five months] and is valid for all T and T_3 homologues in all 24 oils:

TABLE I

Quantitative Presence of Individual Tocopherols in 24 Vegetable Oils mg/kg (% of Total Tocopherols)[a]

Type of oil and weighed sample (mg)	α-T		β-T		γ-T		δ-T		α-T_3	
Avocado 500	64	(77)	–	–	19	(23)	–	–	–	
Cottonseed crude 200	402	(41)	1.5	(0.2)	572	(58)	7.5	(0.8)	–	
Peanut (Ground-nut) 500	169	(51)	5.4	(1.5)	144	(43.5)	13	(4)	–	
Cocoa butter A, nonalkalized, deodorized 500	18.5	(8)	3.9	(1.7)	196	(84.3)	14	(6)	–	
Cocoa butter B nonalkalized, nondeodorized 500	10.9	(5)	–	–	170	(85)	17	(9)	1.8	(1)
Coconut refined 500	–	–	–	–	–	–	2.4	(11)	20	(89)
Maize germ refined 500	134	(22)	18	(3)	412	(68)	39	(7)	–	
Mazola 300	191	(16)	+		942	(79)	42	(3)	23	(2)
Olive refined pure DAB7 500	93	(93)	–	–	7.3	(7)	–	–	–	
Rapeseed refined 500	70	(26)	16	(6)	178	(65)	7.4	(3)	–	
Castor DAB7 300	28	(6)	29	(6)	111	(23)	310	(65)	–	
Safflower 500	223	(84)	7.0	(2.5)	33	(12)	3.9	(1.5)	–	
Mustardseed crude 300	75	(13)	–	–	494	(82)	31	(5)	–	
Sesame refined pure DAB6 500	12	(4)	5.6	(2)	244	(83)	32	(11)	–	
Soybean refined 500	116	(10)	34	(3)	737	(63)	275	(24)	–	
Soy Fraction A Ölmühle Hamburg 500	6069	(7.5)	1171	(1.5)	48006	(59)	26671	(32)	–	
Sunflower refined 500	608	(96)	17	(2.5)	11	(1.5)	–	–	–	
Edible oil A 500	71	(9)	26	(3)	491	(60)	227	(28)	–	
Edible oil B 500	423	(77)	20	(3.5)	80	(14)	30	(5.5)	–	
Edible oil C 500	165	(44)	–	–	63	(17)	–	–	50	(13)
Edible oil I 500	65	(15)	–	–	140	(33)	++		56	(13)
Edible oil II 500	84	(8)	30	(3)	623	(62)	270	(27)	Tr.	
Walnut cold pressed 300	–	–	–	–	263	(85)	46	(15)	–	
Wheat germ cold pressed 200	1179	(54)	398	(18)	493	(23)	118	(5)	Tr.	

[a]All values are averages from three determinations or more; uncorrected base values; weighed samples between 200 and 500 mg; Tr = traces; + = traces of β-T contained in α-T_3; ++ = traces of δ-T contained in γ-T_3. No δ-T_3 was found in any of the oils and γ-T_3 was found only in edible oil samples C [97 mg (26%)] and I [168 mg (39%)]. β-T_3–if present– is included in the γ-T values.

mg T or T_3/kg	N	g	s_{rel}% ±
1 - 10	25	7	46,3
11 - 30	44	11	18,4
31 - 100	45	11	7,9
101 - 1200	45	18	5.2

ANTIOXIDANTS

Natural oils and fats do not usually contain additional artificial antioxidants. Should butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), or ethoxyquine (EMQ) nevertheless be present, the following points must be noted: in the solvent systems used for separating the T and T_3 homologues, BHT migrates near to the solvent front; BHA is located between γ-T and δ-T; and EMQ has identical band to γ-T.

EMQ is the only one of the antioxidants to fluoresce brilliant blue at 366 nm and is therefore easy to recognize. The T and T_3 homologues do not fluoresce. BHA, BHT, and EMQ are easily removed by washing the T(T_3) solution in petroleum ether (bp 50-75 C) with 80% sulfuric acid, which by the way also removes a lot of carotinoids if present (solutions becoming almost colorless).

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