Japan Customs Analysis Methods

No. 107

Quantitative Analysis of Vitamin E in Vegetable Oils

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1. Scope

This analysis method is applied to wheat germ oils and the like for which their vitamin E contents are required.

2. Outline of Test Method

This analysis method is applied for determination of vitamin E in vegetable oils according to the following procedure:

(1) Identifying α-, β-, γ-, and δ- tocopherols contained in an analysis sample using a high performance liquid chromatograph (HPLC) with standard tocopherols as references, and confirming that there is no interference of coexisting substances in the sample to an internal standard to be used in this test.

(2) Determining each of the tocopherols using an internal standard method.

Note that if the addition of tocopherol derivatives is questioned, evaluation of HPLC separation conditions, etc. or a pretreatment such as saponification is required.

3. Apparatus

High performance liquid chromatograph (HPLC), equipped with a detector to measure absorbance at 300 nm (e.g. UV detector). Measurement conditions are as follows:

(a) Separation column, Shodex SIL-5B, 4.6 mm I.D. × 250 mm, or equivalent column
(b) Column temperature, 30 °C
(c) Mobile phase, n-hexane-isopropanol (100:1, v/v) (Both HPLC grade)
(d) Flow rate, 1.0 mL/min
(e) Detection, UV 300 nm
(f) Injection volume, 20 μL

4. Reagents

All chemicals must be JIS special reagent grade or equivalent, unless otherwise specified.

(a) α- tocopherol
(b) β- tocopherol
(c) γ- tocopherol
(d) δ- tocopherol
(e) n- hexane
(f) 2, 2, 5, 7, 8-pentametyl-6-hydroxychroman [or 2-metyl-2-phytyl-6-hydroxychroman]
(g) Isopropanol

5. Preparation of standard solutions for calibration curve

(1) Tocopherol standard stock solution [(1)]

Accurately weigh approximately 25 mg of α-, β-, γ-, and δ-tocopherols into a beaker and dissolve in n-hexane. Transfer it with n-hexane into a 100-mL brown volumetric flask. Dilute to volume with n-hexane.

Note 1) In the case that the contents of tocopherols in the test sample are different, the amounts of standard tocopherols should be adjusted.

(2) Internal standard solution [(2)]

Accurately weigh approximately 25 mg of 2, 2, 5, 7, 8-pentametyl-6-hydroxychroman or 2-metyl-2-phytyl-6-hydroxychroman into a beaker and dissolve in n-hexane. Transfer it with

n-hexane to a 100-mL brown volumetric flask. Dilute to volume with n-hexane. Use this solution as internal standard solution.

Note 2) Confirm the separation of sample ingredients by HPLC, and use an internal standard substance which is not affected by the coexistent substances.

(3) Standard solutions for calibration curve

Using whole pipets, add 1, 2, 3 and 5 mL of the tocopherol standard stock solution (prepared in 5. (1)) to separate 20-mL glass stoppered-Erlenmeyer flasks [3]. To each of the flasks, add 1 mL of the internal standard solution (prepared in 5. (2)) using a whole pipet, and dilute with n-hexane to about 10 mL each.

Note 3) Use brown glass stoppered-Erlenmeyer flasks or cover flasks with aluminum foil.

6. Preparation of Test solution

Accurately weigh [4] a proper quantity of test sample into a 20-mL glass stoppered-Erlenmeyer flask. [3] Add 1 mL of the internal standard solution to the flask using a whole pipette, and dilute with n-hexane to about 10 mL. Then, filter the solution through a membrane filter of a 0.45 μm pore-size. Use the filtrate as test solution for HPLC analysis.

Note 4) Test solution should be prepared by adjusting a concentration of the most abundant tocopherol in the test sample to about 0.1 mg/mL. Sampling amounts of vegetable oils to which vitamin E is not added would be approximately 0.2-0.6 g. However, in case that tocopherols' amounts in the test sample are small, note that an effect of coexistent substances becomes significant.

7. Procedure

7.1. Preparation of calibration curve

Inject 20 μL each of the standard solutions prepared in 5 (3) into HPLC. From the obtained chromatograms, measure the peak areas of the tocopherols and the internal standard substance.

Draw the calibration curves of the tocopherols by plotting the weight ratio (Wx/Ws) of the tocopherols (Wx) to the internal standard substance (Ws) against the peak area ratio (Ax/As) of the tocopherols (Ax) to the internal standard substance (As).

7.2. Determination of tocopherols in test solution

Inject 20 μL of the test solution prepared in 6 into HPLC. Calculate the peak area ratios of the tocopherols to the internal standard substance based on the peak areas of each substance from the obtained chromatogram, and convert them to weight ratios using the calibration curves constructed in 7.1.

Calculate the contents of each of the tocopherols in the test sample using the following formula:

Round off fractions to the second decimal place.

$$\%, \text{each tocopherol} = \frac{(Wx/Ws) \times Ms}{S \times 1000} \times 100$$

Where -

Wx/Ws: Weight ratio of a tocopherol to the internal standard substance, obtained from the calibration curve

Ms: weight of the internal standard substance contained in 1 mL of internal standard solution (mg)

S: Sample weight (g)

Calculate the content of vitamin E in the test samples as the sum of the contents of the tocopherols.

Round off fractions to the first decimal place.

9. References

(1) Takahashi T., Iwase K., Koga T., Sasagawa K. (1998) Reports of the Central Customs Laboratory **38**: 23 (in Japanese).

(2) Satoshi N. *et al.* (1987) J. Jpn. Oil Chem. Soc. **36**: 506 (in Japanese).

(3) Ishiguro M. (1983) Reports of the Central Customs Laboratory **24**: 45 (in Japanese).

(4) Ishiguro M. (1983) Reports of the Central Customs Laboratory **23**: 95 (in Japanese).

(5) Hiromu K. *et al.* (1982) J. Jpn. Oil Chem. Soc. **31**: 456 (in Japanese).

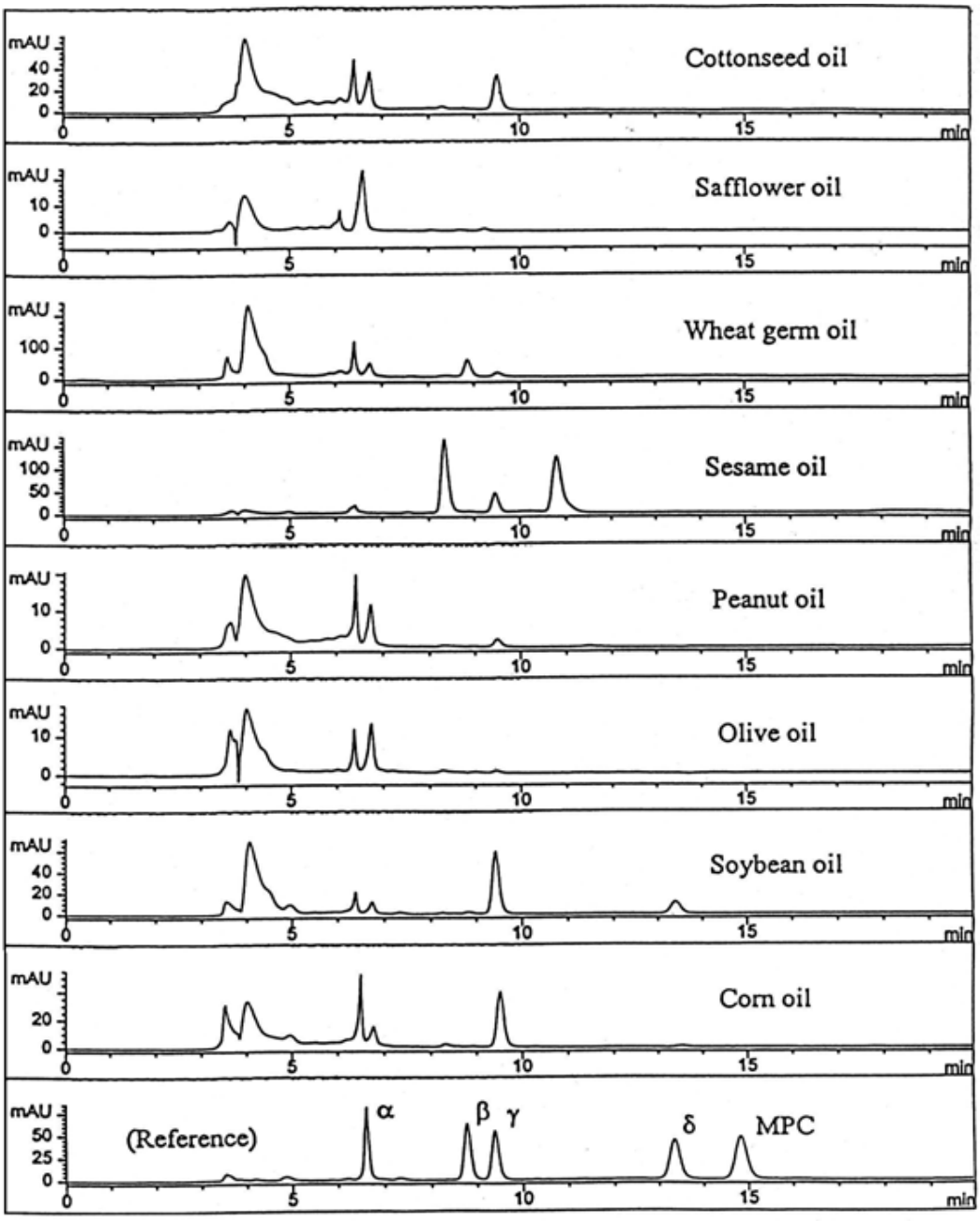
(6) Barrie T., Linda B. (1979) Anal. Biochem. **180:** 368.

(7) Carpenter A. P., Jr. (1979) J. Am. Oil Chem. **56**: 668.

(8) Tsuyoshi F. (1979) J. Jpn. Oil Chem. Soc. **28**: 468 (in Japanese).

(9) ビタミン学実験法 I（日本ビタミン学会編），東京化学同人（1979), pp.27-.
(10) ビタミンハンドブック③ ビタミン分析法（日本ビタミン学会編), 化学同人（1979) pp.27-.

(Reference)



Example: HPLC chromatograms of various oils

Conditions:

Column	SIL-5B, 4.6 mm × 250 mm (30 ºC)
Mobile phase	n-hexane: IPA (100:1); flow rate, 1.0 mL/min
Detector	UV 300 nm