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β-CAROTENE from BLAKESLEA TRISPORA

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β-CAROTENE from *BLAKESLEA TRISPORA*

Prepared at the 87th JECFA (2019) published in FAO Monographs 23 (2019). Superseding specifications prepared at the 61st JECFA (2003), published in FNP 52 Add 11 (2003). A group ADI with β -carotene (synthetic) of 0 – 5 mg/kg bw was established at the 57th JECFA (2001).

SYNONYMS CI Food Orange 5; INS No. 160a(iii)

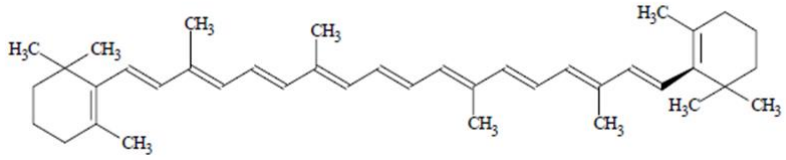
DEFINITION Obtained by a fermentation process using the two sexual mating types (+) and (-) of the fungus Blakeslea trispora. The colour is isolated from the biomass by solvent extraction and crystallised. The colouring principal consists predominantly of trans β-carotene together with variable amounts of cis isomers of β-carotene. Minor amounts of other carotenoids of which γ-carotene accounts for the major part may also be present. The only organic solvents used in the extraction and purification are ethanol, isopropanol, ethyl acetate and isobutyl acetate. The main articles of commerce are suspensions in food grade vegetable/plant oil and water dispersible powders.

Chemical names ß-Carotene, ß,ß-carotene

C.A.S. number 7235-40-7

Chemical formula $C_{40}H_{56}$

Structural formula



Formula weight 536.88

Assay Not less than 96.0% total colouring matter (expressed as ß-carotene).

DESCRIPTION Red to brownish-red crystals or crystalline powder.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility	Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay from 300 – 600nm. The spectrum shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The ratio A_{455}/A_{483} is between 1.14 and 1.18. Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 340 nm. The ratio A_{455}/A_{340} is not less than 15.

PURITY

Sulfated Ash (Vol. 4)	Not more than 0.2%
Carotenoids other than ß-carotene	Carotenoids other than ß-Carotene: Not more than 3% of total colouring matters. See description under TESTS
Residual solvents (Vol. 4)	Ethanol and Ethyl acetate: Not more than 0.8% singly or in combination Isopropanol: Not more than 0.1% Isobutyl acetate: Not more than 1.0% See description in Volume 4
Lead (Vol. 4)	Not more than 2 mg/kg. Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, under “General Methods, Metallic Impurities”.

TESTS

PURITY TESTS

Carotenoids other than β-carotene

Determine by liquid chromatography (see Volume 4) using the following procedure:

Chromatographic system

- HPLC equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler and integrator
- Detector wavelength: 445 nm
- Column: Reverse phase C18; Vydac 218 TP54 (250 x 4.6 mm, 5 µm) or equivalent
- Mobile phase: 99% methanol and 1% tetrahydrofuran containing 50 mg/l of L-ascorbic acid
- Isocratic elution
- Column temperature: 30°
- Flow rate: 0.6 ml/min
- Injection volume: 10 µl

- Temperature of the autosampler: (4pprox.. 15°)
- Run time: 4pprox.. 25 min

Sample preparation

Weigh 25 mg of the sample and dissolve in tetrahydrofuran. Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute 1 ml of the solution to 25 ml in a volumetric flask with mobile phase. Use freshly prepared solutions.

Results

The retention time for β-carotene (all trans isomer) is about 19 minutes corresponding to the largest peak in the chromatogram. The retention time for γ-carotene is about 20 minutes and the peak at about 22 minutes corresponds to the 13-cis isomer.

Γ-Carotene as a % of total β-carotene:

$$= \frac{A_1 \times 100}{A_1 + A_2 + A_3}$$

where

A_1 is the area of the γ-carotene peak

A_2 is the is the area of the all-trans β-carotene peak

A_3 is the combined area of the peaks from the isomers of all-trans β-carotene

METHOD OF ASSAY

Total colouring matters content by spectrophotometry

Proceed as directed under Total Colouring Matters Content – Colouring Matters Content by Spectrophotometry, Procedure 2, using the following conditions:

Sample weight (W): 0.08 g (±0.01 g);

Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml;

Volume of the two pipets: $v_1 = v_2 = 5$ ml;

Specific absorbance of the standard: $A_{1cm}^{1\%} = 2500$;

Wavelength of maximum absorption: λ_{max} about 455 nm.

Calculation

Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, \text{w/w}) = \frac{A \times V_1 \times D}{A_{1cm}^{1\%} \times W}$$

where

A is the absorbance of the twice-diluted sample solution at 455 nm; and

D is the dilution factor $(V_2 x V_3)/(v_1/v_2)$.