Food and Agriculture Organization of the United Nations

World Health Organization

Specifications Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 96th Meeting 2023

Lycopene from *Blakeslea trispora*

This monograph was also published in: Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 96th meeting 2023. FAO JECFA Monographs 31

Lycopene from *Blakeslea trispora*

Specifications revised at the 96th JECFA (2023) and published in FAO JECFA Monographs 31 (2023) superseding specifications prepared at the 67th JECFA (2006). A group ADI "not specified" for lycopene from all sources was established at the 71st JECFA (2009).

SYNONYMS INS 160d(iii)

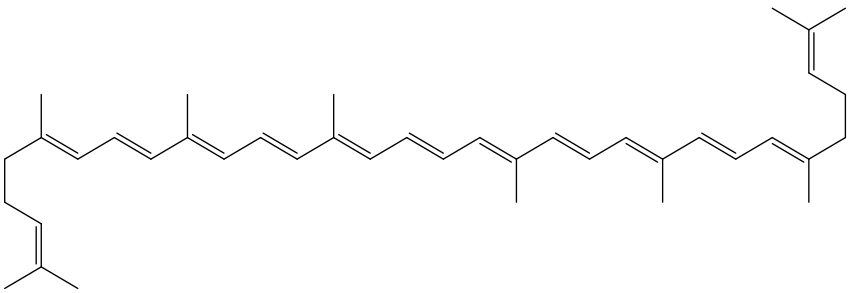
DEFINITION Lycopene from *Blakeslea trispora* is extracted from the fungal biomass and purified by crystallization and filtration using only Isopropanol and isobutyl acetate. It consists predominantly of all-*trans*-lycopene. It also contains minor quantities of other carotenoids. Commercial lycopene preparations intended for use in food are formulated either as suspensions in edible oils or as water-dispersible powders and are stabilised with antioxidants.

Chemical names
Ψ,Ψ-carotene
all-*trans*-lycopene (all-E)-lycopene
(all-E)-2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene

CAS number 502-65-8

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

Structural formula



Formula weight 536.9

Assay Not less than 95% total lycopenes; not less than 90% all-*trans*-lycopene

DESCRIPTION Red crystalline powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION
Solubility (Vol. 4) Insoluble in water, sparingly soluble in tetrahydrofuran (THF)

Test for carotenoids — The colour of the solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 1N sulfuric acid.

Solution in THF — A 1% solution is clear and has intensive red-orange colour

Spectrophotometry (Vol. 4) — A solution in hexane shows an absorption maximum at approximately 470 nm

PURITY

Other carotenoids — Not more than 5%
See description under METHOD OF ASSAY

Loss on drying (Vol. 4) — Not more than 0.5% (40 °C, 4 h at 20 mm Hg)

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Residual solvents (Vol. 4) — Isopropanol: Not more than 0.1%
Isobutyl acetate: Not more than 1.0%

METHOD OF ASSAY

The HPLC method of assay is suitable for determination of total lycopenes (all-*trans*-lycopene and *cis*-lycopene isomers), all-*trans*- lycopene, and other carotenoids. (Note: the predominant *cis* isomer detected in lycopene from *B. trispora* is 13-*cis*-lycopene.)

Reagents (Note: all solvents should be HPLC-grade)
Acetonitrile
Methanol Acetone Hexane
Methylene chloride
Lycopene standard (purity 95% or higher; available from Vitatene S.A.)

Apparatus
VIS or UV-Vis spectrophotometer with a 1-cm light path optical cell HPLC system with either a VIS or UV-Vis detector or a suitable diode array detector, injector, column oven, and integrator
Column: Vydac 218 TP54 5 µm (4.6x250 mm) or equivalent

HPLC conditions

Mobile phase:	acetonitrile/methanol (40:60)
Flow rate:	1 ml/min
Detection:	470 nm
Injection volume:	10 µl
Column temperature:	30 °C
Injector temperature:	10 °C
Run time:	15 min

Standard solution
Weigh accurately about 25 mg lycopene standard into a 100-ml volumetric flask. Dissolve in 10 ml of methylene chloride and add hexane to volume. Pipet 1 ml of the above solution into a 50-ml volumetric flask and add acetone to volume.

Sample solution
Prepare in the same manner as the standard solution.

HPLC analysis
Inject the standard solution into the chromatograph and record the resulting chromatogram. The retention time of all-*trans*-lycopene is approximately 11.5 to 12.5 min. The relative retention time of 13-*cis*-lycopene with respect to all-*trans*-lycopene is 1.25. The relative retention times for other carotenoids with respect to all-*trans*- lycopene are 1.2 for β-carotene and 1.1 for γ-carotene.

Record the total peak area of all-*trans*-lycopene and *cis*-lycopene isomers and calculate the response factor (RF) for lycopene as follows:

$$RF = \frac{A_{st} \times 5000}{W_{st} \times P_{st}}$$

Where

RF is the response factor for lycopene (AU ml/mg);
A_{st} is the total lycopene (all-*trans*-lycopene and *cis*-lycopene isomers) peak area;
5000 is the volume of the volumetric flask in which the standard was dissolved (100 ml) multiplied by dilution (50);
W_{st} is the weight of the standard (mg); and
P_{st} is the purity of the standard expressed as a proportion of lycopene in the lycopene standard (determined as described under Standard purity determination).

Inject the sample solution into the chromatograph and record the following peak areas:
A_1 – all-*trans* lycopene
A_2 – total lycopene (all-*trans*-lycopene + *cis*-lycopene isomers)
A_3 – other carotenoids
A_4 – all carotenoids (all-*trans*-lycopene + *cis*-lycopene isomers + other carotenoids)

Results
Calculate the % of total lycopenes, all-*trans*-lycopene, and other carotenoids as follows:

$$Total\ lycopenes\ \% = \frac{A_2 \times 5000}{W \times RF} \times 100$$

$$All - trans - lycopene\ (\%) = \frac{A_1}{A_2} \times 100$$

$$Other\ carotenoids\ \% = \frac{A_3}{A_4} \times 100$$

W is the sample weight (mg);
RF is the response factor (AU ml/mg); and
5000 is the volume of the volumetric flask in which the standard was dissolved (100 ml) multiplied by dilution (50).

Standard purity determination

Accurately weigh about 20 mg of the lycopene standard into a 100-ml volumetric flask. Dissolve in 10 ml of methylene chloride and add hexane to volume. Pipet 1 ml of the solution into a 100-ml volumetric flask and add hexane to volume. Measure the absorbance in a 1-cm optical cell at the wavelength of maximum absorption (approximately 470 nm). Use hexane as the blank.

Calculation

$$P_{st} = \frac{A_{max}}{345 \times W_{st}} \times 10000$$

where

P_{st} is the purity of the lycopene standard calculated as a proportion of lycopene in the lycopene standard (NOTE: P_{st} equals 1 for a 100% pure standard and is less than 1 for a standard with purity below 100%);
A_{max} is the absorbance at 470 nm;
W_{st} is the weight of the standard (mg);
10000 is the volume of the volumetric flask in which lycopene was dissolved (100 ml) multiplied by dilution (100); and
345 is the absorptivity of lycopene in hexane.