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**β -CAROTENE-RICH EXTRACT from DUNALIELLA
SALINA**

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β-CAROTENE-RICH EXTRACT FROM *DUNALIELLA SALINA*

Prepared at the 87th JECFA and published in FAO JECFA Monographs 23 (2019), superseding specifications prepared at the 84th JECFA (2017) and published in JECFA Monograph 20 (2017). The 84th JECFA (2017) concluded that there was no health concern for the use of β-carotene-rich extract from *D. salina* when used as a food colour in accordance with the specifications established at 84th meeting. The Committee emphasized that this conclusion applies to the use of this extract as food colour, not as food or food supplement.

SYNONYMS

Natural β-carotene, carotenes-natural; CI (1975) No. 75130; CI (1975) No. 40800 (β-Carotene)

DEFINITION

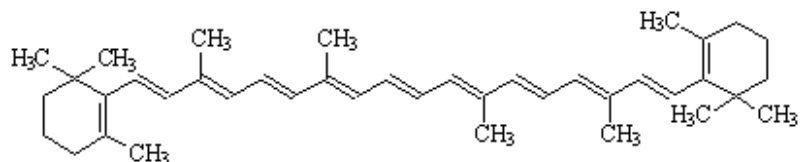
β-Carotene-Rich Extract from *Dunaliella salina* is obtained by extraction from strains of the algae *Dunaliella salina* (syn. *D. bardawil* and *D. Kone*) using the essential oil d-limonene. The extract is then prepared as a suspension in vegetable oil after removal of the essential oil. The main colouring principles are trans- and cis-isomers of β-carotene together with minor amounts of other carotenes including α-carotene, lutein, zeaxanthin and cryptoxanthin. The isomers of β-carotene account for approximately 90% of the carotenes in the product. Besides the colour pigments and vegetable oil, β-Carotene-Rich Extract from *Dunaliella salina* contains lipids and other fat-soluble components naturally occurring in the source material such as fatty acids typically found in food oils, long-chain alcohols, alkenes, and waxes. The main articles of commerce may be further blended with vegetable oil to standardize the colour content.

Class Carotenoid

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

Chemical formula C₄₀H₅₆ (β-Carotene)

Structural formula All-trans-β-Carotene



Formula weight 536.88 (β-Carotene)

Assay Content of carotenes (calculated as β-carotene) is not less than declared
See description under TESTS

DESCRIPTION	Opaque, deep-red, viscous suspension
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u>	Insoluble in water; practically insoluble in ethanol; soluble in hexane
<u>Spectrophotometry</u> (Vol. 4)	A cyclohexane solution of the sample shows maximum absorptions at 448 – 457 and 474 – 486 nm
<u>Colour reaction</u>	A spot of a solution of the sample in toluene (about 400 µg/mL of β-carotene) on a filter paper turns blue 2-3 min after application of a spray or drop of 20% solution of antimony trichloride solution in toluene.
PURITY	
<u>Arsenic</u> (Vol. 4)	Not more than 3 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 Vol. 4 (under “General Methods, Metallic Impurities”).
<u>Lead</u> (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 Vol. 4 (under “General Methods, Metallic Impurities”).
TESTS	
PURITY TESTS	
<u>d-Limonene</u>	Principle Determination of essential oil limonene by gas chromatography (Vol. 4) under the following conditions: Chromatography conditions <ul style="list-style-type: none"> - Column: polyethylene glycol Agilent DB-WAX, or equivalent, 30m x 0.53mm x 1.0 microns - Carrier Gas: High purity Nitrogen, 12kPa head pressure, 5:1 split or splitless - Detector: Flame ionization detector, 200° - Injection: 2.0 µL (split) or 0.5µl (splitless), 150°

- Temp. Program:
 - o Initial: 80°, 5.0 minute
 - o Rate: 10°/minute
 - o Final: 150°, 3 minutes
 - o Total run time: 15 minutes
- Quantitation:
 - o Calculate by: Area
 - o Method: Internal standard
 - o Curve Fit Type: Linear
- Internal standard solution: Prepare a 10.0 mg/mL solution of terpinene in n-hexane.

Sample solution:

Accurately weigh a sample based on the expected quantity of limonene (1.0-2.5g) into a 25mL volumetric flask. Pipette 1 mL of the Internal standard solution into the flask and vortex 15 seconds. Add about 10mL dichloromethane and vortex 15 seconds. Make up to volume with dichloromethane. Mix thoroughly

System Suitability Check

- Perform 2 injections of the blank. No limonene peak should be detected.
- Perform 5 injections of the standard solution containing
- 1.0 mg/mL of limonene and 0.4 mg/mL of terpinene in n- hexane. The coefficient of variation of 5 injections is
- <1.00%. Separation between the limonene and terpinene peaks should be not less than 0.5 min.

Calculations:

$$RF_1 = \frac{A_{istd} \times [STD]}{A_{std} \times [ISTD]}$$

$$[LIM] = \frac{A_{lim} \times DIL \times REF}{A_{istd} \times m}$$

$$[ISTD] = \frac{m_{istd}}{12.5}$$

Where:

RF₁ = Response factor calculated from the calibration standard
 [STD] = Concentration of the standard as % w/w
 [ISTD] = Concentration internal standard as % w/w
 [LIM] = Concentration of limonene in the sample as % w/w
 A_{istd} = Area of the Internal standard peak
 A_{std} = Area of the limonene peak in the calibration standard

A_{lim} = Area of the limonene peak in the sample
DIL = Dilution factor, mL
 m = Sample mass, g
 m_{istd} = Mass of internal standard, g

METHOD OF ASSAY Proceed as directed under Colouring Matters, Total Content by Spectrophotometry (procedure 2) in Volume 4, using the following conditions:

W = amount to obtain adequate absorbance, g

$V_1 = V_2 = V_3 = 100$ mL

$v_1 = v_2 = 5$ mL Solvent: cyclohexane

$A_{1cm}^{1\%} = 2500$;

lambda max = 448 – 457 nm