CAROTENES (Algae)

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). No ADI allocated at the 41st JECFA in 1993. Metals and arsenic specifications revised at the 59th JECFA (2002).

SYNONYMS Natural ß-carotene, carotenes-natural; INS No. 160a(iv); CI (1975) No. 75130; CI (1975) No. 40800 (ß-Carotene)

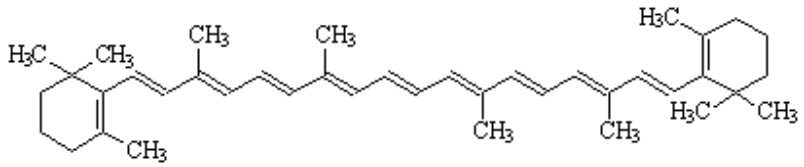
DEFINITION Carotenes (algae) are obtained by solvent extraction of the dried *Dunaliella salina* (syn. *D. bardawil* and *D. Kone*). The main colouring principles are *trans* and *cis* -ß-carotene together with minor amounts of other carotenoids such as alpha-carotene and xanthophylls. Besides the colour pigments, carotenes (algae) may contain lipids, naturally occurring in the source material, food grade vegetable oil, and tocopherol added to retard oxidation of the pigment. The only solvents used for the extraction are carbon dioxide, acetone, methanol, propan-2-ol, hexane, ethanol and vegetable oil.
The main articles of commerce are suspensions in food grade vegetable/plant oil. Others are liquid in food grade oil, dispersion in water using food additive emulsifier, and as a powder using a pulverizing agent such as dextrin. This is for ease of use and to improve stability as ß-carotenes easily oxidize.

Class: Carotenoid

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

Chemical formula: $C_{40}H_{56}$ (ß-Carotene)

Structural formula



all-*trans*-ß-Carotene

Formula weight: 536.88 (ß-Carotene)

Assay: Content of carotenes (calculated as ß-carotene) is not less than declared

DESCRIPTION Red-brown to brown or orange to dark orange solid, suspension, liquid, dispersion or powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water
Spectrophotometry (Vol. 4)	A cyclohexane solution of the sample (1 in 200,000) shows maximum absorptions at 448 -457 and 474 - 486 nm
Colour reaction	A spot of a solution of the sample in toluene (about 400 µg /ml of ß-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

Residual solvents (Vol. 4)	Not more than 50 mg/kg, singly or in combination, of acetone, hexane, methanol, ethanol and propan-2-ol
Tocopherols	Not more than 0.5% See description under TESTS
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Tocopherols

Reagents

- Ethanol: Dehydrate as follows. Add 0.02% each of $KMnO_4$ and KOH to absolute ethanol (Reagent quality) and distil. Discard the first 5% of the distillate and leave at least 5% in the distilling flask.
- Petroleum ether (bp. 30-60^o): Distil prior to use.
- Ferric chloride Solution: Dissolve 1 g of ferric chloride in and make up to 500 ml with dehydrated ethanol. Store in a non-actinic glass flask.
- alpha,alpha'-Dipyridyl Solution: Dissolve 1 g of alpha,alpha'-dipyridyl in and make up to 220 ml with dehydrated ethanol. Store in a non-actinic glass flask.

Procedure

Transfer 0.1 g of the sample, accurately weighed, into a 25 ml volumetric flask. Add a few ml of petroleum ether, and swirl to dissolve the sample. Add approximately 10 ml of ethanol. Boil petroleum ether by heating carefully on a steam bath. Make up to volume with ethanol (first cool the flask and its contents to room temperature). Pipet an aliquot into a second volumetric flask, and make up to volume with ethanol. The aliquot and flask size should be such that on making up to volume this second time the solution contains approximately 15 mg tocopherol per 100 ml. Pipet 2 ml of the solution into a third 25 ml volumetric flask. Add 1 ml of ferric chloride solution and begin timing, using a stopwatch. Immediately add 1 ml of alpha,alpha'-dipyridyl solution and mix thoroughly by swirling. Dilute to volume with ethanol and mix

again by shaking vigorously. Approximately 9.5 min after the timing began, fill the spectrophotometer cell. Exactly 10 min after the addition of ferric chloride, read the absorbance vs. ethanol at 520 nm. The readings should be between 0.2 and 0.8. Measure absorbance of a blank. For the blank use 2 ml of ethanol instead of the sample solution.

% Total Tocopherols is

$$\frac{AD \times 28.2}{L \times CD \times 10}$$

where:
AD = Absorbance of sample - Absorbance of blank;
28.2 = Factor for alpha-, beta-, gamma- and delta- tocopherols;
L = Length of the spectrophotometer cell in cm;
CD = Concentration of the sample expressed as grams of sample in 100 ml of ethanol solution of which 2 ml was taken before adding ferric chloride solution

METHOD OF ASSAY

Proceed as directed under *Colouring Matters, Total Content by Spectrophotometry* in Volume 4, using the following conditions:

W (g) = amount to obtain adequate absorbance;
$V_1 = V_2 = V_3 = 100$ ml; $v_1 = v_2 = 5$ ml;
$A^{1\%}_{1\ cm} = 2500$; and lambda $_{max}$ = 448 - 457 nm