PAPRIKA EXTRACT
(TENTATIVE)

New tentative specifications prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008). No ADI was allocated at the 69th JECFA (2008).

Information required on batches of commercially available products:

- *analytical data on composition*
- *levels of capsaicinoids*
- *levels of arsenic*

SYNONYMS INS No. 160c, Capsanthin, Capsorubin

DEFINITION Paprika extract is obtained by solvent extraction of the dried ground fruit pods of *Capsicum annuum.* The major colouring compounds are capsanthin and capsorubin. Other coloured compounds, such as other carotenoids are also present. The balance of the extracted material is lipidic in nature and varies depending on the primary extraction solvent. Commercial preparations may be diluted and standardised with respect to colour content using refined vegetable oil.

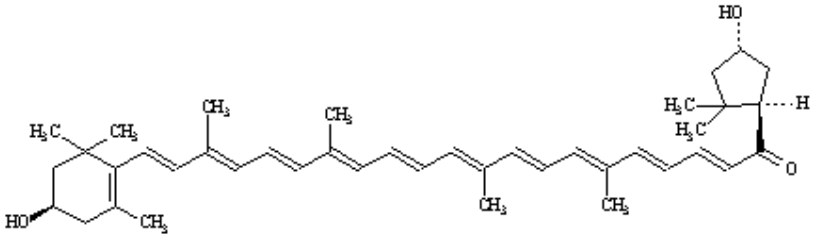
Only methanol, ethanol, 2-propanol, acetone, hexane, ethyl acetate and supercritical carbon dioxide may be used as solvents in the extraction.

Chemical names Capsanthin: (3R, 3'S, 5'R)-3,3'-dihydroxy-β,κ-carotene-6-one
Capsorubin: (3S, 3'S, 5R, 5'R)-3,3'-dihydroxy-κ,κ-carotene-6,6'-dione

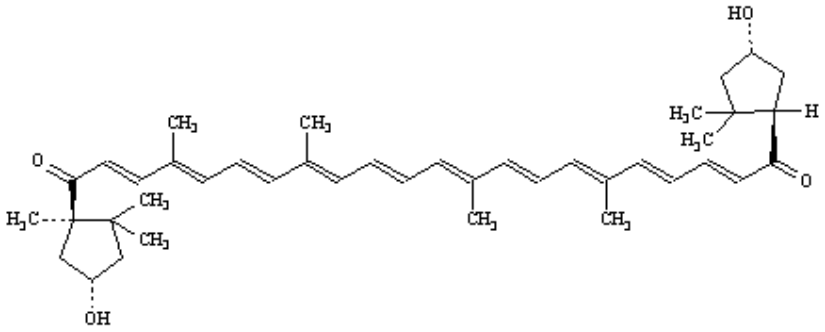
C.A.S number Capsanthin: 465-42-9
Capsorubin: 470-38-2

Chemical formula Capsanthin: $C_{40}H_{56}O_3$
Capsorubin: $C_{40}H_{56}O_4$

Structural formula



Capsanthin



Capsorubin

Formula weight	Capsanthin: 584.85 Capsorubin: 600.85
Assay	Total carotenoids: not less than declared. Capsanthin/capsorubin: Not less than 30% of total carotenoids.

DESCRIPTION

Dark-red viscous liquid

FUNCTIONAL USE

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility	Practically insoluble in water, soluble in acetone
Spectrophotometry	Maximum absorption in acetone at about 462 nm and in hexane at about 470 nm.
Colour reaction	To one drop of sample add 2-3 drops of chloroform and one drop of sulfuric acid. A deep blue colour is produced.
High Performance Liquid Chromatography (HPLC)	Passes test. See Method of assay, Capsanthin/capsorubin

PURITY

Residual solvents (Vol. 4)	Ethyl acetate, methanol, ethanol, acetone, 2-propanol, hexane: Not more than 50 mg/kg either singly or in combination
Capsaicinoids	*Information required on levels in commercial products* See description under TESTS
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine by the atomic absorption hydride technique. 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”).
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption/ICP technique 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

Capsaicinoids

Capsaicinoids are determined by reversed-phase HPLC (Volume 4 under “Chromatography”) using a reference standard to allow quantification.

Preparation of standard

Prepare all standard solutions in ethanol and keep out of direct sunlight.

- Standard solution A,150 µg/ml: Accurately weigh and transfer 75 mg of N-vanillyl-n-nonenamide, >99 % (CAS Registry Number 2444-46-4) into a 500 ml volumetric flask, dissolve and dilute to volume. Mix thoroughly.
- Standard solution B, 15 µg/ml: Pipet 10 ml standard solution A into a 100 ml volumetric flask, dilute to volume, and mix well.
- Standard solution C, 0.75 µg/ml: Pipet 5 ml of standard solution B into 100 ml volumetric flask, dilute to volume, and mix well.

Preparation of sample

Accurately weigh up to 5 g extract into a 50 ml volumetric flask, do not allow the extract to coat the sides of the flask. Add 5 ml acetone (ACS Grade) to the flask and swirl until the sample is completely dispersed. Ensure the extract has not coated the bottom of flask when neck is at a 45° angle. Slowly add ethanol (95% or denatured) with mixing until the solution becomes cloudy. Dilute to volume and mix well. Directly pipet 5 ml sample mixture into a 10 ml syringe attached to a 6 ml C-18 SEP-PAK cartridge. Take care to avoid coating of sample on the sides of syringe. Allow the aliquot to pass through the SEP-PAK and collect the eluent in a 25 ml volumetric flask. Rinse the SEP-PAK with three 5 ml portions of ethanol, and collect in the flask. Dilute to volume with ethanol and mix. Filter through a 0.45 µm syringe filter and collect in a glass vial.

Apparatus

Liquid chromatograph equipped with a 20 µl sample loop injector, a fluorescence detector and/or ultraviolet detector and integrator.
Column: LC-18 (150 x 4.6 mm id, 5 µm)
Detector:
Fluorescence - Excitation 280 nm and emission 325 nm
UV Detector - 280 nm
Mobile phase: 40% acetonitrile and 60% deionised H_2O containing 1% Acetic acid (v/v).
Flow rate: 1.5 ml/min

Procedure

Inject 20 µl of the sample solution in duplicate. Inject the appropriate standard solution (Standard solution C is appropriate for samples expected to contain low levels of capsaicins) prior to the first sample injection and after every 6 sample injections. Purge the column with 100% acetonitrile for 30 min at 1.5 ml/min after no more than 30 sample injections. Equilibrate with mobile phase prior to further

determinations.

Calculations

Calculate individual capsaicinoids (µg/ml) as follows:

Nordihydrocapsaicin: $C_N = (N/A) \times (Cs/RN)$
Capsaicin: $C_C = (C/A) \times (Cs/RC)$
Dihydrocapsaicin: $C_D = (D/A) \times (Cs/RD)$

Total capsaicins (µg/ml) = nordihydrocapsaicin + capsaicin + dihydrocapsaicin

where

A = average peak area of standard;
N, C, and D = average peak areas for respective capsaicinoids (nordihydrocapsaicin, capsaicin and dihydrocapsaicin) from duplicate injections;
Cs = concentration of std in µg/ml;
$C_{N,C,D}$ = concentration of compound in extract expressed as µg/ml;
RN, RC, and RD = response factors of respective capsaicinoids relative to standard.

Response factors:
Nordihydrocapsaicin (N) UV: RN = 0.98; FLU: RN = 0.92
Capsaicin (C) UV: RC = 0.89; FLU: RC = 0.88
Dihydrocapsaicin (D) UV: RD = 0.93; FLU: RD = 0.93
N-vanillyl-n-nonenamide UV: R = 1.00; FLU: R = 1.00
Relative retention times: Nordihydrocapsaicin 0.90; N-vanillyl-n-nonenamide 1.00, Capsaicin 1.00; Dihydrocapsaicin 1.58

Capsanthin/capsorubin

Determine the total carotenoids in paprika extract by spectrophotometry.

Accurately weigh 300 to 500 mg of sample, and transfer quantitatively to a 100 ml volumetric flask. Dilute with acetone to volume, dissolve by shaking and leave to stand for 2 min. Pipet 1 ml of this extract into another 100 ml volumetric flask, dilute to volume with acetone, and shake well. Transfer a portion to the spectrophotometer cell, and read the absorbance A at 462 nm. Adjust the sample concentration to obtain an absorbance between 0.3 and 0.7.

Determine total pigment (%) as capsanthin and capsorubin

$$\text{Total} = \frac{a}{2100} x \frac{10000}{W}$$

where

a = absorbance of sample
2100 = $A^{1\%}_{1cm}$ for capsanthin/capsorubin in acetone at 462 nm
W = weight of sample (g)

Determine the identity and relative purity of paprika extract by reversed-phase HPLC. See Volume 4 under “Chromatography”. The sample is saponified to release the parent hydroxy-carotenoids from the extracts prior HPLC analysis.

Sample preparation

Dissolve 0.2 g of the sample in acetone, quantitatively transfer into a 500 ml separatory funnel and add enough acetone to make up to 100 ml. Add 100 ml diethyl ether and mix well. Remove any insoluble particles by filtration. Add 100 ml of KOH-methanol (20%) and leave the solution for one hour. Shake periodically. Remove the aqueous phase and wash the organic phase several times with distilled water until the washings are neutral. Filter through a bed of anhydrous Na_2SO_4 and evaporate to dryness in a rotary evaporator at a temperature below 35°. Dissolve the pigments in acetone and make up to 25 ml in a volumetric flask. Keep the samples refrigerated until analysis by HPLC. Thoroughly disperse the samples, e.g. by sonication, and filter through a 0.45 µm filter before analysis.

Chromatography

Filter acetone (HPLC grade) and deionised water and de-gas before use.
Column: Reversed-phase C-18 (250 x 4 mm i.d.)
Precolumn: Reversed-phase C-18 (50 x 4 mm i.d.)
Mobile phase: Program a gradient acetone/water as follows:

Time (min)	Acetone (%)	Water (%)
-10 (pre-injection)	75	25
0	75	25
5	75	25
10	95	5
17	95	5
22	100	0
27	75	25

Flow rate: 1.5 ml/min
Detector: Diode array detector, store spectra in the range of 350-600 nm.
Detection wavelength: 450 nm
Injection volume: 5 µl

Identify peaks by comparing the peaks obtained with known standards and quantify the individual carotenoids. Saponified carotenoids will elute in the same order, with capsorubin and some minor carotenoids eluting first and β-carotene in last place. The order of elution is:

- Neoxanthin
- Capsorubin
- Violaxanthin
- Capsanthin
- Antheraxanthin
- Mutatoxanthin
- Cucurbitaxanthin A (Capsolutein)
- Zeaxanthin
- Cryptocapsin
- β-Cryptoxanthin
- β-Carotene

Calculate the percent of each peak using the total area of the peaks in the chromatogram. Sum the percentages of capsanthin

and capsorubin to get the total value.