ANNATTO EXTRACTS (SOLVENT-EXTRACTED NORBIXIN)

Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI for bixin of 0 – 12 mg/kg bw and a group ADI for norbixin and its disodium and dipotassium salts of 0 – 0.6 mg/kg bw expressed as norbixin were established at the 67th JECFA (2006). The colouring matters bixin and norbixin derived from annatto extracts (solvent-extracted bixin; solvent-extracted norbixin; aqueous-processed bixin; alkali-processed norbixin, acid-precipitated; and alkali-processed norbixin, not acid-precipitated) are included in the ADIs for bixin and norbixin. All previous ADIs for annatto extracts were withdrawn.

SYNONYMS

Annatto C, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(ii)

DEFINITION

Solvent-extracted norbixin is obtained from the outer coating of the seeds of the annatto tree (Bixa orellana L.) by washing with one or more of the following food grade solvents: acetone, methanol, hexane, ethanol, isopropyl alcohol, ethyl acetate, alkaline alcohol or carbon dioxide followed by solvent removal, crystallization and drying. Aqueous alkali is added to the resultant powder, which is then heated to hydrolyse the colouring matter and cooled. The aqueous solution is filtered, and acidified to precipitate the norbixin. The precipitate is filtered, washed, dried and milled, to give a granular powder.

Solvent-extracted norbixin contains several coloured components; the major colouring principle is *cis*-norbixin, a minor colouring principle is *trans*-norbixin; thermal degradation products of norbixin may also be present as a result of processing.

Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name

cis-Norbixin: 6,6'-Diapo-Ψ,Ψ-carotenedioic acid
cis-Norbixin dipotassium salt: Dipotassium 6,6'-diapo-Ψ,Ψ-carotenedioate
cis-Norbixin disodium salt: Disodium 6,6'-diapo-Ψ,Ψ-carotenedioate

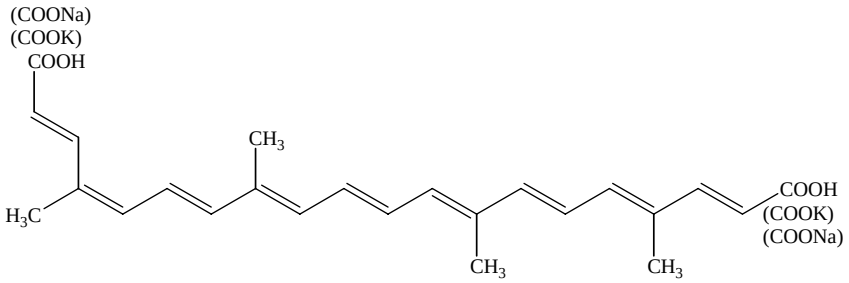
C.A.S. number

cis-Norbixin: 542-40-5
cis-Norbixin dipotassium salt: 33261-80-2
cis-Norbixin disodium salt: 33261-81-3

Chemical formula

$C_{24}H_{28}O_4$, $C_{24}H_{26}K_2O_4$, $C_{24}H_{26}Na_2O_4$

Structural formula



cis-Norbixin

Formula weight: 380.5 (acid), 456.7 (dipotassium salt), 424.5 (disodium salt)

Assay: Not less than 85 % colouring matter (expressed as norbixin)

DESCRIPTION

Dark red-brown to red-purple powder

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in alkaline water, slightly soluble in ethanol

UV/VIS absorption (Vol. 4): The sample in 0.5% potassium hydroxide solution shows absorbance maxima at about 453 nm and 482 nm.

Thin Layer Chromatography: Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250 μm, size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10 µl to the plate. Allow to dry and develop using a mixture of n-butanol, methyl ethyl ketone and 10% aqueous ammonia (3:2:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Bixin and norbixin appear as yellow spots with R_f values of about 0.50 to 0.45, respectively. Spray with 5% sodium nitrite solution and then with 0.5 mol/l sulfuric acid and the spots immediately decolourise.

PURITY

Residual solvents (Vol. 4):

Acetone:	Not more than 30 mg/kg
Methanol:	Not more than 50 mg/kg
Hexane:	Not more than 25 mg/kg
Ethanol:	Not more than 50 mg/kg, singly or in combination
Isopropyl alcohol:	
Ethyl acetate:	

Arsenic (Vol. 4): Not more than 3 mg/kg
Determine using an ICP-AES/AAS-Hydride technique. Alternatively, determine arsenic using Method II of the Arsenic Limit Test. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an AAS ICP-AES technique appropriate to the specified level. The selection of the sample size and method of sample

preparation may be based on the principles of the method described in Volume 4.

Mercury (Vol. 4) — Not more than 1 mg/kg
Determine using cold vapour atomic absorption technique. Select sample size appropriate to the specified level.

METHOD OF ASSAY Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 1, using 0.5 % potassium hydroxide as solvent. Measure the absorbance at the A_{max} of about 482 nm. The specific absorbance ($A^{1\%}_{1\ cm}$) is 2870.