ANNATTO EXTRACTS (OIL-PROCESSED BIXIN)

(TENTATIVE)

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). Due to the lack of toxicity data, no ADI was established at the 67th JECFA (2006). All previous ADIs for annatto extracts were withdrawn.

Information required on chemical characterisation of the non-colouring matter components of commercial products.

Note: The tentative specifications will be withdrawn unless the requested information is received before the end of 2008.

SYNONYMS Annatto D, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(i)

DEFINITION Seeds from the annatto tree (*Bixa orellana* L) are abraded in hot vegetable oil to remove colouring matter from the surface of the seeds. The oil is sieved to remove seeds.

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

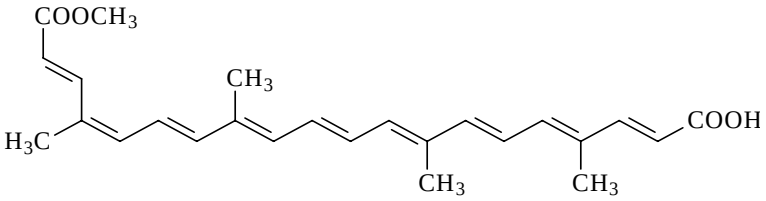
Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name *cis*-Bixin: Methyl (9-*cis*)-hydrogen-6,6'-diapo-Ψ,Ψ-carotenedioate

C.A.S. number *cis*-Bixin: 6983-79-5

Chemical formula $C_{25}H_{30}O_4$

Structural formula



cis-Bixin

Formula weight 394.5

Assay Not less than 10 % colouring matter (expressed as bixin)

DESCRIPTION Dark red-brown to red-purple oil

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, slightly soluble in ethanol
UV/VIS absorption (Vol. 4)	The sample in acetone shows absorbance maxima at about 425, 457 and 487 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

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 2, using 10 ml tetrahydrofuran to dissolve the sample and acetone in place of cyclohexane. Measure the absorbance at the A_{max} of about 487 nm. The specific absorbance ($A^{1\%}_{1\,cm}$) is 3090.