ANNATTO EXTRACTS (ALKALI-PROCESSED NORBIXIN, NOT ACID-PRECIPITATED)

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 All previous ADIs for annatto extracts were withdrawn.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 G, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(ii)

DEFINITION

Alkali-processed norbixin (not acid-precipitated) is prepared by removal of the outer coating of the seeds of the annatto tree (*Bixa orellana* L) with aqueous alkali. The bixin is hydrolysed to norbixin in hot alkaline solution. The precipitate is filtered, dried and milled to give a granular powder. Extracts contain mainly the potassium or sodium salt of norbixin as the major colouring matter.

Alkali-processed norbixin (not acid-precipitated) 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

(COONa)
(COOK)
COOH

CH₃
CH₃
COOH
(COOK)
(COONa)

cis-Norbixin

Formula weight 380.5 (acid), 456.7 (dipotassium salt), 425 (disodium salt)

Assay Not less than 15% colouring matter (expressed as norbixin)

DESCRIPTION Dark red-brown to red-purple powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

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: $250~\mu m,\, size: 5~x~20~cm))$ for 1 h at $110^\circ.$ 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 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.