BEET RED

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not specified' was established at the 31st JECFA (1987)

SYNONYMS Beetroot Red; INS No. 162

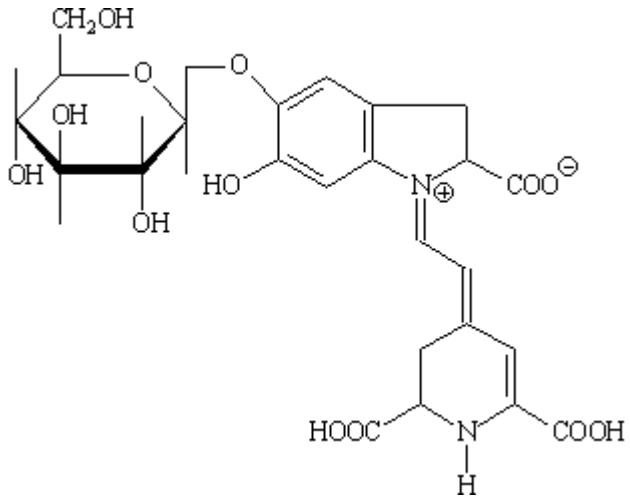
DEFINITION Obtained from the roots of red beets (*Beta vulgaris* L var rubra) as press juice or by aqueous extraction of shredded beet roots; composed of different pigments all belonging to the class betalaine; main colouring principle consists of betacyanins (red) of which betanine accounts for 75-95%; minor amounts of betaxanthine (yellow) and degradation products of betalaines (light brown) may be present; the betanine content in extracts of beetroot will suffer a progressive degradation which is accelerated by raising the pH, temperature and water activity; it is therefore expected that all commercial products will slowly lose their colour and alter their shade according to the conditions of storage.
Besides the colour pigments the juice or extract consists of sugars, salts and/or proteins naturally occurring in red beets. The solution may be concentrated and some products may be refined in order to remove most of the sugars, salts and proteins. Food grade acids (e.g., citric, lactic, L-ascorbic) may be added as pH controlling agents and stabilizers and carriers (e.g., maltodextrin) may be added as aids for manufacturing dry powders.

Chemical names [S-(R^*,R^*)-4-[2-[2-Carboxy-5-(ß-D-glucopyranosyloxy)-2,3-dihydro-6-hydroxy-1H-indol-1-yl)ethenyl]-2,3-dihydro-2,6-pyridine-dicarboxylic acid; 1-[2-(2,6-dicarboxy-1,2,3,4-tetrahydro-4-pyridylidene) ethylidene]-5- ß-D-glucopyrano-syloxy)-6-hydroxyindolium-2- carboxylate

C.A.S. number 7659-95-2 (betanine)

Chemical formula Betanine: $C_{24}H_{26}N_2O_{13}$

Structural formula



Formula weight Betanine: 550.48

Assay Content of red colour (expressed as betanine) is not less than 0.4%.

DESCRIPTION

Red or dark red liquid, paste, powder or solid.

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in or miscible with water; insoluble in or immiscible with ethanol
Colour reaction	Addition of an aqueous 10% w/v sodium hydroxide solution to an aqueous solution of the sample successively changes the colour from red to reddish violet to yellow.
Spectrophotometry (Vol. 4)	Betanine in water at pH 5.4 has an absorbance maximum at about 530 nm and at pH 8.9 exhibits a broadened maximum at about 545 nm.
Thin layer chromatography	Passes test See description under TESTS

PURITY

Nitrate	Not more than 2 g nitrate anion/g of red colour (as calculated from assay) See description under TESTS
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead	Not more than 2 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.”
Basic colouring	To 1 g of the sample add 100 ml of 1% sodium hydroxide solution, and mix well. Extract 30 ml of this solution with 15 ml of diethyl ether. When extracted wash the ether layer twice with 5 ml of dilute acetic acid TS; the dilute acetic acid layer does not produce a colour.
Other acidic colouring matters	To 1 g of the sample add 1 ml of ammonia TS and 8 ml of water, and shake well. Discard an oily layer when separated. Proceed as directed under *Determination by Paper Chromatography (Ascending chromatography)*, using 2 µl of the solution as the sample solution, and a mixture of pyridine and ammonia TS (2:1 by volume) as the developing solvent. Stop the development when the solvent front has advanced about 15 cm from the point of application. No spot is observed at the solvent front after drying under daylight, or, if any spot is observed, it shall be decolourized when sprayed with a solution of stannous chloride (2 parts of stannous chloride by weight in 5 parts of water).

TESTS

IDENTIFICATION TESTS

Thin layer chromatography

(a) On cellulose plates (0.25 mm) with Sørensen's phosphate buffer (pH 5.6) as solvent, Beet Red colour gives a number of spots in various colours (yellow, orange, red, purple, violet). Betanine appears as a purple spot with an R_f value of about 0.7.
Sørensen's phosphate buffer (pH 5.6):
- Solution A: 1/15 M potassium dihydrogen phosphate: Dissolve 9.08 g of KH_2PO_4 in water and dilute to 1000 ml.
- Solution B: 1/15 M disodium hydrogen phosphate: Dissolve 11.88 g of $Na_2HPO_4 \cdot 2H_20$ in water and dilute to 1000 ml.
Sørensen's phosphate buffer is composed of a mixture of solutions A and B in the following proportions: 94.8 parts of solution A + 5.2 parts of solution B.
(b) On cellulose plates (0.10 mm) in the solvent (2 g sodium citrate + 78.5 ml water + 21.5 ml ammonia TS), betanine follows the front of the solvent as distinct from acidic water-soluble synthetic dyes. In this solvent betanine is yellow.

PURITY TESTS

Nitrate

Apparatus
A suitably sensitive potentiometric instrument, such as a pH/mV meter, with nitrate - selective electrode and reference electrode as prescribed by the manufacturer.

Solutions
- Standard nitrate solution (10,000 mg/l): Dissolve 16.31 g of potassium nitrate (KNO_3), previously dried at 105º, 24 h in 1000 ml of water
- Buffer solution: Dissolve 6.66 g of aluminium sulfate octahydrate, $Al_2(SO_4)_3 \cdot 8H_2O$, 3.12 g of silver sulfate (Ag_2SO_4), 1.24 g of boric acid (H_3BO_3) and 1.94 g of sulfamic acid (NH_2HSO_3) in 900 ml water, adjust to pH 3.0 with 1 M sulfuric acid and dilute with water to 1000 ml
- Diluted buffer solution: Dilute the Buffer solution with an equal amount of water
- Calibration solutions: Dilute the standard solution with the Diluted buffer solution in order to prepare the following solutions: 0, 100, 200, 300, 400 and 500 mg nitrate/l.

Procedure
Accurately weigh about 0.5 g of the sample in a conical flask, add 50 ml of Diluted buffer solution and dissolve by swirling.
Measure the potential of the calibration solutions and also of the sample solution. Plot the calibration curve from the potential figures against the corresponding nitrate concentrations using antilog paper with the nitrate concentrations along the linear axis. From the calibration curve read the nitrate concentration of the sample.

Calculation

$$Nitrate\ content = \frac{a}{200 \times w \times A}\ g / g\ colouring\ matters$$

where
a = nitrate concentration of sample, mg/l
w = weight of sample
A = % red colour as calculated from assay

METHOD OF ASSAY

Dissolve a quantity of Beet Red accurately weighed in buffer TS (pH 5) and dilute to a suitable volume with the buffer solution (V ml in total); the maximum absorption shall be within the range of 0.2 to 0.8. Centrifuge the solution if necessary, and measure the absorption, correcting for a blank composed of Buffer TS (pH 5). The colour content is calculated on the basis of the maximum absorption A (at about 530 nm), using the specific absorbance for betanine, A (1%, 1 cm) = 1120.

$$\%\ Red\ colour = \frac{A \times V}{1120 \times L \times W}$$

where
A = maximum absorption
V = volume of test solution measured in ml
L = length of cell measured in cm
W = weight of sample in g.