Food and Agriculture Organization of the United Nations

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SPIRULINA EXTRACT (TENTATIVE)

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SPIRULINA EXTRACT (TENTATIVE)

New specifications prepared at the 86th JECFA (2018), published in FAO JECFA Monograph 22 (2018). A temporary ADI "not specified" was established at the 86th JECFA (2018).

Information Required on:

- *Full compositional characterization of commercial products in both liquid and powder forms.*
- *Full compositional characterization of the aqueous extract before formulation/standardization.*
- *Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data).*
- *Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).*

SYNONYMS INS 134; Spirulina colour

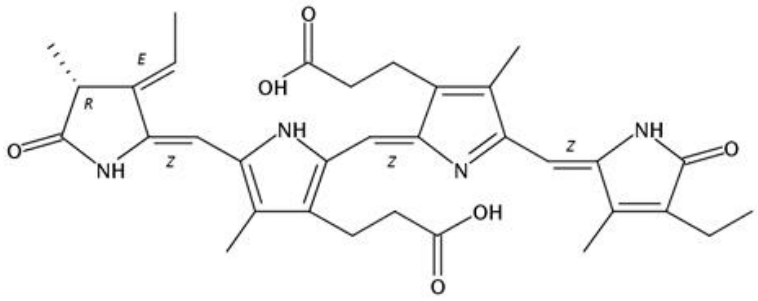
DEFINITION Spirulina extract is obtained by aqueous extraction of the biomass of *Arthrospira platensis*, an edible cyanobacterium. The organism is cultivated and harvested under conditions that prevent the growth of other cyanobacteria and the production of microcystins. The material extracted from the biomass is further treated by steps that may include pH adjustment, centrifugation, filtration, concentration, sterilization, drying, and dilution to the desired degree of pigment concentration. The main colouring principles are two phycobiliproteins, C-phycocyanin and allophycocyanin, which are water-soluble pigment-protein complexes where the chromophore is covalently bonded to the protein. Extracts may also contain trace amounts of chlorophyll, beta-carotene, and other carotenoids. Spirulina extract may contain peptides, other proteins, carbohydrates and minerals. Commercial products are formulated in liquid and powder forms.

C.A.S. number 20298-86-6

(Phycocyanobilin; 3-[(2Z,5E)-2-[[3-(2-carboxyethyl)-5-[(Z)-[(3E,4R)-3-ethylidene-4-methyl-5-oxopyrrolidin-2-ylidene]methyl]-4-methyl-1H-pyrrol-2-yl]methylidene]-5-[(4-ethyl-3-methyl-5-oxopyrrol-2-yl)methylidene]-4-methylpyrrol-3-yl]propanoic acid)

Chemical formula $C_{33}H_{38}N_4O_6$ (Phycocyanobilin)

Structural formula



Phycocyanobilin

Formula weight 586.68 (Phycocyanobilin)

Assay Total phycocyanins as the sum of C-phycocyanin and allophycocyanin not less than declared.

See description under TESTS

DESCRIPTION Clear blue liquid or blue powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water. Insoluble in ethanol.

Colour Value Not less than declared (15 to 300 for powdered products on the dried basis and 10 to 70 for liquid products).

See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 6% for the powdered product (105°, 4h)

Arsenic (Vol. 4) Not more than 1 mg/kg

Determine using a method 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 Vol. 4 (under “General Methods, Metallic Impurities”).

Cadmium (Vol. 4)	Not more than 1 mg/kg Determine using a method 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 Vol. 4 (under "General Methods, Metallic Impurities").
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method 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 Vol. 4 (under "General Methods, Metallic Impurities").
Mercury (Vol. 4)	Not more than 1 mg/kg Determine using a method 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 Vol. 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	Total (aerobic) plate count: less than 1000 CFU/g Yeast and moulds: less than 100 CFU/g Coliforms: absent in 10 g *Salmonella spp*.: absent in 25 g *S. aureus*: absent in 10 g
Microcystins	Less than 0.5 µg/g as microcystin-LR (dried basis) See description under TESTS

TESTS

PURITY TESTS

Microcystins	Principle Determine microcystins by enzyme linked immunoassay (ELISA) under the following conditions: Reagent Methanol/water (75:25, v/v) Equipment Use a commercially available ELISA kit with cross reactivity for microcystin-LR and other microcystins.

Sample preparation
In the absence of other instructions provided by the ELISA kit manufacturer, follow the procedure presented here.

Dry an appropriate amount of spirulina extract. Homogenize 3.0 g of the dried material in 20.0 ml of the methanol/water reagent for 20 minutes. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Transfer the supernatant into a glass flask. Add 10.0 ml of the methanol/water reagent to the homogenizer and homogenize the residue for 30 seconds. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Combine the supernatants and dilute with water to a concentration within the range indicated by the ELISA kit manufacturer.

Procedure
Follow the instructions provided by the ELISA kit manufacturer.

Colour Value

For the purpose of this specification, Colour Value is based on the absorbance of a buffered solution at 618 nm.

Reagent
Sodium phosphate buffer (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure
Transfer 330 mg of spirulina extract into a 100 ml volumetric flask and dilute to volume with water. Transfer 10 ml of the solution into a second 100 ml volumetric flask and dilute to volume with the sodium phosphate buffer (100 mM, pH 6.0). Determine the absorbance (A_{618}) of the solution in a 1-cm cell at 618 nm with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference.

Calculate the Colour Value of the spirulina extract as follows:

Colour Value = $A_{618} \times 100 / W_1$
Where
W_1 is the weight of spirulina extract taken, in g

METHOD OF ASSAY

Principle

Determine total phycocyanins as the sum of C-phycocyanin and allophycocyanin under the following conditions:

Reagent

Sodium phosphate buffer (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure

Transfer 100 mg of spirulina extract into a 25 ml volumetric flask and dilute to volume with sodium phosphate buffer (100 mM, pH 6.0). Sonicate the mixture for 30 minutes maintaining the temperature at 8°. Incubate at 30° for 8 h, shaking manually every hour. Mix the contents of the flask and transfer to a centrifugation tube; centrifuge at 3500 rpm for 4 minutes. Determine the absorbance of the supernatant in a 1-cm cell at 620 nm (A_{620}) and 650 nm (A_{650}) with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference. The dilution should be adjusted with additional buffer, if needed, to obtain absorbance values of 0.2 to 0.6 at 620 nm.

Calculate the C-phycocyanin content of the spirulina extract (%, w/w) as follows:

$$T_{cPC} = (0.162 \times A_{620}) - (0.098 \times A_{650}) \times V_1 \times 100 / W_1$$

Where
W_1 is the weight of spirulina extract taken, in mg
V_1 is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the allophycocyanin content of the spirulina extract (%, w/w) as follows:

$$T_{aPC} = (0.180 \times A_{620}) - (0.042 \times A_{650}) \times V_1 \times 100 / W_1$$

Where
W_1 is the weight of spirulina extract taken, in mg
V_1 is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the total phycocyanin content of the spirulina extract as follows:

$$T_{PC} = T_{cPC} + T_{aPC}$$