Food and Agriculture Organization of the United Nations

95th JECFA - Chemical and Technical Assessment (CTA), 2022

SPIRULINA EXTRACT

Chemical and Technical Assessment (CTA)

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1. Summary

This Chemical and Technical Assessment summarizes data and information on spirulina extract submitted to the Joint FAO/WHO Expert Committee on Food Additives (JECFA or Committee). Review of spirulina extract as a food colour was requested by the Forty-ninth Codex Committee on Food Additives (CCFA). Spirulina extract was reviewed at the Eighty-sixth and Ninety-fifth JECFA meetings.

At the Eighty-sixth JECFA meeting, the Committee evaluated data necessary for the assessment of safety, dietary intake and specifications related to the use of spirulina extract as a colouring additive in a variety of food categories (FAO and WHO, 2019). The Committee established an ADI "not specified" for spirulina extract based on the absence of toxicity in repeated-dose animal studies conducted with spirulina extract and dried spirulina (Evaluation, 2019) and drafted specifications for spirulina extract. The specifications were made tentative and the ADI was made temporary because the sponsor did not provide the information the Committee needed to fully describe the material. Therefore, the following additional information was requested following the Eighty-sixth JECFA meeting:

- 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).

The Committee evaluated new compositional data and analytical methods received in response to the above requests. Compositional data were provided for commercial liquid and powder products, the aqueous extract before formulation/standardization, and the biomass before extraction. The data were obtained from production samples and commercial articles analysed by company laboratories or competent third-party laboratories using recognized official methods. Analytical methods and validation data were provided for identifying spirulina extract and distinguishing it from other blue food colours and for the determination of total microcystins.

At the Ninety-fifth JECFA meeting, the tentative specifications were revised by adding the new identity tests and total microcystin test and the tentative status was removed. The temporary status of the ADI "not specified" was also removed. This chemical and technical assessment summarizes the evaluation.

2. Description

Spirulina extract is a food colour obtained from the dried or fresh biomass of *Arthrospira platensis* (also commonly called *Spirulina platensis*), an edible cyanobacterium, by extraction with water followed by separation, filtration, and evaporation of the water to various degrees. Commercially available spirulina extract is produced as liquid or powder formulations which have a range of pigment concentrations based on the intended use of the products.

Spirulina extract contains two blue-coloured phycobiliproteins, C-phycocyanin and allophycocyanin, which are types of water-soluble fluorescent proteins found in several types of algae and other photosynthetic organisms that include cyanobacteria (sometimes referred to as blue-green algae), eukaryotic algae, red algae, and cryptomonads. The chromophore in the two phycocyanins is phycocyanobilin (Figure 1), which is covalently linked to the protein backbones and is brilliantly coloured and highly fluorescent (Glazer & Fang, 1973).

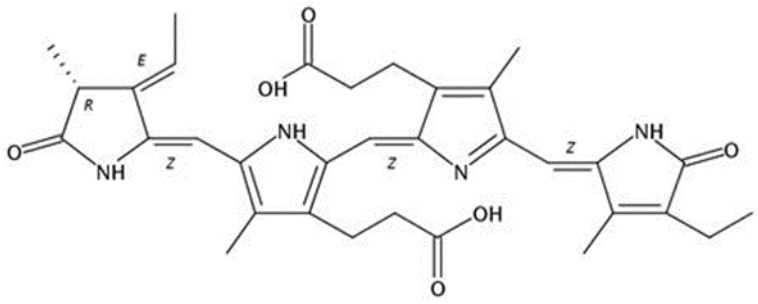

Figure 1. Phycocyanobilin

3. Manufacturing

Spirulina extract is produced from *A. platensis* cultivated in open or covered ponds or in bioreactors using growth media and conditions intended to control for the presence of contaminating microorganisms (including those known to produce toxins such as microcystins). Proper identification of *A. platensis* requires light microscope examination and biochemical analyses. The following physical characteristics may be useful for identity testing: filament length and shape (primarily coiled or helical, though straight forms may also occur), filament structure (composed of many cells with clear and visible transverse cross walls), width of cells making up the filament, and occurrence of gas vacuoles within the cells. An important biochemical marker for the identification of *A. platensis* is the content of γ-linolenic acid (GLA; 18:3) which is virtually absent in other species of cyanobacteria. Arthrospira strains have a significant proportion of γ-linolenic acid, less than 0.5% of total fatty acid as α-linolenic acid (ALA), a low content of 16:1 fatty acids, and a very low content of 16:2 fatty acids (Food Chemicals Codex, 2018).

To produce spirulina extract, the harvested biomass (dried or fresh) is first subjected to water extraction; food grade acidity regulators may be used to regulate the pH. Water extraction is followed by centrifugation and filtration to remove cell debris and most of the water-insoluble components, such as oils, oil-soluble components, and water-insoluble fibres. The resulting spirulina extract formulations contain proteins, carbohydrates, minerals, and two phycocyanin (phycobiliprotein) components that impart the blue colour (Jespersen et al, 2005). Other water-soluble components of the biomass, including carbohydrates, minerals and additional proteins not associated with phycobiliprotein, also remain dissolved in the filtrate.

Following extraction, the filtrate is concentrated to the desired pigment concentration. The manufacturing process also includes pasteurization and/or sterilization by thermal or non-thermal treatment. Additional physical processes may be used according to the final desired formulation, such as standardization and/or drying. The phycocyanin content in the final product, whether liquid or powder, may be subject to dilution with food grade carriers and diluents to lower pigment concentrations. Further enrichment of the pigment with a combination of separation and filtration steps may also be performed to obtain extracts of higher pigment concentrations. The intended forms of spirulina extract are either an aqueous liquid or a powder form; the powder form is derived from the liquid extract upon drying and may be mixed with approved food grade carriers for commercial formulations. In addition, a liquid form may in turn be derived from powder upon reconstitution at desired concentrations as needed.

4. Chemical Characterization

4.1 Composition of the food additive

The primary colouring principles in spirulina extract are C-phycocyanin (CAS No. 11016-15-2; EINECS No. 234-248-8; ~ 30 kDa), also known as phycocyanin C, and allophycocyanin (no CAS number assigned; ~ 105 kDa) in various ratios, with C-phycocyanin occurring in higher amounts. The coloring component phyocyanobilin (Figure 1) has CAS No. 20298-86-6, molecular weight 586.68 g/mol, and chemical formula $C_{33}H_{38}N_4O_6$. The chemical name of phycocyanobilin is (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).

Table 1 summarizes compositional data provided by the sponsor for the aqueous extract before formulation/standardization, commercial powder formulations, and commercial liquid formulations. The data were obtained from production samples and commercial articles analysed by company laboratories or competent third-party laboratories using recognized official methods.

All three materials contain proteins complexed to phycocyanobilin plus additional proteins, carbohydrates including sugars, ash (carbonates, sulfates, and phosphates from the medium typically used to grow the spirulina biomass (Markou et al, 2021), and water. Other components include fats and fibre. The aqueous extract before formulation and standardization can contain up to 90 g/100 g water as well as small amounts of the photosynthesis components carotenoids and chlorophylls (ref) which are largely removed during production of the commercial products by the water extraction and filtration processes. The powder and liquid products both contain carriers and other substances commonly added to foods (see Table 1).

The total content of C-phycocyanin and allophycocyanin in spirulina extract varies depending on the desired colour effect and degree of dilution of the extract. Phycobiliprotein concentrations in spirulina

extract range from 15-30 g/100 g in powder products to 1.7-13 g/100 g in liquid products, as the sum of C-phycocyanin and allophycocyanin. Colour value, which indicates phycocyanin content, ranges from 103-190 in powder products to 14-55 in liquid products (see Table 1).

In addition, the sponsor provided information on the spirulina biomass. The biomass consists of 64% protein, 18% dietary fibre (of which is 81% soluble fibre), 12.5% ash (2/3 $NaKHPO_4$ and 1/3 $CaSO_4$, $CaCO_3$, and $MgCO_3$), 2% organic acids such as tartaric acid, carotenoids, and chlorophyll.

Table 1. Spirulina extract components and potential impurities in (1) the aqueous extract before formulation and standardization, (2) powder formulations, and (3) liquid formulations.

Description	**Spirulina aqueous extract before formulation/ standardization**	**Spirulina extract powder formulations**	**Spirulina extract liquid formulations**
Components			
Phycobiliproteins (C-phycocyanin and allophycocyanin)	20.6-70.1 g/100 g	15-30 g/100 g	1.73-8.13 g/100 g
Colour value (E10%, 1 cm, 618-620 nm)	Not reported	103-190	14-55
Protein (total)	60.0-92.4 g/100 g	31.8-63.8 g/100 g	5.5-15.4 g/100 g
Total carbohydrates - Titrable acid calc as tartaric (pH 7.0) - Titrable acid calc as citric (pH 8.1) - Sugars - D-glucose - D-fructose - Sucrose - Maltose	0.1-13.3 g/100 g 0.07 g/100 g 0.21 g/100 g <0.10 g/100 g <0.10 g/100 g <0.10 g/100 g	17.8-63.8 g/100 g 0.14-3.33 g/100 g 0.44-6.40 g/100 g 1.3-16.44 g/100 g <0.1-16.89 g/100 g 0.14-18.9 g/100 g 1.19 g/100 g	53.2-63.0 g/100 g 0.03-0.11 g/100 g 0.10-0.64 g/100 g 0.29-19.7 g/100 g 0.77-19.4 g/100 g 21.4-57.3 g/100 g
Fat or lipids, fatty acids	<0.1-0.4 g/100 g	<0.3-<0.6 g/100 g	<0.05-<0.6 g/100 g
Fibre	<0.50-10.4 g/100 g	0.88-6.13 g/100 g	0.10-4.9 g/100 g
Ash (calcium, magnesium, potassium, and sodium carbonates, nitrates, phosphates, and sulfates)	1.7-15.2 g/100 g	1.78-9.7 g/100 g	0.4-2.1 g/100 g
Total carotenoids Total chlorophyll	Not reported	Not detected Not detected	Not reported
Water	0.4-89.2 g/100 g	0.94-7.09/100 g	29.0-43.3 g/100 g
Added carriers and other components	N/A	Trisodium citrate, sucrose or sucrose syrup, glucose syrup dried, maltodextrin, D-trehalose	Sucrose syrup, invert sugar, D-trehalose, sodium citrate, citric acid, mono- and digly-cerides of fatty acids, vegetable oil, trisodium citrate, sunflower oil, sugars
Potential impurities			
Heavy metals - Lead (Pb)	<0.015-0.06 mg/kg	0.029-0.165 mg/kg	<0.015-0.086 mg/kg

- Arsenic (As) - Mercury (Hg) - Cadmium (Cd)	<0.04-1.080 mg/kg <0.01-0.047 mg/kg <0.01-0.168 mg/kg	0.011-0.05 mg/kg <0.01-0.006 mg/kg 0.008-0.084 mg/kg	<0.04-<0.1 mg/kg <0.005-<0.05 mg/kg <0.005-0.081 mg/kg
Microcystins and nodularin	<25 µg/kg (<0.025 µg/g)	<25 µg/kg (<0.025 µg/g)	<25 µg/kg (<0.025 µg/g)
Microbiological impurities		Aerobic plate count 710 cfu/g Total plate count 290-6350 cfu/g Total viable count <100 cfu/g Bacillus not detected Coliform bacteria <10-<100 cfu/g E. coli not detected Listeria not detected Salmonella spp. negative Staph aureus negative Staph E not detected Yeasts <10 cfu/g Molds <10 cfu/ g Coagulase not detected	 Total plate count <1000 cfu/g Total viable count <100 cfu/g Bacillus aureus <10 cfu/g Coliform bacteria <10-<100 cfu/g E coli not detected in 1 g Listeria not detected in 25 g Salmonella spp. not detected in 25 g Staph aureus <10 cfu/g and not detected in 25 g Yeasts <100 cfu/g Molds <100 cfu/g Enterobacteriaceae <10 cfu/g CI Perfringens <10 cfu/g Total Clostridia <10 cfu/g
Aflatoxins		Not detected	
Melamine		Not detected	
β-Methylamino-L-alanine (BMAA)		Not detected	
Polynuclear aromatic hydrocarbons		Not detected	
Pesticides		Not detected	

4.2 Possible impurities (including degradation products)

The eighty-sixth JECFA established specifications for the following impurities.

- Arsenic ≤ 1 mg/kg
- Cadmium ≤ 1 mg/kg
- Lead ≤ 1 mg/kg
- Mercury ≤ 1 mg/kg
- Total (aerobic) plate count < 1000 CFU/g

- Yeast and moulds < 100 CFU/g
- Coliforms absent in 10 g
- *Salmonella spp*. absent in 25 g
- *Staphylococcus aureus* absent in 10 g
- Microcystins < 0.5 µg/g as microcystin-LR (dried basis)

All specification limits are met by commercial powder and liquid forms of spirulina extract according to the data in Table 1. In addition, the following impurities have not been detected in the powder form: aflatoxins, melamine, β-methylamino-L-alanine (BMAA), polynuclear aromatic hydrocarbons, pesticides, and other microbial contaminants (see Table 1).

4.3 Analytical methods

Analytical methods used to assess the physical and chemical properties of spirulina extract include spectrophotometric methods to determine the identity (colour value) and purity (as total phycocyanin content). Calculation of total phycocyanin content is based on the absorbance of spirulina extract samples at 620 and 650 nm and use of published extinction coefficients for phycocyanin C and allophycocyanin (Yoshikawa & Belay, 2008). Similarly, the identification (colour value) of the material is calculated using the absorbance of buffered solutions at 618 nm. This characteristic is reportedly used by food manufacturers to assess the purity of spirulina extract for use as a food colour without measurement of or conversion to phycocyanin (phycobiliprotein) content. Fluorometry and precipitation methods are used to distinguish spirulina extract from other blue food colours.

Analytical methods used to assess the purity of spirulina extract include a liquid chromatograph-mass spectrometry method for the determination of total microcystins as microcystin-LR. The method is based on methods reported in the scientific literature (Foss & Aubel, 2015). An enzyme linked immunoassay (ELISA) limit test for microcystin also may be used (Carmichael & An, 1999; Food Chemicals Codex, 2022). Other analytical methods for purity determinations are based on standard methods, published in the Combined Compendium of Food Additive Specifications FAO JECFA Monographs 1, Vol 4 (JECFA, 2006). Commercial products may be analyzed for heavy metals (lead, arsenic, mercury, and cadmium) using methods appropriate to the specified levels.

4.4 Rationale for proposed specifications

The proposed specifications for spirulina extract are intended to identify and establish the purity of the products of commerce based on the manufacturing method (aqueous extraction) and the use as a food colour. The identity tests include measurement of the colour value by spectrophotometric absorbance at 618 nm and fluorometry and precipitation tests to distinguish spirulina extract from other blue food colours. The purity is established based on the phycocyanin content, loss on drying (powder form), and limits for microbiological contaminants, heavy metal impurities, and microcystins.

5 Functional Use

5.1 Technological function

Spirulina extract is intended for use as a blue food colour.

5.2 Food categories and use levels

Spirulina extract is used for colouring foods such as flavoured dairy products, cheese, dairy desserts and ice cream, non-dairy ice cream (e.g. sherbet), processed fruits and vegetables, baked goods and baking mixes, alcoholic and non-alcoholic beverages and beverage bases, breakfast cereals, cocoa products, confectionary (including soft and hard candy, chewing gum), egg products, gravies and sauces, herbs and spices, condiments, and soups and soup mixes. Spirulina extract is also used as a colouring agent in food supplements (GSFA category 13.6) including nutritional supplements, vitamins and minerals. Use levels may be as low as 50 mg/kg. The intended food uses and use levels reported in the survey range between 400–40,000 mg/kg depending on the colour strength.

6 Reactions and Fate in Foods

No reactions of spirulina extract with food matrices are known to occur or are expected when used as a food colour.

7 References

Evaluation of certain food additives: eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations; 2019 (WHO technical report series; no. 1014).

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