Food and Agriculture Organization of the United Nations

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Specification Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 87th Meeting 2019

BLACK CARROT EXTRACT

This monograph was also published in: Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 87th meeting 2019. FAO JECFA Monograph 23 (2019)

BLACK CARROT EXTRACT

(TENTATIVE)

New tentative specifications for black carrot extract as the powder form were prepared at the 87th JECFA and published in JECFA Monograph 23 (2019). The 87th JECFA did not conclude on the safety of black carrot extract or establish an ADI.

Information required:
Data regarding a full characterization of the protein, carbohydrate, lipid, fibre, mineral, and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

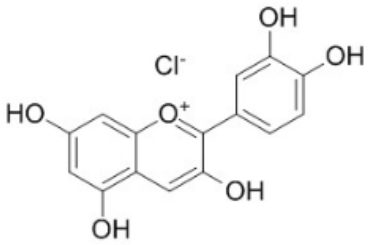
SYNONYMS INS No. 163(vi), purple carrot extract, black carrot colour, purple carrot colour, black carrot anthocyanins, purple carrot anthocyanins

DEFINITION Black carrot extract is a food colour obtained from black, purple, or red carrot (Daucus carota L. ssp. Sativus). The principal colouring components are five anthocyanins formed from the aglycone cyanidin substituted at the central hydroxyl position with a sugar moiety consisting of galactose, glucose, and/or xylose. Three of the five anthocyanins are acylated with p-coumaric, ferulic, or sinapinic acids. Anthocyanins formed from other aglycones (malvidin, pelargonidin, and peonidin) are present in minor amounts along with other polyphenols. Other components include proteins, carbohydrates, lipids, fibre, minerals, and water. Black carrot extract is produced by aqueous acidic extraction of crushed, ground, or milled carrot roots followed by fermentation to reduce sugars. Methanol or ethanol may be produced during the fermentation step. The pigments may be concentrated by ultrafiltration, reverse osmosis, or adsorption onto a polymeric resin followed by desorption with ethanol, isopropyl alcohol, and/or water. The concentrate is spray-dried with a carrier such as maltodextrin, dextrin, or gum to produce the powdered product.

Chemical names, formulas, and C.A.S. numbers

Cyanidin 3-p-coumaroylxylosylglucosylgalactoside, $C_{41}H_{45}O_{22}^+$
C.A.S. 142506-21-6
Cyanidin 3-feruloylxylosylglucosylgalactoside, $C_{42}H_{47}O_{23}^+$
C.A.S. 142561-99-7
Cyanidin 3-xylosylgalactoside, $C_{26}H_{29}O_{15}^+$
C.A.S. 142506-19-2
Cyanidin 3-xylosylglucosylgalactoside, $C_{32}H_{39}O_{20}^+$
C.A.S. 142561-98-6
Cyanidin 3-sinapoylxylosylglucosylgalactoside, $C_{43}H_{49}O_{24}^+$
C.A.S. 142630-71-5

Structural formula



Cyanidin chloride

Assay	Anthocyanin content not less than 3%.
DESCRIPTION	Red or purplish-red powder with characteristic odour.
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Soluble in water, ethanol, and isopropyl alcohol.
Spectrophotometry (Vol. 4)	Maximum wavelength approximately 518 nm Determine the UV-visible absorption spectrum of the sample dissolved in water, pH 3.
Colour reaction	Add 0.1 g of sample to 50 ml of acidified water (pH 3 – 3.5) and shake to mix. Filter if necessary. The red or purplish-red solution will turn blue or dark green upon addition of sodium hydroxide TS.
PURITY	
Residual solvents (Vol. 4)	Methanol, not more than 50 mg/kg Ethanol, not more than 50 mg/kg Isopropyl alcohol, not more than 50 mg/kg
Arsenic (Vol. 4)	Not more than 3 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 method described in Volume 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 method described in Volume 4 (under “General Methods, Metallic Impurities”).
Lead (Vol. 4)	Not more than 2 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 method described in Volume 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 method described in Volume 4 (under "General Methods, Metallic Impurities").

Colouring matters

The major colouring principals shall correspond to the five anthocyanins derived from cyanidin as defined above. Determine by liquid chromatography.
See description under TESTS

TESTS

PURITY TESTS

Colouring matters

Determine colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 Hypersil ODS, 250 mm x 3 mm i.d., 5 µm particle size or equivalent
- Eluent A: Formic acid/water (10:90)
- Eluent B: Formic acid/water/acetonitrile (10:40:50)
- Injection volume: 20 µl
- Column temperature: 40°
- Detector: UV-visible/diode array at 518 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	88	12
1.0	88	12
26.0	70	30
35.0	0	100
38.0	0	100
43.0	88	12
46.0	88	12

Reagents: HPLC grade

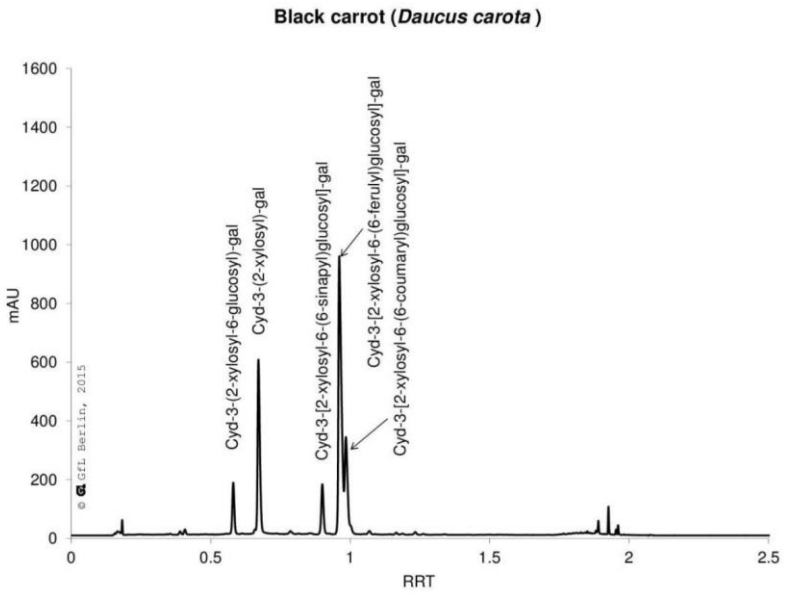
Internal standard:

- Pelargonidin-3-glycoside chloride (C.A.S. 18466-51-8) – BOC Sciences, Cat. No. 18466-51-8 or equivalent

Preparation of internal standard solution:
Weigh accurately about 2 mg of standard and dissolve in 2 ml of 1.2 M hydrochloric acid. Store at -20° for up to two months. Thaw shortly before analysis.

Sample preparation:
Weigh accurately 1.0±0.02 g of sample and dissolve in 5 ml of water. Centrifuge if necessary and filter through a 0.45 µm filter. Dilute the solution if necessary to avoid saturating the HPLC detector. Transfer 500 µl to an HPLC vial and mix with 10 µl of internal standard solution.

Procedure:
Inject the sample solution. Obtain the retention times of the colouring matters components relative to the pelargonidin-3-glycoside chloride internal standard. Identify the individual components by comparing the retention times to the reference chromatogram.



Reference chromatogram for colouring matters in Black carrot extract.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and water acidified to pH 3.0 with citric acid as the solvent.

Absorptivity (a) = 30.0 l/(g·cm) and wavelength of maximum absorbance = 518 nm.