Food and Agriculture Organization of the United Nations

87th JECFA - Chemical and Technical Assessment (CTA), 2019
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BLACK CARROT EXTRACT

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1. Summary

This Chemical and Technical Assessment summarizes data and information on black carrot extract (INS No. 163(vi)) submitted to the 87th meeting of the Joint FAO/WHO Expert Committee on Food Additives) (JECFA, 2019) upon request by the 50th session of the Codex Committee on Food Additives (CCFA, 2018). New tentative specifications for black carrot extract as the powder form were prepared at the 87th meeting. The 87th meeting did not conclude on the safety of black carrot extract or establish an ADI.

2. Description

Black carrot extract is a food colour obtained from black, purple, or red carrot (*Daucus carota* L. ssp. *sativus*). The colour of the powder form of black carrot extract is red or purplish-red. The liquid is red at pH 1-3 and becomes purplish-red as the pH increases.

Other names for black carrot extract include purple carrot extract, black carrot colour, purple carrot colour, black carrot anthocyanins, and purple carrot anthocyanins.

3. Methods of manufacture

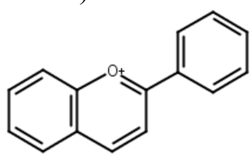
Black carrot extract is produced by aqueous acidic extraction of crushed, ground, or milled carrot roots followed by fermentation to decrease sugars. Methanol or ethanol may be produced during the fermentation step. The anthocyanins are concentrated by ultrafiltration, reverse osmosis, and/or adsorption onto a polymeric resin followed by desorption with ethanol, isopropyl alcohol, and/or water. The finished product may be a liquid or powder. The concentrate is spray-dried with a carrier such as maltodextrin, dextrin, or gum to produce the powdered product.

4. Chemical characterization

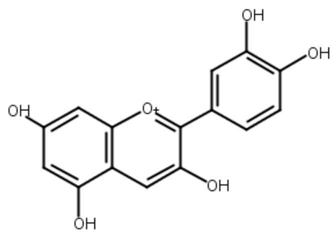
4.1 Composition

The main colouring components of black carrot extract 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. The anthocyanin content in the powder form is specified to be not less than 3%.

All anthocyanins are structurally based on the 2-phenyl-1-benzopyrylium chromophore (flavylium cation, C.A.S. 14051-53-7):

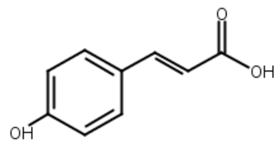


The five main anthocyanins in black carrot extract all contain cyanidin (C.A.S. 13306-05-3) as the chromophore:

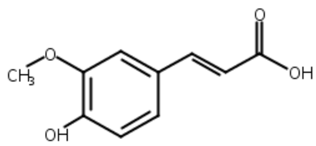


Other components of the five main anthocyanins in black carrot extract are the following:

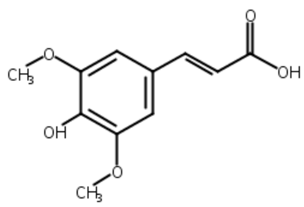
- *p*-Coumaric acid (*p*-hydroxycinnamic acid, C.A.S. 7400-08-0)



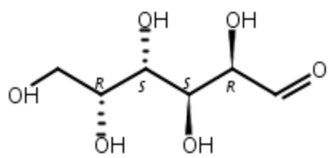
- Ferulic acid (C.A.S. 1135-24-6)



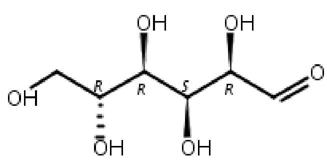
- Sinapinic acid (C.A.S.530-59-6)



- Galactose (C.A.S. 59-23-4)



- Glucose (C.A.S. 50-99-7)



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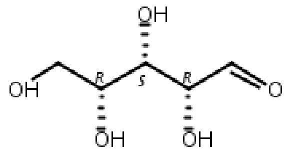
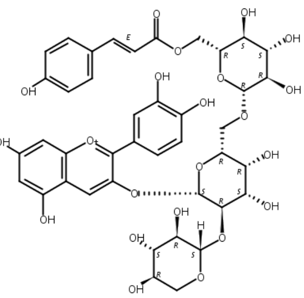
- Xylose (C.A.S. 58-86-6)

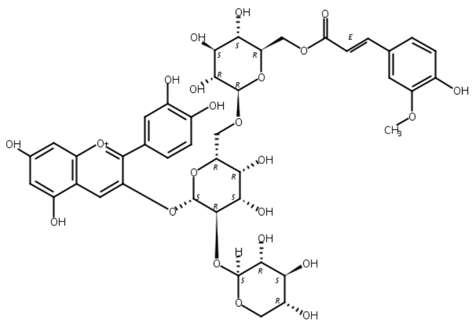


The five main anthocyanins in black carrot extract have the following chemical structures.

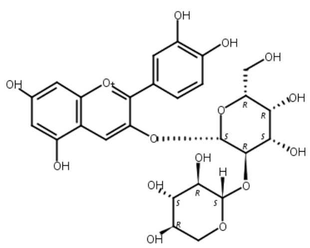
- Cyanidin 3-*p*-coumaroylxylosylglucosylgalactoside, $C_{41}H_{45}O_{22}^{+}$
  - C.A.S. 142506-21-6
  - IUPAC name: (2R,3S,4S,5R,6R)-6-[[(2R,3R,4S,5R,6S)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4-dihydroxy-5-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]methoxy]-3,4,5-trihydroxyoxan-2-yl]methyl (E)-3-(4-hydroxyphenyl)prop-2-enoate



- Cyanidin 3-feruloylxylosylglucosylgalactoside, $C_{42}H_{47}O_{23}^{+}$
  - C.A.S. 142561-99-7
  - IUPAC name: [(2R,3S,4S,5R,6R)-6-[[(2R,3R,4S,5R,6S)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4-dihydroxy-5-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]methoxy]-3,4,5-trihydroxyoxan-2-yl]methyl (E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate
  -



- Cyanidin 3-xylosylgalactoside (cyanidin 3-lathyroside), $C_{26}H_{29}O_{15}^{+}$
  - C.A.S. 142506-19-2
  - IUPAC name: (2S,3R,5R)-2-[(2S,5R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl]oxyoxane-3,4,5-triol



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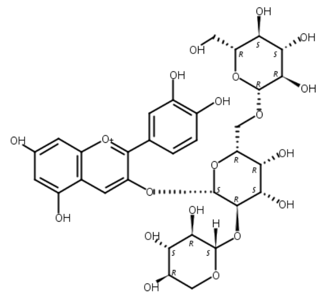
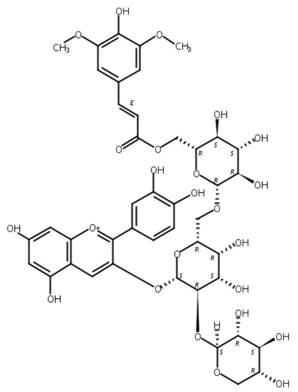
- Cyanidin 3-xylosylglucosylgalactoside, $C_{32}H_{39}O_{20}^{+}$
  - C.A.S. 142561-98-6
  - IUPAC name: Not available



- Cyanidin 3-sinapoylxylosylglucosylgalactoside, $C_{43}H_{49}O_{24}^{+}$
  - C.A.S. 142630-71-5
  - IUPAC name: [(2R,3S,4S,5R,6R)-6-[[(2R,3R,4S,5R,6S)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4-dihydroxy-5-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]methoxy]-3,4,5-trihydroxyoxan-2-yl]methyl (E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoate



4.2 Possible impurities (including degradation products

Possible impurities in black carrot extract include methanol (not more than 50 mg/kg), ethanol (not more than 50 mg/kg), isopropyl alcohol (not more than 50 mg/kg), arsenic (not more than 3 mg/kg), cadmium (not more than 1 mg/kg), lead (not more than 2 mg/kg), and mercury (not more than 1 mg/kg).

4.3 Analytical methods

The specifications monograph cites test methods included in Volume 4 of the FAO Combined Compendium of Specifications (JECFA, 2006). Colouring matters are identified by a reversed-phase high-performance liquid chromatography method using pelargonidin-3-glycoside chloride (C.A.S. 18466-51-8) as the internal standard. Total colouring matters content is determined by visible spectrophotometry using water acidified to pH 3.0 with citric acid as the solvent, following procedure 1 in Volume 4 (under "Specific Methods, Food Colours").

5. Functional uses

Black carrot extract is intended for use in colouring dairy-based desserts, processed fruit products, processed vegetable products, confectionery, chewing gum, cereals, pastas and noodles, cereal/starch-

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based desserts, processed rice and soy products, cakes, cookies, pies, preserved egg products, condiments (vinegar, mustard), sauces and gravies, dietetic foods and dietary supplements, non-alcoholic beverages, and alcoholic beverages.

6. Reactions and fate in foods

Anthocyanins are characterised by their high reactivity with other substituents in foods and susceptibility toward hydration and oxidation which result in the formation of brown or colourless degradation products (EFSA, 2013; Jackman et al., 1987; Kammerer, 2016; Scotter and Castle, 2004; Solymosi et al., 2015). Factors influencing the intensity and stability of anthocyanins include pH, chemical structure, concentration, copigmentation and self-association, enzymatic degradation, metal complexing, temperature, light, oxygen, acetaldehyde, amino acids, ascorbic acid, sugars and their degradation products, and sulfur dioxide (EFSA, 2013; Giusti and Wrolstad, 2003; Kammerer, 2016; Kim et al., 2012).

In general, anthocyanins are most stable at pH 1-3 (Jackman et al., 1987; Scotter and Castle, 2004). As the pH increases, anthocyanins become susceptible to nucleophilic attack on the C2 site of the flavylium cation by water (Kammerer, 2016). (The flavylium molecule is historically represented as a cation because anthocyanins were first isolated as chlorides from strongly acidic solutions (Bueno et al., 2012)). Intramolecular copigmentation in the form of acylation occurs in some anthocyanins. Acylation results in higher hydration constants due to stacking of the acyl moiety with the anthocyanin backbone, which sterically hinders nucleophilic attack by water and increases stability at higher pH (Kammerer, 2016). Similar but less effective interactions occur by intermolecular copigmentation with other compounds such as food substances and self-association of the anthocyanin molecules themselves (Hubbermann et al., 2006; Kammerer, 2016). For example, the presence of saccharides may increase or decrease anthocyanin stability depending on their concentrations (Kammerer, 2016). These copigmentation phenomena are unique to anthocyanins (Brouillard et al., 2010; Kammerer, 2016).

Ascorbic acid in the presence of oxygen and amino acids, iron or copper iron ions, phenols, or sugar derivatives may interact with and decolour anthocyanins (Kammerer et al., 2016; Kırka et al., 2006; Scotter and Castle, 2004). Under aerobic conditions, anthocyanins may react with ascorbic acid via condensation-type mechanisms (Jackman et al., 1987). Anthocyanins also form adducts with sulfites, which are often used as antimicrobial preservatives, due to reversible nucleophilic addition to the anthocyanidin backbone (Kammerer, 2016). This interaction discolours the pigment and simultaneously confers high heat stability on the glycosidic bond (Kammerer, 2016; Scotter and Castle, 2004).

Black carrot extract is produced by the aqueous acidic extraction of crushed, ground, or milled roots of black, purple, or red carrot followed by fermentation to decrease sugars (Algarra et al., 2014; Assous et al., 2014; Kamiloglu et al., 2015; Kammerer et al., 2004; Kırka et al., 2006; Montilla et al., 2011; Smeriglio et al., 2018; Turker et al. 2004). Acetic, citric, formic, hydrochloric, or trifluoroacetic acids are used for acidification (Algarra et al., 2014; Assous et al., 2014; Ersus and Yurdagel, 2007; Esatbeyoglu et al., 2016; Gras et al., 2015; Montilla et al., 2011). Ultrasound processing has also been used for extraction (Agcam et al., 2017; Gras et al., 2015). The resulting anthocyanins may be concentrated by ultrafiltration, reverse osmosis, or adsorption onto a polymeric resin followed by desorption with ethanol, isopropyl alcohol, and/or water (Esatbeyoglu et al., 2016; Gras et al., 2016; Montilla et al., 2011).

Recently the anthocyanins in black carrot extract ihave been analysed by solid-phase extraction, UV-visible spectrophotometry, liquid chromatography with UV-visible/photodiode array detection, and liquid chromatography-mass spectrometry (Algarra et al., 2014; Assous et al., 2014; Giusti and Wrolstad, 2001; Gras et al., 2015; Grassmann et al., 2007; Kammerer et al., 2004; Lee et al., 2005; Montilla et al., 2011; Smeriglio et al., 2018; Zadernowski et al., 2010). Five main black carrot anthocyanins have been identified (see Section 4) (Agcam et al., 2017; Algarra et al., 2014; Assous et al., 2014; Giusti and Wrolstad, 2003; Gras et al., 2015; Kamiloglu et al., 2015; Kammerer et al., 2004; Montilla et al., 2011; Smeriglio et al., 2018; and Zadernowski et al., 2010).

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Three of the five anthocyanins in black carrot extract are stabilized by intramolecular co-pigmentation through acylation with *p*-coumaric, ferulic, or sinapinic acids (Giusti and Wroland, 2003; Gras et al., 2016; Kammerer, 2016; Malien-Aubert et al., 2001; Montilla et al., 2011; Smeriglio et al., 2018). Increased stability may be accomplished by spray-drying with a carrier such as maltodextrin, dextrin, or gum (Assous et al., 2014; Ersus and Yurdagel, 2007; Kammerer, 2016; Montilla et al., 2011).

The anthocyanin pigments in black carrot extract begin degrading above pH 3 and change in colour from red to blue with increasing pH (Assous et al., 2014; Kammerer et al., 2004). The pigments are stable in peach and apricot nectars heated to 80 ºC but are less stable in orange juice during both heating and storage (Kırka et al., 2006). Exposure to 90 ºC heat results in rapid degradation of the anthocyanins (Gras et al., 2016; Kırka et al., 2006). Refrigerated storage at 4 ºC increases their stability in fruit juices and nectars (Kırka et al., 2006). However, freezing and thawing black carrots reduces anthocyanin stability compared to fresh carrots (Zadernowski et al., 2010). Light also may degrade black carrot anthocyanins during storage of processed foods (Kammerer, 2016).

Some food colours from natural sources may have undesirable odour or flavour characteristics (Giusti and Wrolstad, 2003). Black carrot extract was evaluated for its sensory properties as a food colour in hard candy and jelly and was found to be acceptable at levels up to 0.5% (Assous et al., 2014).

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7. References

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