Food and Agriculture Organization of the United Nations

89th JECFA - Chemical and Technical Assessment (CTA), 2020

JAGUA (GENIPIN-GLYCINE) BLUE

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1. Summary

This Chemical and Technological Assessment (CTA) reports data and information on Jagua (Genipin-Glycine) Blue submitted to the 84th and 89th meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) upon request by the 48th and 51st sessions of Codex Committee on Food Additives (CCFA) (Rep. 16/FA – Appendix XIV and Rep. 19/FA - Appendix X).

At the 84th meeting the Committee was asked to evaluate all data necessary for the assessment of safety, dietary intake and specifications related to the use of Jagua (Genipin-Glycine) Blue as a food colour in a variety of food categories. At the 89th meeting, the Committee reviewed additional data on characterization of the low molecular weight components and a validated method for their analysis. This document discusses published information regarding identity, manufacturing, specifications, and uses relevant to *Genipa americana*, Jagua, and Jagua (Genipin-Glycine) Blue and incorporates information received after the 84th meeting.

2. Description

The *Genipa americana,* L. is a small to medium-sized tree ranging from 8 to 20 m in height carrying horizontally growing branches with glossy dark green leaves at the end (UNCTAD, Nations Conference on Trade and Development, 2005). It belongs to the Rubiaceae family and is native to central and tropical South America (Djerassi et al., 1960; Ueda et al., 1990). The genipa plant yields round to ovoid-shaped medium-sized to large ovoid edible berries, 9–15 cm in length and 7–9 cm in width (Ojeda, 1966; Silva et al., 1998; UNCTAD, 2005). The central cavity of the berry fruit contains flat dark brown seeds 10–12 mm in length. This fruit is referred to as jagua fruit, chipara, guayatil, maluco, caruto or huito (Ramos-de-la-Peña et al., 2015) in Spanish and as genipap in English.

When the pulp of the unripe jagua fruit is exposed to air, it turns dark blue in colour. The blue colour has been widely used by various ethnic groups of South America to colour their skin, and as a natural dye for colouring clothes and ceramics (Ojeda, 1966; UNCTAD, 2005). The jagua fruit has also been explored for its potential anti-inflammatory properties (Wang et al., 2012). It is used in beverage production, as a substitute for pectin (Fernandez & Rodrigues, 2012), and in traditional medicine. The aromatic flesh of the russet-brown ripe jagua fruits is consumed fresh; in Venezuela it is known as "caruto" or used to prepare syrup as an ingredient of beverages, liqueurs, and spirits (Ojeda, 1966).

The unripe jagua fruits contain high level of a cyclopentan-[C]-pyran skeleton class of compounds, called iridoids (Dinda et al., 2007 a, b). A total of 14 iridoids have been identified and quantified in the

unripe jagua fruit (Bentes & Mercadante, 2014), and genipin in its aglycone form is one of them (CAS No. 6902-77-8; IUPAC: methyl (1R,4aS,7aS)-1-hydroxy-7-(hydroxymethyl)-1,4a,5,7a-tetrahydrocyclopenta[c]-pyran-4-carboxylate). Methods for extraction and purification of both geniposide and genipin from genipap have been reviewed (Ramos-de-la- Peña et al., 2014, 2016). Iridoids have been associated with a wide range of biological activities, such as anti-inflammation (Villasenor, 2007**)**

Genipin is unique, among iridoids in its ability to react with primary amines present in amino acids and proteins, in the presence of oxygen, to produce water-soluble blue pigments (Touyama et al., 1994a; Lee et al., 2009; Cho et al., 2006; Fujikawa et al., 1987; Paik et al., 2001; Park et al., 2002). Genipin has been also shown to be a good cross-linker for proteins, such as collagen, gelatine and chitosan moieties (Yang et al., 2011). The blue pigments are proposed as the product of oxygen radical-induced polymerization and dehydrogenation of intermediary pigments that result in the formation of blue high molecular weight, water-soluble dimers (Touyama et al., 1994b). Touyama et al. (1994a, b) showed that the cross-linking activity is the result of a complex multi- route substitution reactions including, both the substitution of an ester group of genipin with a secondary amide, and the formation of intermediates derived from genipin, by elimination of molecules of water and the replacement of an oxygen atom with –N-methyl groups. These reddish pigments have the characteristics of pseudo-azulenic-methyl/carboxymethyl-pyridine structure or dimers. In the presence of oxygen these pigments rapidly turn to blue polymers.

3. Method of manufacture

Jagua (Genipin-Glycine) Blue is produced by pressing the juice from the ground pulp of the peeled, unripe fruit of *Genipa americana*, followed by extraction with water and filtration of the juice. The genipin content is determined and a stoichiometric amount of glycine is being added. The mixture is heated at 70°C for two hours, until the blue colour is completely formed. The liquid is further centrifuged, concentrated and/or dried. Residue of unreacted genipin is considered an impurity of Jagua (Genipin-Glycine) Blue. A powdered form commercial product is obtained after concentrating the Jagua (Genipin-Glycine) Blue to 20° Brix, mixed with a food-grade carrier, spray dried, ground and sieved.

4. Chemical characterisation

4.1 Chemical formulae and characteristics

Blue Polymer (Figure 1)
CAS number: 1314879-21-4; Formula: $C_{27}H_{25}O_8N_2$;10-12; Melting Point: > 300°C; Colour: Deep Blue; soluble in water; insoluble in hexane, chloroform, ethyl ether, ethyl acetate, acetone, ethanol and methanol.

Dimer 1 (Figure 2)
CAS number: 1313734-13-2; Formula: $C_{28}H_{28}N_2O_8$; Molecular Weight: 522; Melting Point: > 300°C; Colour: Deep Blue; soluble in methanol and water; sparingly soluble in ethanol; insoluble in hexane, chloroform, ethyl ether, ethyl acetate, acetone.

Dimer 2 (Figure 3)
CAS number: 104359-67-3; Formula: $C_{27}H_{25}N_2O_8$; Molecular Weight: 505; Melting Point: > 300°C; Colour: Deep Blue; strongly soluble in water; sparingly in ethanol and methanol; insoluble in hexane, chloroform, ethyl ether, ethyl acetate, acetone.

Dimer 3 (Figure 4)
CAS number: 1313734-14-3; Formula: $C_{27}H_{24}N_2O_8$; Molecular Weight: 505; Melting Point: > 300°C; Colour: Deep Blue; strongly soluble in water; insoluble in hexane, chloroform, ethyl ether, ethyl acetate, acetone, ethanol and methanol.

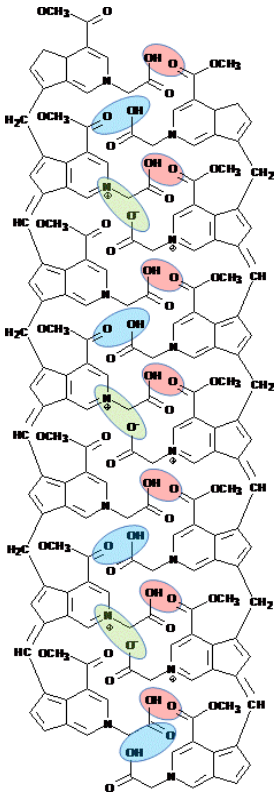

Figure 1. Blue polymer

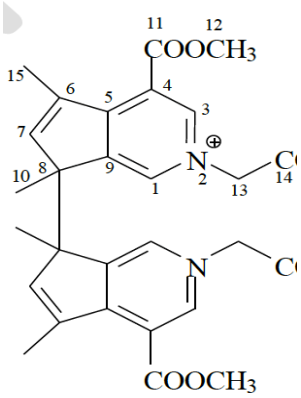

Figure 2. Dimer 1

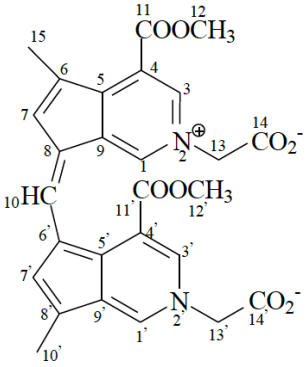

Figure 3. Dimer 2

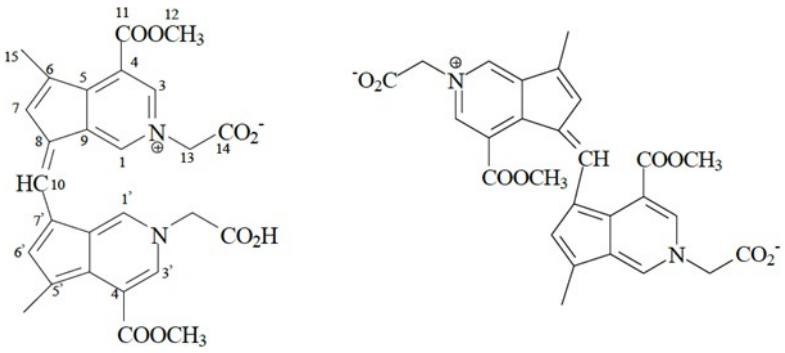

Figure 4. Dimer 3

4.2 Composition

The powdered commercial Jagua (Genipin-Glycine) Blue product consists of 30-40% blue polymer, minor amounts of the three dimers, along with high quantities of sugars, carbohydrates (25-30%), modified starch (20-22%), protein (ca. 7%), ash, water (ca. 5%), and fat based on bromatological analyses from five independent batches.

Identity specifications proposed by the sponsor include solubility, UV-vis (maximum wavelength in water approximately between 590-594 nm) and IR (assignable bands at: 3393, 2949, 1726, 1630 and 1540 cm-1, respectively) and HPLC-UV (identity of Rt of the blue polymer). Purity specifications include: loss on drying (< 5%), water insoluble matter (< 0.2%), ether-extractable matter (< 0.2%), unreacted genipin (< 0.3%), limits for lead (2 mg/kg), arsenic (1mg/kg), a limit for microbiological contaminants including aerobic plate count (< 1000 CFU/g), total coliform (< 10 cfu/g), E. Coli (absent in 25 g of sample), Coagulase positive S. aureus (absent in 1 g of sample), and yeasts and moulds (< 10 CFU/g).

4.3 Possible impurities (including unreacted substances)

Impurities of Jagua (Genipin-Glycine) Blue are unreacted genipin, heavy metals and microbiological contaminants. The sponsor provided results from ten independent commercial lots of Jagua (Genipin-Glycine) Blue, to support the proposed specifications for the possible impurities.

According to the data provided by the sponsor, Jagua (Genipin-Glycine) Blue is stable, and no decomposition products are expected under normal storage conditions.

5. Analytical methods

The proposed method, both for qualitative and quantitative determination of the blue polymer in Jagua (Genipin-Glycine) Blue, is based on a reversed phase chromatographic method with UV-vis detection at 590 nm (HPLC-UV-vis), with high selectivity for complex natural products, isomers and closely related compounds (http://phx.phenomenex.com/lib/5732_I.pdf). The sponsor also provided details of the methods used to obtain a pure in-house reference standard of the blue polymer for the quantitation of Jagua (Genipin-Glycine) Blue. The sponsor provided details of the HPLC-UV- vis method to calculate residual unreacted genipin in Jagua (Genipin-Glycine) Blue.

The procedure described in Volume 4 of Combined Compendium of Food Additive Specifications (Food Colours, Procedure 1) for the measurement of % Total Colouring Matters by Spectrophotometry using water as solvent was included.

The analytical methods for metallic impurities are described in Volume 4 of Combined Compendium

of Food Additive Specifications (General Methods, Metallic Impurities).

6. Rationale for proposed specifications

The identity assays for commercial Jagua (Genipin-Glycine) Blue include: solubility in water, maximum absorbance at 590 nm, IR spectrum and the HPLC retention time of the peak corresponding to the blue polymer in Jagua (Genipin-Glycine) Blue.

The purity of the commercial product is established based on loss on drying, water insoluble matter, ether-extractable matter, limits of genipin and heavy metals impurities, and on the % Total Colouring Matter and % blue polymer.

7. Functional uses

7.1 Technological function

Jagua (Genipin-Glycine) Blue is intended for use as food colour. It impart a blue colour, or tints of blue when mixed with other food safe colours.

7.2 Food categories and use levels

Jagua (Genipin-Glycine) Blue is proposed for addition to the food categories (Table 1) as listed in CODEX STAN 192-1995, Annex B.

Table 1- Food categories and maximum proposed levels for use of Jagua (Genipin-Glycine) Blue

Food Category		**Maximum Level (%)**	**Suggested Use (%)**
Category Number	**Codex standard title**		
01.0	**Dairy Product and Analogous**		
01.1.2	Dairy-based drinks, flavoured and/or fermented (Chocolate milk, cocoa, eggnog, drinking yogurt,whey-based drinks, etc.)	GMP	0.003 – 0.03
01.7	Dairy-based desserts (fruit and/or flavoured yogurt, pudding, etc.)	GMP	0.003 – 0.03
03.0	**Edible ices, including sherbet and sorbet**	GMP	0.003 – 0.06
05.0	**Confectionery**		
05.1.4	Cocoa and Chocolate products	GMP	0.003 – 0.03
05.2.1	Hard Candy	GMP	0.003 – 0.03
05.2.2	Soft Candy	GMP	0.003 – 0.03
05.2.3	Nougats and Marzipans	GMP	0.003 – 0.03

05.3	Chewing gum	GMP	0.003 – 0.03
05.4	Decorations (for bakery wares), toppings (non-fruit), sweet sauces	GMP	0.006 – 0.06
14.0	**Beverages, excluding dairy products**		
14.1.1.2	Table water and soda waters	GMP	0.001 – 0.01
14.1.2	Fruit and vegetable juices	GMP	0.001 – 0.01
14.1.3	Fruit and vegetable nectars	GMP	0.001 – 0.01
14.1.4	Water-based flavoured drinks, including: sport, energy, electrolyte andparticulate drinks.	GMP	0.001 – 0.01

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