Food and Agriculture Organization of the United Nations

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Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 84th meeting 2017

Jagua (Genipin-Glycine) Blue

(Tentative)

This monograph was also published in: *Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 84th meeting 2017. FAO JECFA Monographs 20*

JAGUA (GENIPIN-GLYCINE) BLUE (TENTATIVE)

New specifications prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017). No ADI was established at the 84th JECFA (2017)

Information Required on:

- *Characterization of the low molecular weight components of the blue polymer*
- *A validated method for the determination of dimers*
- *Data for levels of dimers from five batches of the commercial product*

SYNONYMS Jagua blue

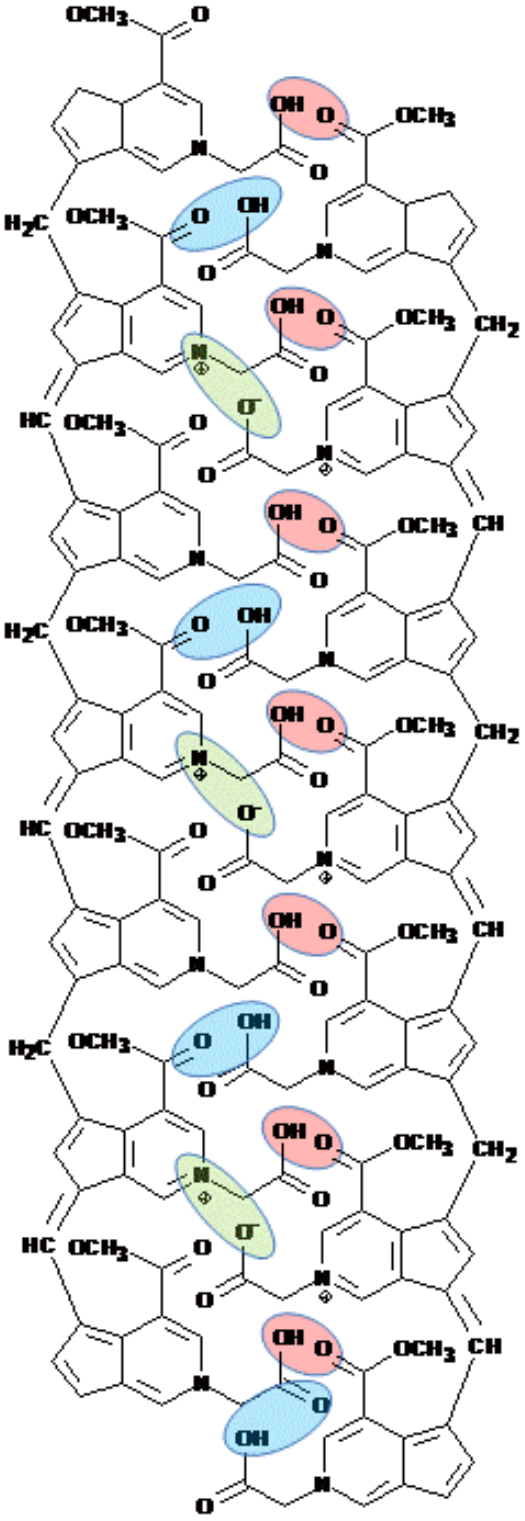
DEFINITION Jagua (Genipin-Glycine) Blue consists mainly of a blue polymer and blue dimers as minor subsidiary colours; it is produced by the reaction between genipin (methyl (1R,4aS,7aS)-1-hydroxy-7-(hydroxymethyl)-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-4-carboxylate) and glycine resulting in the combination of alternating dimeric moieties linked by a methylene bridge.

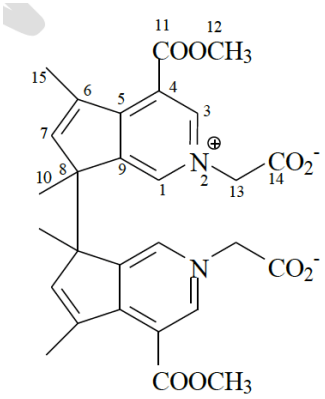
Jagua (Genipin-Glycine) Blue is produced by a two-step process. In the first step the unripe fruit of *Genipa americana* is peeled, ground to pulp, pressed for the juice, and extracted with water. The extracted juice, is filtered, and checked for genipin content. In the next step the jagua extract is treated with a stoichiometric amount of glycine and heated at 70° for two hours. The resulting liquid containing the Jagua (Genipin-Glycine) Blue is centrifuged and concentrated. A liquid product is obtained by concentrating the Jagua (Genipin-Glycine) Blue up to 20-50° Brix and formulating with glycerine or other food grade additives. Alternatively, a powder product is obtained after concentrating the Jagua (Genipin-Glycine) Blue 20° Brix and mixing with a food-grade carrier such as maltodextrin or modified food starches, spray drying, or using other drying technologies and sieving.

C.A.S. number 1314879-21-4
(Blue Polymer)

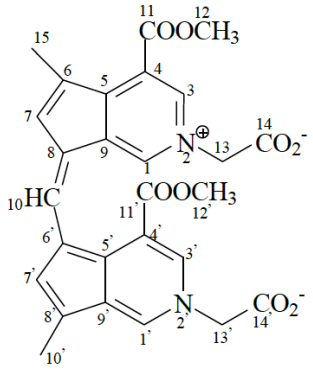
Chemical formula $(C_{27}H_{25}O_8N_2)_n$
(n is typically 10-12)

Structural formula



Molecular weight	Approximately 6000 Da (number average molecular weight, approximately a lognormal distribution between 4500 and 9500 Da).
Assay	Not less than 20% and not more than 40% as the polymer by HPLC Not less than 95% of % Total Colouring Matters See Methods of Assay
C.A.S. Number	1313734-13-2 (Dimer 1)
Chemical formula	$C_{28}H_{28}N_2O_8$ (Dimer1)
Structural formula	 Dimer 1
Molecular weight	522 (Dimer1)
C.A.S. Number	104359-67-3 (Dimer 2)
Chemical formula	$C_{27}H_{25}N_2O_8$ (Dimer 2)

Structural formula



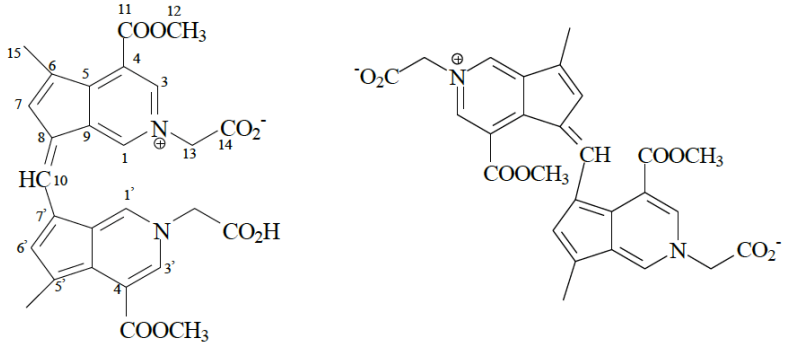
Dimer 2

Molecular weight 505 (Dimer 2)

C.A.S. Number 1313734-14-3 (Dimer 3)

Chemical Formula $C_{27}H_{24}N_2O_8$ (Dimer 3)

Structural Formula



Dimer 3

Molecular weight 505 (Dimer 3)

Assay *Information required for Dimer 1, Dimer 2 and Dimer 3*

DESCRIPTION Blue to black, liquid or in powder; odourless

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water. Practically insoluble in hexane and ethanol.
Spectrophotometry (Vol.4)	The UV-Visible absorption spectrum of a sample dissolved in water shows absorption maximum between 590-594 nm.
HPLC	The retention time of the blue polymer is approximately at 10 min ($\lambda_{detector}$=590 nm); it is the main peak in the chromatogram of Jagua (Genipin-Glycine) Blue. See Method of Assay.
Infrared Spectrum	Infrared spectrum of the sample obtained by using potassium bromide disk corresponds to the reference spectrum

PURITY

Loss on drying (Vol 4)	Not more than 6% (at 105°, to constant weight).
Water insoluble matter (Vol. 4)	Not more than 0.2%
Ether-extractable Matter (Vol. 4)	Not more than 0.2%
Genipin	Not more than 0.3% See description under TESTS
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 method described in Volume 4
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
Microbiological Criteria (Vol 4)	Aerobic Plate Count: Not more than 1000 CFU/g Total Coliforms : Not more than 10 CFU/g *E. coli*: Absent in 25 g of sample Coagulase positive *S. aureus*: Absent in 1 g of sample Yeasts and moulds: Not more than 10 CFU/g

TESTS

PURITY TESTS

Genipin

HPLC Analysis of Genipin in Jagua (Genipin-Glycine) Blue

- Apparatus: HPLC-UV-Vis:
- Column: Phenomenex Luna PFP (PentaFluoroPhenyl, 100Å, 150 x 4.6 mm, 5µm), with guard column, or equivalent.
- Flow Rate: 1 mL/min
- Injection volume: 10 µL
- Eluents: A) 0.1% acetic acid in water; B) Methanol, HPLC grade
- Detector: UV at 240 nm for peak identification and quantitation
- Temperatures:
  - T_{Column}: 30°
  - $T_{Detector}$: 30°
- Retention time: approximately 22 minutes
- Standard: Genipin (>98%, HPLC grade)

Gradient:

Time (min)	% Solvent A	% Solvent B
0	95	5
15	65	35
22	65	35
27	0	100
29	0	100
31	95	5
41	95	5

Sample Preparation:
Dissolve 10 mg of Jagua (Genipin-Glycine) Blue in ethanol:water (1:1) in a 10 mL volumetric flask. Transfer 6 mL of the above solution to a 10 mL volumetric flask and fill to the mark with a methanol:water (1:1) solution. Filter with 0.45 µm nylon filter and inject onto the HPLC column.

Quantitation:

Construct the linear standard curve for quantitation of genipin using the equation:

$$Peak\ Area = Slope \times [Genipin], \mu g/mL + Intercept$$

Use the following for quantitation

$$\%Genipin = \frac{(Area\ at\ 240\ nm - Intercept)}{Slope} \times \frac{10}{(6 \times mg\ of\ sample)}$$

METHOD OF ASSAY

The percentage of the blue polymer in Jagua (Genipin-Glycine) Blue is calculated by HPLC (Vol. 4).

The contribution to colour from the blue polymer is calculated as %Total Colouring Matters by spectrophotometry (Vol. 4).

Preparation of In-House Reference Standard

Equipment: Glass column; resin Sephadex LH-20; RP-C18 Column.
Solvents: water, methanol.

STEP 1: Wash about 5 g of Jagua (Genipin-Glycine) Blue powder, at least twice, with 200 mL ethyl acetate and discard the supernatant.

STEP 2: Extract the residue from Step 1 with 500 mL methanol, at least five times. Separate the remaining solid material and save for Step 4. Reduce the volume of the supernatant under vacuum. Load this on to a Sephadex LH-20 Column.

STEP 3: Elute with methanol:H_2O (50:50) to obtain two coloured fractions:
Fraction 1 (about 600 mg). Reduce the volume using vacuum and save this fraction for Step 5.
Fraction 2 (about 200 mg). Load this fraction on to a RP-C18 Column. Elute with a MeOH gradient (50% up to 100%) in order to obtain 3 coloured fractions corresponding to dimers 1, 2 and 3.

STEP 4: Load the residue from Step 2 (about 1.2 g) on to the Sephadex LH-20 column. Elute with MeOH:H_2O (9:1).

STEP 5: Combine the first blue band of Step 4 with Fraction 1 of Step 3. Run the combined fraction on a RP C-18 column chromatography separation, and elute with MeOH:H_2O (2:1). Combine the three blue bands obtained. Lyophilize, and label the residue as In-House Reference Standard.

Preparation of the Standard Solution
Accurately weigh 10 mg of the In-house Reference Standard. Transfer to a 20 mL volumetric flask and dissolve with deionized water. Prepare a minimum of five point standard curve by serially

diluting the Reference Standard Stock with deionized water in duplicate.

Qualitative and quantitative determination of blue polymer in Jagua (Genipin-Glycine) Blue

Apparatus:

- HPLC- Photodiode Array (PDA)/UV-Vis
- Column: Phenomenex Luna PFP (PentaFluoroPhenyl, 100 Å, 150 x 4.6 mm, 5 µm) with guard column, or equivalent.
- Flow Rate: 1 mL/min
- Injection volume: 10 µl
- Eluents: A) Water; B) Methanol, HPLC grade
- Detector: PDA/UV-Vis (wavelengths: 230-800 nm; 590 nm for peak identification and quantitation)
- Temperatures:
    - T_{Column}: 40°
    - $T_{Detector}$: 40°
- Sample: Room Temperature
- Retention Time of interest: 10.3 min

Gradient:

Time (min)	% Solvent A	% Solvent B
0	80	20
5	80	20
6	0	100
10	0	100
11	80	20
20	80	20

Sample Preparation:
Prepare three independent samples dissolving 10 mg each of Jagua Blue in deionized water (10 mL volumetric flask). Filter before injection onto column.

Quantitation:
Refer to the standard curve described in the “Method of Assay” to calculate the (%) concentration of the polymer in Jagua blue using the following formula:

$$\%\ Polymer = \frac{Area\ at\ 590\ nm\ \pm Intercept}{Slope} \times \frac{100}{1000} \times \frac{10}{w}$$

Where Intercept and Slope are the values obtained from the standard curve and w is the weight (mg) of the sample.

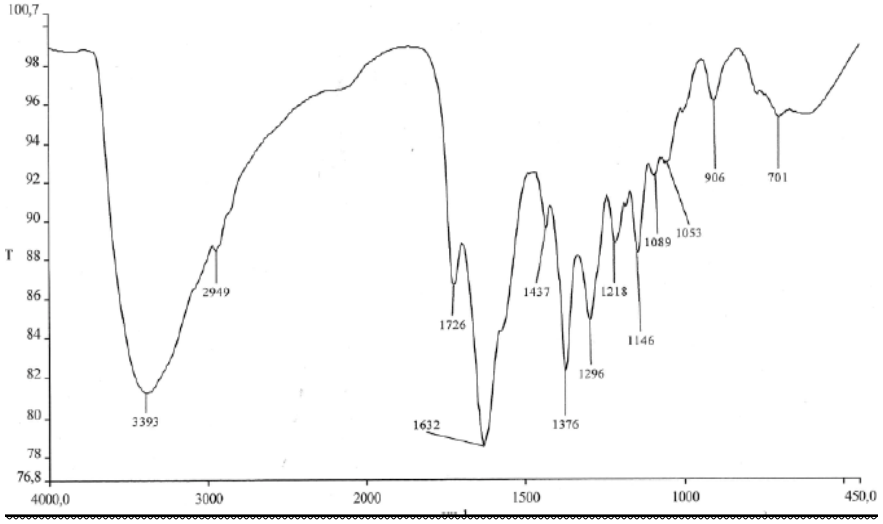
% Total Colouring Matters

Determine % of Total Colouring Matters by Spectrophotometry using water as solvent, as described in Volume 4 of Combined Compendium of Food Additive Specifications (Food Colours, Procedure 1). a=14.911 L/(g*cm) and $E^{1\%}_{1cm}$= 149.1 of the polymer at the wavelength of maximum absorbance (592 nm), respectively.

$$\%\ Total\ Colouring\ Matters = 100 \times \frac{Abs \times 1L}{a \times 1cm} \times \frac{F}{w}$$

Where:

- Abs is the absorbance of the sample solution at 592 nm
- a is the absorptivity of the standard solution (L/g*cm)
- F is the dilution factor
- W grams of the sample.



IR Spectrum of the blue polymer in Jagua (Genipin-Glycine) Blue shows characteristic bands at: 3393, 2949, 1726, 1630 and 1540 cm^{-1}.