Memorandum

Date: **August 16, 2023**

From: Division of Color Certification and Technology, Office of Cosmetics and Colors
Color Technology Branch (HFS-106)

To: Division of Food Ingredients, Office of Food Additive Safety
Regulatory Review Branch, Team 1 (HFS-255)
Attention: Shayla West-Barnette

Subject: CAP 0C0317: Ecoflora, SAS, c/o Exponent, Inc.:
Jagua (genipin-glycine) blue for use as a color additive in various foods. Submissions dated January 31, 2019 and November 1, 2018.

Introduction

The Office of Cosmetics and Colors, Color Technology Branch (OCAC CTB) has reviewed the chemistry data in CAP 0C0317, dated January 31, 2019, and CMF 000036, dated November 1, 2018, from Ecoflora, SAS, c/o Exponent, Inc. Ecoflora is petitioning the FDA to amend 21 CFR part 73 to allow for the use of jagua (genipin-glycine) blue as a color additive in the following foods: flavored milk, dairy drinks and substitutes, dairy and dairy alternative yogurt, ice cream, frozen dairy and dairy alternative desserts, puddings, gelatins, ices, sorbets, ready-to-eat multi-colored cereals, flavored potato chips, tortilla, corn, and other chips, candy, chewing gum, non-alcoholic fruit based/flavored drinks, nutritional beverages, smoothies, flavored cream cheese-based spreads, icings, frostings, jams, syrups, and fruit toppings and fillings.

Ecoflora previously submitted a cover letter dated May 8, 2005 requesting a color additive master file, a draft petition dated March 8, 2014, and a draft petition dated November 1, 2018.

OCAC CTB concludes that the petitioner has provided all relevant information concerning the color additive's name, identity, and composition. The petition adequately identifies all possible impurities in the color additive and requests exemption from batch certification. The petition includes suitable methods for enforcing the color additive requirements, adequate stability data, and an appropriate label for the color additive.

1. Identity

The petition identifies the proposed color additive as jagua (genipin-glycine) blue. It is prepared from the juice of the unripe fruit of *Genipa americana*. The fruit is washed with water, disinfected with quaternary ammonium compounds, alcohol (70%) or acetic acid (15%), and stored under refrigeration (less than 10 °C) for up to five days. The fruit is then cut and the pulp removed from the shells, which are discarded. The pulp is crushed and the juice is collected by filtering with water. The juice contains genipin, an iridoid, which is mixed with an equivalent amount of glycine, an amino acid, at 70 °C for at least two hours (Figure 1). The dark blue liquid is concentrated by evaporation and pasteurized to produce the liquid form of the color additive or is spray dried after mixing with a food-grade carrier (e.g., modified starch) to produce the powder form of the color additive.

The petition states that *G. americana* belongs to the Rubiaceae family. Common names for the plant include juito, huito, and genipa (Spanish), genipap and genipa (English), bois de fer (French), and genipapo (Portuguese). *G. americana* is widely distributed throughout the tropical and subtropical areas of South and Central America. The fruit is large; the average weight is approximately 200 g and the average size is 8.15 cm long and 6.63 cm in diameter. The petition states that only the unripe fruit is used to produce jagua (genipin-glycine) blue.

The chemical name for genipin (CAS No. 6902-77-8) is methyl (1R,4aS,7aS)-1-hydroxy-7-(hydroxymethyl)-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-4-carboxylate). Iridoids, of which genipin is an example, are found in a wide variety of plants. Glycine (CAS No. 56-40-6) is 2-aminoacetic acid and is a common building block of proteins. The intermediate product (center compound in Figure 1) has the chemical name (1S,4aS,7aS)-1,4a,5,7a-tetrahydro-1-hydroxy-7-(hydroxymethyl)-4-(methoxycarbonyl)-2H-cyclopenta[c]pyridine-2-acetic acid (CAS No. 1011270-09-9).

The principal coloring component in the proposed color additive is a polymer (Compound 1 in Table 1) formed from repeating dimeric units. The repeating units contain two genipin moieties previously reacted to add glycine units as side chains (see center compound in Figure 1). The petitioner proposed that two polymer units attach through an ionic bond established between the cationic nitrogen and a carboxylate group to form the polymer, as depicted in Figure 1. The color additive also contains three minor dimer coloring components (Compounds 2, 3, and 4 in Table 1) that are structural units of the polymer.

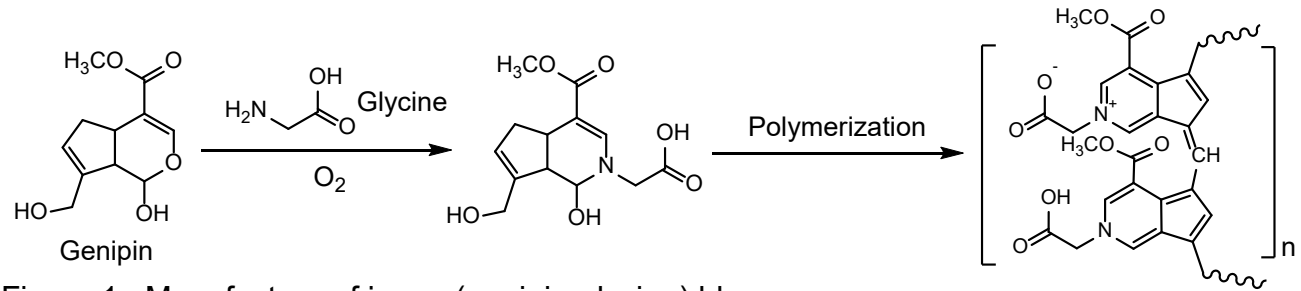
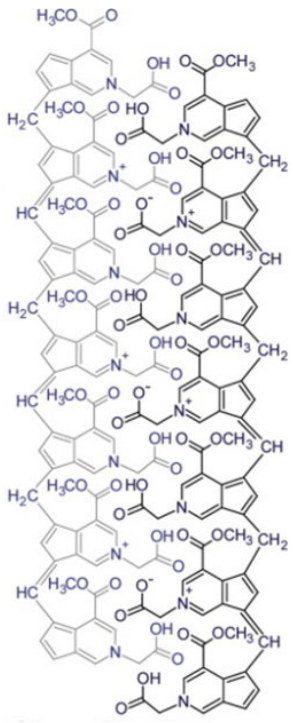
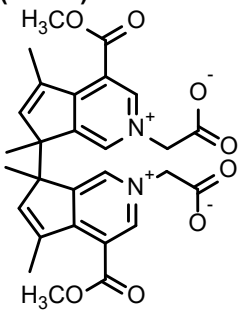
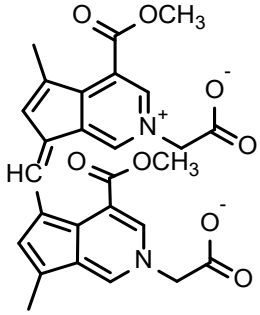
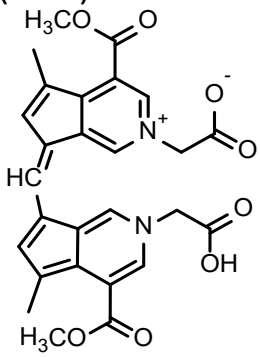


Figure 1 Manufacture of jagua (genipin-glycine) blue

All coloring components have been isolated, identified and quantified by the petitioner using high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, and mass spectrometry (MS). The chemical names, molecular formulas, molecular weights, and CAS numbers for the polymer and dimer components are given in Table 1.

Table 1 Coloring components of jagua (genipin-glycine) blue

Component	Chemical name	Molecular formula	Molecular weight (Da)	CAS No.
Compound 1 (polymer) 	Glycine reaction products with 2-carboxymethyl)-5-([2-(carboxymethyl)-4-(methoxycarbonyl)-5-methyl-2H-cyclopenta[c]pyridin-7-yl]methylene]-4-methoxycarbonyl)-7-methyl-5H-cyclopenta[c]pyridinium inner salt homopolymer and methyl (1R,4aS,7aS)-1-4a,5,7a-tetrahydro-1-hydroxy-7-(hydroxymethyl) cyclopenta[c]pyran-4-carboxylate_from jagua extract	$(C_{27}H_{25}O_8N_2)_{10\text{-}12}$	~6,000	1314879-21-4
Compound 2 (dimer) 	7,7'-Bi-7H-cyclopenta[c]pyridinium, 2,2'-bis(carboxymethyl)-4,4'-(methoxycarbonyl)-5,5',7,7'-tetramethyl-, bis(inner salt)	$C_{28}H_{28}N_2O_8$	520	1313734-13-2

Compound 3 (dimer) 	5*H*-Cyclopenta[c]pyridinium,2-(carboxymethyl)-5-[[2-(carboxymethyl)-4-(methoxycarbonyl)-5-methyl-2*H*-cyclopenta[c]pyridin-7-yl]methylene]-4-(methoxycarbonyl)-7-methyl-, inner salt	$C_{27}H_{24}N_2O_8$	504	104359-67-3
Compound 4 (dimer) 	7*H*-Cyclopenta[c]pyridinium, 2-(carboxymethyl)-7-[[2-(carboxymethyl)-4-(methoxycarbonyl)-5-methyl-2Hcyclopenta[c]pyridine-7-yl]methylene]-4-(methoxycarbonyl)-5-methyl-, inner salt	$C_{27}H_{24}N_2O_8$	504	1313734-14-3

The petitioner proposed the following identity for jagua (genipin-glycine) blue:

(a) Identity. Jagua (genipin-glycine) blue

(1) The color additive Jagua (genipin-glycine) blue is prepared from the juice of unripe Jagua (*Genipa americana*) fruits and formed by the reaction between genipin, an iridoid present in the fruit juice, and glycine.

(2) Color additive mixtures for food use made with Jagua (genipin-glycine) blue may contain only those diluents that are suitable and are listed in this subpart as safe for use in color additive mixtures for coloring foods.

OCAC CTB recommends the following identity for jagua (genipin-glycine) blue:

(a) *Identity.* (1) The color additive jagua (genipin-glycine) blue is prepared from the juice of the unripe fruit of *Genipa americana* by reacting the genipin in the juice with glycine using mild heat. The color additive contains a polymer as the principal coloring component and three dimers as minor coloring components.

(2) Color additive mixtures for food use made with jagua (genipin-glycine) blue may contain only those diluents that are suitable and are listed in this subpart as safe for use in color additive mixtures for coloring foods.

2. Physical, Chemical, and Biological Properties

The petition includes physical, chemical, and spectrophotometric properties for the establishment of the identity of the color additive.

The polymer shown as compound 1 in Table 1 is the primary source of the blue color of jagua (genipin-glycine) blue. The dimeric coloring components (compounds 2, 3, and 4 in Table 1) are considered by the petitioner to be subsidiary colors but OCAC CTB concludes that they are minor coloring components. Jagua (genipin-glycine) blue is highly soluble in water, and aqueous solutions have a wavelength of maximum absorbance near 590 nm (Figure 2).

The following particle size measurements of jagua (genipin-glycine) blue were included in the petition: surface area, 0.28-0.35 m^2/g; superficial diameter, 17.4-21.4 µm; and volumetric diameter, 37.3-61.6 µm. The melting point of the final product is 184-190 °C.

OCAC CTB concludes that this information is satisfactory.

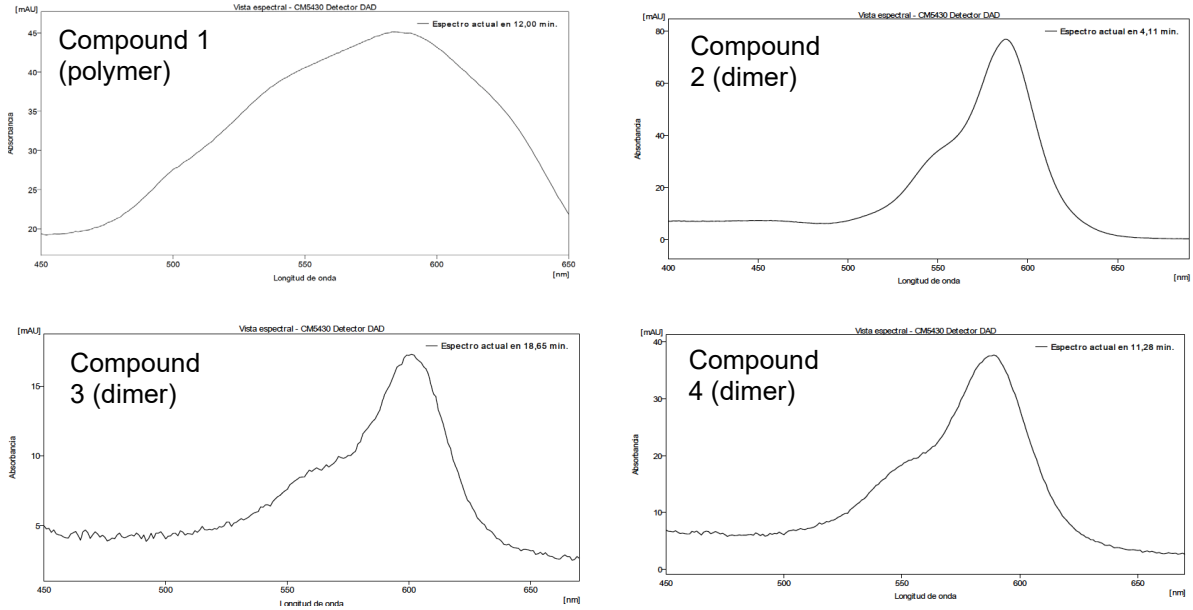


Figure 2 UV-visible spectra of components of jagua (genipin-glycine) blue

3. Chemical Specifications

The petitioner provided complete chemical analyses of five batches of the powder form of jagua (genipin-glycine) blue using appropriate analytical methods. Ranges of the results are shown in Table 2. The five batches identified in Table 2 include two batches used for toxicity testing. In addition, the petitioner provided results for three other batches used for toxicity testing. The compositions of the additional three batches are consistent with the results in Table 2.

The color value or E10% assay, as defined in the petition, is calculated from the visible absorption spectrum at 590 nm of a solution prepared by dissolving 15 mg of jagua (genipin-glycine) blue in 40 g of water. Based on the color values, the polymer concentration in the five batches is 31-35%.

Table 2 shows that in addition to the coloring components, the powder form of the color additive contains carbohydrates that include modified starch used as a carrier, fat, protein, moisture, and ash (inorganic constituents). Impurities include arsenic, cadmium, mercury, lead, and various microorganisms.

The petitioner also provided complete chemical analyses of three batches of the liquid form. Ranges of the results are shown in Table 2. The content of the liquid form is similar to the content of the powder form except the liquid form does not contain modified starch.

Table 2 Jagua (genipin-glycine) blue analytical results.

Analyte	Results for five powder batches	Results for three liquid batches
Absorbance at 590 nm of 15 mg jagua (genipin-glycine) blue / 40 g of water Color value (E10%)	0.91-1.02 243-273	0.63-0.76 167-202
Polymer (%)	31-35	25-32
Minor coloring components (dimers) (%)	0.139-0.197	0.028-0.039
Total carbohydrates (%) Modified starch (%)	81-84 20-26	30-32
Genipin (ppm)	<10	<10
Glycine (ppm)	<20	<20
Total fat (%)	0.2-2	0.1-0.16
Protein (%)	5-10	5-5.35
Ash (%)	4-7	5-8
Moisture (%)	4-5	57-58
Arsenic (ppm) Cadmium (ppm) Mercury (ppm) Lead (ppm)	<0.05 <0.01 <0.01 0.05-0.24	<0.02 <0.01 <0.01 <0.02
Escherichia coli Coagulase positive *Staphylococcus aureus* Coliform count colony-forming units (CFU)/g Yeast and mold (CFU/g) Aerobic mesophiles microorganisms (CFU/g)	Absent Absent <10 <10 <10	Absent Absent <10 <10 <10

The petitioner proposed the following chemical specifications for jagua (genipin-glycine) blue.

Components and attributes	Powder form	Liquid form
Appearance	Dark blue	Dark blue
Color value (E10%)	240-280	120-240
Polymer	20-40%	10-35%
Minor coloring components (%)	<0.40%	<0.40%
Carbohydrates	≤85%	≤60%
Modified starch	20-30%	N/A
Total protein	≤10%	≤10%
Total fat	≤10%	≤10%
Moisture	≤10 %	≤70 %
Ash	≤10 %	≤10 %
Heavy metals (As, Pb, Cd, Hg)	≤1 ppm	≤1 ppm
Genipin	≤0.005%	≤0.005%
Glycine	≤0.020%	≤0.020%
Aflatoxines (B1, B2, G1, G2)	≤0.01 ppm	≤0.01 ppm
Fumonisine (B1, B2)	≤0.5 ppm	≤0.5 ppm
E. coli	Negative	Negative
Aerobic plate count	<1000 CFU/g	<1000 CFU/g
Yeast and mold	<300 CFU/g	<300 CFU/g
Coagulase positive *S. aureus*	Negative	Negative
Total coliform	<10 CFU/g	<10 CFU/g

Unreacted genipin is a potential impurity in jagua (genipin-glycine) blue and was assessed by the petitioner using a high-performance liquid chromatography-diode array detection (HPLC-DAD) method. Residual genipin was not found at levels above the petitioner's limit of quantitation (LOQ) of 10 ppm, and the petitioner recommended a specification of ≤0.005% (50 ppm) for genipin. However, OCAC CTB recommends a specification of ≤0.002% for genipin, which corresponds to not more than 20 mg/kg (20 ppm), because this specification is consistent with the petitioner's analytical results. The petitioner has been consulted about the 20 mg/kg (20 ppm) specification and concurs with it.

Although the petitioner provided analytical data and a proposed specification for glycine, OCAC CTB concludes that a specification is not needed because glycine is a widely occurring amino acid. OCAC CTB also concludes that specifications are not needed for the coloring components or carbohydrates, modified starch, fat, protein, moisture, and ash content in jagua (genipin-glycine) blue. A microbiologist in the Office of Food Additive Safety, Division of Food Ingredients concludes that specifications do not need to be included in the proposed regulation for aflatoxins, fumonisin, *E. coli*, aerobic plate count, yeast and mold, coagulase positive *S. aureus*, as well as total coliform, based on the manufacturing process described in the petition and the source from which the color additive is derived.

Therefore, OCAC CTB recommends the following specifications for jagua (genipin-glycine) blue.

(b) *Specifications.* Jagua (genipin-glycine) blue must conform to the following specifications and must be free from impurities, other than those named, to the extent that such other impurities may be avoided by good manufacturing practice:

(1) Lead (as Pb), not more than 1 milligram/kilogram (mg/kg) (1 part per million (ppm)).
(2) Arsenic (as As), not more than 1 mg/kg (1 ppm).
(3) Mercury (as Hg), not more than 1 mg/kg (1 ppm).
(4) Cadmium (as Cd), not more than 1 mg/kg (1 ppm).
(5) Genipin, not more than 20 mg/kg (20 ppm).

4. Manufacturing Process Description

The petition describes the starting materials, equipment, and processes used for the manufacture of jagua (genipin-glycine) blue. The petitioner reported that only water is used for extracting the juice from the jagua fruit.

The petition describes the selection criteria for the unripe fruit and how the fruit is disinfected with quaternary ammonium compounds, alcohol (70%) or acetic acid (15%). The fruit is stored under refrigeration (less than 10 °C) for up to five days prior to cutting up and obtaining the juice. The pulp is removed from the shells using mechanical filters and the shells are discarded. The pulp is then crushed, and the juice is collected by filtering with water. The quantity of genipin in the juice is measured and the stoichiometric amount of glycine is added. The mixture is heated at 70 °C for at least two hours and the reaction mixture is checked periodically to assess the presence of residual genipin and glycine. When they are no longer detected, the blue mixture is centrifuged and the liquid layer is retained. For the powder form of the color additive, the liquid layer is concentrated by evaporation under vacuum to 20 °Brix (sugar content), spray dried after adding modified starch, sieved, and stored in 90 micron-thick metallic bags manufactured from polyethylene terephthalate-10 (PET), bioriented polypropylene-20 (“Metallic Bopp”), and low-density polyethylene-70 (LDPE). For the liquid form, the liquid layer is concentrated to 45-65 °Brix, pasteurized, and stored in 1 L polyethylene high-density (PEHD) containers.

The final product is analyzed for polymer and dimer content, color content, moisture (powder form), genipin and glycine content, pH, heavy metals, and microbials. The petitioner has submitted supporting data (Attachment 24) to confirm that there is no concern about formation of Maillard-type compounds from the reaction between the acids and sugars in the fruit and the added glycine during the heating process.

OCAC CTB concludes that this information is satisfactory.

5. Stability Data

The stability of jagua (genipin-glycine) blue was evaluated by measuring the color loss of the powder and liquid forms following storage or heat and by measuring the microbial content of both forms.

For the powder form, five one hundred gram quantities of the color additive were placed in metallic bags similar to the bags used for storage of the final product. The bags were tightly sealed and stored at room temperature (18-34 °C) for 48 months.

Color changes during the 4-year storage time were monitored by spectrophotometric and HPLC-DAD analyses of the principal coloring component using visible absorbance and polymer content as parameters, respectively. Average percentages of color conservation for the five batches over the 4-year period were 100.5 ± 3.9 and 101.4 ± 3.9 for each method. No new peaks were observed in the chromatograms, indicating that no new substances formed. Product recovery of ≥90% of original color content throughout the shelf life was considered acceptable by the petitioner. Jagua (genipin-glycine) blue powder was found to be stable for 48 months.

The petitioner also conducted an accelerated stability test by heating powder samples from a representative batch of jagua (genipin-glycine) blue at 176 °C (approximately 350 °F) for up to 20 minutes. For the initial 0-minute measurement, a 2 g sample was taken directly from the storage bag. Four additional 2 g samples were weighed into porcelain crucibles and placed in a muffle furnace preheated to 176 °C. Measurements at 5, 10, 15, and 20 minute intervals were made in triplicate.

The stability of the heated powder form was evaluated by monitoring the concentration of the principal coloring component using HPLC-DAD and by monitoring possible degradation products using ultra-high-performance liquid chromatography with high resolution mass spectrometry detection (UHPLC/HR-MS). The results showed that no degradation products such as smaller components were present after heating. The color additive had the same composition after heating, except minor polymerization of the principal coloring component to higher molecular weight components was observed as heating time progressed.

Microbial stability was tested on a batch of the powder form over a 3-year period. Total coliform, *E. coli,* aerobic plate count, yeast and mold, and coagulase positive *S. aureus* count did not change from the original values. The water activity at the end of the test was 0.4 (Aw ≤0.85 in food products will inhibit the growth of organisms).

Based on these tests, the petitioner recommends a 3-year shelf life for the powder form of the color additive.

For the liquid form, the stability of the color additive was evaluated by measuring the color loss following storage and the microbiology content of two different batches. The liquid products were stored at 4 ºC in 1 L PEHD containers for approximately 6 months. The color values for both liquid batches did not show significant change from the initial

values during the testing period. The *E. coli*, aerobic plate count, yeast and mold, and coagulase positive *S. aureus* count also did not change from the original values. *Salmonella* serovars and *Listeria monocytogenes* were negative.

The petitioner also evaluated the thermal stability of the liquid form. Four 2 g samples were weighed into porcelain crucibles and heated at 176 ºC for up to 20 minutes as described for the powder form. The stability of the color additive was evaluated by monitoring the concentration of the principal coloring component by HPLC-DAD. The results showed that the color additive had the same composition after heating.

Based on these tests, the petitioner recommends a 4-month shelf life for the liquid form of the color additive.

The petitioner also tested the stability of jagua (genipin-glycine) blue in food matrices. Table 3 shows the representative food products used to test the stability of the color additive in each proposed food category. Further details are shown in Table 4. An HPLC-DAD method was used to quantify the principal coloring component in these food matrices to determine the stability of the proposed color additive during the shelf lives of the products. These stability data are in Appendix III, parts B-G (included in the January 31, 2019 submission package).

Stability studies for each representative food product were conducted in triplicate at each concentration and storage condition or temperature. Stabilizers such as carboxymethyl cellulose or carrageenan were used in the dairy products. Cookies and crackers were baked at 176 °C, and the dairy products were heated at 47 °C and 72 °C. Photos of food product samples and the color additive extracted from each sample at the beginning of each stability study, at different intervals during the study, and after the end of each study were submitted by the petitioner. The petitioner stated that no new substances formed in the food matrices during the stability studies.

OCAC CTB concludes that the petitioner has fully addressed the stability of the powder and liquid forms of the color additive as well as its stability in food products.

Table 3 Summary of stability data for jagua (genipin-glycine) blue in various foods

Food categories	**Proposed uses**	**Representative food in stability testing**	**Color loss at the end of test (temperature, °C)**
Flavored milk	Flavored milk	Flavored milk	2% (4)
Dairy drinks and substitute	Milk shakes		
Dairy drinks and substitutes	Milk substitutes	Soy milk	No color loss (4 and 25)
	Other dairy drinks		
Yogurt	Yogurt, regular and Greek Yogurt, dairy alternative	Yogurt	9% (4)

Desserts	Ice cream, frozen dairy, and dairy alternative desserts, pudding, gelatins, ices, sorbets	Ice cream	6% (4)		
Fats and oils	Cream cheese-based spreads, flavored	Cream cheese	No color loss (4)		
Sweet baker products	Cakes and cookies	Cookies	2% (25)		
Savory snacks and crackers	Crackers, flavored	Cracker	8% (25)	12% (35)	16% (45)
	Potato chips, favored tortilla, corn, and other chips	Potato chips	3% (25)	5% (35)	7% (45)
Cereal, ready-to-eat (RTE)	RTE cereal, multi-colored	RTE cereal	1% (25)	2% (35)	12% (45)
Candy	Candy containing chocolate	Chocolate dragee	2% (4)	3% (25)	3% (35)
	Candy not containing chocolate	Hard candy	0.4% (20)	1% (25)	
		Gummies	3% (4)	3% (25)	4% (35)
	Chewing gum	Chewing gum	2% (20)	3% (25)	
Jams, syrups, toppings	Icing and frosting Fruit toppings, fillings, and jam Syrups/toppings for beverages, desserts, and breakfast syrup	Jelly powder	3% (25)	5% (35)	7% (45)
		RTE jelly	No color loss (4)		
Non-alcoholic beverages	Soft drinks	Soft drink	17% (4)	18% (25)	
	Sport drinks	Sport drink	30% (4)	47% (25)	
	Energy drinks	Energy drink	20% (4)	15% (25)	
	Fruit based drinks (including fruit flavored drinks), smoothies	Fruit-based drink	44% (4)	47% (25)	
	Nutritional beverages (ready-to-drink and powdered)	Nutritional beverage (powdered)	2% (25)	5% (35)	11% (45)
	Flavored waters (carbonated and still)	Flavored water	20% (4)	22% (25)	
	Enhanced or fortified waters	Vitamin water	28% (4)	33% (25)	
	Beverage powders/concentrates	Beverage powder	4% (25)	5% (35)	9% (45)

Table 4 Shelf life and length of study for each representative food used in stability testing

Food Matrix	**Shelf Life**	**Study type or storage conditions**	**Study length**
Skim and flavored milk	<12-14 days	4 °C	28 days
Soy milk	6 months (after opened 7-10 days)	Normal conditions[2]	29 days
Yogurt	1-2 weeks	4 °C	22 days
Ice cream	2-6 months	-18 °C	6 months
Cream cheese	1-2 weeks	4 °C	22 days
Cookies	2 months (60 days)	Normal conditions[1]	62 days
Crackers	8 months (240 days)	Accelerated conditions[3,a]	84 days
Seasoned potato chips	2 months (60 days)	Accelerated conditions[3,a]	85 days
Cereal	12 months (365 days)	Accelerated conditions[3,a]	82 days
Chocolate dragee	12 months (365 days)	Accelerated conditions[3,b]	84 days
Hard candy	12 months (365 days)	Normal conditions[1]	85 days
Gummies	12 months (365 days)	Accelerated conditions[3,b]	92 days
Chewing gum	12 months (365 days)	Normal conditions[1]	63 days
Jelly powder	12 months (365 days)	Accelerated conditions[3,a]	83 days
RTE Jelly	4 weeks	4 °C	43 days
Soft drink	6-9 months	Normal conditions[2]	42 days
Sport drink	9 months	Normal conditions[2]	79 days
Energy drink	12-24 months	Normal conditions[2]	70 days
Fruit-based drink	6-12 months	Normal conditions[2]	49 days
Nutritional beverages (powder)	12 months	Accelerated conditions[3,a]	84 days
Flavored water	2 months	Normal conditions[2]	28 days
Vitamin water	3 months	Normal conditions[2]	42 days
Powder beverage/concentrate	12 moths	Accelerated conditions[3,a]	85 days

[1] Normal conditions at 20 °C and 25 °C
[2] Normal conditions at 4 °C and 25 °C
[3] Accelerated study was done to establish the predicted shelf life of the color additive at 20 °C and 25 °C
[a] At 25 °C, 35 °C, and 45 °C
[b] At 4 °C, 25 °C, and 35 °C

6. Uses and Restrictions

The petition requests the use of jagua (genipin-glycine) blue in the following food categories: flavored milk, dairy drinks and substitutes, dairy and dairy alternative yogurt, ice cream, frozen dairy and dairy alternative desserts, puddings, gelatins, ices, sorbets, ready-to-eat multi-colored cereals, flavored potato chips, tortilla, corn, and other chips, candy, chewing gum, non-alcoholic fruit based/flavored drinks, nutritional beverages, smoothies, flavored cream cheese-based spreads, icings, frostings, jams, syrups, and fruit toppings and fillings. Figure 3 shows the final color shades obtained when different levels of the color additive are used in representative foods.

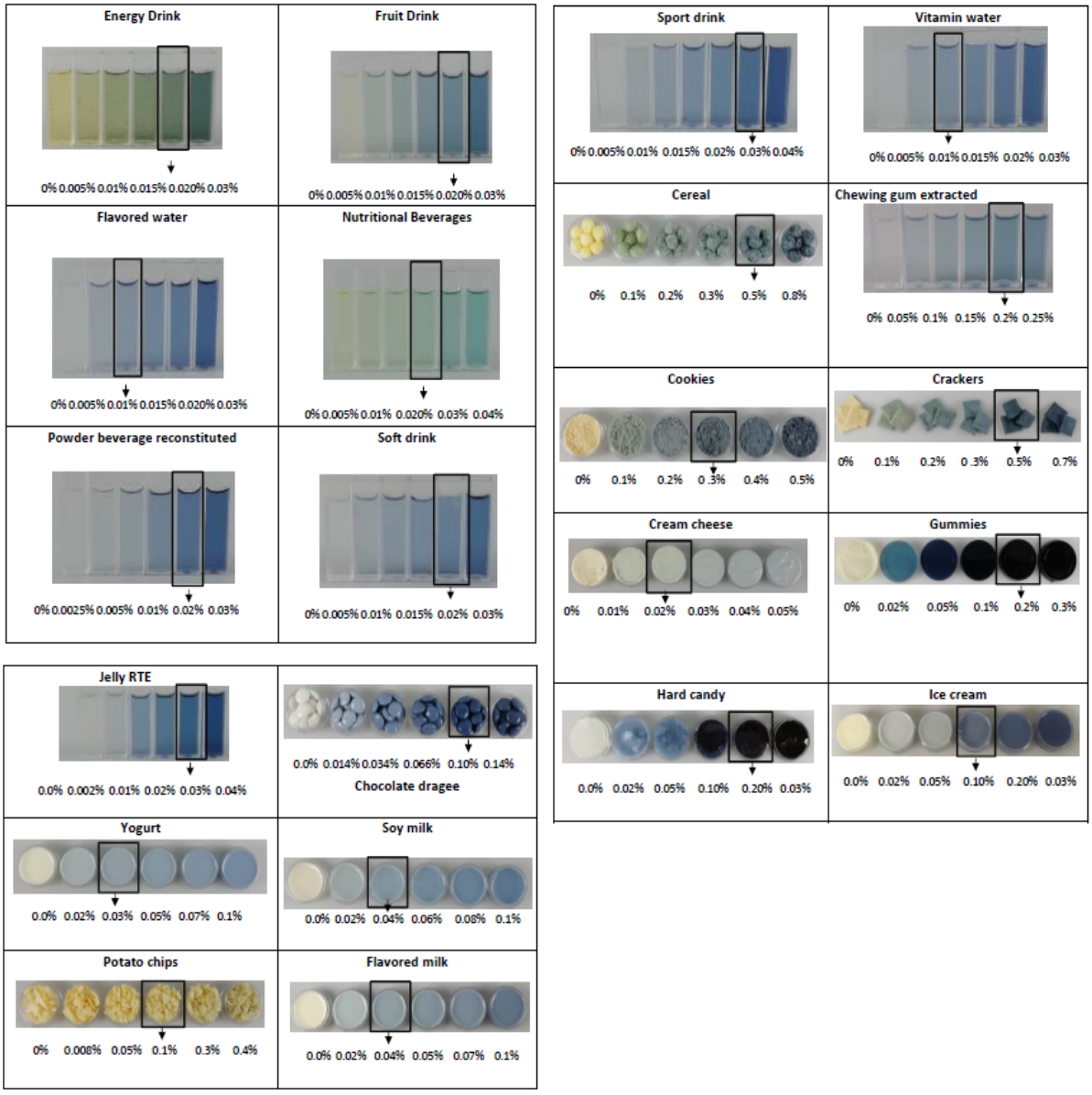


Figure 3 Different levels of jagua (genipin-glycine) blue in representative categories of food products. The selected levels with the arrow correspond to the recommended maximum use levels based on commercial references and consumer preferences.

The petitioner stated that jagua (genipin-glycine) blue will be used in food products consistent with good manufacturing practices (GMPs). The color additive may be used without any limitation and its use is self-limiting. OCAC CTB notes that the food products were shown to darken when excess color is used. The petitioner stated that excess use in a product will increase the production cost without adding any coloring benefit.

OCAC concludes that this information is satisfactory for describing the proposed uses of the color additive at levels consistent with GMP.

7. Labeling Requirements

The petition includes a proposed label for jagua (genipin-glycine) blue with expiration dates, requirements for storage conditions, and the ingredients used to produce the color additive. The proposed label also recommends storing the color additive protected from direct sunlight and moisture and states that the color additive is for food use but does not include the specific uses.

OCAC CTB concludes that the proposed label satisfies the requirements of 21 CFR 70.25.

8. Analytical Methods for Enforcing Chemical Specifications

The petition includes a spectrophotometric method to assay the color additive (Attachment 9) and HPLC-DAD methods for quantification of the polymer and dimer components (Attachments 4, 5, 10, and 29.). The petition also includes methods for the determination of glycine, genipin, and carbohydrates, protein, fat, moisture, heavy metals (As, Cd, Hg, and Pb), and microbiological impurities in the color additive (Attachments 6, 11, 26, 27, and 28).

OCAC CTB concludes that the petitioner has provided appropriate analytical methods.

9. Qualitative and Quantitative Determination of the Color Additive in Products

The petition includes an HPLC-DAD method for the determination of the color additive in different food products.

OCAC CTB concludes that this information is satisfactory.

10. Identification and Determination of Any Substance Formed in or on Products Because of the Use of the Color Additive

The petitioner is not aware of any substance that could form in food products as a result of use of the color additive.

OCAC CTB concludes that no constituents of concern are formed from the degradation of jagua (genipin-glycine) blue in the proposed food products.

11. Tolerances and Limitations

The petitioner did not propose a tolerance or limitation for jagua (genipin-glycine) blue.

12. Exemption from Batch Certification

The petitioner requested that jagua (genipin-glycine) blue be exempted from batch certification.

OCAC CTB concludes that batch certification of jagua (genipin-glycine) blue is not necessary for the protection of the public health.

13. Proposed Regulation

OCAC CTB has discussed the proposed regulation elsewhere in this review.

14. Summary

OCAC CTB concludes that the petitioner has provided the chemistry data needed for listing jagua (genipin-glycine) blue as a color additive.

OCAC CTB recommends the following identity for jagua (genipin-glycine) blue:

(a) *Identity*. (1) The color additive jagua (genipin-glycine) blue is prepared from the juice of the unripe fruit of *Genipa americana* by reacting the genipin in the juice with glycine using mild heat. The color additive contains a polymer as the principal coloring component and three dimers as minor coloring components.

(2) Color additive mixtures for food use made with jagua (genipin-glycine) blue may contain only those diluents that are suitable and are listed in this subpart as safe for use in color additive mixtures for coloring foods.

OCAC CTB recommends the following specifications for jagua (genipin-glycine) blue:

(b) *Specifications.* Jagua (genipin-glycine) blue must conform to the following specifications and must be free from impurities, other than those named, to the extent that such other impurities may be avoided by good manufacturing practice:

(1) Lead (as Pb), not more than 1 milligram/kilogram (mg/kg) (1 part per million (ppm)).
(2) Arsenic (as As), not more than 1 mg/kg (1 ppm).
(3) Mercury (as Hg), not more than 1 mg/kg (1 ppm).
(4) Cadmium (as Cd), not more than 1 mg/kg (1 ppm).
(5) Genipin, not more than 20 mg/kg (20 ppm).

Nebebech Belai -S

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cc: HFS-100 (Katz, Manga)
HFS-105 (Bowes)
HFS-106 (Barrows)

NBelai: CAP0C0317_C_Memo_Draft.doc
Drafted: HFS-106: NBelai: 2/1/21
Edit/Comments: JBarrows: 2/11/21
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Edit/Comments/Init: JBarrows: 5/10/21, 7/26/21, 3/24/22, 4/13/22, 4/25/22
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Edit/Comments in response to OFAS review: NBelai: 11/2/22, 11/15/22
Edit/Comments: SHice: HFS-255: 12/6/22
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