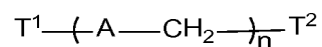


REIVINDICACIONES

- Una composición colorante que comprende un polímero de Fórmula 4

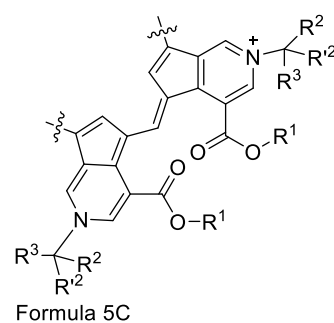
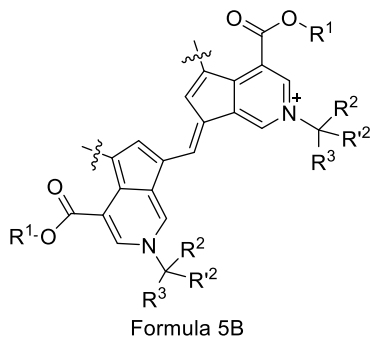
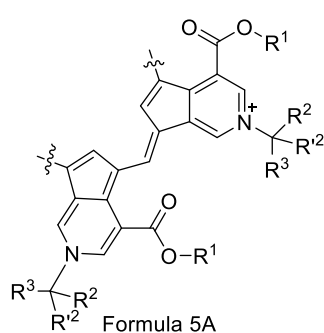


Fórmula 4

su isómero geométrico o su sal,

donde n es un número entero del 2 al 20;

donde cada A se selecciona de forma independiente de un grupo que comprende la Fórmula 5A, Fórmula 5B, Fórmula 5C, su isómero geométrico, su sal, y una combinación de estos:



Donde:

R¹ es hidrógeno, metilo, etilo, propilo, isopropilo, butilo, sec-butilo, isobutilo o terbutilo;

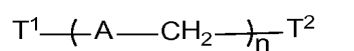
R² and R¹² son independientemente hidrógeno o C₁₋₁₀ alquilo;

R³ es hidrógeno o COOH;

Y donde T¹ es hidrógeno o un grupo metilo; y T² es hidrógeno o A-T¹, siendo A y T¹ como se describen arriba.

Y un diluyente seleccionado del grupo que consiste en agua, metanol y etanol.

2. Un polímero de Fórmula 4:

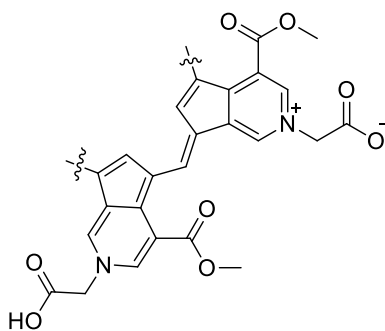


Fórmula 4

su isómero geométrico o su sal,

donde n es un número entero del 2 al 20;

donde cada A es de la Fórmula 5'A (Me), su isómero geométrico del mismo, su sal, y una combinación de estos:



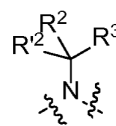
Formula 5'A (Me)

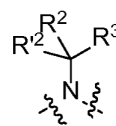
Donde:

T¹ es hidrógeno o un grupo metilo; y T² es hidrógeno o A-T¹, siendo A y T¹ como se describen arriba.

3. El polímero de la Reivindicación 2, en donde el peso molecular numérico promedio de dicho polímero (Mn) es aproximadamente 6,000.

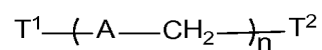
4. El polímero de la Reivindicación 2, en donde el espectro IR presenta los siguientes picos ($\pm 5 \text{ cm}^{-1}$): 3393, 2949, 1726, 1630 y 1540 cm^{-1} .
5. Un producto que comprende (a) el polímero de la Reivindicación 2 y (b) un alimento o un producto alimenticio cualquiera, un medicamento, un producto nutracéutico o un producto cosmético.
6. Un dispositivo médico teñido con el polímero de la Reivindicación 2.



7. La composición colorante de la Reivindicación 1, donde  representa un residuo de aminoácido, donde R^3 es COOH y R^2 y/o R^{12} representa la(s) cadena(s) lateral(es) del aminoácido, y donde se selecciona el aminoácido de un grupo que comprende Glicina, Alanina, Valina, Leucina, Isoleucina, Serina, Cisteína, Treonina, Metionina, Prolina, Fenilalanina, Tirosina, Triptófano, Licina, Arginina, Aspartato, Glutamato, Asparagina, Glutamina, Taurina, Carnitina, Ornitina y Citrulina, donde además el aminoácido puede estar en configuración D o L.
8. La composición colorante de la Reivindicación 7, en donde el aminoácido es glicina.
9. Un producto alimenticio que comprende un alimento y la composición colorante de la Reivindicación 7.
10. Un medicamento o producto nutracéutico que comprende un ingrediente activo o droga o un ingrediente nutracéutico y la composición colorante de la Reivindicación 7.
11. Un producto cosmético que comprende la composición colorante de la Reivindicación 7.
12. Un dispositivo médico teñido por la composición colorante de la Reivindicación 7.

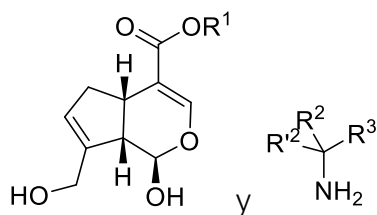
13. Un método de preparación de la composición colorante de la Reivindicación 7, que comprende las etapas de:
 - (a) mezclar el zumo del fruto de la *Genipa americana* y un aminoácido bajo condiciones de calor, en donde el aminoácido se selecciona del grupo que incluye Glicina, Valina, Licina, Metionina, Prolina, Tirosina, Triptófano, y/o alguna combinación de estos;
 - (b) eliminar el azúcar de la mezcla de (a); y
 - (c) aislar una composición colorante del producto libre de azúcar de (b),donde la intensidad de la composición colorante obtenida usando el método es mayor a la intensidad de una composición colorante obtenida de una mezcla de pasos (a) sin eliminar el azúcar.
14. El método de la Reivindicación 13, en donde se elimina el azúcar en el paso (b) mediante fermentación, cromatografía en columna, HPLC, cromatografía de exclusión por tamaño, filtración por osmosis inversa, ultrafiltración, microfiltración, diálisis, separación mediada por resina XAD4, separación mediada por resina XAD7, o alguna combinación de estos.
15. El método de la Reivindicación 13, en donde la eliminación o remoción de azúcar de la mezcla (a) comprende las etapas de:
 - (i) esterilizar la mezcla del paso (a) bajo condiciones de calor;
 - (ii) inocular la mezcla esterilizada del paso (b) con una levadura o bacteria;
 - (iii) incubar la mezcla inoculada del paso (c) bajo condiciones de fermentación para producir un producto de fermentación; y
 - (iv) aislar una composición colorante del producto de fermentación del paso (iii);en donde la temperatura se mantiene en aproximadamente 30°C bajo condiciones de fermentación.
16. El método de la Reivindicación 13, en donde el aminoácido es glicina.
17. El método de la Reivindicación 13, en donde la composición colorante es azul, verde azul, verde o morado.

18. Una composición colorante que comprende un polímero de Fórmula 4:



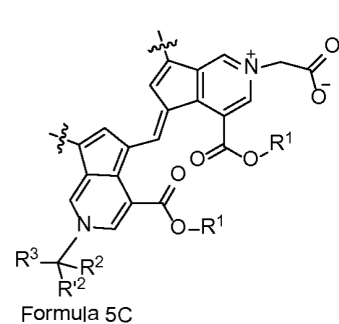
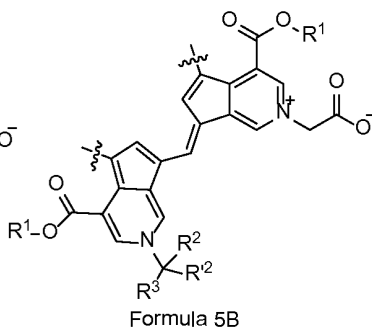
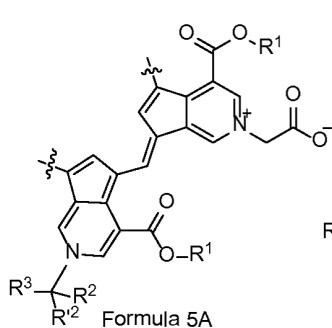
Fórmula 4

su isómero geométrico o su sal, en donde el polímero de Fórmula 4 se conforma por la reacción de:

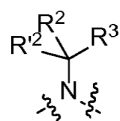


donde n es un número entero del 2 al 20;

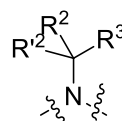
donde cada A se selecciona de forma separada a partir del grupo que comprende la Fórmula 5A, Fórmula 5B, Fórmula 5C, su isómero geométrico, su tautómero, su sal, y una combinación de estos:



Donde R¹ es metilo; y



representa una amina alcalina primaria o



representa un residuo

de aminoácido, donde R^3 es COOH y R^2 y/o R^1 representa la(s) cadena(s) lateral(es) del aminoácido, y donde se selecciona el aminoácido de un grupo que comprende Glicina, Alanina, Valina, Leucina, Isoleucina, Serina, Cisteína, Treonina, Metionina, Prolina, Fenilalanina, Tirosina, Triptófano, Licina, Arginina, Aspartato, Glutamato, Asparagina, Glutamina, Taurina, Carnitina, Ornitina y Citrulina, en los que el aminoácido puede estar en configuración D o L.

y donde T^1 es hidrógeno o un grupo metilo; y T^2 es hidrógeno o $A-T^1$, siendo A y T^1 como se describen arriba.

Y un diluyente seleccionado del grupo que consiste en agua, metanol y etanol.

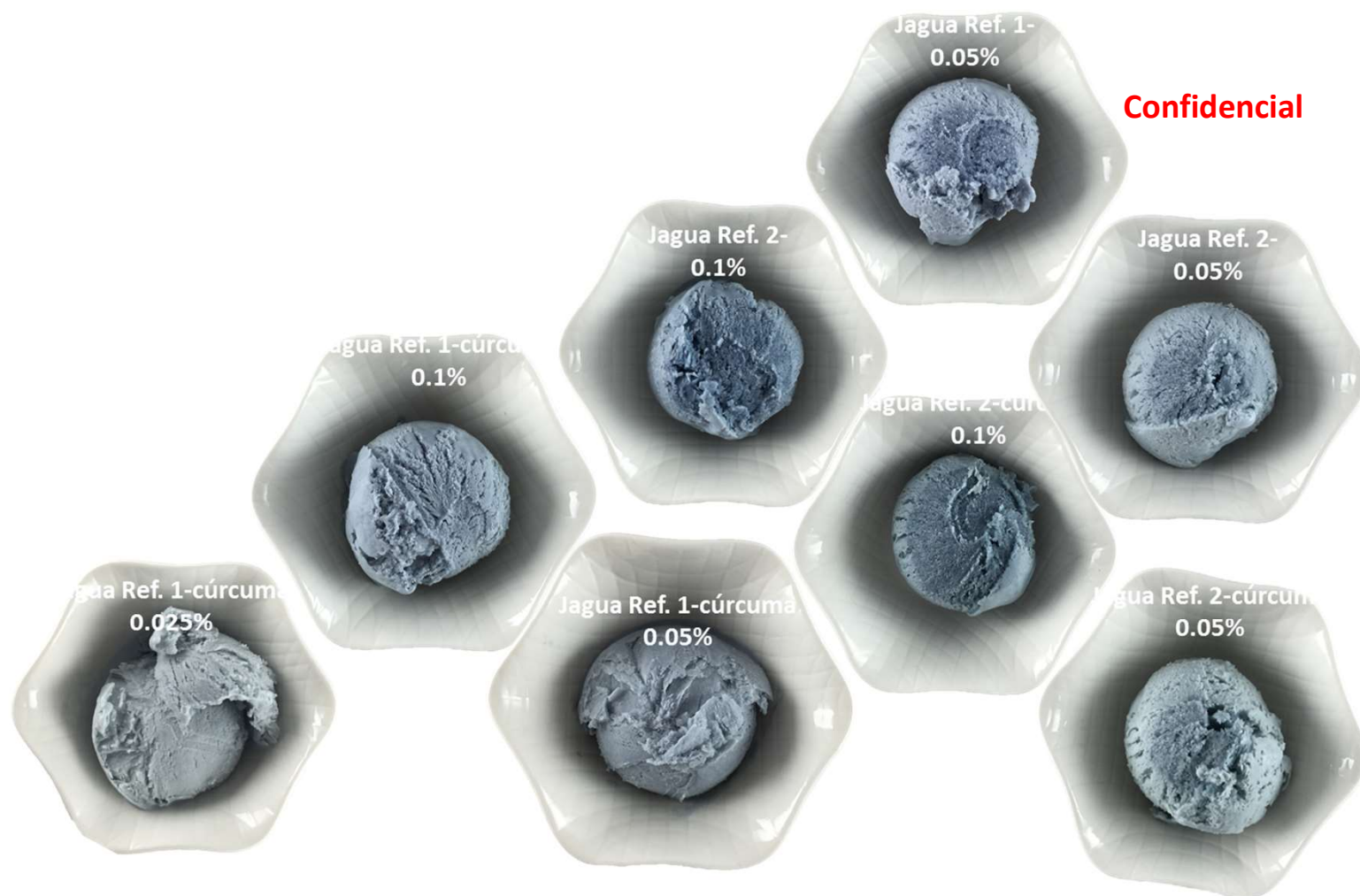


Confidencial

Ejemplos de aplicaciones del color azul (componente principal y responsable de la coloración: polímero)

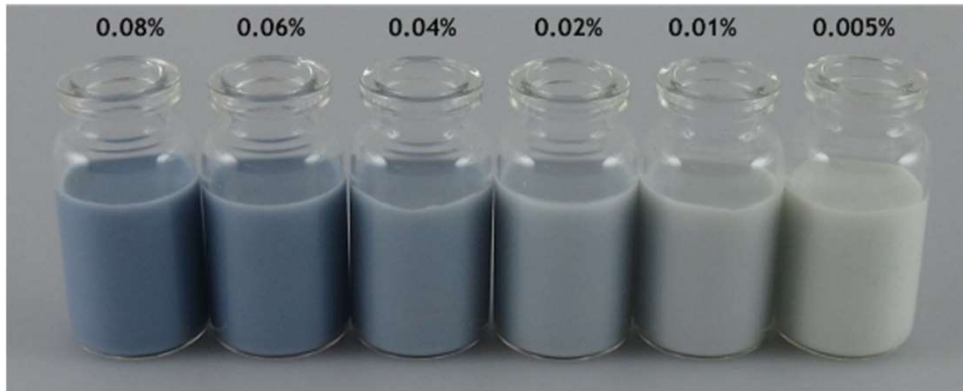
Confidencial

Azul de jagua VC-7684

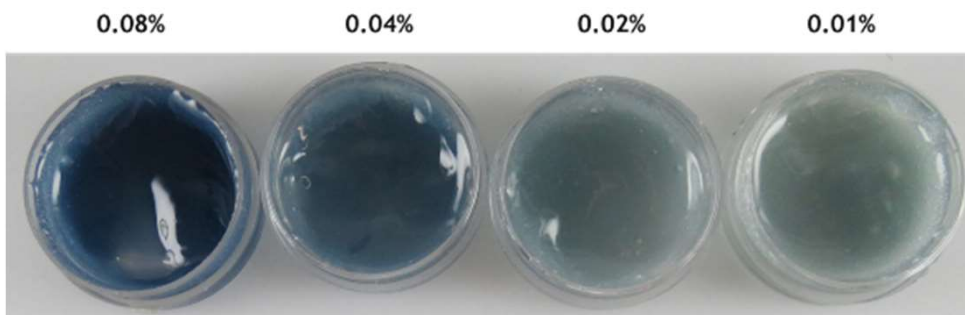


Azul de jagua

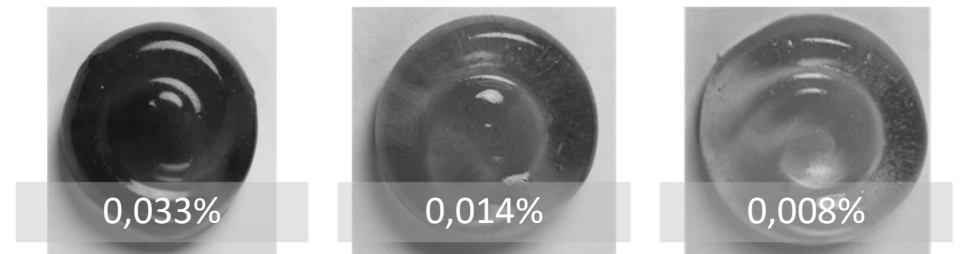
Leche saborizada



Gelatina

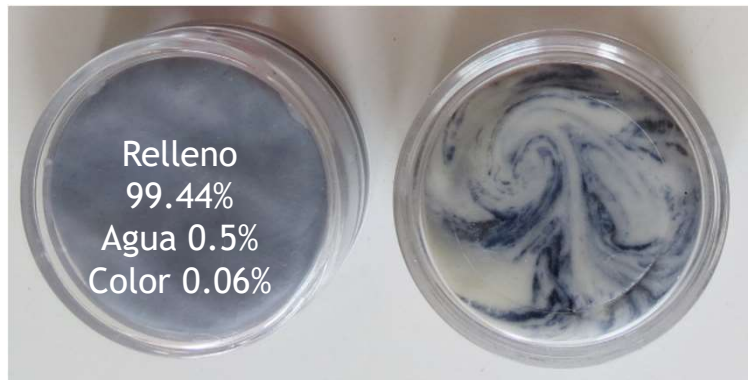


Gomitas



Confidencial

Rellenos de galletas (grasa y azúcar)



Crema



Confidencial

Cubierta de Cupcake



Malteada



Bebidas tipo gaseosa

Confidencial



Aplica para refrescos,
bebidas energéticas y
bebidas isotónicas



Cristales de azúcar



Chocolate Blanco en diferentes
concentraciones



Crema de manos



Splash



Gel antibacterial

Confidencial



Jabón líquido



Jabón

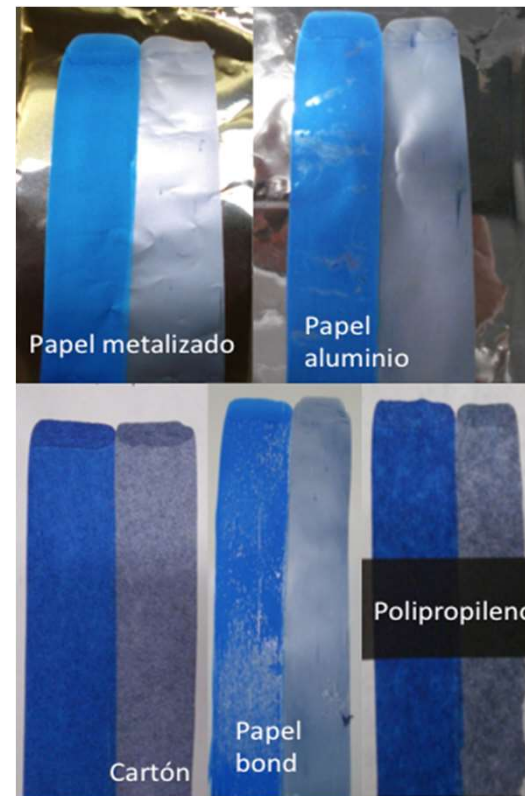


Nota: En algunas aplicaciones el color se ve verde, porque la base tiene una tonalidad amarilla y la mezcla de azul y amarillo es verde.

Confidencial

Aplicación en tintas de impresión, superficies varias

Aplicación en tintas de impresión, lengüeta



Farmacéutico: Pastillas



Confidencial

Textil: Teñido de telas





Señor

DIRECTOR DIRECCIÓN DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente N° 15-304877
Solicitud de Patente de Invención titulada “COMPUESTOS DE
COLORANTE DERIVADOS DE GENIPINA O MATERIALES QUE
CONTIENEN GENIPINA”.

Respuesta a oficio por artículo 45

NELSON URREGO, identificado con cédula de ciudadanía No. 79.574.305 de Bogotá, obrando como Apoderado de la sociedad **ECOFLOA S.A.S.**, según lo acredita el documento de poder adjunto, me permito dar respuesta a las observaciones de carácter técnico relacionadas con la patentabilidad para la concesión de la solicitud de la referencia en los términos del artículo 45 de la Decisión 486.

Reivindicaciones relativas a uso

La anterior reivindicación 6 ha sido modificada para cumplir con los requerimientos del artículo 14 de la Decisión 486.

De la solicitud de patente



Reivindicaciones

Para facilitar la concesión, adjuntamos un nuevo capítulo reivindicatorio de 1-18 reivindicaciones que acoge todas las recomendaciones realizadas por la Examinadora y que cumple con todos los requisitos establecidos en la Decisión 486.

Todas las modificaciones encuentran sustento suficiente en la descripción presentada inicialmente.

Sobre las reivindicaciones de método, la Examinadora requiere agregar condiciones o especificaciones, ya de lo contrario se consideran como no patentables. Aunque no estamos de acuerdo, en la nueva reivindicación 10 de método se indica que el proceso se desarrolla que el jugo y el aminoácido Genipa americana se mezclan bajo condiciones calor. De igual manera para las reivindicaciones 12 y la 13, que especifica aún más las condiciones de proceso. Estas modificaciones incorporan condiciones y especificaciones en los métodos de las anteriores reivindicaciones 12-15, ahora reclamaciones 10-13. Por lo tanto, estas nuevas definiciones caracterizan suficientemente los métodos reivindicados.

El soporte de las modificaciones de estas reivindicaciones de método se encuentra, sin referirse únicamente a estos apartes, en los párrafos 171, 229 y 232 de la publicación internacional de esta solicitud, en el ejemplo 9, los párrafos 208-212 y la table 2.

La Examinador solicita soporte suficiente para los productos y dispositivos reclamados. La solicitud presentada contiene información suficiente sobre los productos alimenticios, productos farmacéuticos, productos nutracéuticos,

productos cosméticos y dispositivos médicos mencionados en las reivindicaciones. Por ejemplo, el párrafo 140 en la solicitud presentada incluye varios productos alimenticios que pueden colorearse según la composición colorante reivindicada, incluidos productos lácteos, productos de panadería, refrescos, confitería, bebidas, alimentos para mascotas, alimentos para animales de granja, alimentos para plantas, yogur, pudín, sopa, etc. (Publicación WO '275, párrafo 140). La solicitud también incluye listas extensas de productos farmacéuticos (por ejemplo, tabletas, cápsulas de gel, pomadas, cápsulas, polvos, líquidos, geles y jarabes), productos nutracéuticos (por ejemplo, vitaminas, suplementos vitamínicos y mezclas dietéticas), productos cosméticos (por ejemplo, geles de ducha, jabón, maquillaje, esmalte de uñas, lápiz de labios, enjuague bucal, pasta de dientes, loción y crema de afeitar) y dispositivos médicos (por ejemplo, dispositivos quirúrgicos, hilos quirúrgicos, tijeras quirúrgicas, y guantes) que pueden ser coloreados por la composición colorante reivindicada. (Publicación WO '275, párrafos 141-144). En consecuencia, la solicitud en estudio incluye información y soporte suficientes para los productos y dispositivos reclamados.

Además, el solicitante proporciona Brauch, J.E., et al., Food Res. Int'l, 89: 391-398 (2016) ("Brauch") y un archivo con fotografías de los productos probados que comprenden la composición reivindicada, que muestran las aplicaciones e información adicional para las composiciones de colorantes reivindicadas y que apoyan aún más las reivindicaciones de productos y dispositivos incluidos.

En vista de las nuevas reivindicaciones presentadas con este memorial y las pruebas que también se anexan, solicitamos el retiro de las objeciones realizadas y la concesión de la patente para la invención objeto de la solicitud de la referencia.



Luego de este memorial pagaremos la tasa correspondiente a Examen de patentabilidad para estudio de las nuevas reivindicaciones.

Del señor Director,

NELSON URREGO

T.P. 93.909 del C. S. de la J.

C.C. No. 79.574.305 de Bogotá

Adjunto. Nuevo capítulo reivindicatorio de 1 - 18 reivindicaciones.
 Pruebas de productos.
 Artículo científico de los inventores.

MXB.



Jagua blue derived from *Genipa americana* L. fruit: A natural alternative to commonly used blue food colorants?



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pH

Storage and thermal treatment

ABSTRACT

Due to consumers' increasing health awareness, food industry aims at replacing synthetic dyes by natural counterparts. The substitution of blue synthetic dyes is particularly challenging since current natural alternatives such as phycocyanin (*Spirulina*) suffer from poor stability. Jagua blue (produced from *Genipa americana* L. fruit) might represent a potential novel blue pigment source. However, only little is known about its color properties, and application in food systems. Therefore, the blue color and the stability of Jagua blue were assessed for the first time and compared to commonly used colorants, namely, *Spirulina*, brilliant blue FCF (Blue no. 1), and indigo carmine (Blue no. 2). The reaction rate of Jagua blue was independent of its concentration, confirming thermal degradation to follow first-order kinetics. Between pH 3.6 and 5.0, the color hue of Jagua blue solutions was similar to that of Blue no. 2. However, Jagua blue revealed markedly higher storage stabilities ($t_{1/2} = 86\text{--}105$ days) than Blue no. 2 ($t_{1/2} \leq 9$ days) and was less susceptible to acidic pH of 3.6 ($t_{1/2} = 86$ days) than *Spirulina* ($t_{1/2} = 70$ days). High negative b^* values (blueness) of colored gelatin gels were only obtained for Jagua blue and *Spirulina*, and the former exhibited higher light stabilities ($t_{1/2} = 15$ days) than *Spirulina* gels ($t_{1/2} = 4$ days). Our findings indicate Jagua blue to be a most promising alternative to synthetic dyes, providing relevant information regarding potential food applications.

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1. Introduction

Color is considered as one of the most important attributes in food. Nowadays, there is a growing interest for the replacement of artificial colorants by natural counterparts since consumption of several synthetic dyes has been blamed to be harmful to human health, e.g. by promoting hyperactivity in children (McCann et al., 2007). Very few natural blue food colorants are commercially available to replace artificial dyes, such as brilliant Blue FCF (Blue no. 1) (1) and indigo carmine (Blue no. 2) (2) (Fig. 1). Moreover, current natural alternatives such as phycocyanin (*Spirulina*) (3), a blue extract from the dried biomass of the cyanobacteria *Spirulina* (syn. *Arthrospira platensis* and *A. maxima*)

approved for food use (Byrne, 2011; Watson, 2013) or previously suggested ferric anthocyanin chelates (Buchweitz, Brauch, Carle, & Kammerer, 2013a, 2013b; Newsome, Culver, & van Breemen, 2014; Sigurdson & Giusti, 2014), are mostly suffering from substantially poorer stabilities and do not match desired color hues and intensities (Newsome et al., 2014).

In recent years, *Genipa americana* L. fruit, also known as Genipa, Genipap, Jagua, or Huito, has been reported to be a potential novel pigment source for producing blue tints (Echeverri-Lopez, Zapata-Porras, & Torres-Roldan, 2010; Cano et al., 2014; Wu, Ford, & Horn, 2009). The tree originates from the Amazonian region and naturally grows throughout the tropical and partly subtropical rainforests of Latin America (UNCTAD, 2005). Its unripe fruit has been reported to contain high levels of iridoids, such as genipin (4), being essential for the blue color formation resulting from its reaction with primary amine sources (Fig. 1) (Bentes & Mercadante, 2014; Fujikawa et al., 1987). This color reaction has been previously used for the preparation of a water-soluble blue colorant, presently marketed as Jagua blue only in Colombia (Invima Comisión Revisora, 2014), by adding an unprocessed raw juice of the genipin-rich unripe fruit pulp to defined amounts of pure glycine (5) (Echeverri-Lopez et al., 2010). Besides Jagua blue, a similar *Genipa*-based blue colorant has been proposed by blending

Abbreviations: $(\text{area}^N_{580-750\text{nm}})_t$, blue color intensity at a given time t , area under the absorption curve of the spectra in the range between 580 and 750 nm; $\text{area}\%$, blue color hue, ratio of $(\text{area}^N_{580-750\text{nm}})_t$ and total area under the absorption curve; $E_{1\%}^{1\text{cm}}(1\%)$, color value; N , normalized, absorption spectra normalized to the minimum absorption between 350 and 520 nm; Jagua blue, colorant produced from unripe fruit juice of *G. americana* L. and glycine; *Spirulina*, phycocyanin from the dried biomass of the cyanobacteria *Spirulina*; Blue no. 1, brilliant blue FCF; Blue no. 2, indigo carmine.

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E-mail addresses: johanna_brauch@uni-hohenheim.de, johanna_brauch@gmx.de (J.E. Brauch).

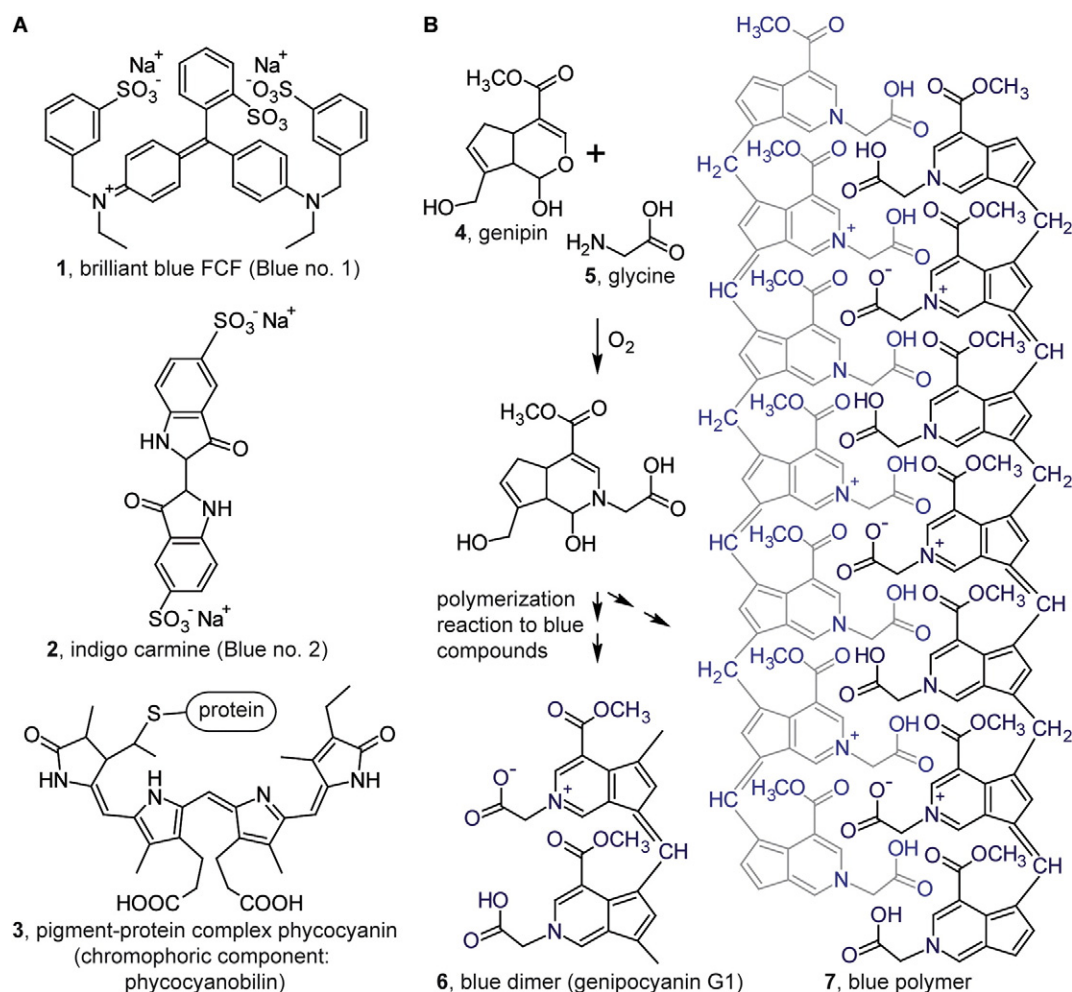


Fig. 1. (A) Chemical structures of brilliant blue FCF (Blue no. 1), indigo carmine (Blue no. 2), and phycocyanin (*Spirulina*). (B) Two suggested chemical structures of Jagua blue pigments: a blue dimer (Fujikawa et al., 1987) derived from the polymerization reaction of genipin and glycine (modified according to Bentes & Mercadante, 2014) and a blue polymer (according to Cano, Echeverri-Lopez, Gil-Romero, Correa-Garcés, & Zapata-Porras, 2014).

juice from ripe *Genipa* fruit with other amino acid- or protein-rich edible juices such as watermelon juice (Wu et al., 2009). Although not approved for EU and US markets, this colorant has been reported to be already in use in the US as color additive “fruit juice” (Title 21 CFR, Code of Federal Regulations, § 73.250) under the trade name “Huito”, or “acid-stable-blue” (Brauch, 2016; Wu & Horn, 2013). Cano et al. (2014) recently suggested the *Genipa* fruit-derived colorant to be composed of a complex mixture of polymers, where one polymer (CAS 1314879-21-4) (7) mainly imparting the blue color was prevailing. In addition, a blue dimer (6) has also been previously described (Fujikawa et al., 1987). Due to the high stability already reported for blue iridoid-based colorants obtained from *Gardenia jasminoides* J. Ellis fruit (Gardenia blue) (Jespersen, Strømdahl, Olsen, & Skibsted, 2005; Paik, Lee, Cho, & Hahn, 2001), blue colorants obtained from *G. americana* L. fruit quickly gained popularity. Thus, several companies currently aim at introducing the fruit and derived products into markets outside of Latin America, where they are most likely considered as novel food and food ingredients (Regulation (EC) No 258/97), respectively (UNCTAD, 2005; WTO, 2006). However, detailed reports on color evaluation and stability of Jagua blue are scarce to date.

The main goal of the present study was to evaluate for the first time the potential of Jagua blue as a natural blue colorant by investigating its color properties at food relevant pH values, and in a gelatin-based food model. The results should be compared to those obtained for Blue no. 1, Blue no. 2, and *Spirulina*. Moreover, the currently unknown kinetic order

of thermal degradation of Jagua blue should be determined using aqueous Jagua blue solutions. For this purpose, color hues and their stabilities were examined by monitoring CIE $L^*a^*b^*$ color parameters as well as wavelengths at maximum absorption in the visible range ($\lambda_{\max}$), and decreases in absorbance at $\lambda_{\max}$ as previously carried out for genipin-based colorants (Echeverri-Lopez et al., 2010; Ghidouche, Rey, Michel, & Galaffu, 2013; Jespersen et al., 2005; Paik et al., 2001). The description of color hues for solutions only defined by these values may be inaccurate since intensities may vary according to their differing molecule types. Therefore, sample colors were investigated by additional calculations of initial color values [$E_{\text{cm}}^{\lambda_{\max}}(1\%)$] as well as so-called area $_{580-750\text{nm}}^N$ (color intensity) and area% (color hue) values. The determination of the latter two color parameters has been recently suggested as a suitable method to precisely define the color of blue ferric anthocyanin chelates (Buchweitz et al., 2013b).

2. Materials and methods

2.1. Chemicals, reagents, and color samples

Sodium acetate, glacial acetic acid, and potassium sorbate were purchased from Merck (Darmstadt, Germany). Gelatin and crystalline sucrose were from Ruf Lebensmittelwerk (Quakenbrück, Germany) and Südzucker (Mannheim, Germany), respectively. All further chemicals were at least of analytical grade and obtained from Merck and VWR

(Darmstadt, Germany). Ultrapure water (Milli-Q system) from EMD Millipore (Bedford, MA) was used throughout.

Jagua blue, a standardized *G. americana* L. fruit-derived spray dried powder, was provided by Ecoflora (Sabaneta, Colombia). The sample was produced according to a previously published procedure (Echeverri-Lopez et al., 2010). It complied with the quality control parameters specified by the producer to ensure product uniformity. More details on the composition of the colorant, in particular the main polymer imparting the blue color (CAS 1314879-21-4) (7) (Fig. 1), may be found in the US patents 20140350127 and 20130345427 (Cano et al., 2014; Echeverry, Gil, & Vargas, 2013). Powders of phycocyanin (*Spirulina*, 21 CFR § 73.530), brilliant blue FCF (Blue no. 1, 21 CFR § 74.101 /E 133), and indigo carmine (Blue no. 2, 21 CFR § 74.102 /E 132) were kindly donated by Sensient Colors Europe (Geesthacht, Germany).

2.2. Storage and heating experiments of blue color solutions

Blue no. 1, Blue no. 2, Jagua blue, and *Spirulina* powders were dissolved in water to final concentrations of 4, 40, 400, and 14,600 mg/L (w/v), respectively. These concentrations were chosen to obtain similar maximum absorptions in the visible range (0.2–0.5 AU). Aliquots of 100 μ L of the aqueous stock solutions were combined with 1700 μ L of sodium acetate buffer solutions at pH 3.6, 4.0, 4.5, and 5.0, respectively. Samples were stored at room temperature in the dark for 1 h prior to verifying pH values. The storage experiment was carried out by depositing the solutions for 50 days at 20 ± 2 °C in the dark.

For heating experiments, Jagua blue powder was dissolved in water to obtain concentrations of 120, 300, and 750 mg/L (w/v; natural pH 5.5). Aliquots of 1 mL of these samples were heated in Eppendorf tubes in a water bath at 50, 60, 70 and 90 °C, respectively, for up to 14 h. At 1-h intervals, samples were cooled in an ice bath to stop thermal color degradation.

Aliquots of samples from storage and heating experiments were used for subsequent color analysis applying spectrophotometric measurements.

2.3. Color analysis of blue color solutions by spectrophotometric measurements

Spectrophotometric measurements were carried out from 350 to 800 nm in 2-nm steps using a Power Wave XS plate reader (BioTec Instruments, Winooski, VT, USA) equipped with Gen5 Software. Aliquots of 250 μ L of samples from storage and heating experiments were measured in 96-well polystyrene plates (VWR, Darmstadt, Germany). Prior to storage and measurements, samples for storage were covered with a transparent polyester film (Brand, Wertheim, Germany) to avoid water evaporation.

Initial blue color was expressed by the wavelength at maximum absorption ($\lambda_{\max}$). The method of Buchweitz et al. (2013b) was used to further characterize the color intensity, and the color hue of the samples. Accordingly, for color intensities, $(\text{area}^N_{580-750\text{nm}})_t$ values [AU*nm] were determined by calculating the blue area under the absorption curve of the normalized (N) spectra ranging from 580 to 750 nm at a given time t , as illustrated for $t = 0$ in Fig. 2. Color hues were obtained by calculating area% values [%], being the ratio of $\text{area}^N_{580-750\text{nm}}/\text{area}^N * 100\%$, where area^N represented the total area under the absorption curve of the normalized spectra. Further details on calculation of $\text{area}^N_{580-750\text{nm}}$ and area% may be found in the study of Buchweitz et al. (2013b). Noteworthy, initial color intensities were displayed as $(\text{area}^N_{580-750\text{nm}})_0$ per μ g colorant (Table 1) since different concentrations were necessary to obtain color solutions exhibiting similar maximum absorbance.

Besides the characterization of the samples by $\lambda_{\max}$, $\text{area}^N_{580-750\text{nm}}$, and area%, initial color values of the colorants were calculated as $E_{1\text{cm}}^{1\%}(1\%) = A * 10/m$ according to Yang, Zhou, Wei, Zhu, and Fan (2012), where A represented the maximum absorption in the visible

range and m is the mass [g] of the colorant. For this purpose, additional spectrophotometric measurements were carried out by using a GENESYS 10S UV–Vis spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA) equipped with VISIONlite version 2.0 software. Prior to measurements, Blue no. 1 and Blue no. 2 were adjusted with water to concentrations of 0.02 g/L (w/v), while Jagua blue and *Spirulina* were dissolved in water to concentrations of 0.20 and 0.60 g/L (w/v), respectively (natural pH 5.5–5.9). Aliquots of 1 mL of the color solutions were measured in semi-micro cuvettes (Brand, Wertheim, Germany), and absorption spectra were recorded from 350 to 800 nm in 2-nm steps.

2.4. Degradation kinetics of blue color solutions

Blue color stability of stored and heated samples was expressed as the ratio of $(\text{area}^N_{580-750\text{nm}})_t / (\text{area}^N_{580-750\text{nm}})_0 * 100\%$ (relative color retention), where $(\text{area}^N_{580-750\text{nm}})_t$ was the blue color intensity after a given period t and $(\text{area}^N_{580-750\text{nm}})_0$ was the initial blue color intensity prior to storage and thermal treatment. Half-life ($t_{1/2}$) of the samples was calculated from color intensity values according to first-order kinetics as described by Buchweitz et al. (2013b). Briefly, the natural logarithms of the $(\text{area}^N_{580-750\text{nm}})_t / (\text{area}^N_{580-750\text{nm}})_0$ ratios were plotted against the storage times [days] or heating times [hours], being $\ln[(\text{area}^N_{580-750\text{nm}})_t / (\text{area}^N_{580-750\text{nm}})_0] = -k * t$. The $t_{1/2}$ values [$t_{1/2} = (\ln 2) / k$] were subsequently calculated from the slope of the linear regression ($-k$).

2.5. Characterization of blue colored gelatin gels by CIE $L^*a^*b^*$

The mixture of the gelatin-based gels was prepared as previously described (Buchweitz et al., 2013a). Subsequently, 10 mL aliquots of the aqueous stock solutions were added to 100 g of the gelatin mixture at 79 °C. Aliquots of 10 g of the blue gels were filled into Petri dishes (35/10 mm) (Greiner bio-one, Frickenhausen, Germany). Prior to closing and sealing the Petri dishes with Parafilm®, samples were cooled to room temperature. Samples were placed in a climate chamber (Binder, Tuttlingen, Germany) and stored under controlled temperature (25 °C) and illumination (visible light, L36 W/840 and UV-light, LT36W/009) (Osram, Munich, Germany). Positions of the samples were rotated twice per week to ensure uniform light exposure. Furthermore, blue gels were stored at 25 °C in the dark.

Color and its stability in gel samples was assessed by monitoring CIE $L^*a^*b^*$, chromaticity C^* , and hue angle h° at selected storage days using a reflection colorimeter (Chromameter CR-300) (Konica Minolta, Osaka, Japan). The equipment was calibrated with a standard white tile ($L^* = 97.43$, $a^* = 0.01$, $b^* = 1.64$) and set for illuminant D65, and a 2° observer angle for all samples.

Additional blue colored gel samples were used to measure their respective initial water activity (a_w ; AquaLab CX-2) (Gemini B.V., Apeldoorn, Netherlands), soluble solid content (refractometer, RX-5000) (Atago, Tokyo, Japan), and pH value (one measurement per sample). Obtained values were expressed as the average calculated from all samples. Soluble solid content and pH value of the samples were determined to be 63.6 ± 0.4 °Brix and 5.6 ± 0.1 , respectively.

For characterization of color stability, total color differences $[\Delta E_{ab}^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}]$ between different samples at the beginning and after storage were determined. Additionally, the half-life ($t_{1/2}$) of the gel samples was calculated according to first-order kinetics from negative b^* values, representing the blue color loss. The $t_{1/2}$ values were calculated from the slope ($-k$) of the linear regression from $\ln(b^*/b^*_0) = -k * t$ as also described by Buchweitz et al. (2013a).

2.6. Statistical analysis

All samples were prepared in triplicate with two repeated measurements for each replication. Results were expressed as mean

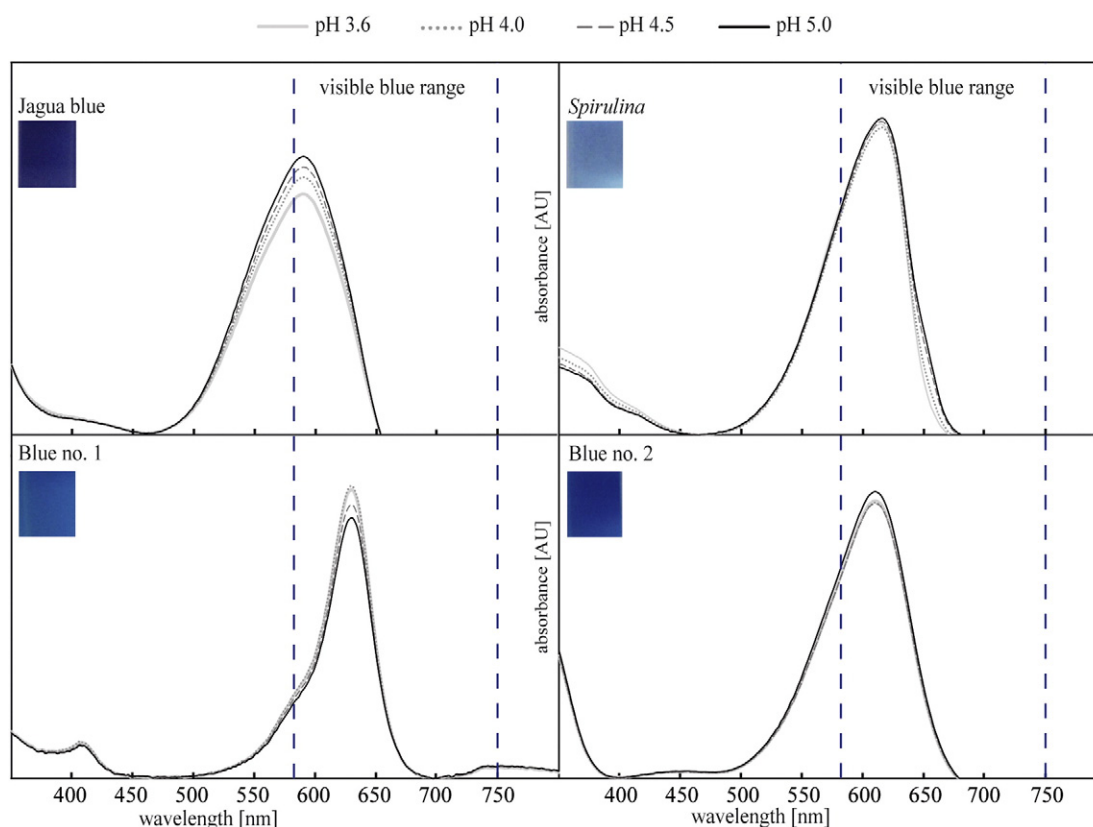


Fig. 2. Normalized absorption spectra of color solutions prepared with Jagua blue, *Spirulina*, Blue no. 1, and Blue no. 2 at different pH values. The visible blue color range is indicated by the dotted lines.

values $\pm$ standard deviation ($n = 3$) calculated from the mean values obtained from the samples prepared in triplicate. In order to determine significant differences of means at $P < 0.05$, analysis of variance (ANOVA) comprising the Tukey test was carried out with JMP 6 (SAS, Cary, NC, USA).

Table 1

Initial wavelengths of maximum absorption ($\lambda_{\max}$),^a color hues (area%),^a and intensities [(area^N_{580–750nm})₀]^a of blue color solutions at different pH values as well as color values [$E_{1\text{cm}}^{\max}(1\%)$]^a.

pH	Color solution			
	Jagua blue	<i>Spirulina</i>	Blue no. 1	Blue no. 2
	$\lambda_{\max}$ [nm]			
3.6	588.0 $\pm$ 0.0d	616.0 $\pm$ 0.0b	629.3 $\pm$ 1.2a	609.3 $\pm$ 1.2c
4.0	588.7 $\pm$ 1.2d	616.0 $\pm$ 0.0b	628.7 $\pm$ 1.2a	608.7 $\pm$ 1.2c
4.5	589.3 $\pm$ 1.2d	616.0 $\pm$ 0.0b	629.3 $\pm$ 1.2a	609.3 $\pm$ 1.2c
5.0	588.7 $\pm$ 1.2d	617.0 $\pm$ 1.4b	629.3 $\pm$ 1.2a	610.0 $\pm$ 0.0c
	area% [%]			
3.6	51.1 $\pm$ 0.0 g	71.7 $\pm$ 0.0d	85.8 $\pm$ 0.2a	69.2 $\pm$ 0.0e
4.0	51.4 $\pm$ 0.1f	72.9 $\pm$ 0.1c	85.5 $\pm$ 0.1a	69.2 $\pm$ 0.1e
4.5	51.4 $\pm$ 0.0f	73.6 $\pm$ 0.0b	85.4 $\pm$ 0.5a	69.1 $\pm$ 0.0e
5.0	51.2 $\pm$ 0.0 g	73.6 $\pm$ 0.0b	85.4 $\pm$ 0.4a	69.0 $\pm$ 0.1e
	(area^N_{580–750nm})₀ [(AU*nm)/μg]			
3.6	5.5 $\pm$ 0.1 h	0.2 $\pm$ 0.0i	359.8 $\pm$ 6.2ab	120.5 $\pm$ 2.6d
4.0	5.9 $\pm$ 0.1 g	0.2 $\pm$ 0.0i	366.3 $\pm$ 7.2a	119.9 $\pm$ 3.3d
4.5	6.2 $\pm$ 0.0f	0.3 $\pm$ 0.0i	344.8 $\pm$ 4.6bc	119.9 $\pm$ 3.5d
5.0	6.4 $\pm$ 0.0e	0.3 $\pm$ 0.0i	331.0 $\pm$ 6.6c	125.5 $\pm$ 12.9d
	$E_{1\text{cm}}^{\max}(1\%)$			
	29.5 $\pm$ 0.1	6.1 $\pm$ 0.1	1359.1 $\pm$ 18.5	451.5 $\pm$ 39.7

^a Mean values $\pm$ standard deviation ($n = 3$). Significant differences between values in the same column are indicated by different letters ($P < 0.05$).

3. Results and discussion

3.1. Impact of pigment type and pH on color appearance of blue color solutions

Wavelength of maximum absorption ($\lambda_{\max}$) of each pigment type remained unaffected by the different pH values (3.6–5.0) of the color solutions (Fig. 2, Table 1). Color solutions of Jagua blue (B) (Fig. 1) and the synthetic colorant Blue no. 2 (2) exhibited lowest $\lambda_{\max}$ values of 589 and 609 nm, respectively. By contrast, $\lambda_{\max}$ values of *Spirulina* (3) and the synthetic Blue no. 1 (1) color solutions were significantly higher (616 and 629 nm, respectively).

Based on the pigment type, color solutions differed in their initial blue color hues (area%) and intensities [(area^N_{580–750nm})₀] (Table 1). For Jagua blue color solutions, area% values of 51% and (area^N_{580–750nm})₀ values ranging from 5.5 (pH 3.6) to 6.4 (AU*nm)/μg (pH 5.0) reflected intense blue hues with a faint violet tint. By contrast, area% values of 73% for the *Spirulina* color solutions shifted to more bluish color hues. However, they exerted up to 24-fold lower color intensities [0.3 (AU*nm)/μg] than the Jagua blue samples. The synthetic variants Blue no. 1 and Blue no. 2 showed highest blue color intensities of 331.0–366.3 (AU*nm)/μg and 119.9–125.5 (AU*nm)/μg, respectively. While Blue no. 1 samples showed the most blue color hues (area% = 85%), those of Blue no. 2 (69%) were comparable to *Spirulina* and Jagua blue. Color values $E_{1\text{cm}}^{\max}(1\%)$ followed (area^N_{580–750nm})₀ values throughout the whole experiment. For example, Jagua blue exhibited markedly higher $E_{1\text{cm}}^{\max}(1\%)$ values (29.5) than *Spirulina* (6.1). Noteworthy, blue color intensity of *Spirulina*-based colorants may vary, depending on the method used for their purification (Rito-Palomares, Nuñez, & Amador, 2001).

The impact of pH on color appearance was less pronounced than that of the pigment type. Significant differences in area% values were only determined for the natural pigment sources (Table 1). However,

their slight changes in color hues were not visually perceivable. While color intensities determined for *Spirulina* and Blue no. 2 were not affected by the pH, those of Jagua blue and Blue no. 1 changed significantly in the studied pH-range between 3.6 and 5.0. Both Jagua blue and Blue no. 1 possess acidic groups, i.e., carboxyl and sulfonate groups, respectively (Fig. 1), that may dissociate in aqueous solution. Thus, the higher color intensity of Jagua blue samples at pH 5.0 [6.4 (AU*nm)/μg] was most likely supported by the dissociated form of its pigments. By contrast, Blue no. 1 exhibited its maximum color intensity under acidic conditions [359.8 (AU*nm)/μg, pH 3.6], where the pigment has been reported to change from its anionic to the neutral form (Flury & Flühler, 1994). Noteworthy, pH-dependent differences in initial color intensities observed in our study were below 13%, thus not impeding industrial application of the two colorants to food products within the low acidic to acidic range.

Previously, intense blue color hues have been reported for ferric anthocyanin chelate solutions when revealing area% values in the range of 55–70%, while those having lower area% values reflected blue hues with a faint violet tint (Buchweitz et al., 2013b). By contrast, a more greenish blue tint has been described for color solutions containing Blue no. 1 (Khera, Munro, & Radomski, 1979), as confirmed by the high area% values determined in our study. Nevertheless, Jagua blue color solutions have been shown to contain complex mixtures of different polymeric components (Cano et al., 2014). Geniposide, the glycosylated form of genipin being the major iridoid in unripe *G. americana* L. fruits (Bentes & Mercadante, 2014), has been reported to react with glycine to purple pigments (Cano et al., 2014). Hydrolyzing the glycosidic bond of geniposide in the presence of a β-glucosidase allows the reaction of genipin with glycine, yielding blue pigments. Thus, the faint violet tint of Jagua blue used in the present study may be avoided by the addition of β-glucosidase to the unripe fruit pulp, favoring the blue pigment formation upon the addition of glycine.

3.2. Degradation kinetics of Jagua blue

The determination of degradation kinetics is essential to predict the color loss of Jagua blue. In previous studies (Ghidouche et al., 2013; Jespersen et al., 2005; Paik et al., 2001), degradation kinetics has been determined by calculating kinetic parameters via zero, first and second order, and subsequently searching the best fitting model by comparing the respective correlation coefficients (R^2). In the present study, first-order kinetics was assumed for Jagua blue. This was further investigated by calculating its thermal degradation rates from color intensity values at different concentrations and temperatures. A reaction rate being independent of the initial concentration is the clear indication of first-order kinetics [$t_{1/2} = \ln(2) / k$].

Pigment concentrations of Jagua blue under isothermal conditions did not affect half-life ($t_{1/2}$) (Table 2), thus confirming the assumed first-order degradation. As expected, $t_{1/2}$ values of the color solutions decreased with increasing temperatures from 211.9 h at 50 °C to 13.5 h at 90 °C. The effect of thermal treatment on color hue was negligible, showing variations in area% values ($\Delta\text{area}\%$) between 1.6% and 3.8%, most likely being a result of the slight hypsochromic shifts (1.3–2.2 nm) observed. Even at the harshest heat treatment (90 °C), the intense blue color of the Jagua blue samples was retained.

The exact pigment composition of iridoid-based colorants has been reported to strongly depend on the reaction conditions (e.g., pH, temperature, genipin:amino acid ratio) used for their production (Cho et al., 2006; Lee, Lim, Bhoo, Paik, & Hahn, 2003; Wu et al., 2009; Yang et al., 2012). This may have a great influence on the degradation mechanism of these colorants, thus hampering comparison of their color stabilities. Moreover, studies on the thermal stability of blue colorants derived from *G. americana* L. fruit juice and its reaction products with glycine as used in this experiment are scarce. Exceptionally high thermal stabilities have previously been reported for iridoid-based blue colorants obtained from *Gardenia jasminoides* J. Ellis fruits (Jespersen et al.,

Table 2

Kinetic data^a of Jagua blue color solutions at different concentrations during thermal treatment at various temperatures assuming first-order decay.

Jagua blue concentration [mg/L]	Temperature [°C]			
	50	60	70	90
Half-life, $t_{1/2}$ [h]				
750	212.7 ± 18.1a	143.6 ± 29.1b	35.3 ± 3.5c	14.6 ± 1.5d
300	201.2 ± 24.1a	149.6 ± 27.1b	39.3 ± 2.9c	13.2 ± 2.7d
120	221.7 ± 26.2a	147.6 ± 13.6b	(87.6 ± 56.0)*	12.7 ± 3.9d
Average	211.9	150.5	37.3**	13.5
Correlation coefficient, R^2				
750	0.9542 ± 0.0384	0.9544 ± 0.0273	0.9267 ± 0.0135	0.9603 ± 0.0193
300	0.9419 ± 0.0336	0.9500 ± 0.0248	0.9356 ± 0.0131	0.9462 ± 0.0135
120	0.9228 ± 0.1112	0.9103 ± 0.0415	(0.7358 ± 0.3055)*	0.9517 ± 0.0087
Average	0.9396	0.9383	0.9311**	0.9527
$\Delta\text{area}\%$ [%]				
750	−1.7 ± 0.2	−2.3 ± 0.5	−1.5 ± 0.1	−3.5 ± 0.1
300	−2.4 ± 0.1	−3.3 ± 0.0	−1.7 ± 0.0	−3.9 ± 0.1
120	−2.7 ± 0.2	−3.6 ± 0.1	(−1.6 ± 0.1)*	−3.9 ± 0.1
Average	−2.3	−3.0	−1.6**	−3.8

^a Mean values ± standard deviation ($n = 3$). Significant differences between values in the same column are indicated by different letters ($P < 0.05$). $\Delta\text{area}\%$, color hue shift, initial area% – area% after thermal treatment.

* Value of low quality due to low R^2 .

** Average calculated without the value of concentration 120 mg/L.

2005; Paik et al., 2001). However, the color decay of *Gardenia* pigments (*Gardenia* blue) has been suggested to follow a zero or not simply a first-order reaction. In contrast and in accordance with our study, Ghidouche et al. (2013) reported that the blue color decay of the so-called “fruit juice color blend” Huito containing *Genipa* fruit juice and further edible juices was best fitted with first-order kinetics when stored under daylight exposition.

Jespersen et al. (2005) reported only 65% of residual absorbance after storage of a phycocyanin solution derived from *Spirulina platensis* for 5 h at 55 °C (pH 5), most likely due to thermal denaturation of the protein-based pigment above 50 °C. In a further study, the blue color decay of blue ferric anthocyanin chelates has been assessed at 50–80 °C by using ($\text{area}_{580-750\text{nm}}^N$)_t values (Buchweitz et al., 2013b). Jagua blue solutions in our study, however, showed an impressively higher pigment stability ($t_{1/2} = 150.5$ h, 60 °C) than that reported for blue ferric anthocyanin chelate solutions based on a purple carrot extract ($t_{1/2} = 4.0$ h, 60 °C, pH 5.0).

3.3. Storage stability of blue color solutions at different pH values

Blue color hue (area%) of all color solutions investigated remained stable throughout the entire storage period of 50 days at 20 °C in the dark ($\Delta\text{area}\% = -3.1$ –2.5). However, based on relative color retentions (Table 3), Jagua blue solutions were considerably less stable (71–75%) than those of Blue no. 1 (95–96%), which exhibited a poor fit to the first-order kinetics model (correlation coefficient, $R^2 = 0.76$ –0.85). As also previously reported (Khera et al., 1979; Kuramoto, Lachman, & Cooper, 1958), Blue no. 2 was unstable in aqueous solution, and the blue color faded within one week. Only at the highest pH investigated, 3% of initial color intensity was retained until the end of storage, resulting in a half-life ($t_{1/2}$) of 9 days. In accordance to previous results (Chaiklahan, Chirasuwan, & Bunnag, 2012), the color degradation of *Spirulina* solutions followed first-order reaction kinetics ($R^2 = 0.90$ –0.94). While degradation kinetics of Jagua blue was only marginally affected by the pH, *Spirulina* samples showed significantly higher $t_{1/2}$ values

Table 3

Color retention,^a half-life values ($t_{1/2}$, first-order decay),^a and corresponding correlation coefficients (R^2)^a of blue color solutions during 50 days of storage in the dark (20 °C) at different pH values.

pH	Color solution			
	Jagua blue	<i>Spirulina</i>	Blue no. 1	Blue no. 2
Color retention after 50 days [%]				
3.6	70.9 ± 0.1a	64.9 ± 0.6f	94.9 ± 0.9ab	0.0 ± 0.0
4.0	71.6 ± 0.0a	76.0 ± 0.5d	94.7 ± 0.3ab	0.0 ± 0.0
4.5	73.4 ± 0.0a	84.7 ± 0.4bc	95.2 ± 1.1ab	0.0 ± 0.0
5.0	75.0 ± 0.3a	87.9 ± 0.3a	95.5 ± 0.4a	2.7 ± 0.6
$t_{1/2}$ [d]				
3.6	85.9 ± 0.3 cd	69.8 ± 0.3ef	501.6 ± 120.1ab	n.a.
4.0	88.7 ± 0.5bcd	107.0 ± 2.7d	448.3 ± 48.8ab	n.a.
4.5	96.7 ± 0.7abc	168.6 ± 6.1bc	492.0 ± 126.8ab	n.a.
5.0	104.6 ± 2.5a	219.7 ± 10.5a	514.8 ± 70.1ab	8.6 ± 0.7
R^2				
3.6	0.9281 ± 0.0144	0.9357 ± 0.0064	0.8545 ± 0.0091	n.a.
4.0	0.9433 ± 0.0029	0.9135 ± 0.0079	0.8281 ± 0.0153	n.a.
4.5	0.9489 ± 0.0015	0.8970 ± 0.0012	0.7908 ± 0.0558	n.a.
5.0	0.9550 ± 0.0030	0.8985 ± 0.0135	0.7565 ± 0.0320	0.9805 ± 0.0051

^a Mean values ± standard deviation ($n = 3$). Significant differences between values in the same column are indicated by different letters ($P < 0.05$). n.a., not available due to color loss within 7 days of storage.

when stored at pH 5.0 (220 days) compared to a storage at pH 3.6 (70 days). Thus, at pH 3.6, Jagua blue was shown to exhibit a higher color stability ($t_{1/2} = 86$ days) than *Spirulina*. This may be due to the proteinaceous nature of the latter (phycocyanin, Fig. 1) that has been reported to lose its color by acid-induced protein denaturation (Chaiklahan et al., 2012). By contrast, Jagua blue pigments are composed of a pyridine-based structure (Fig. 1), being less susceptible to acidic pH conditions.

Studies investigating storage stability of Jagua blue are scarce and have generally been carried out by simply monitoring the decrease in absorbance at $\lambda_{\max}$. Recently, Jagua blue prepared from an unripe *G. americana* L. fruit juice and glycine has been found to degrade markedly faster (36% absorbance reduction) under acidic conditions (pH 3.0), compared to low acidic conditions (pH 5.0, 18% absorbance reduction) when stored for 15 days at 20 °C (Echeverri-Lopez et al., 2010). In accordance with our results, drastically lower color stabilities compared to Jagua blue have been determined for Blue no. 2 under the same storage conditions. Thus, Jagua blue might represent a promising natural alternative for Blue no. 2, revealing a similar blue color hue and generally higher pigment stability.

3.4. Initial color appearance of blue colored gelatin gels

Based on the results obtained for blue color solutions, color stabilities of Jagua blue, *Spirulina*, Blue no. 1 and Blue no. 2 should be assessed in a real food matrix possessing reduced water activity ($a_w = 0.865 \pm 0.010$). For this purpose, the colorants were applied to a gelatin-based gel.

Initial gel colors markedly differed depending on the pigment type applied. Blue hues were observed for gels containing Jagua blue and *Spirulina*, while those produced with Blue no. 1 and Blue no. 2 exhibited turquoise green and blue hues, respectively. Accordingly, Jagua blue and *Spirulina* samples exhibited slightly positive a^* values (redness) of 1.2 and 2.3, respectively (Fig. 3). By contrast, greenish blue color hues of gelatin gels dyed with both synthetic colorants were characterized by markedly negative a^* values (greenness) of -8.2 to -11.5 . Intense blue colors as determined by distinct negative b^* values (blueness) of -9.6 and -20.6 were only observed when gelatin mixtures were colored with Jagua blue and *Spirulina*, respectively. Thereby, identical hue angles h° (277°) for both gelatin gels were obtained being in the blue range of the $L^*a^*b^*$ color space (Table 4, Fig. 3). Due to their high negative a^* values, h° values of gelatin gels containing Blue no. 2 and Blue no.

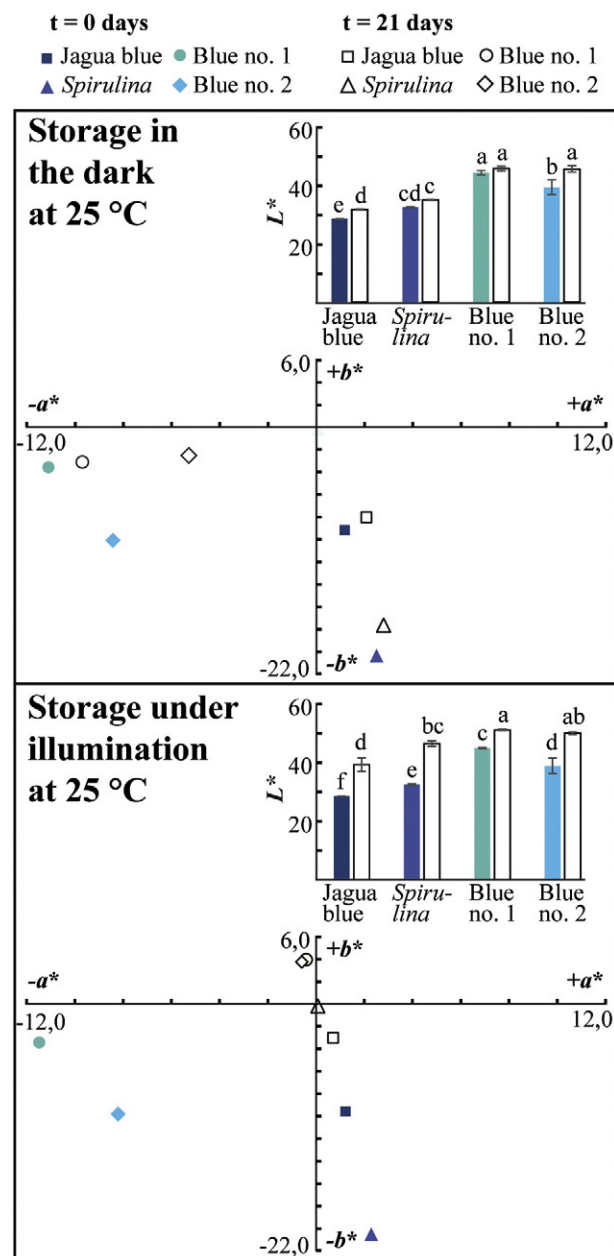


Fig. 3. Color values a^* and b^* as well as lightness (L^*) of blue colored gelatin gels before and after 21 days of storage at 25 °C in the dark and under illumination, respectively.

1 were located in the bluish green (229°) and green range (198°), respectively. Initial L^* and C^* values of colored gels ranged from 28.6 (Jagua blue) to 44.9 (Blue no. 1) and 9.7 (Jagua blue) to 20.7 (*Spirulina*), respectively. Since pigment concentrations differed, gels were not further compared by their initial lightness (L^*) and chromaticity (C^*) values.

3.5. Storage stability of blue colored gelatin gels

As expected, the color degradation of all gels investigated depended on storage conditions. Markedly higher increases in lightness ΔL^* (6–14) and decreases in chromaticity ΔC^* [-7 –(-21)] were determined for the illuminated samples compared to those stored in the dark [$\Delta L^* = 1$ –6; $\Delta C^* = -1$ –(-7)] (Fig. 3, Table 4). Additionally, blue color losses, expressed by negative b^* values, were stronger during illuminated storage ($\Delta b^* = 7$ –20) than in the dark ($\Delta b^* = 1$ –7) (Fig. 3).

Table 4

Hue angle (h°),^a chromaticity (C^*),^a and total color differences (ΔE_{ab}^*)^a as well as kinetic data (first-order decay)^a of blue gelatin gels during 21 days of storage under different conditions at 25 °C.

Storage condition	Gelatin gel			
	Jagua blue	<i>Spirulina</i>	Blue no. 1	Blue no. 2
h°				
Dark, day 0	277.1 ± 0.9a	276.9 ± 1.1a	197.8 ± 1.1c	229.0 ± 7.7b
Dark, day 21	284.3 ± 0.6a	278.9 ± 1.4a	197.8 ± 1.3b	204.5 ± 6.6b
Illumination, day 0	277.2 ± 0.8a	276.4 ± 1.2a	196.6 ± 0.3c	229.1 ± 0.3b
Illumination, day 21	284.0 ± 6.1a	295.6 ± 7.4a	95.9 ± 0.5b	99.0 ± 1.5b
C^*				
Dark, day 0	9.2 ± 0.2c	20.5 ± 0.4a	11.6 ± 0.4bc	13.2 ± 2.8b
Dark, day 21	8.3 ± 0.0bc	17.8 ± 0.6a	10.2 ± 0.4b	5.9 ± 1.3c
Illumination, day 0	9.7 ± 0.4c	20.7 ± 0.8a	12.0 ± 0.2bc	12.9 ± 2.6b
Illumination, day 21	3.1 ± 0.9ab	0.2 ± 0.2b	4.0 ± 0.0a	3.8 ± 0.5a
ΔE_{ab}^*				
Dark	3.5 ± 0.1b	3.7 ± 0.3b	2.1 ± 0.0b	10.4 ± 2.3a
Illumination	12.6 ± 2.6b	24.8 ± 1.1a	14.7 ± 0.1b	19.2 ± 4.4ab
$t_{1/2}$ [d]				
Dark	118.1 ± 17.7ab	103.7 ± 8.3b	145.4 ± 11.3a	11.1 ± 1.1c
Illumination	14.5 ± 3.2a	4.1 ± 0.6b	n.a.	n.a.
R^2				
Dark	0.6845 ± 0.0491	0.6854 ± 0.0780	0.8217 ± 0.0621	0.9928 ± 0.0047
Illumination	0.9776 ± 0.0072	0.8836 ± 0.0390	n.a.	n.a.

^a Mean values ± standard deviation ($n = 3$). Significant differences between values in the same row are indicated by different letters ($P < 0.05$). n.a., not available due to color loss within 2 days of storage; $t_{1/2}$, half-life values calculated from negative b^* values.

Thus, comparatively low total color differences (ΔE_{ab}^*) of 2–10 were determined for gels stored in the dark, while up to 7-fold higher ΔE_{ab}^* values were obtained for those exposed to light, which was unambiguously perceivable by visual inspection. Most interestingly, blue color loss of gels colored with Jagua blue, *Spirulina*, and Blue no. 1 showed a low correlation to the first-order kinetics model when stored in the dark ($R^2 = 0.68$ – 0.82), while under illumination color decay was assumed to follow first-order kinetics.

Different pigment types had the strongest influence on blue color stability of the gels. As exemplified by half-life ($t_{1/2}$) values calculated from negative b^* values, the blue color of the *Spirulina* containing gel degraded 4-fold faster than that of the Jagua blue-based gel after light exposure (Table 4). Due to significantly higher decreases in negative b^* values and positive a^* values (Fig. 3), the color hue change of the *Spirulina* sample was more pronounced ($\Delta h^\circ = 19$) than that of Jagua blue ($\Delta h^\circ = 7$) (Table 4). Interestingly, turquoise color already faded after 2 days of storage under illumination in gels containing synthetic pigments. This observation was confirmed by marked changes in a^* and b^* values towards the yellowish range (Fig. 3). While Blue no. 2 has already been reported to be unstable to light and reducing sugars (Kuramoto et al., 1958; Pasiás, Asimakopoulou, & Thomaidis, 2015), Blue no. 1 is known for its good light stability (Hajratwala, 1974). Further study is required to elucidate whether the gelatin gel matrix or the low pigment concentration of Blue no. 1 in the gel may have contributed to the faster color degradation when stored under illumination.

The half-life of the Jagua blue gel stored under illumination ($t_{1/2} = 14.5$ days) was similar to those that have been reported for gels containing ferric anthocyanin chelates prepared with red cabbage juice (10.4–16.9 days) (Buchweitz et al., 2013a). However, an even higher color stability has been described for illuminated ferric anthocyanin chelates-based gels derived from a purple carrot extract. Noteworthy,

the mode of illumination applied in the previous study (visible light) differed from our experiments (visible and UV-light). Additionally, the proteinaceous gel matrix might have contributed to the faster color degradation of Jagua blue. By contrast, it might have supported color stabilities of the ferric anthocyanin chelates. For instance, positively charged gelatin is known for its interaction with negatively charged phenolic compounds (González-Neves, Favre, & Gil, 2014).

4. Conclusion

In conclusion, Jagua blue pigments were characterized by a color hue similar to the synthetic food colorant Blue no. 2. Compared to Blue no. 2, Jagua blue displayed markedly higher color stabilities in both aqueous solutions under acidic to low acidic conditions and a gelatin-based gel. However, the color hue of Jagua blue did not match that of Blue no. 1. Nevertheless, since Jagua blue is composed of several different polymers displaying a variety of blue hues, further studies are required to investigate their individual color properties, aiming at complete conformity to Blue no. 1. Although the color hue of *Spirulina* solutions was comparable to that of Blue no. 1, its susceptibility to acidic pH imparted by its proteinogenic nature has to be considered when applying to food. First-order kinetics suggested for the thermal degradation of Jagua blue may be instrumental to predict color decay in dyed food.

According to the results presented, Jagua blue may be considered a promising natural alternative for coloring at acidic to low acidic pH ranges. Noteworthy, since Jagua blue is produced from a plant-derived raw material, variations in its composition, and thus color intensity may naturally occur. Therefore, clearly defined quality parameters have been specified for Jagua blue to ensure consistent color and batch uniformity of the final preparation. Finally, in order to meet the legal requirements for introducing Jagua blue on the EU and US markets, further investigations with focus on safety assessments should be performed as specified in the Commission Recommendation 97/618/EC under the Novel Food Regulation (EC) No 258/97 and in 21 CFR (Code of Federal Regulations) Part 71 by the FDA (Food and Drug Administration), respectively.

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