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THE AMAZING COLORS OF PERUVIAN BIODIVERSITY: SELECT PERUVIAN PLANTS FOR USE AS FOOD COLORANTS

Los Colores Maravillosos de la Biodiversidad Peruana: Plantas Peruanas Selectas con Uso Para Colorantes de Alimentos

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ABSTRACT

The increasing consumer demand for more nutritious foods, naturally sourced ingredients, and cleaner labels is pushing the food and cosmetic industries to transition from the use of artificial colorants towards naturally sourced alternatives. In this context, the industry is continuously searching for sources of more stable colorants, with special interest in plant sources. The vibrant biodiversity found in Peru represents an exciting economic opportunity. In this review, we highlight select Peruvian crops with excellent potential for use as colorant sources for industrial applications, with some sources being extensively studied and others receiving attention in more recent years. Purple corn, a crop native to the Andes region, is a rich source of pigments with great stability and a long history of use in different applications around the world. Colored-fleshed potatoes, underutilized Andean crops, can express different colors due to their assorted pigment profiles. Sauco, the Peruvian elderberry, has strong antioxidant activity and a unique pigment profile that gives it its characteristic black-purple color. Berberis species, a diverse class of shrubs with highly-pigmented berries, can be directly used as color additives without the need of extraction

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procedures. Huito, an understudied fruit native to the Amazon, is naturally colorless, but it can turn blue when exposed to oxygen or amino acids and can express different hues depending on the source of the primary-amine group. Overall, purple corn, colored-fleshed potatoes, sauco, Berberis species, and huito are promising Peruvian sources of natural colorants for food and cosmetic applications due to their versatility, stability, and attractive color characteristics.

Keywords: amazing colors | biodiversity | peruvian plants | foods colorants

RESUMEN

La creciente demanda de los consumidores de alimentos más nutritivos, ingredientes de origen natural y etiquetas más limpias está empujando a las industrias de alimentos y cosméticos a pasar del uso de colorantes artificiales a alternativas de origen natural. En este contexto, la industria busca continuamente fuentes de colorantes más estables, con especial interés en las fuentes vegetales. La vibrante biodiversidad que se encuentra en el Perú representa una emocionante oportunidad económica. En esta revisión, destacamos cultivos peruanos seleccionados con excelente potencial para su uso como fuentes de colorantes para aplicaciones industriales, con algunas fuentes que se estudian ampliamente y otras que reciben atención en años más recientes. El maíz morado, un cultivo nativo de la región de los Andes, es una rica fuente de pigmentos con gran estabilidad y una larga historia de uso en diferentes aplicaciones alrededor del mundo. Las papas de pulpa coloreada, cultivos andinos subutilizados, pueden expresar diferentes colores debido a sus perfiles de pigmentos variados. Sauco, la baya del saúco peruana, tiene una fuerte actividad antioxidante y un perfil de pigmento único que le da su característico color negro-púrpura. Las especies de Berberis, una clase diversa de arbustos con bayas altamente pigmentadas, se pueden usar directamente como aditivos de color sin necesidad de procedimientos de extracción. Huito, una fruta poco estudiada originaria del Amazonas es naturalmente incolora, pero puede volverse azul cuando se expone al oxígeno o a los aminoácidos y puede expresar diferentes tonalidades según la fuente del grupo de aminas primarias. En general, el maíz morado, las papas de pulpa coloreada, el sauco, las especies de Berberis y el huito son fuentes peruanas prometedoras de colorantes naturales para aplicaciones alimentarias y cosméticas debido a su versatilidad, estabilidad y atractivas características de color.

Palabras clave: colores sorprendentes | biodiversidad | plantas peruanas | colorantes alimentarios

1. INTRODUCTION

Color is an important aspect of life as it impacts perception and senses (Sigurdson et al., 2017). This influence is epitomized when eating. The color of food and beverages may alter the perceived odor intensity (Zellner & Whitten, 1999), flavor identity (Zampini et al., 2007), sweetness perception (Johnson et al., 1982), and overall acceptability of a product (Johnson et al., 1982; Spence, 2015). Color also indicates that a product is ready for consumption such as with banana peel colors and brown spots being used to indicate ripeness (Mendoza & Aguilera, 2006). The food industry is therefore motivated to deliver food with the proper color–a goal oftentimes achieved by the addition of dyes and pigments. Color additives may be used to standardize natural crops, to compensate for color performance and flavor expectations in products as seen with addition of red colorants to develop a pink hue in strawberry yogurt, or to create new products such as blue raspberry flavor (Sigurdson et al., 2017).

Although safety regulation is robust, consumers are worried about the safety of artificial colorants. One of the most prevalent concerns is a link between childhood hyperactivity and artificial color consumption as reported in the Southampton study (McCann et al., 2007). In a toxicology review by Kobylewski and Jacobson (2012), research was presented showing linkages between the artificial food dyes and allergies, hyperactivity, genotoxicity, and cancer. However, additional research is needed and limitations including bias and study duration exist among many of the current studies (Kobylewski & Jacobson, 2012). Despite continued approval on the safety of artificial colorants by regulatory agencies, consumer concerns and social clean-label trends are leading to the use of naturally derived pigments for coloring food (Sigurdson et al., 2017). These naturally derived colorants generally have favorable consumer perceptions, but it is challenging for food manufacturers to match the hues, stability, and vibrant colors of artificial pigments with the current portfolio of available naturally sourced pigments (Wrolstad & Culver, 2012).

These new consumer trends and the limitations of the existing naturally sourced pigments represent an exciting opportunity for countries with an ample plant diversity such as Peru. In this review, we aim to highlight purple corn, colored-fleshed potatoes, sauco, Berberis species, and huito—pigment sources with potential for industrial applications and the production of naturally sourced colorants.

2. PURPLE CORN

Purple corn (*Zea mays L.*), also known as purple maize, is a crop native to the Andes regions of Peru and has been widely cultivated and consumed throughout the Andean region of South America, mainly Peru, Ecuador, Bolivia, and Argentina. Its deep purple shade has led to its pigments being used to color food and beverages (Luna-Vital et al., 2017; Chatham, Howard, and Juvik, 2020). For example, in South America, purple corn extracts are widely applied as colorants in two of the most popular homemade dessert and beverages–mazamorra and chicha morada, respectively (FAO, 2013). Other countries have also shown interest in using this rich source of pigments to color food with purple corn color being recognized by the European Union with the code E-163 and the same code for the Japanese legislation. Imports of port purple corn and its color products are growing. In 2017, Peru exported over 396,000 kilos of purple corn valued at US$645,769, an increase from $602,248 in 2016 (AGAP, 2017).

2.1 Purple Corn Pigments

The main class of pigments present in purple corn is the water-soluble anthocyanins. Additionally, the water-insoluble but alcohol-soluble pholobaphenes have been reported in purple corn (Grotewold et al., 1994; Lee and Harper, 2002). These two pigments partially share the same biosynthetic route in purple corn plants as both are derived from a flavanone intermediate (Figure 1).

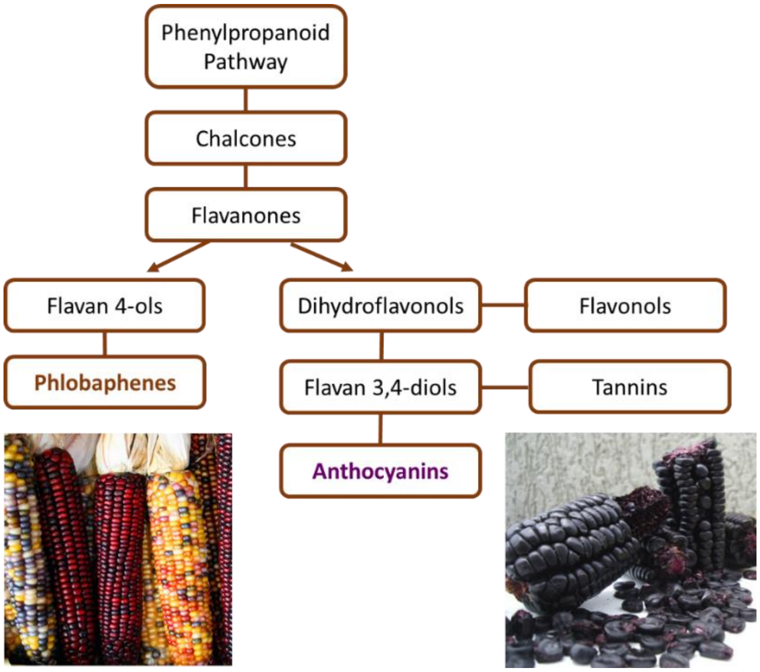

Figure 1. Scheme of the biosynthetic pathway of purple corn phlobaphenes and anthocyanins (adapted from Grotewold, 2005).

2.2 Purple Corn Anthocyanins

Anthocyanins are one of the major sources of color which provide the purple reddish hue to purple corn. Anthocyanin content in purple corn ranges from 6.8mg/g fresh weight to 82.3 mg/g fresh weight depending on the section analyzed (Cevallos-Casals and Cisneros-Zevallos, 2003; Wu et al., 2006; Li et al., 2008). This content was higher than most of the known anthocyanin-rich plants based on fresh weight such as blueberries (1.3 to 3.8 mg/g) (Cevallos-Casals and Cisneros-Zevallos, 2003; Wu et al., 2006), strawberries (0.21±0.03 mg/g) (Wang and Lin, 2000; Wu et al., 2006), red cabbage (3.22±0.41 mg/g) (Ahmadiani et al., 2014; Wu et al., 2006), eggplants (8.57 mg/g) (Wu et al., 2006), and chokeberries (14.80 mg/g) (Kulling and Rawel, 2008; Wu et al., 2006). The levels of pigment reported in purple corn have been especially high in the inedible leaf and cob regions (Paulsmeyer, Vermillion, and Juvik 2022; Nankar et al. 2016). The amount of anthocyanin in the cobs ranged from 0.49% to 4.60 % of the dry or fresh weight, respectively, roughly 2 to10 times more than that found in the kernel (Li et al., 2008). Recently, a study on the total anthocyanin content in different purple corn tissues reported that the leaf had the highest average anthocyanin content of 913.56 mg anthocyanins per kg maize, followed by cob (608.65 mg/kg), tassel (588.59 mg/kg), silk (511.82 mg/kg), seeding (500.91 mg/kg), husk (225.78 mg/kg), anther (196.39 mg/kg), and kernels (7.91 mg/kg) containing the lowest concentration of anthocyanins of the tissues (Paulsmeyer, Vermillion, and Juvik 2022). Extraction of purple corn pigments for color usage is usually achieved by soaking the ground purple corn materials into polar solvents, such as water, ethanol, methanol, acetone, and their mixtures (Lao and Giusti, 2018; De Nisi et al., 2021).

The anthocyanin profile of purple corn has been well characterized, with 6 major and 17–20 minor anthocyanins identified (Aoki et al., 2002; De Pascual-Teresa et al., 2002; González-Manzano et al., 2008; González-Paramás et al., 2006; Jing and Giusti, 2005; Jing et al., 2007; Li et al., 2008; Cuevas Montilla et al., 2011; Pedreschi and Cisneros-Zevallos, 2007; Zhao et al., 2008; Žilić et al., 2012, Haggard et al., 2018; Paulsmeyer, Vermillion, and Juvik, 2022; Chatham et al., 2018; Lao and Giusti 2016). The six major purple corn anthocyanins are cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, and their malonic acid derivatives on the 6’’ position of the glucose moiety. Minor anthocyanins include the diglucoside-derivatives (Žilić et al., 2012), dimalonyl-derivatives (Aoki et al., 2002; Jing et al., 2007; Montilla et al., 2011), and rutinose-derivatives of the main anthocyanidins (Žilić et al., 2012). Additionally, other less common pigments are the major anthocyanins linked to succinic acid and catechin (González-Manzano et al., 2008; González-Paramás et al., 2006; Li et al., 2008; Montilla et al., 2011; Paulsmeyer, Vermillion, and Juvik, 2022). Two unique flavanol-anthocyanin condensed forms, catechin-(4,8)-pelargonidin 3,5-diglucoside and afzelechin-(4,8)-pelargonidin 3,5-diglucoside, were found in Apache Red purple corn (Chatham et al., 2018). Delphinidin-3-glucoside was also detected in purple corn, but it was not universally reported (Žilić et al., 2012).

3. COLORED-FLESHED POTATOES

Potato (*Solanum tuberosum*, belonging to the nightshade family) is a starchy, tuberous crop with cultivars in assorted colors, shapes, and sizes. It was originally domesticated in South America, and a large variety of wild species found in the Andes of Peru and Bolivia were brought into cultivation as a staple food several thousands of years ago (Hawkes, 1992; Salaman et al., 1985). The potato was introduced outside of the Andes region in the 1700s to Europe and later to North America (Ochoa, 1990). Today, it is planted in more than 100 countries and ranks as the world’s fourth most important crop following maize, wheat, and rice (FAO, 2009; Hendley, 2006).

3.1. Potato varieties and pigmented cultivars

About 5000 potato varieties are recorded worldwide, with vast genetic diversity among cultivars of white-, yellow-, orange-, red-, purple-, and blue-fleshed varieties (Burlingame et al., 2009; Kaspar et al., 2013). The flesh may be partially or solidly pigmented and sometimes only the skin is pigmented. White- and yellow-fleshed potatoes are the most well-known and are rich sources of carotenoids, with yellow-fleshed cultivars containing 10 times more carotenoids than white-fleshed ones (Brown et al., 2005). Other pigmented varieties, such as red-, purple-, and blue-fleshed have gained consumer interest due to a higher concentration of anthocyanins (Brown, 2005; Lachman and Hamouz, 2005). Studies have reported higher contents of polyphenols in red- and purple-fleshed cultivars than in those with white flesh (Hamouz et al., 2011) with purple-fleshed having a reported 4.68 g gallic acid equivalents /kg dry weight and yellow fleshed potatoes having 2.96 g gallic acid equivalents /kg dry weight (Lachman et al., 2008). The total anthocyanin content in red and purple potato varieties differs based on the cultivar with Lachman et al. (2009) reporting 0.7 to 74.3 mg cyanidin-3-glucoside equivalents/100g fresh weight, Hamouz et al. (2011) reporting 135.3 to 573.5 mg cyanidin/ kg fresh weight, and Lachman and Hamouz (2005) reporting 6.9 to 35 mg/ 100g fresh weight for red potatoes and 5.5 to 17.1 mg/100 g fresh weight for purple varieties. Anthocyanin content of purple-fleshed potatoes was higher than that of red-fleshed potatoes (Ezekiel et al., 2013; Lewis et al., 1998) with Lewis et al. (1998) reporting anthocyanin content of purple flesh and red flesh potatoes at 368 mg/100g fresh weight and 22 mg/100g fresh weight, respectively.

3.2 Anthocyanins in colored-fleshed potatoes

Pigmented potato cultivars derive their color from anthocyanins, and the type of anthocyanins varies by the color of the potatoes' skin and flesh. Anthocyanins contained in the potatoes with pigmented flesh have been investigated by many researchers due to their antioxidant activity and as alternatives to artificial colorants. In purple and red-fleshed varieties, approximately over 98 % of the total anthocyanins were acylated (Lachman & Hamouz, 2005), an important distinction as acylated anthocyanins generally have better stability than non-acylated anthocyanins (Wrolstad and Culver, 2012).

In red-fleshed potatoes, most pigments were found to be acylated glucosides of pelargonidin with pelargonidin-3-rutinoside-5-glucoside acylated with *p*-coumaric acid reported as the most abundant (Rodriguez-Saona et al., 1998; Naito et al., 1998; Lewis et al., 1998) and peonidin-3-rutinoside-5-glucoside acylated with *p*-coumaric acid being reported in lesser amounts (Lewis et al., 1998). A large collection of purple-fleshed potato cultivars with entirely or partially colored flesh have been studied, such as Purple Peruvian, All Blue, Shetland Black, Vitelotte, Purple Majesty, and Purple Mackintosh with reports showing different anthocyanin profiles based on the cultivar (Reyes et al., 2004; Lachman and Hamouz, 2005; Li et al., 2012). In Andean purple potato extracts, five anthocyanidins were present (pelargonidin, cyanidin, petunidin, peonidin, and malvidin) with petunidin and peonidin reported as the most predominant and many of these anthocyanins being acylated with either caffeic, *p*-coumaric, or ferulic acids (Giusti et al., 2014). In blue-fleshed potato varieties, derivatives of petunidin, malvidin, and peonidin all acylated with *p*-coumaric acid were predominant (Hillebrand et al., 2009; Eichhorn and Winterhalter, 2005).

4. SAUCO, THE PERUVIAN ELDERBERRY

The genus Sambucus L. (elderberry) belongs to the Adoxaceae family and has been used as a medicinal plant for hundreds of years. The two most known species around the world are the European elderberry (*S. nigra* L.) and the American elderberry (*S. canadensis* L.). Yet, *Sambucus peruviana* is a Peruvian elderberry species commonly known as "sauco" or "Andean elderberry" (Pangestu et al, 2020). It is recognized at the species level due to its geographical isolation from other Sambucus plants or treated as subspecies of *S. nigra* L. due to their morphological similarities (Applequist, 2015). This plant is native to Central and South America and can grow in the southern hemisphere between 2800 and 3900 meters of elevation (Porras-Mija et al., 2020). This berry has attracted attention due to its high in vitro antioxidant capacity and rich anthocyanin content that creates its characteristic black-purple color.

4.1 Botanical characteristics

Sauco is a large perennial shrub or a small deciduous tree typically around 3-6 meters tall but with the capabilities to grown up to 12 meters in a suitable environment. Its small, bright white flowers bloom between September to February, and its purplish-black, small fruits mature between January and July. The fruits of sauco are between 7 and 12 mm and have a fleshy, juicy, sweet taste. Although its trunk, fruits, and flowers all have high economic values, this species has not been commercially exploited in Peru (other than by small local business) (Mostacero León et al., 2017).

4.2 Anthocyanins in sauco

Total anthocyanin content of sauco ranges between 0.45 and 0.99 mg cyanidin-3-glucoside equivalents/g fresh weight depending on its growth region (Porras-Mija et al., 2020). Though barely morphologically distinguishable from *S. nigra* and *S. canadensis*, sauco has a unique anthocyanin profile. The most predominant anthocyanin in sauco was cyanidin-3-glucoside, taking over 35 % of the total anthocyanin content, followed by cyanidin-3-lathyroside (28.6 %), cyanidin-3-sambubioside (23.5 %) and cyanidin-3-galactoside (2.7 %) (Zhou, 2021). Comparatively, *S. nigra* lacked cyanidin-3-galactoside and cyanidin-3-lathyroside, while *S. canadensis* was more abundant in 3,5-diglycosides and acylated anthocyanins (Zhou et al., 2020).

Aligned with their anthocyanin profiles, sauco displayed different color expression patterns and tinctorial capacity compared to *S. nigra* and *S. canadensis*. The aqueous extract of sauco displayed orange-red tones at acidic pH salmon pink tones at mildly acidic pH, and brownish purple tones at neutral to alkaline pH (Figure 2). Sauco extract consistently exhibited higher color intensity than the other two elderberry species under acidic pH (pH 1–4) and was capable of resembling the color hue of FD&C No. 40 in a wider pH range (Zhou, 2021). This could be explained by the considerable amount of cyanidin-3-lathyroside in sauco as glycosylation with lathyrose is characterized by a lower visual detection threshold as compared to other types of glycosylation (Zhao et al., 2014).

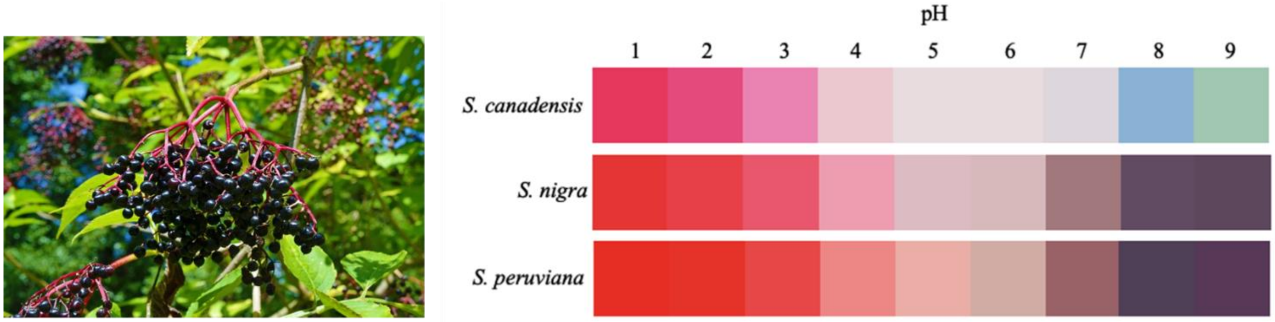

Figure 2. Sambucus peruviana, and a representation of the colors obtained (color swatches) with anthocyanin extracts of 3 *Sambucus* species in pH 1-9 buffers.

Studies have revealed high potential of applying sauco anthocyanin extract as a natural food colorant, especially for dyeing acidic food. Pangestu et al. (2020), demonstrated that sauco extract produced a bright, intense red color to model beverages. The half-life of the chroma of these model beverages colored with sauco was up to 23 weeks, and the addition of copigments such as chlorogenic acid and ferulic acid intensified the color and significantly extended the half-life to 49 weeks in the case with ferulic acid (Pangestu et al., 2020). Overall, anthocyanin extracts of sauco, especially with the addition of ferulic acid, showed great potential on enhancing the color of commercial food and beverage.

5. BERBERIS SPECIES

The genus *Berberidaceae* is well studied and characterized, having an extensive distribution around the world. To date, this genus includes ~500 species (Wan et al., 2017) with ~100 distributed in South America and 32 found in Peru (Ulloa & Sagástegui, 2006). Out of those in Peru, 14 are endemic and include *Berberis armata* Citerne, *Berberis barbeyana* C.K. Schneid, *Berberis beauverdiana* C.K. Schneid, *Berberis buceronis* J.F. Macbr, *Berberis citernei* Ahrendt, *Berberis cliffortioides* Diels, *Berberis dryandriphylla* Diels, *Berberis flexuosa* Ruiz & Pav., *Berberis hochreutineriana* J.F. Macbr, *Berberis humbertiana* J.F. Macbr, *Berberis monosperma* Ruiz & Pav., *Berberis podophylla* C.K. Schneid, *Berberis*

Tomentosa Ruiz & Pav., and *Berberis weberbaueri* C.K. Schneid. (Ulloa & Sagástegui, 2006). The plants consist of spiny, deciduous evergreen shrubs with yellow wood, 3–6 mm long flowers with six petals and sepals (usually with the same color) in alternating whorls (Khosrokhavar et al., 2010), and small red or blue berries (5–15 mm). The leaves of the long shoots do not participate in photosynthesis but transform into tri-pointed spines and, finally, into short shoots with several leaves (1–10 cm long, simple and entire or with spiny margins) that participate in photosynthesis (Perveen & Qaiser, 2010). In the life cycle of Berberis, there are sexual and asexual reproduction processes that allow the plant to survive in harsh conditions. The reproductive organs of the flower are protected from rain by three inner concave sepals and six petals that completely enclose the anthers and stamens (Peterson et al., 2005).

5.1 Anthocyanins in Berberis species

The anthocyanins of three Berberis species grown in Peru were recently characterized and studied as natural colorants. These included *Berberis boliviana* Lechler, *Berberis commutata* Eichler, and *Berberis humbertiana* J.F. Macbr. *Berberis boliviana* Lechler (depicted in Figure 3) is a wild species that grows especially in the Mesoandean region between 2000 and 4200 m of elevation in Peru. There is information about this species in Peru since conquest time when this plant was named as quisca-quisca, meaning thorny plant (Cobo, 1653). It grows around fields as a protective fence mainly because of the sharp thorns. It has small, edible, red-purple berries with a length of 7.05±0.355 mm and weight of 0.103±0.02 grams on average. The fruits are available from March until May each year and have a dark purple color produced by an abundance of monomeric anthocyanins. Some Peruvian traditions mention that these fruits were used as a natural coloring shampoo by Inca maidens to wash and care for their hair. Additionally, the fruit is used as a soft purple colorant for fibers. Using the pH-differential method, the anthocyanin content in berries was 7g cyanidin-3-glucoside equivalents /100g of monomeric anthocyanins of seedless berries (Del Carpio-Jiménez et al., 2011). Anthocyanins were characterized by high pressure liquid chromatography with photodiode array and mass spectrophotometer detectors. Ten anthocyanins and five aglycones (cyanidin, malvidin, petunidin, peonidin, and delphinidin) were identified. Anthocyanins were identified as petunidin-3-glucoside (24.4 %), delphinidin-3-glucoside (24.1 %), malvidin-3-glucoside (22.1 %), cyanidin-3-glucoside (10.2%), petunidin-3-rutinoside (7.15 %), malvidin-3-rutinoside (4.9 %), cyanidin-3-rutinoside (3.8 %), delphinidin-3-rutinoside (2.6 %), peonidin-3-glucoside (1.1 %), and peonidin-3-rutinoside (0.9 %) (Del Carpio-Jiménez et al., 2011).

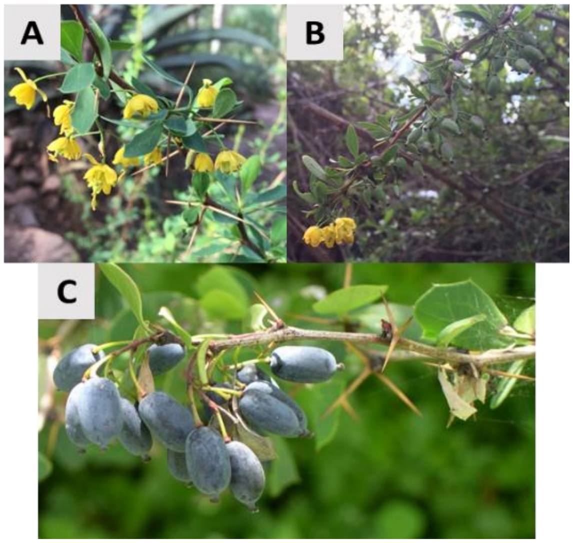

Figure 3. Picture of flowers (A) and unripe fruits (B) and ripe fruits (C) of *Berberis boliviana* Lechler (Del Carpio-Jiménez, 2021).

Berberis commutata Eichler (depicted in Figure 3) is a wild berry that grows in Peru, preferable in the Mesoandean region between 2000 and 4200 m of elevation. It is a spiny, deciduous evergreen shrub with sharp thorns, yellow flowers grouped in small hanging bunches, and many dark purple berries of 10±0.81 mm in length and 0.318±0.02 grams in weight–larger berries than the other Berberis species. Common names for the berries include t'ancar, ch'eqche, and huancachu. The full-ripened, purple fruits are available from February until late May each year and have anthocyanins and phenolics as the main phytochemical constituents. Four aglycones (delphinidin, cyanidin, petunidin, and malvidin) and seven anthocyanins were found in *Berberis commutata* fruits: delphinidin-3-glucoside

(35.8%), delphinidin-3-rutinoside (16.4 %), cyanidin-3-glucoside (16.2 %), petunidin-3-glucoside (18 %), petunidin-3-rutinoside (4.5%), malvidin-3-glucoside (5.1 %) and delphinidin-pentoside-hexoside (2.5 %) (Del Carpio-Jiménez, 2021).

Berberis humbertiana J.F. Macbr (depicted in Figure 4), is an endemic wild berry that grows in central and south Peru between 3000 and 4200 m of elevation (Ulloa and Sagástegui, 2006). This species is also a spiny shrub with sharp thorns and has tiny, dark purple berries of 4.34±0.53 mm in length and 0.040±0.01 grams in weight. The flowers are yellow, and the fruits are available from March until May each year. The anthocyanin content of the berries is high, quantified at approximately 4g/100g of monomeric anthocyanins in seedless berries. Five aglycones (delphinidin, cyanidin, petunidin, peonidin and malvidin) and nine anthocyanins were found in the *Berberis humbertiana* fruits: delphinidin-3-glucoside (42.7 %), petunidin-3-glucoside (19.1 %), malvidin-3-glucoside (16.4 %), cyanidin-3-glucoside (8.7 %), cyanidin-3-rutinoside (3.1 %), petunidin-3-rutinoside (3.1 %), peonidin-3-glucoside (2.5 %), delphinidin-3-rutinoside (2.3 %) and malvidin-3-rutinoside (2.2 %)

Figure 4. Picture of flowers (A) and ripe fruits (B) of *Berberis commutate* Eichler (Instituto de Ecología Regional- FCN – UNT, Argentina). Picture of ripe fruits of *Berberis humbertiana* J.F. Macbr (C) (Del Carpio-Jiménez, 2021)

5.2. Berberis anthocyanins used as yogurt colorants

Wallace and Giusti (2008) incorporated powder from *Berberis boliviana* Lechler into yogurt samples containing 3 different fat levels. The color of yogurt at 20 mg cyanidin-3-glucoside equivalents/100 g yogurt was similar to commercial blueberry yogurt. The addition of ground *B. boliviana* berries rich in anthocyanins achieved color characteristics similar to artificially colored commercial brand blueberry yogurt. Due to the remarkably high content of monomeric anthocyanins in *B. boliviana* dried berries, there was no need for industrial pigment extraction as their addition produced a bright, stable, and acceptable color in yogurt systems. Pigment half-lives were 125 and 104 days for nonacylated anthocyanins at 10 and 20 mg cy-3-glu equivalents/100 g yogurt. In another study, the pH, color, and antioxidant activity of the lyophilized extract of anthocyanins from *B. humbertiana* and *B. boliviana* fruits in yogurt were studied. Freeze-dried ethanolic extracts (96 %) acidified with citric acid (pH 3.5) were incorporated into commercial yogurt at concentrations of 80 mg/50 g and 100 mg/50 g of yogurt. The systems maintained an acidic pH, achieved a coloration similar to commercial yogurt, and produced stronger antioxidant activity (Del Carpio-Jiménez, 2021).

6. HUITO

Genipa americana L., also known commonly as “Huito”, “Jagua”, “Genipap” or “Genipapo” in addition to many other names (Brauch et al., 2016; Wu and Horn, 2013), is a tree native to the Amazon region in South America (Brauch et al., 2016; Francis, 1993). It can also be found in areas with moist and warm climates in Mexico, Central America, and Jamaica (Francis, 1993). Unripe huito fruits are small and firm with a green skin color, while ripe fruits are larger, softer, and have a more yellow/red skin color (Bentes and Mercadante, 2014), When the fruit is cut and the interior flesh is exposed to oxygen, the fruit becomes blue and continues to get darker and bluer with time (Bentes et al., 2014).

Traditionally, huito fruit has been used to dye textiles and ceramics (Ramos-De-La-Peña, 2015; Bentes and Mercadante, 2014) and as a skin dye as the blue color can develop through the interaction genipin, an iridoid naturally present in the fruit, with amino acids on the skin (Neri-Numa et al., 2018). Unripe (immature, green) fruits are used most often for dyeing (Brauch et al., 2016; Francis, 1993; Bentes and Mercadante, 2014), while ripe (mature) fruits are used mostly for medicine, such as treating ulcers and wounds (Francis, 1993), and for consumption (Bentes and Mercadante, 2014). In addition to the fruit's many uses, the leaves and trunk of the huito tree are also important resources that can serve many functions.

Figure 5. Picture of the Huito tree and fruits, showing a comparison of the unripe (A) and ripe (B) fruits (pictures by Pierina Tuesta, and from Bentes and Mercadante, 2014).

6.1 Genipin and Geniposide

The active pigment in huito that allows for color development is an iridoid in the monoterpenes class called genipin. It is naturally colorless and is found in both *Genipa americana* L. (genipap fruit) and *Gardenia jasminoides Ellis* (gardenia fruit) (Neri-Numa et al., 2017; Bentes and Mercadante, 2014). According to Neri-Numa et al. (2017), genipin is characterized by a "cyclopentanoid unit fused with a dihydropyran ring whose hydroxyl group at the C1 position of the genipin pyran ring can be substituted by 1–2 moieties forming the genipin glycosides genipin-1-O-β-glucoside (geniposide) and genipin-1-O-β-D-gentibioside". Both the genipap and gardenia fruits contain genipin and geniposide, with geniposide predominant in the gardenia fruit (Bentes and Mercadante, 2014). However, for the blue pigments to form, geniposide must be hydrolyzed by β-glucosidase which removes the sugar and allows for the genipin aglycone to bind with a primary amine group, as seen in Figure 6 (Bentes and Mercadante, 2014; Neri-Numa et al., 2017; Bellé et al., 2018).

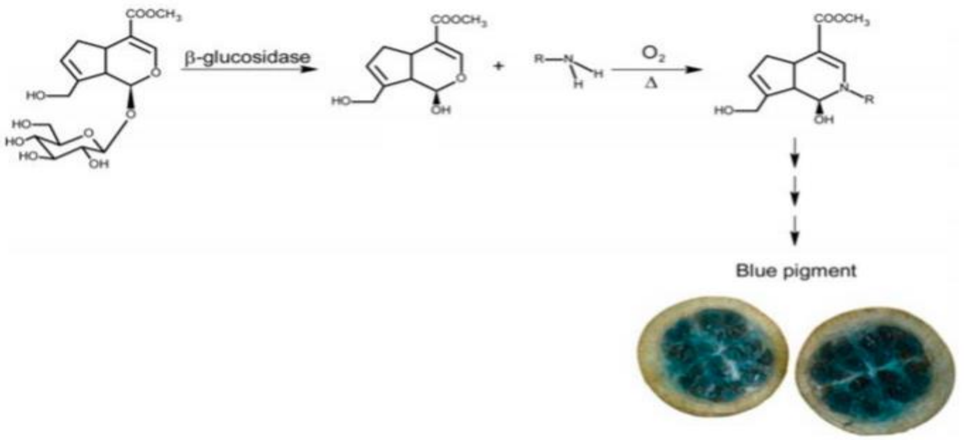

Figure 6. Conversion of geniposide to genipin through hydrolysis with β-glucosidase followed by blue pigment formation. Adapted from Bentes and Mercadante (2014).

Because genipap fruit contains more genipin than geniposide (1–3% genipin) in contrast to gardenia fruit which has more geniposide (0.17 % genipin) (Ramos-De-La-Peña et al., 2014), the genipap fruit can develop more blue pigments without the addition of β-glucosidase (Neri-Numa et al., 2017). Genipin can be extracted with organic solvents, ultrasound, pressurized liquid extraction, enzymes, high-pressure processing, and cold-mechanical/sonic enzyme-assisted extraction (Ramos-De-La-Peña et al., 2015; Náthia-Neves et al., 2017; Bellé et al., 2018, Ramos-De-La-Peña et al., 2014).

6.2 Role of the primary amine group source

The source of the primary amine group that binds with genipin impacts the color expression. However, due to the complexity of the reaction, it is still unclear why some sources are preferable. Pure amino acids have been shown to express different colors, such as blue, green, purple, brown, black, red, yellow, and orange, based on the experimental conditions (Wu et al., 2013; Cano et al., 2019). For example, glycine produced blue or purple while arginine produced red or brown under specific experimental conditions (i.e. pH, temperature) (Wu et al., 2013; Cano et al., 2019). Fruit and vegetable juices are a preferable source of amino acids for natural colorants made from huito because they could be considered as natural by consumers. Watermelon juice, lychee juice concentrate, banana puree, and celery juice can all produce blue with fresh huito. The color characteristics of the formed blue pigment include vibrant blue, gray/blue, and purple/blue, with watermelon juice being a preferable amino acid source due to the production of a dark blue color with huito (Wu et al., 2013). In addition to the source of primary amine group, the ratio of primary amine to genipin group also impacts the color produced. It has been suggested that there is a maximum limit up to where further addition of the primary amine source will not increase absorbance (Wu et al., 2013; Brauch, 2016; Lee et al., 2003). However, this ratio may change if β-glucosidase is added to the solution which would favor the formation of free genipin to interact with primary amine groups (Brauch, 2016; Cho et al., 2006).

6.3. Reaction mechanism

Although the reaction mechanism of pigment formation with genipin is not completely elucidated (Neri-Numa et al., 2017), understanding genipin's crosslinking reaction with primary amine groups can help better predict how pigments may be forming. There are two main stages during the genipin-primary amine group interaction. As seen in Figure 7, the first reaction involves a secondary amide substituting for an ester group on the genipin molecule, and the second reaction is a nucleophilic attack (Ramos-De-La-Peña et al., 2014).

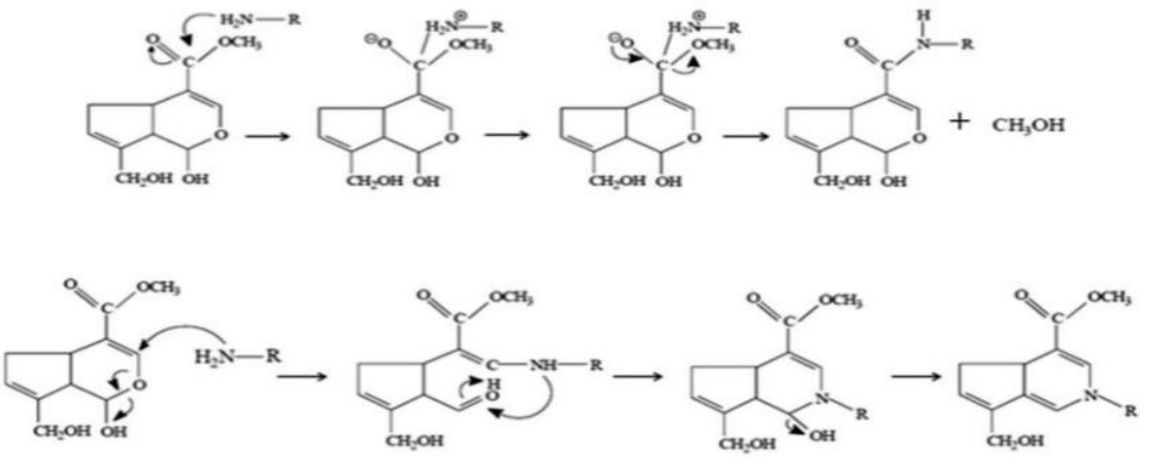

Figure 7. Reaction of genipin crosslinking with primary amine groups (Adapted from Ramos-De-La-Peña et al., 2014)

This nucleophilic attack of the primary amine group occurs at the C-3 carbon of the genipin molecule. A secondary amine attack then occurs, forming at the gap of the dihydropyran ring where the intermediate amine group was formed after the nucleophilic attack (Ramos-De-La-Peña et al., 2014). One proposed mechanism for pigment formation from genipin with amino acids is pictured in Figure 8.

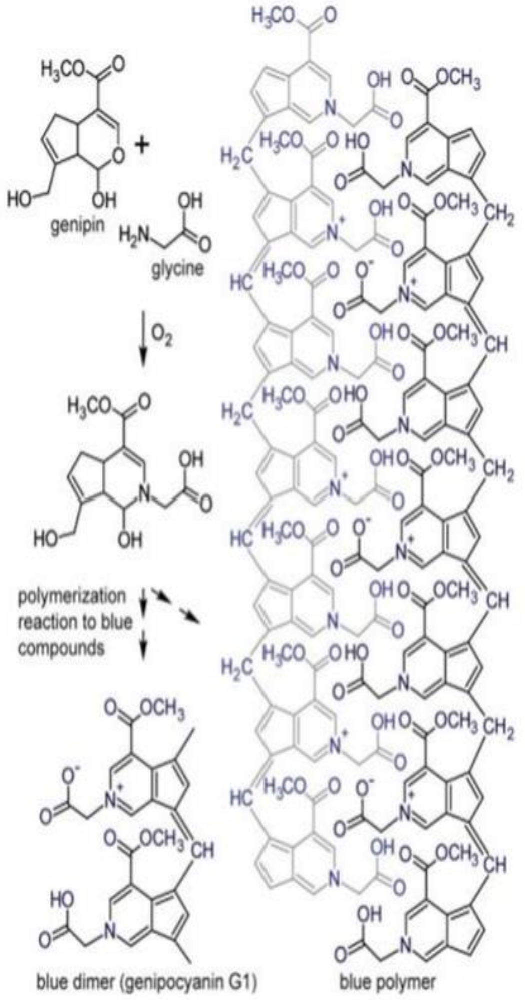

Figure 8. Proposed polymerization reaction mechanism of genipin with glycine (Adapted from Brauch et al., 2016).

Every primary amine group source has its own molecular structure that produces a unique compound after binding. Additionally, compounds can form monomers, dimers, or polymers (Brauch et al., 2016), increasing the possibilities of binding reactions. Cano et al. (2019) identified polymers of pure amino acids for glycine, lysine, valine, methionine, and proline using chromatography and proposed molecular configurations for genipin with the amino acids.

6.4 Huito's pigment stability

Huito is a promising source of blue color due to its enhanced stability to conditions such as pH and temperature. Huito color is stable at acidic pH, and production of the blue color is favored in mildly acidic pH (Wu et al., 2013; Brauch et al., 2016). Blue pigment formation can occur within pH 3–8 but is preferable at pH 4–6 (Wu et al., 2013; Brauch et al., 2016; Neri-Numa et al., 2018). It has also been suggested that the pigments formed from genipin are heat stable, with color development increasing with higher temperatures (Wu et al., 2013; Brauch, 2016). Although promising, studies on the stability of this pigment to light and temperature are limited and show conflicting results (Neri-Numa et al., 2018; Wu et al., 2013; Brauch et al., 2016). The formation and stability of blue pigments from huito and primary amine groups is largely dependent on the conditions of the experiment and the combination of pH with temperature (Neri-Numa et al., 2018; Wu and Horn, 2013). More research is needed to elucidate pigment formation mechanisms and their stability in food matrices.

7. CONCLUSION

Market trends show that naturally sourced colorants are here to stay. Although artificial alternatives will continue to be an attractive option, consumers' demand for clean labels and healthier foods will continue to drive the industry to look for alternatives. Peruvian biodiversity offers exciting new opportunities to the food and cosmetic industry due to the diverse plant materials available. Additional research is needed to fully understand the behavior and capabilities of the pigments obtained from these promising plants.

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Author Contributions

Gonzalo Miyagusuku-Cruzado (GMC), Danielle M. Voss (DMV) and M. Monica Giusti (MMG) conceptualized the manuscript, coordinated the work with all authors, and prepared the original draft. Carla Del Carpio-Jiménez (CdCJ) contributed most content related to Berberis. Fei Lao (FL) and Pu Jing (PJ) contributed most of the content related to Purple Corn. Kai Zhang contributed most of the content related to colored potatoes. Yucheng Zhou contributed most of the content related to sauco. Sydney Grouge contributed most of the content related to huito. GMC and MMG supervised the work. GMC, MMG and DMV reviewed the complete manuscript and DMV edited the final copy of the manuscript. MMG acquired the funding and approved the document for submission.

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