Purple pigment from *Peltogyne mexicana* heartwood as a potential colorant for food

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Abstract *Peltogyne mexicana* heartwood might be a novel purple pigment source. The results of the present study demonstrate that the purple pigment is an important source of phenolic compounds (698.22 ± 2.99 mg GAE/g) and flavonoids (48.01 ± 0.51 mg EPE/g). UV–Vis spectrum and color parameters (L^* a^* b^*) showed that purple pigment has different shades of purple–red (H° value 19.32 ± 0.02 in methanol and 22.85 ± 0.01 in ethanol) depending on the solvent and the pH. Also, the purple pigment did not exhibit acute oral toxicity at a single dose (2000 mg/kg body weight). No mutagenicity was observed in the Ames test with three *Salmonella typhimurium*

strains. The purple pigment exhibited considerable coloring properties with a wider range of citric acid-dependent color hues in gelatin (H° from 280.3 to 319.9 and from 68.0 to 88.1), and higher color intensity than commercial anthocyanin. Minor variations in the hue were found in yogurt, for purple pigment with H° values from 317.5 to 315.0, and commercial anthocyanin from 82.6 to 88.7 and 276.9 to 295.5. However, purple pigment required lower concentrations to achieve superior effects. For gelatin and yogurt samples, similar variations in the color parameters L^* , a^* , b^* , and pigment degradation were observed for purple pigment and commercial anthocyanin in the stability assay.

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Graphical abstract

Keywords *Peltogyne mexicana* heartwood · Purple pigment · Acute toxicity · Mutagenicity · Color stability

Abbreviations

ANT	Anthocyanin
MI	Mutagenic index
NMNG	<i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
OQ	Oxide-4-nitroquinone
PA	Picrolonic acid
PP	Purple pigment

Introduction

Color is one of the most important attributes associated with the sensorial quality of food, and the general appearance is a determining factor of the product acceptance by consumers (Rodríguez-Sánchez et al. 2017). Food colorants are critical in the development of a new product since they are used to homogenize the color, correct or improve natural color variations, and endow color to colorless foodstuffs to make them more attractive to the consumer (Giménez et al. 2015).

Synthetic dyes have been widely used in foods because of their excellent technical characteristics such as high stability, a variety of shades, high solubility, and practical use (Arici et al. 2016). However, for some years, their use has been a concern because various investigations have shown their toxicities, which have mainly been related to the development of allergies, attention-deficit/hyperactivity disorder in children, and some have been shown to be genotoxic (Stevens et al. 2013).

In addition, currently, there is a widespread desire for the consumption of healthier and less processed food. Natural pigments such as anthocyanins, betalains, and carotenoids have been demonstrated to be safe and even provide beneficial health effects including antioxidant (Mojica et al. 2017; Pérez-Loredo et al. 2016), antimicrobial (Cisowska et al. 2011), anti-inflammatory (Szymanowska et al. 2015), and anticarcinogenic (Bontempo et al. 2015). In addition to their organoleptic features, colorants have an important role in contributing significantly to the functional properties of food products (Rodríguez-Sánchez et al. 2017).

Because of the negative effects of synthetic colorants on human health, interest in the study and exploitation of new sources of natural pigments has been increasing. *Peltogyne mexicana* Martínez, best known as Palo Morado by the local population, is an endemic tree in Guerrero State, Mexico that belongs to the *Caesalpiniaceae* family. The hardness and attractive purple color of its heartwood have been exploited by native people to construct wooden crafts, furniture, and souvenirs (Navarro-Martínez et al. 2005), and as a result of these activities, several heartwood residues have been generated that are not economically usable. It has been reported that a purple pigment can be extracted from Palo Morado heartwood with methanol, which owes its color to peltomexicanin, a peltogynoid quinone methide compound (Robinson and Robinson 1935) that has antioxidant activity (Gutiérrez-Macías et al. 2016) and is structurally related to flavonoids. On the other hand, quinone methide compounds have shown anticarcinogenic activity (Bolton 2014). The attractive dyeability and antioxidant-based bioactivity of the purple pigment from the heartwood of Palo Morado have created interest in its use as a novel food colorant to substitute Federal Food, Drug, and Cosmetic Act (FD&C) Red No. 3, Red No. 40, Blue No. 1, and Blue No. 2 synthetic pigments.

However, one of the main disadvantages of natural pigments in food matrices is their high susceptibility to chemical reactions that occur during storage (Chung et al. 2016). This high reactivity depends on the structure of the compound, pH, temperature, concentration of oxygen, light, presence of metals and enzymes, as well as interaction with other components of the food matrix such as lipids, proteins, sugars, and organic acids (Gutiérrez-Zúñiga et al. 2014; Mojica et al. 2017). Stability assessments of natural pigments in food matrices contribute to determining the physical and chemical factors that can improve or diminish their functionality in food. This strategy allows the application of appropriate technologies to increase their stability during the shelf-life of the product.

Additionally, the use of new compounds as food additives should be supported by exhaustive toxicity studies to ensure the safety of their consumption. Currently, there are no previous studies on the toxicity of purple pigment from the heartwood of Palo Morado. Thus, the objectives of this study were to determine the acute toxicity of purple pigment in mice using the method proposed by the Organization for Economic Cooperation and Development (OECD 2001) guidelines and the Ames genotoxicity test was also performed. In addition, the use and stability of the purple pigment as a colorant in two food matrices, yogurt,

and gelatin, were evaluated and compared with those of a commercial anthocyanin.

Materials and methods

Chemicals

Analytical grade: hydrochloric acid (Fermot, Mexico), methanol (Meyer, Mexico), propylene glycol (Drotasa, Mexico), Folin–Ciocalteu reagent (Hycel). Aminoanthracene, citric acid, dimethyl sulfoxide (DMSO), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, oxide-4-nitroquinone, and picrolonic acid were supplied by Sigma-Aldrich. Commercial anthocyanin powder (Enocyanin 3%) was supplied by Jobari Colors and Flavors (Mexico).

Plant material

Wastes from Palo Morado (*P. mexicana* Martinez) heartwood were collected in Guerrero State (17°09'32"N latitude and 99°30'51"W longitude). The plant was identified at the Post-Graduate College of Chapingo, Mexico and a voucher specimen (Registration number 51,392) was deposited at the CHAP Herbarium.

Experimental animals

The acute toxicity experiment was carried out on male and female ICR mice (weighing 25 ± 5 g) according to protocols established by the OECD Test Guidelines (2001). The animals were housed in polypropylene boxes under standard environmental conditions on a 12 h light–dark cycle and were provided food and water ad libitum. The purple pigment was administered using a mixture of distilled water:propylene glycol (4:1) as the vehicle.

Pigment extraction

The purple pigment was obtained according to the method reported by Gutiérrez-Macías et al. (2016), which is briefly described. The waste of Palo Morado heartwood was crushed into a fine powder, sieved ($355 \mu\text{m} < \text{particle size} < 150 \mu\text{m}$), and then it was extracted in the dark with methanol (MeOH in 1% hydrochloric acid [HCl]) at a solvent:solid ratio of 95:5 (v/w) at 20 ± 2 °C for 24 h in a 250 mL flask with magnetic stirring. The solution was filtered and concentrated using a rotatory evaporator (R-200, Büchi, Flawil, Switzerland) at 40 °C, which yielded a purple amorphous powder, which was subsequently subjected to toxicity and stability tests.

Purple pigment characterization

Total phenolic content

The total phenolic content was determined in the purple pigment by Folin–Ciocalteu assay following the methodology proposed by Singleton et al. (1999). Briefly, 200 μL of Folin–Ciocalteu reagent were mixed with 400 μL of Na_2CO_3 (20%), 400 μL of sample and 3000 μL of distilled water, then vortexed and kept in the dark for 30 min. The absorbance was measured at 765 nm at the end of the incubation time in a spectrophotometer (DR5000; Hach, Mexico State, Mexico). The results were reported as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

Total flavonoid content

The total flavonoid content was determined using the method described by Xu and Chang (2007). For this purpose, 250 μL of the sample was mixed with 1250 μL of distilled water and 75 μL of 5% NaNO_2 . After 6 min, 150 μL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and 500 μL of 1 M NaOH were added and allowed to react for 5 min. The volume of the mixture was adjusted to 2.5 mL with distilled water. At the end of the reaction, the absorbance was measured at 510 nm. The results were reported as milligrams of (–)-epicatechin equivalents per gram of sample (mg EPE/g).

UV–Vis spectrum

Purple pigment solutions were prepared at 1 mg/mL in methanol, acidified methanol (0.1 M HCl), alkalized methanol (0.1 M NaOH), and ethanol. The UV–Vis spectra were recorded in a range of 200–700 nm using a spectrophotometer (DR5000 HACH, Mexico, Mexico).

CIE $L^*a^*b^*$ color parameters

The color attributes were expressed as L^* , a^* , and b^* parameters, where L^* represents lightness ($L^* = 0$ or 100 yields black or indicates diffuse white, respectively). It is possible to obtain chroma (C^*) and hue angle (H°) from the a^* and b^* parameters. C^* indicates color intensity, which is the distance of a color from the original shade. The H° expressed as degrees, ranged from 0° to 360°, where 0° (red) is located on the $+a^*$ axis, then rotating anticlockwise to 90° (yellow) for the $+b^*$ axis, 180° (green) for $-a^*$, and 270° (blue) for $-b^*$ (Nontasan et al. 2012). L^* , a^* , b^* color parameters were obtained from purple pigment solutions at 1 mg/mL in methanol, acidified methanol (0.1 M HCl), alkalized methanol (0.1 M NaOH), and ethanol, which were measured using a colorimeter

(CFEZ1005, HunterLab, Reston, VA, USA, D65 illuminator and 10° observer angle). The H° and C^* values were calculated using Eqs. 1 and 2.

$$H^\circ = \tan^{-1} \left[\frac{b^*}{a^*} \right] \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

where H° is the hue angle and C^* is the chroma.

Acute toxicity

The acute toxicity experiment was performed according to the OECD 423 guideline for testing of chemicals and in compliance with the Official Mexican Standard for the Care and Use of Laboratory Animals (NOM-062 1999), and protocols were reviewed and approved by the Bioethical Committee of National School of Medicine and Homeopathy with Act number ENMH-CB-0094-2014.

Groups of male and female mice ($n = 3$) were orally administered single 2000 mg/kg doses of the purple pigment or vehicle. The animals were observed for signs of toxicity or death every 30 min after administration for the first 4 h, and then every day for the next 14 days. Consumption of food and water, as well as the weight of the animals, were monitored weekly. After concluding the experimental period, the animals were euthanized, and a gross necropsy of the principal organs was performed. The experiment was repeated with a second group of animals (both sexes) to obtain a total of six animals per group. The median lethal dose (LD_{50}) value was determined according to the globally harmonized classification system (GHS).

Mutagenicity assay

The mutagenic potential of the purple pigment was evaluated using Ames test with three histidine mutant strains of *Salmonella typhimurium*: (His-) TA98, TA100, and TA102. For this purpose, we used the method described by Gutiérrez-Zúñiga et al. (2014). Briefly, 100 μ L of each culture was mixed with 2 mL soft agar (0.6%) in sterile tubes, and different concentrations of purple pigment dissolved in dimethyl sulfoxide (DMSO; 100, 50, and 25 μ g/plate) were added. The evaluation was carried without and with metabolic activation by 500 μ L enzymatic fraction liver S9 mix from male Wistar rats treated with 10% Aroclor-1254 (Ames et al. 1973). Then, the content of each tube was poured onto medium Vogel–Bonner plates and incubated at 37 °C for 48 h. All experiments were carried out in triplicates. The revertant colonies were determined using a colony counter (Fisher Scientific 7-910, Montreal, Quebec, Canada). The reversion rate was compared with that of control plates treated

with and without mutagen. The positive control was 2-aminoanthracene (AA) used at a concentration of 10 μ g/plate as a mutagen to the TA98, TA100, and TA102 strains and the addition of S9 fraction. Picrolonic acid (PA, 50 μ g/plate), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NMNG, 10 μ g/plate) and oxide-4-nitroquinone (OQ, 10 μ g/plate) were used to induce mutations for the TA98, TA100, and TA102 strains, respectively, in the absence of the S9 fraction. The mutagenic index (MI) it was calculated as the ratio of revertant colonies of the tested samples divided by the revertant colonies of the control. The sample was non-mutagenic when the MI was < 2 for at least one of the tested concentrations (Bernstein et al. 1982; Margolin et al. 1981).

Purple pigment as colorant in food matrices

Effect of the pH on the stability of purple pigment in aqueous dispersion

Purple pigment dispersions were prepared with a concentration of 0.25 mg/mL, adjusting the pH to 3, 4, 5, 6 and 7 with 0.01 M phosphate buffer solutions. Three milliliters of each dispersion were taken and placed in tubes that were closed hermetically and kept in a water bath at 70 °C (accelerated test). Samples were taken every 10 min for 60 min and two additional samples at 75 and 90 min. The absorbance readings were taken at the corresponding maximum wavelength for each pH.

Effect of temperature on the stability of the purple pigment dispersion

It was necessary to select a pH in which a quantifiable change was determined during performance for the kinetic of temperature stability. In accordance with the foregoing, pH 6 was selected to carry out the stability kinetics of the purple pigment in aqueous dispersion at different temperatures. The choice was made considering the correlation coefficient and the degradation constant determined in the previous section.

A dispersion of the purple pigment was prepared at a concentration of 0.25 mg/mL adjusting at pH 6, where the dispersion presented greater stability. Three milliliters of each dispersion were taken and placed in hermetically sealed tubes that were kept in a water bath at 40, 50, 60 and 70 °C. The absorbance was read at the maximum wavelength corresponding to the selected pH, every 10 min for 60 min and two additional readings at 75 and 90 min.

Gelatin formulation

The base gelatin was formulated like commercial gelatins: gelatin 2 g, sucrose 25 g, citric acid 0.063, 0.126, 0.252 g (for purple pigment), and 0.063 g (for anthocyanin), and hot water up to 100 g. The purple pigment and citric acid were added at varying concentrations for different shades using propylene glycol as the carrier. Also, different concentrations of anthocyanins per 100 g of gelatin and 0.063 g citric acid per 100 g of product were added. In all cases, the final weight of gelatin was adjusted to 100 g with distilled water.

Yogurt formulation

The purple pigment was added to the commercial natural yogurt sample using propylene glycol as the carrier at concentrations of 10, 15, 20, 30, 40, 50 and 60 mg/100 g of yogurt. For comparison, a commercial pigment anthocyanin was used at concentrations of 40, 48, 56, 64, 80, 112 and 160 mg/100 g of yogurt.

Color sample measurement

In addition to L^* , a^* , b^* color parameters and H° and C^* values of the food matrices, the total color difference (ΔE) was determined to establish the magnitude of the color change along with the stability study using Eq. 3.

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad (3)$$

where ΔE is the total color difference between a sample at the beginning (L^*_0 , a^*_0 , b^*_0 color parameters) and during the period of stability (L^* , a^* , b^* color parameters).

Pigment quantification in gelatin

To quantify the pigment, 3 g of the gelatin samples were placed in tubes with 3 mL MeOH (50%, pH 3.8), stirred for 5 min using a vortex (vortex mixer VM-300, Taipei, Taiwan) at 3150 rpm), and then the absorbance was read at 550 nm using an ultraviolet–visible (UV–Vis) DR5000 spectrophotometer (HACH, Mexico, Mexico). The anthocyanin gelatin samples were re-dissolved in a water bath at 30 °C, the pH was adjusted to 4.7, and the absorbance was measured at 524 nm. The concentration of the pigment was obtained using standard curves constructed with concentrations of 80, 65, 50, 40 and 20 $\mu\text{g/g}$ for the purple pigment gelatin and 640, 480, 320, 160 and 80 $\mu\text{g/g}$ for anthocyanin gelatin samples.

Pigment quantification in yogurt

To quantify pigments in the yogurt, 3 g of each pigmented sample were extracted with 7 mL acidified MeOH (2% HCl), shaken for 10 min, and then centrifuged in a refrigerated centrifuge for 45 min at $1280 \times g$ (Dynamica Velocity 14R, London, UK). The absorbance of the supernatant was read at 542 and 532 nm for purple pigment and anthocyanin samples, respectively. The absorbance values were interpolated in the standard curves to quantify the pigments using concentrations of 350, 250, 125, 62.5, 31.3 and 15.3 $\mu\text{g/mL}$ for the purple pigment and 1000, 750, 500, 250, 125 and 62.5 $\mu\text{g/mL}$ for the anthocyanin yogurt samples.

Selection of matrices for purple pigment stability assessment

Different concentrations of the purple pigment and citric acid generated a variety of hues in the gelatin matrix and, therefore, samples for stability evaluation were selected based on color preference determined by a panel of 30 untrained judges. For the yogurt, the color was determined using a similar method to that used for the different purple pigment concentrations, and a shift was observed in the color intensity. Therefore, the intermediate concentration of the purple pigment was chosen.

Pigment stability assessment

Samples of 30 g of gelatin and yogurt dyed with purple pigment and anthocyanin were stored at 4 °C in polyethylene terephthalate (PET) containers. Color and pigment concentrations were measured for the first 4 days and then every 8 days for the next 4 weeks. We calculated the ΔE values and residual pigment percentage (%RP) using Eqs. 3 and 4, respectively. Experiments were carried out in triplicate.

$$\%RP = \frac{[C]_t}{[C]_0} \times 100 \quad (4)$$

where $[C]_t$ and $[C]_0$ are the pigment concentration at time t and the beginning of the stability test, respectively.

Statistical analysis

Each experiment was performed in triplicate, and the results were expressed as means $\pm$ standard deviations. Statistical differences were calculated using a one-way analysis of variance (ANOVA), and Tukey's multiple range tests were used to compare means, differences were considered significant at $P < 0.05$. Data were processed

Fig. 1 Mutagenic effect of purple pigment (PP) from Palo Morado's heartwood using Ames test with three histidine (His⁺) mutant strains of *Salmonella typhimurium*: His⁺ a TA98, b TA100, and c TA102 with and without S9 fraction. Control (-), basal revertants; vehicle, DMSO; Control (+) with S9 fraction, AA (10 µg/plate), mutagenic to TA98, TA100, and TA102; Control (+) without S9 fraction, PA (50 µg/plate), mutagenic to TA98; MNNG (10 µg/plate), mutagenic to TA100; and OQ (10 µg/plate), mutagenic to TA102

using the SAS statistical software (v 6.0, SAS Institute SA de CV, Mexico).

Results and discussion

Purple pigment characterization

Total phenolic and total flavonoid content

The total phenolic content (TPC) in purple pigment was 698.22 ± 2.99 mg GAE/g and the total flavonoid content (TFC) was 48.01 ± 0.51 mg EPE/g. The TPC found in the purple pigment is comparable to the reported concentration for extracts obtained with ethanol and water from *Erythrophloeum suaveolens* wood: 581 and 646 mg GAE/g, respectively, and are higher than the concentrations of the wood extracts from *Distemonanthus benthamianus* (490 and 150 mg GAE/g) and *Pterocarpus soyauxii* (283 and 108 mg GAE/g) (Saha et al. 2013). Phenolic compounds are produced as secondary metabolites in plants in response to stress situations such as physical, chemical and microbiological damages, as well as by the decrease of nutrients (Moreira et al. 2017).

Although the heartwood of trees does not represent such an attractive source of bioactive compounds, since the leaves, fruits, flowers and roots are easier to manipulate, the heartwood has a wide variety of extractive compounds, principally polyphenols that shows powerful bioactive properties (Saha et al. 2013): antioxidant, anti-inflammatory, anti-carcinogenic, hypoglycemic and hypolipidemic properties (Acosta-Estrada et al. 2014). According to this, the purple pigment represents a potential source of phenolic compounds that can give a nutraceutical value to the food matrices even when it has been added as a food colorant.

UV-Vis spectrum

In the UV-Vis spectrum of the purple pigment in different solvents (Figure S1, supplementary material) a maximum absorption is observed in the ultraviolet region of the spectrum, characteristic of the phenolic compounds ($\lambda = 285\text{--}291$ nm). The maximum absorption bands in methanol, ethanol and methanol-HCl 0.1 M are at 538, 538, 534 nm. Also, a low intensity band was observed close to 570 nm using methanol as solvent. On the other hand, changes in the UV-Vis spectrum occurs with methanol-NaOH 0.1 M, a band about 337 nm and a second band close to 617 nm were observed, probably due to the formation of degradation products by the hydroxylation, oxidation and ring-cleavage, with the consequent formation of polyhydroxybenzoic acids, and further degradation results in low molecular weight phenolic carboxylic acids (Wang and Zhao 2016).

Table 1 Mutagenic index of purple pigment (PP) from Palo Morado heartwood in mutant strains of *Salmonella typhimurium*: (His+) TA98, TA100, and TA102

PP (μg/mL)	<i>Salmonella typhimurium</i> strain					
	TA98		TA100		TA102	
	With S9	Without S9	With S9	Without S9	With S9	Without S9
25	1.07 ± 0.43	1.11 ± 0.19	1.02 ± 0.34	1.05 ± 0.28	0.93 ± 0.22	1.02 ± 0.26
50	1.17 ± 0.43	0.94 ± 0.11	1.01 ± 0.35	0.97 ± 0.27	1.06 ± 0.18	0.97 ± 0.26
100	1.12 ± 0.27	0.98 ± 0.23	0.95 ± 0.30	1.03 ± 0.28	1.03 ± 0.17	1.00 ± 0.25
Control (+)	80.09 ± 9.32	6.64 ± 2.41	20.03 ± 5.27	18.55 ± 3.85	4.73 ± 0.71	4.69 ± 0.25

CIE $L^*a^*b^*$ color parameters

Analysis and characterization of natural pigments are carried out using UV–Vis spectroscopy, mainly. However, the CIE $L^*a^*b^*$ color space represents a practical tool for determining the color properties of pigments in different systems (Giménez et al. 2015). The purple pigment is an extract that consists mainly of compounds of phenolic nature, whose stability varies depending on the pH, as UV–Vis spectrum analysis showed. Purple pigment in methanol and ethanol showed positive a^* (greenness–redness) and b^* (blueness–yellowness) values, also it showed red hue (H° values 19.32 ± 0.02 and 22.85 ± 0.01 , respectively). In acidified methanol the hue turned to brilliant red (H° value 35.14 ± 0.19). However, in alkalized methanol color changed to green, represented with negative a^* value (-7.35) and H° value of 297.76 ± 0.01 (Figure S2, Table S1, Supplementary Material).

Acute toxicity

The purple pigment from Palo Morado heartwood did not alter the behavior and body weight, or cause organ damage or death at a dose of 2000 mg/kg during the experimental period (14 days). In addition, the water and food consumption were not affected by administration of the purple pigment. According to the specifications of the GHS, the purple pigment presented an $LD_{50} > 2000$ mg/kg in both sexes and, therefore, belongs to category 5.

Mutagenicity assay

The purple pigment did not exhibit His⁺ revertants $\geq$ twofold those of the control blank and vehicle control, and a dose–response effect was not found (Fig. 1). The calculated mutagenic index values for the purple pigment were from 0.925 to 1.168 and 0.938 to 1.114 with and without the S9 fraction, respectively (Table 1). The results obtained demonstrate that the purple pigment had no mutagenic effects on the three strains of *S. typhimurium* tested.

Purple pigment as colorant in food matrices

Effect of the pH on the stability of purple pigment in aqueous dispersion

Accelerated tests were performed to evaluate pH and temperature effects on purple pigment stability. According to Fig. 2 there was a significant loss of purple pigment at 70 °C to pH 7, while at acidic pH (3–6), the percentage of residual pigment concentrations greater than 50% were obtained and there are not significant differences ($P > 0.05$) between pH 3, 4 and 6.

Four mathematical models were evaluated to calculate the degradation constant of the kinetics at pH 3, 4, 5, 6 and 7 and at 70 °C: first order, second order, first order in two stages, and fractioned models (Table S2 from Supplementary Material). Fractioned model is suitable for kinetic studies where a significant concentration of reactants remains at the end of the assay after a long reaction time (Ahmed et al. 2004). The stability kinetics were best fitted to the fractioned model ($0.9099 < R^2 < 0.09355$) (Table S3 from Supplementary Material). The degradation constants of the purple pigment dispersions were similar in the range of pH 3–7, there is no significant difference ($P > 0.05$) between any of the degradation constants in each treatment at 70 °C ($0.0350 < k < 0.0395$).

The stability of the anthocyanins decreases as the pH of the medium increases. The highest stability is below pH 3, preferably at pH 2, whereas at a pH higher than 5 the degradation constant increases significantly (Hou et al. 2011). In contrast, purple pigment from *P. mexicana*, showed a similar stability within a range of pH 3–6, being a little less stable at pH 7. Great stability at pH > 3 is an attractive feature in the food area, since most food products have pH range within 4–7.

Effect of temperature on the stability of the purple pigment dispersion

As shows Fig. 3, the loss of the purple pigment increased as the temperature increased. At 40 °C more than 90% of the purple pigment is preserved, but at 70 °C the residual

Fig. 2 pH stability for purple pigment in aqueous dispersion expressed as residual pigment (%) after heating at 70 °C for 90 min. Different capital letters denote significant difference ($P < 0.05$)

Fig. 3 Thermal stability of the purple pigment in aqueous dispersion at pH 6 expressed as residual pigment (%) after heating for 90 min. Different letters denote significant difference between means ($P < 0.05$)

concentration decreases to 61.2%. The concentration of purple pigment remains similar between 50 and 60 °C.

Due to the considerable remaining concentration of the pigment after long periods of heat treatment, the fractionated model (Ahmed et al. 2004) was used for its better adjustment of the data ($0.9099 < R^2 > 0.9355$). The degradation constant of the pigment dispersions at 40, 50, 60 and 70 °C increased as the temperature was increased ($0.019 < k > 0.043$), finding a significant difference ($P < 0.05$) between the degradation constants at the different temperatures evaluated (Table S4 Supplementary Material).

The degradation constants and temperatures were related by the Arrhenius equation. The activation energy (E_a) determines the minimum amount of energy required to produce a chemical reaction (energy barrier). When the temperature increases, the number of molecules with energy greater than the activation energy is increased,

which increases the reaction rate (Giménez et al. 2015). According to the above, high E_a values indicate that a minor temperature change is enough to degrade the colorant more rapidly than low E_a values. To determine the activation energy, a graph of the negative natural logarithm of the degradation constant was constructed with the inverse of the temperature (°K) (Figure S3 from Supplementary Material). The activation energy of the purple pigment dispersion was 18.98 kJ/mol ($R^2 = 0.9984$). Activation energy value for purple pigment results lower than those obtained by Hou et al. (2011), who evaluated the stability of black rice anthocyanins, and they determined E_a values between 23.99 and 68.16 kJ/mol. Also, Giménez et al. (2015) reported E_a value for the betaxanthins of *Opuntia* 43.7 kJ/mol (30–90 °C) and for curcumin 23.7 kJ/mol (30–90 °C).

The thermal stability of anthocyanins is related to their molecular structure, the copigmentation phenomenon that occurs during heating, as well as in the presence of other compounds that favor their stability (Hou et al. 2011; Castañeda-Ovando et al. 2009). In a similar way, the purple pigment that present monomers anthocyanin like, is polymerized under specific conditions of pH and temperature, which are more stable than the monomeric units. On the other hand, there are some studies that report the formation of aggregates of pigments in concentrated solutions or, when they are subjected to a heat treatment (Behera et al. 2005).

Gelatin and yogurt matrices dyed with purple pigment

Gelatin samples dyed with purple pigment showed a^* values of -7.1 ± 0.1 to 24.4 ± 0.1 and b^* values of 3.7 ± 0 to 17.9 ± 0 . H° values were 280.3 ± 0.1 to 319.9 ± 0.1 and 68.0 ± 0.2 to 88.1 ± 0.1 , corresponding to blue–purple hues. It was further noted that higher concentration of the purple pigment decreased the brightness value L^* and increased the C^* value or color saturation. Moreover, increasing the concentration of citric acid (0, 63, 126, and 252 mg/100 g) changed the shades from blue to purple, which is characteristic of the color change exhibited by purple pigment when the pH of the medium is modified (Table 2).

Samples pigmented with anthocyanins (Table 2) showed a^* values of 3.7 ± 0 to 18.6 ± 0.1 , b^* values from 14.9 ± 0.9 to 19.8 ± 0 , and H° from 12.0 ± 0 to 66.1 ± 0.1 , corresponding to red hues. It was noted higher concentrations of anthocyanin decreased the brightness, L^* (from 55.5 ± 0 to 27.0 ± 0.5), and increased the color saturation, C^* (from 17.8 ± 0 to 21.5 ± 0.1).

A considerable variety of natural pigments have been evaluated for potential use in gelatins, jellies, gummies, and similar foods. However, few studies have been

Table 2 Gelatins pigmented with purple pigment from Palo Morado heartwood and commercial anthocyanin

Citric acid		Purple pigment (mg/100 g)			
(mg/L)		4	6	8	12
63
	$H^{\circ} = 280.3 \pm 0.1$	$H^{\circ} = 310.9 \pm 0.2$	$H^{\circ} = 315.2 \pm 0.1$	$H^{\circ} = 319.9 \pm 0.1$	
	$C^{*} = 7.0 \pm 0.1$	$C^{*} = 10.9 \pm 0.1$	$C^{*} = 17.0 \pm 0.1$	$C^{*} = 18.8 \pm 0.2$	
126
	$H^{\circ} = 68.5 \pm 0.2$	$H^{\circ} = 298.3 \pm 0.1$	$H^{\circ} = 303.9 \pm 0.1$	$H^{\circ} = 310.6 \pm 0.1$	
	$C^{*} = 8.3 \pm 0$	$C^{*} = 14.0 \pm 0.1$	$C^{*} = 17.7 \pm 0.1$	$C^{*} = 24.0 \pm 0.1$	
252
	$H^{\circ} = 88.1 \pm 0.1$	$H^{\circ} = 291.1 \pm 0.1$	$H^{\circ} = 297.9 \pm 0.1$	$H^{\circ} = 301.9 \pm 0.1$	
	$C^{*} = 12.6 \pm 0.1$	$C^{*} = 18.9 \pm 0.1$	$C^{*} = 25.0 \pm 0.1$	$C^{*} = 28.64 \pm 0.1$	
Citric acid		Anthocyanin (mg/100 g)			
(mg/L)		8	16	24	32
63
	$H^{\circ} = 12.0 \pm 0$	$H^{\circ} = 23.5 \pm 0$	$H^{\circ} = 37.1 \pm 0$	$H^{\circ} = 45.2 \pm 0$	
	$C^{*} = 17.8 \pm 0$	$C^{*} = 18.2 \pm 0$	$C^{*} = 17.6 \pm 0$	$C^{*} = 19.0 \pm 0$	
63
	$H^{\circ} = 53.3 \pm 0.1$	$H^{\circ} = 58.8 \pm 0$	$H^{\circ} = 60.3 \pm 0.2$	$H^{\circ} = 66.1 \pm 0.9$	
	$C^{*} = 19.5 \pm 0$	$C^{*} = 20.8 \pm 0$	$C^{*} = 21.5 \pm 0.1$	$C^{*} = 18.1 \pm 1.5$	

Table 3 Yogurt samples pigmented with purple pigment from Palo Morado heartwood and commercial anthocyanin

Purple pigment (mg/100 g)	10	15	20	30	40	50	60

H°	315.1 $\pm$ 0	316.7 $\pm$ 0.1	315.6 $\pm$ 0.2	315.7 $\pm$ 0	315.6 $\pm$ 0	315.1 $\pm$ 0	317.5 $\pm$ 0
C^*	9.3 $\pm$ 0	13.2 $\pm$ 0	19.0 $\pm$ 0	24.4 $\pm$ 0	26.1 $\pm$ 0	28.4 $\pm$ 0	26.3 $\pm$ 0
Anthocyanin (mg/100 g)	40	48	56	64	80	112	164

H°	82.6 $\pm$ 0.3	88.7 $\pm$ 0.1	276.9 $\pm$ 0.1	280.4 $\pm$ 0.1	289.5 $\pm$ 0.1	290.2 $\pm$ 0.1	295.5 $\pm$ 0.1
C^*	4.9 $\pm$ 0	5.4 $\pm$ 0	6.1 $\pm$ 0	6.5 $\pm$ 0	7.8 $\pm$ 0	9.0 $\pm$ 0	10.8 $\pm$ 0

reported on pigments with purple–blue hues of natural origin for use in food since most studies of anthocyanins, carotenoids, and betalains evaluated pigments with shades ranging between yellow and red. Iridoids are the main pigments with blue hues, and those obtained from *Gardenia jasminoides* J. fruit have gained great popularity in markets because of their high stability (Brauch et al. 2016). Purple pigment from the heartwood of Palo Morado showed attractive hues in the gelatin, which could be used commercially because few natural pigments show the observed colorations.

Yogurt dyed with purple pigment (Table 3) showed a^* values from 6.6 ± 0 to 17.8 ± 0 , b^* values from -19.4 ± 0 to -6.6 ± 0 , and H° from 317.5 ± 0 to 315.1 ± 0 . These values correspond to red–purple hues, and higher concentrations of the dye extract decreased the brightness (L^*) and b^* values, whereas the C^* and a^* values were simultaneously increased and, so, the tone was intensified.

Yogurt samples pigmented with anthocyanin (Table 3) presented a^* values from 4.8 ± 0 to 9.8 ± 0 , b^* values from -4.7 ± 0 to 0.63 ± 0 , and H° values from 82.6 ± 0.3 to 88.7 ± 0.1 and 276.9 ± 0.1 to 295.5 ± 0.1 , corresponding to red hues. Like samples of the purple pigment, the brightness L^* and b^* values decreased with increasing pigment concentration; however, the C^* and a^* values increased.

Several studies have reported the use and stability of natural pigments as colorings in milk matrices such as yogurt and milk (Nontasan et al. 2012; Cerezal-Mezquita et al. 2014, 2015; Ścibisz et al. 2012). According to these investigations, the storage temperature, pH, fat content, and the presence of other phenolic compounds significantly affects the stability of the pigments (Žilić et al. 2016).

Selection of matrices for purple pigment stability assessment

The selected concentrations for purple pigment stability assessment were 4, 6 and 8 mg/100 g, each with three doses of citric acid, 63, 126 and 252 mg/100 g. This additive was used because it has an important effect on the sensory attributes of products such as gelatin by balancing the sweet taste of food. For anthocyanin gellatins, three high concentrations (48, 56 and 64 mg/100 g) were evaluated using a five-point hedonic test, because they produced more intense colorations for this type of formulated product, making them more attractive to consumers.

According to the sensorial evaluation, the preferred gellatins were those with the highest purple pigment and citric acid concentrations of 8 and 252 mg, and 8 and 126 mg, respectively each per 100 g of gelatin. For anthocyanin, the gelatin was formulated with the highest concentrations of 64 and 63 mg anthocyanin and citric acid, respectively per 100 g of gelatin. Based on the results,

Fig. 4 Colour stability assessment (lightness [L^*] —●—, —○—; a^* —▲—, —△—; b^* —◆—, —◇—) of **a** gelatin and **b** yogurt samples pigmented with purple pigment (PP) from Palo Morado heartwood (filled markers) and commercial anthocyanin (ANT, hollow markers) stored at 4 °C for 28 days

these samples were chosen for stability testing of pigments in the gelatin matrix.

The yogurt matrix samples containing a concentration of 20 mg purple pigment/100 g with intermediate C^* and hue H° values were chosen. The anthocyanin samples chosen contained the highest pigment concentration of 164 mg/100 g.

These results show that for the gelatin samples, the selected purple pigment concentration was eightfold less than that used for anthocyanin. In the case of the yogurt samples, the purple pigment concentration used for coloring was 8.2-fold lower than that of anthocyanin.

Pigment stability assessment in gelatin matrices

The evaluation of the stability of gelatin revealed that samples pigmented with purple pigment showed an increasing trend in the lightness L^* and parameter b^* trend. Furthermore, the values were significantly different ($P < 0.05$) at the end of the stability test period with

Fig. 5 **a** Residual pigment (%) and **b** ΔE determination in gelatin (—●—, —○—) and yogurt (—▲—, —△—) samples pigmented with purple pigment (PP) from Palo Morado heartwood (filled markers) and commercial anthocyanin (ANT, hollow markers) stored at 4 °C for 28 days

respect to t_0 , resulting in lighter shades (Fig. 4a). In contrast, parameter a^* remained constant without any significant differences ($P > 0.05$) with respect to t_0 of the stability test. Samples with anthocyanin showed significantly decreasing lightness ($P < 0.05$), whereas parameters a^* and b^* increased significantly ($P < 0.05$) by the end of the stability assessment (Fig. 4a).

Furthermore, pigment concentrations in the gelatin matrix were measured during the stability evaluation. Figure 5a shows a significant decrease in the concentration ($P < 0.05$) in both the purple pigment and anthocyanin. At the end of the stability test, the % RP values for the purple pigment and anthocyanin gelatin samples were $80.96 \pm 1.24\%$ and $72.84 \pm 2.98\%$, respectively.

The color differences ΔL^* (brightness), Δa^* (redness), and Δb^* (yellowness) at the beginning (t_0) and at the end (t_{28}) of the stability evaluation were calculated (Table 4) as reported by Trehan et al. (2018). According to the delta values obtained it was found that both samples, purple pigment and anthocyanin, showed higher yellowness values at the end of the pigment stability evaluation.

Table 4 Color differences (ΔL^* , Δa^* , and Δb^*) after 28 days of storage time

Sample	Pigment	ΔL^*	Δa^*	Δb^*
Gelatin	Purple pigment	2.83 ± 0.2	-0.58 ± 0.09	3.08 ± 0.08
	Anthocyanin	-1.50 ± 0.22	2.72 ± 0.06	5.55 ± 0.01
Yogurt	Purple pigment	0.88 ± 0.06	-1.43 ± 0.07	1.83 ± 0.08
	Anthocyanin	0.27 ± 0.05	-0.45 ± 0.01	0.82 ± 0.01

Moreover, the total color difference, ΔE value, was calculated to confirm if changes in color parameters L^* , a^* , and b^* could be perceived by the human eye. According to Choi et al. (2002), color differences between samples and are not perceived when the ΔE has a value < 3 . At the end of the stability evaluation, purple pigment and anthocyanin-dyed gelatin samples showed ΔE values of 4.22 ± 0.02 and 5.48 ± 0.03 , respectively, which were clearly > 3 , at the end of the stability evaluation (Fig. 5b).

The effect of sugars on the stability of anthocyanins and other flavonoids is controversial because some studies affirm their negative effect, whereas others do not (Gutiérrez-Zúñiga et al. 2014; Hubbermann et al. 2006; Maier et al. 2009). Gutiérrez-Zúñiga et al. (2014) determined that neocandenteone, a pigment isolated from *Dalbergia congestiflora* heartwood show good stability in hard candy and gummies with sugar contents of 63.3 and 80% (w/w), respectively.

A stability study of anthocyanins and phenolic compounds from grape pomace pectin and gelatin matrices reported that anthocyanins were more stable in pectin than in gelatin matrix during storage at 6 and 20 °C for 24 weeks (Maier et al. 2009). This result was likely due to the stabilizing effect of polyuronic acids of pectin.

Anthocyanin stability in gelatin, pectin, and agar–agar gels containing citric or tartaric acid and sucrose or fructose was evaluated by Hubbermann et al. (2006). Products with high sugar content showed loss of pigment because, during the heat treatment of sugars and storage, degradation products such as furfural and hydroxymethyl furfural are generated. Moreover, the protein and sugar content and the heat supply promote Maillard reactions, which degrade the pigment.

Changes in color parameters (L^* , a^* , b^*) and %RP of purple pigment gelatins compared to those of anthocyanin samples suggest that the purple pigment was more stable, indicating its potential usefulness in matrices with high sugar and protein content, such as gelatin.

However, it is necessary to consider the heat treatment applied to gelatin samples during preparation because high temperatures are a determining factor in the stability of natural pigments such as *P. mexicana* pigments. High temperatures might favor the degradation of the pigment to colorless compounds, thereby decreasing the dyeing power (Maier et al. 2009).

Pigment stability assessment in yogurt matrices

Variations in the L^* , a^* , b^* parameters of both pigments evaluated in yogurt samples during the stability period are shown in Fig. 4b. The lightness (L^*) and b^* values showed an increasing trend for both pigments, which differed significantly ($P < 0.05$) between the first and the last day. In contrast, the a^* values decreased significantly ($P < 0.05$) in both cases, but this effect was more pronounced with the purple pigment samples than anthocyanin samples, which was detected as clearer and less reddish samples.

Ścibisz et al. (2012) evaluated the stability of anthocyanin blueberry yogurt pigmentation using an extract concentration of 2 g/100 g. A decrease in a^* values and increase in brightness L^* , and b^* values were observed in samples tested, which was like the results of samples dyed with purple pigment and anthocyanin. According to the authors, the changes observed may be due to the conversion of anthocyanins to a colorless or yellow form, which is characteristic of the degradation process. Likewise, peltomexicanin and the other pigments present in purple pigment from Palo Morado heartwood are chemically related to the (+)-peltogynol as flavonoids, which produce colorless to yellow degradation compounds.

Color differences in brightness, redness and yellowness at the beginning (t_0) and at the end (t_{28}) of the pigment stability evaluation are described in Table 4. According to Trehan et al. (2018), positive values of ΔL denote brighter samples than the reference, as can be seen for both pigments, this can be justified by the loss (degradation) of the natural pigments. Kaur et al. (2018) reported lower brightness values (L^*) in pigmented samples of rice compared to their non-pigmented counterparts, which shows the direct effect of the pigment in this color parameter. Also, the ΔE values were 0.03 ± 0.001 to 2.48 ± 0.03 and 0.28 ± 0.01 to 1.09 ± 0.04 for yogurt dyed with purple pigment and anthocyanin samples, respectively, which demonstrates that the color differences in yogurt matrices dyed with both pigments would not be perceived by consumers (Fig. 5b).

The final concentrations of pigment were significantly different ($P < 0.05$) compared to the initial concentrations, resulting in a % RP of $73.1 \pm 2.8\%$ and $76.3 \pm 1.7\%$ for yogurt samples containing the dye extract and anthocyanin, respectively (Fig. 5a). Results showed that the % RP was

lower in the dye extract than it was in the anthocyanin extract, which could be due to a decrease in the recovery percentage of the dye extract when the concentration was determined probably due to a high binding affinity to pigmented matrices.

Wallace and Giusti (2008) evaluated the stability of anthocyanin from red radish, red cabbage, black carrot, and grape skin in yogurt matrix, and discovered that the fat content in the milk matrix affected the final color of the product, which might favor co-pigmentation of anthocyanins. Furthermore, a similar situation could occur with peltomexicanin since it has similar structural characteristics to anthocyanins.

Conclusion

The Palo Morado heartwood is a source of valuable chemical compounds. Presently, the waste produced by its use in manufacturing handicrafts is discarded, but it could be used as a source material for extracting natural pigments with purple tones. The purple pigment from Palo Morado heartwood showed a great capacity for the pigmentation of gelatin and yogurt matrices, requiring lower doses than those of the commercial anthocyanin. Furthermore, the purple pigment also showed a greater range of color tones in gelatins than anthocyanin, depending on the concentration of citric acid, and it colored the yogurt more intensely. Moreover, the purple pigment did not exhibit mutagenic effects or acute toxicity. These properties could be exploited commercially to replace the use of synthetic dyes in the coloration of food products and create valuable products from the wastes of Palo Morado heartwood.

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