Original article

Nutritional and nutraceutical characteristics of white and red *Pithecellobium dulce* (Roxb.) Benth fruits

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Nutritional and nutraceutical characteristics of white and red *Pithecellobium dulce* (Roxb.) Benth fruits.

Abstract – Introduction. *Pithecellobium dulce* is a legume native to tropical America that produces edible arils which can be white or red. The plant is also grown in Asia and, to date, predominantly fruits produced in Asia have been the subject of scientific studies. We studied white and red arils produced in America. **Materials and methods**. White aril and red aril fruits were evaluated in an array of reagent-based assays to determine nutritional and nutraceutical properties. **Results and discussion**. White arils and red arils showed similar physicochemical characteristics, with high content of vitamin C (79.7–82.6 mg·100 g^{-1} fresh weight) and dietary fiber (5.83–6.12% fw). The anthocyanin content of red arils (29.5 mg·100 g^{-1} fw, as cyanidin-3-glucoside equivalents) was similar to that of strawberry. Total phenolics (517 mg·100 g^{-1} fw, as gallic acid equivalents) and antioxidant activities (ABTS, 224 mg; DPPH, 223 mg, as vitamin C equivalents) of red arils were 1.3 times higher than those in white arils. The methanolic extract of red arils showed a higher α-glucosidase inhibition (IC_{50} 2.9 mg·mL^{-1}) than acarbose (IC_{50} 4.9 mg·mL^{-1}). The methanolic extract [(50, 100 and 500) µg per tube] of red and white arils showed positive-strong antimutagenic activities (inhibition in the range 25–70%) in the assay (*Salmonella typhimurium* YG1024 strain, 1-nitropyrene as mutagen, 200 ng per tube). We are reporting for the first time remarkably high characteristics (*i.e.,* antioxidant, inhibition of α-amylase and α-glucosidase and content of dietary fiber) of *P. dulce* fruits, mainly of the red ones; properties which combined permit us to suggest that consumption of these fruits could have beneficial health effects in people with diabetes.

Mexico / *Pithecellobium dulce* / fruits / proximate composition / nutritive value / physicochemical properties / antioxidants / enzyme inhibitors / antimutagens

Caractéristiques nutritionnelles et nutraceutiques des fruits de *Pithecellobium dulce* (Roxb.) Benth. blancs et rouges.

Résumé – Introduction. *Pithecellobium dulce* est une légumineuse originaire d'Amérique tropicale qui produit des arilles comestibles qui peuvent être blancs ou rouges. La plante est également cultivée en Asie et, à ce jour, ce sont principalement les fruits produits en Asie qui ont fait l'objet d'études scientifiques. Nos recherches ont porté sur les arilles blancs et rouges de fruits produits en Amérique. **Matériel et méthodes**. Des fruits à arilles blancs et rouges ont été évalués dans une série de tests à base de réactifs pour déterminer leurs propriétés nutritionnelles et nutraceutiques. **Résultats et discussion**. Les arilles blancs et rouges ont montré des caractéristiques physico-chimiques similaires, à haute teneur en vitamine C [de (79,7 à 82,6) mg·100 g^{-1} de poids frais] et en fibres alimentaires (de 5,83 % à 6,12 % pf). La teneur en anthocyanes des arilles rouges (29,5 mg·100 g^{-1} pf, en équivalents cyanidine-3-glucoside) a été similaire à celle de la fraise. Les phénols totaux (517 mg·100 g^{-1} pf, en équivalents d'acide gallique) et les activités antioxydantes (ABTS, 224 mg ; DPPH, 223 mg, en équivalents de vitamine C) des arilles rouges ont été 1,3 fois plus élevés que ceux des arilles blancs. L'extrait méthanolique des arilles rouges a montré une inhibition de l'α-glucosidase supérieure (CI_{50} 2,9 mg·mL^{-1}) à celle de l'acarbose (CI_{50} 4,9 mg·mL^{-1}). L'extrait méthanolique [(50, 100 et 500) mg par tube] des arilles rouges et blancs a montré de fortes activités antimutagènes (inhibition dans la gamme 25 % à 70 %) lors de l'essai (souche *Salmonella typhimurium* YG1024, 1-nitropyrène comme mutagène, 200 ng par tube). Nous mentionnons pour la première fois les caractéristiques remarquablement élevées (antioxydantes, inhibition de α-amylase et de α-glucosidase et de la teneur en fibres alimentaires) des fruits de *P. dulce*, principalement de ceux à arille rouges ; les propriétés combinées nous permettent de suggérer que la consommation de ces fruits pourrait avoir des effets bénéfiques sur la santé des personnes atteintes de diabète.

Mexique / *Pithecellobium dulce* / fruits / composition glopale / valeur nutritive / propriété physicochimique / antioxydant / inhibiteur d'enzyme / antimutagène

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1. Introduction

The consumption of fruits and vegetables is important for reducing the risk of chronic degenerative diseases [1]. These foods contribute vitamins, minerals, dietary fiber and a wide range of phytochemicals (*e.g.,* phenolics, pigments) with nutraceutical properties (*e.g.,* antioxidant, anticarcinogenic and antihyperglycemic) [1,2]. The beneficial effects of a balanced diet are well known; however, food-related diseases are the main causes of death worldwide. In Mexico, the leading causes of mortality are cardiovascular diseases (51.9%), cancer (28.2%) and diabetes (20.6%) [3].

Pithecellobium dulce is commonly known as guamuchil in Sinaloa (México) and as monkeypod in the United States of America [4]. It is an arboreous tree; a legume of up to 20 m high which is widely distributed in the tropical areas of Mexico and America. This plant was initially introduced into the Philippines and then into India, where it was first described in 1795 [5]. In Mexico *P. dulce* has many traditional uses, such as medical, food, animal feed and timber [6]. *Pithecellobium dulce* leaves contain compounds with potential for diabetes treatment, whereas seeds have protease inhibitors and saponins with anti-inflammatory properties [7]. The monkeypod fruit is a pod composed of sweet edible arils covering black seeds; depending on the tree, arils are white or red. Reports about the nutritional and nutraceutical composition of these fruits are scarce and most of them are studies with fruits collected from Asia, where it is an introduced tree. *Pithecellobium dulce* arils are a good source of vitamin C (120–140 mg·100 g^{-1}), thiamine (222 µg·100 g^{-1}) and pectin (4–5%) but they have low content of calcium (13 mg·100 g^{-1}), phosphorus (54 mg·100 g^{-1}) and iron (1.4 mg·100 g^{-1}) [6–8]. The edible portion of *P. dulce* shows antioxidant, hepatoprotective and better inhibitory activity of the H^+, K^+-ATPase pump than omeprazole, a drug commonly used for gastritis treatment [9, 10]. Ellagic acid, gallic acid, mandelic acid, rutin, quercitrin, naringenin and kaempferol are the principal phenolics in the arils [10]. Most of the published studies about *P. dulce* arils did not specify the color of their fruits and so the nutritional/nutraceutical differences associated with this characteristic were impossible to establish. Thus, the main goal of our research was to evaluate the chemical/nutritional, antioxidant, antihyperglycemic (inhibition of α-glucosidase and α-amylase) and antimutagenic characteristics of *P. dulce* red and white arils.

2. Materials and methods

2.1. Plant material

White and red ripe *P. dulce* fruits were collected in the municipalities of Culiacan and Navolato, Sinaloa, Mexico. White and red arils were recovered from the pods, measured with a caliper (GENERAL, Switzerland) and weighed. Fresh material was used for evaluation of the physicochemical parameters. Arils were freeze-dried, milled and sieved through a number 40 mesh screen (particles smaller than 0.420 mm). The resulting aril flour was stored at –20 °C in plastic bags in darkness until use.

2.2. Phytochemical analyses

Phytochemicals were identified in tube assays: tannins, reactions with $FeCl_3$ (10%), gelatin, gelatin-NaCl and NaCl; flavonoids, using Mg/HCl; alkaloids, reactions with Mayer's, Wagner's or Dragendorff's reagents; saponins, by the foam formation test; triterpenes and/or sterols, the Liebermann-Burchard or Salkowski reactions; volatile coumarins, using paper chromatography and 5% KOH; reducing sugars, by Tollens' reagent; and free anthracenics, using 5% NaOH or magnesium acetate. Thin layer chromatography (TLC) was used for confirmatory tests [11].

2.3. Physicochemical parameters

These parameters were determined by standard methodologies [12]: soluble solids by using an Abbé refractometer (Milton Roy Co., CA, USA); and pH and titratable acidity (0.1 N NaOH) with a digital potentiometer (Orion Research Corp., Cambridge, MA, USA).

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2.4. Proximate composition, starch and dietary fiber

Moisture, lipids, ash and crude fiber were determined by the AOAC methodologies [12]. Protein determination was carried out by the micro-Kjeldahl method using the factor 6.25. Carbohydrates were calculated by difference: carbohydrates = (100 – moisture – proteins – lipids – ash). Dietary fiber and starch were measured with commercial kits ["dietary fiber, total" and "starch (GO/P) assay", respectively] distributed by Sigma (Sigma-Aldrich Co., St. Louis, MO, USA).

2.5. Vitamin C

Vitamin C was determined by HPLC, as described by Gökmen *et al.* [13], using Agilent 1100 equipment with a DAD detector (Agilent Technologies, Palo Alto, CA, USA) and a SPHEROCLONE ODS (2) column (250 mm × 4.6 mm × 5 µm) (Phenomenex, Torrance, CA, USA). The mobile phase was 25 mM monobasic phosphate and detection was carried out at 254 nm.

2.6. Mineral analyses

Samples for mineral analyses were prepared as described by Alcántar-González and Sandoval-Villa [14]. Briefly, fruit samples (0.5 g) were digested overnight in 10 mL concentrated HNO_3. Then, concentrated $HClO_4$ (1.5 mL) and concentrated H_2SO_4 (2 mL) were added; the mixture was heated starting with a low temperature (100–120 °C), in order to reach a complete oxidation, followed by progressive temperature increments up to 300 °C. The digestion was complete when samples showed a clear and transparent color. Mineral content was evaluated with an atomic absorption spectrometer (SpectrAA-220: Varian Inc., Palo Alto, CA, USA), as previously reported [15].

2.7. Fatty acid profile

Fatty acids were extracted and esterified as recommended by Park and Goins [16], with slight modifications. Nonadecanoic acid (C19:0; SUPELCO Inc., Germany) was used as an internal standard and trimethylsilyl chloride for the esterification reaction. Analysis was done with a GC HP 6890 Series II Plus coupled with a Selective Mass Detector 5973N (Agilent Technologies, Palo Alto, CA, USA) and a Quadrex series 007 column (30 m × 0.25 mm internal diameter × 0.25 µm) (Quadrex Corp., Woodbridge, CT, USA).

2.8. Total phenolics

Total phenolics were determined as described by Waterhouse [17]. The aril flour (1 g) was mixed with 10 mL of methanol-water (8:2 v/v) and sonicated for 15 min; the mixture was filtered, the liquid recovered and residue re-extracted as described; recovered liquids were made up to 100 mL with distilled water. Twenty µL of the extract (standard or blank) were mixed with 1.58 mL of deionized water and 0.1 mL of Folin-Ciocalteu reagent; the mixture was allowed to stand for 3 min; a saturated sodium carbonate solution (0.3 mL) was added; the mixture was incubated for 2 h and absorbance was measured at 765 nm (Spectronic® 20 Genesys™, Spectronic Instruments Inc., Rochester, NY, USA). Results were expressed as gallic acid equivalents (GAE) in 100 g of fresh weight of fruit (mg GAE·100 g^{-1} fw).

2.9. Total flavonoids

Fruit extract was prepared as described in section 2.8 [18]. Total flavonoids were quantified by reaction with $AlCl_3$. The extract (0.25 mL) was mixed with 1.25 mL of deionized water and 0.075 mL of 5% solution of sodium nitrite; the mixture was allowed to stand for 5 min, 0.15 mL of $AlCl_3$ was added, then it was allowed to stand for 6 min and mixed with 0.5 mL of 1 M NaOH and 0.275 mL of deionized water. Absorbance was measured at 510 nm and results were expressed as quercetin equivalents (QE) (mg QE·100 g^{-1} fw).

2.10. Condensed tannins

Samples (0.2 g) were extracted with methanol (10 mL) by sonication, as described in

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section 2.8. Condensed tannins were evaluated by reaction with vanillin (0.5%) in HCl (4% in methanol) as described by Price *et al.* [19]. Briefly, the extract (1 mL) was mixed with 5 mL of vanillin (1 mL·min^{-1}) and incubated (30 °C for 20 min). Absorbance was measured at 500 nm and results were expressed as (+)- catechin equivalents (mg CE·100 g^{-1} fw).

2.11. Anthocyanins

Anthocyanins were determined as described by Giusti and Wrolstad [20]. Aril flour (1 g) was extracted with 20 mL of methanol acidified with 1% of concentrated HCl; the sample was sonicated for 10 min; the solution was recovered by filtration and the residue re-extracted; the liquid phases were mixed and used for the analysis: an aliquot of 0.4 mL was mixed with 1.6 mL of pH 1 KCl buffer or with pH 4.5 CH_3COOK buffer; solutions were allowed to stand for 15 min and absorbance (A) readings were obtained at 530 and 700 nm, using distilled water as the blank. Sample absorbance (A_S) was calculated as [$A_S = (A_{530}–A_{700})_{pH\ 1} – (A_{530}–A_{700})_{pH\ 4.5}$]. Anthocyanins were determined as: [anthocyanins (mg·L^{-1}) = ($A_S \times MW \times DF \times 1000$) / ($\varepsilon \times 1$)], where: MW = molecular weight of cyanidin-3-glucoside (449.2 g·mol^{-1}), DF = dilution factor (for this assay, DF = (2 / 0.4) = 5) and ε = molar absorptivity of cyanidin-3-glucoside (26900 mol·L^{-1}). Results were expressed as cyanidin-3-glucoside equivalents (mg C3GE·100 g^{-1} fw).

2.12. Preparation of the methanolic extracts

Methanolic extracts of *P. dulce* arils were used to evaluate the following activities: antioxidant, inhibition of α-amylase and α-glucosidase, and antimutagenic.

Aril flour (1 g) was mixed with 20 mL of methanol, sonicated for 10 min, centrifuged (20,000 *g*, 10 min, 4 °C), the supernatant was recovered, the pellet was re-extracted one more time, and both supernatants were combined. An aliquot of the supernatant was taken to evaluate the antioxidant activity and the other part was concentrated at 39 °C under vacuum (Büchi Waterbath B-48, Brinkmann Instruments Inc., Westbury, NY, USA) to obtain the methanolic extract which was stored at –20 °C in darkness until use.

2.13. Antioxidant activity

2.13.1. ABTS assay

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical was generated by mixing 7 mM ABTS with 2.5 mM potassium persulfate; the mixture was allowed to stand in darkness for 16 h at room temperature, then it was diluted with phosphate-buffered saline, pH 7.4, to get an absorbance reading of 1 to 1.2 at 734 nm [21]. For the assay, methanolic extract (50 µL), or methanol as control, and ABTS radical (1.95 mL) were mixed and incubated in darkness (37 °C for 10 min) and readings were taken at 734 nm [21]. Antioxidant activity (AA) was calculated as percentage of discoloration of the ABTS radical: {%AA = [(A_C–A_S) / A_C] × 100}, where A_C and A_S were the absorbances for the control and the sample, respectively. Results were expressed as vitamin C equivalents (mg VCE·100 g^{-1} fw).

2.13.2. DPPH assay

The antioxidant activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method as recommended by Lim *et al.* [22] with slight modifications. The methanolic extract (0.2 mL) was mixed with 1.8 mL of the DPPH radical (150 µM in ethanol); the mixture was incubated in darkness (room temperature for 30 min) and absorbance was measured at 517 nm. Quantification was done as described in section 2.13.1.

2.14. α-Glucosidase inhibition

Inhibitory activity of α-glucosidase was carried out as described by Pinto *et al.* [23] with slight modifications. In 96-well plates, one hundred µL of *Saccharomyces cerevisiae* α-glucosidase (1 U per mL in phosphate-buffered saline (PBS), pH 6.9) and 50 µL of methanolic extract at different concentrations in PBS, or acarbose as the positive control, were mixed and incubated at 37 °C for

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10 min; then, fifty µL of nitrophenyl-glucopyranoside (5 mM in PBS) were added and the mixture was incubated (37 °C for 10 min). Readings were taken at 405 nm and the percentage of α-glucosidase inhibition (αGI) was calculated as {%αGI = [(A_C–A_S) / A_C] × 100}, where A_C and A_S were the absorbances for the control and sample, respectively; fifty µL of solvent instead of extract were used as control. Additionally, sample interferences were corrected by using a blank, which was prepared by mixing 100 µL of enzyme, 50 µL of extract and 50 µL of PBS. The amount of sample required to achieve 50% of enzyme inhibition (IC_{50}) was calculated using the GraphPad Prism Inc. v. 5.03 software (La Jolla, CA, USA).

2.15. α-Amylase inhibition

The α-amylase inhibition (αAI) was determined as described by Kim *et al.* [24] with slight modifications. The starch hydrolysis was screened in sterile Petri dishes containing 1.5% of agar and 1% of starch. In each plate, three paper-sterile discs (Whatman 1, 6 mm) were placed on agar; then, ten µL of extract per disc [(10, 1 and 0.1) µg·mL^{-1}, which correspond to (100, 10 and 1) µg of extract per disc] and 10 µL per disc of human-saliva α-amylase (10 U·mL^{-1}) (Sigma-Aldrich Co., St. Louis, MO, USA) were added. Petri dishes were incubated (37 °C for 72 h) and flooded with iodine solution (5 mM I_2 in 3% KI). As a negative control, PBS, pH 6.9, was used instead of the extract. The αAI was calculated as the reduction (%) in the inhibitory halo of α-amylase: {%αAI = [(D_C–D_S) / D_C] × 100}; where D_C and D_S were the diameters for the halos of the control and sample, respectively. Acarbose was used as a control.

2.16. Antimutagenic activity

Pithecellobium dulce methanolic extract was dissolved in dimethyl sulfoxide (DMSO) and its activity against the mutagenicity of 1-nitropyrene (1-NP) on *Salmonella typhimurium* YG1024 was determined as described by Kado *et al.* [25].

A dose-response curve for 1-NP mutagenicity was prepared (0–250 µg per tube). Toxicity/mutagenicity of the evaluated extracts was determined and those with a negative result were assayed for antimutagenicity. An extract was considered toxic if it induced a significant reduction or mutagenic if it increased the spontaneous reversion more than two times.

A cocktail was prepared by mixing water, disodium hydrogen phosphate buffer (0.2 M, pH 7.4), KCl-$MgCl_2$ 1.65 M–0.4 M, 0.1 M NADP and 1 M glucose-6-phosphate (1155:1500:60:120:15 v/v). In sterile glass culture tubes (10 mm × 100 mm) kept on ice, 95 µL of cocktail, 100 µL of bacteria (1 × 10^{10} cells·mL^{-1} in PBS), 10 µL of extract (100, 50, 10 and 5) µg·µL^{-1}, values equivalent to (1000, 500 and 100) µg per tube, respectively) or DMSO as control, and 5 µL of 1-NP (40 µg·µL^{-1}, 200 µg per tube) were mixed; the mixture was incubated in darkness with vigorous shaking for 90 min (37 °C). Tubes were placed in an ice bath and removed one at time and the content of every one of them was mixed with 2 mL of soft agar containing 90 nmol of histidine and biotin. Tubes were vortex-mixed and poured onto minimal glucose plates. Plates were incubated in darkness at 37 °C for 48 h, colony revertants were counted and antimutagenic activity was determined as percentage inhibition of the mutagenicity of 1-NP. Each assay was done in triplicate for two independent experiments.

2.17. Statistical analysis

The results are presented as the average of at least three independent experiments. Statgraphics Plus version 5 (Statpoint Technologies Inc., Warrington, VA, USA) was used for the variance analyses and to establish statistical differences by Fisher's test (α = 0.05).

3. Results and discussion

3.1. Phytochemical analysis

The same types of metabolites were identified in white and red arils of *Pithecellobium*

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Table I.
Phytochemical analysis of *Pithecellobium dulce* arils in Mexico. Alkaloids, triterpenes and/or sterols, volatile coumarins and free anthracenics were absent.

Metabolite	Reaction of or with	White arils	Red arils
Tannins	10% $FeCl_3$	Present	Abundant
	Gelatin	Present	Abundant
	Gelatin/NaCl	Present	Abundant
	NaCl	Absent	Absent
Flavonoids	Mg/HCl	Present	Present
Saponins	Foam formation test	Present	Abundant
Reducing sugars	Tollens	Present	Present

dulce (*table I*); however, the concentrations of tannins, flavonoids and saponins were higher for the red arils [9]. Previously, sterols were reported for *P. dulce* arils, contrasting with our finding of their absence in the white and red arils studied.

3.2. Physicochemical and nutritional analyses

The white and red arils studied showed similar average weight and size but a higher variability was registered for the white arils (*table II*). In general, the size was similar to that reported for white *P. dulce* fruits (20 mm) [4]. *Pithecellobium dulce* arils were sweet and slightly acidic; apparently, the acidity for the red arils was slightly higher, characterized by a lower pH than that of white arils but without significant differences in acidity. Contrasting results were reported previously; red arils showed higher acidity (4.8%) than that of white arils (2.4%) [9].

Proximate composition was similar for white arils and red arils, but white arils showed a high level of ash (*table II*). For both arils, the content of proteins was higher than the value previously reported (0.7%), whereas their values for moisture and carbohydrates were similar; 77.9% and 19.9%, respectively [7]. The energy values for both arils were similar to that of cherimoya (*Annona cherimoya*) (75 kcal·100 g^{-1} fw) and banana (*Musa acuminata* Colla) (89 kcal·100 g^{-1} fw) [26]. *Pithecellobium dulce* arils could be considered as a low-energy food, diet consumption of 100 g contributing less than 5% of the recommended caloric intake [27]; the fruits showed an almost null content of starch, and, remarkably, their dietary fiber (*table II*) was higher than in commonly consumed fruits (*e.g.,* apples, 2.45%: mango, 1.6%; orange, 2.4%; guava, 5.4%) [26]. A 100-g portion of *P. dulce* arils contributes 17% for men and 24% for women of the recommended daily intake of dietary fiber [27]. A relationship between the consumption of fiber-rich foods and a decreased risk for coronary heart diseases, diabetes, obesity and certain types of cancer has been identified. In addition to their health benefits, fibers improve the texture and stability of foods; thus, researchers and food producers are interested in new sources of dietary fiber [28]. The content of vitamin C was higher in white arils than in red arils (*table II*); the consumption of a 100-g portion could contribute about 90% and 100% of the suggested daily intake for men and women, respectively [27]; however, the reported values were lower than those previously published (120–140 mg·100 g^{-1}) [7, 8]. Both arils were good sources of potassium and copper, and their concentrations were higher in red arils (*table II*). The main fatty acids in both white arils and red arils were stearic, palmitic and oleic, whereas their concentrations of essential fatty acids (linoleic and linolenic) were low considering the recommended daily intakes (linoleic 11–17 g per day, linolenic 1.1–1.6 g per day) [27].

3.3. Phenolics and antioxidant activity

The content of phenolics in red arils was 1.32 higher than that in white arils,

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Table II.
Phytochemical and nutritional analyses of *Pithecellobium dulce* arils in Mexico.

a) Physical characteristics of fresh fruits (average of 40 measurements ± standard deviation).

Aril	Length	Width 1	Width 2	Whole fruit	Aril	Seed
		(mm)			Weight (g)	
White	23.01 ± 6.04	14.52 ± 4.37	14.7 ± 4.22	2.93 ± 1.97	2.71 ± 1.98	0.22 ± 0.05
Red	19.27 ± 3.00	14.97 ± 2.30	13.60 ± 2.03	1.96 ± 0.57	1.70 ± 0.53	0.26 ± 0.07

b) Chemical characteristics (average of 3 measurements ± standard deviation).

Aril	pH	Titratable acidity (% of citric acid)	Total soluble solids (°Brix)	Moisture (%)	Proximate composition (g·100 g^{-1} fresh weight)						Energy[1] (kcal)
					Proteins	Lipids	Ash	Carbohydrates	Starch	Total dietary fiber	
White	4.70 ± 0.10 a	0.89 ± 0.07	16.7 ± 0.6	77.8 ± 2.0	2.59 ± 0.2	0.15 ± 0.02	0.68 ± 0.09 a	18.75	< 0.1	6.12 ± 0.7	77.46
Red	4.28 ± 0.16 b	0.93 ± 0.05	17.2 ± 0.5	76.6 ± 0.1	2.38 ± 0.2	0.14 ± 0.01	0.51 ± 0.02 b	20.36	< 0.1	5.83 ± 0.9	82.46

Carbohydrates were determined by difference of the other proximate components.
[1]Energy was expressed as kcal·100 g^{-1} fresh weight and calculated using the USDA conversion factors for fruits: carbohydrates 3.6 kcal·g^{-1}, proteins 3.36 kcal·g^{-1} and lipids 8.37 kcal·g^{-1}.

c) Nutritional characteristics.

Aril	Vitamin C (mg·100 g^{-1})	Minerals (adequate intake in mg per day) [27]								
		Ca (1200)	Fe (22)	Mg (340)	P (700)	K (1250) (mg·100 g^{-1})	Na	Zn (15)	Cu (0.75)	Mn (2.3)
White	82.6 ± 5	17.76 ± 2.13 a	0.58 ± 0.02	28.86 ± 3.75	22.30 ± 0.89 a	285.53 ± 17.13	0.92 ± 0.06 a	0.35 ± 0.03	0.15 ± 0.01 a	0.10 ± 0.01
Red	79.7 ± 12	35.1 ± 3.16 b	0.72 ± 0.07	28.08 ± 1.40	16.34 ± 1.14 b	326.45 ± 13.06	1.67 ± 0.05 b	0.41 ± 0.02	0.2 ± 0.02 b	0.12 ± 0.01

Aril	Fatty acids				
	Stearic	Palmitic	Oleic	Linoleic	Linolenic
			(mg/100 g)		
White	61.4 ± 6.5	74.1 ± 11.2 a	25.6 ± 3.0	10.2 ± 1.3 a	10.8 ± 1.5
Red	54.7 ± 5.9	46.9 ± 8.2 b	35.4 ± 10.0	6.0 ± 2.1 b	11.1 ± 1.9

Different letters between rows show significant differences.

Table III.
Phenolic compounds and antioxidant activity of *Pithecellobium dulce* arils in Mexico (results were calculated per 100 g of arils on a fresh weight basis, values are the mean ± standard deviation of three measurements).

Aril	Phenolics				Antioxidant activity	
	Total phenolics (mg Eq gallic acid)	Total flavonoids (mg Eq quercetin)	Tannins (mg Eq catechin)	Anthocyanins (mg Eq cyanidin-3-glucoside)	DPPH	ABTS
					(mg vitamin C)	
White	392.2 ± 5 a	50.0 ± 2.7 a	148.2 ± 48 a	< 1 a	170.9 ± 14 a	155.9 ± 14 a
Red	517.8 ± 42 b	86.6 ± 9.5 b	309.2 ± 49 b	25.9 ± 0.5 b	223.4 ± 12 b	224.8 ± 16 b

Different letters between rows show significant differences.

corroborating previous results [29], as well as tannins, flavonoids and antioxidant activity (*table III*). Total phenolic values of red arils and white arils were higher than those of other tropical fruits (*e.g.,* papaya, 28 mg·100 g fw; orange, 75 mg·100 g fw; guava, 139-178 mg·100 g fw) [22].

Vitamin C interferes with total phenolic determination by the Folin-Ciocalteu assay; vitamin C reactivity is 66.2% of the gallic acid reactivity [30]. *Pithecellobium dulce* arils had a vitamin C content of about 80 mg·100 g^{-1} fw (*table II*) and its contribution to their total phenolic content is lower than 13%, with 52.96 mg GAE·100 g^{-1} fw (*table III*). Based on the vitamin C instability, this calculation must be higher than the real value since vitamin C was measured in fresh fruits and total phenolics in dried aril flours. In general, phenolics are abundant in plants and the Folin-Ciocalteu assay gives a good estimation of the total phenolic content in these organisms [30].

The anthocyanin content of red arils was similar to that of red currants (*Ribes rubrum*, 12.14–22.06 mg·100 g^{-1} fw) [31], cranberries (*Vaccinium macrocarpon*, 19.8–65.6 mg·100 g^{-1} fw) and strawberries (*Fragaria* × *anannasa*, 20.07–39.08 mg·100 g^{-1} fw) [32]. The antioxidant activity for the methanolic extract of red arils was 1.30 and 1.44 times higher than that of white arils, measured by the DPPH and ABTS assays, respectively; this ratio was similar to that found for total phenolics. By the DPPH assay, antioxidant activity of red arils was higher than that in other tropical fruits [*e.g.,* dragon fruit and guava with (13.5 and 218) mg vitamin C Eq·100 g^{-1} fw], but it was lower for the white arils [22]. In the evaluation of antioxidant activity by the ABTS method, activity of red arils and white arils was higher than that reported for *Opuntia* fruits (13–33 mg vitamin C Eq·100 g^{-1} fw) [33] and lower than that of different prune varieties (*Prunus domestica*, 258–604 mg vitamin C Eq ·100 g^{-1} fw) [34].

3.4. Inhibition of α-glucosidase and α-amylase

The activities of α-glucosidase (αGI) and α-amylase (αAI) were evaluated for the methanolic extract of both arils (red and white arils). The αGI and αAI values of red arils were higher than those of white arils (*table IV*). For αGI, activities of both arils were higher than those obtained with different strawberry varieties (IC_{50} approximately 20 mg·mL^{-1}) [23], measured by the same protocol. The IC50 value for αGI of the methanolic extract of red arils was 1.6 times lower than that of acarbose; this means a higher activity for the red arils, as less methanolic extract than acarbose was required to get similar glucosidase inhibition. The IC50 values for ethanolic extracts of sorghum varieties and acarbose are similar [24], being less active than the methanol extract of red arils. Only red arils exhibited αAI at 100 µg per disc; this value was about three times lower than that of acarbose (*table IV*). It has been reported that phenolic-rich fruits can be good inhibitors of the enzymes involved in carbohydrate digestion [2]. Fractions enriched in anthocyanins obtained from fruits show good inhibitory activity of α-glucosidase, whereas those high in tannins are better against α-amylase [35]. On the other hand, Wiese *et al.* have shown a high affinity

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Table IV.
Inhibitory activity of the methanolic extracts of *Pithecellobium dulce* arils against α-glucosidase and α-amylase (%) in Mexico (values are the mean ± standard deviation of three measurements). Acarbose was used as a positive control.

Aril	α-glucosidase IC_{50} ($mg \cdot mL^{-1}$)	α-amylase (%)		
		Extract concentration in µg per disc		
		100	10	1
White	16.7 ± 7.0 a	0 a	0 a	0 a
Red	2.9 ± 0.1 b	33.3 ± 4.7 b	0 a	0 a
Acarbose	4.9 ± 0.9 c	100 c	41.3 ± 11.9 b	8.0 ± 2.7 b

Different letters between rows show significant differences.

Table V.
Inhibition (%) of the 1-nitropyrene (200 ng per tube) mutagenicity by the methanolic extracts of the *Pithecellobium dulce* arils in Mexico (values are the mean ± standard deviation of three measurements).

Aril	Concentration of methanolic extract (µg per tube)		
	50	100	500
White	30.7 ± 11.8 a	40.8 ± 11.3 a	70.9 ± 1.9 a
Red	25.6 ± 5.3 a	40.3 ± 10.0 a	65.3 ± 2.8 b

The tester strain was *Salmonella typhimurium* YG1024.
Different superscript letters between rows show significant differences.
The number of spontaneous revertants was 35 ± 8 and that of the induced revertants was 402 ± 84.

between anthocyanins and the salival α-amylase [36]. Consequently, the αGI and αAI of the red arils could be associated with their high content of anthocyanins and tannins.

It has been established that use of inhibitors of glucosidase (*e.g.,* acarbose) reduces postprandial hyperglycemia, delays the risk of progression of diabetes by 36% and reduces cardiovascular accidents by 49% [37]. Postprandial hyperglycemia induces several physiological conditions such as oxidative stress, increment in the concentration of inflammation factors, high cardiac frequency and endothelial dysfunction, among others [38]. It has been suggested that combination of glucosidase inhibitors and antioxidants is a good therapeutic/preventive option for the conditions mentioned [39]. The methanolic extracts of *P. dulce* red arils showed high αGI and antioxidant activity; these properties substantiate their consumption as a good preventive/therapeutic strategy for people at risk for type 2 diabetes; additionally, the high dietary fiber of *P. dulce* fruits could be important to potentiate the health benefits mentioned.

3.5. Antimutagenic activity

The methanolic extracts of red arils and white arils were toxic at 1000 µg per tube for *Salmonella typhimurium* YG1024, reducing the number of spontaneous revertants by approximately 40% (data not shown); at lower concentrations (500, 100 and 50 µg per tube), methanolic extracts were neither toxic nor mutagenic. Thus, the antimutagenicity of both methanolic extracts was evaluated up to 500 µg per tube. The methanolic extracts of red and white arils showed a dose-response activity against 1-nitropyrene mutagenicity (red arils 25.6% – 65.3%; white arils 30.7% – 70.9%) (*table V*); by considering the classification of Wall *et al.* [40], methanolic extracts were strong antimutagens at 500 µg per tube. Nitroaromatic compounds are classified as carcinogens; 1-nitropyrene

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is considered a public health concern since it has been found in foods [41]. The antimutagenic activities for the methanolic extracts of both types of *P. dulce* fruits (at 500 and 100 µg per tube) were higher than those reported for the same concentrations of an aqueous extract of *Randia echinocarpa* fruits, showing 32% – 53% and 5% – 17%, respectively [42], and for a phenolic extract of *Phaseolus vulgaris* seeds (35% inhibition at 500 µg per tube) [43].

4. Conclusions

The *Pithecellobium dulce* white and red arils showed a similar phytochemical profile and nutritional composition (*e.g.,* proteins, lipids, fiber and vitamin C). However, the best nutraceutical characteristics (*e.g.,* phenolics, antioxidant activity and inhibitory activities of α-amylase and α-glucosidase) were found for red aril fruits; this is the first report of these properties. The remarkably nutraceutical characteristics of red arils, combined with their high content of dietary fiber, permit us to suggest that consumption of these fruits could provide health benefits, *e.g.,* for prevention or treatment of chronic degenerative diseases such as diabetes, in addition to their nutritional contribution.

It is well known that fruit composition is influenced by geographic and environmental factors. Thus, future studies must be done in order to establish the locality and harvesting season effects on the composition and biological activities registered for the *P. dulce* fruits. Moreover, *in vivo* studies are required to demonstrate the nutraceutical contribution of *P. dulce* fruit consumption.

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Características nutricionales y nutracéuticas de los frutos de *Pithecellobium dulce* (Roxb.) Benth. blancos y rojos.

Resumen – Introducción. *Pithecellobium dulce* es una leguminosa originaria de la América tropical que produce vainas comestibles que pueden ser blancas o rojas. La planta se cultiva también en Asia y, actualmente, son principalmente las frutas producidas en Asia las que son objeto de estudios científicos. Nuestras investigaciones se centraron en las vainas blancas y rojas de los frutos producidos en América. **Material y métodos**. Los frutos de vainas blancas y rojas se evaluaron en una serie de pruebas basadas en reactivos para determinar sus propiedades nutricionales y nutracéuticas. **Resultados y discusión**. Las vainas blancas y rojas mostraron características físico-químicas similares, con alto contenido en vitamina C [de (79,7 a 82,6) mg·100 g^{-1} de peso fresco] y en fibras alimentarias (de 5,83 % a 6,12 % pf). El contenido en antocianos de las vainas rojas (29,5 mg·100 g^{-1} pf, en equivalentes de cianidina-3-glucosida) fue similar al de la fresa. Los fenoles totales (517 mg·100 g^{-1} pf, en equivalentes de ácido gálico) y las actividades antioxidantes (ABTS, 224 mg; DPPH, 223 mg, en equivalentes de vitamina C) de las vainas rojas fueron 1,3 veces mayores que en las vainas blancas. El extracto metanólico de las vainas rojas mostró una inhibición de α-glucosidasa superior (CI_{50} 2,9 mg·mL^{-1}) a la de la acarbosa (CI_{50} 4,9 mg·mL^{-1}). El extracto metanólico [(50, 100 y 500) µg por tubo] de las vainas rojas y blancas mostró positive-fuertes actividades antimutagénicas (inhibición de entre el 25% y el 70%) durante el ensayo (cepa *Salmonella typhimurium* YG1024, 1-nitropireno como mutágeno, 200 ng por tubo). Mencionamos por primera vez las características especialmente elevadas (antioxidantes, inhibición de α-amilasa y de α-glucosidasa y contenido en fibras alimentarias) de los frutos de *P. dulce*, sobre todo aquellos de vainas rojas; las propiedades combinadas nos permiten deducir que el consumo de estos frutos podría tener efectos beneficiosos en la salud de los diabéticos.

México / *Pithecellobium dulce* / frutas / composición proximal / valor nutritivo / propiedades fisicoquímicas / antioxidantes / inhibidores de enzimas / antimutágeno

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