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Chemical characterization, polyphenol content and antioxidant capacity of two pitahaya ecotypes (*Hylocereus* spp.)

UNIVERSIDAD NACIONAL DE COLOMBIA

Caracterización química, contenido de polifenoles y capacidad antioxidante de dos ecotipos de pitahaya (*Hylocereus* spp.)

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Keywords:
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Antioxidant capacity
Bioactive compounds
DPPH
Functional foods
Polyphenols

ABSTRACT

Pitahaya has originated worldwide interest due to its content of bioactive compounds with proven beneficial effects on health, acting as antioxidants against free radicals. This study aimed to evaluate the nutraceutical potential of the peel and pulp of the red (*Hylocereus monacanthus*) and yellow (*Hylocereus megalanthus)* pitahaya ecotypes for nutritional formulation purposes. Two pitahaya ecotypes were analyzed, obtaining a methanolic extract of the peel and edible part to perform the proximal chemical analysis, the phytochemical screening, and determine antioxidant activity by the DPPH, ABTS, and IC_{50} methods. Flavonoids, tannins, quinones, among other bioactive compounds, were identified. Yellow pitahaya presented higher content of polyphenols and higher antioxidant activity by the ABTS method, while the average inhibition percentage for both ecotypes was 93% by DPPH method. IC_{50} was higher for the edible part of red pitahaya with 1.68 mg mL^{-1}. Both ecotypes have a high content of polyphenols and a high antioxidant capacity, which agree with those found in different studies such as those of Colombia, Brazil and Korea, being as high or even higher than most varieties of citrus fruits in Peru. Future studies should consider the inclusion of other metabolites and bioactive substances such as betalains due to their antioxidant activity. Both pitahaya ecotypes are rich in antioxidants, bioactive compounds, have low energy density, and may be suitable for food prescriptions as a functional ingredient in food industry.

Palabras clave:
ABTS
Capacidad antioxidante
Compuestos bioactivos
DPPH
Alimentos funcionales
Polifenoles.

RESUMEN

La pitahaya ha suscitado el interés mundial debido a su contenido de compuestos bioactivos con comprobados efectos benéficos para la salud, actuando como antioxidantes frente a los radicales libres. El objetivo de este estudio fue evaluar el potencial nutracéutico de la cáscara y pulpa de los ecotipos pitahaya roja (*Hylocereus monacanthus*) y amarilla (*Hylocereus megalanthus)*, con fines de formulación nutricional. Se analizaron dichos ecotipos de pitahaya, obteniéndose un extracto metanólico de la cáscara y parte comestible de ambos ecotipos a fin de realizar el análisis químico proximal, la marcha fitoquímica, y determinar actividad antioxidante por los métodos DPPH, ABTS e IC_{50}. Se identificaron flavonoides, taninos, quinonas, entre otros compuestos bioactivos. La pitahaya amarilla presentó mayor contenido de polifenoles y mayor actividad antioxidante por el método ABTS, mientras que el porcentaje de inhibición promedio para ambos ecotipos fue del 93% por el método DPPH. El IC_{50} fue mayor para la pulpa de pitahaya roja con 1,68 mg mL^{-1}. Ambos ecotipos presentan un alto contenido de polifenoles y una alta capacidad antioxidante, los cuales concuerdan con los encontrados en distintos estudios como los de Colombia, Brazil y Corea, siendo tan alta o incluso superior a la de la mayoría de las variedades de cítricos en Perú. Futuros estudios deberían considerar incluir a otros metabolitos y sustancias bioactivas como las betalainas debido a su actividad antioxidante. Ambos ecotipos de pitahaya son ricos en antioxidantes, compuestos bioactivos, y de bajo aporte calórico, recomendándose su uso en prescripciones alimentarias y en la industria de alimentos como ingrediente funcional.

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Natural antioxidants are substances that can be part of our diet, capable of preventing the adverse effects of reactive oxygen species (ROS) -free radicals-, by inhibiting or interrupting the reactions in which they participate (Olugbami *et al*., 2014). ROS are produced during cell metabolism or by exposure to oxidizing compounds. These free radicals are related to non-communicable diseases and pathologies, such as cancer and cellular aging. A high amount of ROS leads to significant oxidation of various macromolecules, causing damage to cells and tissues (Seyidoglu and Aydin, 2016).

Polyphenols are antioxidant compounds of plant origin, which include flavonoids, phenolic acids, tannins, and lignans, among others, that show a protective effect against the harmful impacts of free radicals. These phenolic compounds can be found in a varied diet, rich in fruits and vegetables, abundant in essential nutrients such as vitamins, minerals, and antioxidants necessary for the body (Seyidoglu and Aydin, 2016). A diet rich in polyphenols, based on the daily consumption of fruits and vegetables has a beneficial effect on the prevention of non-communicable diseases (NCDs) (Williamson, 2017).

Pitahaya or dragon fruit is an exotic, non-climacteric fruit (Ortiz-Hernández *et al*., 2012), but it may behave as climacteric when collected at a high maturation state (Vásquez-Castillo *et al*., 2016). It shows various functional/nutraceutical characteristics (Joshi and Prabhakar, 2020), having antioxidant, antiproliferative, anti-inflammatory, chemopreventive and antidiabetic properties (Joshi and Prabhakar, 2020; Kim *et al*., 2011). These properties are explained by its high content of polyphenols and secondary metabolites such as steroids, triterpenes, tannins, and flavonoids (Ibrahim *et al*., 2018). Pitahaya species (*Hylocereus spp*.) are tropical fruits native to Mexico, Central, and South America. However, it is currently produced in many Asian countries, Australia, Israel, and the USA (Verona-Ruiz *et al*., 2020). Pitahaya fruit's pulp is white, red or fuchsia with edible black seeds has a gelatinous consistency and a sweet taste (Ibrahim *et al*., 2018). Regarding its nutritional value, the fruit is rich in vitamins (mainly vitamin C), minerals (especially magnesium, potassium and phosphorus), antioxidants and fiber, but their levels differ among varieties or species (Ibrahim *et al*., 2018; Verona-Ruiz *et al*., 2020). Researchers in Korea, focusing on the antioxidant and antiproliferative properties of pitahaya, found marked differences in the polyphenol and flavonoid contents of red and white pitahaya pulp and peel. Red pitahaya peel and white pitahaya peel contained similar polyphenols and flavonoids levels, while red pitahaya pulp contained more polyphenols and flavonoids than white pitahaya pulp (Kim *et al*., 2011).

Despite being popular as a health food in many countries (Ibrahim *et al*., 2018), its production and consumption are not widespread in Peru (Ramos, 2017). Research in this country about local pitahaya species as functional foods is scarce, despite the great global interest that this fruit has arisen lately (Verona-Ruiz *et al*., 2020). Many ecotypes -differentiated and locally adapted varieties- are found among pitahaya species (Ortiz-Hernández *et al*., 2012); thus, it is expected to find differences in their chemical composition, nutritional value and antioxidant capacity. In addition, there is a research gap on the evaluation of the nutraceutical potential of the *Hylocereus megalanthus* "yellow pitahaya" and *Hylocereus monacanthus* "red pitahaya" species.

In this context, this research aimed to chemically characterize the fruit, determine the polyphenol content and evaluate the antioxidant capacity of both pitahaya ecotypes for nutritional formulation purposes.

MATERIALS AND METHODS

The protocol for this research proyect was submitted to the Ethics and Research Committee of the Faculty of Health Sciences at *Universidad Peruana de Ciencias Aplicadas* , Lima Peru. They exempted the protocol from further revision and its execution was approved based on the documents FCS/203-09-18 , FCS/CEI 210-09-19, and FCS/CEI 024-02-20.

Physicochemical characterization, total phenolic content, and ABTS radical scavenging capacity assays were carried out at *La Molina Calidad Total Laboratorios - UNALM*. Phytochemical screening, DPPH radical scavenging capacity and the IC50 assays were performed at the *Instituto de Investigación de Bioquímica y Biología Molecular de la Universidad Nacional Agraria La Molina*.

Plant materials

The samples of *Hylocereus megalanthus* "yellow pitahaya" and *Hylocereus monacanthus* "red pitahaya" were obtained from *Mercado Modelo de Frutas*, Lima, Peru, between Jan 2019- Jan 2020. The yellow pitahaya ecotype was selected in its 5 stage of maturity, indicated by the peel yellow color, with slightly greenish nipple tips, based on a visual maturity scale for yellow pitahaya (ICONTEC, 1996). The red pitahaya ecotype was selected in its full maturity stage, indicated by the peel red-purple color in 75-100% of the fruit (Osuna-Enciso *et al*., 2011). Fruits that showed bruises and deterioration were excluded. Botanical identification of the fruits as *Hylocereus monacanthus* (Hort. Ex Lem) Britton & Rose (red pitahaya) and *Hylocereus megalanthus* (K. Schum. Ex Vaupel) Ralf Bauer (yellow pitahaya) was carried out in the Natural History Museum of the *Universidad Nacional Mayor de San Marcos* (UNMSM).

After washing and drying, the weight of each fruit was recorded. Then, the peel and the pulp were weighed separately. The edible part (pulp and seeds) was homogenized using a mortar, evenly distributed on Petri dishes and frozen at -20 °C for 48 h. Afterwards, the samples were lyophilized at -60 °C for 2 days, obtaining the material for the extraction process. Later, the peels were cut into 2 mm slices and dried using a vacuum oven at 40 °C until there was no difference in weight. The dried peels were ground with a blender and sieved using a 300-µm sieve (Standard Mesh N° 50), obtaining the pitahaya peel flour ready for the extraction step.

For the proximal chemical analysis, the edible part of the fruits (pulp and seeds) was separated from the peel, placed in separated aluminum trays, frozen at -20 °C, and then lyophilized for 48 h. This material was used for the determination of the chemical composition of both pitahaya ecotypes.

Reagents

Analytical grade chemicals were used in all the assays and analyses. 2,2-diphenyl-1-picrylhydrazy (DPPH), gallic acid, 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Folin-Ciocalteu reagent and methanol reagent and HPLC grade, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, potassium persulfate, sodium carbonate, Shinoda, Mayer, Dragendorff,Lieberman Burchard, Borntrager, Gelatine, and FeCl3, Rosenheim, Kedde and Ninhydrin reagents were purchased in Merck (Merck KGaA, 64271 Darmstadt , Germany).

Extraction procedure

The extraction technique was based on the method reported by Lock de Ugaz (1994) with modifications. First, 10 g of the lyophilized homogenate of the edible part and the peel flour were macerated separately in 100 mL methanol at room temperature for 7 days, stirring for about 2 min daily. Then, the mixture was filtered using Whatman paper # 4 and the methanolic extract was obtained (100 mL). The methanolic extract -obtained as described above- was used for the phytochemical screening, determination of the DPPH radical scavenging capacity and the IC_{50} assay.

A second methanolic extract was prepared to determine the ABTS radical scavenging capacity and the total phenolic content (Folin-Ciocalteu assay). Thus, 25 g of the lyophilized homogenate of the edible part and of the peel flour were weighed and homogenized with 25 mL of methanol (80%), constantly stirring to obtain a uniform consistency. Then, the mixture was transferred into a 50 mL centrifuge Falcon tube and macerated for 20 to 24 h at 4 °C. After that time, the sample was concentrated by centrifuging at 4000 rpm ((KENDRO Labofuge 400R) for 30 min, and then the extract was filtered using Whatman paper # 4. The supernatant was transferred to 1.5 mL Eppendorf tubes, avoiding the light. The samples were stored at -18 and -20 °C until the antioxidant capacity (ABTS) analysis was performed.

Physicochemical characterization

To determine physicochemical characteristics, 400 g of each fresh pitahaya ecotype was used. Crude fiber (NTP 205.003:1980 Revised in 2011) (INACAL, 2011), ash (AOAC 9030.05), moisture (AOAC 925.10), fat (AOAC 922.06), protein (AOAC 978.04), (AOAC International, 2016), carbohydrate (by difference), energy provided by proteins, carbohydrates, and fat, and total energy content (Collazos *et al*., 1993) were quantified. The results from each determination were reported as a single value.

Phytochemical screening

The methodology proposed by Lock de Ugaz (1994) was followed to determine phytochemicals present in pitahaya

pulp and peel. The test was performed in triplicate for each extract. Figure 1 shows the flow diagram with the steps for this analysis. The methanolic extract was partitioned into five fractions. These five fractions were evaluated by qualitative reactions to screen for specific phytochemicals, namely: tannins, amino acids and flavonoids (fraction A), steroids and quinones (fraction B), cardenolides, steroids and alkaloids (fraction C), leucoanthocyanidins, cardenolides, steroids and alkaloids (fraction D), and flavonoids and leucoanthocyanidins (fraction E). The color intensity of the precipitate formation was used as an analytical response to these tests.

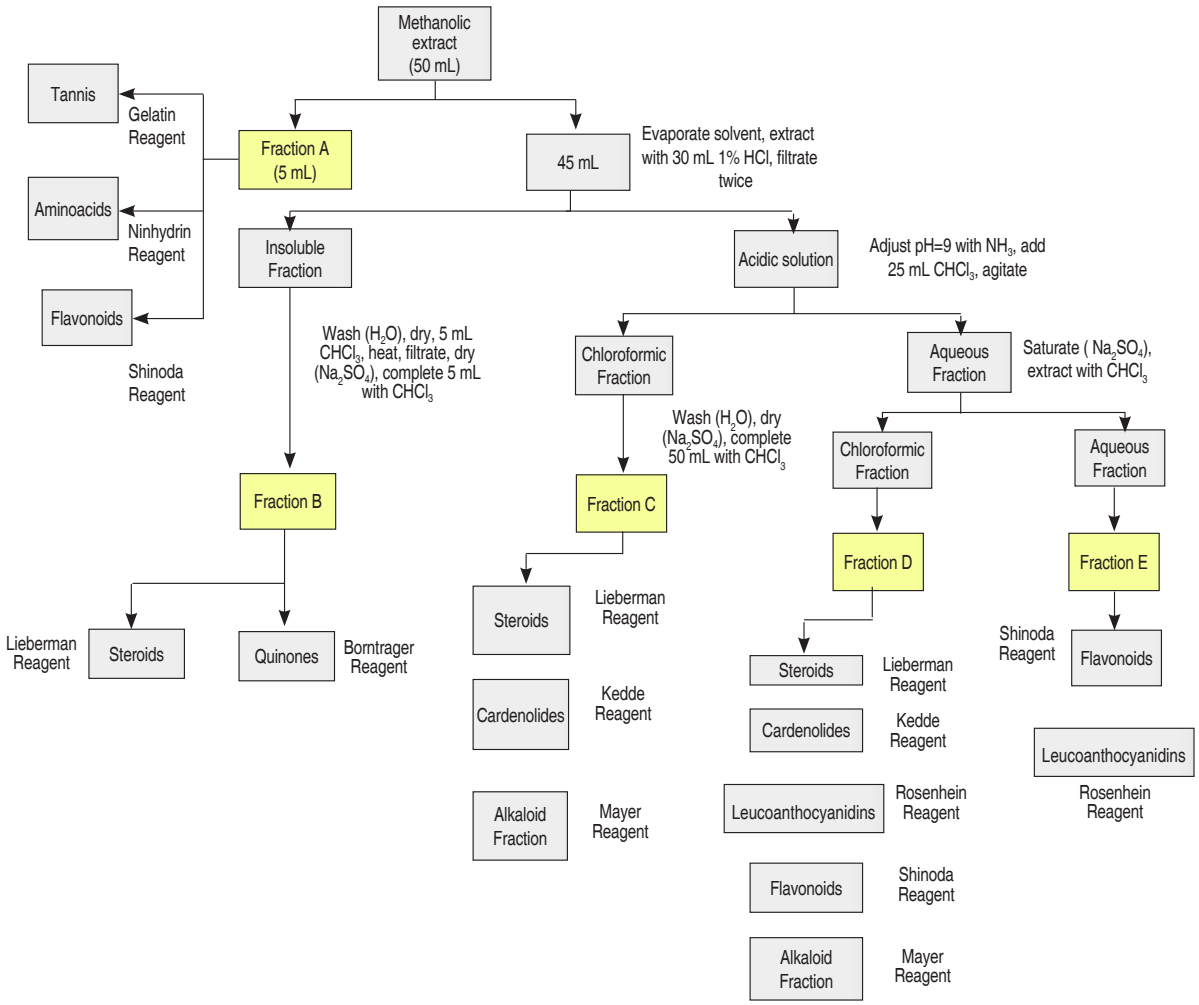

Figure 1. Steps for the phytochemical screening based on the methanolic extracts of yellow and red pitahaya.

Total phenolic content

The determination of total phenolic compounds or polyphenols in pitahaya pulp and peel was performed using a modified Folin-Ciocalteu method (Singleton and Rossi Jr, 1965). For the calibration curve, 0.1 mL aliquots of 10, 20, 40, 60, 80 mg mL^{-1} gallic acid standard stock solution (SIGMA) (10 mg mL^{-1}) were mixed with 8.5 mL of distilled water, followed by the addition of 1.0 mL of sodium carbonate (Na_2CO_3) (20% v:v). Mixtures were incubated for 5 min at 20 °C, and then 0.5 mL Folin–Ciocalteu reagent was added. Mixtures were stirred vigorously and then incubated - under constant agitation- for 30 min in darkness at 20 °C. Absorbance was measured using a spectrophotometer (Spectrum Pharo 300 Merck) at 760 nm. The same procedure was applied to test the pitahaya samples, replacing the gallic acid solution for the methanolic extracts of the samples. The total phenolic content of pitahaya extracts was calculated as

mg gallic acid equivalent (GAE)100 g^{-1} of dry sample and determined from the standard curve of gallic acid and reported as a single value.

DPPH radical scavenging capacity

DPPH radical scavenging capacity was performed in triplicate for each extract using Blois's method (Blois, 1958). A 0.3 M DPPH methanolic solution was prepared. Then, 2 mL of the pitahaya extracts (5% v/v) were added to 0.8 mL of the DPPH solution. Samples were incubated for 30 min at 20 °C. Gallic acid was used as a positive control at 31.3 µg mL^{-1}.

The decrease in absorbance of pitahaya test mixtures (due to quenching of DPPH free radicals) was determined at 517 nm, and the percentage of inhibition was calculated according to the equation:

$$\%\text{inhibition}=\left(\frac{\text{Ac}-(\text{Am}-\text{Abm})}{\text{Ac}}\right)\times 100$$

Where Ac is the absorbance of the reagent blank (DPPH+methanol), Am is the absorbance of the sample+DPPH, and Abm is the absorbance of the sample blank (sample+methanol).

IC_{50}

IC_{50} is a parameter widely used to measure and compare the antioxidant activity of test samples. For this study, the IC_{50} value is the concentration of the pitahaya test mixture required to quench 50% of the initial DPPH radicals (Ordoñez-Gómez *et al.*, 2018).

IC_{50} was obtained from the linear regression between the percentage of inhibition (which represents the antioxidant activity of the samples) in the ordinate versus the concentration of the samples (µg mL^{-1}) in the abscissa.

ABTS radical scavenging capacity

ABTS method (µmol Trolox eq g^{-1}) was used to determine the hydrophilic antioxidant capacity (Arnao *et al.*, 2001). The assay is based on the ability of radical scavenging compounds to reduce the blue-green radical cation (ABTS • +) to a non-colored form.

The extent of discoloration is calculated relative to the Trolox antioxidant standard. Reagent A was prepared with ABTS at a concentration of 7.84 mg mL^{-1} in distilled water. Reagent B was prepared with potassium persulfate at a concentration of 1.32 mg mL^{-1} in distilled water; both solutions were stored in the dark at 20 °C. The chromogenic radical (ABTS2+) stock solution was prepared, mixing equal volumes (1:1) of reagents A and B. The mixture was allowed to react for 12 h in the dark at 20 °C. Then, 1 mL of the ABTS stock solution was taken and diluted with 65 mL of methanol (80%). The absorbance of the prepared solution was read at 734 nm. It was corrected by adding methanol (80%) or stock solution. The reading was taken again at the same absorbance until it was within the range 1.1±0.02.

A standard Trolox curve was made, by preparing a series of Trolox standard solutions that contains different Trolox concentrations and a constant volume of ABTS stock solution, using methanol (80% v:v) as diluent.

To determine the radical scavenging capacity of the pitahaya samples, 150 µL of the sample was mixed with 2850 µL of the radical ABTS solution. A mixture of the standard solution and methanol was used as a blank. The reaction took place at 20 °C for 30 min, and the absorbance was measured at 734 nm in a Spectroquam UV/BIS Pharo 300 spectrophotometer. Finally, the hydrophilic antioxidant capacity quantified was expressed in µmol Trolox eq g^{-1} of sample, and reported as a single value.

Statistical analyzes

DPPH antioxidant activity results were expressed as mean±standard deviation of the three repetitions. The results were compared by means of the Wilcoxon test for two-tailed paired samples, with statistical significance determined at $P<0.05$. Statistical analyzes were carried out using STATA v.15.The results for the physicochemical characterization, total phenolic contents, and ABTS radical scavenging capacity assays were not available in triplicate.

RESULTS AND DISCUSSION

Physicochemical characterization

Table 1 shows the physicochemical characterization of *Hylocereus monacanthus* (red ecotype) and *Hylocereus megalanthus* (yellow ecotype) based on the proximate analysis results. The pulp of *Hylocereus megalanthus* showed the highest protein content (2.2%). The peel of both ecotypes had a higher percentage of moisture and crude

fiber than the edible part. *Hylocereus monacanthus* showed a slightly higher percentage of crude fiber (pulp: 2.3%, peel: 0.9%) than *Hylocereus megalanthus* (pulp: 2.0%, peel: 0.8%). These values are lower than the crude fiber values reported for *Hylocereus polyrhizus* (11.35 %) (Cordeiro *et al*., 2015). The fat content varied from 0.1 to 0.6% in the edible part of the red and yellow ecotypes, respectively. In all cases, the energy provided by carbohydrates exceeded 80%. Thus, based on the results of the present study, local ecoytypes of yellow and red pitahaya had a carbohydrate content (between 10-19% carbohydrates) similar to those in apples, pears, peaches, sweet granadilla, and guava (Ministerio de Salud del Perú, 2017). The energy content values of the edible part for *Hylocereus megalanthus* and *Hylocereus monacanthus* were different (85.4 kcal $100g^{-1}$ of sample and 55.3 kcal 100 g^{-1} of sample, respectively), being the latter value comparable with the values of the edible part reported by researchers in Brazil (Jeronimo and Costa Orsine, 2015) for *Hylocereus undatus* (53.68 kcal 100 g^{-1} sample), and those reported for pears and apples (Ministerio de Salud del Perú, 2017). The average energy content of the edible part (70.35 kcal 100 g^{-1} sample) is comparable to those in grapes, figs and cherimoya, being lower than those in banana and lucuma (Ministerio de Salud del Perú, 2017). In general, the low energy content of pitahaya, particularly the red ecotype, makes it appropriate for low-calorie diets (Jeronimo and Costa Orsine, 2015) . The values for percentage of moisture, proteins, lipids, and carbohydrates for *Hylocereus monacanthus* (85.7%, 1.2%, 0.1%, and 12.4%, respectively) and *Hylocereus megalanthus* (79%, 2.2%, 0.6%, and 17.8%, respectively) in the present study are very similar to those reported for *Hylocereus undatus* (Jeronimo and Costa Orsine, 2015). According to Verona-Ruiz *et al*. (2020), *Hylocereus megalanthus* has a higher percentage of soluble solids and is sweeter than *Hylocereus monacanthus*, which correlates with the percentage of carbohydrates of both species in this study.

The average (peel and pulp) protein content and fat content for both ecotypes are similar to those reported by Verona-Ruiz *et al*. (2020) for *Hylocereus megalanthus* and *Hylocereus undatus*; however, the yellow ecotype in the present study showed higher values. Researchers in Ecuador reported that *Hylocereus megalanthus* seeds are a good source of omega 6 fatty acids, mainly linoleic acid (69.98%) (Altuna *et al*., 2018). Scarce information regarding *Hylocereus monacanthus* was found in the literature. More studies are needed that focus on the fatty acid composition of the seeds of local pitahaya ecotypes, as they could be used as a raw material to extract healthy oils with functional properties.

Phytochemical screening

Table 2 shows the results of the phytochemical screening of the methanolic extracts of the peel and the pulp of *Hylocereus monacanthus* and *Hylocereus megalanthus*

Table 1. Physicochemical characteristics of yellow and red pitahaya

Physicochemical characteristics	Yellow ecotype[a]		Red ecotype[b]	
	Peel	Pulp	Peel	Pulp
Carbohydrates (g $100g^{-1}$ sample)	11.7	17.8	6.8	12.4
Total Energy (kcal $100g^{-1}$ sample)	54.1	85.4	30.8	55.3
% kcal from carbohydrates	86.5	83.4	88.3	89.7
% kcal from fat	1.7	6.3	0.0	1.6
% kcal from protein	11.8	10.3	11.7	8.7
Protein (g $100g^{-1}$ sample) (factor: 6.25)	1.6	2.2	0.9	1.2
Fat (g $100g^{-1}$ sample)	0.1	0.6	0.0	0.1
Moisture (g $100g^{-1}$ sample)	84.4	79	90	85.7
Ash (g $100g^{-1}$ sample)	2.2	0.4	2.3	0.6
Crude fiber (g $100g^{-1}$ sample)	2.0	0.8	2.3	0.9

[a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus.*

The presence of tannins, steroids, flavonoids, amino acids, cardenolides and leukoanthocyanidins, and traces of alkaloids was qualitatively determined for both *Hylocereus megalanthus* and *Hylocereus monacanthus*. For the yellow ecotype, the peel and pulp showed more presence of triterpenoids, while leukoanthocyanidins and cardenolides were found mainly in the peel. For the red ecotype, the peel and pulp contained more cardenolides and flavonoids. Compared to the phytochemical screening results for a local ecotype of *Hylocereus undathus* (Figueroa and Mollinedo, 2017), both studies had positive results for flavonoids and negative results for anthraquinones. Likewise, alkaloids were positively detected in *Hylocereus undathus;* however, only traces of them were identified in the samples in this study. These differences in the qualitative identification of phytochemicals in pitahaya may be due to the use of different solvents for the extraction, the variability between species, and the geographical origin of the samples. Pitahaya extracts rich in bioactive compounds have been studied due to their therapeutic properties. Flavonoids, tannins, and terpenoids are reported to have antimicrobial properties, while triterpenoids and steroids possess anticancer activity (Ibrahim *et al*., 2018). Terpenoids also show anti-diabetic properties (Joshi and Prabhakar, 2020).

On the other hand, polyphenols, flavonoids (including leukoanthocyanidins), alkaloids, amino acids, and steroids in *Hylocereus spp.* could be responsible for the hepatoprotective properties of the fruit (Ibrahim *et al*., 2018), while cardenolides are well-known bioactive compounds, showing anticancer and cardiotonic properties (Verma *et al*., 2016). Future research should include betacyanins determination, especially for red pitahaya due to their antioxidant properties (Joshi and Prabhakar, 2020).

Table 2. Qualitative analysis of the methanolic extracts of yellow and red ecotypes of pitahaya.

Fraction	Reagent	Secondary metabolite	Red pitahaya[a]		Yellow pitahaya[b]	
			Peel	Edible part	Peel	Edible part
A	NINHYDRIN	Aminoacids	-	+	+ + +	+ + +
	SHINODA	Flavonoids	+	+	+	-
	GELATIN	Tannins	+ +	+ + +	+ + +	+ + +
	$FeCl_3$	Tannins	+ +	+ +	+ + +	+ + +
B	BORNTRAGER	Anthraquinones	-	-	-	-
	LIEBERMAN BURCHARD	Steroids (S) Triterpenoids (T)	S (++)	S (++)	S(+++), T (++)	S (+++), T (+++)
C	KEDDE	Cardenolides	-	-	++	-
	LIEBERMAN BURCHARD	Steroids (S) Triterpenoids (T)	-	-	S (+)	-
	MAYER	Alkaloids	-	-	-	-
D	SHINODA	Flavonoids	-	+	-	-
	ROSENHEIN		-	-	-	-
	KEDDE	Cardenolides	+ + +	+ + +	-	-
	LIEBERMAN BURCHARD	Steroids (S) Triterpenoids (T)	S (+)	-	S (++)	S (+/-)
	MAYER	Alkaloids	-	-	(+/-)	-
E	SHINODA	Flavonoids	+	+	(+/-)	-
	ROSENHEIM	Leucoanthocyanidins	(+/-)	(+/-)	+ + +	(+/-)

[a]*Hylocereus monacanthus*, [b]*Hylocereus megalanthus*, * (-): Negative, (+): Mildly positive, (++): Moderately positive, (+++): Markedly positive, (+/-): Traces.

Total phenolic content

Table 3 shows the total polyphenols content in the methanolic extracts of the red and yellow ecotypes of pitahaya determined by the modified Folin-Ciocalteu method (Singleton and Rossi Jr, 1965).

The peel of *Hylocereus monacanthus* showed a higher total phenolic content (0.68 mg GAE g^{-1} of sample), in contrast to the value obtained in the peel of *Hylocereus megalanthus* (0.43 mg GAE g^{-1} of sample). When comparing the phenolic content in the pulp, *Hylocereus megalanthus* showed a higher value (0.48 mg GAE g^{-1} of sample) than *Hylocereus megalanthus* (0.32 mg GAE g^{-1} of sample). The polyphenols content in the methanolic extracts of the pulp of the yellow pitahaya is about 12% higher than that of the peel; meanwhile, the polyphenols content of the red pitahaya peel is about twice the amount found in the pulp.

Table 3. Total phenolic content of yellow and red ecotypes of pitahaya

Values expressed on	Total phenolic content			
	Yellow pitahaya[a]		Red pitahaya[b]	
	Peel	Edible part	Peel	Edible part
Dry basis[a] (mg GAE g^{-1} sample)	0.43	0.48	0.68	0.32

[a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus*.

Researchers in Colombia (Daza *et al*., 2014) assessed the total phenolic content in the ethanolic extract of the pulp, peel, and seeds of *Cereus triangularis* (yellow pitahaya). They reported the sample as yellow pitahaya but *Cereus triangularis* is actually a synonym of *Hylocereus trigonus*. This duplicity is explained by the challenges in the classification of pitahaya plants. They were classified initially under the genus *Cereus*, but the genus *Hylocereus* (synonym of *Selenicereus*) is currently used (The Plant List, 2013). Further, morphological and genetic heterogeneity in pitahaya by hybridization among species and varieties caused taxonomical confusion to identify them at the species level (Abirami *et al*., 2021).

Daza *et al*. (2014) reported 102±1.2, 77.6±0.4 and 202.7±1.1 mg GAE g^{-1} of dry sample, of peel, pulp and seeds, respectively. After comparing their results with the values in the present study, there are considerable differences in the phenolic contents for pulp (102 GAE g^{-1} of dry sample versus 0.48 mg GAE g^{-1} of dry yellow pitahaya pulp, and 0.32 mg GAE g^{-1} of dry red pitahaya pulp); these values are 212 to 316-fold higher than those in the present study. The same applies to results of the peel (77.6 mg GAE g^{-1} of dry sample versus 0.43 mg GAE g^{-1} of yellow pitahaya peel, and 0.68 mg GAE g^{-1} of red pitahaya peel), presenting values 115 to 182-fold higher than those in this study. It is not clear whether these differences could be explained by the methodology (variations in the duration of the extraction procedure and the use of a different solvent) (Daza *et al*., 2014), the species and maturation stage, and/or the geographical origin of the cultivar (Ibrahim *et al*., 2018; Som *et al*., 2019). In a recent study on a red-pulp pitahaya species in Australia (Suleria *et al*., 2020), researchers reported values of total phenolic content for the ethanolic peel extracts (0.45±0.12 mg GAE g^{-1} of sample), and they are similar to the results of this study (0.43 mg GAE g^{-1} of yellow pitahaya peel, and 0.68 mg GAE g^{-1} of red pitahaya peel).

Antioxidant activity

ABTS radical scavenging capacity

A high antioxidant activity was found by the ABTS method in both ecotypes; nonetheless, the yellow pitahaya ecotype presented the highest values in both the peel and the pulp, 731.68 and 579.46 µmol Trolox eq g^{-1} of sample respectively, as shown in Table 4.

The higher antioxidant capacity in the pulp compared to peel found in the red pitahaya ecotype is consistent with the results reported by researchers in Malaysia (Mohd Adzim Khalili *et al*., 2012) for the methanolic extracts of the peel and pulp of red pitahaya (*Hylocereus* sp.), based on the ABTS method.

Table 4. Radical scavenging capacity of yellow and red ecotypes of pitahaya

Values expressed on	ABTS Radical scavenging capacity			
	Yellow pitahaya[a]		Red pitahaya[b]	
	Peel	Edible part	Peel	Edible part
Dry basis (µmol Trolox eq g^{-1} sample)	731.68	579.46	364.50	565.62

[a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus*.

The pulp is more relevant for nutritional purposes, and for both ecotypes, the values of antioxidant activity in the pulp were very similar (579.46 and 565.62 µmol Trolox eq g^{-1} of sample, for yellow pitahaya and red pitahaya, respectively) and can be considered to have a high antioxidant capacity.

For the yellow pitahaya ecotype, the antioxidant activity of the peel was 26.7% higher than the pulp antioxidant activity. It is likely that the peel of *Hylocereus megalanthus* had more antioxidant compounds than the pulp; in fact, leucoanthocyanidins were qualitatively detected in the peel but not in the pulp. On the contrary, for the red pitahaya ecotype (*Hylocereus monocanthus*) was found that the peel (364.5 µmol Trolox eq g^{-1} of sample) showed 36% less antioxidant activity than the pulp (565.6 µmol Trolox eq g^{-1} of sample).

Colombian researchers determined antioxidant capacity by the ABTS method in the ethanolic extracts of the peel, pulp, and seeds of yellow pitahaya (*Hylocereus megalanthus* Haw), finding a higher antioxidant capacity in the peel compared to the pulp (without seeds); nevertheless, the seeds showed the highest antioxidant capacity (Torres-Grisales *et al*., 2017). The edible part -the pulp with the seeds- was analysed in this study; however, the peel of *Hylocereus megalanthus* still showed a higher antioxidant activity than the pulp and seeds together.

DPPH radical scavenging capacity

As shown in Table 5, the pulp and peel methanolic extracts of both ecotypes presented values of 93% of DPPH radical inhibition, with no significant difference (P>0.05) when comparing the values of peel vs. pulp for red and yellow pitahaya.

Table 5. DPPH radical scavenging capacity and IC50 in the yellow and red pitahaya ecotypes.

Sample	DPPH radical scavenging capacity (%) Mean±standard deviation	IC_{50} (mg mL^{-1})
YPP[a]	93.31±0.71	2.8
YPF[a]	93.14±3.70	1.68
RPP[b]	93.16±1.48	2.53
RPF[b]	93.62±3.04	2.67

YPP=yellow pitahaya peel, YPF=yellow pitahaya pulp, RPP=red pitahaya peel, RPF=red pitahaya pulp, [a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus*.

The DPPH test results in this study are higher than the results reported by researchers in South Korea (Kim *et al*., 2011), for the methanolic extracts of the peel and edible part of the red pitahaya (56.8±5.6% and 33.2±1.8%) and white pitahaya (68.1±2.8% and 23.8±3.3%) respectively. On the other hand, Colombian researchers (Torres-Grisales *et al*., 2017) reported an 8% lower antioxidant capacity (85.0±0.2%) in the ethanolic extract of yellow pitahaya pulp (*Hylocereus megalanthus* Haw). The difference between their results and those in this study for yellow pitahaya could be explained by the inclusion of the seeds in the edible part, use of a different solvent for the extraction and probably by genus and species variations.

The high radical scavenging capacity of the samples in the present study could be due not only to the presence of phenolic compounds but also to other metabolites present such as betalains and their derivatives (in the case of red pitahaya) (Kim *et al*., 2011).

IC_{50}

Results were also expressed as IC_{50} (mg mL^{-1}) (Table 5), which correspond to the amount of extract required to reduce DPPH radical by 50%; thus, the lower the IC_{50}, the higher the antioxidant capacity of the extract (Olugbami *et al*., 2014).

The IC_{50} values for the peel and edible part of the pitahaya samples in this study were 2.80 mg mL^{-1} and 1.68 mg mL^{-1} for *Hylocereus megalanthus* and 2.53 mg mL^{-1} and 2.67 mg mL^{-1} for *Hylocereus monacanthus*. The antioxidant activity of pitahaya samples in this study was slightly lower, except for the yellow pitahaya pulp, that showed a higher antioxidant capacity in contrast to aguaymanto, which was obtained from four different areas in Peru (1.86, 2.04, 2.24, and 2.36 mg mL^{-1}) (Teixeira et al., 2016).

On the other hand, a study carried out in Peru (Ordoñez-Gómez *et al*., 2018), the methanolic extracts of various citrus fruits presented, in most cases, higher IC_{50} values and lower antioxidant capacity than those found in the pitahaya ecotypes samples of this study.

Thus, the results of the present study confirm the potent antioxidant capacity of the yellow and red local ecotypes of pitahaya, which is as high or even higher than the antioxidant capacity in most citrus varieties in Peru. The dissemination of these findings may be helpful to promote the consumption of local pitahaya ecotypes, their prescription in people diets; and their utilization as raw materials in food processing due to their nutraceutical properties.

Betalains were not included in the phytochemical screening of this study. Nevertheless, they should be included in future research in order to complement these results. Statistical analysis was only applied to the DPPH radical scavenging capacity assay, since the results for all the other analyses were not available in triplicate; this was due to a methodological limitation of the study. Finally, the stage of maturity of the fruits for each ecotype was not the same; however, it is an important variable to standardize to obtain accurate results when comparing characteristics of both species.

CONCLUSIONS

Local red and yellow pitahaya ecotypes show a high nutraceutical potential and can be used in dietary prescriptions. The low carbohydrate content (12.4-17.8%) and the low energy content (55.3–85.4%) of the pulp make both species, particularly the red pitahaya, a good option for inclusion on and low sugar diets.

The high antioxidant capacity of the local ecotypes of *Hylocereus megalanthus* and *Hylocereus monacanthus* is explained by their high content of total polyphenols. Both species show similar IC_{50} values to those reported for other locally-produced fruits with high antioxidant capacity. The presence of other bio-active compounds in the yellow and red pitahaya extracts, such as tannins, steroids, flavonoids, amino acids, cardenolides, leukoanthocyanidins, and triterpenoids, indicate a high nutraceutical potential. Future research could focus on the quantitive determination of these bio-active molecules to establish the nutraceutical potential of these fruits more accurately, including betalains.

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REFERENCES

Abirami K, Swain S, Baskaran V, Venkatesan K, Sakthivel K and Bommayasamy N. 2021. Distinguishing three Dragon fruit (*Hylocereus spp*.) species grown in Andaman and Nicobar Islands of India using morphological, biochemical and molecular traits. Scientific Reports 11(1): 1–14. https://doi.org/10.1038/s41598-021-81682-x

Altuna JL, Silva M, Álvarez M, Quinteros MF, Morales D and Carrillo W. 2018. Yellow pitaya (*Hylocereus megalanthus*) fatty acids composition from ecuadorian Amazonia. Asian Journal of Pharmaceutical and Clinical Research 11(11): 218–221. https://doi.

org/10.22159/ajpcr.2018.v11i11.24922

AOAC International. 2019. Official Methods of Analysis (OMA) (21st Edition). AOAC International. https://www.aoac.org/official-methods-of-analysis-21st-edition-2019

Arnao MB, Cano A and Acosta M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 73(2): 239–244. https://doi.org/10.1016/S0308-8146(00)00324-1

Blois MS. 1958. Antioxidant determinations by the use of a stable free radical. Nature 181(4617): 1199–1200. https://doi.org/10.1038/1811199a0

Collazos C, White PL, Viñas E, Alvistur E, Urquieta R and Vasquez J. 1993. Composición de los alimentos de mayor consumo en el Peru (6ta Edición). Ministerio de Salud, Instituto Nacional de Nutrición. Lima. Perú.

Cordeiro MHM, Da Silva JM, Mizobutsi GP, Mizobutsi EH and Da Mota WF. 2015. Caracterização física, química e nutricional da pitaia-rosa de polpa vermelha. Revista Brasileira de Fruticultura 37(1): 20–26. https://doi.org/10.1590/0100-2945-046/14

Daza L, Herrera A, Murillo E and Méndez J. 2014. Evaluación de propiedades antioxidantes de parte comestible y no comestible de pitahaya, uchuva y mangostino. Biotecnología en el Sector Agropecuario y Agroindustrial 12(1): 98–105.

Figueroa SL and Mollinedo O. 2017. Actividad antioxidante del extracto etanólico del mesocarpio del fruto de *Hylocereus undatus* "pitahaya" e identificación de los fitoconstituyentes (Tesis de Título Profesional). Universidad Norbert Wiener, Perú.64 p. http://repositorio.uwiener.edu.pe/handle/123456789/925

Ibrahim SRM, Mohamed GA, Khedr AIM, Zayed MF and El-Kholy AAES. 2018. Genus *Hylocereus*: Beneficial phytochemicals, nutritional importance, and biological relevance—A review. Journal of Food Biochemistry 42(2): 1–29. https://doi.org/10.1111/jfbc.12491

INACAL - Instituto Nacional de Calidad. 2011. NTP 205.003.1980. INACAL. https://salalecturavirtual.inacal.gob.pe:8098/detalle. aspx?id=23607&idtv=5407

ICONTEC - Instituto Colombiano de Normas Técnicas y Certificación. 1996. Norma Técnica Colombiana. NTC 3554. Frutas frescas. Pitahaya amarilla. https://tienda.icontec.org/gp-frutas-frescas-pitahaya-amarilla-ntc3554-1996.html

Jeronimo MC and Costa Orsine JV. 2015. Chemical and physical-chemical properties, antioxidant activity and fatty acids profile of red pitaya [*Hylocereus Undatus* (Haw.) Britton & Rose] grown in Brazil. Journal of Drug Metabolism & Toxicology 06(04): 6–11. https://doi.org/10.4172/2157-7609.1000188

Joshi M and Prabhakar B. 2020. Phytoconstituents and pharmaco-therapeutic benefits of pitaya: A wonder fruit. Journal of Food Biochemistry 44(7): 1–15. https://doi.org/10.1111/jfbc.13260

Kim HJ, Choi HK, Moon JY, Kim YS, Mosaddik A and Cho SK. 2011. Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content. Journal of Food Science 76(1): 1–8. https://doi.org/10.1111/j.1750-3841.2010.01908.x

Lock de Ugaz O. 1994. Investigación fitoquímica. Métodos en el estudio de productos naturales. (Segunda Ed). Fondo Editorial, PUCP.

Ministerio de Salud del Perú. 2017. Tablas peruanas de composición de alimentos. 10ma Edición. Instituto Nacional de Salud, Centro Nacional de Alimentacion y Nutrición. http://www.ins.gob.pe/insvirtual/images/otrpubs/pdf/Tabla%20de%20Alimentos.pdf

Mohd Adzim Khalili R, Che Abdullah AB and Abdul Manaf A. 2012. Total antioxidant activity, total phenolic content and radical scavenging activity both flesh and peel of red pitaya, white pitaya and papaya. International Journal of Pharmacy and Pharmaceutical Sciences 4(2): 113–122.

Olugbami JO, Gbadegesin MA and Odunola OA. 2014. *In vitro* evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. African Journal of Medicine and Medical Sciences 43(Suppl 1): 101–109.

Ordoñez-Gómez ES, Reátegui-Díaz D and Villanueva-Tiburcio JE. 2018. Total polyphenols and antioxidant capacity of peel and leaves in twelve citrus. Scientia Agropecuaria 9(1): 123–131. https://doi.org/10.17268/sci.agropecu.2018.01.13

Ortiz-Hernández YD, Livera-Muñoz M, Carrillo-Salazar JA, Valencia-Botin AJ and Castillo-Martínez R. 2012. Agronomical, physiological, and cultural contributions of pitahaya (*Hylocereus spp.)* in Mexico. Israel Journal of Plant Sciences 60(3): 359–370. https://doi.org/10.1560/IJPS.60.3.359

Osuna-Enciso T, Ibarra-Zazueta ME, Muy-Rangel, MD, Valdez-Torres JB, Villarreal-Romero M and Sergio Hernández-Verdugo. 2011. Calidad postcosecha de frutos de pitahaya (*Hylocereus undatus* Haw.) cosechados en tres estados de madurez. Revista Fitotecnia Mexicana 34(1): 63–72.

Ramos E. 2017. ¿Por qué la pitahaya no surge todavía como fruto de exportación en Perú? Agencia Agraria de Noticias. https://agraria.pe/noticias/por-que-la-pitahaya-no-surge-todavia-como-fruto-de-13943

Seyidoglu N and Aydin C. 2016. Stress, natural antioxidants and future perspectives. In Salanta LC. (Ed.). The health benefits of foods - current knowledge and further development (p. 13). Intechopen. https://doi.org/10.5772/intechopen.91167

Singleton VL and Rossi Jr. JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16(3): 144–158.

Som AM, Ahmat N, Abdul Hamid HA and Azizuddin NM. 2019. A comparative study on foliage and peels of *Hylocereus undatus* (white dragon fruit) regarding their antioxidant activity and phenolic content. Heliyon 5(2): e01244. https://doi.org/10.1016/j.heliyon.2019.e01244

Suleria HAR, Barrow CJ and Dunshea FR. 2020. Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. Foods 9(9): https://doi.org/10.3390/foods9091206

Teixeira BJ, Mercedes I and Ataurima A. 2016. Evaluation of the content of phenolic compounds and antioxidant capacity of the ethanol extracts of the fruits of aguaymanto (*Physalis peruviana* L.). Revista de la Sociedad Química del Perú 82(3): 272-279. http://revistas.sqperu.org.pe/index.php/revistasqperu/article/view/58/50

The Plant List. 2013. *Cereus triangularis* Haw. http://www.theplantlist.org/tpl1.1/record/kew-2712460

Torres-Grisales Y, Melo Sabogal DV, Torres-Valenzuela LS, Serna-Jiménez JA and Sanín Villarreal A. 2017. Evaluation of bioactive compounds with functional interest from yellow pitahaya (*Selenicereus megalanthus* Haw). Revista Facultad Nacional de Agronomia Medellin 70(3): 8311–8318. https://doi.org/10.15446/rfna.v70n3.66330

Vásquez-Castillo W, Aguilar K, Vilaplana R, Viteri P, Viera W and Valencia-Chamorro YS. 2016. Calidad del fruto y pérdidas poscosecha de pitahaya amarilla (*Selenicereus megalanthus* Haw.) en Ecuador. Agronomia Colombiana 34(1): S1081–S1083. https://doi.org/10.15446/agron.colomb.v34n1supl.58279

Verma SK, Das AK, Cingoz GS and Gurel E. 2016. In vitro culture of *Digitalis* L. (Foxglove) and the production of cardenolides: An up-to-date review. Industrial Crops and Products 94: 20–51. https://doi.org/10.1016/j.indcrop.2016.08.031

Verona-Ruiz A, Urcia-Cerna J and Paucar-Menacho L. M. 2020. Pitahaya (*Hylocereus spp.*): Culture, physicochemical characteristics, nutritional composition, and bioactive compounds. Scientia Agropecuaria 11(3): 439–453. https://doi.org/10.17268/sci.agropecu.2020.03.16

Williamson G. 2017. The role of polyphenols in modern nutrition. Nutrition Bulletin 42(3): 226–235. https://doi.org/10.1111/nbu.12278