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Composition, physico-chemical properties and antioxidant capacity of *Renealmia alpinia* (Rottb.) Maas fruit

Composición, propiedades físico-químicas y capacidad antioxidante del fruto *Renealmia alpinia* (Rottb.) Maas

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Abstract

Composition and bioactive properties of x'kijit fruit [*Renealmia alpinia* (Rottb.) Maas] pulp and peel is unknown so far. Therefore, the objective of this study was to evaluate physicochemical, and nutritional characteristics, bioactive compounds and antioxidant activity of peel, pulp of the fruit (x'kijit fruit). The fruit pulp was higher in soluble solids (10.53°Brix) and pH (6.2), while the peel had lower pH (3.9) and soluble solids (2.3°Brix). The protein and fat contents were higher in pulp with values 4.2 and 8.6%, respectively. Vitamin C, flavonoids, phenolic compounds, and carotenoids were higher in pulp (28.3 mg ascorbic acid.100 g^{-1} FW, 567.8 mg catechin.100 g^{-1} FW, 102.4 mg GAE.100 g^{-1} FW, and 332 µg of carotenoid.100 g^{-1} FW, respectively) than peel. However, the peel was higher in anthocyanins (181.4 mg of cyanidin.100 g^{-1} FW), yielding an antioxidant activity of 23.3mMFe^{2+}.100 g^{-1}. The x'kijit fruit demonstrated to have an important nutritional composition and a high antioxidant activity.

Keywords
x'kijit fruit • nutritional characteristics • antioxidant activity

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Resumen

Hasta el momento se desconoce la composición y propiedades bioactivas de la pulpa y piel del fruto x'kijit [*Renealmia alpinia* (Rottb.) Maas]. Por lo anterior, el objetivo de este estudio fue evaluar las características fisicoquímicas, nutrimentales, compuestos bioactivos y la actividad antioxidante en la pulpa y piel del x'kijit. La pulpa del fruto presentó mayores valores de sólidos solubles (10,53°Brix) y pH (6,2), mientras que en la piel fueron más bajos pH (3,9) y sólidos solubles (2,3°Brix). Los contenidos de proteína y grasa fueron más elevados en pulpa, 4,2 y 8,6 %, respectivamente. La vitamina C, flavonoides, compuestos fenólicos y carotenoides fueron más elevados en la pulpa que en la piel (28,3 mg de ácido ascórbico 100 g^{-1} BH (Base Húmeda); 567,8 mg catequina 100 g^{-1} BH; 102,4 mg GAE.100^{-1} BH y 332 µg 100 g^{-1}, respectivamente). Sin embargo, en la piel las antocianinas fueron mayores (181,4 mg de cianidina 100 g^{-1} BH), proveyendo una actividad antioxidante de 23,3 mM Fe^{2+} 100 g^{-1}. La fruta X'kijit demostró poseer una importante composición nutrimental y una alta actividad antioxidante.

Palabras clave

x'kijit´• características nutrimentales • capacidad antioxidante

Introduction

In Mexico, there are more than 23,000 plant species; which are used by rural communities for food, medicine, utensils, alcoholic and non-alcoholic drinks, paper, fiber, and fuel (11). The edaphoclimatic characteristics of the Sierra Norte region of the state of Puebla in Mexico, led to the presence of numerous exotic and medicinal plant species (23). Among the exotic fruits found in this region is *Renealmia alpinia (*Rottb.) Maas*,* (x'kijit in the Totonaco language), which is one of only four species of the *Renealmia* genera.

The x'kijit fruit typically grows from lowland evergreen jungles up to 1,500 m, and it is an herbaceous plant of 2-6 m high, within florescence of 12-50 cm in length.

The fruit is an ovoid capsule, 3-4 cm long and 1.5-2 cm in diameter. In the immature stage, the fruit peel is red, but turns black when ripe. The pulp has a yellow color with a considerable amount of black seeds (20).

In rural communities of Veracruz, Chiapas, Oaxaca and Puebla, the x'kijit fruit is used for medicine against nausea and vomiting, to prepare ink, and seed oil for frying (22). But in the literature reviewed there are few studies about the evaluations of this fruit for human consumption and other uses. Therefore, the aim of this study was to determine the content of bioactive compounds and antioxidant activity of the pulp and peel of *Renealmia alpinia* (Rottb.) Maas, in order to contribute to the study of composition and applications in industry.

Materials and methods

Fruits and materials

Ripe x'kijit [*Renealmia alpinia* (Rottb. Maas)] fruits were obtained from Cuetzalan del Progreso locality in the Sierra Norte of Puebla, Mexico, in two differents years (November 2015 and 2016). Fruits were selected with no apparent physical and decay damage, washed carefully with distilled water and stored at 8°C in polystyrene bags until the analysis (1). Sodium 2.6-dichloroindophenolate (DCIP), metaphosphoric acid, ascorbic acid, Folin-Ciocalteu reagent, Gallic acid, catechin, 2,4,6-Tripyridyl-s-Triazine (TPTZ), ferric chloride ($FeCl_3$), Sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), Sodium hydroxide (NaOH), Sodium carbonate (Na_2CO_3), and calcium carbonate ($CaCO_3$), were purchased from Sigma–Aldrich (MO, USA). Ethanol, methanol, glacial acetic acid, hydrochloric acid, acetone, and sodium acetate were purchased from J. T. Baker (PA, USA).

Physicochemical analyses

The pulp, peel, and seeds were manually separated. The weights of each part of the fruits were determined with an Ohaus, Explorer Pro analytical balance (NY, USA).

The pH, total soluble solids, and titratable acidity of the fruits peel and pulp were evaluated according to 981.12, 932.12, and 942.15 AOAC methods (2), respectively.

The *L**, *a** and *b** color parameters were determined in the pulp (puree) and peel of the fruits in reflectance mode with a Colorflex M6405 colorimeter (Virginia, USA). Hue angle ($\tan^{-1}(b^*/a^*)$) and Chroma ($(a^{*2} + b^{*2})^{½}$) values were calculated. Moisture, protein, fat, and ash contents were determined according to 925.25, 12.1.07, 31.04.02, and 31.012 of the AOAC methods, respectively (2), and carbohydrates were calculated by difference. All measurements were performed in triplicate.

Bioactive compounds and antioxidant activity

Each replicate of pulp or peel (0.5 g) was mixed with 25 mL of absolute ethanol or metaphosphoric acid (for vitamin C determination). The mixture was stirred for 30 min at medium speed and filtered to obtain a clear extract. The extract was used to quantify ascorbic acid, flavonoids, total phenols, and antioxidant activity of the x'kijit pulp and peel.

Ascorbic acid was determined by the reduction of 2.6-dichloroindophenol sodium salt (DCIP) according to the method of Julián-Loaeza *et al.* (2011), with some modifications. One milliliter of the mixture was placed in an amber tube and mixed with 1 mL of acetate buffer and 1 mL of 2, 6-dichloroindophenol solution (30 ppm). The mixture was stirred for 1 min and evaluated at 515 nm with a UV-Vis Jenway spectrophotometer model 6405 (Staffordshire, UK). The results were reported as mg of ascorbic acid. 100 g^{-1}.

Carotenoid content was evaluated according to Lichtenthaler and Wellburn (1983). One gram of x'kijit pulp and peel were macerated with 5 mL of acetone solution (80% v/v), 2 g of calcium carbonate and 2 g of sea sand; the mixed was centrifuged at 1.096 g at room temperature for 10 min. The absorbance was evaluated at 470, 645, and 662 nm and acetone was used as blank. The contents were calculated according to the following expressions:

$$C_a = 11.75\, A_{662} - 2.35\, A_{645}$$

$$C_b = 18.61\, A_{645} - 3.96\, A_{662}$$

$$C_c = 1.000 A_{470} - 2.27 C_a - (81.4 C_b)/227$$

where:
A = absorbance
C_a = chlorophyll *a*, C_b is the chlorophyll *b*
C_c = carotenoid content (mg of carotenoids.100 g^{-1} of FW).

The flavonoid content was determined using 0.5 mL of extract mixed with 0.5 mL of $NaNO_2$ (1.5%), 1 mL of $AlCl_3$ (3%), and 1 mL of NaOH (1 N). The mixture was stirred for 5 min and determined at 490 nm.

The result was reported as mg of catechin100 g^{-1} of fresh weight (14). Total phenols were analyzed according to the method by Gao *et al.* (2004) with some modifications. One milliliter of the extract was placed in an amber glass tube and mixed with 1 mL of Folin-Ciocalteau reagent (0.1 N). After 3 min, 1 mL of Na_2CO_3 (0.05%) was aggregated.

The mixture was left to stand for 30 min in the dark at room temperature and the absorbance was determined with a UV-Vis spectrophotometer at 765 nm and reported as mg of Gallic Acid equivalent to GAE.100 g^{-1} FW.

The monomeric anthocyanin in the peel and pulp was made with pH differential method with some modifications (15). One gram of each sample was mixed with 50 mL of methanol/hydrochloric acid (99:1) (v/v). The mixture was stirred for 3 h and filtered. The extracts obtained were diluted with a buffer solution (pH 1.0 and 4.5). The absorbance was evaluated at 520 and 700 nm with a UV-Vis spectrophotometer. The concentration of monomeric anthocyanin was calculated as cyanidin-3-glucoside equivalents (mg. g^{-1}) with the following equation:

$$\textbf{Anthocyanin (mg. 100g}^{-1}\textbf{)} = \textbf{A*MW*DF*1000*SF*(}\varepsilon\textbf{*b)}^{-1}$$

where:

A = (Absorbance 520nm - Absorbance 700nm) $_{pH\ 1.0}$ - (A520nm - A700nm) $_{pH\ 4.5}$;

MW (molecular weight) = 449.2 mol^{-1} for cyanidin-3-glucoside (cyd-3-glu)

DF = dilution factor, ε (molar extinction coefficient = 26 900 $L.mol^{-1}$)

SF = (Relationship: Solvent (L)/ Fruit Weithg (100 g^{-1}).

The ferric reducing antioxidant power (FRAP) was quantified according to the method of Ghasenzadeh *et al.* (2012). A portion of the extract (0.75 mL) was mixed with 2.75 mL of FRAP reactive (10 mL of acetate buffer plus, 1 mL of TPTZ (30 mM) and 1 mL of $FeCl_3$ (60 mM), and incubated at room temperature for 5 min. The mixture was shaken for 1 min and the absorbance was read at 630 nm. The FRAP was quantified as a reduction of iron ions (iron sulfate), and reported as mM Fe^{2+}.100 g^{-1}.

Analysis of data

Results data of physicochemical, nutritive and functional properties was reported as the average of three replicates of each collected season (2015 and 2016). Due to the considerable differences between pulp and peel results were not statistically analyzed among fruit components (peel and pulp).

Results and discussion

Physicochemical characteristics

The x'kijit fruits weighed between 10 and 13 g, with contents of 9.3 ± 2.1, 22.3 ± 3.8% and 68.5 ± 5.2% of seed, pulp and peel, respectively. This is typical of fruits in the cactaceae genera (25). The x'kijit fruit is described as an ovoid capsule (3-4 cm of longitude and 1.5-2.0 cm in diameter) with numerous seeds embedded in the pulp (photo, page 381), a similar description was reported by Gómez-Betancur and Benjumea (2014).

The pH and titratable acidity of the peel were 3.9 and 0.33±0.05 % (citric acid), respectively; these values are similar to other fruits, such as bananas and apples.

The total soluble solids content in the pulp (10.5%) and peel (2.3%) were very low compared to other fruits (28) (table 1, page 381).

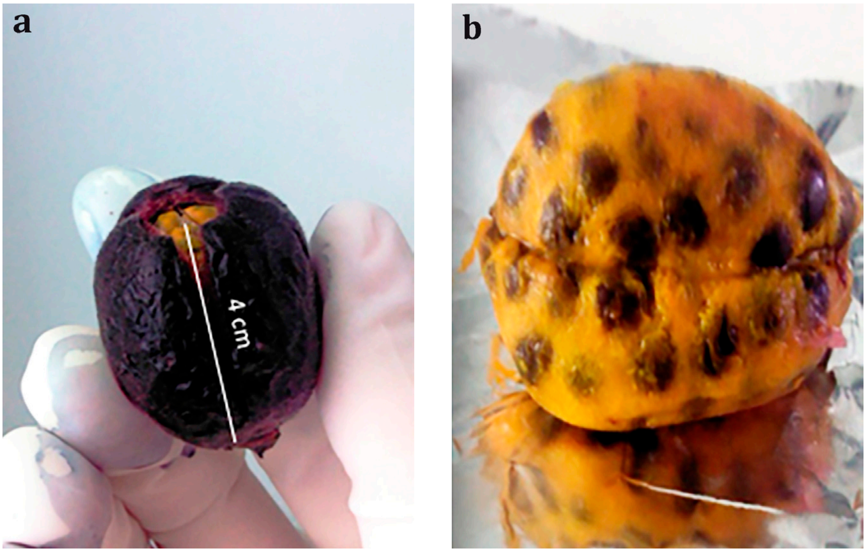

Photo. Whole (a) and pulp and seeds (b) of x'kijit (*Reanealmia Alpinia*) fruit.
Foto. Fruto (a) y pulpa y semillas (b) del x'kijit (*Reanealmia Alpinia*).

Table 1. Physicochemical characteristics of pulp and peel of x'kijit [*Renealmia alpinia* (Rottb.) Maas] fruit [a].
Tabla 1. Características fisicoquímicas de la pulpa y piel del fruto x'kijit [*Renealmia alpinia* (Rottb.) Maas] [a].

Characteristics	Pulp	Peel
pH	6.2 ±0.0	3.9 ±0.0
Total soluble solids (%)	10.53±0.2	2.3 ±0.1
Titratable acidity (%)	0.33 ±0.05	0.37±0.2
*L**	49.1 ±0.0	8.2 ±0.2
*a**	19.8 ±0.0	23.0 ±0.4
*b**	72.7 ±0.3	7.7 ±0.4
Hue	81.9 ±5.5	20.2 ±2.8
Chroma	75.5 ±6.2	22.1 ±3.1

[a] Average (n = 6) ± standard deviation. / [a] Promedio (n=6) ± desviación estándard.

The *L**, *a**, and **b* color parameters in pulp were 49.1, 19.8, and 72.7, respectively (table 1, page 381). The Hue angle (82°) indicates an intense yellow color of the pulp and the Chroma value (76) shows high intensity and purity (photo, page 381). The peel presented low *L**, Hue and Chroma values in comparison with the pulp, these results are related to a dull, red color (table 1 and photo, page 381).

Bioactive compounds and antioxidant activity

The x'kijit pulp presented a higher concentration of protein, fat, and carbohydrates than other fruits (table 2). The average protein contents in fruits with high amounts of seeds, comparable to the x'kijit, such as *Annona diversifolia* and *Psidium guajava,* were 0.97% and 2.2%, respectively (14). Fat in x'kijit pulp was high, similar to *Psidium guajava* (27). The carbohydrate content in pulp was higher in contrast to the peel; both values were same to other fruits (28). The moisture content was higher in the peel than in the pulp; this may be attributed to the elevated amount of protein and fat in the pulp.

Color of the fruits is a parameter related to the content of bioactive compounds (9, 16, 18). The x'kijit yellow pulp and the red peel indicated the presence of bioactive compounds such as flavonoids, carotenoids and anthocyanins (table 2).

The pulp had a medium vitamin C content, consistent with those reported by Contreras *et al.* (2011), and Sánchez Moreno (2006). The ascorbic acid in pulp was about five times higher than in peel (table 2); this content was analogous to other tropical fruits, such as yellow mombin (6). In addition, the contents of ascorbic acid were higher than those reported in tomato, mountain papaya, prickly pear, and tree tomato (3, 8, 18).

Flavonoids are attributed different health benefits, such as arterial dilator, hepatoprotective, antioxidant, choleretic, and antifungal characteristics, among others (24).

Table 2. Nutritional and bioactive components of pulp and peel of x'kijit [*Renealmia alpinia* (Rottb.) Maas] fruit [a].

Tabla 2. Compuestos nutrimentales y bioactivos de la pulpa y piel del x'kijit [*Renealmia alpinia* (Rottb.) Maas] fruit [a].

Proximate composition[b] (%)	**Pulp**	**Peel**
Water	76.60± 2.40	94.80±0.60
Protein	4.20± 0.10	0.70±0.00
Fat	8.60± 0.10	2.50±0.40
Carbohydrates	10.30± 2.20	1.90±0.20
Ash	0.40± 0.10	0.10±0.00
Antioxidant compounds		
Vitamin C (mg of ascorbic acid. 100 g^{-1})	28.30± 4.00	5.90± 0.40
Flavonoids (mg of catechin. 100 g^{-1})	567.80±12.1	55.80± 8.70
Phenolics (mg of GAE. 100 g^{-1})	102.40±10.50	59.90± 1.20
Carotenoids (μg 100 g^{-1})	332.00±21.70	0.59± 0.24
Anthocyanins (mg of cyanidin.100 g^{-1})	ND	181.40±19.40
Antioxidant activity (FRAP) (mM Fe^{2}+. 100 g^{-1})	1.9±0.50	23.3 ± 0.10

[a] Mean (n = 6) ± standard deviation. [b] Fresh weight (FW). /[a] Media (n=6) ± desviación estándard. [b] Peso Fresco (PF).

Flavonoids of x'kijit pulp were higher than those in several mango varieties (19) while the phenolics compounds were similar to other fruit contents (21).

These antioxidant compounds evaluated in pulp were ten and two times higher than in peel, respectively (table 2, page 382).

The carotenoid in the x'kijit pulp (332 μg 100 g^{-1}) was higher than in the peel (0.59 μg 100 g^{-1}) (table 2, page 382); this compound provides pulp a deep yellow pigmentation (4). Anthocyanins were not detected in pulp, but the peel showed a high concentration (181.4 mg of cyanidin.100 g^{-1}) (table 2, page 382), these results are comparable to those reported in other peels (skin) and whole fruits (5, 7, 26). Thus, the x'kijit pulp and peel have several uses in food, cosmetics, pharmaceutical and medicine, as those practiced by local people in rural communities (24).

Antioxidant properties

The FRAP value on the peel was superior to the pulp (23.3 mM Fe^{2+}. 100 g^{-1}) (table 2, page 382), probably it is due to the peel's high anthocyanins content. Moreover, this antioxidant property is considered in the intermediate and high levels compared to other fruits (30, 31).

Conclusion

It is estimated that this study is the first to characterize the x'kijit fruit, that includes physicochemical properties and nutritive and functional components. The pulp of x'kijit is rich in protein and fat. Besides it has strong antioxidant potential due to their high content of flavonoids, carotenoids in pulp and anthocyanins, phenolic compounds in peel. X'kijit fruits are a new nutritional alternative, a natural source of pigments and bioactive compounds with antioxidant properties.

These properties could help elucidate commercial uses and products of the fruit components in the pharmaceutical and food industries.

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