



***Vaccinium leucanthum* SCHLECHTENDAHL FRUIT, A NEW SOURCE OF DIETARY FIBER AND ANTIOXIDANT COMPOUNDS**

FRUTO *Vaccinium leucanthum* SCHLECHTENDAHL, UNA NUEVA FUENTE DE FIBRA DIETÉTICA Y COMPUESTOS ANTIOXIDANTES

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Abstract

Vaccinium leucanthum Schltdl. is a tree growing wild in the State of Hidalgo, Mexico, and its fruit is a small edible berry known as “cahuiche”. Its physicochemical parameters, nutritional composition, antioxidant compounds (ascorbic acid, phenolic compounds, flavonoids, and anthocyanins), and antioxidant capacity (ABTS^{•+}, DPPH[•] and FRAP) were quantified in this work. HPLC-DAD was used to identify phenolic compounds and anthocyanins. The total dietary fiber was 8.36 g/100g fresh weight (f.w.). Total phenolics content was 1,090mg of gallic acid equivalents/100 g f.w. and total anthocyanins content was 267 mg equivalents of cyanidin-3-glucoside/100g f.w. Radical scavenging capacity by ABTS^{•+} and DPPH[•] were 1,035 and 1,293 μ mol of Trolox equivalents/100 g f.w., respectively. While the assay of FRAP showed 1,558 μ mol of Fe²⁺ equivalents/100 g f.w. Gallic acid was the major phenolic acid and cyanidin-3-glucoside was the most abundant anthocyanin. Cahuiche could be considered as a fruit with functional properties due to its high content in dietary fiber, phenolic compounds, and antioxidant capacity.

Keywords: antioxidant capacity, bioactive compounds, chromatography analysis.

Resumen

El *Vaccinium leucanthum* Schltdl., es un árbol silvestre que crece en el Estado de Hidalgo, México, y su fruto comestible es conocido como “cahuiche”. Los parámetros fisicoquímicos, composición nutrimental, compuestos antioxidantes (ácido ascórbico, fenoles, flavonoides y antocianinas) y la capacidad antioxidante (ABTS^{•+}, DPPH[•] y FRAP) fueron cuantificados en este trabajo. Se utilizó HPLC-DAD para identificar los compuestos fenólicos y antocianinas presentes. El contenido de fibra dietética total fue de 8.36 g/100g de peso fresco (p.f.). El contenido de compuestos fenólicos fue de 1,090 mg equivalentes de ácido gálico y el contenido de antocianinas fue de 267 mg equivalentes a glucósido-3 de cianidina/100 g p.f. La capacidad de captación de radicales por ABTS^{•+} y DPPH[•] fue de 1,035 y 1,293 μ mol equivalentes de Trolox/100 g p.f., respectivamente. El ensayo de FRAP presentó 1,558 μ mol equivalentes de Fe²⁺/100 g p.f. El ácido gálico fue el compuesto fenólico más abundante mientras que la glucósido-3 de cianidina fue la antocianina más abundante. El cahuiche podría ser considerado como un fruto con propiedades funcionales debido a su alto contenido en fibra dietética, compuestos fenólicos y capacidad antioxidante.

Palabras clave: capacidad antioxidante, compuestos bioactivos, análisis cromatográfico.

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1 Introduction

The Ericaceae family comprises about 13 genera of shrubs and heathers, including the genus *Vaccinium*, the most important member, presenting fleshy and juicy berries consumed locally where their production exists (Hancock *et al.*, 2003). In Mexico, some species of *Vaccinium* are grown, such as *V. corymbosum*, *V. confertum* Kunth., *V. consanguineum* Klotzch., and *V. leucanthum* Schlechtendahl, being the former (blueberry) the most studied. On the other hand, *V. leucanthum* Schlechtendahl (cahuiche), whose distribution has been mainly reported in Hidalgo, Puebla and Veracruz, Mexico (Fuentes *et al.*, 2013), is a wild edible fruit of local consumption without commercial exploitation, and, as far as we know, it has not been reported scientific data about its chemical, nutritional, and antioxidant composition.

The chemical composition of red fruits depends on the type of crop and species, soil type and environmental conditions, plant nutrition, flowering season and type of harvesting, as well as post-harvest storage (Zorenc *et al.*, 2016).

Several studies have shown that diets rich in plant foods including red fruits provide protective effects against cardiovascular, inflammatory and some types of cancer. Also, in recent years, health benefits from the consumption of red fruits of the genus *Vaccinium* have gained visibility due to their high antioxidant capacity related to a high content of phenolic compounds, especially flavonoids and anthocyanins (Zoratti *et al.*, 2015). In addition, their consumption provides a low energy intake, but a high content of dietary fiber, vitamins, organic acids and carotenoids (Manach *et al.*, 2005; Nile and Park, 2014).

For these reasons, the aim of this work was to know the physicochemical properties, nutritional composition, antioxidant compounds, antioxidant capacity and identification of phenolic compounds including anthocyanins of the wild fruit *Vaccinium leucanthum* by *in vitro* assays.

2 Materials and methods

2.1 Plant material

Vaccinium leucanthum Schldl. fruit (cahuiche) was collected in “Huasca de Ocampo”, Hidalgo, (2,300 m altitude, 20° 12' 10" latitude N and 98° 35'

55" longitude W) Mexico, in November 2016. The weather was semi-cold, winter season, mean minimum temperature of 7 °C and a mean maximum temperature of 18 °C. The fruits at physiological maturity stage without external injuries were selected and the whole fruits were used for the analyses. Subsequently, the fruits were stored at -70 °C before their lyophilization at -55±1 °C and 0.04 mbar.

2.2 Physicochemical properties

2.2.1 Diameter and mass

Diameter and mass of cahuiche were determined using a digital vernier caliper (ABSOLUTE, Mexico) and an analytical balance (OHAUS, China). Measurements were randomly performed on 60 fresh fruits, reporting the results as the means in mm and mg ± standard deviation respectively.

2.2.2 Color

The CIE L*, a*, and b* values were determined using a spectrophotometer (Konica-Minolta CM-508d, Japan) with 1 cm of path length optical glass cell (20 g of fresh fruit). The equipment was set to measure total transmittance using illuminant C, and a 2° observation angle. The color values were expressed as *L* (whiteness or brightness/darkness), *a* (redness/greenness) and *b* (yellowness/blueness) (Maskan, 2001).

2.2.3 pH, total soluble solids, and titratable acidity

The pH was measured with a digital pH meter (Hanna Instruments Woonsocket, RI, USA). Total soluble solids (TSS, in °Brix) were determined using a digital refractometer (PR-101, Atago Palette, Tokyo, Japan), and titratable acidity was determined by the AOAC method (942.15) based on titration of the juice with 0.1 M NaOH using phenolphthalein as indicator (AOAC, 2000); titratable acidity was reported in g of citric acid per 100 g of fresh weight (f.w.).

2.3 Nutritional composition

2.3.1 Energetic value

The energetic value was calculated based on the theoretical energy content of protein (4 kcal/g), ethereal extract (9 kcal/g) and carbohydrates (4 kcal/g) contained in 100 g f.w. (Manzi, *et al.*, 2001).

2.3.2 Proximate composition analysis

Moisture, ash, lipid, protein and carbohydrate contents were determined by the Official Methods of AOAC (methods: 920.151, 940.26, 920.85, 959.48 and 923.03 respectively).

2.3.3 Total dietary fiber

The determination of total dietary fiber (TDF) was performed using the gravimetric enzymatic method using SIGMA Total Dietary Fiber Assay Kit (SIGMA, TDF100), according to the manufacturer's specifications. Slight modifications were made for soluble and insoluble dietary fiber determinations according to Asp *et al.* (1998). It consisted of filtering and washing with boiling deionized water the insoluble dietary fiber (IDF). Then the filtrate was mixed with 95% ethanol at 60 °C and it was let stand for 60 min in order to precipitate the soluble dietary fiber (SDF). The precipitate was filtered and weighed after overnight drying at 105 °C in a hot air oven. Total dietary fiber (TDF) was calculated as the sum of IDF and SDF.

2.3.4 Determination of reducing sugars

Reducing sugars (RS) were determined according to the method reported by Miller (1959), which is based on the use of 3,5-dinitrosalicylic acid (DNS) to oxidize RS reducing itself giving a colored reaction. The results were reported as glucose equivalents per 100/g of fresh weight (GE/100 g f.w.).

2.3.5 Elemental spectrometric analysis

Iron, copper, zinc, manganese, boron, sodium, phosphorus, potassium, calcium and magnesium were determined after an $\text{HClO}_4/\text{HNO}_3$ digestion (Jones and Case, 1990), using inductively coupled plasma optical emission spectrometry (ICP-OES, GBC, 932-AA Model, Australia).

2.4 Bioactive compounds

The concentration of the bioactive compounds was performed using the whole fruit; 10 g of fruit was triturated and macerated with 250 mL of ethanol during 24 hours at room temperature in an amber screw cap bottle (GL 45). The mixture was centrifuged at 3,400 rpm, 4 °C, for 20 min and the supernatant was filtered (Whatman No. 1 filter paper). The filtrate was used to determine bioactive compounds concentration and antioxidant capacity.

2.4.1 Ascorbic acid content

The ascorbic acid content of the fruit was determined according to the colorimetric method described by Dürüst *et al.* (1997). Briefly, 0.1 mL of mixture filtrated fruit was added with 0.1 mL of acetate buffer and 0.8 mL of 2, 6-dichloroindophenol sodium salt hydrate (DCPI). The absorbance of the mixture was measured at 520 nm (Spectrophotometer Power Wave XS UV-Biotek, KC Junior Software, USA). Ascorbic acid was used as a reference standard and the results were expressed as mg of ascorbic acid per 100 g of fresh weight (mg AA/100 g f.w.).

2.4.2 Total phenolics content

Total phenolics content of the sample was determined according to Geogé *et al.* (2005). Folin-Ciocalteu reagent was diluted 1:1 with distilled water (v/v) and 0.5 mL was mixed with 0.5 mL of sample. After 1.5 mL (2%, w/v) sodium carbonate and 2.7 mL of deionized water were added and incubated for 1 h at room temperature. The absorbance of the mixture was measured at 765 nm. Gallic acid was used as a reference standard and the results were expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g f.w.).

2.4.3 Total flavonoids content

Aluminum chloride colorimetric assay (Zhishen *et al.*, 1999) was used to measure total flavonoids content. An aliquot (1 mL) of extract or quercetin standard solution was added to a 10 mL volumetric flask containing 4 mL of water. A volume of 0.3 mL of 5% (w/v) NaNO_2 and 0.3 mL of 10% (w/v) AlCl_3 were added. After 6 min, 2 mL of 1 molL^{-1} NaOH was added and the total volume was raised to 10 mL by the addition of water. The solution was mixed and the absorbance was measured using a blank reagent at 510 nm. The total flavonoids content was expressed as mg of quercetin equivalents per 100 g of fresh weight (mg QE/100 g f.w.).

2.4.4 Total monomeric anthocyanins content

Monomeric anthocyanins content was determined using the pH-differential method of Giusti and Wrolstad (2001), using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 molL^{-1}), and sodium acetate buffer, pH 4.5 (0.4 molL^{-1}). Absorbance was measured at 510 and 700 nm. Total anthocyanins content was calculated using a molar extinction

coefficient of $26,900 \text{ Lcm}^{-1} \text{ mol}^{-1}$ and a molecular weight of 449.2 g mol^{-1} of cyanidin 3-glucoside (predominant anthocyanin).

2.5 Antioxidant capacity

2.5.1 ABTS⁺ radical scavenging capacity

Antiradical capacity was measured according to Re *et al.* (1999). The cation (ABTS⁺) was produced by reacting 7 molL^{-1} ABTS⁺ stock solution with 2.45 molL^{-1} potassium persulfate in the dark at room temperature for 16 h before being used. The ABTS⁺ solution was diluted with deionized water to reach an absorbance of 0.70 ± 0.01 at 754 nm. Absorbance was measured at 754 nm after the addition of 0.1 mL of sample into 3.9 mL of diluted ABTS⁺ solution and incubating per 7 min at room temperature. The scavenging capacity was expressed as μmol of Trolox Equivalent Antioxidant Capacity (TEAC) per 100 g of fresh weight ($\mu\text{mol TEAC}/100 \text{ g f.w.}$).

2.5.2 DPPH[•] radical scavenging capacity

Antiradical capacity was measured with DPPH[•] radical as described by Brand-Williams *et al.* (1995). An ethanolic solution (7.4 molL^{-1}) of the stable DPPH[•] radical was prepared. Then 0.3 mL of sample was taken into vials and 2.7 mL of DPPH[•] solution was added and it was left to stand at room temperature for 1 h. The solution was stirred and centrifuged at 3,000 rpm per 10 min. Finally, the absorbance was measured at 520 nm and μmol equivalents of Trolox per 100 g of fresh weight ($\mu\text{mol TE}/100 \text{ g f.w.}$) were obtained.

2.5.3 FRAP assay of reducing antioxidant power

The FRAP solutions were prepared as described by Benzie and Strain (1996). The FRAP reagent was prepared as follows: acetic acid buffer pH 3.6, $1 \times 10^{-2} \text{ molL}^{-1}$ TPTZ (2,4,6-tripyridyl-s-triazine) solution, and $2 \times 10^{-2} \text{ molL}^{-1}$ $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; solutions were mixed in this order at a volume ratio of 10:1:1. Then 0.03 mL of the sample was taken into vials, 0.9 mL of the FRAP reagent, and 0.09 mL of H_2O were added. The absorbance at 595 nm was measured against a blank reagent after 10 min of incubation at room temperature. The results were expressed as μmol equivalents of Fe^{2+} per 100 g of fresh weight ($\mu\text{mol Fe}^{2+}\text{E}/100 \text{ g f.w.}$).

2.6 Extraction and identification of compounds

2.6.1 Crude extract

Five grams of lyophilized *V. leucanthum* fruit were triturated in a porcelain mortar and macerated using 60% (v/v) ethanol-aqueous (solid-liquid ratio 1:25) at room temperature, in darkness during 24 hours. The extraction solution was filtered through a No. 1 Whatman filter paper, then it was concentrated using a rotary evaporator under reduced pressure (Büchi R-3000, Flawil, Switzerland) at 35 °C. Then, the extract was freeze-dried (Freeze Dry System, FreeZone 6, Labconco, USA.) and cold storage. It was obtained $1.7 \pm 0.1 \text{ g}$ of dry extract per each 5 g of *V. leucanthum* lyophilized fruit.

2.6.2 High-performance liquid chromatography analysis

The crude extract of *V. leucanthum* fruit was analyzed as described by Garzón *et al.* (2010). The sample was filtered through a $0.45 \mu\text{m}$ membrane and $20 \mu\text{L}$ was injected into an HPLC-DAD (Agilent, 1100 Series, Waldbronn Germany) using a C18 column ($250 \times 4.6 \text{ mm}$, $5 \mu\text{m}$ particle size, ZORBAX Eclipse XDS-C18, Agilent). The mobile phase consisted of 5% (v/v) formic acid in water (solvent A) and 5% (v/v) formic acid in acetonitrile (solvent B) at a flow rate of 0.3 mL/min. The linear gradient elution was as follows: 0-20 min 20% B, and 20-30 min 20-58% B. Absorbance spectra were recorded every 1.2 nm from 200 to 700 nm. Detection was performed at 320 nm for phenolic acids and at 520 nm for anthocyanins; all the results were compared with standards which were treated with the same method. The spectra were analyzed using HP Agilent, ChemStation, California software, USA.

2.7 Statistical analysis

All values were obtained in triplicate and expressed by mean \pm standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA), and differences among means were compared using the Tukey-Kramer test ($P \leq 0.05$), using the Sigmasat® Software V4.0 (Systat Software Inc., Ca., USA).

Table 1. Physicochemical parameters of *Vaccinium leucanthum* fruit.

Parameters	Values	Parameters	Values
Color			
L*	46.56±0.99	pH	3.08±0.10
a*	21.26±1.02	Soluble solids (°Brix)	14.67±0.38
b*	2.62±0.60	Titrateable acidity (%)	1.06±0.02

Table 2. Proximal analysis and nutrimental composition of *Vaccinium leucanthum* fruit.

Parameters		Microelements (mg/kg fresh weight)	
Energetic value (kcal/100g fw)	78.77±0.73	Iron	114.20±0.95
Moisture (%)	73.35±0.37	Copper	11.30±0.26
Ashes (%)	0.61±0.01	Zinc	37.01±0.10
Lipids (%)	0.40±0.00	Manganese	37.10±0.20
Protein (%)	1.07±0.01	Macroelements (g/100 g fresh weight)	
Carbohydrates (%)	24.57±0.71	Sodium	0.77±0.02
		Phosphorus	0.32±0.04
Total dietary fiber (%)	8.35±0.21	Potassium	0.81±0.02
Insoluble dietary fiber (%)	7.10±0.17	Calcium	0.94±0.03
Soluble dietary fiber (%)	1.25±0.03	Magnesium	0.29±0.02

Values are expressed in fresh weight and as mean ± standard deviation (SD).

3 Results and discussion

3.1 Physicochemical parameters

Cahuiche is a tiny and globose berry, presenting a diameter of 6.75±0.4 mm, weighing 180±12.6 mg each fruit. Cahuiche showed a dark red color at the stage of maturity studied in this work. Color parameters L*, a*, and b* are shown in Table 1, the parameter L* indicates brightness, the parameter a* in positive values indicates red color and the parameter b* in negative values represents a blue color. The color of fruits depends on the composition and concentration of phenolic compounds, among which are anthocyanins, these have the capacity to produce a range of colors including orange, red, yellow and blue (Rui *et al.*, 2011; Zielinska and Michalska, 2016).

Physical and chemical characteristics of cahuiche fruit like other *Vaccinium* fruit, are highly dependent on the climatic conditions and several factors including a decrease in rainfall, soil conditions, temperature and altitude above sea level (Zorenc *et al.*, 2016). This fruit can be considered acid since the pH value was lower than 4.5. Low pH and moisture in fruits are intrinsic factors which confer protection against microorganisms during postharvest

and storage (Hamad, 2012).

Total soluble solids (TSS) content and titrateable acidity are parameters whose ratio is important in the ripening of fruits (Crisosto *et al.*, 1995). In this work the fruits were used at the fully ripe stage, presenting a TSS/acidity ratio of 13.8, which is in the range of some berries as strawberry, cherry and mullberry (Mahmood *et al.*, 2012; Ercisli and Orhan, 2008). A higher value of total soluble solids is related to greater sweetness and superior eating quality of a fruit (Chang and Chang, 2010). The cahuiche, like other berries such as strawberries or blackberries, are non-climacteric fruits that must be harvested once they are ripe and fully colored. This is due to the fact that this type of berries does not exhibit an increase in respiration rate or ethylene production once they have been harvested, and that when harvested in some state of immaturity, they do not undergo sufficient changes in the TSS/acidity ratio to be suitable for fresh consumption (Shin *et al.*, 2008; Ornelas-Paz *et al.*, 2013; Crecente-Campo *et al.*, 2012).

3.2 Nutritional composition

Nutritional composition of cahuiche is presented in Table 2. The energy content in cahuiche is related to the high carbohydrate content since 71 out of 78.77

kcal correspond to this component. Even though, cahuiche contains around 8.36 ± 0.2 g/100 g f.w. of total dietary fiber, in which $15 \pm 1\%$ corresponded to soluble dietary fiber and $85 \pm 1\%$ to insoluble dietary fiber. Because its beneficial effects in human health, dietary fiber is considered as a functional component, having in cahuiche a high concentration of it, which exceeds the values reported in other berries (Hancock *et al.*, 2003; Kosmala *et al.*, 2014).

Reducing sugars (RS) content in cahuiche fruit was 226 ± 3.0 mg GE/100 g f.w. These results can be compared with reports in some fruits such as grape (*Vitis vinifera* 'Cabernet Sauvignon'; 125 mg GE/100 g; Xu *et al.*, 2015), raspberry (*Rubus ideaus* L.; 417 mg GE/100 g) and madroño (*Arbutus unedo* L.; 1,566 mg GE/100g) (Alonso *et al.*, 2011).

The main sources of micro and macro elements are vegetables, legumes, and some fruits. The concentration of such compounds may vary depending on the vegetal matrix (Murphy *et al.*, 2012). Macro and microelements found in cahuiche are shown in Table 2.

The microelements concentration in cahuiche fruit were higher comparing them with blueberry reports (iron: 1.5-6 mg/kg; copper: 0.3-0.6 mg/kg; zinc: 0.6-1.2 mg/kg, and, manganese: 12-39 mg/kg), nevertheless, macroelements, concentration was lower than blueberry (sodium: 0.11-0.22 g/100 g; phosphorus: 1.0-1.5 g/100 g; potassium: 5.6-8.0 g/100 g; calcium: 1.5-3.5 g/100 g, and, magnesium: 0.6-1.0 g/100 g) (Nile and Park, 2014).

These components are important for several physiological and biochemical processes in humans since a deficiency or toxicity can affect water and electrolyte balance, as well as metabolic catalysis and oxygen binding (Fernandez-Panchon *et al.*, 2008). Based on the FAO daily intake recommendations (FAO/WHO, 2000), these results indicate a consumption of 100 g of cahuiche provides 100 percent of the daily microelements and macroelements recommended intake (except zinc with 30%, phosphorus and potassium with 40% each one).

3.3 Bioactive compounds

The concentration of ascorbic acid in cahuiche was 102.3 ± 3.4 mg AA/100 g f.w. This result was higher than those reported in other red fresh fruits such as strawberry (*Fragaria ananassa*), raspberry (*Rubus idaeus*) and blackberry (*Rubus fruticosus*), which show a range of ascorbic acid concentrations between 10 and 50 mg/100 g (Kalt, *et al.*, 1999; Benvenuti, *et al.*, 2004; Luna-Ramírez, *et al.*, 2017).

The recommended daily intake of vitamin C varies from 25 to 75 mg AA for children and adults, and 130 ± 5 mg AA for pregnant and lactating women (Monser, 2000). Considering these requirements, 100 g of cahuiche would contribute 100% of the daily intake of vitamin C.

On the other hand, cahuiche presented $1,090.3 \pm 15.2$ mg GAE/100 g f.w. for total phenolics content, 112.0 ± 3.6 mg QE/100 g f.w. for flavonoid concentration and 267.1 ± 7.1 mg cyanidin-3-glucoside/100 g f.w. for total anthocyanins.

These results were similar or higher than those reported in some fresh berries such as *Vaccinium corymbosum* ranging between 280 and 400 mg GAE/10 g (Contreras *et al.*, 2015; Kraujalytė *et al.*, 2015) *Rubus fruticosus* ranging between 143-319 mg GAE/100 g of total phenols and 117-150 mg of anthocyanins (Luna-Ramírez *et al.*, 2017; Pérez-Grijalva *et al.*, 2018), and *Fragaria* × *ananassa* containing 230 mg/100 g of anthocyanins (Morales-Delgado *et al.*, 2014).

In addition, the results of phenolic compounds can be comparable with other extracts or processed red fruits like *Vitis labrusca* containing 1,089 mg GA/100 g (Carmona-Gómez *et al.*, 2018) and ultrasonicated *Rubus fruticosus* juice, showing 1,343 mg GA/100 g (Luna-Ramírez, *et al.*, 2017). Even though, the concentration of bioactive compounds can vary due to different reasons, such as soil acidity, pH, solar radiation, postharvest management, and storage, even comparing fruits of the same genus and species (de Souza *et al.*, 2014).

According to the obtained results, the content of total phenolic compounds in the cahuiche is followed by the content of total monomeric anthocyanins and finally by the total flavonoids content. This sequence in the values of the concentration of total phenols > anthocyanins > flavonoids was similar to other studies carried out on different fruits of different species such as raspberry (de Souza, *et al.*, 2014) and cranberry (Abeywickrama, *et al.*, 2016).

3.4 Free radical scavenging capacity

Cahuiche showed $1,035.5 \pm 48.8$ μ mol TE/100 g f.w. for ABTS⁺ assay and $1,293.6 \pm 28.0$ μ mol TE/100 g f.w. in the DPPH[•] method. This scavenging capacity can be related to a high content of phenolic compounds, such as flavonoids and anthocyanins, compounds that confer important antioxidant capacity (Teleszko and Wojdylo, 2015).

Table 3. Phenolic acids and anthocyanins in crude extracts of *Vaccinium leucanthum* fruit.

Compounds	Retention time (min)	Concentration (mg/g dry weight)
Chlorogenic acid	7.97	1.24
Gallic acid	8.67	2.19
Caffeic acid	13.48	0.2
p-Coumaric acid	20.03	0.12
Malvidin-3-O-glucoside	10.85	2.13
Cyanidin-3-glucoside	14.79	3.26
Petunidin-3-glucoside	15.45	2.28

These values can be compared with those reported in the literature in different *Vaccinium* (*Vaccinium* spp.) and other fresh berry fruits such as blackberry, raspberry, strawberry, and cherry, ranging between 132 and 588 $\mu\text{mol TE}/100\text{ g}$ for ABTS⁺ assay, and between 828 and 2,367 $\mu\text{mol TE}/100\text{ g}$ for DPPH assay (de Souza *et al.*, 2014; Luna-Ramírez *et al.*, 2017).

For FRAP determination, cahuiche showed values of $1,558.1 \pm 5.8\ \mu\text{mol Fe}^{2+}\text{E}/100\text{g f.w.}$ This result can be compared with other studies in different species of *Vaccinium* like *V. corymbosum*, *V. uliginosum* and *V. eschenaultii* (Kraujalytė *et al.*, 2015; Nagulsamy *et al.*, 2015).

These antioxidant capacities can be related with the presence of phenolic compounds in the *V. leucanthum* extract, for this reason, an identification by HPLC-DAD was performed.

3.5 Identification of phenolic compounds by HPLC-DAD

Table 3, show four phenolic acids and three anthocyanins identified in *Vaccinium leucanthum* crude extract. The major phenolic acid concentration was gallic acid, with 2.19 mg/g dry weight (d.w.). The phenolic acids composition and content of *V. leucanthum* fruit can be compared with other berries and fruits of *Vaccinium* genus like *V. myrtillus* and *V. corymbosum* (Moze *et al.*, 2011; Coklar and Akbulut, 2017).

Meanwhile, cyanidin-3-glucoside was the major anthocyanin concentration with 3.26 mg/g d.w. The identification and content of anthocyanins in *V. leucanthum* fruit can be compared with other studies conducted in *V. meridionale* and *V. uliginosum*, where different conformations and monomers of such anthocyanins have been found (Garzón *et al.*, 2010; Wang *et al.*, 2014).

The total content of monomeric anthocyanins identified by HPLC-DAD was 67%, while phenolic

acids was 33% of the total content, showing approximately a 2:1 ratio between them. This behavior was comparable to that reported by Moze *et al.*, (2011) in bilberry (*Vaccinium myrtillus*) and blueberry (*Vaccinium corymbosum*), where 72.5% correspond to monomeric anthocyanins and 27.5% correspond to phenolic acids, as well as with Teleszko and Wojdylo, (2015), in chokeberry (*Aronia melanocarpa*) and blackcurrant (*Ribes nigrum*), where the content of monomeric anthocyanins is higher than phenolic acids in a 2:1 ratio (65% anthocyanins: 35% phenolic acids) and 10:1 ratio (90% anthocyanins: 10% phenolic acids) respectively.

Conclusions

This study establishes for the first time, the basis for the characterization of *Vaccinium leucanthum* fruit (cahuiche) a wild fruit endemic to Mexico, regarding its physicochemical properties, nutrimental composition, and antioxidant capacity. The phenolic acids and anthocyanins content in cahuiche can be comparable with other red fruits. Bioactive compounds and antioxidant capacity in cahuiche were higher than other fruits cited in scientific literature. The consumption of cahuiche can represent a potential benefit for human health as a berry with functional properties because of its important content in micro and macroelements, dietary fiber, bioactive compounds like ascorbic acid and phenolic compounds mainly gallic acid and cyanidin-3-glucoside, and due to its high antioxidant capacity.

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Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
d.w.	dry weight
FRAP	Ferric-Reducing Antioxidant Power
f.w.	fresh weight
HPLC-DAD	High-Performance Liquid Chromatography-Diode Array Detector
TEAC	Trolox Equivalent Antioxidant Capacity

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