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Morphological and Nutritional Characterization of Wild Edible Blackberries (*Rubus* spp.) from Sinaloa, Mexico

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Abstract. Wild blackberries (*Rubus*) are fruits that grow in inaccessible high mountain areas, which has made it difficult to study their chemical and nutritional composition. The objective of this research was to evaluate the nutritional composition and the macro- and micro-nutrient profile of wild species of fruits of the *Rubus* genus collected in Sinaloa, Mexico. Botanical samples of wild *Rubus* were identified and deposited in the Herbarium of the Institute of Biology of the UNAM. Subsequently, the chemical composition the profile of carbohydrates, lipids and minerals were evaluated. Wild *Rubus* species were identified as *R. liebmannii*, *R. coriifolius* and *R. palmeri*. In addition, it was found that *R. liebmannii* is the first time it has been recorded for the state of Sinaloa. Likewise, the results show that carbohydrates represent the largest proportion of the macro-components (85 %, glucose and fructose); they have a high content of fatty acids (such as linolenic acid) and are rich sources of minerals (such as K, Ca and Mg). The results of this research could be relevant to be used in the genetic improvement of commercial species that currently exist in the market.
Keywords: *Rubus*; wild blackberries; macro-nutrients; minerals; fatty acids.

Resumen. Las zarzamoras silvestres (*Rubus*) son frutos que se desarrollan en zonas de alta montaña poco accesibles, lo que ha dificultado el estudio de su composición química y nutricional. El objetivo de la presente investigación fue evaluar la composición nutricional y el perfil de macro y micronutrientes de especies silvestres de frutos del género *Rubus* colectadas en Sinaloa, México. Muestras botánicas de *Rubus* silvestres fueron identificadas y depositadas en el Herbario del Insituto de Biología de la UNAM. Posteriormente se les evaluó la composicón química, el perfil de perfil de carbohidratos, lípidos y minerales. Las especies silvestres de *Rubus* fueron identificadas como *Rubus liebmannii*, *Rubus coriifolius* y *Rubus palmeri.* Además, se encontró que *Rubus liebmannii*, es la primera vez que se registra para el estado de Sinaloa. Asimismo, los resultados muestran que los carbohidratos representan la mayor proporción de los macro-componentes (85 %, glucosa y fructosa); tiene un alto contenido de ácidos grasos (como ácido linolénico) y son fuentes ricas de minerales (como K, Ca y Mg). Los resultados de la presente investigación podrían ser de relevancia para ser utilizados en el mejoramiento genético de especies comerciales que actualmente existen en el mercado.
Palabras clave: *Rubus*; zarzamoras silvestres; macronutrientes; minerales; ácidos grasos.

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Introduction

The genus *Rubus* L. is a large and complex group of plants in the family Rosaceae naturally distributed in fresh and tropical regions worldwide with an uncertain number of species since some studies estimate 250–330 species, but others up to 750 species [1-3]. In Mexico, Rzedowski and Calderón de Rzedowski [4] catalogued 61 *Rubus* species, but recently, Rodríguez-Bautista et al. [5] reported only 42 species of this genus using bioinformatic tools. The most studied *Rubus* species in Mexico are mainly located in the Trans-Mexican Volcanic Belt and the Sierra Madre del Sur [4-6], though some exploratory research for the Sierras Madre Occidental has provided new records of wild *Rubus* for Sinaloa and Durango and some of these species are underutilized as a seasonal food source by local human communities [7,8]. However, all these investigations mention the need for more studies on this group's systematics, taxonomy, evolution, and ecology for Mexico and the world.

Several authors mention that *Rubus* species, and other Rosaceae species, are difficult to delimitate because of their easily hybridization among *Rubus* species, frequent apomixis, phenotypic plasticity and the lack of a consensus on the morphology of the genus [9-11]. The morphology of the *Rubus* species is multifaceted and varied since they can be shrub or woody plants, prostrate or erect, with compound leaf blades and serrated-toothed margins [12,13], which are considered useful characters to generate taxonomic discriminations when distinguishing these from other Rosaceae species [14]. However, within *Rubus*, the most relevant criteria are those that show the least phenotypic variation, highlighting among their flowers and fruits, as well as some constant characteristics of leaves, stems and roots [6,10].

The high variability is not exclusive to anatomical structures in *Rubus* plants since this diversity is also found in the phytochemical profile of nutritional compounds and secondary metabolites of leaves, roots, and fruits [15-20]. The macro- and micronutrients reported in *Rubus* fruits are well known in some popular commercial-domesticated *Rubus*, such as *R. fruticosus* (common blackberry), *R. coreanus* (bokbunja or Korean berry) and *R. idaeus* (common raspberry), which include a large variety of amino acids, vitamins, carbohydrates, lipids, and minerals, as well as their phytochemical profile [16,20-22].

The chemical profile of bioactive compounds (including tannins, flavonoids, and phenolic acids) of wild *Rubus* species from the "Chara Pinta (Tufted-Jay) Sanctuary" in Sinaloa, Mexico, is well documented by previous works [18-20]. However, the composition of nutritional, macro- and micronutrients remains unexplored. This study aims to evaluate the nutritional composition and the macro- and micronutrient profiles of wild *Rubus* berries from Sinaloa, Mexico.

Experimental

Materials and methods
Plant material

A search for individuals of the genus *Rubus* L. was carried out in 3 sites with anthropogenic alterations within the "Chara Pinta Sanctuary" (23º48'48.4 " N, 105º50'15.1" W), in the ejido lands of El Palmito, Concordia, Sinaloa, Mexico, during the spring-summer 2015 season (June 29-30, 2015). The collection sites were the Cabins of the Chara Pinta Sanctuary (CP, 2172 m a.s.l.), the Road to El Mirador (CM, 2163 m a.s.l.) and kilometer 200 of Federal Highway 40 (CF, 2013 m a.s.l.) (Fig. 1). Specimens with fertile stems (which presented leaves and inflorescences with fruits in different degrees of maturity and apical flowers or buds) and sterile stems were collected, taking care that these came from the central part of the plant, since in this region it is where it presents less phenotypic variability of the same structures, as different previous studies have established [14]. The plant material was pressed and dried at 50 ºC for 72 h. The material was analyzed and herbarized with the support of the National Herbarium of the Institute of Biology of the National Autonomous University of Mexico (MEXU) staff. About 1.0 kg of only ripe fruits of two from three *Rubus* species was collected and immediately freeze-died at -80 °C. However, for one of them, the collected fruits were insufficient for the subsequent nutritional evaluation.

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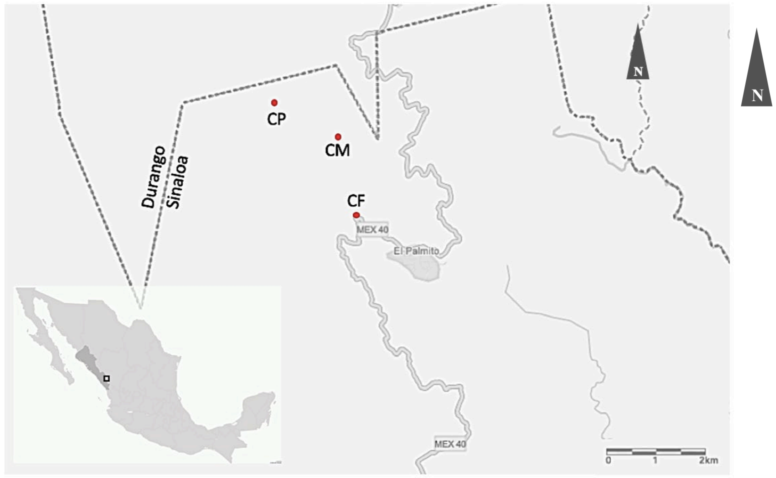

Fig. 1. Map of the wild blackberry collection sites in El Palmito, Sinaloa, Mexico. CP, Cabins of the Chara Pinta Sanctuary; CM, Road to El Mirador; CF, Federal Highway México 40.

Identification of wild blackberry species

The characters present in the exsiccates were compared with those of specimens previously identified and deposited in the MEXU, as well as with those described in the following floristic consultation sites: Tropicos (www.tropicos.org); KEW: World Checklist of Selected Plant Families (apps.kew.org); Catalog of Life (www.catalogueoflife.org); Global Biodiversity Information Facility (data.gbif.org); The New York Botanical Garden Virtual Herbarium (data.gbif.org); JSTOR Plant Science (plants.jstor.org); Encyclopedia of Life (www.eol.org); Biodiversity Heritage Library (www.biodiversitylibrary.org); and the Mesoamerican Flora project (http://www.tropicos.org/Project/FM).

Proximate composition of berries

Protein, moisture, fat, and ashes contents were determined on a dry basis according to the AOAC methods [23]. An amount of 0.2 g of freeze-dried fruits was digested with 1.625 g of catalyst (KSO_4:$CuSO_4$, 10:1, w/w) and 5 mL of H_2SO_4 for 6 h at 550 ºC. Then, 10 mL of distilled water was added, before adding 15 mL of H_3BO_3 and 3 drops of methyl red as an indicator. The colour of the mix was adjusted with 40 % NaOH and titrated with 0.08 N HCl. For moisture, 1.0 g of dried samples were held at 130 ºC for 1 h to remove remained moisture. The crude lipid content was estimated in 1.5 g of dried fruits in a Soxhlet system using petroleum ether and Whatman paper #1. Finally, ash content was measured in 1.0 g of freeze-dried fruits in a muffle at 550 ºC for 12 h. Carbohydrates were estimated by weight difference.

Extraction of macro-nutrients

Freeze-dried powder (360 g) of both *Rubus* fruits were mixed with 1 L of acidified methanol (80:0.3, MeOH:trichloroacetic acid, v/w) at 4 ºC for 24 h, then filtered with Whatman paper #1. These steps were repeated, and filtered fractions were collected together. Briefly, the MeOH was extracted by rotary evaporation in a Büchi equipment (Labortechnick AG., Switzerland). The aqueous part was exposed to ethyl acetate (1:1, v/v) and shacked vigorously for 2 min in a separatory funnel twice. The oily and watery phases were concentrated by rotary evaporation. The non-polar fraction (NPF) was kept at -20 ºC under its posterior evaluation. For carbohydrate isolation, the defatted samples were freeze-dried at -80 ºC, 5.0 g of this were resuspended in 100 mL of acidified water (0.3 % TCA), and exposed to 100 g of Amberlite XAD-7 adsorption resin (Sigma-Aldrich, St.-Louis, USA) for 30 min. A glass column of 300 × 25 mm was packed with the mix and washed with 500 mL of acidified water. The pH of the liquid was adjusted to 7.0 and freeze-died under its analysis.

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Profile of carbohydrates

The carbohydrate-enriched fractions (CEF) were profiled according to the procedure reported by Xiang et al. [24], with some modifications. Briefly, 10 mg of CEF were solubilized with 50 µL of the derivatizing *N*,*O*-bis(trimethylsilyl)trifluoroacetamide **(**BSTFA, 33027, Sigma-Aldrich, St.-Louis, USA) and 50 µL of pyridine (27047, Sigma-Aldrich, St.-Louis, USA), sonicated for 5 min and heated at 70 ºC for 4 h. Afterwards, 1.5 mL of HPLC grade MeOH was used to resuspend the mix. A volume of 5 µL was injected in a GC-MS Agilent system (GC6890N-MS5973, Agilent Technologies, Inc., La Jolla, USA) and carried out with helium gas through a QUADREX 007-23 (30 m × 0.25 mm ID, 0.25 µm film thickness, Quadrex Corp., Woodbridge, USA). The injector temperature was 250 ºC, while the oven was at 60 ºC and the temperature gradient of 5 ºC/min until 200 ºC and heated up 10 ºC/min until 275 ºC, remaining constant at 275 ºC as far as 60.5 min. Mass detector was operated in the electron impact mode with 70 eV of energy. The temperatures for the detector and the quadrupole were 245 ºC and 150 ºC, respectively. The resulting data were compared with the NIST/EPA/NIH database Version 2.0 (NIST 08 Mass Spectral Library, Gaithersburg, USA). The obtained results were presented as relative percentages (%) of carbohydrates.

Profile of lipophilic compounds

The lipophilic profile was performed following the method described by Ahumada-Santos et al. [25]. About 5.0 mg of FEF were solubilized in 1 mL of hexane HPLC degree and injected in an Agilent GS-MS system (GC6890N-MS5973, Agilent Technologies, Inc., La Jolla, USA) through a QUADREX 007-23 (30 m × 0.25 mm ID, 0.25 µm film thickness, Quadrex Corp., Woodbridge, USA), using helium gas as a carrier. A volume of 5 µL was injected at 250 ºC of vaporizer temperature, the oven started at 60 ºC and the gradient was increased 5 ºC/min until 200 ºC and heated up 10 ºC/min until 275 ºC, remaining constant at 275 ºC as far as 60.5 min. The equipment mass detector was used in the electron impact mode with 70 eV of energy. Temperatures for the detector and the quadrupole were 245 ºC and 150 ºC, respectively. The results were compared with information compilated in the NIST/EPA/NIH database Version 2.0 (NIST 08 Mass Spectral Library, Gaithersburg, USA). Results were expressed as relative percentages (%) of the identified lipophilic compounds.

Profile of minerals

The mineral profiling was done following the procedure of Frías-Espericueta et al. [26], with slight adaptations. About 1.0 g of freeze-dried powder of both fruits was calcinated at 550 ºC for 2 h into a muffle. Ashes were solubilized in 5 mL of concentrated HNO_3 (trace metal grade) and kept at room temperature for 1 h. Then, the mix was filtered and made up to 50 mL with Milli-Q water. Finally, 1.0 mL of sample was injected in a Varian SpectrAA 220 atomic absorption spectrophotometry (Varian, Palo Alto, USA) and monitored with the Varian AA software version v3.10 (Varian, Palo Alto, USA). Analysis of certified reference material (DOLT-4, National Research Council Canada) gave recovery acceptable values of 95–115 % in all analyzed metals. Results were expressed as mg/100 g dw.

Statistical analysis

Data were analyzed by one-way analysis of variance using the Statgraphics Centurion XVI.I statistical package (Statgraphics Technologies, UK). Tukey's test was used to compare mean values +/- standard deviation under a statistical difference ($p<0.05$). All experiments were done in triplicate ($n=3$).

Results and discussion

Description of collected *Rubus*

The following *Rubus* species were described and identified in the Chara Pinta Sanctuary, Sinaloa (Fig. 2):

Rubus liebmannii Focke, Abh. Nat. Ver. Bremen 4: 158. 1874

TYPE: MEXICO, Oaxaca, Cerro de Zempoaltepec. 1842. Holotype: Liebmann s.n.; 1841-1843; Mexico (C).

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Perennial stems, 2–3 m tall; shoots erect, terminal part arched, abruptly angulate, scarcely hairy and armed with some compact curved spines; trimeric, deciduous leaves (Fig. 2**(A.1)**); petioles hairy, prickly, 4–6 cm long; stipulate tiny, hoggy, 8–10 mm long; leaflets irregular, fine and pronouncedly serrated, green and pubescent on both sides, terminal leaflet oval or elliptic, acuminate, with 8–10 veins on each side, 7–10 cm long, its petiole 2–3 cm long; lateral leaflets 4–5.5 cm long, with petioles approximately 2 mm long (Fig. 2**(A.2)**); densely hairy and spiny flower stems, small, curved spines; terminal and axillary panicles, short, sloping; tomentose stems and pedicels with small glands; cinereo-tomentose calyx, glanduliferous; sepals extended or reflexed; oval, pink petals (Fig. 2**(A.3)**); fruits 1 cm long, black; numerous, tomentulous drupelets (Fig. 2**(A.4)**).

Material examined: IBUNAM: MEXU: 1417228. *Rubus liebmannii* Focke ROSACEAE Shrub 2.5 m; spiny, semi-woody, hairy, brown, semiglabre stems; alternate, trifoliate, spiny leaves, biserrulous margin; inflorescence in corymb: pink, pentameric, perfect flowers. El Palmito, Concordia, Sinaloa, Mexico. 23°35’22.5” N, 105°52’09.5” W, 2172 m a.s.l. 06/30/2015. Collector: Oscar Abel Sánchez-Velázquez. Determined: Biol. Gilda Ortíz-Calderón / Dr. Oscar Abel Sánchez-Velázquez.

This species had not been previously reported for the state of Sinaloa. This blackberry is also known as “tunita de cerro”, “zarza” and “zarzamora” in Spanish and “citun-zarza” and “tsitubi” in Purepecha. Its geographical distribution is reported in the forested areas of Michoacán, Hidalgo, Durango, Jalisco, and Guanajuato. Ecologically, like many *Rubus* species, they are important plants as colonizers of new sites for the development of other associated species of primary vegetation [4]. Some anatomical parts (leaves, stems and fruits) of this plant are used in traditional medicine for diarrhea, colds, dysentery, and insomnia [27]. This is a species of blackberry endemic to Mexico that is not under any category of protection under Mexican law and that had not been reported for the state of Sinaloa.

Rubus coriifolius Liebm. Vidensk. Meddel. 1852: 157. 1853.

TYPE: MEXICO, Veracruz, Mirador. 1853. Type: Liebmann s.n.; Sep; Mexico: Alpatlahua, 7000'(C).

Perennial stems, 1.5-2 m tall; subrepent buds, pubescent, rarely with small thorns; stems angled, with long, recurved and compact spines, puberulent at the base; leathery, persistent leaves, pentameric leaflets (sterile stems) (Fig. 2**(B.1)**); semibristled stipules, 4–5 mm long, pubescent; pubescent petioles, rarely with glands intermixed with villi, petioles and central vein spines, 5–7 cm long; leaflets elongated-ovate 5–10 cm long, obtuse at the base, accumulated at the apex, roughly biserrated, dark green, puberulent, underside densely pubescent, with prominent veins (Fig. 2**(B.2)**); petiole of the average follicle 3–4 cm long, those of the lateral leaves approximately 2.5 cm long and those smaller than 4–6 mm long; alternate floral branch leaves; leaflets 5–7 cm long, 2.5-3 cm wide; petiole of the middle leaflet 12–16 mm long, those of the lateral leaflets 2–5 mm long; terminal and axillary panicles, the main frequently 30 cm long; scattered, tomentose branches, often with interspersed glandular villi; lanceolate, sharp bracts; sepals thick, ovate, acuminating or acute, tri-veined, tomentose, at the end reflexed; petals obovate, longer than sepals, pink or white (Fig. 2**(B.3)**); small fruits, red or black when approaching maturity, glabrous; 8–20 drupelets, ovoid, glabrous, gradually fall apart (Fig. 2**(B.4)**).

Material examined: IBUNAM: MEXU: 1417229. *Rubus coriifolius* Liemb. ROSACEAE Shrub 3 m; climbing branches; spiny, semi-woody and herbaceous stems, hairy, glandular; alternate leaves, tri or pentafoliate, spined, biserrulous margin; hairy-glandular inflorescence, corymb: perfect white, pentameric flowers. El Palmito, Concordia, Sinaloa. 23°34’09.7” N, or 105°50’57.1” W. 2013 m a.s.l. 06/30/2015. Collector: Dr. Oscar Abel Sánchez-Velázquez. Determined: Biol. Gilda Ortíz-Calderón / Dr. Oscar Abel Sánchez-Velázquez.

Rubus coriifolius is a plant distributed in the mountainous systems of highlands forestry coverage in Mexico and even in Central America and Venezuela [28]. Stems, leaves and fruits are used in traditional herbalism for antimicrobial and antiparasitic purposes [29].

Rubus palmeri Rydberg, sp. Nov.

TYPE: MEXICO, Durango, San Ramón. 1906. Isotype: T: Edward Palmer - 78. (MO/BC: MO-197903/A: 1927515). Perennial stems, reclining on bushes or walls, 2–4 m long, angled, hairy, tomentose or glabrous, the oldest, armed with strong recurved spines 5–8 mm long; pentafoliated or trifoliate leaves (Fig.

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2**(C.1)**); petioles, petioles and central veins hairy and heavily armed with curved spines; subulated stipules; 8–10 mm long; leaflets rounded-oval or obovate, abruptly short and acuminate at the apex, rounded or bordered at the base, 5–10 cm long, light green, burr and irregularly serrated, with oval teeth, sparsely hairy in the upper part, rather dense villi on the underside; about 10 lateral veins on each side (Fig. 2**(C.2)**); inflorescences with many flowers, frequent foliaceous, villous-tomentose bracts, some glands and armed with recurved stingers; sepals are ovate, apiculate, tomentose on both sides, about 7 mm long; white, obovate petals, 12–15 mm long (Fig. 2**(C.3)**); dark purple fruits, large, juicy; glabrous drupelets, 20–35; fall, 3 mm long, strongly reticulated (Fig. 2**(C.4)**).

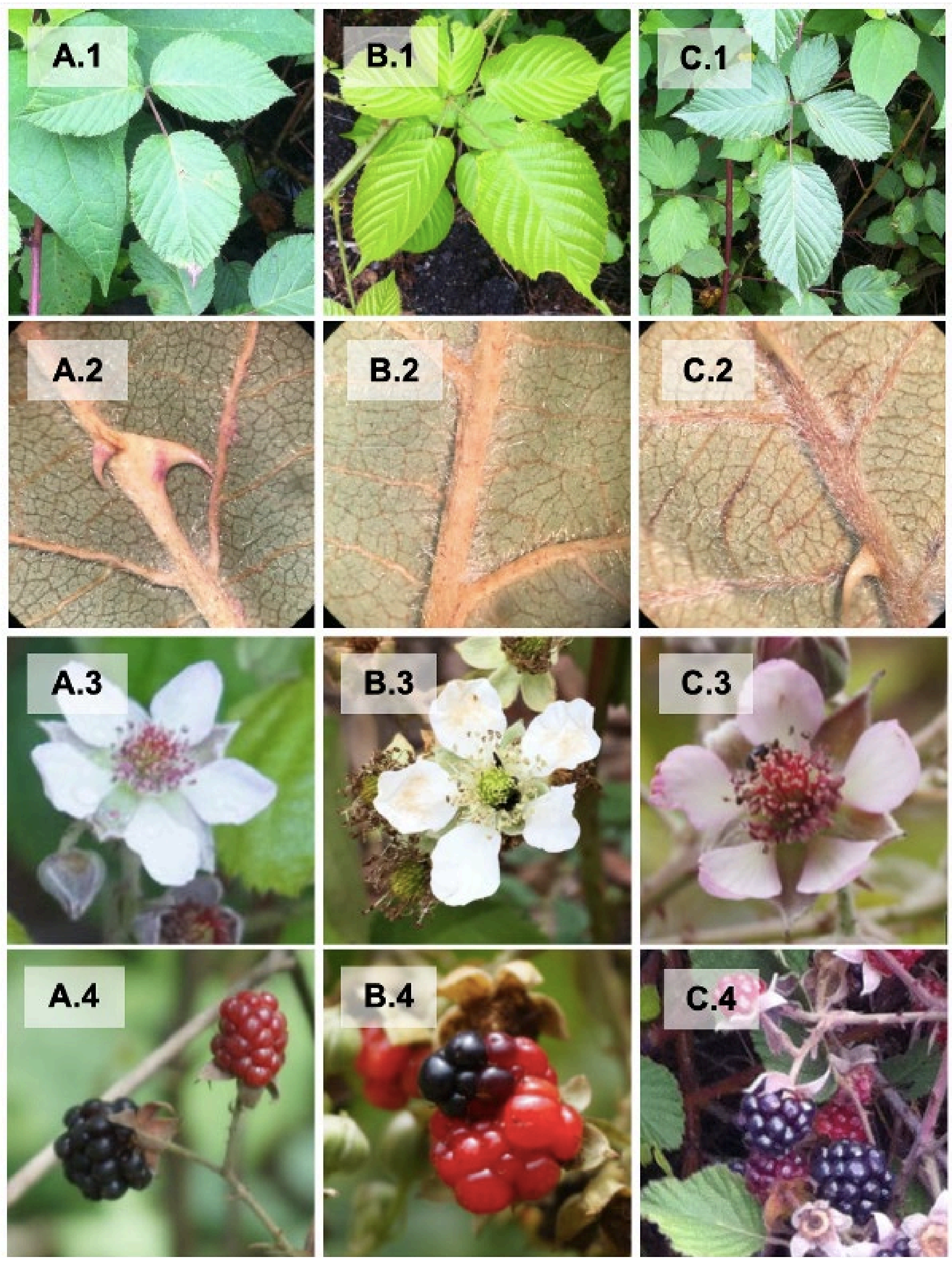

Fig. 2. Illustration of some anatomical parts used for the identification of wild blackberries from the Chara Pinta Sanctuary. A. *R. liebmannii*; B. *R. coriifolius*; C. *R. palmeri*; leaves **(1)**; underside of leaflets **(2)**; flowers **(3)**; fruits **(4)**.

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Material examined: IBUNAM: MEXU: 1417230. *Rubus palmeri* Rybd. ROSACEAE Shrub 2 m; hanging and creeping branches; spiny, semi-woody, hairy stems; alternate leaves, tri or pentafoliate, spined, biserrulous margin; hairy inflorescence, corymb: white, pentameric, perfect flowers. El Palmito, Concordia, Sinaloa. 23°34'55.7" N, or 105°51'21.4" W. 2163 m a.s.l. 06/29/2015. Collector: Dr. Oscar Abel Sánchez-Velázquez. Determined: Biol. Gilda Ortíz-Calderón / Dr. Oscar Abel Sánchez-Velázquez.

Rubus palmeri is a species native to northwestern Mexico and other states such as Chiapas, San Luis Potosí, Tamaulipas, and Veracruz, in addition to being distributed in Guatemala [28]. Fruits of this species are used to make jams, tamales, and sweets, while their leaves are used as medicines for parasitic diseases and gastrointestinal system pain [4].

Despite their morphological peculiarities, these three species are evolutionarily related to each other [5]. *Rubus liebmannii* and *Rubus coriifolius* belong to the *Floribundi* subgenus, while *Rubus palmeri* is classified as part of *Sapidi*. The criteria for this robust grouping are more related to the characteristics of inflorescences, such as the number and arrangement of flowers, as well as the presence and abundance of glandular trichomes and spines [30]. Other related subgenres are *Adenotrichi* and *Duri*, in which most of the tropical species described in the American continent are found [31].

The El Palmito Sanctuary region has a temperate sub-humid (Cwa) climate with a marked rainy season between July and September. Peaks of more than 2,000 m a.s.l dominate the relief. And deep ravines that converge in perennial water flows. That is why the vegetation is intimately linked to a high mountain orography, typical of the Sierra Madre Occidental, where botanical communities such as mountain mesophyll forest, pine forest and mixed oak-pine forest stand out, and to a lesser extent it can be found riparian and gallery vegetation [32]. The dominant plant species belong to the genera *Pinus* and *Quercus*; however, it is also frequent to find individuals from other genera such as *Salix*, *Abies*, *Juniperus*, and *Cupressus*. While secondary vegetation is made up of species of *Datura*, *Argemone*, *Salvia*, *Ipomoea*, *Lopezia*, *Castilleja*, *Geranium*, *Sida*, *Oxalis*, among many others, including *Rubus coriifolius* and *Rubus palmeri* [7,32]. Human economic activities have modified the local ecosystems during the last one hundred years, generating environmental conditions for foreign and exotic flora, like *Hypoestes phyllostachya*, *Ricinus cummunis*, *Ipomoea quamoclit*, *Taraxacum officinale*, *Trifolium repens*, and *Melinis repens*, which indicates that there is a flow of species from other latitudes to these sites, either deliberately or accidentally [8].

Therefore, the collection sites have some representative characteristics of the different types of vegetation in the Chara Pinta Sanctuary. The Cabins site (CP) is a deforested area of approximately 5 ha of gentle inclination and bordered by a canopy of Nearctic affinity (*Pinus herrerai*, *Pinus engelmannii*, *Pinus lumholtzii* and *Pinus douglasiana*). Human influence in CP is high due to the change in land use for recreational purposes, whose occupation is restricted to no more than 50 people, but this figure is rarely reached. *Rubus liebmannii* individuals were found at this site, forming corridors, at least 30 m long, on the margin of a low-traffic dirt road. Additionally, a *Rubus coriifolius* specimen was identified at this location.

The path to the viewpoint (CM) is a crossing point to the Mirador, located between the cabins and the community of El Palmito (Table 1). In this place are present individuals of *Pinus engelmannii* and *Pinus douglasiana*, as well as *Quercus urbanii*, *Arbutus xalapensis* and *Conostegia xalapensis*. Mixed and dispersed individuals of *Rubus liebmannii* and *Rubus palmeri* (RP) were observed at this site. Deforestation of plants with a diameter greater than 1.5 m is still active here. Secondary vegetation is also made up of some introduced grasses and herbaceous plants.

The last site consists of a point of convergence between Federal Highway Mexico 40 and a constant flow (CF) waterfall. The flora present here corresponds to gallery vegetation and orographic shade, with species such as *Alnus glabrata*, *Dendropanax arborens*, *Arbutus madrensis*, *Casimiroa edulis*, *Clethra hartwegii*, etc. In addition, you can find minor species of *Cuphea*, *Castilleja*, *Cosmos*, *Adiantum*, by others, as well as introduced species such as *Leonotis nepetifolia*, *Lysamachia arvensis* and *Melinis repens* [8]. Individuals of RC and RP were found in interspersed communities on the roadside at a distance of no more than 10 m between the convergence of the highway and the stream.

The reproductive season of wild *Rubus* in tropical ecosystems generally occurs a few weeks before the rainy season. The simultaneous co-fructification of one or more species facilitates zoochory by sharing dispersers in common with each other [33]. This phenomenon favors plant succession with native flora and the recruitment of invasive or non-native species and, due to the characteristics of *Rubus* fruits, it is

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one of the genera that has increased its distribution to new lands around the world and has caused different impacts on colonized ecosystems [33].

The ease with which wild *Rubus* species can colonize new pristine and degraded ecosystems, the morphological plasticity of some taxonomic characters, apomixis, and interspecific hybridization makes it difficult to keep floristic listings for *Rubus* up to date. Maintaining constant and consecutive studies of the genus in the Chara Pinta Sanctuary can be useful to know the ecological relevance of these species to maintain the quality of the ecosystem, as well as compile characteristics with possible agronomic interest that allow us to enhance the knowledge of these fruits could increase their interest for human nutrition purposes.

Table 1. Presence of *Rubus* species at collection site.

Site	Geographical Location	Species		
		RL	RC	RP
CP	N 23°35’22.5”, W 105°52’09.5”	X	X	
CM	N 23°34’55.7”, W 105°51’21.4”.	X		X
CF	N 23°34’09.7”, W 105°50’57.1”		X	X

RL, *Rubus liebmannii*; RC, *Rubus coriifolius*, RP, *Rubus palmeri*; CP, Cabins Chara Pinta; CM, Road to El Mirador; CF, Federal Highway México 40.

Proximate composition

Results from the chemical composition analysis of raw dried fruits of *R. liebmannii* and *R. palmeri* are displayed in Table 2. The protein content for *R. liebmannii* was 5,402 mg/100 g dw, while for *R. palmeri* it was 5,019 mg/100 g dw. It is known that the proteins and polypeptides in *Rubus* fruits are present in low amounts, but with a rich profile of amino acids [34], additionally, the nitrogenous content decreases with the maturity level of fruits [35]. In some grown *Rubus* berries, such as *R. coreanus*, *R. ideaus* or *R. fruticosus* [16,36], the protein content is about 5–7 % dw, while in wild *Rubus* species from upper latitudes showed protein proportions of 3.3–4.4 % dw, like *R. ellipticus* and *R. niveus* [37], although, for berries of the wild *R. amabilis*, proteins can represent up to >10 % dw [38]. However, tropical blackberries such as *R. adenotrichus* and *R. ulmifolius* presented values of 5.9 % and 6.56 % dw in ripe fruits [37]. It means that wild tropical and cultivated blackberries could have similar protein content.

Table 2. Proximate composition and mineral profile of *R. liebmannii* and *R. palmeri* fruits.

Macro-nutrients	Composition (mg/100 g dw)	
	Rubus liebmannii	***Rubus palmeri***
Protein	5,402 ± 424^{a}	5,019 ± 537^{a}
Lipids	6,433 ± 398^{b}	7,239 ± 520^{a}
Ashes	2,503 ± 211^{b}	2,929 ± 110^{a}
Carbohydrates	85,749 ± 738^{a}	84,892 ± 615^{a}

Micro-nutrients			
Minerals	**RDA (mg/day)**		
Potassium (K)	3,500	592.8 ± 31.9^{a}	480.0 ± 33.9^{b}
Calcium (Ca)	1,200	188.9 ± 7.1^{b}	205.1 ± 9.5^{a}
Magnesium (Mg)	350	104.6 ± 3.8^{b}	165.4 ± 19.7^{a}
Sodium (Na)	2,000	61.4 ± 2.5^{b}	83.8 ± 8.5^{a}
Manganese (Mn)	75	10.7 ± 1.0^{a}	7.3 ± 0.6^{b}
Iron (Fe)	15	4.4 ± 0.3^{a}	4.2 ± 0.3^{a}
Zinc (Zn)	10	1.8 ± 0.1^{b}	2.4 ± 0.1^{a}
Copper (Cu)	2	0.7 ± 0.0^{a}	0.8 ± 0.1^{a}

RDA, recommended daily allowance minerals in adults (RDI, 2006). Mean ± S.D. Similar letters at same line indicate non statistical differences (p<0.05). n=3.

For *R. liebmannii* the lipid compounds represented 6,433 mg/100 g dw and for *R. palmeri* 7,239 mg/100 g dw, with differences (p<0.05) between them. Most of the oily portion of *Rubus* berries is found in the dermis and seed [21,34]. Therefore, *R. palmeri* could have a greater quantity of lipids because it has more oily structures in the fruitlessness (around >30 drupeols/drupelet) compared to fruits of *R. liebmannii* (<25 drupeols/drupelet). Our results indicate that *R. liebmannii* and *R. palmeri* have a higher content of lipids compared to other *Rubus* berries, such as wild *R. ellipticus*, *R. ulmifolius*, and *R. niveus* with 1.1–4.7 % dw [37]; wild genotypes of raspberry (*R. idaeus*) with 2.1–3.47 % dw; and grown raspberries and blackberries with 3.3–4.2 % dw [16,21,39]. The presence of a number of oily anatomical structures in our *Rubus* fruits could contribute to the superior content of lipids in these. However, also external circumstances may affect their synthesis and concentration to protect the fruits against biotic and abiotic environmental conditions.

Ash content was 2,503 and 2,929 g/100 g dw correspondingly for *R. liebmannii* and *R. palmeri*, with statistical differences (p<0.05) between them. Ashes represent the minerals contained within fruit structures and may contribute to fruit nutraceutical properties [16]. These results are within the range reported for wild *R. amabilis* [37,38] from fresh highlands, but lower than tropical wild or grown *Rubus* with ash contents of 3.0–4.4 % dw [35,40]. This phenomenon is a natural process determined by factors such as mineral availability in soil, diversity of micro- and macro-elements, or the metabolism of fruits during the ripening process [35,41]. Thus, *Rubus* fruits are an excellent source of microelements that could contribute to reaching the required daily dose of minerals [16,37]. Individual minerals will be discussed in more detail in the next section.

The total carbohydrates contents were estimated at 85,749 and 84,982 mg/100 g dw for *R. liebmannii* and *R. palmeri*, respectively, without statistical differences (p<0.05) between them. It is known that the carbohydrates represent the major macro-compounds in *Rubus* berries (>80 % dw), so our results coincide with the reported for wild blackberries genotypes, such as *R. imperialis*, *R. ellipticus* or *R. idaeus* [37,38], but are lower than the >90 % dw reported in some grown *Rubus* genotypes [16]. The carbohydrates represent the sum of free carbohydrates, fiber, and starch [35,42], and their structural and metabolic role is crucial for the maturity process, synthesis of secondary metabolites, and sensory properties for the dispersing animals, but also carbohydrates represent 40–80 % of total energy requirement in humans [43].

Mineral composition

In both *Rubus* species, the blackberries presented the following mineral distribution: K<Ca<Mg<Na<Mn<Fe<Zn<Cu. However, their contents were not the same in these berries (Table 2).

Potassium (K) was the major mineral in both fruits, since in *R. liebmannii* its content was 592.9 mg/100 g dw, while for *R. palmeri* was 480.0 mg/100 g dw, being this last statistically low (p<0.05). The potassium content in our fruits was lower than that reported for *R. ellipticus*, *R. niveus* and *R. ulmifolius* fruits (680–920 mg/100 g dw) [37], but higher than amounts reported for raspberry cultivars (103–152 mg/100 g dw) [44,45], as well as fruits of *R. amabilis* (113.3 mg/100 g dw) [38]. The recommended daily dose of K is 2500 mg per day, so the intake of 100 g of dry fruit of *R. liebmannii* or *R. palmeri* could contribute with the 16.9 % and 13.7 % of the K required in adults.

Table 3. Lipid and carbohydrate profiles of wild *Rubus* fruits.

Profile	**RT (min)**	***Rubus liebmannii***	***Rubus palmeri***
Lipid compounds		**Proportion in NPF (%)**	
C16:0, palmitic acid	30.6	10.3 ± 0.4^{b}	15.2 ± 0.3^{a}
C18:0, stearic acid	41.1	0.9 ± 0.1^{a}	0.6 ± 0.0^{b}
C18:1, oleic acid	39.0	25.6 ± 1.2^{b}	30.7 ± 1.5^{a}
C18:2, linoleic acid	39.8	15.1 ± 1.4^{a}	6.3 ± 0.8^{b}
C18:3, linolenic acid	38.9	28.0 ± 0.1^{b}	30.2 ± 0.1^{a}
α-tocopherol	52.6	12.5 ± 1.1^{a}	9.0 ± 1.8^{b}
β-sitosterol	53.9	6.5 ± 0.5^{b}	8.0 ± 0.3^{a}
Carbohydrates		**Proportion in CEF (%)**	
D-fructose	30.31	48.7 ± 0.5^{b}	49.7 ± 0.5^{a}
D-altrose	31.74	7.2 ± 1.7^{a}	6.8 ± 0.4^{a}
D-galactose	34.40	18.0 ± 0.6^{a}	17.8 ± 1.0^{a}
D-xylose	34.51	6.7 ± 0.9^{a}	5.5 ± 0.4^{b}
D-glucose	39.96	19.8 ± 0.7^{a}	21.0 ± 0.7^{a}

RT, retention time; NPF, non-polar fraction; CFE, carbohydrate enriched fraction. Mean ± S.D. Similar letters at same line indicate non statistical differences (p<0.05). n=3.

Calcium (Ca) content in *R. liebmannii* and *R. palmeri* were 188.9 and 205.1 mg/100 g dw, respectively, having differences (p<0.05) between them. The calcium concentration in *R. palmeri* was closer to the highest values reported by Castilho Moro et al [45] for raspberry cultivars in tropical cultivars (162–175 mg/100 g dw), similar to *R. imperialis* (190–202 mg/100 g dw) [39], but our two fruits had less Ca than the reported for *R. idaeus* from cold latitudes, *R. ellipticus*, *R. ulmifolius* and *R. niveus*, ranged around 243–620.5 mg/100 g dw [37]. For adults, 100 g of dried berries of *R. liebmannii* and *R. palmeri* could represent 15.7 % and 17.1 %, respectively, of the recommended daily allowance (RDA) of Ca [46].

Magnesium (Mg) was the third majority element in both species with values of 104.6 and 165.4 mg/100 g dw, respectively for *R. liebmannii* and *R. palmeri* being statistically different (p<0.05). *R. liebmannii* presented Ca amounts lower than subgenus *Ideaobatus* cultivars and wild species (119–162 mg/100 g dw) [37,45]. On the other hand, the Mg content of *R. palmeri* is higher than raspberries and *R.*

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ulmifolius (149–162 mg/100 g dw), but inferior to the reported for *R. niveous* (179 mg/100 g dw) [37,45]. In adults is necessary to consume around 300 mg of Mg/day, so 100 g of our dried fruits may represent 29.9 % and 47.3 % of the Mg recommended daily dose.

Sodium (Na) concentrations in *R. liebmannii* and *R. palmeri* were 61.4 and 83.8 mg/100 g dw, respectively, being different ($p<0.05$). Our values are included in the ranged Na content of 56.3-89.4 mg/100 g dw reported for several *Rubus* fruits [37]. The Na requirement is around 2,000 mg/day, so 100 g of dried fruits of *R. liebmannii* and *R. palmeri* could represent only 3.1 % and 4.2 %, respectively.

Manganese (Mn) content was 10.7 and 7.3 mg/100 g dw for *R. liebmannii* and *R. palmeri*, respectively, having differences ($p<0.05$). This microelement values were higher in our fruits compared to other *Rubus* fruits, that ranged in 1.4–2.4 mg/100 g dw [37]. The Mn contents from *R. liebmannii* could represent 14.3 %, while *R. palmeri* may be the 9.7 % of this mineral RDA consuming 100 g dry fruits.

Iron (Fe) contents for *R. liebmannii* and *R. palmeri* were 4.4 and 4.2 mg/100 g dw. This trace element has values between 1.0 and 4.2 mg/100 g dw [37,39,45,47]. In contrast to other microelements, Fe content increase with the fruit ripening process [47]. In cultured blackberries, the Fe content represents only 8 % of the RDA [16], but 100 g of dried *R. liebmannii* and *R. palmeri* could represent more than 29.3 % and 30 % of the RDA, respectively. Iron is an essential mineral for human health and plays a key role in a wide variety of metabolic processes, such as the transport of oxygen, hormones, and connective tissue production, among others [48].

Zinc (Zn) content was 1.8 and 2.4 mg/100 g dw for *R. liebmannii* and *R. palmeri*, respectively, with differences ($p<0.05$). Zn concentration in these fruits resulted lower than species such as *R. ellipticus*, *R. ulmifolius* or *R. niveus* (8.1–17.6 mg/100 g dw), but the concentration of Zn in *R. palmeri* is ranged from 2.1–3.0 mg/100 g dw of species as *R. amabilis* and *R. idaeus* [37,38,45]. A portion of 100 g of dried *R. liebmannii* or *R. palmeri* could represent 18 % and 24 % of the Zn RDA, respectively.

Lastly, the copper (Cu) amount in *R. liebmannii* was 0.7 mg/100 g dw and 0.8 mg/100 g dw for *R. palmeri*. This trace element is reported in other *Rubus* berries in ranges of 0.1–0.6 mg/100 dw [37,39,45], the reason why the Cu of our fruits is within the usual concentrations for the *Rubus* berries. The RDA of Cu is relatively low (2 mg/day), thus, consuming 100 g dw of *R. liebmannii* or *R. palmeri*, could contribute to the 35 % and 40 %, respectively, necessary for an adult.

Lipid compounds

The profile of lipid compounds is resumed in Table 3. Five long-chain fatty acids (LCFA) were found in both *Rubus* fruits, including two saturated fatty acids (C16:0, C18:0), one monounsaturated (C18:1), and two polyunsaturated fatty acids (C18:2, C18:3). Palmitic or hexadecenoic acid (C16:0) was reported at 10.3 % and 15.2 % of the NPF for *R. liebmannii* and *R. palmeri*, respectively, having differences ($p<0.05$) between them. Bushman et al. [34] reported in seeds of five *Rubus* genotypes, proportions of 1.9–3.5 % of palmitic acid; Caidan et al. [38] reported in *R. amabilis* 0.27 % for hexadecenoic acid of the total oil content; and in raspberry varieties Celik & Ercisli [21] found proportions of 4.9–8.3 % for this saturated fatty acid. It means that our fruits had, at least, 0.2–1.8 times more proportions of C16:0. This is the most abundant saturated fatty acid in foods and the human body, and it could be synthesized endogenously via *de novo* lipogenesis [49]. Palmitic acid plays a key role in many physiological processes, but it should also be noted that it can also have adverse health effects, especially when combined with other risk factors such as heart disease and a high predisposition to cancer development [49–51].

Stearic or octadecanoic acid (C18:0) for *R. liebmannii* was 0.9 %, while for *R. palmeri* was statistically lower ($p<0.05$) being only 0.6 % of the NPF. In both species, this was the minor fatty acid. In *R. idaeus*, some genotypes presented non-detectable levels of C18:0, but in those who managed to be identified they did not show quantities of stearic acid >1.2 % [21]; in seed-subproducts from five *R. fruticosus* cultivars, the range of octadecanoic acid was 0.8–3.3 % [34]; and in *R. amabilis* this fatty acid showed values of 0.15 % of total oil content [38]. Stearic acid is known to be less abundant than other saturated LCFA in foods, like palmitic acid [49], and we confirmed this fact in *R. liebmannii* and *R. palmeri* NPF.

Oleic acid (C18:1) is an omega-9 fatty acid, monounsaturated and observed in *R. liebmannii* 25.6 % of the NPF, while for *R. palmeri* it was 30.7 %. This fatty acid represents the major fatty compound in *R. palmeri* and the second most abundant in berries of *R. liebmannii*, agreeing with other *Rubus* berries. Therefore, fruits of a Tibetan blackberry showed a C18:1 content of 8.61 % of the total fatty fraction, while in seed oils from cultured blackberries and raspberries, Dimića et al. [52] and Radocaj et al. [53] reported proportions of 3.43–3.53 %, and Bushman et al. [34] recorded percentages of 10.4–17.3 in seed from five varieties of caneberries (*Rubus* spp.). According to the last, *R. liebmannii* and *R. palmeri* presented higher proportions of this vital fatty acid. In the human body, oleic acid has modulatory effects on health and chronic degenerative diseases [54], thus the regular consumption of these fruits could provide a valuable amount of omega-9 fatty acids.

Linoleic acid (C18:2) is an omega-6 fatty acid, polyunsaturated with a content of 15.1 % in the NPF for *R. liebmannii*, while for *R. palmeri* it was statistically lower ($p<0.05$), representing 6.3 %. The C18:2 had values of 42.2–52.6 % of oily proportion in cultured raspberries [21], whereas, for varieties of cranberry this fatty acid was determined in concentrations of 53.1–63.7 % of oily content in seeds. Having said this, we can see that our fruit has very low levels of C18:2 compared to the grown genotypes of some *Rubus* berries. This content has beneficial effects for medical and nutritional purposes, including neuro and cardioprotection, antimicrobial activity, and anticarcinogenic effects. It aids in the synthesis of beneficial lipoproteins while reducing high-density lipoprotein levels [55].

The last fatty acid identified was a-linolenic acid (C18:3), an omega-3 fatty acid polyunsaturated that represented 28.0 % of the NPF of *R. liebmannii*, and 30.2 % of *R. palmeri*. This omega-3 was the main oily compound in *R. liebmannii* and the second most abundant in *R. palmeri*, having statistical differences ($p<0.05$) between them. Hence, our fruits have linolenic acid levels in the range reported for grown *Rubus* fruits, which reach the 15.2–31.2 % of the total oily portion [21,34,38]. a-Linolenic acid is an essential fatty acid for humans and other animals, and it is fundamental to many cellular functions [56]. Together with linoleic acid, this is the main fatty acid in plants and other *Rubus* berries [21,53], but in our case, oleic and linolenic acids represented >58 % of the oil content.

In addition to fatty acids, two lipid phytocompounds were identified in *R. liebmannii* and *R. palmeri* NPF (Table 3). A type of vitamin E, α-tocopherol, was identified in *R. liebmannii* in a proportion of 12.5 % of the FNP, meanwhile, in *R. palmeri* it was statistically lower ($p<0.05$) with values of 9.0 %. α-tocopherol is also reported in other *Rubus* berries, such as some cultivars of *R. fruticosus* in proportions of 0.8–1.3 mg/100 g of fruit pomace [16] and 1.6-12.6 % for cranberry seeds [34]. It is because in our fruits, the α-tocopherol has relatively high levels of the potent lipid antioxidant [57]. α-tocopherol is considered a homologous of vitamin E and exhibits antioxidant activity in the human body protecting polyunsaturated fatty acids against free radicals [58]. This lipophilic compound is absorbed from several food sources, and accumulated in cell membranes before scavenging radicals *via* delivering an H^+ to quench free oxygen and other radicals, but the interactions "O-H" has been shown, at least, 10 % weaker than other antioxidant phytochemicals. However, the tocopheryl radical produced is less reactive than the products of polyphenols [59]. It is for all the above that *R. liebmannii* and *R. palmeri* may be considered good sources of this priceless dietary component.

β-sitosterol was also detected in NPF of *R. liebmannii* and *R. palmeri* of 6.5 % and 8.0 %, respectively, having statistical differences ($p<0.05$). β-sitosterol is a phytosterol that occurs naturally in *Rubus* fruits and other plants with important roles related to cellular structures and membrane functionality [60,61]. Blackberry pomaces made from grown varieties presented three types of sterols (campesterol, stigmasterol and β-sitosterol), of which, β-sitosterol is the majoritarian (0.43 g/100 g dw) [53]. But also, have been reported in other berries, such as *R. suavissimus* [59], *R. glaucus* [60] and *R. idaeus* [60]. This phytosterol has shown antineoplastic activities, improves the permeability of the skin barrier, reduces cholesterol levels, and promotes the synthesis of hyaluronic acid [62,63].

Carbohydrate profile

The carbohydrate profile was formed by a mix of an aldopentose, a ketohexose, and some aldohexoses (Table 3). D-fructose was the main carbohydrate in the CEF from *R. liebmannii* and *R. palmeri* with proportions of 48.7 % and 49.7 %, respectively, having differences ($p<0.05$) among them. During fruit ripening Acosta-Montoya et al. [35] and Lefèvre et al. [44] reported concentrations of fructose between 2.5–10.4 g/100 g dw in *R. adenotrichus* and 18.3 mg/100 g dw in *R. idaeus*, respectively. Compared to these wild and commercial *Rubus* genotypes, our fruits seem to show, at least, twice as much fructose. This ketohexose is the major and most important carbohydrate in the human diet, only after glucose [43]. Interestingly, the fructose content is not only favored by the ripening stage but also is influenced by the fruit size, since berries

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of *R. hirsutus* showed statistical differences ($p<0.05$) comparing large fruits with medium or small [35,42]. The berries from *R. palmeri* are 0.5–1.0 cm bigger than *R. liebmannii* fruits, and this size difference could be one of the possible reasons for the D-fructose in these.

D-altrose was at proportions of 7.2 % and 6.8 % in the CEF from *R. liebmannii* and *R. palmeri*, respectively. The altrose is an uncommon aldohexose not reported previously in fruits of this genus. But Tako et al. [64] reported this unusual sugar as deoxy shape in some mushrooms with folk medical properties. But also, the altrose could be wrongly identified as an isomer of other aldohexoses such as D-glucose, D-mannose, or D-galactose, which have been recorded in *Rubus* fruits [42]. More studies are required on this compound to confirm its presence in these fruits.

D-galactose was found to be 18.0 % and 17.8 % of the CEF for *R. liebmannii* and *R. palmeri*, respectively. D-galactose was also identified in *R. idaeus* as part of hetero-polysaccharides or in form of D-galacturonic acid [43]. Galactose is an important compound of cell walls in fruits and other plant anatomical structures, but in nutraceutical terms, this plays an important role as part of anthocyanin moieties [40]. The linked galactose to anthocyanidins influences the bioavailability, metabolism and bioactivity in the human body [65].

D-xylose represented the lowest proportion of individual carbohydrates in *R. liebmannii* with 6.7 %, and 5.5 % for *R. palmeri*, with statistical differences ($p<0.05$) between them. This aldopentose has been recorded in other *Rubus*, such as grown *R. idaeus* [43]. However, it has not been previously quantified in other *Rubus* berries. Xylose is present in *Rubus* fruits glucosiding or acyling aglycones like cyanidin or delphinidin [66]. Furthermore, the xylosyl, an acyl group linking a hexose and an aglycone, allows holding the stability of anthocyanins through the intestine biotransformation before joining the bloodstream [67].

Finally, the D-glucose occupied 19.8 % of the CEF from *R. liebmannii*, while for *R. palmeri* it represented 21.0 %, as far as this aldohexose was the second most frequent in these fruits. This aldohexose was reported in fruits of wild *R. adenotrichus* as the main individual at contents about 117 mg/g of dw [35]. Though, as Pentelidis et al. [68] claim in their work, the fructose content is predominant in *Rubus* berries, and it is refuted by Lefèvre et al. [44] in Russian-grown raspberries. Glucose is the most abundant monosaccharide in nature, with many important functions in plants: most of the organoleptic attributes of *Rubus* fruits are due to this aldohexose, since the sweetness, odor, or flavor is favored for this, and the main sugar in anthocyanins of these red fruits [42,43,69]. Glucose stands out in the nutritious properties of *Rubus* berries because it is the primary source of fuel for body metabolism, but their antioxidant effects are less known as working in synergy with natural antioxidants such as α-tocopherol, tannins and flavonoids [18,69].

Conclusions

Specimens of *Rubus liebmannii*, *Rubus coriifolius*, and *Rubus palmeri* were well identified by their anatomical attributes in the forested lands of the Chara Pinta Sanctuary, Sinaloa, Mexico. *Rubus liebmannii* was recorded in Sinaloa for the first time. The proximate composition of two wild blackberries (*R. liebmannii* and *R. palmeri*) was described for the first time. The protein content in these fruits was higher than other popular *Rubus* berries. The carbohydrates represented the major macro-compounds (>85 % dw) made up of >50 % fructose and glucose but also reported altrose for the first time for *Rubus* berries. Ashes in both species resulted similarly to the reported for highland *Rubus* berries. The mineral profile showed a similar trend in both fruits (K<Ca<Mg<Na<Mn<Fe<Zn<Cu). Potassium and calcium content in 100 g dw of our *Rubus* fruits may contribute to the >15 % of the RDI, but for Mg, Fe or Cu represented at least 29 % of the RDI. Two saturated, monounsaturated and two polyunsaturated fatty acids were identified in these fruits, a-linolenic acid being the majority in the two berries. Also, the α-tocopherol and β-sitosterol were identified. The studied *R. liebmannii* and *R. palmeri* fruits showed a quite rich profile and quantity of nutrients, which, in some cases, were greater than those reported in the popular blackberries or raspberries. The attractive characteristics of these fruits could be considered for the genetic improvement of existing crops or for exploiting these berries as new cultivars in the forestlands of Sinaloa.

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