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Comparación taxonómica, fisicoquímica, fenólica y antioxidante en especies de frutos silvestres altoandinos: *Rubus* y *Hesperomeles*

Taxonomic, physicochemical, phenolic and antioxidant comparison in species of high Andean wild fruits: *Rubus* and *Hesperomeles*

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Resumen

Actualmente, el ser humano cuida más su salud. Es bien sabido que el consumo de frutas previene algunas enfermedades degenerativas como el cáncer y la diabetes. Por otro lado, la biodiversidad altoandina es rica en diversos recursos que han sido infravalorados hasta el momento. En el presente estudio, se compararon la caracterización botánica, las propiedades físicas y químicas, los compuestos fenólicos y la capacidad antioxidante de cuatro frutos silvestres altoandinos no climatéricos de las especies *Rubus* (siraca negra y siraca roja) y *Hesperomeles* (pacra y capachu), recolectados en la provincia de Andahuaylas, región de Apurímac en Perú, entre 3600 y 3900 m s. n. m. Se realizó la identificación taxonómica según el catálogo de angiospermas y gimnospermas por ubicación geográfica. Los polifenoles se determinaron por el método espectrofotométrico basado en la utilización del reactivo de Folin-Ciocalteau, y la capacidad antioxidante, por el método de decoloración del reactivo DPPH. Los datos por triplicado se analizaron a través de un análisis de varianza de una vía (ANOVA) y una prueba de rangos múltiples de diferencia significativa mínima (LSD). Los resultados presentaron diferencias significativas ($p < 0.05$) en todas las propiedades estudiadas. El índice de madurez y el contenido de fenoles incidieron directamente en la capacidad antioxidante. Los cuatro frutos silvestres estudiados presentaron valores altos en polifenoles y actividad antioxidante. Por lo tanto, deberían ser considerados en programas de mejoramiento genético y extensión en campo para promover su consumo, lo cual favorecería una alimentación saludable y nutritiva.

Palabras clave: actividad antioxidante DPPH, *Hesperomeles*, índice de madurez, *Rubus*, polifenoles.

Abstract

Currently, humans are taking better care of their health. It is known that fruit consumption prevents degenerative diseases such as cancer and diabetes. On the other hand, the high Andean biodiversity is rich in various resources that have been undervalued up to now. In the present study, the botanical characterization, physical and chemical properties, phenolic compounds, and antioxidant capacity were compared in four non-climacteric high Andean wild fruits of the species *Rubus* (black siraca and red siraca) and *Hesperomeles* (pacra and capachu), collected in the Peru province of Andahuaylas, Apurimac region, between 3600 and 3900 m.a.s.l. The taxonomic identification was performed according to the catalog of angiosperms and gymnosperms by geographical location. Polyphenols were determined by the spectrophotometric method based on the use of the Folin–Ciocalteu reagent, and the antioxidant capacity by the DPPH reagent discoloration method. Data in triplicate were analyzed by a one-way analysis of variance (ANOVA) and a multiple-range test of least significant difference (LSD). The results showed significant differences ($p < 0.05$) in all properties studied. The maturity index and phenolic content directly affected the antioxidant capacity. The four wild fruits studied presented high values of polyphenols and antioxidant activity. Therefore, they should be considered in genetic improvement and field extension programs to promote their consumption, which would encourage healthy and nutritious eating.

Keywords: antioxidant activity DPPH, *Hesperomeles*, maturity index, *Rubus*, polyphenols.

Introduction

Nowadays, human beings are more conscious about their health. It is known that some degenerative diseases can be prevented by consuming natural products; the global demand for this type of food is increasing. Biodiverse environments are a rich source of vegetables; however, historical references indicate that some fruits have been underutilized and undervalued (Teixeira *et al*., 2019). With the arrival of the first settlers in the Andean region, different and adverse ecosystems were discovered, and to feed themselves, they used the wild sources they found, simultaneously developing a process of domestication of genetic resources (Rubio *et al*., 2019). In Latin America, wild fruits have been used since pre-Hispanic times as food and as a source of traditional medicine to cure certain diseases empirically (Hernández *et al*., 2019).

Their health benefits are attributed to polyphenols, which are secondary metabolites of plants that determine sensory and nutritional properties; they have also attracted attention due to their antioxidant capacity (Rodriguez *et al*., 2021; Toshima *et al*., 2021). The maturity stage of fruits has a direct and proportional influence on phenols and their antioxidant capacity. Different traditional and modern methods are used for the quantification of these parameters (Barrientos *et al*., 2019).

Based on the above, the objective of this study was to determine the botanical, physicochemical, phenolic, and antioxidant characterization of four Peruvian non-climacteric high Andean wild fruits. Taxonomic identification was performed, several physicochemical properties were determined, phenolic compounds were quantified by the spectrophotometric method based on the use of the Folin-Ciocalteau reagent, and the antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent decolorization method. These procedures were chosen for their versatility and precision (Toshima *et al*., 2021).

Materials and methods

Vegetable material

Four wild fruits: siraca negra (*Rubus sparciflorus* J. F Macbr.), siraca roja (*Rubus urticifolius* Poir), pacra (*Hesperomeles palsensis* C. Scheneider), and capachu (*Hesperomeles escalloniifolia* Schltdl) (Figure 1), were collected when they reached maturity in the plant; they had low levels of respiration and ethylene production because they are non-climacteric fruits. These were collected in September and October 2020 in the Kankarhuay forest (13°34'17.9"S, 73°16'23.2"W), between 3600 and 3900 m of altitude, located in the village center of Cotahuacho Alto, district of Pacucha, province of Andahuaylas, Apurimac Region, Peru (Figure 2). The samples were transported in a container conditioned at -4 °C, then frozen at a temperature of -24 °C (LG LM57, South Korea) until used in the analyses, these were developed as soon as possible in the laboratory.

Botanical characterization

The taxonomic identification of the native fruits was carried out by a specialist in specimens and products of wild flora and fauna, MSc. Hugo Dueñas Linares, with a certificate of registration authorized by the Ministry of Agriculture of Peru. The description of the vegetative and reproductive characteristics was made according to the register for the flora of Peru in the department of Apurimac, according to the catalog of angiosperms and gymnosperms.

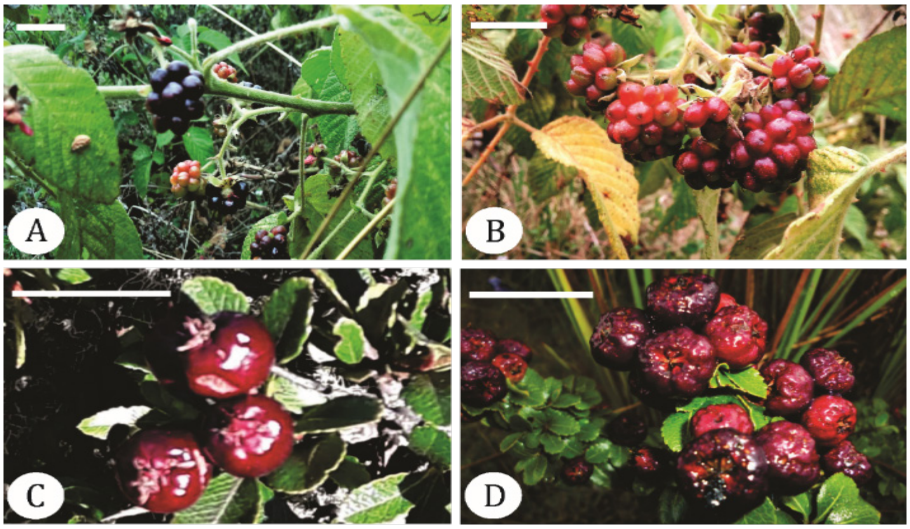

Figure 1. High Andean wild fruits of the genus *Rubus* and *Hesperomeles*. A: black siraca, B: red siraca, C: pacra, and D: capachu. The white bar indicates 1 cm.

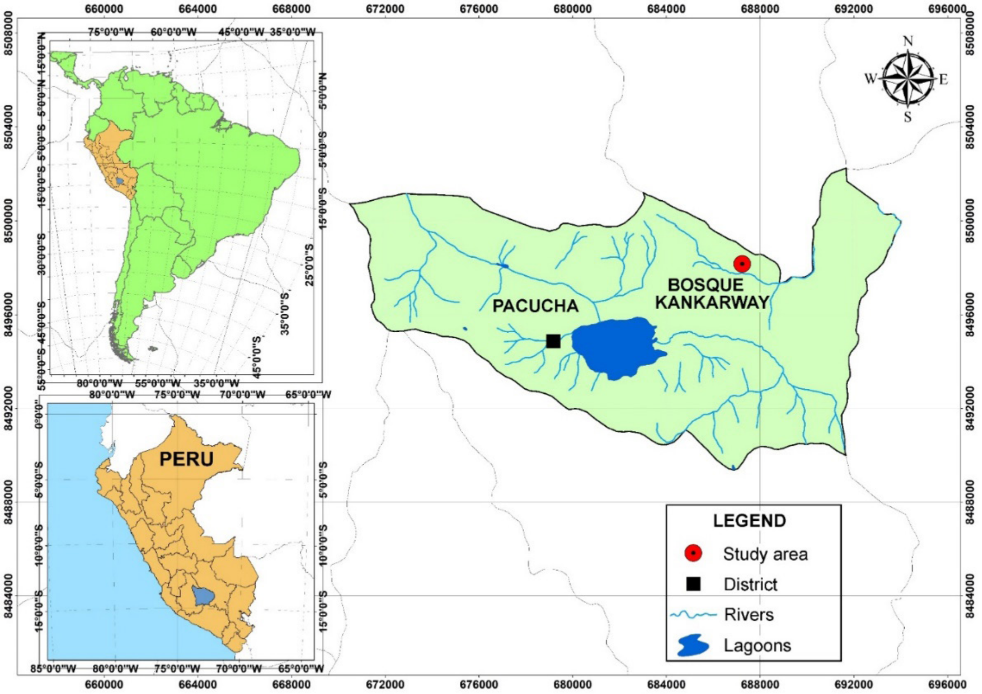

Figure 2. Geographical location of the wild fruit collection site.

Physicochemical characterization

Moisture, pH, total soluble solids, acidity, maturity index, and color were determined in the four wild fruits. Water content was determined using the gravimetric method. Hydrogen potential was determined using a potentiometer (Schott Instruments Lab. 850), according to established protocols for this type of samples. Total soluble solids were quantified at room temperature (20 °C) using a table refractometer with temperature correction (ABBE ISOLAB, Germany). A drop of each wild fruit was used to perform the measurements, the results were expressed in °Bx. 5 g of fruit juice were diluted in 50 mL of distilled water, and the total acidity expressed as citric acid was determined by potentiometric titration with a 0.1 N NaOH solution, which was standardized before use. The fruits were harvested at their optimum ripeness stage; the degree of ripeness was considered by the color of the peel and its change from green to the desired color. Then, the ripeness index was calculated by the relationship between total soluble solids and titratable acidity. Color parameters L^*, a^* and b^* were measured using a Minolta C-400 colorimeter.

Phenolic compounds

The total polyphenol content was measured according to the Folin-Ciocalteu reagent spectrophotometric method (Singleton and Rossi, 1965), for which 0.02 g of the sample were dissolved in a methanol solution and filtered on a 0.22 µm membrane. Then, 3.2 mL of water, 200 µL of the sample, 200 µL of Folin-Ciocalteu reagent, and 400 µL of sodium carbonate solution were mixed. The absorbance was measured in a spectrophotometer at 760 nm after the mixture had rested for 30 min. Gallic acid dissolved in 80 % (v/v) methanol was used as a standard. The total content of phenolic compounds was expressed as mg of gallic acid equivalents. Measurements of the sample extracts were repeated three times.

Antioxidant capacity

The method developed by Brand-Williams *et al.* (1995) was used. A 5 g sample of fresh wild fruit was weighed, and 20 ml of 80 % methanol was added and mixed for 15 min at 800 rpm. The extract obtained was stored for one day in the absence of light, then centrifuged at 3000 rpm for 20 min, an aliquot of the supernatant was taken and stored at 4 °C protected with aluminum foil, then 150 µL of the extract and 2850 µL of diluted DPPH solution (24 mg/100 ml methanol) were taken. A blank solution was prepared with 150 µL of 80 % methanol to obtain a correction factor due to dilution. The mixture was left to react in the dark for 30 min at 20 °C. The antioxidant compounds in the sample reacted with the stable DPPH radical in methanol solution, while the radical absorbed light at 515 nm in a spectrophotometer (GENESYS 150, USA).

Statistical analysis

The statistical design corresponded to a completely randomized design, in which each treatment corresponded to a type of fruit. The significant difference in the results was analyzed through a one-way analysis of variance (ANOVA), where the total variance was decomposed into the variance of the treatments and the variance of the error, followed by a Fisher's mean comparison with a significance level of 5 %; the statistical analysis was performed with the statistical package STATGRAPHICS Centurion XVI.

Results

Botanical characterization

The botanical characterization was developed according to the record for the flora of Peru in the department of Apurimac, the results are shown in Table 1. The four specimens correspond to officially accepted taxonomic positions.

R. sparciflorus J. F Macbr (black siraca)

The shrubby and fickle plant measures 2 to 3 m high, the fruits are of embryonic origin, polydrupe and polyspermic, black, with ovoid shape and soft texture. It fructifies from January to February and from September to October; the flowers appear in November and are dark pink, axillary, have racemose inflorescence, and pinkish corolla; the leaves are simple, smooth, pinnate and alternate, and have serrated margins; the stems are woody, thorny, branch-hugging, aerial and perennial; and the root is a taproot, underground, and semi-woody.

Uses: The flowers have medicinal uses as the so-called *qayapanakuy* in the Quechua language, its translation in English is "the cure of fright"; the leaves and fruits are used to cure stomach ailments, internal heat, scurvy, and other health issues. From a nutritional point of view, the fruits have a sweet and sour taste, and the stems are used as a natural protective barrier in farmers' fields.

Table 1. Taxonomic identification of the wild fruits studied

Scientific name	Common name	Family	Genus
Rubus urticifolius Poir.	Black siraca	*Rosaceae*	*Rubus*
Rubus sparciflorus J. F Macbr.	Red siraca	*Rosaceae*	*Rubus*
Hesperomeles palsensis C.Scheneider	Pacra	*Rosaceae*	*Hesperomeles*
Hesperomeles escalloniifolia Schltdl	Capachu	*Rosaceae*	*Hesperomeles*

R. urticifolius Poir (red siraca)

The shrubby and fickle plant measures 4 to 4.5 m high, the fruits are polydrupes, polyspermic and heart-shaped, with firm consistency, red color, acidulous flavor, and aromatic odor. They fructify at the beginning of September, then, from February to April; the flowers appear in September and are light pink, have an alternate arrangement, panicle inflorescence, and pinkish corolla; the leaves are compound, imparipinnate, with serrated margins, leathery consistency, and pinnate and obtuse base; the stems are thorny, perennial, of semi-woody consistency, branching, and grow in an aerial habitat; the root is of embryonic origin, with pivotal form, semi-woody consistency, underground, and perennial.

Uses: The fruits are used to reduce flu-like symptoms, they are also attributed with properties to relieve menstrual cramps; the flowers are used in aromatherapy to reduce stress and in infusions. The fruits of sweet and sour taste are consumed fresh, in jams, soft drinks, among other homemade products; the stems are used as a natural protective barrier on farms.

H. palsensis C. Scheneider (pacra)

It is a tree with a woody stem that reaches a height of up to 4 m; the fruit is embryonic, in drupe, polyspermic, and has a trichomose cell wall, a circular shape, hard consistency, bright reddish color, and little sweet and sour taste. It fructifies between March and May. The flower is white bell-shaped, with alternate arrangement, inflorescence in raceme, and its flowering period begins in December; the leaves are of simple type, petiolate, ovate in shape, with cuspid apex, serrulate margins, alternate arrangement, hairy coriaceous consistency, pinnate venation and a base with slightly widened sheath; the stem is woody without modifications, trichomatous, with sympodial branching, and of aerial and perennial habitat; the root is underground embryonic, with a pivotal shape, semi-woody consistency and perennial.

Uses: The fruits, which are not very sweet and sour, are only consumed fresh; the stems are used as a natural protective barrier in the farmers' fields and as a source of firewood for burning in artisanal kitchens.

H. escalloniifolia Schltdl (Capachu)

It is a dense tree armed with simple thorns and woody stems that reach up to 3 m in height; the fruits are of embryonic origin, in knob and polyspermic, of spherical shape, fleshy, and reddish-purple, they fructify between March and June and in September in smaller quantity; the flower is white cremate, in clusters, with inflorescence of dicasio compound, dialipetal actinomorphic corolla, and blooms from December to February; the leaves are of alternate type, lanceolate-ovate shape, with serrated margins, serrated apex, petiolate, pinnate, with leathery consistency, and acute base; the stem is modified with protruding nodes, woody aerial and erect, perennial and with sympodial branching; and the root is pivot-shaped, woody and underground perennial.

Uses: The sweet and sour fruits are consumed to reduce flu-like symptoms, and the stems are used as a natural protective barrier in farmers' fields and as a source of firewood for combustion in artisanal kitchens.

Physicochemical characterization

The average values of the physicochemical properties are shown in Table 2. Moisture had high values between 73.70 and 82.82 %, characteristic for fruits coming from shrubs. PH was acid in all samples, being the red siraca the most acidic (0.78 ± 0.04 % of citric acid). The total soluble solid content was measured in °Bx, noting that similar values were present, which varied between 5.20 and 9.60. On the other hand, a higher maturity index was observed in the *Hesperomeles* species (29.63 and 20.11); in the case of the *Rubus* genus, lower values were observed (10.83 and 12.14). Varied colors were observed in the fruits, showing that black and red siraca have a color equal to their common name, while pacra and capachu have a reddish color.

Phenolic compounds

Black siraca was the richest source of phenolic compounds (15.60 mg gallic acid equivalent (GAE)/g), followed by red siraca, pacra, and capachu (Table 3).

Antioxidant capacity

Capachu was the wild fruit with the highest antioxidant capacity (22.05 µmol Eq*Trolox (ET)/g), followed by pacra, black siraca, and red siraca (Table 4).

Figure 3 shows a comparison by species (*Rubus* and *Hesperomeles*), taking into consideration the maturity index with phenolic compounds and antioxidant capacity. In the wild pacra fruit, the higher maturity index (29.63) was close related to a high content of polyphenols (13.07 mg GAE/g) and a high antioxidant capacity (16.22 µmol ET/g), a similar behavior was observed in the capachu fruit. On the other hand, a lower maturity index in black siraca and red siraca caused a decrease in the antioxidant capacity and phenolic compounds of these species.

Discussion

The genus *Rubus* is widely distributed in the plant kingdom, its pigmented fruits contain bioactive compounds that prevent various degenerative diseases; several genotypes of *Rubus* in a state of consumption maturity have been reported to

Table 2. Physicochemical properties of the wild fruits

Property (± s)	Black siraca	Red siraca	Pacra	Capachu
Moisture (%)	82.82 ± 0.03a	82.60 ± 0.02b	78.61 ± 0.02c	73.70 ± 0.04d
pH	2.60 ± 0.07a	2.18 ± 0.08b	3.06 ± 0.17c	4.00 ± 0.15d
Total soluble solids (°Brix)	9.60 ± 0.23a	8.80 ± 0.19b	5.20 ± 0.26c	8.00 ± 0.50d
Acidity (% citric acid)	0.78 ± 0.04a	0.80 ± 0.02b	0.18 ± 0.01c	0.40 ± 0.05d
Maturity index	12.14 ± 0.70a	10.83 ± 0.20b	29.63 ± 0.60c	20.11 ± 0.45d
Lightness L*	22.10 ± 0.01a	63.30 ± 0.02b	61.00 ± 0.03c	20.30 ± 0.04d
Chroma	20.31 ± 0.02a	44.74 ± 0.02b	37.84 ± 0.01c	39.86 ± 0.02d
Hue	224.00 ± 0.02a	334.00 ± 0.01b	335.00 ± 0.02c	249.00 ± 0.01d

Note: In each row there is no significant statistical difference between values sharing the same letter (Fisher's test with 95 % confidence).

Table 3. Content of phenolic compounds in black siraca, red siraca, pacra and capachu

Wild fruits	mg GAE/g fresh fruit (± s)
Black siraca	15.60 ± 0.40a
Red siraca	14.52 ± 0.27b
Pacra	13.07 ± 0.30c
Capachu	9.00 ± 0.21d

Note: In each column there is no significant statistical difference between values sharing the same letter (Fisher's test with 95 % confidence).

Table 4. Antioxidant capacity in black siraca, red siraca, pacra and capachu

Wild fruits	µmol ET/g fresh fruit (± s)
Black siraca	9.63 ± 1.20a
Red siraca	7.39 ± 1.50b
Pacra	16.22 ± 1.40c
Capachu	22.05 ± 1.30d

Note: In each column there is no significant statistical difference between values sharing the same letter (Fisher's test with 95 % confidence).

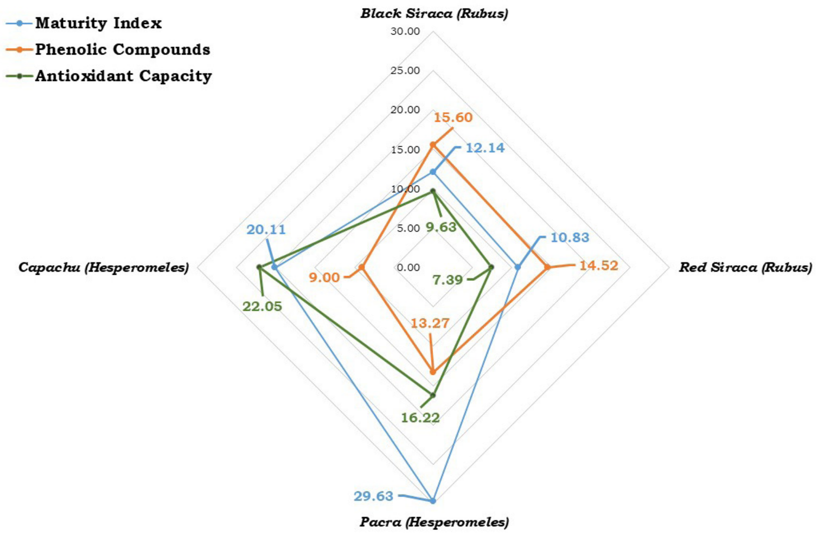

Figure 3. Comparison by species of the maturity index with phenolic compounds and antioxidant capacity.

have these qualities. In addition, the taxonomic identification of several specimens has been carried out, among which blackberry (*Rubus adenotrichus* Schltdl) stands out (Rubio *et al.*, 2019; He *et al.*, 2020). Counts of the chromosomes of these species have also been performed, which serves for their conservation and valuation of genetic (Rodríguez *et al.*, 2018). In the present study, such counting was not developed, but it is the next challenge for future research in high Andean specimens.

On the other hand, plants of the genus *Hesperomeles* are found in high-altitude ecosystems, which favors the presence of an important antioxidant capacity (Mejía *et al.*, 2016). The wild fruits studied in the present research, developed above 3600 m of altitude, would corroborate this statement. Recently, Guevara *et al.* (2022) also reported high levels of antioxidants in wild Andean blueberry fruits, demonstrating that higher altitude environments may increase the production of bioactive compounds as a protective mechanism against environmental stress factors such as UV radiation and low temperatures.

The high-water content in fruits decreases their storage life and sugar content; in the case of the studied *Rubus* and *Hesperomeles* species, lower moisture contents were observed, which is an advantage compared to other common fruits (Rubio *et al.*, 2019). Sweetness is quite valued in the processing of different products and is closely related to high values of total soluble solids (Lončarević *et al.*, 2018; Petkovsek *et al.*, 2020), as is the case of the data reported in the present investigation.

The maturity indexes were within the ranges reported by various authors (values close to 10 refer to pleasant flavors), the wild fruits studied presented higher values than this standard (Vergara *et al.*, 2016; Rubio *et al.*, 2019). This could be attributed to the process of conversion of organic acids into sugars. It is known that in fruits, malic and citric acids are found in higher quantities, and in the case of sugars, glucose, and fructose. Therefore, the flavor is affected by the content of saccharides and organic acids (Petkovsek *et al.*, 2016).

Regarding the L* value, lower values were observed in black siraca and capachu, contrary to what happened in the case of red siraca and pacra. This could be due to the fact that, as ripening advances, the L* value decreases, so there is a trend toward darkening. The absence of negative values indicates a distancing from the green color in the fruit structure. The appearance of pigments is evident during the ripening of fruits (Vergara *et al.*, 2016). The observed results agree with those of Rawat *et al.* (2023) in the ripening of *Mahonia nepalensis*, where a similar decrease in L* and increase in pigments over time was observed. This was attributed to the degradation of chlorophylls and the accumulation of phenolic compounds.

The results for polyphenols in the wild fruits studied varied between 9.00 and 15.60 mg GAE/g. *Rubus* species presented higher values than *Hesperomeles* species, being statistically different between them ($p < 0.05$). The contents of phenolic compounds reported in the present investigation exceeded the values found by Wang and Lin (2000) in blackberry (2.04 - 2.48 mg GAE/g) and raspberry (2.08 - 2.67 mg GAE/g) samples, Bobinaitė *et al.* (2012) reported 2.79 - 7.15 mg GAE/g in improved raspberries and 1.08 - 2.70 mg GAE/g for *Rubus hirsutus Thumb*. The presence of phenolic compounds can be influenced by different variables, including climatic conditions, crop type, wild fruit species, and the phenolic stage. Their isolation is conditioned by the type of solvent used (Valencia and Guevara, 2013).

According to Schulz *et al.* (2019), the predominant phenolic acids in *Rubus* species are gallic acid and 3,4-dihydroxybenzoic acid.

The antioxidant potential of *Rubus* species ranged from 7.39 to 9.63 µmol ET/g determined by the DPPH method; in *Hesperomeles* species, it ranged from 16.22 to 22.05 µmol ET/g, showing a significant statistical difference among the four wild fruits ($p < 0.05$). The results for *Rubus* are below the values reported by Rubio *et al.* (2019), which varied between 15.35 and 65.70 µmol ET/g, as mentioned by Paredes *et al.* (2010) who reported values between 25.30 and 35.50 µmol ET/g for *Rubus fructicosus* and *Rubus ideaous*, the same happens with Valencia and Guevara (2013) who reported 39.02 µmol ET/g for *Rubus fructicosus L.* Similar results were found by Cuevas *et al.* (2010). The antioxidant capacity values for *Hesperomeles* species are similar to those reported by the authors described above; quite close to those reported by Hernández *et al.* (2019) for *Prunus serotina subsp. capuli (Cav).* McVaugh at a very ripe stage (32.80 µmol ET/g). Likewise, they are in the range found for Japanese wild raspberry, blackberry, and *Rubus*, whose values varied between 4 and 35 µmol ET/g (Toshima *et al*., 2021).

As mentioned above, the species studied in the present work are perfect candidates for re-evaluation and genetic improvement due to their high levels of polyphenols and antioxidant capacity. In the *Rubus* and *Hesperomeles* genera, it has been observed that the higher the maturity index, the higher the content of phenolic compounds and antioxidant capacity. An analysis of red raspberries (*Rubus idaeus*) during ripening showed significant increases in phenols and antioxidant activity due to the accumulation of antioxidant secondary metabolites during the ripening of these wild berries (Lebedev, 2022). Similar studies in other wild berries support this trend. For example, wild blackberries (*Rubus ulmifolius*) exhibited higher levels of phenols and flavonoids and higher antioxidant activity in ripe fruits compared to immature fruits (Castro *et al*., 2022).

Johnson and Mejía (2012) mentioned that there is a positive correlation between the content of phenolic compounds and the antioxidant capacity, which would indicate that the content of polyphenols and the maturity indexes in the studied fruits directly influence their antioxidant activity.

Conclusions

The taxonomic identification of the wild fruits showed that the black siraca and red siraca belong to the genus *Rubus*, species *Rubus urticifolius* Poir and *Rubus sparciflorus* J. F Macbr., respectively; on the other hand, the fruits pacra and capachu correspond to the genus *Hesperomeles*, species *Hesperomeles palsensis* C. Scheneider and *Hesperomeles escalloniifolia* Schltdl, respectively.

The physicochemical properties showed marked differences between the studied fruits, highlighting that the fruit pacra presented the lowest acidity. The fruit color was varied, showing that the fruits of black and red siraca have a color equal to their common name, while pacra and capachu have a reddish color.

The polyphenol content was higher in the black siraca fruit, followed by red siraca, pacra, and capachu. A high antioxidant capacity was observed in the pacra and capachu fruits, followed by the wild red and black siraca fruits, which have similar values between them. Similar results have been reported in wild fruits of the genera *Rubus* and *Hesperomeles*.

The maturity index was related to the higher content of phenolic compounds and antioxidant capacity in the wild fruits pacra and capachu; on the contrary, a lower maturity index affected the values of black siraca and red siraca.

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