Physicochemical Properties, Bioactive Compounds, and Antioxidant Activity of Andean Fruits: Optimization of Extraction by Response Surface Methodology

Propiedades fisicoquímicas, compuestos bioactivos y actividad antioxidante de frutas andinas: optimización de la extracción mediante metodología de superficie de respuesta

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Abstract

Native fruits from the Peruvian Andes, such as *ushpa* (*Vaccinium floribundum* Kunth) and *sachon* (*Hesperomeles obtusifolia* (Pers.) Lindl.), grow in high-altitude environments that favor the accumulation of bioactive compounds. However, limited characterization has restricted their sustainable utilization. This study analyzed the physicochemical properties, total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), vitamin C (VC), and antioxidant activity (AA) of both fruits. It also evaluated the effects of solvent type (ethanol and methanol) and concentration (70% and 80%), and extraction time (60, 90, and 120 minutes) on extraction efficiency using response surface methodology. Both fruits exhibited higher levels of bioactive compounds and antioxidant activity compared to other berry species. Optimal conditions for *ushpa* (64.86% methanol, 139.68 minutes) and for *sachon* (64.86% ethanol, 90 minutes) yielded TPC = 3,587 and 948 mg GAE/100 g, TFC = 1,821 and 701 mg CE/100 g, TAC = 252 and 8 mg C3G/100 g, and AA = 563 and 501 µmol TE/g, respectively. These findings support further research and potential valorization of these native fruits.

Keywords
andean berries • paramo • polyphenols • vitamin C • antioxidant activity • RSM

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Resumen

Los frutos nativos de los Andes peruanos, como *ushpa* (*Vaccinium floribundum* Kunth) y *sachon* (*Hesperomeles obtusifolia* (Pers.) Lindl.), crecen en ambientes de altura que favorecen la acumulación de compuestos bioactivos. Sin embargo, la caracterización limitada ha restringido su uso sostenible. Este estudio evaluó las propiedades fisicoquímicas, contenido total de fenoles (TPC), contenido total de flavonoides (TFC), contenido total de antocianinas (TAC), vitamina C (VC) y actividad antioxidante (AA) de ambos frutos. Asimismo, se analizó el efecto del tipo (etanol y metanol) y concentración (70% y 80%) de solvente y tiempo de extracción (60, 90 y 120 minutos) mediante metodología de superficie de respuesta. Ambas frutas presentaron niveles superiores de compuestos bioactivos y actividad antioxidante en comparación con otras especies de bayas. Las condiciones óptimas para *ushpa* (64,86% de metanol, 139,68 minutos) y *sachon* (64,86% de etanol, 90 minutos) generaron valores de TPC = 3 587 mg y 948 GAE/100 g, TFC = 1 821 y 701 mg CE/100 g, TAC = 252 y 8 mg C3G/100 g, y AA = 563 y 501 µmol TE/g, respectivamente. Estos resultados respaldan futuras investigaciones y posible valorización de estos frutos nativos.

Palabras clave
bayas andinas • páramo • polifenoles • vitamina C • actividad antioxidante • RSM

Introduction

Peru, a globally recognized megadiverse country, is home to more than 780 edible plant species, including 525 native fruits (MIDAGRI, 2022). Within its diverse ecosystems, the Andean *paramo,* a high-mountain ecosystem located between 3,500 and 4,200 meters above sea level, stands out for its rich plant biodiversity and extreme climatic conditions. These conditions have favored the development of native fruit species with unique sensory and functional properties that remain largely unexplored (Torres-Guevara *et al.*, 2020).

Compared with countries such as Ecuador (Guevara-Terán *et al.*, 2022), Brazil (Rigolon *et al.*, 2020), Chile (Oyarzún *et al.*, 2020), Cameroon (Bayang *et al.*, 2021), and China (Liu *et al.*, 2022), where native fruits have been extensively characterized, studies in the Peruvian Andes remain scarce. For example, Torres-Guevara *et al.* (2023) identified 39 native fruit species in northern Peru, most of which are still unknown in commercial markets. Berries were predominant among them, widely recognized for their richness in vitamin C and phenolic compounds that confer strong antioxidant activity (Bezerra *et al.*, 2024).

Two native berries from the Andean paramo, *ushpa* (*Vaccinium floribundum Kunth*) and *sachon* (*Hesperomeles obtusifolia (Pers.) Lindl.*), have long been consumed by local communities for both nutritional and medicinal purposes. Despite their ethnobotanical relevance, scientific studies on these species are scarce and mostly limited to preliminary assessments of their phytochemical composition (Torres-Guevara *et al.*, 2020).

In this context, the present study provides a comprehensive analysis of the physicochemical traits (soluble solids and pH), total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), vitamin C (VC), and antioxidant activity (AA) of *V. floribundum* and *H. obtusifolia* from the Peruvian Andean *paramo*. Recognizing that efficient extraction processes depend on multiple interrelated variables, this study applies multivariate optimization to identify the best extraction conditions.

We hypothesize that both species exhibit high levels of bioactive compounds and antioxidant activity, and that extraction parameters significantly influence their recovery. These findings provide insights into the bioactive potential of these native species and support future research and valorization.

Materials and Methods

Chemicals and Reagents

All chemicals used in this study were of analytical grade or higher. Gallic acid, the Folin-Ciocalteu reagent, and quercetin standard were purchased from Millipore-Sigma (Steinheim, Germany). HPLC-grade methanol, along with analytical-grade or higher ethanol and methanol were sourced from Supelco (Bellefonte, PA, USA). The ascorbic acid standard was obtained from Merck (Darmstadt, Germany). 6-Hydroxy-2,5,7,8 -tetramethylchroman-2-carboxylic acid (Trolox) was acquired from Sigma-Aldrich (Steinheim, Germany).

Sample Collection

In April 2023, 1 kg of mature fruits of both *sachon* (*H. obtusifolia*) and *ushpa* (*V. floribundum*) were collected from the *paramo* ecosystem in the locality of Totora, Pacaipampa district, Ayabaca province, Piura region, Peru, at an elevation of 3,000-3,700 meters above sea level. The characteristics of this ecosystem, along with the distribution of these species and other relevant information, are described in Torres-Guevara *et al.* (2023). Species identification was based on previous studies by botanist Fidel A. Torres-Guevara. These species (figure 1) were selected because, although they are not commonly commercialized, they are widely used by local communities for nutritional and medicinal purposes.

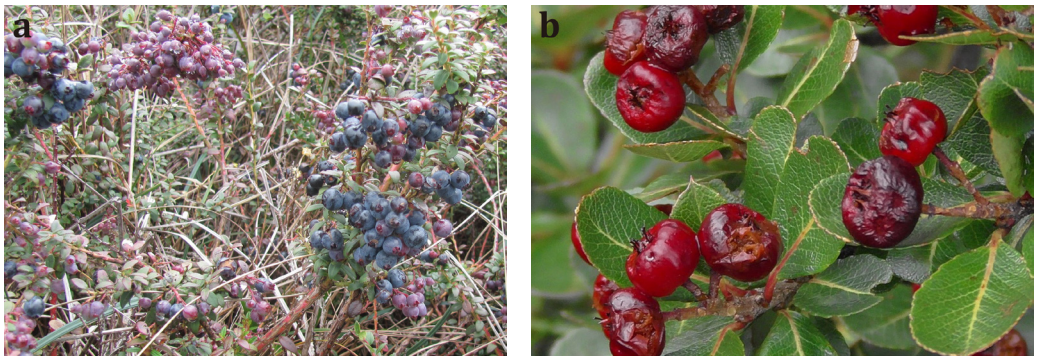

Figure 1. Fruit-bearing shrubs of (a) *V. floribundum* Kunth and (b) *H. obtusifolia* (Pers.) Lindl.

Figura 1. Frutales de (a) *V. floribundum* Kunth y (b) *H. obtusifolia* (Pers.) Lindl.

Physicochemical Analyses

Fruits were washed, dried, and homogenized. Juice was filtered and analyzed for pH (pHep-HI98107, HANNA Instruments, Italy) and total soluble solids (°Brix) using a HI96801 digital refractometer (HANNA Instruments, Italy). Analyses were conducted in quintuplicate at room temperature. Color parameters (L*, a*, b*) were measured in the CIELab* color space using a FRU WR-10QC colorimeter (Shenzhen Wave Optoelectronics Technology Ltd., China), with measurements taken at five random points on each sample's surface.

Extraction and Chemical Analysis

Samples were freeze-dried (BK-FD10PT, Biobase, China), ground (IKA M20 universal mill, Germany), and sieved using a Ro-Tap RX29-16 shaker (WS Tyler, USA). Particle fractions between 150 and 300 µm were collected, packaged, and stored at -20°C in a refrigerated incubator (FOC 215I, Velp Scientifica, Italy).

Extract Preparation

Extracts were prepared by maceration with agitation, adapting the method from a previous study (León-Roque *et al.*, 2023). The evaluated factors were: solvent type (ethanol or methanol), solvent concentration (70% or 80%), and extraction time

(60, 90, or 120 minutes). Briefly, 0.5 g of sample was mixed with 10 mL of solvent and extracted at room temperature using a vortex mixer (Labnet International, USA). Extracts were centrifuged (5000×g, 15 minutes, 4°C) in a Bio RS-24 multi-rotator (Boeco, Germany), and the supernatants were stored at -20°C in 2 mL amber tubes. Before chromatographic analysis, extracts were filtered through 0.45 µm PTFE syringe filters.

Phenolic Compounds

TPC was determined using the Folin–Ciocalteu method adapted from Magalhães *et al.* (2010). Absorbance was measured in triplicate at 765 nm using a Genesys 30 UV-Vis spectrophotometer (Thermo Scientific, USA), and results were expressed as mg gallic acid equivalents (mg GAE) per 100 g of dry basis (d.b.).

TFC was measured by the aluminum chloride colorimetric assay described by Abderrahim *et al.* (2015). Absorbance was recorded in triplicate at 415 nm, and results were expressed as mg catechin equivalents (mg CE) per 100 g d.b.

TAC was quantified using the pH differential method described by Giusti & Wrolstad (2001). Absorbance was recorded at the visible maximum wavelength (λ_vis-max) and at 700 nm. Analyses were performed in triplicate, and results were expressed as mg cyanidin-3-glucoside equivalents (C3G) per 100 g d.b.

Vitamin C

VC concentration was determined in triplicate by HPLC-DAD (Shimadzu LCMS-2020) using ascorbic acid as the standard. A stock solution of 500 ppm was prepared, and calibration was constructed from 0-250 ppm. Chromatographic detection was performed at 254 nm using a Shim-pack GIST C18 (5 µm, 150 × 4.6 mm) column at a flow rate of 0.5 mL/min and an injection volume of 10 µL. The column temperature was maintained at 30°C, and the mobile phase consisted of a methanol-water (80/20 v/v, solvent B) for 10 minutes. Extracts were filtered through 0.45 µm syringe filters into 2 mL vials, which were then placed in the autosampler. Results were expressed as mg VC per liter d.b., and data were processed using LabSolutions software.

***In Vitro* Antioxidant Activity**

AA was determined in triplicate using the ABTS radical cation assay described by Re *et al.* (1999), and results were expressed as µmol Trolox equivalents (TE) per gram d.b.

Experimental Design

The effects of solvent type, solvent concentration, and extraction time on TPC, TFC, TAC, and AA were evaluated using response surface methodology (RSM), with 12 randomized runs and 36 total experiments. Data were fitted to a second-order polynomial equation, and analysis of variance (ANOVA) was applied to assess the significance of individual factors and their interactions. Regression coefficients were used to establish the direction of the effects, where positive values reflected increases in the response, while negative values indicated decreases. A Pareto chart of standardized effects illustrated the magnitude and importance of each factor.

Since maximizing one response variable may compromise another, global optimization was conducted using a multiple-response strategy based on the desirability function. This approach converts each response into a standardized scale (0-1), where 1 represents the optimal condition.

Vitamin C content was excluded from the optimization process, as it was not detected in most methanol-based extractions of both *ushpa* and *sachon*.

Data Analysis

Physicochemical differences between *ushpa* and *sachon* were assessed using Student's t-test ($\alpha = 0.05$). The effects of extraction treatments were analyzed via ANOVA ($\alpha = 0.05$) followed by Tukey's test for multiple comparisons. Statistical analyses were conducted with Minitab 18.0 (Minitab Inc., USA), while optimization analyses were performed using Statistica 12 (Stat Soft Inc., USA) ($\alpha = 0.05$).

Results and Discussion

Physicochemical Characteristics

The physicochemical analysis of *ushpa* and *sachon* fruits revealed significant differences ($p < 0.05$) in color parameters (figure 2(a-c), page XXX), with higher values in *sachon* (L*: 46.44 ± 0.21, a*: 2.18 ± 0.55, b*: 1.03 ± 0.21) compared to *ushpa* (L*: 45.88 ± 0.47, a*: -2.38 ± 0.20, b*: 0.50 ± 0.17).

Lower L* values have been reported in other berries, such as 18.6 in *Rubus ulmifolius*, 20.0 in *Aristotelia chilensis* (Mattson *et al.*, 2022), 31.1 in *Rubus* sp., 27.2 in *Vaccinium* sp. (Rigolon *et al.*, 2020), and 25.7 in *Vaccinium myrtillus* L. (Vega *et al.*, 2023). The L* value tends to decrease during fruit ripening, leading to a darker appearance, although high L* values have also been recorded in ripe fruits such as *Fragaria vesca* L. (56.7) and *Prunus avium* L. (62.9) (Vega *et al.*, 2023).

Regarding total soluble solids (°Brix) content (figure 2(d), page XXX), no significant differences were observed between *ushpa* (12.90 ± 3.13) and *sachon* (13.14 ± 3.08) ($p > 0.05$). Both fruits showed values comparable to other berries, such as *Vitis vinifera* L. (16.9) and *Rubus idaeus* (7.0-11.0) (Frías-Moreno *et al.*, 2021; Gomes *et al.*, 2021). These values indicate a sweetness level similar to that of widely commercial fruits, suggesting good potential for direct consumption or industrial processing.

Several authors have noted that °Brix content can vary depending on cultivar, climate, ripening stage, and agronomic practices (King *et al.*, 2021). Saad *et al.* (2022) reported values ranging from 3.8 to 8.2 in *Fragaria × ananassa*, depending on the maturity stage. Seki *et al.* (2024) found values of 8.1 and 10.0 in red and white *Fragaria* sp., respectively. Cuesta-Riaño *et al.* (2022) reported values of 5.7 and 7.8 in *Rubus glaucus* Benth and *Rubus adenotrichos*, respectively. Aliman *et al.* (2020) documented high variability in °Brix among *Vaccinium myrtillus* L. cultivars depending on the harvest year.

With respect to pH (figure 2(e), page XXX), both *ushpa* and *sachon* exhibited values close to 3.1, with no significant differences between them ($p > 0.05$). This similarity is attributed to shared environmental factors in the paramo ecosystem that influence acidity. For example, environmental conditions have been shown to affect the quality, including the acidity, of both cultivated and wild apples collected from different locations in China (Li *et al.*, 2021). Other berries exhibit a range of pH values: 3.6 in grapes, 2.9-3.1 in *Rubus idaeus* (Frías-Moreno *et al.*, 2021), 3.09-3.5 in different *Aronia mitschurinii* harvests (King *et al.*, 2021), 3.1 in *Rubus glaucus* Benth and 3.2 in *Rubus adenotrichos* (Cuesta-Riaño *et al.*, 2022), and 3.2-3.6 in *Vaccinium myrtillus* L. (Aliman *et al.*, 2020).

Chemical Analysis and Antioxidant Activity

Table 1 (page XXX), presents the results for *ushpa* and *sachon* extracts, which confirmed our hypothesis of their high levels of TPC, TFC, TAC, VC, and AA, highlighting their strong bioactive potential. Comparative values for other berries are provided in Table S1.

TPC, TFC and TAC

Ushpa displayed higher TPC (3,004.62 mg GAE/100 g d.b. vs. 772.15 mg GAE/100 g d.b.) and TFC (1,661.10 mg CE/100 g d.b. vs. 564.08 mg CE/100 g d.b.), exceeding levels found in other berries, including *Vaccinium corymbosum* L. (Aliman *et al.*, 2020) and *Vaccinium myrtillus* L. (Vega *et al.*, 2023). A study on *Vaccinium floribundum* in Ecuador reported much lower TPC (Monge-Sevilla *et al.*, 2024), highlighting the high regional variability in phenolic content. While *sachon* exhibited considerable TPC and TFC levels, they were lower than those reported for *Aronia mitschurinii* (King *et al.*, 2021), *Aristotelia chilensis* (Mattson *et al.*, 2022), and *Sorbus aucuparia* L. (Orsavová *et al.*, 2023).

Ushpa (240.50 mg C3G/100 g d.b.) also had significantly higher TAC than *sachon* (4.46 mg C3G/100 g d.b.), consistent with the dark blue pigmentation of *ushpa*. TAC values in *ushpa* surpassed those reported for *Rubus ulmifolius* (Mattson *et al.*, 2022), *Rubus idaeus* (Frías-Moreno *et al.*, 2021), *Sorbus aucuparia* L. (Orsavová *et al.*, 2023), and *Vitis vinifera* L. (Gomes *et al.*, 2021).

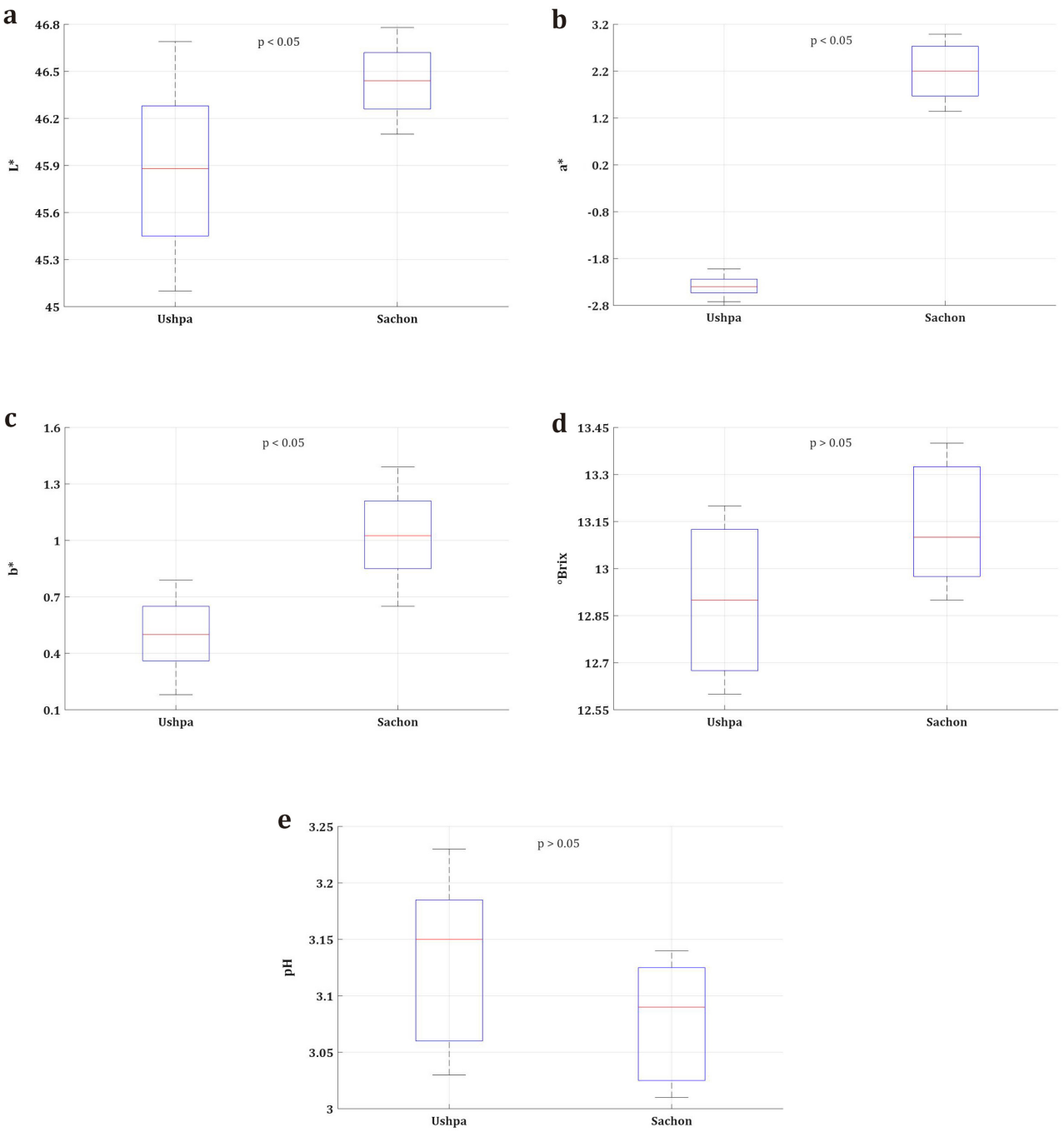

Figure 2. Values of (a) L*, (b) a*, (c) b*, (d) °Brix, and (e) pH in *ushpa* and *sachon*.

Figura 2. Valores de (a) L*, (b) a*, (c) b*, (d) °Brix, y (e) pH en *ushpa* y *sachon*.

Differences in metabolite content can be attributed to factors such as climate, altitude, and ripening stage (Aliman *et al.*, 2020; Guevara-Terán *et al.*, 2022). Studies on *Vaccinium floribundum* have shown that fruits harvested at higher elevations exhibit greater phenolic content due to increased solar exposure (Guevara-Terán *et al.*, 2022).

Table 1. TPC, TFC, TAC, VC, and AA of *ushpa* and *sachon* extracts.
Tabla 1. TPC, TFC, TAC, VC, and AA de extractos de *ushpa* y *sachon*.

Treatment	TPC (mg GAE/100 g d.b.)		TFC (mg CE/100 g d.b.)	
	Ushpa	*Sachon*	*Ushpa*	*Sachon*
70% methanol for 60 minutes	2,438.99 ± 16.93^{g}	782.98 ± 2.01^{d}	1,647.31 ± 38.43^{cdef}	492.22 ± 0.39^{g}
70% ethanol for 60 minutes	3,075.57 ± 78.40^{bcd}	926.48 ± 23.14^{a}	1,713.55 ± 9.78^{bc}	663.86 ± 10.38^{a}
80% methanol for 60 minutes	2,933.87 ± 85.95^{de}	812.61 ± 8.94^{bcd}	1,683.52 ± 27.67^{bcd}	499.93 ± 3.55^{fg}
80% ethanol for 60 minutes	2,981.49 ± 21.78^{cde}	848.03 ± 5.33^{b}	1,567.34 ± 31.62^{fg}	579.67 ± 8.42^{d}
70% methanol for 90 minutes	3,224.85 ± 54.50^{b}	597.11 ± 10.21^{h}	1,662.93 ± 38.46^{bcde}	511.54 ± 3.28^{f}
70% ethanol for 90 minutes	3,148.19 ± 49.30^{bc}	738.84 ± 7.60^{e}	1,742.83 ± 51.33^{b}	630.71 ± 0.60^{b}
80% methanol for 90 minutes	3,042.08 ± 22.12^{cd}	759.96 ± 1.02^{cd}	1,652.63 ± 25.93^{efg}	565.18 ± 2.23^{f}
80% ethanol for 90 minutes	2,894.93 ± 136.95^{de}	795.18 ± 19.19^{d}	1,614.83 ± 11.38^{defg}	614.26 ± 2.52^{c}
70% methanol for 120 minutes	2,705.57 ± 32.24^{f}	832.93 ± 10.59^{bc}	1,686.13 ± 30.51^{bcd}	544.43 ± 5.70^{e}
70% ethanol for 120 minutes	3,122.61 ± 26.33^{bc}	781.23 ± 9.61^{d}	1,642.56 ± 10.65^{cdef}	649.11 ± 7.29^{a}
80% methanol for 120 minutes	3,650.03 ± 63.52^{a}	648.37 ± 18.56^{g}	1,829.97 ± 2.44^{a}	452.93 ± 6.37^{h}
80% ethanol for 120 minutes	2,846.72 ± 44.33^{ef}	697.60 ± 13.08^{f}	1,545.44 ± 11.13^{g}	616.74 ± 4.52^{bc}

Treatment	VC (mg /L d.b.)		TAC (mg C3G/100 g d.b.)		AA (µmol TE/g d.b.)	
	Ushpa	*Sachon*	*Ushpa*	*Sachon*	*Ushpa*	*Sachon*
70% methanol for 60 minutes	475.31 ± 5.92^{b}	93.70 ± 4.90^{g}	255.38 ± 0.08^{a}	6.50 ± 0.15^{b}	470.79 ± 0.45^{d}	445.26 ± 0.45^{bc}
70% ethanol for 60 minutes	n.d.^{c}	n.d.^{h}	234.32 ± 0.13^{g}	6.50 ± 0.31^{b}	465.06 ± 1.56^{d}	423.90 ± 3.69^{d}
80% methanol for 60 minutes	525.20 ± 1.58^{b}	121.68 ± 0.81^{g}	239.66 ± 0.13^{e}	2.23 ± 0.08^{g}	504.92 ± 1.35^{b}	399.67 ± 1.19^{e}
80% ethanol for 60 minutes	n.d.^{c}	n.d.^{h}	241.22 ± 0.15^{d}	4.45 ± 0.08^{cd}	400.71 ± 0.45^{g}	284.77 ± 1.19^{i}
70% methanol for 90 minutes	477.88 ± 0.57^{b}	97.82 ± 1.42^{g}	240.11 ± 0.20^{e}	1.96 ± 0.15^{g}	374.92 ± 7.83^{h}	398.36 ± 1.19^{ef}
70% ethanol for 90 minutes	n.d.^{c}	n.d.^{h}	237.61 ± 0.41^{f}	4.41 ± 0.00^{d}	478.87 ± 0.45^{c}	360.33 ± 2.34^{h}
80% methanol for 90 minutes	412.46 ± 0.52^{b}	248.52 ± 22.06^{b}	239.61 ± 0.54^{b}	4.57 ± 0.00^{c}	449.73 ± 2.07^{f}	416.69 ± 1.19^{h}
80% ethanol for 90 minutes	n.d.^{c}	198.01 ± 0.32^{d}	243.09 ± 0.43^{c}	4.68 ± 0.13^{cd}	326.19 ± 2.74^{i}	382.99 ± 0.78^{g}
70% methanol for 120 minutes	470.16 ± 11.77^{b}	176.37 ± 8.42^{e}	237.70 ± 0.20^{f}	3.34 ± 0.13^{ef}	499.97 ± 0.90^{b}	476 ± 0.00^{a}
70% ethanol for 120 minutes	n.d.^{c}	377.02 ± 1.51^{a}	241.13 ± 0.13^{d}	7.66 ± 0.08^{a}	463.50 ± 1.35^{de}	388.20 ± 10.38^{fg}
80% methanol for 120 minutes	1,065.81 ± 134.94^{a}	241.90 ± 2.05^{c}	231.65 ± 0.27^{h}	3.70 ± 0.08^{e}	528.11 ± 0.45^{a}	435.10 ± 5.20^{c}
80% ethanol for 120 minutes	419.32 ± 0.31^{b}	188.37 ± 0.38^{de}	239.93 ± 0.13^{e}	3.25 ± 0.08^{f}	456.2 ± 2.51^{e}	452.56 ± 1.56^{b}

Results are presented as mean ± standard deviation. Different letters within each column indicate statistically significant differences between treatments ($p < 0.05$). Significant differences ($p < 0.05$) in TPC, TFC, TAC, VC, and AA were observed between ushpa and sachon. n.d. = not detected.

Los resultados se presentan como media ± desviación estándar. Letras diferentes dentro de cada columna indican diferencias estadísticamente significativas entre tratamientos ($p < 0,05$). Se observaron diferencias significativas ($p < 0,05$) en TPC, TFC, TAC, VC y AA entre ushpa y sachon. n.d. = no detectado.

Similarly, plant genetics also plays a key role in metabolite biosynthesis and antioxidant activity. For instance, Lebedev *et al.* (2022) reported a strong positive correlation (r = 0.8) between the genetic diversity of *Rubus idaeus* L. and its TPC, TFC, and TAC. However, another study found no correlation between TPC, TFC, TAC, and AA in *Vaccinium macrocarpon* Aiton and its genetic profile (Debnath & An, 2019). This discrepancy may be due to the use of non-specific genetic markers (Lebedev *et al.*, 2022), or to the complex interaction between environmental conditions and genetics, as observed in VC content of *Fragaria × ananassa* Duchesne (Ali & Serçe, 2022).

Vitamin C

Ushpa had significantly higher VC content than *sachon* (568.92 *vs.* 200.48 mg/L d.b.). This difference may be due to bioactive compounds in *sachon* being more strongly bound within its solid matrix, requiring more intensive extraction methods (Cuesta-Riaño *et al.*, 2022; Mattson *et al.*, 2022).

VC levels in *ushpa* were higher than those in *Vaccinium floribundum* and *Rubus glabratus* Kunth (Monge-Sevilla *et al.*, 2024), but lower than those in *Sorbus aucuparia* L. and *Rubus idaeus* (Frías-Moreno *et al.*, 2021; Orsavová *et al.*, 2023). VC content is influenced by genotype and environmental factors such as altitude and sunlight (Bayang *et al.*, 2021). In *Vaccinium floribundum*, VC content increased with elevation (Guevara-Terán *et al.*, 2022).

Antioxidant Activity (AA)

AA measured with ABTS was significantly higher in *ushpa* than in *sachon* (449.47 *vs.* 401.03 µmol TE/g d.b.), consistent with findings in other fruit species, such as *Opuntia ficus-indica*, *Myrica rubra*, *Vaccinium floribundum*, and *Rubus glabratus* (El Mannoubi, 2021; Liu *et al.*, 2022; Monge-Sevilla *et al.*, 2024).

However, although *ushpa* exhibited greater AA, the difference with *sachon* was smaller than expected, possibly due to the presence of other antioxidant compounds not measured in this study, such as tannins (Varo *et al.*, 2021). For example, in *Vaccinium corymbosum* L., the cultivar Jewel contained more resveratrol but had lower AA than Windsor, while Millenia had the highest VC yet lower AA than both (Varo *et al.*, 2021).

AA levels, like TPC and TFC, are influenced by altitude, solar exposure, and fruit variety, with higher AA reported in *Vaccinium floribundum* harvested at greater elevations (Guevara-Terán *et al.*, 2022).

It is also important to note that AA results can vary depending on the assay (table 1, page XXX). For instance, Vega *et al.* (2023) reported that using the DPPH method, *Vaccinium myrtillus* L. showed the highest AA, followed by *Fragaria vesca* L. and *Prunus avium* L. However, when the FRAP assay was used, the ranking partially reversed, with strawberries showing the highest AA, followed by blueberries and cherries.

Effect of Extraction Conditions

Certain extraction parameters had a significant impact on the outcomes, consistent with our hypothesis. However, as detailed below, the magnitude and direction of these effects varied depending on the fruit species and the metabolite or response assessed.

Influence of Solvent Type and Concentration

The solvent type significantly impacted the extraction of bioactive compounds (Figure S1 and Figure S2), with methanol showing greater efficacy in extracting TFC and maximizing AA in *ushpa*, while in *sachon*, methanol had a negative effect on TPC and TFC but a positive effect on TAC and AA ($p < 0.05$). This variability is likely due to the relative polarity of solvents, with methanol being slightly more polar than ethanol, which favors the extraction of polar compounds such as anthocyanins but may hinder the extraction of non-polar compounds (More & Arya, 2021).

Previous studies also highlight these trends. In *Opuntia ficus-indica*, 80% ethanol outperformed methanol in the extraction of polyphenols (El Mannoubi, 2021). However, in *Băbească neagră* peels, ethanol also enhanced TAC extraction (Serea *et al.*, 2023), whereas for TFC, ethanol was more efficient in the peel of *Opuntia ficus-indica*, while methanol was superior in the pulp (El Mannoubi, 2021). These results underscore that solvent effectiveness also depends on the fruit part and the specific class of metabolites.

Solvent concentration also showed significant effects ($p < 0.05$). In *ushpa*, it positively affected TFC, while in *sachon*, it positively influenced TFC, TAC, and AA. Moreover, in *ushpa*, the interaction between solvent type (ethanol) and concentration was significant for both TPC and TFC.

More diluted mixtures allow for greater solvent penetration into the plant matrix, thereby promoting phenolic release. However, high ethanol concentrations reduce polarity, hindering polar compound extraction. This relationship is not linear; for example, Serea *et al.* (2023) reported that 50% ethanol was optimal for TAC in *Băbească neagră* peels, whereas 85% was optimal for TPC.

Interestingly, pure methanol also enhanced TPC, TFC, and AA, as observed in seeds of *Carica papaya* L. (Robles-Apodaca *et al.*, 2024), *Passiflora edulis*, and *Nephelium lappaceum* L. (Sai-Ut *et al.*, 2023). The addition of water increases polyphenol ionization, promoting their solubilization. For instance, in *Vaccinium floribundum*, 20% ethanol improved TAC extraction compared to 60% (Pérez *et al.*, 2021). In *Sambucus nigra* L., 45% ethanol was suitable for TPC extraction (Pascariu *et al.*, 2024).

Influence of Extraction Time

Extraction time had variable effects depending on the species and the bioactive compound evaluated. In *ushpa*, longer extraction times positively affected TAC and AA, while in *sachon*, extraction time had both linear and quadratic effects on AA. However, prolonged extraction tended to negatively impact TPC, particularly in *ushpa*, and both species exhibited a decline in TPC over extended extraction periods. This suggests that while longer extraction may improve yields, it can also lead to compound degradation due to exposure to oxygen, light, or heat.

The nature of this response also depends on the interaction between time and solvent. For example, in *ushpa*, extraction with methanol at short times was particularly favorable for TAC, whereas prolonged methanol use reduced both TPC and TFC. These results align with findings in *Carica papaya* L. seeds, where methanol exhibited a non-linear behavior depending on both time and concentration. At longer extraction times, the highest TFC yields were obtained when using either pure water (0% methanol) or absolute methanol (100%) as the extraction solvent (Robles-Apodaca *et al.*, 2024).

The literature reports similarly variable results. In *Passiflora edulis* and *Nephelium lappaceum* L., time had no effect on TPC at a fixed ethanol concentration (Sai-Ut *et al.*, 2023). However, in *Băbească neagră* peels, a short extraction (25 minutes) with 85% ethanol reduced TAC and AA, while a longer duration (≥44 minutes) and 64% ethanol improved AA (Serea *et al.*, 2023). Similarly, in *Malpighia emarginata* DC. residues, TAC extraction was more efficient with low ethanol concentrations but progressively declined with extended extraction times (Cerino *et al.*, 2023).

Global Desirability Optimization

Given the varied effects of extraction conditions on bioactive compounds, global desirability optimization was applied (Figure S3). For *ushpa*, the optimal conditions determined were 64.86% methanol and an extraction time of 139.68 minutes, resulting in estimated values of TPC = 3,586.8 mg GAE/100 g d.b., TFC = 1,820.8 mg CE/100 g d.b., TAC = 251.87 mg C3G/100 g d.b., and AA = 562.76 µmol TE/g d.b. In contrast, *sachon* exhibited optimal conditions with 64.86% ethanol and 90 minutes of extraction, achieving TPC of 948.37 mg GAE/100 g d.b., TFC of 701.06 mg CE/100 g d.b., TAC of 7.84 mg C3G/100 g d.b., and AA of 500.71 µmol TE/g d.b.

Previous studies have reported equally variable optimal conditions. For instance, in *Băbească neagră* grape peels, using 85% ethanol for 52.14 minutes yielded TPC and TAC values ranging from 24.67 to 43.97 mg/g and 1.71 to 2.74 mg C3G/g, respectively (Serea *et al.*, 2023). In passion fruit and rambutan seeds, maximum TPC and AA were achieved with 67% and 54% ethanol, respectively, after 186 and 221 minutes (Sai-Ut *et al.*, 2023). In *Carica papaya* L. seeds, extraction with 100% methanol for 6 hours resulted in 6.17 mg GAE/g (TPC) and 52.75 mg QE/g (TFC) (Robles-Apodaca *et al.*, 2024). In *Malpighia emarginata* DC. residues, TAC of 2.54 mg/g was reached with 12% ethanol and 25 minutes of extraction (Cerino *et al.*, 2023).

Conclusions and Future Directions

This study provides the first systematic characterization of the physicochemical, chemical, and antioxidant properties of two native Andean fruits from northern Peru: *ushpa* and *sachon*. Both species showed physicochemical parameters (color, °Brix, and pH) comparable to commercial berries, and remarkably high TPC, TFC, TAC, and AA values, often surpassing those of widely consumed fruits. These traits likely reflect their adaptation to harsh, high-altitude environments with intense solar radiation, which promotes the accumulation of secondary bioactive metabolites.

The study of extraction conditions revealed significant, species-specific effects on each attribute. This complexity led to the use of a multivariate optimization approach based on global desirability, which identified specific extraction conditions for maximizing bioactive compound recovery and antioxidant activity.

These findings not only provide academic insights but also highlight the potential of *ushpa* and *sachon* as sources of functional ingredients for the food, nutraceutical, and cosmetic industries. However, the limited scientific knowledge about native species in Peru, particularly regarding their chemical composition and functionality, remains a barrier to their integration into the agri-food chain. Future research should include proximate composition and detailed phytochemical profiling through chromatographic techniques to identify and quantify individual metabolites. Additionally, while the in vitro antioxidant tests used here offer an initial assessment, future studies should incorporate cellular, animal, or clinical models to better evaluate their real-world functionality.

Supplemmentary material

https://docs.google.com/document/d/19J7wvCNfUOnINnw7pwlwWhhTfAJae_Dw/edit?usp=sharing&ouid=111310786017351827239&rtpof=true&sd=true

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Ethics Declarations

The authors declare no conflict of interest. All experiments were conducted in strict compliance with regulations for genetic resources established by the local authority, the National Forest and Wildlife Service (SERFOR).