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Chemical Composition and Polyphenol Compounds of *Vaccinium floribundum* Kunth (Ericaceae) from the Volcano Chimborazo Paramo (Ecuador)

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Abstract: Mortiño (*Vaccinium floribundum* Kunth) is considered a "superfruit" due to its antioxidant capacity and possible health benefits. To date, there is no known study that addresses the biochemical characterization of mortiño berries from the paramo of the Chimborazo volcano (Ecuador). So, the aim of this research was to evaluate for the first time the effect of the stage of development of the mortiño berries (two stages) and environment of origin (three sampling areas) on fruit quality. Polyphenol compounds were identified by high-performance liquid chromatography (HPLC) coupled to electrospray ionization mass spectrometric (ESI-MS^n) and quantified by high-performance liquid chromatography with a diode array detector (HPLC-DAD). Moreover, antioxidant properties ($ABTS^{\bullet+}$, and DPPH), sugar and organic acids, and minerals were examined. The main organic acids were quinic and citric acid, while glucose, fructose, sucrose, mannose, and sorbitol were the main sugars determined in the mortiño fruits. The main constituents of the mortiño berries included hydroxycinnamic acids (5-*O*-caffeoylquinic acid), flavonols (quercetin 3-hexoside, quercetin 5-hexoside, quercetin 3-pentoside, and quercetin-3-*O*-rhamnoside) and anthocyanins. Seven anthocyanins were identified: glycosides of cyanidin, delphinidin, petunidin, peonidin, and pelargonidin. The research confirms that the mortiño berries produced in the Ecuadorian paramo area are a valuable source of polyphenolics, rich in sugars and organic acids, and can be classified as a good source of microelements.

Keywords: antioxidant activity; polyphenolic; anthocyanins; mortiño; minerals

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1. Introduction

Mortiño (*Vaccinium floribundum* Kunth)—also known as Andean blueberry—is a deciduous perennial shrub endemic to the high Andes of South America and it is considered a "superfruit" due to its antioxidant capacity and possible health benefits [1,2]. This species is particularly common in the Andes from Venezuela to Bolivia and has been used since immemorial times. The berry is consumed fresh, dried, in sausages, jellies, jams, desserts, and in a special beverage called "colada morada" [3,4]. Mortiño berries are used by local communities in Ecuador for medical uses (allegedly as an aliment for rheumatism, fevers, colics, common colds, hangovers, and liver and kidney problems), as well as for ornamental and other uses as dye, fodder, or firewood [5]. One of the current limitations of *V. floribundum* fruit production is that the plant has not been domesticated and the technical difficulties to cultivate it, in addition to the continuous fragmentation experienced by mortiño populations due to anthropogenic processes such as deforestation, productive land reconversion, and overexploitation [6]. Mortiño berries can be found at local markets in

the northern Andes (Colombia and Ecuador), where these fruits are usually collected from wild plants. Actually, with the advancement in food processing technologies, commercial mortiño products are available in the markets in different presentations such as capsules, powder, and wines, known as Wine of the Andes [7].

Mortiño, which can be found in Ecuador in the paramos at high altitudes ranging from 3000 to 4500 m above sea level (masl) [3], is a source of anthocyanins, proanthocyanidins, and polyphenolic compounds, which have been shown to possess antioxidant and anti-inflammatory properties, as well as lipid accumulation inhibition activity in adipocytes [8]. Freire [9] and Coba et al. [10] reported that berries of mortiño have relatively high concentrations of sugar, antioxidants such as vitamin C and the vitamin B complex, and minerals such as potassium, calcium, and phosphorus. The concentration of phytonutrients is influenced by many factors, such as variety, state of maturity, location, environmental conditions, agricultural practices, and pre-/postharvest handling [11–13]. During fruit ripening several biochemical and physiological processes take place, producing changes in fruit quality parameters [14]. Thus, due to oxidative stress in advanced stages of development, an increase in the content of phenol levels and a high content of antioxidant compounds have been observed [10]; however, during berry storage, the content of anthocyanins is reduced, as temperature is the main factor for destabilization of molecular structure [15]. Studies carried out show that the lyophilized extract of *Vaccinium floribundum* does not present toxicity and could be safely included as an ingredient in food. Because of its functional properties, the mortiño extract can also be handled as an antioxidant or natural colorant in the food industry, or even for the development of nutraceuticals in the pharmaceutical industry [16]. Thus, Guijarro-Fuertes et al. [17] developed bread with healthier properties by adding mortiño pulp. Moreover, it has even been used for the synthesis of nanoparticles and solar cells [18]. Additionally, the mortiño bagasse also contains a high amount of gallic acid, chlorogenic acid, caffeic acid, epicatechin, and coumaric acid, which makes it a very interesting source of antioxidant compounds [19]; the berries present as well antimicrobial activity, which makes the blueberry a potential source of bioproducts that can be used to develop new antimicrobials [1]. In addition, medicinal properties have been attributed to *V. floribundum*, such as potential applications in managing the symptoms of diabetes [20] and protection against oxidative stress [21].

Biochemical, nutraceutical and functional evaluation of *mortiño* plant material is essential to acknowledge its potential as a health-promoting aliment. To our knowledge, there are no studies that have addressed the biochemical characterization of *Vaccinium floribundum* from the paramo of the Chimborazo volcano (Ecuador).

For all the above-mentioned reasons, the objective of this research was the novel evaluation of the effect of the stage of development of mortiño berries (two stages) and location of origin (three sampling areas) on: (i) antioxidant activity, (ii) mineral composition, (iii) profile of sugars and organic acids, (iv) total phenolic content, and (v) anthocyanin profile and non-anthocyanin phenolic profile of mortiño (*Vaccinium floribundum* Kunth) produced in the volcano Chimborazo paramo (Ecuador). This information can be used to improve the market for mortiño, which can provide sustainable economic opportunities for farmers, and can be useful in promoting the conservation and sustainable use of this natural resource.

2. Materials and Methods

2.1. Plant Material

Three different local habitats with presence of *V. floribundum* were sampled in the paramo of the Chimborazo volcano in the central Andes of Ecuador. The sampling areas were to observe "mortiño" individuals selected based on verbal information from park rangers. In each study area, different adult plants were randomly selected.

Fresh berries of *V. floribundum* were harvested in three sampling areas: Culebrillas, Polylepis, and Cubillín, in the paramo of the Chimborazo volcano (Ecuador), the native habitat of the species. All growing environments showed loamy-sandy texture soils, with

fairly poor percentages of organic matter (0.90% in Polylepis and Culebrillas, and 0.80% in Cubillín), acidic pH values of 5.27 (Culebrillas and Polylepis) and 5.63 (Cubillín). The predominant vegetation in the three monitored areas was the herbaceous paramo, and only Cubillín also showed an Alpine steppe (alpine grassland).

Berries were randomly picked from different parts of wild bushes on mountain slopes at an altitude between 3500 and 4100 masl. More information about each environmental area is shown in Table 1 and Figure 1. The berries were classified according to their maturity state into: (i) Stage 7: Fruit development; the berries began to develop anthocyanins, which was identified by their reddish coloration from the apical to the basal part of the fruit, and (ii) Stage 8: Ripening or fruit maturity; 100% of cluster berries shows a purple epicarp [22].

Table 1. Sampling areas where the mortiño berries were harvested in the paramo of the Chimborazo volcano, Ecuador.

Locality Name	Province	Coordinates	Altitude (m)	Vegetation Type	Mean Temperature(°C)	Mean Precipitation(mm)
Culebrillas	Bolívar	01°34.20′ S 78.55.5′ W	4000	Herbaceous paramo	3.1	967
Polylepis	Chimborazo	01°32.41′ S 78°53.5′ W	4076	Herbaceous paramo	3.1	967
Cubillín	Chimborazo	01°45′ S 78°31′ W	3500	High mountain forest	7.0	1000

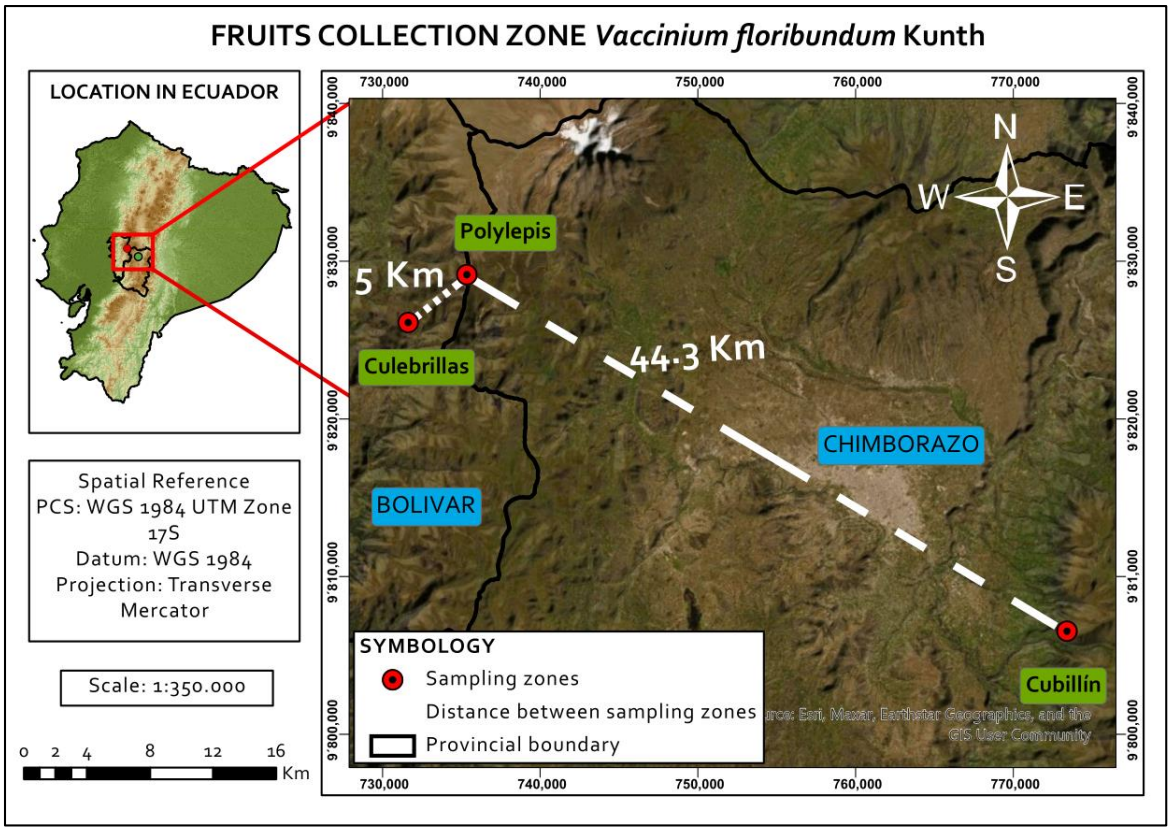

Figure 1. The three paramo zones where the mortiño berries were harvested.

2.2. Sample Preparation

After sorting, the berries were cleaned by removing leaves, stems, and damaged berries and were washed with drinking water to reduce the microbial load, dirt, and organic matter. Then, the berries were immediately frozen with liquid nitrogen and later

freeze-dried in an Alpha 2–4 freeze drier (Alpha 2–4; Christ, Osterode am Harz, Germany) for 24 h at a pressure reduction of 0.220 mbar. The temperature in the drying chamber was −25 °C, while the heating plate reached 15 °C. At the end of freeze-drying, the samples were powdered, and vacuum packed at −20 °C until analyzed. Moisture content in freeze-dried mortiño was 5 g 100 g^{-1}. For conversion from DM to FW, the moisture contents in fresh and freeze-dried mortiño were used.

2.3. Extraction Procedure for Total Polyphenols Content (TPC) and Antioxidant Activity (AA)

The extraction procedure for TPC and AA quantification was prepared as described by Wojdyło et al. [23]. The extractions were performed in triplicate.

2.3.1. Quantification of Total Polyphenols Content (TPC)

The TPC was determined using the Folin–Ciocalteu colorimetric method described by Singleton et al. [24], with some modifications. The absorbance of the blue complex formed was read at 765 nm using a UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). Calibration curves, with a concentration range between 0 and 0.25 g GAE L^{-1}, were used for the quantification of TPC and showed good linearity ($r^2 \geq 0.996$). All determinations were performed in triplicate, and results were expressed as milligrams of gallic acid equivalent per 100 g of sample dry matter (mg of GAE 100 g^{-1} of DM).

2.3.2. Determination of Antioxidant Activity by Two Different Methods

ABTS Method

The ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay was performed according to Re et al. [25] with some modifications. The absorbance was measured by UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). All determinations were performed by triplicate, and the results were expressed in millimoles of Trolox per kilogram of sample dry matter (mmol Trolox kg^{-1} of DM).

DPPH Method

The radical scavenging activity was evaluated using the DPPH$^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. [26], with a modification in the reaction time. The decrease in absorbance was measured at 515 nm using a UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). All determinations were performed by triplicate, and the results were expressed in millimoles of Trolox per kilogram of sample dry matter (mmol Trolox kg^{-1} of DM).

Calibration curves in the range 0.01–5.00 mmol Trolox L^{-1} were used for the quantification of the two methods of antioxidant activity, both showing good linearity ($r^2 \geq 0.998$).

2.4. Determination of Sugars and Organic Acids Profile

Organic acids and sugars profile were identified and quantified according to Hernández et al. [27], with some modifications. Briefly, half a gram of freeze-dried mortiño berry was mixed with 5 mL of phosphate buffer (50 mmol L^{-1}) pH 7.8; the mixture was homogenized, centrifuged, and filtered. Then, 10 µL of the supernatant was injected into a Hewlett Packard (Wilmington, DE, USA) series 1100 high-performance liquid chromatography equipped with a refractive index detector for sugars detection, and UV–Vis detector for organic acids analysis. A Supelcogel TM C-610H column (30 cm × 7.8 mm) with a pre-column (Supelguard 5 cm × 4.6 mm; Supelco, Bellefonte, PA, USA) was used for the analyses of both organic acids and sugars. Absorbance of organic acids was measured at 210 nm. Analyses were run in triplicate and the results expressed as g kg^{-1} dry matter (DM).

2.5. Minerals Analysis

To determine mineral content in the mortiño berries weighed accurately to a weight of 0.1 g of freeze-dried powdered into 75 mL Teflon (TFM) vessels. Then, 4 mL HNO_3 (69 vol.%) and 2 mL of ultra-high-purity deionized water were added and left to stand for 15 min to pre-digest the samples. Next, samples were microwave (CEM Mars One 240/50, Matthews, NC, USA) digested. The quantification of macro-elements [calcium (Ca), sodium (Na), potassium (K), and magnesium (Mg)] and micro-elements [iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn)] was carried using an Inductively Coupled Plasma Mass Spectrometer (ICPMS-2030, Shimazu, Kyoto, Japan). Calibration curves were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.998$). The analyses were run in triplicate and results expressed as g kg^{-1} and mg kg^{-1} for macro and micro-elements, respectively.

2.6. Identification and Quantification of Phenolic Compounds by HPLC-DAD-ESI-MS^n

2.6.1. Extraction and Determination of Phenolic Compounds Non-Anthocyanin

Extraction method: Samples (50 mg) were mixed with 1 mL of extractant (methanol/water (80:20, *v*/*v*) + 1% formic acid), and this mix was stirred during 5 min. After that time, the samples were centrifuged for 10 min at 12,000 rpm and 4 °C. The supernatant was filtered through a 0.45 µm PTFE filter (Millipore Billerica, MA, USA) and then was stored at −20 °C until further use. All extractions were carried out in triplicate.

Phenolic compounds non-anthocyanin: For the analysis of the different samples of mortiño berries, HPLC-ESI-DAD-MS^n Ion Trap (Agilent 1100 series System) was used, which allows us to make successive breaks of the precursor ion for its identification of unknowns. Chromatographic separation was carried out on a C18 column (Poroshell 120, 100 mm × 3 mm i.d., 2.7 µm particle size). The mobile phases consisted of two solvents: water/formic acid (95:1, *v*/*v*) as solvent A and acetonitrile as solvent B at a flow rate of 1 mL min^{-1}. For the determination of polyphenols, the gradient started with 5% B to reach 60% B at 37 min, at 40 min the percentage of B increased to 98% and was maintained for 2 min before returning to the initial conditions. The injection volume was 20 µL. Relative quantification of the phenolic compounds present in the samples was performed by chromatographic comparison with pure standards (caffeic acid, rutinoside quercetin, pelargonidin and cyanidin), and their absorbance spectra at four wavelengths emitted at 280, 320, 360, and 520 nm through a diode array UV detector (DAD) integrated in the HPLC and connected on-line to the mass spectrometer.

2.6.2. Identification and Quantification of Anthocyanins

Extraction method: Anthocyanins extraction and analysis were determined using the method proposed by Hong et al. [28] with some modifications. Briefly, half a gram of freeze-dried samples of mortiño berries was mixed by shaking in an orbital bath, with 4 mL cold extractant [methanol/water/formic acid (80:19.9:0.1, *v*/*v*/*v*)] for 10 min. The mixture was sonicated by ultrasonic bath for 10 min. Next, the samples were centrifuged for 10 min, 4000 rpm at 4 °C, and the supernatants were collected; the pellets residue was re-extracted twice using the same steps, being the supernatants definitely combined. Following, two milliliters of the supernatant were filtered through a 0.45 µm nylon Millipore membrane filter, and then stored at −20 °C until further use. All extractions were carried out in triplicate.

Analytical method: Anthocyanins profile was determined by high-performance liquid chromatography triple quadrupole mass spectrometer (LC-MS/MS 8050; Shimadzu, Kyoto, Japan). The molecules were ionized using Atmospheric Pressure Ionization (Electrospray Ion-ization-ESI). Chromatographic separations were performed with a C18 column (Mediterranean SEA 18, 10 mm × 0.21 mm i.d., 2.2 µm particle size) from Teknokroma (Barcelona, Spain). The mobile phase A consisted of 0.1% (*v*/*v*) formic acid (FA) in water (Milli-Q), and the mobile phase B consisted of 0.1% (*v*/*v*) formic acid in acetonitrile (ACN) at a flow rate of 0.4 mL min-1, an injection volume of 10 µL and oven tempera-

ture of 50 ° C. The gradient condition was 0–2 min 5% B, 2–10 min 95% B, 10–11 min 95% B, 11–12 min 5%B, and 12–16 min 5% B. For the quantification of the anthocyanins (Delphinidin 3-*O*-glucoside; Cyanidin-3,5-di-*O*-glucoside; Cyanidin 3-*O*-glucoside; Cyanidin-3-*O*-arabinoside; Petunidin-3-*O*-glucoside; Peonidin-3-*O*-glucoside; Pelargonidin-3-*O*-rutinoside), a stock of 100 ppm was made, and from it, a calibration plot of concentrations 0.1, 0.3, 0.5, 0.8 and 1 ppm was obtained. All analyses were performed in triplicate.

2.7. Statistical Analyses

The data were subjected to one-way analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. The confidence interval was 95%, and the significant difference was defined as $p < 0.05$. Principal component analysis (PCA) was used to reduce this complex dataset to a lower dimension and reveal simplified structures and relations between localities and states of maturity. To perform these statistical analyses, the software XLSTAT Premium 2016 (Version 9-Addinsoft, New York, NY, USA) was used.

3. Results and Discussion

Since the processed experimental data are so diverse, the results are differently presented into main subheadings.

3.1. Antioxidant Activity (AA) and Total Polyphenol Content (TPC)

The results for AA and TPC obtained in mortiño berries are summarized in Table 2. These parameters are important from a functional point of view because oxidative stress is reported to be the key factor for many diseases such as cardiovascular, hypertension, atherosclerosis, neurodegenerative or cancer, mainly caused by an imbalance between reactive oxygen species (ROS) and the antioxidative defense system [29]. The AA of mortiño berries was evaluated using two spectrophotometric assays: ABTS and DPPH, as each antioxidant compound has different mechanism of action. Significant differences were observed for "sampling area", "stage" and the interaction "sampling area x stage" factors. The highest value of AA by ABTS method was found in mortiño berries collected in the zone of Culebrillas (65.74 mmol Trolox kg^{-1} DM) while for DPPH method the highest value was obtained in mortiño berries collected in Polylepis (77.22 mmol Trolox kg^{-1} DM). These results were slightly higher than those reported by Vasco et al. [3] in mortiño berries and in Andean blackberry, and that those reported by Garzon et al. [4] for Colombian bilberries (*V. meridionale*). Furthermore, our AA values were higher compared to the values reported by Sellappan et al. [30] for other *Vaccinium* species as *V. corymbosum* L. hybrids and *V. ashei*. On the other hand, and with respect to the stage of fruit development, the AA for both methods decreased during fruit development, ranging from 63.34 (stage 7) to 39.74 (stage 8) mmol Trolox kg^{-1} DM for ABTS method, and from 72.16 (stage 7) to 55.37 (stage 8) mmol Trolox kg^{-1} DM for DPPH method. The same trend was reported by Esquivel-Alvarado et al. [31] for berries of *V. consanguineum, V. poasanum and V. floribundum* collected between 3000 and 3200 masl. Analyzing the interaction "sampling area x stage" factors (Table 2), it can be seen that the highest AA content, measured by both methods, was found in the mortiño fruits collected in the Polylepis and Culebrillas areas (>4000 masl). The results indicate that mortiño berries have a great inhibitory activity against free radicals, as can be seen when contrasted with other reports in the literature. Therefore, it can be concluded that mortiño berries are an accessible source of antioxidants.

Table 2. Antioxidant activity (mmol Trolox kg^{-1} dry matter, DM) and total polyphenol content [mg gallic acid equivalent (GAE) 100 g^{-1} DM] in Mortiño berries as affected by sampling area and stage of fruit development.

Factor	ABTS	DPPH	TPC
	(mmol Trolox kg^{-1} DM)		(mg GAE 100 g^{-1} DM)
	ANOVA Test †		
Zone	***	***	***
Stage	***	***	***
Sampling zone x Stage	***	***	***
	Tukey's multiple range test ‡		
	Zone		
Polylepis	47.76 b	77.22 a	2231.45 b
Cubillín	41.12 c	44.85 c	1649.69 c
Culebrillas	65.74 a	69.22 b	2651.57 a
	Stage ¥		
Stage 7	63.34 a	72.16 a	2559.12 a
Stage 8	39.74 b	55.37 b	1796.02 b
	Study zone × Stage		
Polylepis * Stage 7	79.53 a	86.64 a	3089.38 a
Polylepis * Stage 8	16.00 e	67.80 b	1373.52 e
Cubillín * Stage 7	41.43 d	50.97 c	1717.46 d
Cubillín * Stage 8	40.81 d	38.72 d	1581.91 d
Culebrillas* Stage 7	69.07 b	78.86 a	2870.53 b
Culebrillas* Stage 8	62.42 c	59.57 bc	2432.62 c

† NS: not significant at $p < 0.05$; * and ***, significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column was not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; the berries began to develop anthocyanins; Stage 8: Ripening or maturity of fruit; 100% of cluster berries epicarp is purple.

With respect to the TPC, significant differences were also observed for the "sampling zone", "stage" and the interaction "sampling zone × stage" factors for $p < 0.001$. For the "sampling area" factor, the highest values were found for mortiño berries collected in Polylepis and Culebrillas, ranging those from 2231.45 (Polylepis) to 2651.57 (Culebrillas) mg GAE 100 g^{-1} DM; while the lowest values were obtained in the location of Cubillín at lower altitude (values 0.67- fold lower). For the "stage" factor, mortiño berries presented the highest levels of TPC in stage 7 (2559.12 mg GAE 100 g^{-1} DM); and for the interaction "sampling zone × stage" factors, the highest levels were found for the "Polylepis × Stage 7" (3089.38 mg GAE 100 g^{-1} DM) and the "Culebrillas × Stage 7" (2870.53 mg GAE 100 g^{-1} DM). TPC decreased during fruit development by 0.70-fold for stage 8 (Table 2). A similar trend was reported by Esquivel-Alvarado et al. [31] for *V. floribundum* collected at an altitude of 3200 m, who reported values from 1787 mg GAE 100 g^{-1} DM for early fruit development stage to 1441 mg GAE 100 g^{-1} DM for the late fruit development stage. This trend can be attributed to changes such as the hydrolysis of glycosides, the oxidation of phenols by polyphenol oxidases, and the polymerization of free phenols [32]. In the current study, the content of total polyphenol was higher than those reported by Prior et al. [11], who reported mean TPC values for *V. corymbosum.*, *V. angustifolia*, and *V. myrtillus* of 347, 398, and 525 mg GAE 100 g^{-1} FW, respectively, and were also higher than those found in other fruits as pomegranate (777 to 1660 g GAE kg^{-1} DM), plum (440 mg GAE 100 g^{-1} FW) and strawberry (238 mg GAE 100 g^{-1} FW) [3,33], whereas were lower than those reported by Llerena et al. [34] for blackberry (6352.28 mg GAE 100 g^{-1} DM).

In this study, total polyphenols content and antioxidant activity were clearly influenced by altitude. The higher the altitude, the higher the contents of TPC and AA presented by mortiño berries. These results demonstrate that altitude is an important factor affecting the antioxidant activity and total polyphenols content in *V. floribundum*. Similarly, previous

studies have indicated that AA and TPC levels can vary significantly depending on the geographical location of the mortiño plants, as well as the growing conditions such as altitude, radiation, and temperature [1]. The high content of TPC in mortiño berries can significantly contribute to the use of this material as a source of natural antioxidants.

3.2. Sugars and Organic Acids Profile

Table 3 summarizes the effects of environmental origin and stage of fruit development on organic acids (OA) and sugars profile and content. The main organic acids found in *V. floribundum* were quinic acid, followed by citric and malic acids. The same main organic acids were reported by Wang et al. [35] in 10 populations of *Vaccinium uliginosum* from the Changbai Mountains of China. The "Sampling zone" factor significantly ($p < 0.001$) affected the three acids content.

Table 3. Organic acids and sugar content (g kg^{-1} dry matter, DM) in Mortiño berries as affected by location and stage of fruit development.

Factor	Organic Acids				Sugars			
	Citric	Malic	Quinic	Sucrose	Glucose	Fructose	Mannose	Sorbitol
ANOVA Test †								
Zone	***	***	***	***	***	***	***	***
Stage	***	***	***	***	NS	***	***	***
Zonex Stage	***	***	***	***	***	***	***	***
Tukey's multiple range test ‡								
Zone								
Polylepis	41.98 b	22.30 c	108.39 c	9.96 b	110.05 a	60.80 b	31.80 b	106.46 c
Cubillín	65.05 a	28.35 b	199.48 a	61.42 a	71.75 b	50.61 b	35.82 b	190.55 a
Culebrillas	58.06 a	36.71 a	155.99 b	13.05 b	106.94 a	79.19 a	134.04 a	157.76 b
Stage ¥								
Stage 7	63.10 a	26.22 b	177.32 a	41.56 a	91.91	39.08 b	79.30 a	178.97 a
Stage 8	46.96 b	32.02 a	131.92 b	14.73 b	100.58	87.99 a	55.14 b	124.20 b
Zone × Stage								
Polylepis * Stage 7	50.76 b	10.21 e	153.20 b	12.90 bc	116.43 a	64.35 b	34.14 cd	145.12 b
Polylepis * Stage 8	33.20 c	34.96 b	63.57 c	7.02 c	103.66 ab	57.25 b	29.45 cd	67.80 c
Cubillín * Stage 7	75.98 a	25.23 cd	216.84 a	104.74 a	60.04 c	24.19 c	21.00 d	217.02 a
Cubillín * Stage 8	54.11 b	31.47 bc	182.12 ab	18.10 b	83.46 bc	77.03 b	50.64 c	164.07 b
Culebrillas * Stage 7	62.56 ab	19.04 de	161.91 b	7.04 c	99.27 ab	28.69 c	182.76 a	174.77 ab
Culebrillas * Stage 8	53.56 b	54.37 a	150.07 b	19.07 b	114.61 a	129.69 a	85.32 b	140.75 b

† NS: not significant at $p < 0.05$; * and ***, significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column were not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; the berries began to develop anthocyanins; Stage 8: Ripening or maturity of fruit; 100% of the epicarp of the cluster berries is purple.

Quinic and citric acids predominated over malic acid in all environmental areas. This result agreed with that obtained by Mikulic-Petkovsek et al. [36], who indicated that fruit of the Ericaceae family generally contained very little malic acid. Mikulic-Petkovsek et al. [37] reported that wild bilberry fruits (*V. myrtillus*) from high altitude (up to 636 masl) had more organic acids content, compared with bilberry fruit from lower altitudes (up to 217 m). While mortiño berries collected in Cubillín (at an altitude of 3500 m and at a mean temperature of 7 °C) showed the highest content of total OA (287.88 g kg^{-1}), Culebrillas and Polylepis (at more than 4000 m altitude and at a mean temperature of 3.1 °C) showed total OA contents of 250.76 g kg^{-1} and 172.67 g kg^{-1}, respectively. It is important to note

that changes in organic acids in response to temperature also depend on other factors as plant age or fruit type. The temperature at which fruits are grown affects both their titratable acidity and the content of stored organic acids [38]. Our results agree with those found by Mikulic-Petkovsek et al. [37]. However, the results obtained in this study suggest that above 3500 m of altitude the content of organic acids decreases strongly; from 3500 m of altitude (Cubillín) to 4076 (Polylepis), the total AO content decreased to 60%.

Different studies indicate that the organic acid content of the flesh of fruits is affected by environmental factors and cultivation practices such as temperature, light intensity, cultivar, rootstock, mineral nutrition, water availability, and fruit load/pruning. Nonetheless, how these factors alter metabolism to bring about changes in organic acid content is in most cases uncertain [39]. Thus, Wang et al. [35] reported that in *V. uliginosum* the organic acid content was not related to altitude. These differences may be due to a combination of different growing environments (e.g., microclimate), genetics, or other environmental factors. The "stage" and the interaction "zone x stage" factors also affected significantly ($p < 0.001$) the organic acid content (Table 3). Organic acids accumulate in the flesh of many types of fruits at certain stages of their development [38]. Organic acids are related to maturation, in particular malic acid which confers a bitter taste. Thus, the results obtained in this study showed that malic acid increases with maturation, ranging from 26.22 g kg^{-1} DM (Stage 7) to 32.02 g kg^{-1} DM (Stage 8), while both quinic and citric acids decreased. Ayaz et al. [40] also reported in *V. artostaphylos* and *V. myrtillus* that the level of malic acid increased gradually during the maturation of fruits. Compared to previous results reported by Kafkas et al. [41] for blackberries and Correia et al. [42] for Highbush blueberries (*V. corymbosum*), our study showed much lower contents of malic acid for *V. floribundum*.

Regarding the sugar content, glucose, fructose, sucrose, mannose, and sorbitol were the main sugars determined in the fruits of mortiño (Table 3). The sugar content was significantly ($p < 0.001$) affected by the "location", "stage" and the interaction "location × stage" factors. The higher values of glucose and fructose were obtained in the areas of higher altitudes (>4000 m), Polylepis, and Culebrillas. Additionally, Wang et al. [35] reported that the higher the location altitude (>1200 m considered high altitude), the higher the contents of glucose and fructose in bog bilberry (*V. uliginosum*). On the other hand, during fruit development of mortiño, fructose and glucose increased while sucrose, mannose, and sorbitol decreased; the low sucrose content may be due to enzymatic hydrolysis or its transformation into other sugars during the ripening process. Our values agreed with the results reported by Kalt and McDonal [43] for lowbush blueberry (*V. angustifolium*), Correia et al. [42] for highbush blueberry (*V. corymbosum*), and Ayaz et al. [40] for *V. arcostaphylos* and *V. myrtillus*. However, Mikulic-Petkovsek et al. [37] indicated that bilberries grown at low altitude sites (217 m considered low altitude) contained higher levels of total sugars compared to bilberries grown at higher altitudes (636 m considered high altitude). Additionally, since the sucrose content of mortiño was quite low, this fruit should be recommended for low-carbohydrate diets.

Sorbitol is a sugar alcohol characteristic of higher plants. It is a major final product of photosynthesis and, together with sucrose, represents the main forma of carbon translocated in some fruit species [36]. While the highest sorbitol content (190.55 g kg^{-1} DM) was obtained in mortiño fruits grown at low altitudes (Cubillín), the lowest content was found in those fruits grown at higher altitudes (>4000 m). Though Mikulic-Petkovsek [36] also detected sorbitol in chokeberry, rowanberry, and eastern shadbush, this sugar was not detected at all in other berry species [44]. Our results suggest that climate factors such as altitude and temperature play an important role in the sugar content of mortiño berries. Likewise, Cobo et al. [45] also observed this variability in the chemical composition of *V. floribundum* fruits, as a result of climatic and geographic influences.

3.3. Mineral Content

The content of the macronutrients and micronutrients was significantly affected by the "growing environment" and the interaction "growing environment × stage" factors.

Only the macronutrients potassium (K), sodium (Na), and magnesium (Mg) and the micronutrient iron (Fe) were significantly affected by the "stage" factor (Table 4). While the highest quantity of macronutrients (K > Ca > Mg > Na) was found in the mortiño fruits grown in Polylepis, the highest content of micronutrients (Fe > Mn) was observed in fruits grown in Cubillín. Unfortunately, to date there are no studies that provide complete information on the content of minerals in mortiño fruits, and neither how these contents can be influenced by the factors previously outlined such as altitude, stage of development of the fruit, temperature, etc. Vasco et al. [3] reported that mortiño berries are rich in potassium; a serving of 100 g could provide 13% of the recommended adequate intake (AI) of 4.7 g/day for adults. Our results revealed that the macronutrient (K, Na, and Mg) contents decrease with fruit development, while calcium (Ca) and iron (Fe) are the only macro and micronutrients that increase with fruit development. Several studies showed that Ca is an effective pressure-lowering agent [46]; thus, a high Ca content can be beneficial for health. Likewise, it was observed that mortiño immature berries (development stage 7) grown at high altitudes (>4000 m) had a higher potassium content (10.13 and 8.27 g kg^{-1} DM for Polylepis and Culebrillas, respectively). Our results indicated that the mineral content in mortiño fruits is clearly influenced by the growing conditions, the state of fruit development, and the altitude. Karlsons et al. [47] studied the mineral composition of four species of *Vaccinium* (*V. corymbosum*, *V. myrtillus*, *V. macrocarpon*, and *V. oxycoccos*), and reported that the berries of these species were characterized by having a high content of Fe, Ca, Mg, and Mn. In our study, mortiño berries showed levels of macro and micronutrients comparable to those obtained by Karlsons et al. [47] and by Miljkovič et al. [48] in Serbia for *V. myrtillus*. The mineral composition shown by mortiño fruits indicates that these berries are an excellent source of K, Ca, and Fe. In addition, due to their low levels of sodium, mortiño fruits could be properly recommended for low-sodium diets.

3.4. Identification and Quantification of Phenolic Compounds Non-Anthocyanin and Anthocyanins

A total of sixteen different compounds, nine non-anthocyanin (Table 5) and seven anthocyanins (Table 6) have been identified in mortiño berries. To make the discussion easy to follow, phenolic compounds non-anthocyanin and anthocyanins were discussed separately. Quantification of each identified compound is shown in Table 7.

Table 4. Minerals content (g or mg kg^{-1} dry matter, DM) in Mortiño berries as affected by environmental zone and stage of fruit development.

Factor	K	Na	Ca	Mg	Cu	Mn	Fe	Zn
	Macro-Elements (g kg^{-1})				Micro-Elements (mg kg^{-1})			
ANOVA Test †								
Zone	**	***	***	***	**	***	***	***
Stage	**	***	NS	***	NS	NS	***	NS
Zonex Stage	**	***	***	***	**	***	***	***
Tukey's multiple range test ‡								
Zone								
Polylepis	8.85 a	0.30 a	4.88 a	1.24 a	5.63 a	47.63 b	80.95 b	29.61 a
Cubillín	6.83 b	0.24 b	1.80 c	0.41 c	4.74 b	93.05 a	126.89 a	14.40 c
Culebrillas	8.10 a	0.22 b	3.47 b	0.93 b	4.84 ab	22.96 c	70.90 b	17.79 b
Stage ¥								
Stage 7	8.52 a	0.28 a	3.18	0.92 a	5.11	56.76	70.84 b	21.60
Stage 8	7.33 b	0.23 b	3.58	0.79 b	5.03	52.34	114.99 a	19.60

Table 4. *Cont.*

Factor	K	Na	Ca	Mg	Cu	Mn	Fe	Zn
	Macro-Elements (g kg^{-1})				Micro-Elements (mg kg^{-1})			
Zone × Stage								
Polylepis * Stage 7	10.13 a	0.36 a	3.92 b	1.28 a	5.06 ab	48.97 b	69.81 bc	32.42 a
Polylepis * Stage 8	7.57 b	0.25 bc	5.84 a	1.20 a	6.20 a	46.30 b	92.10 b	26.80 a
Cubillín * Stage 7	7.15 b	0.27 b	1.87 c	0.43 c	5.02 ab	93.04 a	61.29 c	15.82 bc
Cubillín * Stage 8	6.50 b	0.21 bc	1.72 c	0.39 c	4.42 b	93.07 a	192.49 a	12.99 c
Culebrillas * Stage 7	8.27 ab	0.19 a	3.74 b	1.06 a	5.22 ab	28.26 c	81.41 bc	16.55 bc
Culebrillas * Stage 8	7.93 b	0.24 ab	3.20 bc	0.81 b	4.46 b	17.67 c	60.38 c	19.02 b

† NS: not significant at $p < 0.05$; *, **, and ***. significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; the berries began to develop anthocyanins; Stage 8: Ripening or fruit maturity; 100% of cluster berries epicarp is purple.

Table 5. Phenolic compounds (non-anthocyanin) identified by HPLC-DAD-ESI-MS^n in Mortiño berries.

Peak No.	[a] Rt (min)	[b] MS/MS (*m*/*z*)	Name of Compounds [c]	Chemical Family
P1	7.3	353,191	3-*O*-Caffeoylquinic acid	Hydroxycinnamic Acid
P2	9.1	337,163	3-Coumaroylquinic acid	Hydroxycinnamic Acid
P3	9.9	707,353,191	5-*O*-Caffeoylquinic acid	Hydroxycinnamic Acid
P4	12.6	335,179	Caffeoylshikimic acid	Hydroxycinnamic Acid
P5	15.9	433,323	Caffeic acid derivate	Hydroxycinnamic Acid
P6	16	463,301	Quercetin 3-hexoside	Flavonols
P7	17.8	463,301	Quercetin 5-hexoside	Flavonols
P8	18	433,301	Quercetin 3-pentoside	Flavonols
P9	18.1	447,301	Quercetin-3-O-rhamnoside	Flavonols

[a] Rt = retention time; [b] MS/MS = tandem mass spectrometry; [c] Compounds were numberer by their elution time.

Table 6. Anthocyanins identified by HPLC-ESI-MS^n in Mortiño berries.

Peak No.	[a] Rt (min)	Molecular Ion [M + H] (*m*/*z*)	[b] MS/MS (*m*/*z*)	Name of Compounds [c]
An1	2.3	627	303,465	Delphinidin 3-*O*-glucoside
An2	4.3	611	287,449	Cyanidin-3,5-di-*O*-glucoside
An3	4.8	449	287,213,137	Cyanidin 3-*O*-glucoside
An4	4.9	419	287,137,213	Cyanidin-3-*O*-arabinoside
An5	4.9	479	317,302,274	Petunidin-3-*O*-glucoside
An6	5.1	463	301,286,201	Peonidin-3-*O*-glucoside
An7	5.0	579	271,433	Pelargonidin-3-*O*-rutinoside

[a] Rt = retention time; [b] MS/MS = tandem mass spectrometry; [c] Compounds were numberer by their elution time.

Table 7. Phenolic compounds quantified in Mortiño berries as affected by sampling zone and stage of fruit development (mg 100 g^{-1} DM).

Factor	Hydroxycinnamic Acid						Flavonols			Anthocyanins							∑Total Polyphenols
	P1	P2	P3	P4	P5	P6	P7	P8	P9	An1	An2	An3	An4	An5	An6	An7	
ANOVA Test †																	
Zone	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Stage	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Zone × Stage	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Tukey's multiple range test ‡																	
Zone																	
Polylepis	1.56 c	1.56 c	291.68 c	140.89 c	69.73 c	61.40 c	75.54 c	35.08 b	24.34 c	0.07 a	0.04 a	6.48 b	12.30 b	0.00 b	0.35 b	0.03 a	721.14 c
Cubillín	247.37 a	95.05 b	595.05 b	343.98 a	221.44 a	165.64 b	224.88 b	149.97 a	367.79 b	0.00 b	0.001 c	10.71 a	18.69 a	0.91 a	0.67 a	0.006 c	2442.21 b
Culebrillas	192.67 b	142.15 a	2032.71 a	196.16 b	138.23 b	196.03 a	281.66 a	1.29 c	509.90 a	0.00 b	0.02 b	6.08 b	11.47 b	0.01 b	0.12 c	0.01 b	3708.58 a
Stage ¥																	
Stage 7	179.22 a	93.33 a	577.24 b	271.86 a	183.31 a	174.01 a	228.07 a	122.94 a	339.93 a	0.00 b	0.001 b	4.46 b	9.74 b	0.06 b	0.42 a	0.008 b	2184.64 a
Stage 8	115.18 b	65.85 b	1369.06 a	182.17 b	102.96 b	108.03 b	159.98 b	1.29 b	261.43 b	0.05 a	0.04 a	11.06 a	18.57 a	0.54 a	0.34 b	0.02 a	2396.65 a
Zone × Stage																	
Polylepis * Stage 7	1.56 d	1.56 d	581.80 b	280.22 bc	137.89 cd	105.71 c	124.17 c	68.88 b	47.40 c	0.00 b	0.00 c	5.55 c	10.44 c	0.00 c	0.70 b	0.02 b	1365.96 c
Polylepis * Stage 8	1.56 d	1.56 d	1.56 c	1.56 e	1.56 e	17.08 d	26.91 d	1.29 c	1.29 c	0.15 a	0.09 a	7.42 c	14.17 bc	0.00 c	0.00 e	0.04 a	76.32 d
Cubillín * Stage 7	302,36 a	110.06 b	667.27 b	363.20 a	235.87 a	172.4 b	226.14 b	298.64 a	370,36 b	0,00 b	0.00 c	6.77 c	12.97 bc	0.19 b	0.47 c	0.00 d	2766.40 b
Cubillín * Stage 8	192,39 bc	80.05 c	522.84 b	324.75 ab	207.01 ab	159.23 b	223.62 b	1.29 c	365.23 b	0.00 b	0.002 c	14.65 a	24.41 a	1.63 a	0.87 a	0.01 c	2118.03 b
Culebrillas * Stage 7	233,75 b	168.36 a	482.64 b	172.14 d	176.16 bc	244.28 a	333.90 a	1.29 c	602.03 a	0.00 b	0.004 c	1.06 d	5.81 d	0.00 c	0.08 de	0.00 d	2421.57 b
Culebrillas * Stage 8	151,59 c	115.95 b	3582.78 a	220.19 cd	100.31 d	147.78 bc	229.42 b	1.29 c	417.78 b	0.00 b	0.05 b	11.10 b	17.12 b	0.01 c	0.15 d	0.02 b	4995.60 a

† NS: not significant at $p < 0.05$; * and ***, significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; berries began to develop anthocyanins; Stage 8: Ripening or fruit maturity; 100% of the epicarp of cluster berries is purple.

The nine non-anthocyanin phenolic compounds were classified into two chemical families: (i) hydroxycinnamic acid (5 compounds), and (ii) flavonols (4 compounds). The content of the phenolic compounds non-anthocyanins was significantly affected by the "growing zone", "stage" and the interaction "growing zone x stage" factors (Table 7). Five hydroxycinnamic acids were detected, four caffeoyl acid derivates, and one coumaroylquinic acid. Baenas et al. [16] also identified in mortiño berries purchased at a local market in Machaci, Ecuador, the same four caffeoyl acid derivates, but at very low concentrations compared to our values; yet they could not identify the presence of coumaroylquinic acid. Furthermore, the highest content of hydroxycinnamic acids was noted for mortiño berries grown in Culebrillas (2701.92 mg 100 g^{-1} DM), followed by Cubillín (1502.89 mg 100 g^{-1} DM), while the lowest content was presented by mortiño fruits grown in Polylepis (505,42 mg 100 g^{-1} DM). From the hydroxycinnamic acids, the isomer of chlorogenic acid, 5-*O*-caffeoylquinic acid, was the most representative in mortiño berries. Our results agree with previous studies carried out by Vasco et al. [3] in *V. floribundum*, by Garzón et al. [4] in *V. meridionale*, by Prencipe et al. [49] in *Vaccinium* berries and by Baenas et al. [16] in *V. floribundum*. Moreover, Wojdyło et al. [50] reported that hydroxycinnamic acids such as 5-caffeoylquinic and caffeoylquinic acid are good sources of antioxidants in vitro that protect low-density lipoprotein (LDL) from oxidation and therefore, supposedly prevent various age-related diseases. Therefore, the consumption of mortiño berries would reduce the risk of cardiovascular disease since its antioxidants lower low-density lipoprotein (LDL) cholesterol levels.

The flavanol glycosides were the second group after the hydroxycinnamic acid derivatives that contributed to the final concentration of polyphenols in mortiño berries. Additionally, those fruits grown in Culebrillas presented the highest levels of flavonols, the quercetin 3-hexoside, quercetin 5-hexoside, and quercetin-3-*O*-rhamnoside were significantly high (Table 7). Other studies also reported these flavonols as the predominant ones in mortiño berries [3,16]. Garzón et al. [4] reported in *V. meridionale* that 93% of the total flavonoids are represented by quercetin derivatives. High content of flavonols may well reflect plant responses to biotic and abiotic stresses or just acclimation to environmental stressors such as heat, cold, UV radiation, drought, salinity, or an attack of herbivores or pathogens [51]. In this study, the highest content of flavonols and hydroxycinnamic acids was obtained for mortiño fruits at the early stages of fruit development (stage 7); the only exception was the 5-*O*-Caffeoylquinic acid, which showed a higher content in the late stage of development. According to Garzón et al. [4], a fairly common feature in the *Vaccinium* family is the presence of quercetin glycosides and hydroxycinnamic acids. Our results confirm that fruit maturity stage and altitude definitely influence the content of non-anthocyanin phenolic compounds; in such a way that above 4000 m of altitude there is a strong reduction in the content of these phenolic compounds. Jaakola and Hohtola [52] reported that flavonol accumulation in fruit skin, as a result of sunlight exposition, is well documented and is the most important environmental factor inducing flavonol biosynthesis, just like that; fruits with sun-exposed peel have higher levels of anthocyanins and flavonols than those grown in the shade. In the literature, there is a great variability regarding the non-anthocyanin phenolic compounds in mortiño berries and in other several species of the *Vaccinium* genus. This variability is due to several factors such as stage of maturity, agronomic factors, cultivars and varieties, geographic region, storage conditions, ripeness, and climate, among others [3,16,31].

Anthocyanins are coloring pigments that give a wide range of colors such as orange, red, purple, and blue in flowers, seeds, fruits, and vegetative tissues [53]. Blueberry and bilberry (*Vaccinium* spp.) are one of the richest sources of anthocyanins [3,16]. In this study, seven anthocyanins have been identified in mortiño berries: glycosides of cyanidin (peaks An_2, An_3, and An_4), delphinidin (peak An_1), petunidin (peak An_5), peonidin (peak An_6) and pelargonidin (peak An_7) (Table 6). Baenas et al. [16] and Esquivel-Alvarado et al. [31] reported the presence of six and five anthocyanins in mortiño berries, respectively, namely derivatives of delphinidin and cyanidin. Garzón et al. [4] and Vasco et al. [3] reported

that Colombian bilberry and Andean blueberry contained only cyanidin and delphinidin glycosides. To our knowledge, the current study identifies the presence of petunidin, peonidin, and pelargonidin in *V. floribundum*. In addition, the anthocyanins contents were significantly affected by the "growing environment", "stage" and the interaction "growing environment x stage" factors (Table 7). Analyzing the environmental area factor, it was observed that the predominant anthocyanins were cyanidin-3-*O*-arabinoside (ranging from 18.69 mg 100 g^{-1} DM of Cubillín to 11.47 mg 100 g^{-1} DM of Culebrillas), followed by cyanidin 3-*O*-glucoside (ranging from 10.71 mg 100 g-1 DM of Cubillín to 6.08 mg 100 g^{-1} DM of Culebrillas). Likewise, higher contents of anthocyanins were shown when mortiño berries reached the late developmental stage (stage 8), being the cyanidin derivatives, followed by petunidin and peonidin, the main anthocyanins. The mortiño fruits showing the highest anthocyanin contents were those grown at an altitude of 3500 m and in a more advanced developmental stage (stage 8). Furthermore, our results indicate that the anthocyanin content slightly decreases above 3500 m of altitude.

It is fairly known that temperature plays a vital role in the synthesis of anthocyanins, and these are more prone to oxidation and relatively unstable. However, the mechanisms are not well understood [54]. Low temperature induces anthocyanin synthesis in various species [55]. However, the accumulation of anthocyanins in cold temperatures is light dependent; in the absence of light, low temperatures prevent anthocyanin biosynthesis. The regulation of cold induction of anthocyanins and the role of light are not well understood yet [52]. The higher the solar radiation at high altitudes, the greater the influence on the secondary metabolite profiles [52]. Li et al. [56] reported that warm weather was related to low levels of anthocyanins, and cool weather was associated with the rapid accumulation of anthocyanins in fruit skin. Maier and Hoecker [57] suggested that high light intensity stimulates anthocyanins production in most plants. In view of the results obtained in this research, well-designed long-term studies are necessary to better understand the plant–environment interaction regarding anthocyanin biosynthesis.

3.5. Principal Component Analysis (PCA)

PCA was used because it is one of the beneficial statistical tools for analyzing several samples and variables in order to establish their differences and similarities. Figure 2 shows that 64.19% of the total variance in the data are represented by PC1 and PC2. Of these two top principal components, PC1 described 42.29% of the total variation and PC2 explained 21.90% of the variation. It is important to note that the higher the distance between two parameters, the lower their correlation. Considering F1 as the dimension that explained the main differences among growing areas and fruit developmental stage Culebrillas-red and Culebrillas-green were positively linked with hydroxycinnamic acids (3-coumaroylquinic acid, and 5-caffeoylquinic acid), flavonols (quercetin derivatives), (antioxidant activity (ABTS), TPC, and organic acids (quinic and malic acids). Instead, Cubillín-red and Cubillín-green were positively linked with anthocyanins (cyanidin 3-*O*-glucoside, cyanidin-3-*O*-arabinoside, pelargonidin-3-*O*-rutinoside, and peonidin-3-*O*-glucoside), hydroxycinnamic acids (caffeoylshikimic acid), flavonols (quercetin-3-pentoside) and minerals (Mn and Fe).

On the other hand, whereas Polylepis-red was negatively linked with anthocyanins (cyanidin-3,5-di-*O*-glucoside, petunidin-3-*O*-glucoside, and delphinidin 3-*O*-glucoside), minerals (Cu) and sugar (fructose), Polylepis-green was positively linked with minerals (Ca, Na, Zn, K, and Mg), antioxidant activity (DPPH) and sugars (glucose).

The biplot (Figure 2) showed that Culebrillas-red and Cubillín-green were laid relatively close to each other along the *X*-axis (PC1). Polylepis-red had large negative scores on the PC2, and it was quite separated from the other locations across PC1. Polylepis-green had large positive scores on the PC2 axis, and it was opposed to Polylepis-red.

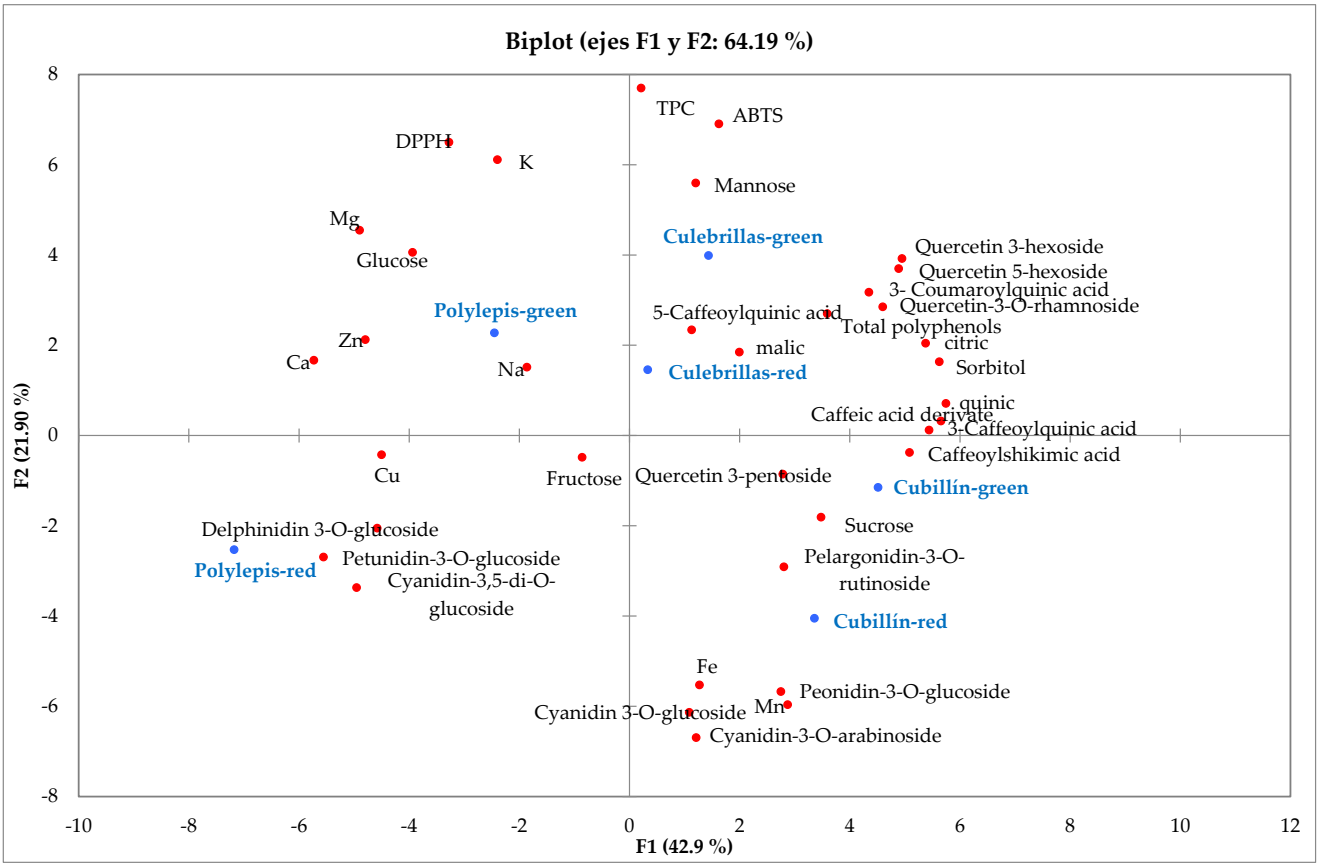

Figure 2. Biplot of principal components analysis (PCA) means showing the relationship among phytochemical parameters and effects of environmental zones (Polylepis, Cubillín and Culebrillas) and stage of development of the fruit of mortiño berries (stage 7 green and stage 8 red).

The results clearly indicated that the sampling zone and fruit developmental stage factors had a crucial effect on the chemical composition and polyphenol compounds of *Vaccinium floribundum*. Therefore, more research considering both the growing environmental factor and the stage of development of the fruit is a must to obtain mortiño berries with high contents of bioactive compounds, becoming an exceptional ingredient to be used in both the cosmetic and pharmacological industries, as well as in the agri-food one.

4. Conclusions

The present study investigated the chemical composition and polyphenol compounds of *Vaccinium floribundum* produced in the volcano Chimborazo paramo, Ecuador. The study was carried out in three growing areas (Polylepis, Culebrillas, and Cubillín) located above 3500 m of altitude and with mortiño berries showing two different stages of fruit development. Despite the fact that the altitude at which this mountain fruit species is found is the main limitation for observation and monitoring, the research confirms that the mortiño berries produced in the Ecuadorian paramo area are a valuable source of polyphenols, rich in sugars and organic acids and can be classified as a good source of microelements, an excellent source of K, Ca and Fe. In addition, due to their low sodium levels, mortiño berries could be recommended for low-sodium diets. The main constituents of mortiño berries include hydroxycinnamic acids (5-*O*-caffeoylquinic acid), flavonols (quercetin derivatives), and anthocyanins. Three anthocyanins (petunidin, peonidin, and pelargonidin) were reported for the first time in mortiño berries, which have never been before identified and quantified in *V. floribundum*. Overall, our data indicate that altitude and stage of fruit development significantly affect mortiño berries quality. This research may successfully contribute to improving market sales for the mortiño and, at the same

time, can provide sustainable economic opportunities for farmers. Finally, our contribution to improving knowledge about this wild fruit species will help improve the sustainability and preservation of this rich natural resource.

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