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Agro-Morphological and Physicochemical Evaluation of Blueberry Varieties (*Vaccinium corymbosum* L.) in the Ecuadorian Andes

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Abstract

Blueberries are in high demand across the world due to their nutritional characteristics. The objective of this study was to conduct an agro-morphological and physicochemical characterisation of blueberry varieties (*Vaccinium corymbosum* L.) in the high Andean valleys of Ecuador, aiming to evaluate their productive performance and agro-industrial potential. The introduced varieties Eureka Maxx, Eureka Gold and Biloxi were studied across different agricultural systems and substrates under a randomised block design. The agro-morphological characterisation included variables such as latent buds, vegetative shoots, flowering onset and yield. Eureka Maxx grown in pots had the highest productivity (37.1%). In the physicochemical analysis, which considered diameter, firmness, pH, acidity, soluble solids, moisture and colour, it was observed that Eureka Maxx and Eureka Gold outperformed Biloxi in size (23.7 mm), firmness (15.2 N), and concentration of soluble solids (11.9 Brix). In general, plant growth and development are influenced by the environmental conditions of the site and crop management practices. Our results show that the varieties Eureka Maxx and Eureka Gold are viable alternatives for blueberry production in the high Andean valleys of Ecuador, to replace the Biloxi and Emerald varieties.

Keywords: Andean valleys; environmental conditions; fruit production plant growth; phenology

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1. Introduction

American blueberry (*Vaccinium corymbosum* L.) has established itself globally as a highly sought-after functional food due to its nutritional profile, which includes antioxidants (flavonoids and anthocyanins), vitamins C and K, and minerals such as calcium, iron and magnesium [1,2]. These properties have driven its positioning in markets across Europe and the United States, where the consumption of "superfoods" continues to expand [1]. According to [3], superfoods are those that contribute to improving human health, including blueberries due to their phenolic compounds (particularly the abundance of anthocyanins) and their antioxidant and anti-inflammatory properties [4].

The increase in demand for blueberries has prompted the development of new varieties that meet quality standards and consumer preferences and extend postharvest shelf life [5]. Australia has become a reference point for varietal innovation [1], following the example of the United States, which leads the world in the development of blueberry varieties. Genetic improvement assisted by biotechnological tools has reduced the time required to obtain new varieties by utilising specific molecular markers and genes of interest [6].

In Latin America, Peru leads the commercial production of blueberries, with approximately 225,000 tonnes produced over 22,000 hectares, followed by Mexico and Chile, with the latter producing around 160,000 tonnes of blueberries on an area of 18,000 hectares [1]. Peru's competitiveness is attributed not only to production volumes but also to the physical quality of the fruit (firmness), which is crucial for international markets [7]. In Ecuador, blueberries are cultivated in pots both outdoors and in greenhouses in high Andean valleys [5], primarily the Biloxi and Emerald varieties. However, these have shown limitations under the environmental conditions of the inter-Andean valleys, which are drier and warmer. Contrary to the indications of [8], Biloxi produces fruit with low soluble solids content and firmness, which reduces shelf life and complicates postharvest handling. Emerald, despite having larger fruit, is susceptible to diseases and shows low tolerance to acidic soils (pH 4.5–5.5), characteristics commonly found in Ecuador's inter-Andean valleys [9]. These limitations compromise productivity and harm Ecuador's competitiveness with its neighbouring countries.

The introduction of new varieties is a strategic priority in order for Ecuador to remain competitive in the market. Evaluating the growth, development and productivity of new varieties under local agro-ecological conditions is essential to ensure sustainability and respond to international market demands [10]. Potentially beneficial blueberries for Ecuador include Eureka Maxx and Eureka Gold, which are blueberry varieties developed in Australia with good plant vigour, high productivity and fruit of medium to large size, excellent firmness and sensory quality [11]. These varieties have recently been introduced to the country and are cultivated in pots under semi-controlled conditions, necessitating an evaluation of their adaptation to local conditions as well as their yield and fruit quality potential [1,2].

Selecting varieties for introduction to new environmental conditions (climate and soil) is critical, as it enables an understanding of their adaptation, productivity and fruit quality, thus ensuring competitiveness with other producing countries [1,7] and strengthening Ecuador's blueberry industry [5]. The implementation of the International Union for the Protection of New Varieties of Plants (UPOV) protocol [12] has enabled the evaluation of new blueberry varieties, ensuring their distinctiveness, uniformity and stability. Adaptation is assessed through the development and growth of the plant by conducting phenological studies using the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale [12], as this facilitates crop expansion and agronomic management (nutrition, pest control, maturity status, crop cycle and so on) [5].

Historically, phenological studies in fruit crops used the Fleckinger scale [13]. Subsequently, ref. [14] developed a decimal scale for cereals, which was refined by ref. [15] through the BBCH scale, widely used to describe the phenology of various crops [15,16]. This system assigns codes to phenological stages based on the plants' morphological characteristics.

The physicochemical characterisation of fruits is fundamental in determining quality, ripeness and suitability for fresh consumption or industrial processing [17]. Key characteristics include soluble solids content, pH, titratable acidity, firmness, weight, equatorial diameter and dry matter [8]. Total soluble solids content (°Brix) is an indicator of sweetness and sugar concentration and it is related to fruit development and market requirements [8].

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Meanwhile, pH and titratable acidity provide information on acid balance and are determining parameters of flavour and fruit stability [8]. Fruit firmness reflects handling resistance, shelf life and the ability to maintain integrity during postharvest [8]. Weight and equatorial diameter measurements allow for fruit classification by size according to market requirements [18]. Lastly, dry matter provides information on texture and the behaviour of the fruit during storage and processing [19]. Local agricultural strategies have focused on optimising the yield and resistance to biotic and abiotic stress of blueberry varieties; however, increasingly rigorous demands regarding classification systems based on fruit size, firmness, soluble solids, and flavour compel breeding programmes to prioritise enhancing these quality parameters without neglecting crop productivity. The fundamental challenge lies in the imperative need to integrate criteria regarding phenotypic uniformity, flavour stability, and extended postharvest shelf life [20]. Furthermore, blueberry marketing is heavily constrained by technical and physiological limitations related to fruit firmness and durability during storage. The progressive loss of fruit firmness, caused by cell wall degradation, is the primary source of significant losses. This issue, combined with a lack of postharvest handling protocols tailored to the specific genetic traits of the cultivars grown in Ecuador and other countries in the region, drastically limits distribution times and access to distant markets [20,21].

One of the major constraints on blueberry productivity in Ecuador is the lack of implementation of more intensive cultivation systems. Consequently, the evaluation of suitable substrates to increase production levels and management under controlled climatic conditions are cited as key factors in improving crop productivity [5,22]. Hence the importance of continuing to develop management processes for new varieties adapted to Ecuador's production conditions and more intensive systems.

The strengthening of the blueberry industry in Ecuador is supported by new technologies, such as the introduction of varieties [2,5]. The successful implementation of novel eco-friendly strategies generates employment in rural areas and promotes community development [23], contributing to the protection of natural resources and fostering responsible agriculture at both social and environmental levels [5,24]. This study aims to morpho-agronomically characterise the new blueberry varieties introduced to Ecuador's inter-Andean valleys, which are cultivated under semi-controlled conditions (greenhouses), following the phenological observations according to the BBCH scale.

2. Materials and Methods

2.1. Study Location and Crop Management

The study was conducted under semi-controlled conditions (plastic greenhouses) at two experimental sites: EcuaBlue and SierraBlue, located in the Yaruquí parish in the canton of Quito at an altitude of 2300 m above sea level. In the canopy, the average temperature is 18 °C and average relative humidity is 78%.

The blueberry varieties included in the study of reproductive phenology and yield were Eureka Gold and Eureka Maxx, which originated from in vitro seedlings transplanted at the start of the study, and the production corresponds to the yield (3 months) of the young plants. The Biloxi variety was included only for the physicochemical characterisation of the fruit since this variety has been in production for more than 4 years. The agronomic management of the varieties at EcuaBlue was conventional, characterised by synthetic inputs, while at SierraBlue, agronomic practices were geared towards reducing the use of synthetic inputs, favouring natural and/or biological inputs and prioritising sustainable practices with low environmental impact.

2.2. Plant Growth and Development

The growth and development of the blueberry varieties were assessed through reproductive phenology (from latent bud to fruit maturity) utilising the BBCH scale [16], which is detailed in Table 1.

Table 1. BBCH phenological scale for the reproductive phase of blueberry varieties.

Phase	Code	Description	Evaluation Moment
Dormant Bud	(00)	Buds are closed and show no visible signs of growth or development.	Number of latent (dormant) buds recorded in each experimental unit at the start of the study (#).
Swollen Bud	(03)	Buds begin to activate. They swell due to the accumulation of reserves and water, preparing for sprouting.	When 10% of the plants in the experimental unit have at least 1 swollen bud (d).
Flower Bud	(51)	Flower buds are formed and visible but remain closed.	When 10% of the plants in the experimental unit have at least 1 flower bud (d).
Flowering	(65)	Flowers are fully open and functional. Stigmas are receptive to pollination.	When 10% of the plants in the experimental unit have at least 1 functional flower (d).
Fruit development	(70)	Fruits begin to develop from fertilised ovaries. Initial fruit growth is evident.	When 10% of the plants in the experimental unit have at least 1 visible fruit (d).
Fruit maturity	(89)	Fruits reach their characteristic size and colour according to species and variety. Fruits are ready for harvest and consumption.	When 50% of the plants in the experimental unit have fruits ready for harvest (d).

Note: # = number; d= days. Source: [16].

2.3. Agro-Morphological and Physicochemical Characterisation of Blueberries

The agro-morphological characterisation of the blueberry varieties was conducted based on the UPOV TG/137/4 (2009) guidelines developed by ref. [12]. For the physicochemical characterisation of the fruits, the Association of Official Agricultural Chemists (AOAC, 2005) protocols and ISO (1999) standards were utilised. The following parameters were evaluated: size, equatorial diameter, firmness, colour, dry matter, pH, soluble solids and titratable acidity (Table 2). Fruit colour was determined using the Royal Horticultural Society (RHS) Colour Chart until a colour match was found. At EcuaBlue, two varieties (Factor 1) and two substrate types (Factor 2) were evaluated, whereas at SierraBlue, only the two varieties were assessed (Table 3).

Table 2. Agronomic and physicochemical variables recorded in blueberry varieties.

Variable	Procedure	Method
Fruit set (#)	Determined at the onset of fruit development.	Counting
Yield (g/plant)	Total weight of mature fruits recorded.	Total harvest weight
Fruit size (g)	Determined using a digital calliper or grading sieves with standardised diameter apertures.	USDA, 2016

Table 2. *Cont.*

Variable	Procedure	Method
Equatorial diameter (mm)	The equatorial diameter (midsection) of the fruits is measured using a digital calliper.	Standard ISO 2859-1:1999
Total soluble solids (°Brix)	Determined via refractometry (BOECO, DOE30103; Hamburg, Germany). Direct reading.	AOAC 932.12
Titratable acidity (% citric acid)	The juice is titrated with 0.1 N NaOH to determine acidity based on citric acid content.	AOAC 942.15
pH	Measured using a potentiometer (Mettler Toledo; Seven Compac T S 220; Greifensee Switzerland). Direct reading.	AOAC 981.12
Firmness (N)	Fruit firmness is measured with a penetrometer (PCE Group; PCE-Fm200; Meschede, Germany) using a 2 mm diameter probe, applied to the equatorial region of the fruit.	AOAC, 2025 USDA, 2002.
Fruit colour	Determined using the Royal Horticultural Society (RHS) colourimetric charts.	CIELAB System. RHS, 2015
Dry matter (%)	Quantified by oven-drying (BENCH-FOODS; Gold Coast, Australia) the fruit at 60 °C until constant weight is achieved (approximately 24–48 h).	AOAC 934.06

= number.

Table 3. Factors, levels and interactions studied at EcuaBlue and SierraBlue.

	EcuaBlue	**SierraBlue**
Factor	Levels	Treatments
F1: Variety	Eureka Gold	Eureka Gold
	Eureka Maxx	Eureka Maxx
F2: Substrate	1. Peat + coconut fibre and coconut chips	
	2. Peat + perlite	
Interaction (F1 × F2)	1–1 1–2 2–1 2–2	

The experimental unit consisted of 3 plants.

2.4. Statistical Analysis

The agro-morphological evaluation of the plants was conducted at EcuaBlue, where two varieties (Factor 1) and two substrate types (Factor 2) were assessed under a completely randomised block design (CRBD) with a factorial arrangement with four replications. At

SierraBlue, a completely randomised block design was employed (two treatments) with four replications (Table 3).

The variables were analysed via an analysis of variance (ANOVA) using the InfoStat (2020) statistical software. When statistical differences were detected, Fisher's Least Significant Difference (LSD) test at 5% was performed [24].

To determine the variation in the physical and chemical variables of the fruits across varieties, a permutational multivariate analysis of variance (PERMANOVA) test was performed using the 'vegan' package [25] in R and employing Euclidean distances and 1000 permutations. The variables evaluated were acidity, pH, dry matter, equatorial diameter, firmness and soluble solids. Furthermore, a principal component analysis (PCA) was conducted using the R package 'FactoMineR' [26] to visualise the physical and chemical characteristics of the fruits from the three varieties. A second PERMANOVA and PCA were performed in the same way on plant phenology, yield and the physical and chemical characteristics of the fruit exclusively for the two Eureka varieties to further explore their agro-morphological differences.

3. Results and Discussion

3.1. Environmental Conditions

The temperature and relative humidity of the study site throughout the final weeks of 2023 and the first 40 weeks of 2024—representing the study period—are shown in Figure 1.

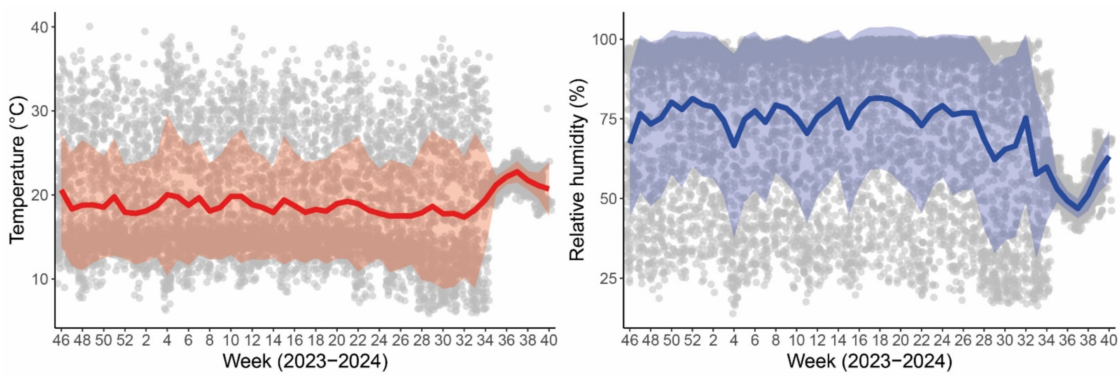


Figure 1. Temperature (T; °C) and relative humidity (RH; %) recorded at EcuaBlue from week 46 in 2023 to week 40 in 2024. Continuous lines represent the average value while the shading represents the standard deviation.

During the 47-week evaluation period, an average temperature of 18.94 °C was recorded, with a minimum of 5.87 °C and a maximum of 40.06 °C. These environmental conditions are favourable for the crop, as temperatures around 20 °C are suitable for the optimal development of blueberry fruit, improving size, sugar accumulation and firmness [27]. On the other hand, temperatures exceeding 30 °C, such as the maximums recorded at the study site, can induce thermal stress, reduce the photosynthetic rate and promote accelerated ripening. This can result in smaller fruits that have a higher concentration of soluble solids due to reduced water content [28]. Furthermore, prolonged exposure to temperatures above 35 °C can cause damage to the fruit cuticle and trigger physiological disorders, negatively affecting postharvest shelf life.

Regarding relative humidity, this parameter averaged 72.15%, with minimum and maximum values of 13.87% and 100%, respectively. These conditions have a direct impact on fruit firmness and quality. Moderate relative humidity, such as that recorded at the study sites, is beneficial for maintaining the cellular hydration of the fruit and preventing excessive losses due to dehydration [6]. However, the maximum levels observed (100%)

during certain periods could generate microclimates conducive to the development of diseases such as the presence of the fungus *Botrytis cinerea*, especially if they coincide with temperate temperatures [6]. Conversely, low humidity conditions (below 30%) can induce water stress, cause excessive hardening or wilting, and alter the accumulation of phenolic compounds; this compromises the sensory and antioxidant properties of the fruit [29].

3.2. Agro-Morphological Characterisation of the Varieties

Figure 2 shows the different reproductive phenological stages of the blueberry crop established during the study according to the BBCH scale.

Figure 2. Phenological stages of the blueberry crop established during the study at EcuaBlue, where 00: Dormant Bud, 03: Swollen Bud, 51: Flower Bud, 65: Flowering, 70: Fruit Development and 89: Fruit Maturity.

Table 4 presents the means for the six phenological stages of the two varieties studied (Eureka Gold and Eureka Maxx) at both sites. At EcuaBlue, the Eureka Gold variety was more precocious in reaching the flower bud and flowering stages, at 17.4 and 27.4 days, respectively, compared to the Eureka Maxx variety (19.1 and 29.1 d). The other phenological stages were statistically identical between the varieties.

Table 4. Average and standard deviation of phenological stages (days) for blueberry varieties evaluated at EcuaBlue and SierraBlue.

Variety	Dormant Bud (Number) *	Swollen Bud (Days) *	Flower Bud (Days)	Flowering (Days)	Fruit Development (Days) *	Fruit Maturity (Days) *
EcuaBlue						
Eureka Gold	42.1 ± 6.1	8.0 ± 1.1	17.4 ± 1.3 b	27.4 ± 1.3 b	76.4 ± 1.0	68.1 ± 5.0
Eureka Maxx	53 ± 14.0	8.3 ± 2.4	19.1 ± 1.7 a	29.1 ± 1.7 a	77 ± 2.5	67.9 ± 4.1
Standard Error	4.7	0.7	0.6	0.63	0.9	1.6
SierraBlue						
Eureka Gold	24 ± 2.9	20.5 ± 4.9	24.7 ± 3.3	79 ± 6.8	95.5 ± 3.8	67.8 ± 9.5 a
Eureka Maxx	29.3 ± 5.0	17.7 ± 4.2	25.7 ± 4.9	79.3 ± 5.7	90.0 ± 3.7	60.5 ± 7.6 b
Standard Error	2.5	2.2	0.6	10.1	2.1	1.4

Averages followed by the same letter are statistically equal (DMS ≤ 5%). * No statistical differences existed.

The varieties' precocity serves as a competitive advantage in markets, as an early entry allows for better pricing [30]. The other phenological stages swollen bud (Sb), flower bud (Fb) and flowering (F) of the varieties were statistically identical (Table 4), with an average of approximately 8 d, 18 d and 28 days respectively. This is consistent with the findings of [30], who reported that plant development following flowering is typically more influenced by environmental factors and agronomic management than by variety genetics. These observations align with local observations made by [5].

At SierraBlue, variety behaviour of Eureka Maxx was more precocious than Eureka Gold (showing an 8-day reduction in the Sb phase). According to [31] and [5], variations in temperature, solar radiation and agronomic management significantly impact plant growth and development. The other phenological stages Fb, F, fruit development (Fd) and fruit maturity (Fm) were similar for both varieties, except for the duration of the flowering-to-harvest period, where Eureka Maxx was more precocious (60.5 d) than Eureka Gold (67.8 d), as observed in Figure 2. This may be explained by the greater efficiency of the Eureka Maxx variety in translocating photo assimilates towards the fruit under the specific conditions of SierraBlue.

When the phenology of blueberry varieties planted in two substrates was evaluated (at EcuaBlue only), plant growth and development were observed to be similar throughout the evaluated period, except for the flowering-to-harvest period (Table 5).

Table 5. Effect of substrate on the phenology and yield of blueberry varieties at EcuaBlue.

Substrate	Dormant Bud (Number) *	Swollen Bud (Days) *	Flower Bud (Days) *	Flowering (Days) *	Fruit Development (Days) *	Fruit Maturity (Days)	Yield (g Plant^{-1})
Peat, coconut fibre and coconut chips (S1)	52.4 ± 13.1	7.8 ± 1.0	18.3 ± 1.5	28.25 ± 1.5	76.9 ± 2.2	66.9 ± 4.0 b	1040 ± 60 a
Peat + perlite (S2)	42.8 ± 9.1	8.5 ± 2.4	18.3 ± 2.1	28.25 ± 2.1	76.5 ± 2.2	69.1 ± 4.7 a	610 ± 33 b
Standard Error	4.7	0.7	0.6	0.6	0.9	1.6	11.4

Averages followed by the same letter are statistically equal (DMS ≤ 5%). * No statistical differences existed.

In substrate 1 (peat, coconut fibre and coconut chips), the fruit reached maturity 3 days earlier than in substrate 2 (69.1 d), which consisted of peat and perlite (Table 5). This could be attributed to substrate 1's enhanced aeration and drainage, which promotes greater plant physiological activity, as reported by [9] in their study on the growth and development of crops in high-porosity substrates.

Furthermore, the type of substrate had a positive effect on yield (Table 5), with production reaching 1040 g/plant/3 months in substrate 1, compared to 610 g/plant/3 months in substrate 2. This difference underscores the importance of the physical conditions of the substrate on which the blueberry plant develops, as indicated by [5,32], with substrate 1 being the more productive here.

In Table 6, it is observed that the Eureka Maxx variety achieved the highest yield per plant at 1098.8 g, nearly double that of the Eureka Gold variety (551.3 g/plant). The results obtained in this study are consistent with other authors' descriptions of the characteristics of Eureka Maxx, which is considered highly productive with significant vegetative development [30].

Table 6. Average yield (g plant^{-1}) of blueberry varieties cultivated at two sites.

	Yield * (g Plant^{-1})	
Variety	EcuaBlue	SierraBlue
Eureka Gold (1)	551.3 a	510.3 a
Eureka Maxx (2)	1098.8 b	356.5 b
Standard Error	111.0	31.0

Averages followed by the same letter are statistically equal (LSD ≤ 5%). * Accumulated yield over 3 months.

Despite Eureka Maxx's precocity, Eureka Gold outperformed it in yield per plant at SierraBlue (510.3 g vs. 356.5 g, respectively), as shown in Table 6. This difference could be due to Eureka Gold adapting better to the environmental, substrate and management conditions. According to [33], varieties with greater stability in their productive behaviour, even if they reach maturity later, exhibit more consistent yields under suboptimal conditions. Conventional management is characterised by the application of synthetic fertilisers and agrochemicals, which favours yield and effective pest (Aphids, Thrips and Mites) and disease (Botrytis, Alternaria and Rust) control. Among the potential negative effects are the accumulation of pollutant compounds in soil, water and the fruit itself [34].

3.3. Physical Characterisation of Blueberry Fruit

The results of the physical characteristics of the blueberry fruits, such as equatorial diameter, firmness and dry matter, show differences between the varieties, as presented in Table 7.

Table 7. Averages of the physical characteristics of blueberry fruits at the two study sites. EcuaBlue (1) and SierraBlue (2).

Parameters	Equatorial Diameter (mm)		Firmness (N)		Dry Matter (g)		Colour	
Location	1	2	1	2	1	2	1	2
Eureka Gold	22.3 ± 1.14 a	25.1 ± 1.35 a	17.7± 1.39 a	13.7 ± 1.46 a	4.3 ± 0.28	4.4 ± 0.11 ab	C203	C203
Eureka Maxx	22.6 ± 2.06 a	24.9 ± 0.66 a	14.3± 0.73 b	15.2 ± 1.03 a	4.4 ± 0.13	4.6 ± 0.17 a	C203	D203
Biloxi	13.7 ± 0.26 b	13.7± 0.29 b	7.3 ± 0.39 c	7.3 ± 0.44 b	4.2 ± 0.04	4.2 ± 0.04 b	C203	C203

Averages followed by the same letter are statistically equal (LSD ≤ 5%).

Based on the results presented in Table 8, there is a marked superiority in the equatorial diameter of the Eureka Gold and Eureka Maxx varieties (22.27 and 22.59 mm, respectively) over Biloxi fruits (13.73 mm). This difference reflects the genetic potential of the new varieties to produce larger-calibre fruits, an attribute highly valued in markets due to consumer acceptance and advantages in fruit packaging [11]. Fruit size is determined by genetic factors, climatic conditions and crop management [27]. The same trend was observed in trials conducted at SierraBlue (Table 7) for these same variables. Fruits grown under organic management show a larger calibre compared to those obtained through conventional management, which could be associated with a greater availability and progressive assimilation of natural soil nutrients, especially where there is a high amount of organic matter (>5%) and microbial activity [35].

Table 8. Averages of chemical characteristics of blueberry fruit, at two study sites: EcuaBlue (1) and SierraBlue (2).

Parameters	Soluble Solids (%Brix)		pH		Titratable Acidity (%)	
Location	1	2	1	2	1	2
Eureka Gold	13.02 ± 0.17 a	11.5 ± 0.1 a	2.94 ± 0.10 a	3.31 ± 0.13 a	0.79 ± 0.17	0.72 ± 0.07 b
Eureka Maxx	11.52 ± 0.46 c	11.6 ± 0.1 a	3.19 ± 0.32 b	2.92 ± 0.10 b	0.79 ± 0.15	0.45 ± 0.05 c
Biloxi	12.20 ± 0.09 b	12.2 ± 0.1 b	2.88 ± 0.10 a	2.88 ± 0.11 b	0.79 ± 0.01	0.96 ± 0.02 a
Standard Error	0.03	0.06	0.06	0.07	0.01	0.01

Averages followed by the same letter are statistically equal (LSD ≤ 5%).

In terms of fruit firmness, Eureka Gold and Eureka Maxx varieties presented the highest values across both study sites, with values ranging from 13.7 to 17.72 N, while Biloxi recorded half the firmness (7.30 N) of the other varieties. These results indicate a more resistant cellular structure in the new varieties, possibly related to a higher accumulation of pectin and lignin in the cell wall, which can extend their postharvest shelf life [8], as observed in Table 7. Firmness is a critical criterion in variety selection, as it prevents damage during postharvest handling by providing better resistance to transport and prolonged storage [36]. Firmness is a critical fruit quality parameter. In blueberries, it varies between 7.8 and 20.7 N, placing Eureka Gold and Eureka Maxx in the upper range of preference for this characteristic. This variable determines the potential for fresh-market commercialisation, shelf life, resistance to transport and handling and, consequently, consumer acceptance [36]. According to [4], organic management allows for slower but more constant plant growth. This translates into greater fruit firmness when compared to conventional management. However, other studies show contrary results, indicating that firmness can be lower in organically managed fruits depending on the cultivar and the edaphoclimatic conditions [4]. While organic management promotes larger fruit size, greater firmness (in certain cultivars and conditions) and higher soluble solids content, it also entails significant disadvantages, such as a more prolonged development cycle and lower yields [35]. This phenomenon is explained by the absence of synthetic fertilisers that can be rapidly absorbed.

Table 7 also presents the dry matter content of the tested varieties. At the SierraBlue location, statistical differences were found between the varieties, with Eureka Maxx containing the highest amount of dry matter in the fruits (4.6 $\pm$ 0.17) than Biloxi. In contrast, at EcuaBlue, there were no statistical differences between the three studied varieties, with an average of 4.3 g. This uniformity can be attributed to the effect of environmental conditions (temperature and relative humidity) during fruit development, as these factors directly influence primary metabolism and the accumulation of soluble compounds. Indeed, ref. [37] mention that homogeneous environments, such as those observed at these cultivation sites, tend to stabilise physiological processes, reducing variability in parameters such as dry matter.

Regarding the fruit colour, Table 7 shows that a constant bluish-purple colouration of medium-dark intensity (C203) was observed in Biloxi and Eureka Gold at both locations, according to the RHS colour chart [38]. This behaviour is consistent with the findings reported by [39], who state that the colour of different varieties remains stable between harvests. In contrast, Eureka Maxx exhibited a variation, moving from C203 at EcuaBlue to an intense bluish-purple (D203) at SierraBlue. This colour variation may be related to the organic crop management employed at both sites, as a study found that organic management increases anthocyanin concentration in blueberries, thereby intensifying pigmentation [39]. The colours matched to the RHS chart [38] show a high degree of comparability with the Nix™ Pro colour sensor device. The results from this sensor constitute a compelling case for its use as a substitute for RHS colour chart systems [40].

3.4. Phytochemical Characterisation of the Blueberry Fruits

The results for the chemical characteristics of the blueberry fruits, including soluble solids, pH and titratable acidity, show statistical differences at the 0.05 level among varieties, as presented in Table 8. Various studies have shown that agronomic management significantly influences the physicochemical parameters of blueberries, such as calibre, pH, acidity, dry matter and firmness [35].

Regarding total soluble solids content in the fruits, Eureka Gold led in sugar content with 13.02 °Brix, followed by Biloxi with 12.2 °Brix and Eureka Maxx with 11.52 °Brix at EcuaBlue (Table 8). This parameter is closely related to sugar accumulation during fruit development and depends on the variety's genetics, agronomic crop management, light availability, water regime and the point of harvest [29].

At SierraBlue, the soluble solids content was highest in Biloxi at 12.2 °Brix, followed by Eureka Maxx with 11.6 °Brix and Eureka Gold with 11.5 °Brix (Table 8). This characteristic in Biloxi may be related to a lower dilution of soluble compounds due to smaller fruit size, indicating a higher concentration of simple sugars, such as glucose and fructose, organic acids, amino acids and minerals [36]. These types of compounds enhance the fruit's sensory value on one hand and, on the other, act as osmoregulatory and antioxidants, contributing to metabolic stability and increased fruit shelf life [19].

Regarding pH, statistical differences were observed between the varieties at both sites. At EcuaBlue, the Eureka Maxx variety had the highest value (3.19), while Eureka Gold (2.94) and Biloxi (2.88) presented similar values. Conversely, at SierraBlue, the pH of Eureka Gold showed the highest value at 3.31, while Eureka Maxx and Biloxi showed lower values (2.92 and 2.88, respectively). Although these parameters are within appropriate ranges, a low pH is associated with higher acidity due to the accumulation of organic acids—mainly citric and malic acids—which influence flavour perception and the microbiological stability of the fruit [40].

Cultivars with lower acidity have a higher pH, such as Eureka Maxx at EcuaBlue and Eureka Gold at SierraBlue; this favours a trend towards a milder and sweeter flavour, which appeals to certain consumer niches [32]. However, an excessively high pH can reduce fruit quality, as it may be affected by detritivore microorganisms, especially under conditions of high humidity and temperature [8]. The pH of organically produced fruits tends to be lower, which is related to higher titratable acidity, resulting in a more intense flavour [4].

On the other hand, titratable acidity (citric acid) at SierraBlue was highest in Biloxi at 0.96%, followed by Eureka Gold (0.72%) and Eureka Maxx (0.45%) as shown in Table 8. This combination of low pH and high acidity in Biloxi reinforces its characteristic acidic profile, placing it in a favourable position for certain market niches and/or industrial applications where greater microbiological stability and a more intense flavour are required [8]. These differences may be related to the varietal expression of tricarboxylic acid metabolism, especially citric acid, the accumulation of which depends on intrinsic genetic and enzymatic factors [8].

At EcuaBlue, the acidity levels of the varieties were statistically equal, with an average of 0.79 (Table 8). This similarity could be explained by similar agronomic management and environmental conditions, which influence metabolic processes such as the activity of enzymes in the tricarboxylic acid cycle [32].

According to [8], blueberries must contain more than 10% soluble solids, exhibit a titratable acidity of 0.3 to 1.3% citric acid and have a pH that fluctuates between 2.25 and 4.25, thereby ensuring optimal quality for the consumer. In general, the values obtained in the present study were within the appropriate physicochemical ranges for commercialisation.

3.5. Physical Characteristics of the Fruit as a Function of the Substrate

The averages of the variables associated with the physical characteristics of blueberry fruits from two varieties cultivated in two types of substrates at EcuaBlue presented statistical differences at the 0.05 level, except for the dry matter content (g) of the fruits, where no statistically significant differences were observed (Table 9).

Table 9. Physical characteristics of blueberries cultivated in different substrates at EcuaBlue.

Substrate	Equatorial Diameter (mm)	Firmness (N)	Dry Matter (g)	Colour
Peat, coconut fibre and coconut chips (S1)	20.06 ± 4.84 a	13.43 ± 5.06	4.3 ± 0.16	C203
Peat + perlite (S2)	19.01 ± 4.16 b	12.76 ± 4.24	4.31 ± 0.21	C203
Standard Error	0.27	0.26	0.06	

Averages followed by the same letter are statistically equal (LSD ≤ 5%).

The average equatorial diameter of the fruit was slightly higher in substrate 1 (20.06 mm) compared to substrate 2 (19.01 mm), as shown in Table 9. This difference can be attributed to the type of substrate: the mixture of peat, coconut fibre and coconut chips offers high water retention and excellent aeration [41]. It is important to note that the substrate is fundamental in either favouring or hindering root development and the absorption of nutrients such as calcium and potassium, which are key to cellular growth and fruit size [42]. This result is relevant from a commercial perspective, given that larger-calibre fruits hold higher market value.

Regarding fruit firmness, no statistical differences were detected between the two types of substrates (Table 9); thus, it can be stated that the substrate type does not alter the cellular structure or the synthesis of fruit wall polysaccharides [36]. It is also important to mention that the perlite present in substrate 2, being an inert and porous mineral, improves aeration and prevents excess moisture [43], which may contribute to maintaining fruit firmness.

Fruit colour presented a value of C203 for both substrates (Table 9) according to the RHS colour chart [38]. This indicates that the composition of the substrates did not influence fruit pigmentation. However, recent studies indicate that substrates with coconut fibre mixtures improve fruit firmness and size, which may be associated with a higher accumulation of anthocyanins that influence colour [9]. Similarly, it has been noted that certain organic substrates can induce metabolic pathways related to pigment synthesis, although without visible changes in the external colouration of the fruit [44].

3.6. Chemical Characteristics of the Fruit as a Function of the Substrate

The averages of the chemical characteristics of blueberry fruits cultivated in two types of substrates showed statistical differences at the 0.05 level (Table 10), suggesting that the substrate influences the chemical composition of the fruits, except for pH.

Table 10. Chemical characteristics of blueberries cultivated in different substrates at EcuaBlue.

Substrate	Soluble Solids (°Brix)	pH	Titratable Acidity (%)
Peat, coconut fibre and coconut chips (S1)	12.33 ± 0.42 a	3.06 ± 0.29	0.88 ± 0.07 a
Peat + perlite (S2)	12.16 ± 0.90 b	2.95 ± 0.16	0.69 ± 0.21 b
Standard Error	0.03	0.05	0.01

Averages followed by the same letter are statistically equal (LSD ≤ 5%).

The soluble solids content (°Brix) of the fruit from plants grown in substrate 1 presented a slightly higher value (12.33) than that in substrate 2 (12.16). Soluble solids are related to sugar content and the sensory quality of the fruit, which can be influenced by the greater water and nutritional availability of substrate 1. The presence of coarse particles, such as coconut chips, allows for rapid drainage and more precise irrigation control—conditions associated with higher sugar content in blueberries [45].

In the case of fruit pH, no differences were observed between the substrates (Table 10), suggesting that both offer suitable conditions for microbiological stability and the fruit's sensory profile [9]. It is noteworthy that substrate 2, composed of sphagnum peat and perlite, possesses a natural acidity (pH 3.5–4.5) that is beneficial for acidophilic species such as the blueberry [46].

A more marked difference was evident in titratable acidity (Table 10); substrate 1 presented a higher value (0.88%) compared to substrate 2 (0.69%). This difference could be explained by higher microbial activity and nutrient mineralisation in substrates with lignified components such as coconut chips [35,45]. Indeed, these characteristics favour the availability of nitrogen and phosphorus, which participate in the biosynthesis of organic acids like citric acid, contributing to the increase in titratable acidity in the fruit [47].

3.7. Agro-Morphological and Physicochemical Differentiation of Varieties

Since the variables 'dry matter', 'flower bud', and 'flowering-to-harvest' showed high and significant correlation with other agro-morphological and physicochemical variables (Figure 3), we removed those variables from following analyses.

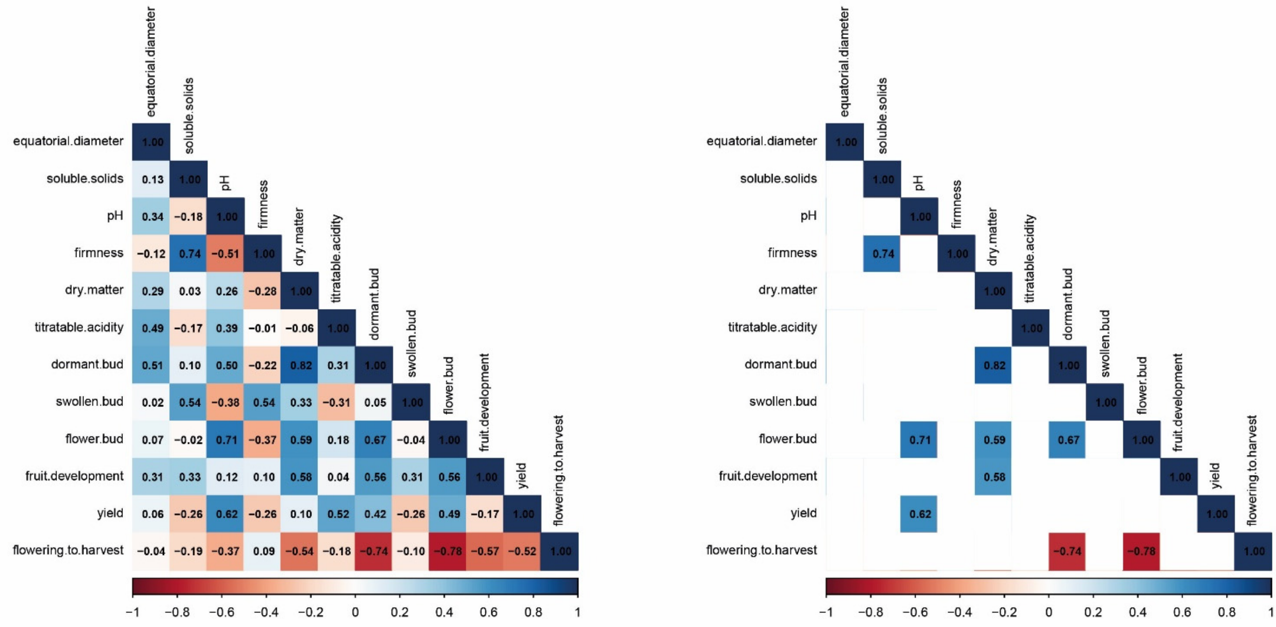
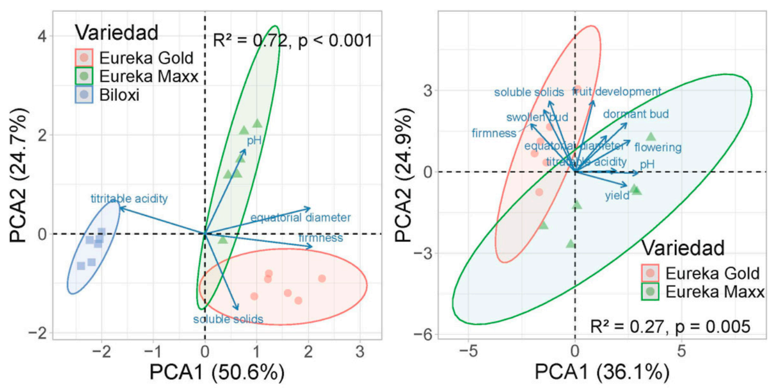


Figure 3. Pearson correlations between pairs of agro-morphological and physicochemical variables. Second panel only shows significant correlations ($p < 0.05$).

The initial PERMANOVA indicated differences between the three varieties ($p < 0.001$). The PCA showed a clear separation between Biloxi and the two Eureka varieties. This separation is apparently explained by the high acidity values of Biloxi. Meanwhile, Eureka Gold and Eureka Maxx present similarities in terms of equatorial diameter and fruit firmness but differences in terms of soluble solids and pH (Figure 4A).



(**A**) (**B**)

Figure 4. Principal component analysis (PCA). (**A**) Principal components of the physical and chemical fruit characteristics of three blueberry varieties; and (**B**) growth and development characteristics of the plant alongside physical and chemical fruit characteristics of two blueberry varieties.

As mentioned in [27], climatic factors cause certain changes that define behavioural differences among blueberry varieties. Indeed, factors such as temperature, humidity and the accumulation of chilling hours are determinants in the behaviour and adaptability of each variety [48]. Similarly, ref. [42] indicate that the cultivation site and the abiotic conditions to which the blueberries are subjected influence various fruit characteristics. Studies conducted by these authors demonstrated that climatic factors primarily influence phenological processes and fruit quality characteristics such as colour and anthocyanin content. However, the authors also emphasise that genotype has the greatest effect on blueberry varieties. This is corroborated in the present study, as Biloxi exhibits a very different pH from the Eureka varieties. Additionally, this same variable (pH) allows for a clear differentiation between Eureka Gold and Eureka Maxx when compared with each other. Although the Biloxi variety adapts to the environmental conditions of the Ecuadorian valleys, it does not achieve the best yields. A study conducted in Colombia with 36-month-old plants and evaluations over 28 weeks demonstrated that despite Biloxi having good quality characteristics (firmness, total soluble sugars and diameter), it does not present competitive yields compared to other varieties such as Sharpblue [11]. This is a relevant factor that illustrates the importance of testing other varieties like the Eureka series, which surpasses Biloxi in several characteristics. In our PCA, the Eureka varieties were located close to one another.

Regarding the second PERMANOVA, differences were detected between the two Eureka varieties (p = 0.02). The PCA also showed a separation between these two varieties. However, bud thickening, firmness and soluble solids were higher for Eureka Gold, while yield and pH were higher for Eureka Maxx (Figure 4B). When considering characteristics related to plant growth and development alongside the physicochemical fruit characteristics of the Eureka varieties, it was found that they differed primarily in yield, the appearance of thickened buds, fruit firmness and soluble solids. In general, it can be stated that the remaining characteristics are very similar.

Ref. [30] indicates that the most important characteristics in a plant breeding programme include the flowering and fruiting periods, yield, texture, shape, firmness, colour, size and Brix degrees of the fruits. In the case of the present study, these characteristics were considered in order to evaluate the blueberry varieties' ability to adapt to the conditions of the inter-Andean valleys. This is because analysing these parameters allows for efficient agronomic management during plant growth and development [8] and ensures high fruit quality for fresh consumption or agro-industrial use [5]. According to [49], both total soluble solids and pH are parameters of great importance for establishing the quality of blueberry varieties; although they vary with climatic conditions, they are more influenced by the genetic load of each variety. In this case, the pH and total solids content of Eureka Gold differed from the other evaluated varieties.

4. Conclusions

The precocity of the varieties was influenced by the evaluation sites. At EcuaBlue, Eureka Gold was more precocious during the vegetative phase, whereas at SierraBlue, Eureka Maxx exhibited this trait. The same behaviour was observed regarding yield: at EcuaBlue, the Eureka Maxx variety achieved the highest yield, while at SierraBlue, it was Eureka Gold, this was due to agronomic management.

The type of substrate also significantly influenced plant phenology and yield, highlighting the importance of crop management in enhancing productivity.

Regarding the physicochemical characterisation of the blueberry fruit, the Eureka Gold and Eureka Maxx varieties presented quality parameters that were superior to those of the Biloxi variety. Both Eureka varieties stood out for their larger calibre, firmness and soluble solids concentration—important attributes for fresh-market commercialisation and market competitiveness.

Looking towards future studies, it is important to conduct permanent evaluations of new varieties under different high-Andean environmental conditions to help identify new materials with improved production levels.

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Abbreviations

The following abbreviations are used in this manuscript:

UPOV	International Union for the Protection of New Varieties of Plants
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie

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