


## Article

# Correlation Analysis of High-Throughput Fruit Phenomics and Biochemical Profiles in Native Peppers (*Capsicum* spp.) from the Primary Center of Diversification

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**Abstract:** The main goal of this work was to investigate the relationship between the fruit morphology and biochemical composition of peppers (*Capsicum* spp.). For that purpose, one hundred native varieties from the Andean region, where the genus *Capsicum* has its origin, were analysed for different phytochemical compounds. In addition, pepper fruits were assessed with the highly precise phenomics tool Tomato Analyzer. The collection showed a broad variability which was more evident within the *C. annuum* group. On average, *C. frutescens* accessions displayed the highest levels of solid soluble content, pH, polyphenols and antioxidant activity. The Tomato Analyzer descriptors under the categories of size, shape index, and latitudinal section, mostly contributed to the variance among *Capsicum* groups. *C. annuum* hold the larger fruits, whereas *C. frutescens* comprised fruits of smaller sizes. The correlation analysis revealed that biochemical traits were negatively correlated with the fruit parameters related to size, suggesting that bigger fruits contain lower amounts of chemical metabolites. The multivariate approximations demonstrated that Andean peppers assorted according to morphometric and colorimetric characteristics, but independently of their species or geographical origin. Groups of valuable native varieties carrying promising traits were identified.

**Keywords:** solid soluble content; acidity; antioxidant capacity; polyphenols; tomato analyzer; correlation analysis



**Citation:** González-López, J.; Rodríguez-Moar, S.; Silvar, C. Correlation Analysis of High-Throughput Fruit Phenomics and Biochemical Profiles in Native Peppers (*Capsicum* spp.) from the Primary Center of Diversification. *Agronomy* **2021**, *11*, 262. <https://doi.org/10.3390/agronomy11020262>

Academic Editor: Susan A.

H. McCallum

Received: 18 November 2020

Accepted: 27 January 2021

Published: 30 January 2021

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## 1. Introduction

The genus *Capsicum* belongs to the Solanaceae family and currently harbors more than 35 species, five of which (*C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L., and *C. pubescens* Ruiz et Pav.) are domesticated [1]. *Capsicum* originates from South America and its genesis apparently occurred in a broad area including Peru, Ecuador and Colombia, along the Andes in north-western South America [2]. According to Carrizo García et al. [2], the subsequent expansion of the genus took place following around the Amazon basin, towards central and south-eastern Brazil, coming back to western South America, and finally northwards to Central America. Therefore, the western Andes of South America remained as a significant center of speciation and northward species dispersal, primarily for *C. annuum* [2,3]. After the voyages of exploration to the New World, peppers moved worldwide, suffering from additional diversification at the secondary centers. These phenomena resulted in the vast phenotypic variability that can be observed nowadays in the form of native varieties or landraces, well adapted to specific agro-climatic conditions and consumers habits [1]. *C. annuum* was the most successful in this expansion, giving rise to sweet and hot pepper varieties spread all over the world. However, *C. pubescens* and *C. baccatum* remained mostly restricted to Andean South America and the Central American highlands, while *C. frutescens* and *C. chinense* are mainly cultivated as spice crops in Africa, Asia and South America [1]. Consequently, landraces exist worldwide and they likely maintain combinations of traits that were left behind when growers selected modern

cultivars. In this context, it is foreseeable that native varieties from the primary centers of *Capsicum* speciation, i.e., Ecuador, Peru and Bolivia, preserved a higher portion of the wealth stored on primitive peppers, and therefore constitute a promising resource for the breeding of new market types with added value.

Peppers are one of the most important vegetables worldwide due to their versatility for cuisine, medicine and industry [4]. The five cultivated *Capsicum* species are today grown globally both as a spice and as a vegetable crop. Sweet, bell peppers have more presence in Europe and North America, while pungent varieties currently dominate the markets in South-Eastern Asia, South America, and Africa [5]. For consumption as a vegetable, most of the pepper diversity was addressed to develop fruits with a sweet taste, large size, blocky shape, thick pericarp and bright colors. In spicy chilli peppers, the predominant selected characters are hotness, fruit size and skin thickness [6]. For industrial food purposes, pepper should be dehydrated and powered, so that water content represents an essential parameter. In turn, pepper powder must possess the adequate spiciness and a specific color [7]. On the other hand, consumers are becoming increasingly interested in tastier vegetables containing healthier components and higher nutritional quality [8]. Many studies have been performed to unravel the biochemical composition of pepper fruits and this crop has demonstrated to be an important source of bioactive compounds, which possess analgesic, anti-obesity, cardio-protective, pharmacological, neurological and dietetic properties [9]. Nevertheless, the search for novel pepper genotypes with tastier fruits, more attractive colours, containing the required pungency and high concentrations of health-promoting components still remains relevant. This might help breeding programs to improve the existing commercial types, expanding the opportunities for new market segments and increasing the profitability of producers.

The organoleptic and bioactive properties of pepper fruits are largely due to the presence of phytochemical compounds. To a large extent, sugars and acids will determine the sensory quality of fruits connected to flavor, while other compounds such as carotenoids and polyphenols are responsible for the visual aspect and health-related attributes of peppers. Many of the functional features of these berries are largely attributed to the antioxidant activity of different metabolites, which play a crucial role in scavenging free radicals responsible for oxidative damage [10]. All these attributes will play a key influence on how consumers perceive the quality of the product and decide their preferences. Apart from adequate characterization of compositional profiles, an extensive and detailed assessment of fruit phenomics will also be essential since fruit size, shape and weight will largely influence the establishment of suitable market niches and the good acceptance of new pepper varieties [11]. Among the lately developed high-throughput phenotyping tools, the Tomato Analyzer (TA) software has emerged as an extremely useful system to perform accurate phenomics assessment in Solanaceae. This freeware allows semi-automatic and highly accurate scoring of a large number of quantitative traits from scanned images of fruit sections [12,13]. Although this software was initially conceived for tomato [14–16], it has been successfully employed to evaluate fruits of eggplant [17,18], and pepper [19–23]. Diversity measured by fruit TA descriptors demonstrated to be higher than that reflected by conventional descriptors. This is due to the fact that automated phenotyping covers a broader dissection of fruit architecture, allowing to measure morphometric attributes which are nearly impossible to obtain manually [16,21,24].

Several works focused on phenotypic characterization of diverse *Capsicum* spp. collections and their biochemical compositions have been conducted to date. However, both assessments are usually accomplished separately. As far as we know, few reports have attempted to establish some correlations between fruit morphology and chemical metabolites and this was never the primary aim of those studies [25–27]. Nevertheless, this knowledge is fundamental, as it will strongly determine the speed at which promising traits could be integrated into commercial pepper types. In a recent work, we employed a small collection of Ecuadorian peppers to draw relationships between the morphometrics of pepper fruits and their content in bioactive compounds [23]. Herein, we analyzed the biochemical

composition of a wider panel of peppers (*Capsicum* spp.), and accurately determined the fruit phenomics by using a high-throughput digital assessment. Linkages between both datasets were determined. For that purpose, a broad collection of native varieties from north-western South America, which likely constitutes the origin of *Capsicum*, was used.

## 2. Materials and Methods

### 2.1. Plant Material

One hundred accessions depicting domesticated species and their ancestors were selected for this work: *C. annuum* (22), *C. annuum* var. *glabriusculum* (1), *C. annuum* wild (4), *C. chinense* (25), *C. frutescens* (12), *C. pubescens* (13), *C. baccatum* (4), *C. baccatum* var. *pendulum* (16), and *C. baccatum* var. *baccatum* (3). Forty-three were from Ecuador, 28 from Peru, and 18 from Bolivia. Eleven *C. annuum* accessions from Mexico were also included for comparative purposes (Table S1). Accessions were kindly provided by the USDA-ARS Plant Genetic Resources Conservation Unit (USA), the Institute for Conservation and Improvement of Valencian Agro-diversity (COMAV, Spain), the Centre for Genetic Resources (CGN, The Netherlands), and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Germany). The accession PM647 was kindly supplied by Dr. Alain Palloix (rest in peace) from the French National Institute for Agriculture, Food, and Environment (INRAE, France). When possible, they were selected to cover broad geographical regions within each country. Data on taxonomical classification and population type of each accession were extracted from genebanks databases. This collection was previously characterized for genetic diversity and molecular markers linked to disease resistance and pungency-related traits [28–30]. *C. annuum* accessions from the collection were split into pungent and non-pungent fruits according to the results of marker MAP1, linked to the *Pun1* gene [31], as previously described [30].

The accessions were cultivated in a greenhouse during 2017 at the Centro de Investigaciones Agrarias de Mabegondo (Mabegondo, A Coruña, Spain) (43°15' N, 8°18' W). Four plants per accession were grown following a completely randomized design. Plants were drip irrigated and fertilized with a mix of nitrogen, phosphorus, and potassium before and after transplanting. Phytosanitary treatments against whiteflies, aphids, and spider mites were applied when necessary.

### 2.2. Conventional Descriptors Assessment

Accessions were characterized by using six conventional morphological descriptors for *Capsicum* following the protocol described by the International Plant Genetic Resources Institute (IPGRI) [32]. These comprised plant height (PHE), plant width (PWI), stem length (SLE), stem diameter (SDI), fruit weight (FEW) and fruit pedicel length (FPL). All parameters were measured in centimeters (cm), except FEW, which was expressed in grams (g). Between ten and twenty-five fruits per accession, depending on their size, were harvested at maturity stage and employed for conventional characterization in the laboratory.

### 2.3. Fruit Characterization with Tomato Analyzer

Mature fruits, from 10 to 25 per accession, were subjected to digital phenotyping. Pepper fruits were longitudinally and transversally cut and scanned with an HP Scanjet G3110 photo scanner (Hewlett-Packard, San Jose, CA, USA) at a resolution of 300 dpi. Stored images (TIF format) were subsequently analyzed using Tomato Analyzer version 4 software [12,13]. A total of 49 fruit parameters, categorized into: basic measurements (7), fruit shape index (3), blockiness (3), homogeneity (3), proximal fruit end shape (4), distal fruit end shape (4), asymmetry (6), internal eccentricity (5), latitudinal section (5), and average color values (9) were automatically recorded (Table S2). A complete description of morphometric and colorimetric descriptors can be found elsewhere [12–14,33]. Default settings were used for all categories, although points were adjusted manually when the software was unable to accurately identify the outline of a trait. Individual measures of each fruit were used to obtain an average value for the corresponding accession.

#### 2.4. Biochemical Methods

Ripe fruits were collected from different plants of the same accession and pooled into two bulks. Each group was homogenized with a blender, and filtered through a gauze. The resulting pepper juice was used for analyses. All analytical measures were performed in triplicate.

##### 2.4.1. Soluble Solid Content, pH, and Titratable Acidity

The soluble solid content (SSC) was determined using a hand refractometer (Shibuya Optical Co., Ltd., Saitama, Japan) and expressed as equivalent °Brix. The pH was measured by direct reading using a pH meter (Crison micropH 2.000, Barcelona, Spain). Titratable acidity was calculated by titrating 25 mL pepper juice against 0.1 N NaOH, to an end point of pH 8.2, as indicated by a pH meter. Titratable acidity was expressed as percentage of acidity, using malic acid as an equivalent. Fresh pepper juice was used directly for these analyses.

##### 2.4.2. Determination of Total Polyphenol Content

The Folin–Ciocalteu method was applied according to Singleton and Rossi [34] with slight modifications. Briefly, 1 mL on pepper juice was centrifuged at  $13,000\times g$  rpm for 5 min. Then, 50  $\mu$ L supernatant was diluted with 750  $\mu$ L distilled water and mixed with 50  $\mu$ L Folin–Ciocalteu reagent. After an incubation time of 3 min, 150  $\mu$ L of 20%  $\text{Na}_2\text{CO}_3$  was added. The mixture was shaken vigorously and incubated for 2 h at room temperature in the dark. Absorbance against a blank was measured at 760 nm using a Thermo Helios spectrophotometer. The standard curve was constructed with gallic acid. The results were expressed as gallic acid equivalents (GAE) in  $\mu\text{g/g}$  fresh weight (FW).

##### 2.4.3. Trolox Equivalent Antioxidant Capacity (TEAC)

The procedure described by Miller and Rice-Evans [35], with slight modifications, was employed. Briefly, 1 mL on pepper juice was centrifuged at  $13,000\times g$  rpm for 5 min. Then, 20  $\mu$ L supernatant was mixed with 980  $\mu$ L of the diluted 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-radical solution. After an incubation time of 15 min at room temperature in darkness, the absorbance was read at 734 nm. The ABTS radical stock solution was prepared by combining 50 mL of 7 mM ABTS and 50 mL of 2.45 mM  $\text{K}_2\text{O}_8\text{S}_2$ . The mixture was placed in the dark at room temperature for 16 h to generate the radical. One milliliter of ABTS stock solution was diluted with approximately 50 mL water and the absorbance was adjusted to 0.9 at 734 nm before use. Trolox was employed for external calibration and results were expressed as  $\mu\text{g}$  Trolox/g FW.

##### 2.4.4. Determination of Moisture Content and Dry Mass

Two bulks of ripe fruits per each accession comprising a variable number between 10 and 25 were independently subjected to freeze-drying in a Telstar-Cryodos-80 lyophilizer (Telstar, Barcelona, Spain) for 24–48 h. Moisture content was calculated as the difference in the sample mass before and after drying, expressed as percentage. Fruit dry weight (FDW) was determined by dividing the total dry matter by the number of fruits at each bulk.

#### 2.5. Data Analysis

Analysis of variance (ANOVA) was performed for conventional descriptors, biochemical and digital parameters. Means, standard deviations, and coefficient of variation (expressed in percentage as the ratio between standard deviation and mean) were used for descriptive analysis of traits. Significant differences among means were detected using Tukey's HSD test ( $p < 0.05$ ). Statistical analyses were performed with Statistical Product and Service Solutions (SPSS) version 17.0 software [36]. Correlations among all evaluated parameters were estimated by using Pearson's test at  $p < 0.05$ , after Bonferroni's adjustment for multiple comparisons [37]. The calculation of coefficients and visualization of correlograms were performed with Rcmdr and Corrplot packages implemented in R 4.0.0



software [38]. Conventional, TA and biochemical traits were comprehensively examined through a principal component analysis (PCA). The similarity across accessions was estimated by agglomerative hierarchical cluster analysis (HCA) using the Ward coefficient. In addition, a Principal Coordinate Analysis (PCoA) for each *Capsicum* spp. was performed. Multivariate analyses were conducted with the computer software SPSS version 17.0 and R 4.0.0 (packages Stats, Vegan, and Ape). Graphical representation of the tree was performed with MEGA X software [39].

### 3. Results

#### 3.1. Biochemical Analysis

Seven parameters were quantified on eighty-two pepper accessions (Table S3). The ANOVA found highly significant differences ( $p < 0.001$ ) among them, although the greatest part of the variance was explained by fruit dry weight and antioxidant activity. The FDW ranged from 0.08 g to 6.63 g, with the lowest values being reached by CGN20808 and CAP524, and the highest by C87, all belonging to *C. annuum*. The average value for Trolox equivalent antioxidant capacity (TEAC) was 615.09  $\mu\text{g}$  Trolox/g FW, varying from 84.86  $\mu\text{g}$  Trolox/g FW in BGV005890 (*C. chinense*) to 4126.63  $\mu\text{g}$  Trolox/g FW in the *C. annuum* accession CAP524. Values for the pH ranged from 4.7 in PI543184 to 6.3 in BGV014749, both *C. chinense* accessions. Total polyphenols content showed an average of 916.36  $\mu\text{g}$  GAE/g FW, varying from 286.06  $\mu\text{g}$  GAE/g FW in BGV005890 (*C. chinense*) to 3706.32  $\mu\text{g}$  GAE/g FW in PI585257 (*C. frutescens*). The *C. annuum* accession CAP524 showed the highest values for antioxidant capacity (4126.62  $\mu\text{g}$  Trolox/g FW), titratable acidity (0.57%) and SSC (11.98 °Brix), while the *C. chinense* accession BGV005890 exhibited the lowest values for those parameters, i.e., TEAC of 84.86  $\mu\text{g}$  Trolox/g FW, acidity of 0.06% and SSC of 3.25 °Brix (Table S1). The average value for the moisture content was 82.06%, the highest being observed for the *C. annuum* accession C87 (90.29%), while the lowest (54.85%) for *C. baccatum* CGN17042.

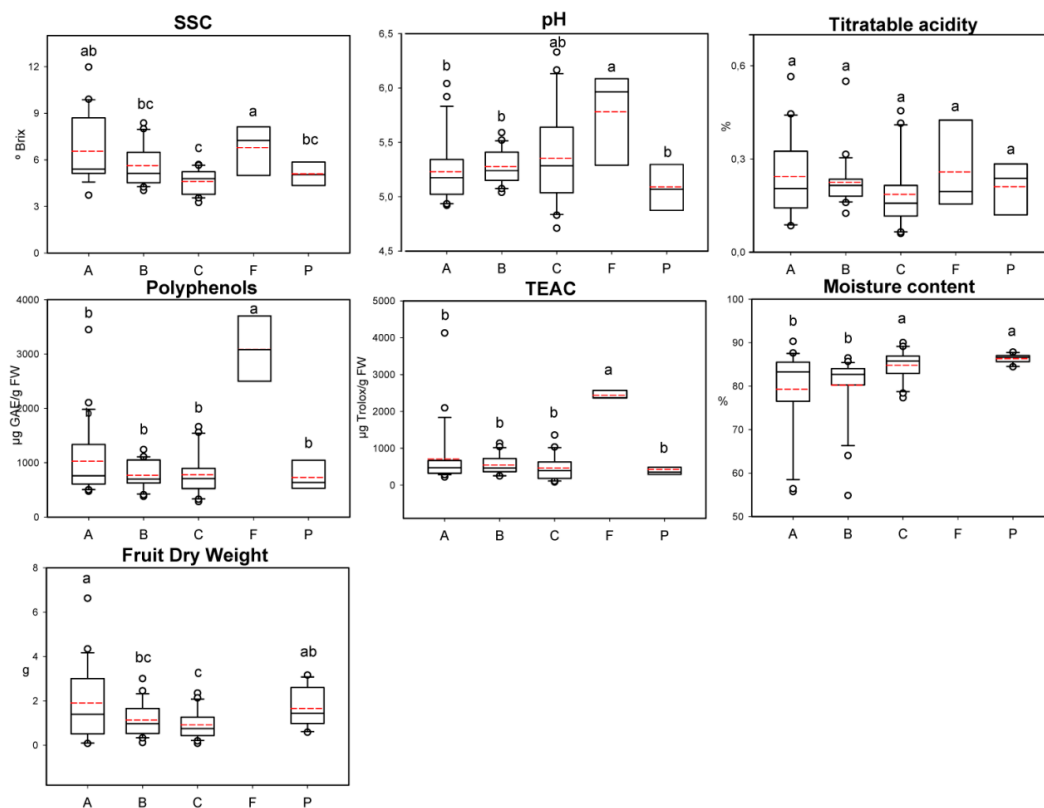
The analysis of variance, considering the species effect, indicated significant differences ( $p < 0.001$ ) among *Capsicum* spp. for all analytical measures, except for titratable acidity (Figure 1). *C. frutescens* showed the highest significant values for total phenols (3117.32  $\mu\text{g}$  GAE/g FW) and antioxidant capacity (2495.44  $\mu\text{g}$  Trolox/g FW). *C. frutescens* and *C. annuum* possessed the highest amounts of SSC, at 7.69 and 6.55 °Brix, respectively, while the greatest values for pH were recorded in *C. frutescens* (5.62) and *C. chinense* (5.38). The greatest percentages of moisture content were observed in *C. pubescens* (86.31%) and *C. chinense* (84.76%), and the highest FDW was recorded for *C. annuum* accessions (average value of 1.90 g) (Figure 1). The broadest variability was observed within the *C. annuum* group, which exhibited highest coefficients of variation for all traits except pH.

#### 3.2. Morphological Assessment with Conventional and TA Descriptors

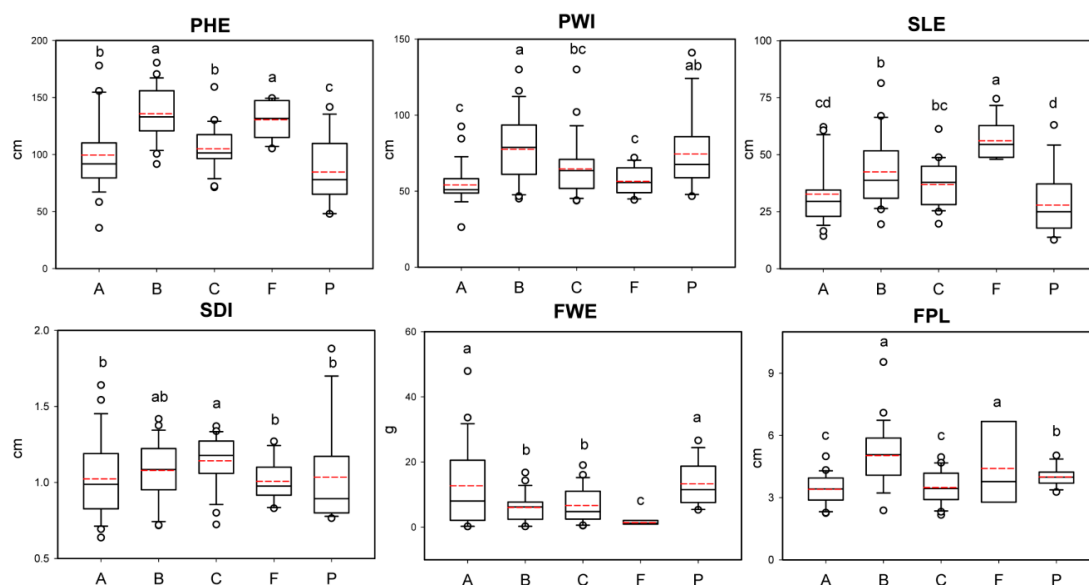
A great diversity was observed within the Andean collection after the evaluation with six quantitative conventional descriptors. The parameters which most contributed to explain the variance among genotypes were PHE, FWE, and FPL (Table S4). Thus, the PHE showed an average value of 110.36 cm, ranging from 35.67 cm in *C. annuum* accession C87 to 180.50 cm in *C. baccatum* var. *pendulum* PI257133. The weightiest fruits were recorded for C87 (*C. annuum*) with an average value of 47.89 g per fruit, while the weightless ones (0.20 g per fruit) were found in *C. annuum* var. *glabriusculum* PM647. The *C. chinense* accession CAP472 possessed the shortest FPL (2.17 cm) whereas the longest were observed for *C. baccatum* CAP1366 (9.54 cm). A coefficient of variation up to 97.92% was recorded for few, while for the remaining traits was lower than 40% (Table S4).

Belonging to a particular *Capsicum* species certainly determined the morphological variation among accessions. Thus, significant differences among the five species were identified for all traits (Figure 2). *C. baccatum* and *C. frutescens* accessions showed the significantly highest plant heights and stem lengths, while the shortest were observed for

*C. pubescens*. A difference of up to 47 g was observed for FWE between the smallest *C. frutescens* and the biggest *C. annuum* fruits (Figure 2).



**Figure 1.** Box plot of biochemical parameters. Twenty-fifth percentile, median, 75th percentile, and range minimum–maximum. Outliers (white circle) were identified as 1.5 times the interquartile range. Red dashed lines represent the mean. Different letters indicate significant differences at  $p < 0.05$ . A = *C. annuum*, B = *C. baccatum*, C = *C. chinense*, F = *C. frutescens*, P = *C. pubescens*. Note that *C. frutescens* is not represented for moisture content and fruit dry weight (FDW) because sufficient fruits were not available for the calculation of those parameters.



**Figure 2.** Box plot of conventional descriptors. Twenty-fifth percentile, median, 75th percentile, and range minimum–maximum. Outliers (white circle) were identified as 1.5 times the interquartile range. Red dashed lines represent the mean. Different letters indicate significant differences at  $p < 0.05$ . A = *C. annuum*, B = *C. baccatum*, C = *C. chinense*, F = *C. frutescens*, P = *C. pubescens*.

The Tomato Analyzer software was employed for the assessment of fruit morphology by image examination of scanned fruit sections. Highly significant differences ( $p < 0.001$ ) were detected among average values, except for proximal angle micro (PAMI), which exhibited differences at  $p < 0.05$  (Table S5). Those attributes related to fruit size and shape, i.e., those under the categories of basic measurements, shape index, and latitudinal section, primarily explained the variance. Minimum values of zero were recorded for proximal indentation area (PIA), distal indentation area (DIA), obovoid (Ob), and H. Asymmetry. Ob (HAob), The coefficient of variation ranged from 1.89% (proximal eccentricity, PEC) to 263.39% (HAob). For each category, the descriptors with largest variability were: Area (75.30%), Fruit Shape Index Internal (48.02%), Distal End Protusion (86.43%), Tomato Pericarp Area (72.23%), Curved Fruit Shape Index (52.84%), Fruit Shape Triangle (32.72%), circular (38.81%), Proximal Indentation Area (116.14%), H. Asymmetry. Ob (263.39%), and Average a Value (AaV) (42.36%) (Table S5). The color analysis demonstrated that all color parameters displayed distinctive variations among accessions. RGB and CIELab colour spaces indicated larger amounts of redness and yellowness components. The Hue value ranged from  $45.91^\circ$  to  $102.87^\circ$  suggesting that the colors in the collection varied from red-orange to yellow-green. The majority of fruits showed moderate lightness (average luminosity value (ALV) = 50.10) and saturation of colors (average chroma (Ach) = 54.16) (Table S5).

As occurred with conventional traits, broad variability was found among the five domesticated species. Forty-four out of 49 TA descriptors exhibited highly significant differences ( $p < 0.001$ ) while divergences at  $p < 0.01$  were observed for HAob and tomato pericarp thickness ratio (TPTR). No significant differences were recorded for PAMI, DIA, and PEC, (Table 1). In general, *C. frutescens* and *C. annuum* showed the lowest and highest values, respectively, for the majority of fruit size features, including those under the basic measurements and latitudinal section categories. Considering the shape, measured as indexes fruit shape index external I (FSIEI), fruit shape index external II (FSIEII), FSI, and fruit shape index internal (FSII), the highest and significantly different values were recorded for *C. annuum* and *C. baccatum* (Table 1). This suggest that pepper fruits from those species displayed much larger height than width, whereas in *C. frutescens*, *C. chinense* and *C. pubescens* fruits, both attributes were not so dissimilar. Accessions from *C. pubescens* displayed the most circular and ellipsoid fruits, while the most rectangular were exhibited by *C. annuum*. The largest variability, according to the coefficient of variation, was recorded for *C. annuum*, which displayed CV higher than 50% for twenty-three traits. Luminosity and average luminosity values indicated that fruits from *C. chinense* accessions possess the brightest colors, while those from *C. pubescens* were the darkest. *C. pubescens* fruits displayed the highest amounts of red colour (higher AaV), while *C. chinense* fruits tended to be the most yellowish (higher Average b Value (AbV)). All species were included in the orange Hue angle range. Values for Chroma did not differ greatly, although significantly more vivid external colors were observed in *C. frutescens* fruits when compared to *C. annuum* (Table 1).

**Table 1.** Analysis of variance for Tomato Analyzer (TA) descriptors. Mean and coefficient variation (in italics) for each *Capsicum* species. Different letters within the same row indicate significant differences at  $p < 0.05$ . See Table S2 for trait acronyms.

| Trait  | Sum of Squares | F value <sup>†</sup> | <i>C. annuum</i> |    | <i>C. baccatum</i> |    | <i>C. chinense</i> |    | <i>C. frutescens</i> |    | <i>C. pubescens</i> |    |
|--------|----------------|----------------------|------------------|----|--------------------|----|--------------------|----|----------------------|----|---------------------|----|
| P      | 429,598.7      | 32.0 ***             | 159.56           | a  | 138.72             | ab | 121.49             | b  | 74.05                | c  | 125.77              | b  |
|        |                |                      | 50.21            |    | 39.36              |    | 36.26              |    | 25.55                |    | 19.51               |    |
| A      | 66,629,009.1   | 34.8 ***             | 1242.11          | a  | 840.47             | b  | 760.86             | b  | 300.00               | c  | 946.34              | ab |
|        |                |                      | 84.79            |    | 59.83              |    | 62.87              |    | 42.02                |    | 38.09               |    |
| WMH    | 14,766.4       | 50.6 ***             | 19.05            | b  | 16.51              | bc | 19.37              | b  | 12.74                | c  | 26.59               | ab |
|        |                |                      | 60.23            |    | 37.33              |    | 41.04              |    | 14.90                |    | 24.65               |    |
| MW     | 13,637.9       | 39.1 ***             | 24.44            | ab | 19.54              | c  | 22.27              | bc | 14.39                | d  | 28.50               | ab |
|        |                |                      | 53.63            |    | 35.34              |    | 35.77              |    | 20.07                |    | 22.28               |    |
| HMW    | 70,333.5       | 37.7 ***             | 56.29            | a  | 52.91              | a  | 43.00              | b  | 25.84                | c  | 40.43               | b  |
|        |                |                      | 49.92            |    | 41.75              |    | 39.75              |    | 26.67                |    | 25.50               |    |
| MH     | 100,288.6      | 44.7 ***             | 62.33            | a  | 56.97              | a  | 45.83              | b  | 26.55                | c  | 43.10               | b  |
|        |                |                      | 50.32            |    | 42.25              |    | 39.89              |    | 26.29                |    | 23.96               |    |
| CH     | 96,081.5       | 42.7 ***             | 64.07            | a  | 58.18              | ab | 47.71              | bc | 28.72                | d  | 45.15               | c  |
|        |                |                      | 49.31            |    | 41.10              |    | 38.31              |    | 24.87                |    | 22.80               |    |
| FSIEI  | 330.4          | 108.6 ***            | 2.69             | a  | 2.96               | a  | 2.18               | b  | 1.84                 | bc | 1.57                | c  |
|        |                |                      | 34.78            |    | 31.89              |    | 40.60              |    | 15.44                |    | 29.06               |    |
| FSIEII | 579.7          | 79.7 ***             | 3.36             | a  | 3.32               | a  | 2.53               | b  | 2.01                 | bc | 1.61                | c  |
|        |                |                      | 51.53            |    | 35.40              |    | 52.56              |    | 19.30                |    | 35.44               |    |
| CFSI   | 789.9          | 73.1 ***             | 3.90             | a  | 3.67               | a  | 2.83               | b  | 2.21                 | bc | 1.78                | c  |
|        |                |                      | 56.95            |    | 37.12              |    | 54.88              |    | 18.62                |    | 33.60               |    |
| PFB    | 11.4           | 74.7 ***             | 1.03             | a  | 0.84               | b  | 0.84               | b  | 0.83                 | b  | 0.82                | b  |
|        |                |                      | 24.42            |    | 17.14              |    | 23.31              |    | 15.50                |    | 17.49               |    |
| DFB    | 0.7            | 7.01 ***             | 0.589            | b  | 0.544              | b  | 0.550              | b  | 0.668                | a  | 0.565               | b  |
|        |                |                      | 29.31            |    | 24.83              |    | 35.05              |    | 13.61                |    | 16.75               |    |
| FST    | 33.0           | 7.6 ***              | 1.895            | a  | 1.647              | ab | 1.807              | a  | 1.249                | b  | 1.492               | ab |
|        |                |                      | 47.00            |    | 31.02              |    | 89.90              |    | 17.21                |    | 26.18               |    |
| E      | 0.2            | 47.4 ***             | 0.098            | a  | 0.079              | b  | 0.079              | b  | 0.067                | b  | 0.065               | b  |
|        |                |                      | 39.65            |    | 32.28              |    | 41.15              |    | 26.55                |    | 25.70               |    |
| C      | 4.7            | 135.6 ***            | 0.313            | a  | 0.319              | a  | 0.245              | b  | 0.219                | b  | 0.157               | c  |
|        |                |                      | 27.40            |    | 27.87              |    | 45.45              |    | 19.64                |    | 49.82               |    |
| R      | 0.5            | 16.9 ***             | 0.418            | c  | 0.423              | c  | 0.428              | bc | 0.467                | ab | 0.476               | a  |
|        |                |                      | 24.30            |    | 19.96              |    | 20.17              |    | 17.59                |    | 13.21               |    |
| SH     | 0.0            | 12.8 ***             | 0.018            | ab | 0.011              | b  | 0.013              | b  | 0.013                | b  | 0.027               | a  |
|        |                |                      | 166.91           |    | 228.81             |    | 197.08             |    | 121.20               |    | 101.67              |    |
| PAMI   | 30,505.5       | 1.1 ns               | 134.39           | a  | 129.38             | a  | 124.94             | a  | 121.95               | a  | 136.91              | a  |
|        |                |                      | 67.99            |    | 52.05              |    | 65.88              |    | 76.47                |    | 68.43               |    |
| PAMA   | 312,234.0      | 27.2 ***             | 99.45            | c  | 84.53              | c  | 107.75             | bc | 134.32               | a  | 128.90              | ab |
|        |                |                      | 62.02            |    | 45.45              |    | 49.49              |    | 27.07                |    | 48.68               |    |
| PIA    | 0.3            | 34.15 ***            | 0.030            | b  | 0.011              | b  | 0.012              | b  | 0.011                | b  | 0.054               | a  |
|        |                |                      | 230.80           |    | 258.98             |    | 220.87             |    | 121.01               |    | 127.85              |    |
| Ob     | 0.1            | 7.4 ***              | 0.004            | c  | 0.015              | ab | 0.022              | a  | 0.009                | bc | 0.007               | c  |
|        |                |                      | 870.35           |    | 362.11             |    | 342.52             |    | 313.75               |    | 476.11              |    |
| Ov     | 5.7            | 71.9 ***             | 0.381            | a  | 0.240              | b  | 0.254              | b  | 0.212                | b  | 0.261               | b  |
|        |                |                      | 40.97            |    | 54.41              |    | 56.73              |    | 60.64                |    | 43.68               |    |
| VAs    | 2.1            | 26.5 ***             | 0.223            | a  | 0.163              | ab | 0.147              | bc | 0.086                | c  | 0.120               | bc |
|        |                |                      | 89.66            |    | 71.02              |    | 79.25              |    | 71.18                |    | 56.92               |    |
| HAob   | 0.3            | 4.7 **               | 0.007            | c  | 0.026              | ab | 0.037              | a  | 0.010                | bc | 0.006               | c  |
|        |                |                      | 993.76           |    | 481.46             |    | 436.67             |    | 326.04               |    | 470.86              |    |
| HAov   | 47.3           | 61.2 ***             | 0.757            | a  | 0.526              | b  | 0.382              | bc | 0.181                | c  | 0.281               | c  |
|        |                |                      | 79.88            |    | 82.77              |    | 83.32              |    | 71.74                |    | 59.60               |    |
| WWP    | 3.9            | 58.8 ***             | 0.277            | b  | 0.392              | a  | 0.391              | a  | 0.370                | a  | 0.356               | a  |
|        |                |                      | 47.07            |    | 33.39              |    | 35.10              |    | 30.95                |    | 27.42               |    |
| DAMI   | 249,026.5      | 13.82 ***            | 87.48            | ab | 77.38              | b  | 98.19              | ab | 108.29               | a  | 117.08              | a  |
|        |                |                      | 76.51            |    | 89.84              |    | 69.80              |    | 41.31                |    | 51.32               |    |
| DAMA   | 450,771.9      | 57.0 ***             | 57.69            | bc | 52.16              | c  | 76.91              | b  | 119.21               | a  | 100.75              | a  |
|        |                |                      | 71.33            |    | 84.33              |    | 65.28              |    | 52.39                |    | 36.42               |    |



Table 1. Cont.

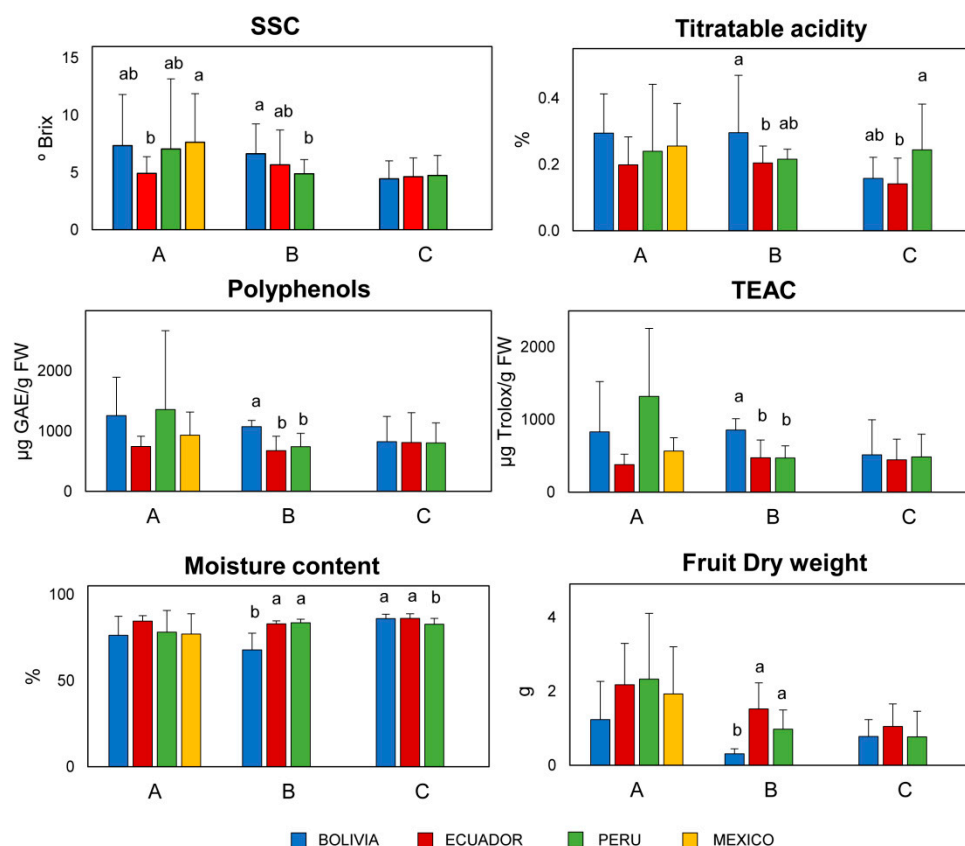
| Trait | Sum of Squares | F value <sup>†</sup> | <i>C. annuum</i> |    | <i>C. baccatum</i> |    | <i>C. chinense</i> |    | <i>C. frutescens</i> |    | <i>C. pubescens</i> |    |
|-------|----------------|----------------------|------------------|----|--------------------|----|--------------------|----|----------------------|----|---------------------|----|
| DIA   | 0.0            | 0.5 <sup>ns</sup>    | 0.004            | a  | 0.004              | a  | 0.004              | a  | 0.005                | a  | 0.003               | a  |
|       |                |                      | 293.85           |    | 131.82             |    | 266.24             |    | 137.72               |    | 216.82              |    |
| DEP   | 5.9            | 42.6 ***             | 0.101            | b  | 0.221              | a  | 0.104              | b  | 0.059                | b  | 0.041               | b  |
|       |                |                      | 187.84           |    | 97.51              |    | 179.04             |    | 143.39               |    | 227.32              |    |
| EC    | 0.2            | 14.8 ***             | 0.733            | b  | 0.748              | ab | 0.755              | ab | 0.768                | a  | 0.762               | a  |
|       |                |                      | 9.19             |    | 6.38               |    | 6.35               |    | 2.90                 |    | 4.36                |    |
| PEC   | 0.0            | 1.4 <sup>ns</sup>    | 0.899            | a  | 0.895              | a  | 0.893              | a  | 0.888                | a  | 0.890               | a  |
|       |                |                      | 9.39             |    | 4.02               |    | 4.17               |    | 1.25                 |    | 0.55                |    |
| DEC   | 0.1            | 5.2 ***              | 0.911            | a  | 0.903              | a  | 0.900              | a  | 0.891                | a  | 0.886               | a  |
|       |                |                      | 11.01            |    | 5.68               |    | 5.22               |    | 2.52                 |    | 1.33                |    |
| FSII  | 557.7          | 78.56 ***            | 3.34             | a  | 3.30               | a  | 2.51               | b  | 1.99                 | bc | 1.63                | c  |
|       |                |                      | 50.51            |    | 35.74              |    | 52.99              |    | 17.90                |    | 34.42               |    |
| ECA   | 0.8            | 51.6 ***             | 0.477            | a  | 0.441              | b  | 0.424              | b  | 0.437                | b  | 0.422               | b  |
|       |                |                      | 14.60            |    | 11.77              |    | 16.52              |    | 7.31                 |    | 9.19                |    |
| LD    | 94,434.3       | 109.1 ***            | 31.88            | a  | 34.82              | a  | 22.61              | b  | 16.39                | bc | 11.70               | c  |
|       |                |                      | 50.12            |    | 44.20              |    | 67.60              |    | 25.84                |    | 67.23               |    |
| TPA   | 6,660,826.0    | 21.1 ***             | 474.05           | a  | 359.14             | ab | 326.27             | b  | 140.59               | c  | 422.15              | ab |
|       |                |                      | 88.91            |    | 60.27              |    | 58.90              |    | 42.69                |    | 36.99               |    |
| TPAR  | 0.1            | 14.2 ***             | 0.417            | b  | 0.424              | ab | 0.434              | ab | 0.439                | a  | 0.439               | a  |
|       |                |                      | 12.84            |    | 8.98               |    | 10.41              |    | 6.88                 |    | 3.59                |    |
| TPT   | 173.5          | 37.6 ***             | 2.72             | b  | 2.61               | b  | 2.76               | b  | 1.98                 | c  | 3.64                | a  |
|       |                |                      | 53.88            |    | 33.27              |    | 33.65              |    | 20.00                |    | 19.47               |    |
| TPTR  | 0.0            | 4.32 **              | 0.212            | a  | 0.206              | a  | 0.206              | a  | 0.204                | a  | 0.203               | a  |
|       |                |                      | 17.80            |    | 10.30              |    | 17.10              |    | 5.87                 |    | 6.72                |    |
| AR    | 104,422.8      | 99.5 ***             | 175.39           | b  | 185.74             | a  | 185.45             | a  | 180.04               | ab | 161.58              | c  |
|       |                |                      | 8.89             |    | 9.04               |    | 9.08               |    | 7.71                 |    | 9.25                |    |
| AG    | 312,572.3      | 101.8 ***            | 87.62            | b  | 100.76             | a  | 109.18             | a  | 87.04                | b  | 64.68               | c  |
|       |                |                      | 20.08            |    | 25.49              |    | 34.29              |    | 33.48                |    | 38.45               |    |
| AB    | 45,985.1       | 40.5 ***             | 42.26            | ab | 40.05              | ab | 47.21              | a  | 36.07                | bc | 29.27               | c  |
|       |                |                      | 29.45            |    | 29.66              |    | 53.24              |    | 39.36                |    | 37.53               |    |
| AL    | 57,469.9       | 104.3 ***            | 102.40           | b  | 106.23             | ab | 109.47             | a  | 101.66               | b  | 89.71               | c  |
|       |                |                      | 10.66            |    | 8.19               |    | 13.90              |    | 11.43                |    | 11.49               |    |
| ALV   | 28,364.8       | 116.9 ***            | 47.80            | b  | 52.17              | a  | 54.21              | a  | 48.52                | b  | 40.99               | c  |
|       |                |                      | 11.62            |    | 14.30              |    | 18.68              |    | 15.77                |    | 16.53               |    |
| AaV   | 23,304.1       | 47.6 ***             | 28.78            | ab | 26.05              | bc | 21.74              | c  | 30.40                | ab | 33.79               | a  |
|       |                |                      | 22.09            |    | 35.27              |    | 72.80              |    | 32.07                |    | 30.71               |    |
| AbV   | 10,312.7       | 44.2 ***             | 43.36            | b  | 48.29              | a  | 47.36              | a  | 46.92                | a  | 41.41               | b  |
|       |                |                      | 11.02            |    | 18.63              |    | 19.53              |    | 9.90                 |    | 13.67               |    |
| AHue  | 38,146.3       | 61.9 ***             | 56.70            | bc | 61.11              | ab | 66.18              | a  | 57.55                | b  | 51.14               | c  |
|       |                |                      | 10.65            |    | 17.83              |    | 27.11              |    | 16.25                |    | 22.24               |    |
| ACh   | 3096.5         | 15.9 ***             | 52.30            | b  | 55.95              | a  | 54.57              | ab | 56.62                | a  | 54.46               | ab |
|       |                |                      | 11.35            |    | 12.15              |    | 15.72              |    | 9.94                 |    | 10.00               |    |

<sup>†</sup>, \*\*, \*\*\* Significant at  $p < 0.01$ ,  $p < 0.001$ , <sup>ns</sup> not significant.

### 3.3. Divergences among Geographical Regions

Differences among Ecuador, Peru, Bolivia and Mexico were analyzed for *C. annuum*, *C. baccatum*, *C. chinense*, and *C. pubescens*, which have a representative number of accessions at each country. In the *C. annuum* group significant differences were only found for SSC, which was higher in peppers from Mexico than in those from Ecuador (Figure 3). *C. chinense* accessions from different countries significantly varied for their titratable acidity and moisture content, with peppers from Peru the ones with the highest acidity and lowest amounts of water. Significant differences for all biochemical traits, except for pH, were observed across *C. baccatum* accessions, with the Bolivian fruits possessing the highest levels of SSC, titratable acidity, polyphenols and antioxidant capacity but, the lowest values for moisture content and dry weight. The biochemical parameters quantified on *C.*

*pubescens* accessions from Ecuador were not significantly distinctive from the homonym peppers growing in Peru (Figure 3).



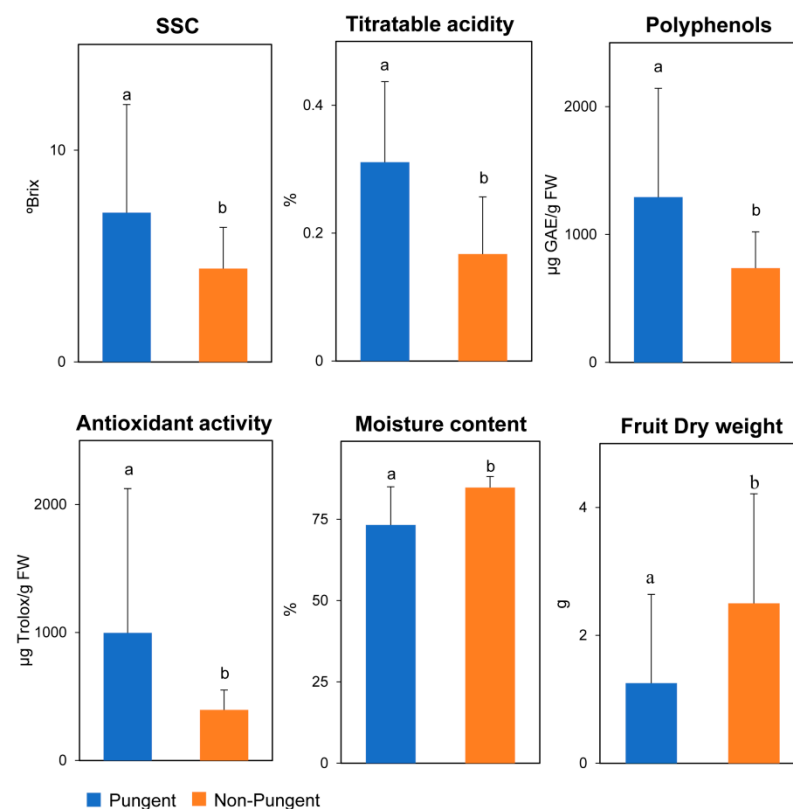
**Figure 3.** Bar plots of biochemical parameters expressed per country for *C. annuum*, *C. baccatum*, and *C. chinense*. Different letters indicate significant differences at  $p < 0.05$ . The absence of letters indicates non-significant differences. Only those parameters showing significant differences among countries are represented. Note that *C. pubescens* was not considered in the figure because significant differences were not observed among countries. A = *C. annuum*, B = *C. baccatum*, C = *C. chinense*.

Significant differences were observed in *C. annuum* for the conventional descriptors PHE, SLE, SDI, FWE, and FPL and 35 out of 49 TA descriptors. Thus, accessions from Mexico displayed the plants with the highest height and stem length, while the weightiest fruits appeared in Peruvian and Ecuadorian peppers (Table S6). Likewise, *C. annuum* peppers from Peru and Ecuador exhibited the largest values for the majority of parameters under the size and latitudinal section categories, although Ecuadorian fruits presented more elongated and rectangular shapes, while peppers from Peru arise as more ellipsoid and circular, as demonstrated by the values of shape index, homogeneity, blockiness and asymmetry. Plant architecture did not significantly vary among *C. baccatum* accessions from the different Andean regions, although Ecuadorian peppers displayed the weightiest fruits (Table S6). Data from scanned fruits revealed significant differences for the majority of attributes, with *C. baccatum* peppers from Bolivia showing the lowest significant values for size, shape, and latitudinal sections. The conventional parameters PHE, SLE, FWE, and FPL significantly diverged among geographical regions in *C. chinense*. Thus, accessions from Ecuador exhibited taller plants than those from Bolivia. Likewise, pepper fruits from the former country had the greatest weight and largest pedicels (Table S6). The TA analysis denoted that *C. chinense* accessions from Ecuador possessed significantly higher values for those parameters associated to fruit size and latitudinal section, although the fruits shape indexes resulted similar across Andean countries. *C. pubescens* plants from Ecuador were significantly taller than those from Peru. On the contrary, Peruvian accessions showed

the heaviest fruits. TA analysis revealed that *C. pubescens* peppers from Peru were larger, although with more roundish shapes. For all four species, the average color values denote that more reddish and darker peppers are prevalent in Ecuador, while Peruvian and Bolivian accessions contained lighter fruits with higher amounts of yellow (Table S6).

### 3.4. Variations between Pungent and Non-Pungent Accessions

All biochemical traits, except for pH, significantly differed among pungent and non-pungent peppers. Putative hot berries possessed greater values for SSC, titratable acidity, polyphenols and antioxidant capacity. However, sweet fruits displayed a higher moisture content and dry weight (Figure 4). Assessment of conventional attributes revealed that pungent types were significantly associated with taller plants and lighter fruits. For TA descriptors, highly significant differences ( $p < 0.001$ ) were observed for 20 parameters and significant differences at  $p > 0.05$  were recorded for 11 traits. On average, pungent peppers had smaller fruits, with thinner pericarps and more roundish shapes (Table 2). Broader variability, expressed as coefficients of variation for each trait, was found within the pungent group. Similarly, a greater variation in CIELab coordinates was evidenced in potentially hot peppers. Significant differences were found for AaV, average hue (AHue) and AChV, suggesting that sweet accessions possessed slightly more redness and brightness fruits (Table 2).



**Figure 4.** Bar plots of biochemical parameters expressed according to the potential pungency of *C. annuum* accessions. Different letters indicate significant differences at  $p < 0.05$ . Only those parameters showing significant differences among countries are represented.

**Table 2.** Analysis of variance for conventional and TA descriptors between pungent and non-pungent *C. annuum* peppers. Only those parameters showing significant differences are represented. PHE = Plant height, FEW = Fruit weight, FPL = Fruit pedicel length, See Table S2 for acronyms of TA descriptors.

| Trait  | Pungent <i>C. annuum</i> |        | Non-Pungent <i>C. annuum</i> |        | Sig.   |
|--------|--------------------------|--------|------------------------------|--------|--------|
|        | Mean                     | CV %   | Mean                         | CV %   |        |
| PHE    | 111.61                   | 31.15  | 90.32                        | 37.77  | 0.05   |
| FWE    | 7.18                     | 141.45 | 17.73                        | 85.64  | <0.001 |
| FPL    | 3.12                     | 26.24  | 3.69                         | 23.80  | <0.001 |
| P      | 113.92                   | 62.90  | 208.77                       | 26.86  | <0.001 |
| A      | 722.51                   | 111.93 | 1802.42                      | 55.41  | <0.001 |
| WMH    | 14.27                    | 70.49  | 24.19                        | 44.08  | <0.001 |
| MW     | 18.34                    | 64.30  | 31.02                        | 35.92  | <0.001 |
| HMW    | 39.83                    | 61.47  | 74.05                        | 26.61  | <0.001 |
| MH     | 43.95                    | 62.38  | 82.15                        | 26.61  | <0.001 |
| CH     | 45.66                    | 60.56  | 83.92                        | 26.41  | <0.001 |
| FSIEI  | 2.54                     | 36.66  | 2.85                         | 32.11  | <0.001 |
| FSIEII | 3.18                     | 55.95  | 3.56                         | 46.71  | 0.019  |
| CFSI   | 3.68                     | 62.11  | 4.13                         | 51.47  | 0.033  |
| PFB    | 0.98                     | 27.64  | 1.07                         | 20.24  | <0.001 |
| DFB    | 0.61                     | 23.23  | 0.57                         | 35.06  | 0.008  |
| FST    | 1.69                     | 41.07  | 2.11                         | 48.21  | <0.001 |
| E      | 0.09                     | 43.08  | 0.10                         | 35.50  | 0.001  |
| C      | 0.30                     | 31.83  | 0.33                         | 21.69  | <0.001 |
| SH     | 0.01                     | 175.79 | 0.02                         | 156.55 | 0.019  |
| Ov     | 0.35                     | 45.79  | 0.41                         | 34.99  | <0.001 |
| VAs    | 0.16                     | 101.96 | 0.29                         | 73.96  | <0.001 |
| HAov   | 0.55                     | 101.43 | 0.99                         | 58.38  | <0.001 |
| WWP    | 0.30                     | 42.74  | 0.26                         | 51.25  | 0.001  |
| DAMI   | 98.01                    | 66.19  | 76.12                        | 88.54  | <0.001 |
| DAMA   | 65.37                    | 62.17  | 49.41                        | 81.29  | <0.001 |
| EC     | 0.74                     | 9.41   | 0.73                         | 8.84   | 0.021  |
| FSII   | 3.13                     | 54.20  | 3.57                         | 46.29  | 0.006  |
| LD     | 28.92                    | 55.12  | 35.08                        | 44.00  | <0.001 |
| TPA    | 240.75                   | 115.09 | 725.64                       | 55.93  | <0.001 |
| TPAR   | 0.43                     | 11.82  | 0.40                         | 12.97  | <0.001 |
| TPT    | 1.95                     | 56.29  | 3.55                         | 38.11  | <0.001 |
| AaV    | 27.94                    | 24.34  | 29.68                        | 19.27  | 0.004  |
| AHue   | 57.43                    | 10.96  | 55.91                        | 10.12  | 0.007  |
| ACh    | 51.77                    | 13.34  | 52.88                        | 8.73   | 0.048  |

### 3.5. Correlation between Biochemical and Morphometric Parameters

Strong and positive significant correlations were recorded between pairs for SSC, titratable acidity, polyphenols and TEAC. These parameters negatively correlated to the moisture content and dry weight, although only the former resulted highly significant (Figure 5). Remarkably, the mean values of SSC, titratable acidity, polyphenols and TEAC showed a particularly stronger negative correlation with the TA descriptors under the categories of basic measurements and latitudinal section (tomato pericarp area (TPA) and thickness (TPT)). However, after Bonferroni correction, only width mid-height (WMH), maximum weight (MW), TPA and TPT remained significant. Conversely, the moisture content and dry weight were positively and significantly inter-related to the same TA parameters (Figure 5). Such results indicate that large fruits possess higher amounts of water, but a reduced levels of bioactive compounds. The network of correlations for TA parameters revealed that some categories were tightly linked and significantly correlated, whereas most of them remained rather independent. Hence, the parameters belonging to the size category were positive and significantly correlated with TPA, TPT, ellipsoid, circular, V. asymmetry (VAs), H. Asymmetry. Ovoid (Ov) (HAov), and the shape index

attributes FSIEI, FSIEII, and curved fruit shape index (CFSI). The homogeneity of pepper fruits displayed significant associations with the internal eccentricity category and shape indexes (FSIEI, FSIEII, and CFSI), even though ellipsoid and circular correlated in a positive manner, while rectangular did it in the opposite way. Fruit weight was positive and significantly correlated to all TA parameters describing size and pericarp features, but also to moisture content and dry weight. On the contrary, a negative connection was established between that parameter and biochemical traits, although only the comparison with titratable acidity was significant. Those results suggest that weightier fruits hold thicker walls but lower amounts of bioactive metabolites. The parameters assigned to the color section were the most independent, exhibiting lack of correlation with the rest of the TA attributes, and being only significantly inter-connected among themselves. Interestingly, the entire coefficients between pairs were positive, except for AaV, which exhibited negative correlations to the other colorimetric traits (Figure 5).

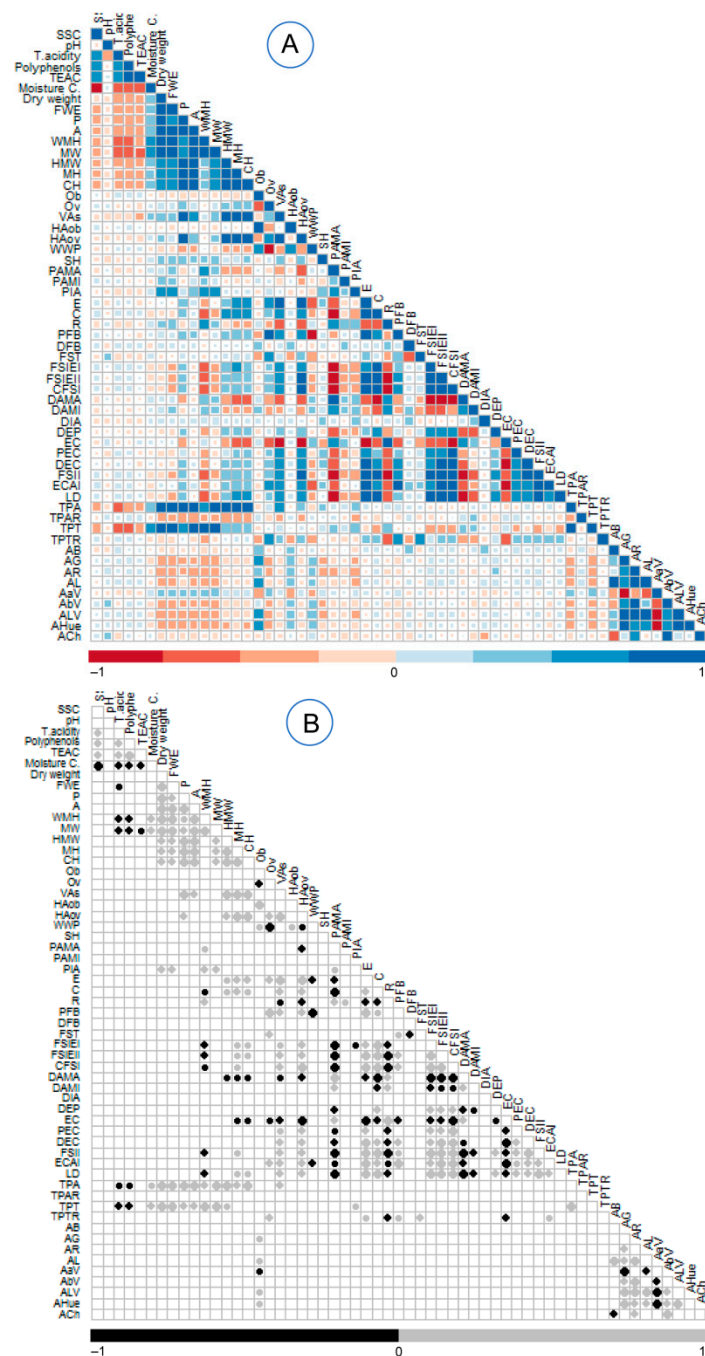
### 3.6. Multivariate Analysis

The principal component analysis resulted in ten principal components with eigenvalues > 1, cumulatively accounting for 89.08% of the total variance. The first component explained 28.3% of the total variance and it was positively and robustly correlated (>80%) to TA attributes related to shape, such as FSIEI, FSIEII, CFSI, circular, FSII, and lobedness degree (LD) (Figure S1). The second component, which accounted for 23.4% of the variance, was positively correlated to traits describing fruit size (perimeter (P), area (A), MW, HMW, and TPA), and weight (FEW and FDW). The third component contributed for 10.2% to the total variance, and the color parameters were clearly the principal traits responsible for the observed variability, with average green (AG), ALV, and AHue being those with a higher degree of positive correlation (Figure S1). Biochemical traits highly contribute to the fifth component, which only explained 5.1% of the variability detected across Andean peppers.

The three principal components were used to project the pepper accessions on two-dimensional plots. Pepper fruits were widely dispersed on the PCA diagrams according to their size, morphology and color (Figure 6). FSII, FSIEI and FSIEII mostly explained the distribution of the accessions on the first axis (PC1). On the other hand, Area and TPA were the main factors discriminating the pepper fruits on the second component (PC2). The arrangement of accessions on the third axis (PC3), primarily responded to the fruit colors. Belonging to a particular *Capsicum* spp. did not predominantly influenced the distribution of pepper accessions on the graphical space, although certain tendencies could be observed. *C. annuum* peppers spread all around the plot, showing the greatest diversity, which encompass fruits with variable shape and size. Hence, morphologies oscillated from highly elongated fruits with large height/width ratio (FSIEII = 7.39) to more roundish fruits (FSIEII = 1.18). Likewise, size varied from huge fruits with areas around 3000 mm<sup>2</sup> and weights of ca. 50 g, to very small fruits (area = 82.6 mm<sup>2</sup>, FEW = 0.20 g). *C. baccatum* and *C. chinense* also exhibited some level of dispersion, although the former tend to concentrate on the positive axis of PC1, while the latter mainly converged on the negative part. This indicates that *C. baccatum* species harbor fruits with more elongated shapes, while round and conical peppers are predominant within the *C. chinense* group (Figure 6). The fruit magnitude was more variable across *C. baccatum* accessions, whose size and weights ranged from 60.21–1985.48 mm<sup>2</sup> to 0.24–16.75 g, respectively. Accessions from *C. pubescens* primarily plotted on the quadrant defined by negative PC1 and positive PC2 axes, denoting that those peppers possess medium sizes and low rates for the height/width indexes. Finally, *C. frutescens* comprised the smallest fruits (average Area = 300 mm<sup>2</sup>, average FWE = 1.41 g), with variable shapes from conical to elongated (average FSIEII = 1.84), which mainly converge on the negative panel delimited by the first and second components (Figure 6). The third component allowed to discriminate accessions according to their colors. Hence, yellow fruits with AHue and ALV values close to 100 and 70, respectively, were only detected for *C. chinense* and *C. baccatum*. The other species displayed red peppers with variable amounts of red color and lightness. Remarkably, the



majority of accessions within the *C. pubescens* group exhibited the most reddish fruits with the lowest lightness (Figure 6).



**Figure 5.** Pearson's rank correlation coefficients between pairs of fruit traits. (A) Correlation coefficients for all pairs of traits. Positive and negative correlations are indicated according to the colored scale. The size of the squares is proportional to the correlation coefficients. (B) Correlation network for most significant traits ( $p < 0.05$ ). Positive correlations are displayed in grey and negative correlations in black. The size of the circles is proportional to the correlation coefficients. SSC = soluble solid content, T. acidity = titratable acidity, TEAC = trolox equivalent antioxidant capacity, Moisture C = moisture content, FWE = fruit weight. Acronyms for TA parameters can be checked at Table S2.

Groups of promising accessions possessing interesting traits were identified with an hierarchical cluster analysis by making use of those parameters highly contributing to the PCA variance. Clustering pattern was independent of *Capsicum* spp. or geographical origin and was primarily based upon fruit morphology, color and biochemical content (Figure 7). Two main groups (I and II) were differentiated. The first one (I) comprised accessions with bigger and heavier fruits, possessing high moisture content but low values for the biochemical parameters. The fruit shapes varied from nearly round (pear-like shape) to triangular or slightly elongated. The sub-group I.1 consisted of *C. pubescens* fruits with manzano type morphology showing an intense red color with low lightness, and *C. chinense* fruits of the habanero type (Figure 7). The sub-group I.2 harbour those fruits with the highest sizes and weights, primarily represented by *C. annuum* accessions. The subdivision of group II allowed to establish two subgroups, which could be additionally partitioned. Hence, the subgroup II.1.1 clustered *C. annuum* and *C. baccatum* wild accessions with the smallest fruits and lowest amounts of water. Interestingly, that group possessed high values for the majority of biochemical components (Figure 7). Subgroup II.1.2, which included primarily *C. chinense* and *C. frutescens*, also grouped small fruits, but with higher moisture content than II.1.1 and more roundish shapes. Yellow peppers of small size and roundish shapes conformed a subgroup within II.1.2. The remaining yellow fruits, mostly *C. chinense* and *C. baccatum*, were included into the sub-group II.2.1, since they showed more elongated morphologies. Sub-group II.2.2 comprised medium-size peppers of elongated forms. It is remarkable within that group the presence of various accessions with the highest fruit shape indices, which measured the ratio of height to width. Pungent *C. annuum* were included into the group II.1.1, whereas the majority of non-pungent accessions were circumscribed to groups I.2 and II.2.2 (Figure 7).

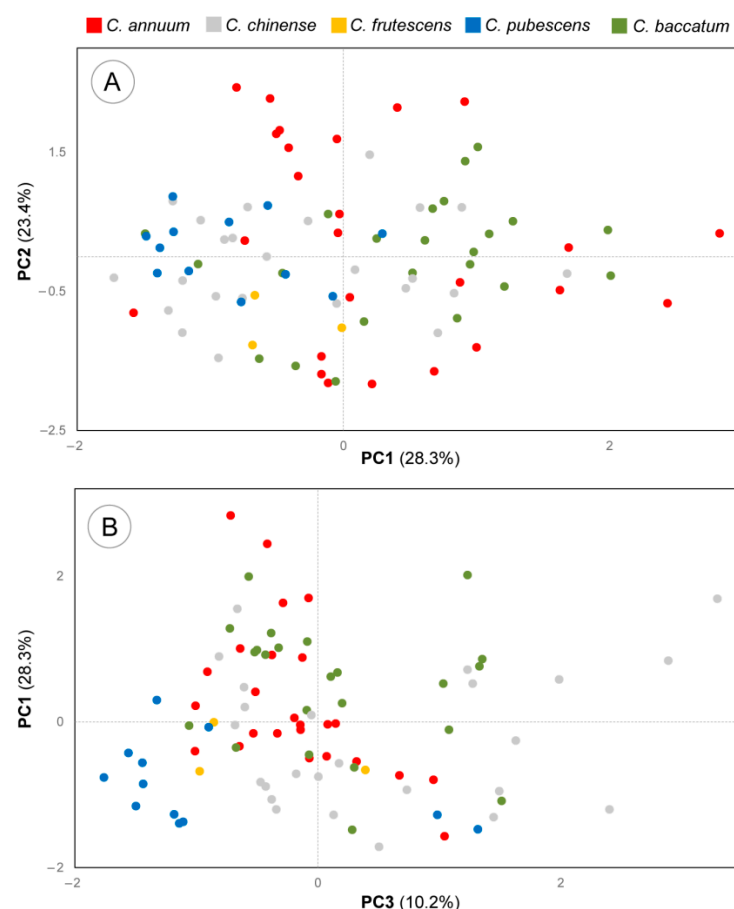
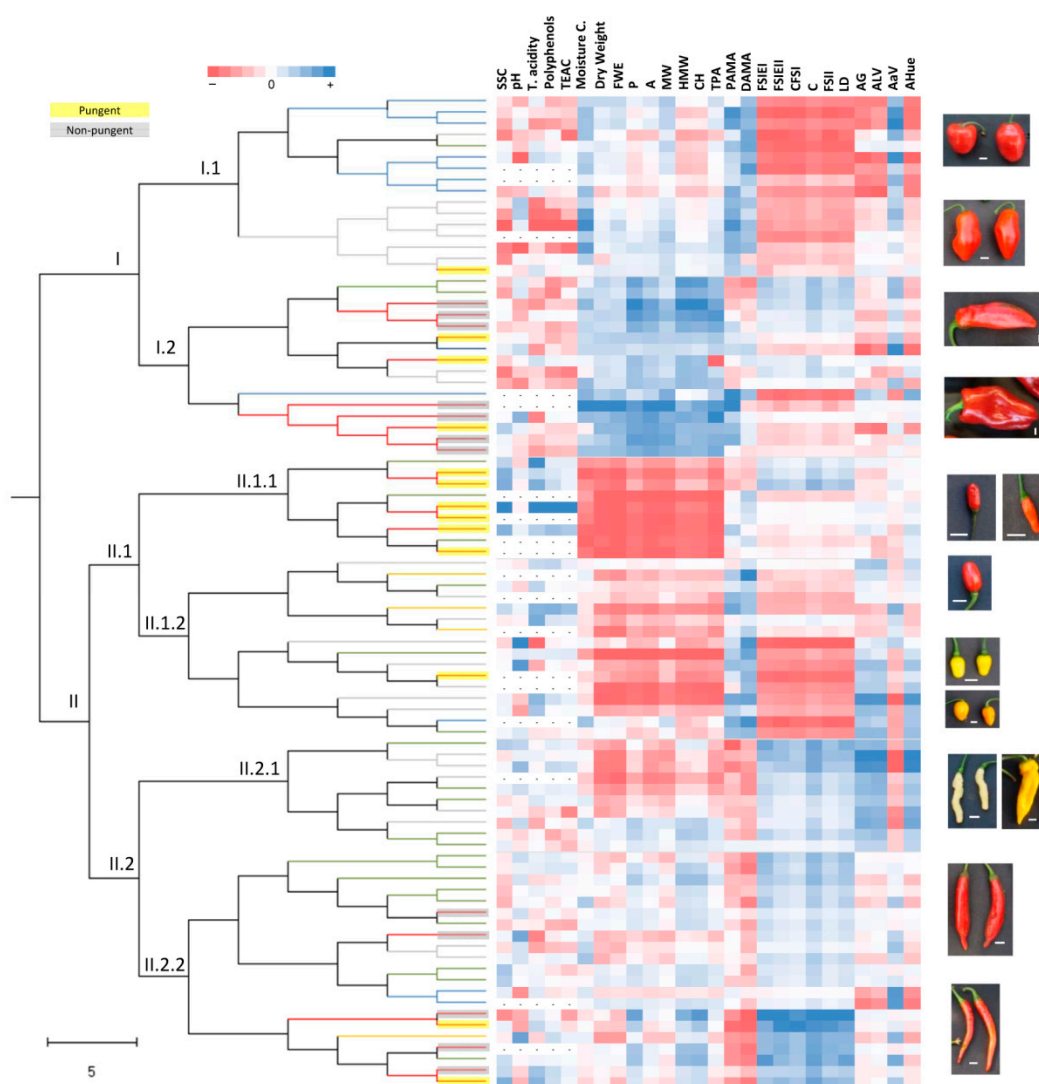


Figure 6. Scatter plot of the PCA analysis. (A) PC1 vs. PC2; (B) PC3 vs. PC1.



**Figure 7.** Cluster analysis based on seven biochemical traits, one conventional fruit descriptor and eighteen TA attributes. Red branches on the dendrogram are *C. annuum*; grey branches, *C. chinense*; yellow, *C. frutescens*; blue, *C. pubescens*; green, *C. baccatum*. The color code matrix represents the variables standardized to z-scores. Pictures are representative accessions for each group. White lines inside the photos indicate 1 cm scale. SSC = soluble solid content, T. acidity = titratable acidity, TEAC = Trolox Equivalent Antioxidant Capacity, Moisture C = moisture content, FWE = fruit weight. Acronyms for TA parameters can be checked at Table S2.

The principal coordinate analysis (PCoA) carried out for each species confirmed the HCA results. Clustering of accessions mainly responded to size and shape, although some correspondences to the original geographical region could be detected (Figure S2). Hence, *C. annuum* peppers from Ecuador and Bolivia had bigger sizes and more elongated shapes than those from Mexico, which conversely displayed higher pungencies. *C. baccatum* fruits from Bolivia were smaller and more roundish than those from Ecuador. Interestingly, all *C. pubescens* accessions were located on the first quadrant, exhibiting fruits of medium-big sizes and roundish shapes.

#### 4. Discussion

Chemical parameters such as SSC, pH or fruit acidity have been investigated to a lesser extent than other nutritional attributes including capsaicinoids, ascorbic acid or phenolic composition and the majority of works have been performed on the most commercially relevant *C. annuum*. However, these components are critical to the overall sensory quality of

fruits and can be employed to determine the end-use and the preferable market for pepper commercialization [25,40]. It is well-known that SSC and acidity increase as ripening progresses, with fully ripe fruits possessing the highest levels of both components [40,41]. Significant differences among accessions and species were observed for those parameters in our *Capsicum* spp. panel, composed of mature fruits. The SSC and titratable acidity ranges observed for *C. annuum* were in agreement with those in previous reports [27,42,43]. Outstanding levels were detected in the accession CAP524, which appeared catalogued as *C. frutescens* by the germplasm bank. However, our earlier genetic analyses classified it as *C. annuum* [29,30], and recent work based on genotyping-by-sequencing suggested that this accession might be considered as a wild *C. annuum* (unpublished data). *C. chinense* showed the poorest performance for those parameters, while *C. frutescens* displayed similar levels to *C. annuum*, although such results must be considered with caution due to the low number of *C. frutescens* accessions assessed in this work. The highest SSC/acidity ratio, which is informative of how tasty the fruits are, was found in the *C. annuum* group, while *C. pubescens* appeared as the least tasty. The SSC mainly comprise sugars derived from degradation of polysaccharides during ripening, while acidity comes from an increasing concentration of organic acids, particularly ascorbic acid [40,44,45]. This acid, together with phenolic compounds are principal contributors to the renowned antioxidant capacity of cultivated peppers [9]. In our report, polyphenols and TEAC showed a large variation across the different accessions, but not among the five *Capsicum* species, which exhibited a shorter range, except for *C. frutescens*. This suggested that domesticated species contained accessions with low, medium and high levels of phenols and antioxidant capacity. Such a conclusion concurred with previous works that frequently quantified the highest and lowest values for those components in accessions from the same species [46–48]. On the other hand, we determined those parameters on fresh fruits, which hindered the comparison to similar works, mostly performed on dry bases [44,46,49]. Indeed, Materska [50] reported that lower levels of phenolic acid derivatives are generally found in lyophilised than in fresh fruits. Nevertheless, our results are in agreement with those previously observed on ripe fresh fruits; although values recorded in our work were slightly lower [51–53]. The accessions of this study with the highest amounts of both parameters (ex. CAP524, CGN20808, PI585257 or PI595907) could be a promising source of antioxidants for the human diet.

A high and significant correlation between polyphenols and TEAC was obtained ( $R^2 = 0.92$ ,  $p < 0.001$ ), in agreement with previous reports [42,46,53]. Similarly, the available literature demonstrated that antioxidant activity and polyphenols tend to concurrently increase with the amounts of ascorbic acid and capsaicin [23,45,48,54]. The latter compounds were not assessed in the present work, however the correlation found between TEAC, phenols and titratable acidity was positive, high and significant ( $R^2 = 0.63$  and  $0.72$ , respectively at  $p < 0.001$ ), suggesting that those accessions with great levels of antioxidant capacity and polyphenols might also contain high amounts of ascorbic acid. In the same way, *C. annuum* peppers catalogued as pungent in our study, which theoretically hold a notable quantity of capsaicinoids, showed significantly higher values for TEAC, phenols and titratable acidity. Therefore, both TEAC and phenols, which are easy to measure parameters, may act as predictors of other attributes, such as capsaicinoids and ascorbic acid, whose quantification is technically more complex.

Water is the most plentiful component in peppers. Its abundance largely influences the post-harvest quality of fruits as well as their market destination [55]. Moisture content varied among the accessions in our panel although the majority oscillated within a range of 70–90%, showing a coefficient of variation of 9.2%. Similar values were reported by other authors [56–58]. Minor variations were recorded among *Capsicum* groups, suggesting that peppers with low and high moisture levels exist across the different species. Frequently, pepper fruits must be dehydrated for commercial purposes, in particular when they are destined to powder production, in which case low levels of water are more appreciated [27]. Percentages of water content lower than 65%, i.e., levels of dry matter

over 35%, were recorded for six accessions in our collection (two *C. baccatum* and four *C. annuum*). These accessions (e.g., CGN17042, CGN20808 or CGN21460) might be explored for the manufacturing of dry peppers. Interestingly, moisture content displayed strong negative correlations ( $p < 0.001$ ) with chemical components, indicating that peppers with higher percentages of water will exhibit lower levels of SSC, acidity, polyphenols and antioxidant capacity. Likewise, an increment in water content would involve a reduction in the potential pungency of fruits. Comparable results were described by Tripodi et al. [27], who reported that pepper types employed for the production of powder contained higher values of dry matter and correlative amounts of capsaicin and other phytochemical traits. Small contrasts were observed among peppers from different geographical regions for their content on chemical compounds. This contradicted the conception that environmental growing conditions largely determine the biochemical composition of fruits [59,60]. However, such absence of differences might be not only be attributed to the comparable climate characteristics of the three Andean countries, but also to the similarities in consumers' preferences, which may have conditioned the farmers' selections and a common evolution in the fruit compositional profiles.

Fifty-five plant and fruit characters were investigated by checking traditional descriptors and highly precise data acquired with a digital tool. Conventional traits describing plant height and fruit weight mostly explained the variability among pepper accessions, as previously reported [61,62]. Regarding the TA analysis, the attributes encompassed into the categories of basic measurements, shape index, and latitudinal section, exhibited the highest contribution to the variance. Similar findings were reported not only for pepper [19,20,23] but also for tomato [16,24] and eggplant [17]. The ANOVA revealed that *C. annuum* statistically diverged from the other species, exhibiting the maximum values for traits related to size, while *C. frutescens* showed the lowest measurements for identical parameters. This is in line with results described by Tripodi and Greco [20] on a large collection of cultivated and wild peppers. Colour parameters also explained a high percentage of the variance within our *Capsicum* collection. This is something expected since the domestication process and subsequent selection procedures led to the generation of cultivated peppers with various ranges of colors, suitable for different uses [63]. The yellow, orange, and red colors in peppers originate from carotenoid pigments produced in the fruit during ripening, which apart from their nutritional value, can act as antioxidants preventing different human diseases [64,65]. Besides, colour will greatly determine whether peppers might be employed as colorants for food or in the cosmetics industry. The majority of Andean peppers possessed a hue angle in the orange range, although some displayed AHue values lower than  $50^\circ$  or higher than  $80^\circ$  which are indicative of intense red or pure yellow, respectively. The former group mainly comprised mainly accessions from *C. pubescens*, while the latter consisted of *C. chinense* peppers. Furthermore, a strong positive interaction was observed between AHue and Luminosity, *C. chinense* fruits being the lightest and *C. pubescens* the darkest. Similar results were reported by Meckelmann et al. [46] in a collection of native chilli peppers from Peru. Although no previous studies have undoubtedly correlated the pepper fruit color to the carotenoid accumulation, it appears that capsanthin, capsorubin and cryptocapsin could be responsible for red colors while the yellow and orange colors come from  $\beta$ -carotene, zeaxanthin and violaxanthin [66,67]. Therefore, those Andean pepper fruits with larger redness or yellowness components might possess notable amounts of carotenoids and they could be potentially novel sources of beneficial compounds for human health, or they might be exploited for the production of natural additives. *C. annuum* accessions were assorted into putative pungent and non-pungent types based on the marker MAP1 [31]. Differences between sweet and hot peppers were found for all biochemical parameters (except pH), for PHE, FWE, FPL, and 31 TA descriptors. In general, pungent types had taller plants, smaller fruits in both length and width, slightly lower shape indexes, less fresh and dry weight but much higher values of SSC, titratable acidity, polyphenols and antioxidant activity. This is in line with data from Hill et al. [68], who identified 17 conserved regions across the



genome of non-pungent peppers, and reported that many of those regions overlapped with QTLs for fruit size and shape, but also with genes involved in the capsaicin biosynthesis.

To date, few works have attempted to evidence the impact of fruit morphology on biochemical compositions [25–27]. However, to investigate the correlation between these two groups of attributes emerge as a mandatory task, since this linkage might affect the speed at which favorable attributes could be introgressed into commercial pepper cultivars. All chemical traits, except moisture content and dry weight, negatively correlated to FWE, TPA, TPT and parameters belonging to basic measurements. Conversely, positive correlations were observed to fruit indexes (FSIEI, FSIEII, CFSI), circular, DFB and color parameters except for AaV. It seems that pepper fruits with higher weights, larger sizes, thicker pericarps, low height/width ratios, and greater proportions of red color contained lower amounts of SSC, titratable acidity, polyphenols and antioxidant activity, but higher levels of water and dry matter. Negative correlations between morpho-agronomic and phytochemical traits were also evidenced by other authors [23,25,27,40]. Fruit weight was strongly and significantly ( $p < 0.05$ ) correlated in a positive manner to TA parameters related to size (P, A, WMH, MW, HMW, MH, and CH), but also to PIA, TPA, and TPT. Negative correlations to fruit shape indexes, internal eccentricity and the majority of color attributes were also detected, although those were not significant. Such results suggested that heavier fruits displayed bigger sizes, thicker pericarps, and more elongated shapes, i.e., lower ratios for height to width. Vilarinho et al. [25] also reported high positive correlations between fruit weight, fruit width and pericarp thickness, supporting the data by Barchi et al. [69], who found that QTLs controlling those traits were linked on chromosome P12.

The network of associations for TA parameters revealed positive and significantly strong correlations between size-based traits and those connected to the pericarp magnitude (TPT and TPA). In addition, HMW, MH and CH showed significant positive correlations to fruit shape indexes, internal eccentricity parameters, ellipsoid and circular. Significant associations among the categories of homogeneity, fruit shape index and internal eccentricity were observed as well. All these results bring to light that smaller fruits possessed shapes that are more roundish and, have thinner walls and lower dimensions of width and height axes. Similar results were obtained in our previous work [23], and also by Tripodi and Greco [20] in a large collection of worldwide peppers. As expected, significant interactions were observed among colors parameters estimated with both RGB and CIELab color spaces implemented into TA software [33]. Unlike previous works, we could not identify strong and significant correlations among ALV, AHue and ACh [25,40,43]. However, the correlation matrix revealed that an increase in lightness significantly occurred with an increase in Hue angle, suggesting that yellow-orange peppers possessed more brilliant colors.

The most effective attributes in discriminating among accessions were determined by principal component analysis. PC1 was positively and strongly correlated to parameters defining the fruit shape, homogeneity, and internal eccentricity while PC2 displayed high coefficients with traits associated to size, latitudinal sections, FWE, and FDW. PC3 was primarily explained by those parameters related to color. Biochemical traits negatively contributed to the first two axes although with very low and non-significant coefficients. These results supported previous works and confirmed that a reduced sub-set of descriptors within the sections of basic measurements, fruit shape and latitudinal section emerged as the most informative, in such a way that they might be used alone to discriminate between *Capsicum* types [19–23]. The representation of accessions on the bi-dimensional space proved that each species was diverse for fruit morphological and colorimetric traits, as accessions of a single group presented a certain degree of dispersion, plotting at different areas of the graph. This was particularly evident for *C. annuum*, which displayed variable accessions from those with large fruits, thick walls, moderate height/width ratios, and triangular shapes (Bell types), to those with tiny, thin-walled fruits, high height/width ratios, and extremely elongated shapes (Cayenne type). Wild *C. annuum* from Mexico (PM647, CGN21526 and CGN22783) exhibited the smallest fruits, conical shapes and grouped together with *C. annuum* accessions CAP524 and CGN20808, which might be

considered closely-related to ancestors. The fruits from *C. chinense* exhibited more variation in size than in shape, with fruit weight ranging from 0.48 to 19.02 g, and shapes oscillating from rectangular forms (Habanero type) to more triangular profiles. *C. baccatum* fruits possessed variable sizes, the wild forms (var. *baccatum*) being the smallest ones. Shapes were mostly elongated, with high height/width ratios. On the contrary, accessions from *C. pubescens* had berries of medium size with roundish shapes. Fruits with yellow and orange colours were mostly observed in *C. chinense* and *C. baccatum*, which also displayed the highest lightness. The smallest values for luminosity were attributed to *C. pubescens* fruits. The fact that most of the variation in fruit features occurred in *C. annuum* was somehow expected. This *Capsicum* represents the most commonly cultivated and consumed around the world and the one that has suffered from more intensive human selection pressures, who greatly modified the morphometry of fruits out of the wild ancestor *C. annuum* var. *glabriusculum* [70].

The clustering analysis reflected similar results to those obtained in PCA, with Andean peppers being arranged independently of their species and according to their fruit morphology, color, and biochemical composition. Hence, roundish peppers of moderate size, high amounts of water and intense red appeared into group I.1, and they predominantly corresponded to *C. pubescens* and *C. chinense* accessions. Cluster I.2 grouped the peppers with the bigger sizes, higher weights, large water contents, triangular shapes and light red colors, the majority (62.5%) of them being *C. annuum*. Yellow peppers were distributed across clusters II.1.2 and II.2.1 and they essentially consisted of *C. chinense* and *C. baccatum* accessions of small size and weight, whose shape varied from almost round to highly elongated. The cluster II.1.1 contained the wild ancestors of *C. annuum* and *C. baccatum*, which displayed the expected tiny and slight fruits, with very low moisture contents, conical shapes and red-orange colors. Interestingly, peppers within this group showed the highest values for biochemical parameters and they were pungent according to the marker associated to the *Pun1* gene. *C. annuum* accessions with highly elongated shapes (Cayenne type) were located in cluster II.2.2 and they displayed both pungent and non-pungent types. These results confirmed our previous work [23] performed on a smaller set of Ecuadorian native landraces and agreed with those by Nankar et al. [22], who observed that a collection of *Capsicum* accessions from the Balkan Peninsula clustered according fruit shape, size and color. However, they differed from those reported by Tripodi and Greco [20] who noticed that a dendrogram obtained with conventional and TA descriptors split accessions according to their *Capsicum* species. Such differences might be attributed to the size and origin of the evaluated panels. On the other hand, these authors found a clear distinction between wild and cultivated species, what is in agreement with our work, at which *C. annuum* wild and *C. baccatum* var. *baccatum* accessions are assorted in separated cluster.

PCoA analyses per species considering the different geographical regions revealed certain divergences in fruit morphology according to the country of origin. Thus, *C. annuum* from Mexico displayed the tiniest and most pungent fruits, while Ecuador and Peru possessed the biggest non-pungent berries. Comparable results were noticed for the *C. baccatum* group, where the smallest fruits corresponding to *C. baccatum* var. *baccatum* accessions, were found only in Bolivia. These observations are in line with our earlier work [29], which concluded that genetic differentiations among *Capsicum* spp. followed a geographic pattern. Hence, higher genetic similarities were detected between Ecuadorian and Peruvian *C. annuum* peppers, which might be a consequence of a more recent gene flow across those regions [71]. The influence of ecogeographic distributions on the patterns of genetic differentiation within wild and domesticated *C. annuum* from Mexico was also detected previously and the might be related to Mexican origin of this *Capsicum* species [72]. Likewise, correlations between genetic variability and geographic arrangements within *C. baccatum* accessions from South America were established by Albrecht et al. [73], who proposed that Ecuadorian and Peruvian accessions likely derived from Bolivian wild-types. All those genetic differences might explain the variable morphology observed

among Andean countries, since at each region fruits would be selected by farmers to meet consumers' preferences and agro-climatic requirements.

## 5. Conclusions

The present work brings to light that native varieties from the Andean region, one of the places postulated to be the origin of the genus *Capsicum*, possess a substantial diversity of pepper fruits with different sizes, shapes, and colors carrying variable amounts of beneficial metabolites. These varieties might be exploited as sources of desirable traits lacking in modern cultivars. Furthermore, negative correlations between fruit geometrics and phytochemical compounds were detected, reinforcing our previous work on a small set of *Capsicum* spp. from Ecuador [23]. Such a contribution is essential in breeding for fruit quality, as it would determine what characters might be independently selected and the speed at which valuable traits could be transferred into commercial peppers.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-4395/11/2/262/s1>, Table S1: List of accessions employed in this work. Na stands for not available, Table S2: List of digital traits measured with Tomato Analyzer software [12,13], Table S3: Analysis of variance for biochemical traits (above). Mean  $\pm$  SD for each accession (below). Last row indicates the average value (Coefficient Variation) for each trait, Table S4: Analysis of variance for conventional descriptors (above). Mean  $\pm$  SD for each accession (below). Last row indicates the average value (Coefficient Variation) for each descriptor. PHE = Plant height, PWI = Plant width, SLE = Stem length, SDI = Stem diameter, FEW = Fruit weight, FPL = Fruit pedicel length, Table S5: Analysis of variance for TA descriptors (above). Mean  $\pm$  SD for each accession (below). Last row indicates the average value (Coefficient Variation) for each descriptor. P = Perimeter, A = Area, WMH = Width mid-height, MW = Maximum width, HMW = Height mid-width, MH = Maximum height, CH = Curved height, FSIEI = Fruit shape index external I, FSIEII = Fruit shape index external II, CFSI = Curved fruit shape index, PFB = Proximal fruit blockiness, DFB = Distal fruit blockiness, FST = Fruit shape triangle, E = Ellipsoid, C = Circular, R = Rectangular, SH = Shoulder height, PAMI = Proximal angle micro, PAMA = Proximal angle macro, PIA = Proximal indentation area, DAMI = Distal angle micro, DAMA = Distal angle macro, DIA = Distal indentation area, DEP = Distal end protrusion, Ob = Obovoid, Ov = Ovoid, VAs = V. Asymmetry, HAob = H. Asymmetry. Ob, HAov = H. Asymmetry. Ov, WWP = Width widest position, EC = Eccentricity, PEC = Proximal eccentricity, DEC = Distal eccentricity, FSII = Fruit shape index internal, ECAI = Eccentricity area index, LD = Lobedness degree, TPA = Tomato pericarp area, TPAR = Tomato pericarp area ratio, TPT = Tomato pericarp thickness, TPTR = Tomato pericarp thickness ratio, AR = Average Red, AG = Average Green, AB = Average Blue, AL = Average Luminosity, ALV = Average L Value, AaV = Average a Value, AbV = Average b Value, AHue = Average Hue, Ach = Average Chroma, Table S6: Mean—Coefficient of Variation (%) for conventional and digital descriptors in *C. annuum*, *C. baccatum*, *C. chinense*, and *C. pubescens* within each geographical region. Different letters within the same column indicate significant differences at  $p < 0.05$ . Only the parameters showing significant differences among regions are represented. See Table S2 for traits' acronyms, Figure S1: Contribution coefficients of each trait to the three Principal Components (PCs), Figure S2: Principal Coordinate Analysis (PCoA) for each *Capsicum* species based on twenty-six biochemical, conventional and TA descriptors. Accessions are represented according to their country of origin.

**Author Contributions:** C.S.: conceptualization and experiment design, J.G.-L., S.R.-M.: experiments and data analysis; C.S.: writing—original draft preparation, C.S.: writing—review and editing, C.S.: supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Spanish Institute for Agricultural and Food Research and Technology (INIA), co-financed by the European Regional Development Fund (FEDER) (Project RTA2015-00042-C02-02), and by Xunta de Galicia (Project ED431C 2018/57).

**Acknowledgments:** We gratefully acknowledge provision of germplasm by USDA, ARS, Plant Genetic Resources Conservation Unit (USA), Center for Genetic Resources (The Netherlands), Institute for Conservation and Improvement of Valencian Agrobiodiversity (Spain) and Leibniz Institute of Plant Genetics and Crop Plant Research (Germany).

**Conflicts of Interest:** The authors declare no conflict of interest.

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