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Sugar and Organic Acid Content Is Dependent on Tomato (*Solanum Lycoperiscum* L.) Peel Color

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Abstract: The sensory properties of fruit and vegetables are a result of taste and aroma caused by many volatile and nonvolatile compounds. The sum of organic acids (malic and citric acids) and soluble sugars (fructose and glucose), as well as their balanced combination and interaction, contributes to the characterization of the tomato flavour. The ratio of sugars and organic acids is the key to the sweetness and sourness of tomatoes. This study aimed to determine the sugar and organic acid content, as well as several physicochemical parameters, of eight tomato landraces from Croatia. All the parameters investigated differed between the tomato landraces. The PLS-DA analysis showed that the most important parameters in tomato landrace discriminatory character are malic acid, fructooligosaccharide content, citric acid, dry matter. The results obtained show a significant positive correlation between tomato dry matter and sugar content. At the same time, fructose and sucrose content is negatively correlated with the green to red hue of tomato peel, as well as positively with the blue to yellow hue, indicating that the sugar content increases with yellow color intensity. The blue to yellow hue of the peel color also positively correlates with citric acid content.

Keywords: landraces; CIELAB; glucose; fructose; malic acid; citric acid; pH; dry matter; correlation

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1. Introduction

The tomato (*Solanum lycopersicum* L.) is an economically important plant cultivated worldwide. Due to its culinary versatility, it is the most widely cultivated vegetable in the world [1]. Tomato belongs to the *Solanaceae* family, which also includes other significant species such as the potato, peppers, eggplants and tobacco [2]. *Solanum* is the largest genus in the *Solanaceae* family, containing 1250 to 1700 species [3]. The Solanum section *Lycopersicon* consists of 13 species or subspecies out of which, the tomato is the only domesticated member [4]. The hypothesis on the origin of tomato domestication was developed by extensive genetic characterization as part of the SolCAP project [5,6] and it is believed that the tomato was domesticated in two waves. First, from *Solanum pimpinellifolium* to *S. lycopersicun* var. *cerasiforme* in Ecuador and Northern Peru, and second, from *S. lycopersicum* var. *cerasiforme* to *S. lycopersicum* var. *lycopersicum* in Central America [7]. The tomato was brought to Europe in the 16th century, which resulted in additional improvements to fruit characteristics [8]. Among evolutionary features, the most significant impacts of domestication are those related to the external appearance of the tomato, such as size, shape, color and fruit firmness [3]. Tomato cultivars show a vast morphological diversity that is controlled by a large number of genetic loci [9].

Plant genetic resources, such as traditional landraces, are the basis of food security and their loss can result in genetic erosion [10]. The lack of taste of commercial tomato varieties, due to breeding efforts focused mainly on yield, has led to an increased interest in traditional tomato landraces; however, traditional tomato landraces can yield less and have a shorter shelf-life compared to commercial varieties [11].

In the everyday language of people, a tomato is a vegetable, while from the botanical point of view, it is a fruit. This topic was a question of debate during the 19th century at the Supreme Court in the USA, with the case of Nix vs. Hedden where the court judged the tomato as a vegetable due to the manner of its use [3].

Tomatoes contain nutritional compounds important for human health such as carotenoids and vitamin E which have antioxidative properties. Carotenoids (lycopene and β-carotene) are precursors of vitamin A and are responsible for the red color of ripe tomatoes [12].

The sensory properties of fruit and vegetables are a result of taste and aromas caused by many volatile and nonvolatile compounds. The sum of organic acids (malic, citric, and oxalic) and soluble sugars (fructose and glucose), as well as their balanced combination and interaction, contribute to the characteristic tomato flavours [13]. The content of organic acids is important not only for the flavour but for the processing and storage of the fruit as well. The organic acids determine the pH of the fruit which, if higher than 4.5, allows the development of spoilage microorganisms [3].

The content of sugars and organic acids depends on the tomato's ripeness. During the ripening of a tomato, a series of biochemical processes take place that converts the unripe, acidic-tasting tomato into a sweet-tasting, aromatic fruit [2]. The total amount of sugar increases during ripening with glucose being predominant in unripe fruits while ripe fruits contain relatively more fructose [14]. After ripening, the sugar content declines again. As with sugars, the amount of organic acid increases during ripening [15]. At all stages, citric acid is the dominant organic acid in tomatoes, however, the content of malic acid may be significant in unripe tomatoes [15]. Moreover, the ratio of malic to citric acid can vary greatly between different tomato cultivars [16]. After ripening, the content of citric acid decreases [17]. Besides ripeness, which can be connected to harvest time, factors influencing the nutritional composition of tomatoes are variety, climate, location and agricultural practices [18].

This study aimed to determine the sugar and organic acid content, as well as the interrelationship with selected physicochemical parameters, in eight tomato landraces from Croatia.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of eight tomato landraces belonging to an ex situ collection from the Institute of Agriculture and Tourism (N 45°13′20.30″, E 13°36′6.49″). As shown in Supplementary Table S1, the seeds from tomato landraces were obtained from different regions across Croatia and have various morphological characteristics. Tomato seedlings were grown in a local nursery and planted in an experimental field of the Institute of Agriculture and Tourism in Poreč, Croatia on 11 May 2021, on black polyethylene (PE) mulch. Weather data is shown in Table S2. The experiment was set up as a randomized complete block design with three replicates. Tomato plants were grown according to standard agronomic practices for tomatoes [19]. All landraces were left to open pollination.

The harvest was carried out from the 9th to the 13th of August. Only fully ripe fruits without any signs of physical damage, or physiological defects, were harvested. From each landrace 8 to 12 tomato fruits were chosen and divided into 4 replications (2 to 3 fruits per replication) and used for further investigation. The tomato fruit pulp, without the placental tissue and seeds from each replication (approximately 500 g), was homogenized by a hand blender and subsequently used for further analyses. For the HPLC analyses, 500 mg of the sample was mixed with 1 mL of 80% methanol in a tube with 2.4 mm metal beads (Omni kit 19–620, Kennesaw, GA, USA) for 1 min at 5 m/s using a bead mill (Omni Bead Ruptor Elite, Kennesaw, GA, USA). The homogenates were left to macerate for 1 h on a rotator (Biosan RS-60, Riga, Latvia) and subsequently centrifuged for 5 min at 16,000× *g*. The extracts were filtered through a 0.22 µm nylon filter before analysis.

2.2. Dry Matter, PH, and Color Analyses in Tomato

Dry matter was determined gravimetrically after air drying (Memmert UF160, Schwabach, Germany) approximately 5 g of tomato sample at 80 °C overnight. The pH analysis was conducted by measuring pH with a Seven2Go S2-Basic portable pH meter (Mettler Toledo, Columbus, OF, USA) in the homogenized tomato pulp. The color of the tomato peel was measured by a MiniScan EZ 4500 Portable Spectrophotometer (HunterLab, Reston, VA, USA). The analyses were carried out on four replicates.

2.3. Tomato Sugar Analysis

The fructooligosaccharide (quantified as inulin), glucose, and fructose content in the tomato samples were analysed using: an HPLC, consisting of a system controller (Shimadzu CBM-40, Kyoto, Japan); a degassing unit (Shimadzu DGU-405, Kyoto, Japan); a solvent delivery unit (Shimadzu LC-20Ai, Kyoto, Japan); an autosampler (Shimadzu SIL-20AC, Kyoto, Japan); column oven (Shimadzu CTO-40S, Kyoto, Japan); and a refractive index detector (Shimadzu RID-20A, Kyoto, Japan). Chromatographic separation was achieved by injecting 10 µL of the sample on a 300 × 8 mm, 9 µm particle size, calcium ion exchange column (Dr. Maisch ReproGel Ca, Ammerbuch, Germany) held at 80 °C using deionized water as the mobile phase (0.6 mL/min, isocratic elution). Retention times, and peak areas of the investigated sugars, were compared to analytical standards for identification and quantification.

2.4. Tomato Organic Acid Analysis

The tomato samples were analysed on an HPLC consisting of two solvent delivery units (Shimadzu Nexera LC-40DX3, Kyoto, Japan); an autosampler (Shimadzu Nexera SIL-40CX3, Kyoto Japan); a thermostated column compartment (Shimadzu Nexera CTO-40C, Kyoto, Japan); and a photodiode array detector (Shimadzu Nexera SPD-M40, Kyoto, Japan).

Chromatographic separation was achieved by injecting 10 µL of the sample on an aqueous C18, 4.6 mm × 250 mm, 2.7 µm core-shell particle size column (Advanced Materials Technology, Wilmington, DE, USA) held at 35 °C with isocratic elution of the mobile phase (25 mM phosphate buffer, pH 2.5) at 0.7 mL/min. Malic and citric acids were identified and quantified against their analytical standards.

2.5. Statistical Analysis

The obtained data were analysed using Analysis of Variance (ANOVA) and, for significant differences ($p \leq 0.05$), a Fischer Least Significant Difference post hoc test was performed. For the differentiation analysis of the investigated tomato landraces, a Partial Least Squares—Discriminant Analysis (PLS-DA) was performed. Pearson's correlations were calculated between all investigated parameters and significant correlations were determined at $p \leq 0.05$.

3. Results

The highest dry matter content was observed in landraces IPT503 and IPT500 (8.36 ± 0.26% and 8.21 ± 0.12%, respectively), while the landraces IPT507, IPT506, IPT501, and IPT504 had the lowest dry matter content, ranging from 6.05 ± 0.2% in IPT504 to 5.77 ± 0.1% in IPT506 (Table 1).

IPT500 showed the highest pH level of 4.41 ± 0.04, which was comparable to the values of IPT507 and IPT501 (4.34 ± 0.08 and 4.33 ± 0.03, respectively). The pH levels found in landraces IPT506, IPT499, IPT502, and IPT504 were not significantly different, ranging from 4.27 ± 0.04 in IPT506 to 4.21 ± 0.03 in IPT504. The lowest pH level was observed in IPT503 (4.04 ± 0.03) (Table 1).

Table 1. Dry matter, pH and CIELAB colorspace values of the investigated tomato landraces.

Landrace	Dry Matter %	PH	*L*	*a*	*b*
IPT499	6.41 ± 0.1 bc [1]	4.25 ± 0.03 bc	43.4 ± 0.2 b	35.9 ± 0.3 a–c	30.9 ± 0.5 cd
IPT500	8.21 ± 0.12 a	4.41 ± 0.04 a	32.2 ± 1.8 c	33.4 ± 0.6 d	22.6 ± 1.3 e
IPT501	5.98 ± 0.11 c	4.33 ± 0.03 a–c	43.0 ± 0.6 b	37.3 ± 0.3 a	30.4 ± 1.1 cd
IPT502	6.90 ± 0.41 bc	4.22 ± 0.03 bc	45.7 ± 3 b	34.7 ± 1.3 b–d	34.7 ± 3.4 bc
IPT503	8.36 ± 0.26 a	4.04 ± 0.03 d	54.3 ± 0.8 a	34.2 ± 1.2 cd	49.2 ± 1.5 a
IPT504	6.05 ± 0.2 c	4.21 ± 0.03 c	46.4 ± 1 b	36.6 ± 0.2 ab	36.5 ± 1.2 b
IPT506	5.77 ± 0.1 c	4.27 ± 0.04 bc	43.5 ± 0.4 b	36.2 ± 0.4 a–c	30.0 ± 0.4 d
IPT507	5.94 ± 0.24 c	4.34 ± 0.08 ab	43.4 ± 0.8 b	37.1 ± 0.3 a	30.9 ± 1.1 cd
p-value	***	***	***	**	***

[1] different letters indicate significant differences in Fischer's Least Significant Difference test; *** $p \leq 0.001$; ** $p \leq 0.01$; *L*—lightness; *a*—green to red; *b*—blue to yellow.

The brightest tomato color, measured as lightness in CIELAB color space, was observed in IPT503, while the lowest brightness was measured in landrace IPT500 (Table 1). At the same time, landrace IPT500 had the least intense red hue characterized by the lowest *a* value, compared to the other investigated landraces, except for IPT502 and IPT503. The most intense yellow hue among the investigated landraces was observed in IPT503 (Table 1).

Significant differences were observed in the sugar content between the samples and the highest content of all the analysed sugars (fructooligosaccharides, fructose, glucose) was found in IPT503. The fructooligosaccharide content varied significantly between the landraces, from the highest value of 0.46 ± 0.03 g/100 g FW found in IPT503, to the lowest value of 0.25 ± 0.01 g/100 g FW found in IPT506. The fructooligosaccharide content of landraces IPT499 and IPT500 was not significantly different from the highest observed value (Table 2).

Table 2. Sugar and organic acid content of tomato accessions.

Landrace	Fructooligo-Saccharides	Fructose	Glucose	Malic Acid	Citric Acid
	g/100 g FW			mg/100 g FW	
IPT499	0.45 ± 0.01 ab [1]	2.22 ± 0.03 bc	2.13 ± 0.03 b	374 ± 23 a–c	507 ± 34 b–d
IPT500	0.40 ± 0.02 ab	2.31 ± 0.11 b	2.13 ± 0.13 b	452 ± 35 ab	443 ± 41 de
IPT501	0.39 ± 0.01 b	2.11 ± 0.04 bc	1.98 ± 0.05 bc	277 ± 65 c	385 ± 41 e
IPT502	0.31 ± 0.01 c	2.11 ± 0.12 bc	1.89 ± 0.17 bc	349 ± 43 bc	479 ± 22 cd
IPT503	0.46 ± 0.03 a	2.64 ± 0.03 a	2.56 ± 0.05 a	430 ± 49 ab	729 ± 20 a
IPT504	0.31 ± 0.01 c	1.87 ± 0.06 d	1.89 ± 0.06 bc	354 ± 36 bc	588 ± 4 b
IPT506	0.25 ± 0.01 d	1.82 ± 0.04 d	1.84 ± 0.06 c	394 ± 36 a–c	573 ± 11 b
IPT507	0.40 ± 0.04 b	2.10 ± 0.07 c	1.97 ± 0.08 bc	484 ± 22 a	563 ± 42 bc
p-value	***	***	***	*	***

[1] different letters indicate significant differences in Fischer's Least Significant Difference test; *** $p \leq 0.001$; * $p \leq 0.05$.

As mentioned, IPT503 had the highest fructose content (2.64 ± 0.03 g/100 g FW), while the lowest fructose content was found in IPT504 and IPT506 (1.82 ± 0.04 g/100 g FW and 1.87 ± 0.06 g/100 g FW, respectively). Fructose content in other landraces ranged from 2.31 ± 0.11 g/100 g FW in IPT500 to 2.1 ± 0.07 g/100 g FW in IPT507 (Table 2).

Glucose was most abundant in IPT503 (2.56 ± 0.05 g/100 g FW). The lowest glucose content was observed in the IPT506 (1.84 ± 0.06 g/100 g FW), followed by IPT502 and IPT504 (1.89 ± 0.17 g/100 g FW and 1.89 ± 0.06 g/100 g FW, respectively).

IPT507 had the highest content of malic acid (484 ± 22 mg/100 g FW), while landraces IPT506, IPT499, IPT500, and IPT503 did not significantly differ in malic acid content (Table 2).

The citric acid content ranged from 729 ± 20 mg/100 g FW in IPT503, to 385 ± 41 mg/100 g FW in IPT501. Landraces IPT507, IPT506, IPT499, IPT502, and IPT504 were in the mid-range, where the content of citric acid varied from 588 ± 4 mg/100 g FW (IPT504) to 479 ± 22 mg/100 g FW (IPT502) (Table 2).

The PLS-DA analysis showed that the most important parameters in tomato landraces character discrimination were malic acid, fructooligosaccharide content, citric acid, dry matter and lightness (*L*) (Figure 1). Based on the PLS-DA, the IPT503 was distinguished as the landrace with the highest dry matter, sugar, citric and malic acid content as well as intense red and yellow hues combined with light peel color (Figure 1). On the other hand, the PLS-DA model indicated that the IPT500 landrace, which is also characterized by high dry matter content, had a darker peel color and low *b* values when compared to the other investigated landraces (Figure 1). The IPT500 landrace is also the only determinate tomato among the investigated landraces. Determinate tomato species are characterized by higher dry matter content compared to indeterminate species [20], which was also the case in our study.

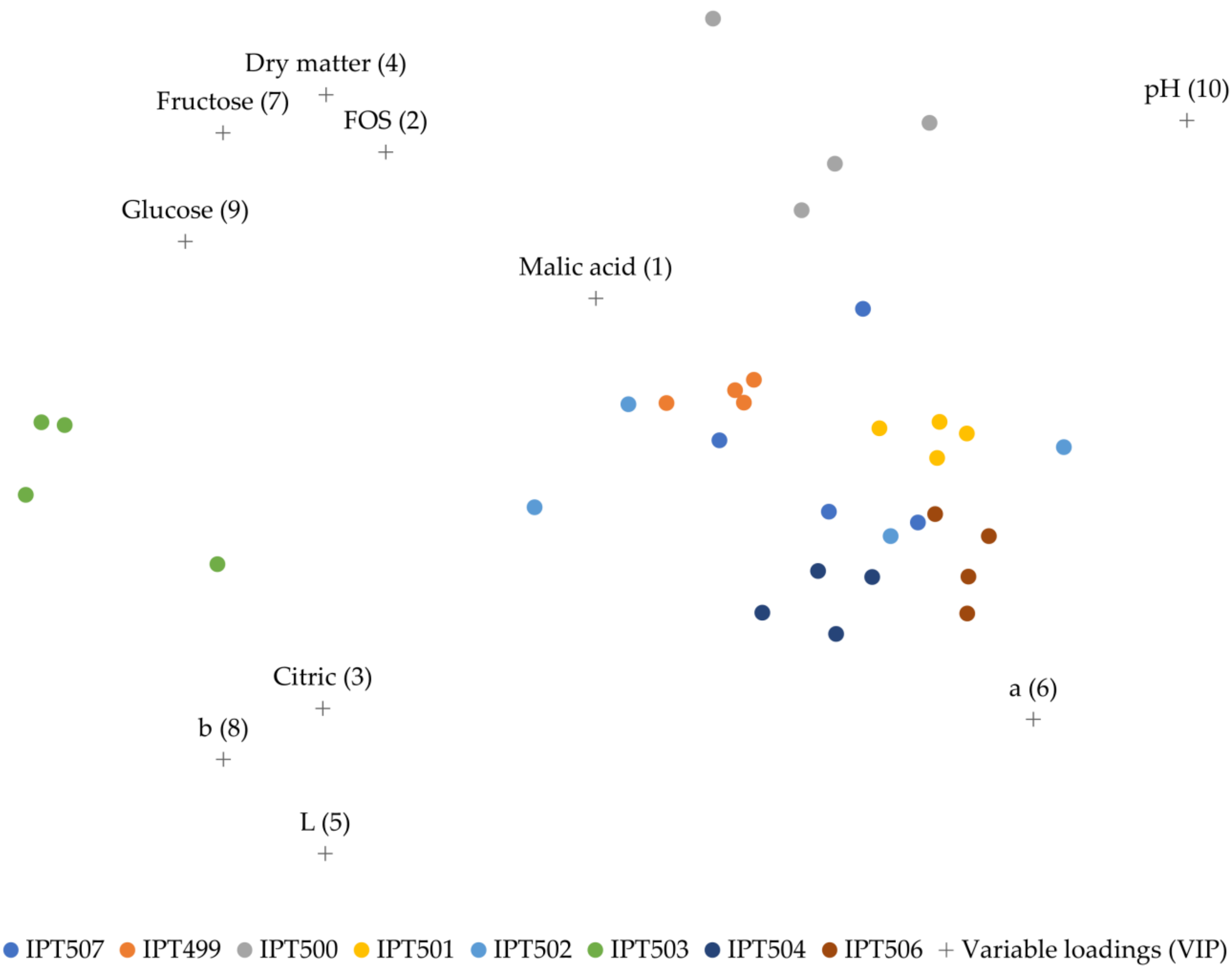

Figure 1. Partial Least Square—Discriminant Analysis of the investigated tomato landraces; *L*- lightness; *a*—green to red; *b*—blue to yellow; FOS—fructooligosaccharides.

A significant positive correlation between tomato dry matter and sugar content was determined. At the same time the dry matter, as well as fructose and glucose content, was negatively correlated with the *a* value (green to red hue), indicating that dry matter content in tomatoes decreased with red color (Table 3). On the other hand, the blue to yellow (*b*) hue was positively correlated with fructose and glucose content, indicating that the yellow tomato peel color was positively associated with sugar content as well as positively correlated with citric acid content (Table 3).

Table 3. Pearson's correlations between the analysed parameters.

Variable	Dry matter	PH	FOS	Fructose	Glucose	Malic Acid	Citric Acid	*L*	*a*	*b*
Dry matter	1.00									
pH	−0.18	1.00								
FOS	0.44 *	−0.14	1.00							
Fructose	0.75 *	−0.22	0.74 *	1.00						
Glucose	0.65 *	−0.33	0.68 *	0.93 *	1.00					
Malic acid	0.14	−0.04	0.04	0.21	0.20	1.00				
Citric acid	0.20	−0.65 *	0.00	0.22	0.37 *	0.40 *	1.00			
L	−0.01	−0.70 *	0.07	0.21	0.35 *	−0.10	0.60 *	1.00		
a	−0.61 *	0.12	−0.15	−0.44 *	−0.38 *	−0.34	−0.13	−0.01	1.00	
b	0.24	−0.76 *	0.21	0.40 *	0.52 *	−0.04	0.64 *	0.93 *	−0.12	1.00

* Significant correlation at $p \leq 0.05$; FOS—fructooligosaccharides; *L*—lightness; *a*—red to green hue; *b*—blue to yellow hue.

4. Discussion

Previous studies have reported that statistically significant differences in the physiochemical variables such as size, weight, pH, sugar content and organic acid content are more consistently influenced by genotype than by different growing methods, environments or cultural practices [21–23].

The dry matter content of tomatoes reported in previous studies [24–26] ranged from 4.07% to 7.85%, depending on the cultivar and the different stages of fruit maturity at harvest. These results are comparable to results obtained in this study (5.77% to 8.36%) and the differences can be attributed to different genotypes.

Our results showed that the landrace IPT503 had the highest fructose and glucose content, and was among others with the highest fructooligosaccharide content, while at the same time, having the highest citric acid and among others with the highest malic acid content. Another characteristic of this landrace is the brightness and intense orange color as observed by the highest lightness and *b* values. Cebolla-Cornejo et al. [22] studied the effects of genotype and environment on the taste and aroma of tomatoes and reported that the content of some sugars and organic acids was significantly genotype dependent, and our study confirmed this effect. The authors reported a range between 1.15 g/100 g and 1.68 g/100 g of fructose per fresh weight, which was approximately two times lower than our results. The results obtained by Rosa-Martinez et al. [26] were comparable to ours, they reported a range of 1.53 g/100 g to 2.35 g/100 g of fructose per fresh weight. The glucose content reported by the mentioned authors ranged from 0.74 g/100 g to 1.29 g/100 g [22] and 1.22 g/100 g to 2.23 g/100 g [26] of glucose per fresh weight, with our results being comparable to the latter range, albeit with a higher maximum and average values.

The contents of organic acids obtained in this study were moderately high compared to values reported by previous studies. Regarding the citric acid content, previously published studies [21,22,26,27] have reported values in a range from 132 mg/100 g fresh weight to 543 mg/100 g fresh weight, which was in line with our values of (385 mg/100 g FW to 588 mg/100 g FW), except for IPT503, which had a 729 mg/100 g fresh weight. However, significant differences were observed when compared to the content of malic acid in previous studies. Studies [22,26,27] reported a malic acid content in tomatoes ranging from 60 mg/100 g fresh weight to 254 mg/100 g fresh weight, while in our study it ranged from 277 mg/100 g FW to 484 mg/100 g FW. The discrepancy between results is likely due to different cultivars used in studies, since malic acid content is cultivar-specific, as noticed by Hernández Suárez et al. [28]. Also, malic acid is sourer, while at the same time having a low impact on titratable acidity compared to citric acid [29]. The average pH values measured in this study were lower compared to earlier studies [21,24], where the authors reported a pH range between 4.25 and 4.78, probably owing to the high organic acid content in our accessions.

Our results showed that there is a positive correlation between the tomato dry matter and sugar content. The tomato dry matter and sugar content were negatively correlated with the *a* value (green to red hue) and at the same time the blue to yellow (*b*) hue was positively correlated with fructose and glucose content indicating that the yellow tomato peel color was positively associated with sugar content as well as positively correlated with citric acid content. The obtained results can be explained by the higher dry matter and sugar content in tomato landraces characterized by the red/yellow peel color compared to the red peel color tomato landraces, as presented in Supplementary Tables S2 and 1, determining the strong positive correlation of the *b* value and negative correlation of the *a* value. The investigated sugars correlated positively with each other. The pH correlated negatively with citric acid while it was not significantly affected by malic acid content. The pH was also negatively correlated with the tomato lightness (*L*) as well as the blue to yellow (*b*) hue.

The red color in tomatoes appears to be a result of the increased biosynthesis of carotenoids (predominantly lycopene) and the depletion of chlorophyll, which is related to the conversion of chloroplasts into chromoplasts during the ripening [30]. According to Selahle et al. [31], the intensity of the red color in tomatoes depends on the relative contents of lycopene and chlorophyll, while the intensity of the yellow color depends on the β-carotene content.

The levels of carotenoids in tomato fruits are affected by a series of factors such as growing conditions and climate [32], but most significantly by the ripening stage and genotype [33]. In previous studies, the *a* value has shown a linear correlation with the ripening stages of the tomatoes [34]. Stinco et al. [35] reported a positive correlation between the color parameter *a* (green to red hue) and lycopene content. Furthermore, Arias et al. [34], Brandt et al. [32] and Stinco et al. [35] reported a positive correlation between the a/b ratio and lycopene content, varying between 0.75 and 0.93. In previous studies the analyses reported a close correlation between the a/b ratio and lycopene content, showing that the a/b ratio is a suitable parameter to characterize the maturity stage of fresh tomatoes. However, it is important to note that some tomato genotypes do not exhibit a red color at all, or only exhibit it partially. There are examples of green, orange and yellow mutants and tomatoes with an external violet color due to the accumulation of both carotenoids and anthocyanins [33]. The results of this study show that the yellow tomato peel color is positively associated with sugar content as well as positively correlated with citric acid content, with the best example of this being landrace IPT503, which had the highest fructose, glucose and citric acid content, while having the lowest *a/b* ratio (Tables 1 and 2).

L, *a* and *b* values of ripe tomatoes obtained by previous studies greatly vary and were highly dependent on the cultivar. *L* values ranged from 30.2 to 65.9, the *a* values ranged from 0.9 to 41.0 and the *b* values ranged from 17.0 to 59.4 [36–38]. These results are comparable to results obtained in this study, although the *a* values were on the higher end of the mentioned range. Young et al. [39] reported that the color of tomatoes is negatively correlated to the total solid content, but positively correlated to the total soluble solids of some tomato lines. Fructose correlated positively with glucose content. Furthermore, it was reported that *a* value (green to red hue) is significantly negatively correlated to pH and positively correlated to citric acid content [39], and these correlations were shown to be insignificant in this study. Causse et al. [40] reported a positive correlation between overall aroma intensity (sugar and acid content) and dry matter content.

5. Conclusions

The present study showed that the studied parameters, including dry matter, sugars, organic acids, peel color and pH differed according to genotype. Based on the developed PLS-DA model, the IPT503 was distinguished from the other tomatoes as the landrace with the highest dry matter, sugar, citric and malic acid content as well as having intense red and yellow hues combined with light peel color. On the other hand, the PLS-DA model result showed that the IPT500 landrace, which is also characterized by high dry matter content, has a darker peel color and less intense red and yellow color when compared to the other investigated landraces. The IPT500 landrace is also the only determinate tomato among the investigated landraces. The results of this study show that the yellow tomato peel color is positively associated with sugar content as well as positively correlated with citric acid content.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030313/s1, Table S1: Tomato landraces used in this study, their origin, and morphological characteristics; Table S2: Weather data during the growing season.

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