

Article

Antioxidant Properties, γ -Aminobutyric Acid (GABA) Content, and Physicochemical Characteristics of Tomato Cultivars

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Citation: Tilahun, S.; Choi, H.-R.; Baek, M.-W.; Cheol, L.-H.; Kwak, K.-W.; Park, D.-S.; Solomon, T.; Jeong, C.-S. Antioxidant Properties, γ -Aminobutyric Acid (GABA) Content, and Physicochemical Characteristics of Tomato Cultivars. *Agronomy* **2021**, *11*, 1204. <https://doi.org/10.3390/agronomy11061204>

Academic Editors: Pedro Javier Zapata and Milena Petriccione

Received: 22 April 2021

Accepted: 11 June 2021

Published: 13 June 2021

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Abstract: Tomatoes are rich in secondary metabolites such as lycopene, β -carotene, phenolics, flavonoids, and vitamin C, which are responsible for their antioxidant activities. A high level of γ -Aminobutyric acid (GABA), a health-promoting functional compound, was also found to accumulate in tomato fruit. In addition to the internal quality attributes, the acceptance of the tomato fruit by consumers is highly dependent on appearance and taste. Hence, we conducted this study to evaluate ‘Tori’, ‘TY VIP’, ‘Mamirio’, and ‘Arya’ tomato cultivars based on their physicochemical characteristics, contents of secondary metabolites, and GABA content. The results have revealed that the tested cultivars were very firm, which renders them the best choice for postharvest distribution of fresh market tomatoes as they resist impacts during harvesting and postharvest operations. Based on total soluble solids (TSS), titratable acidity (TA), and Brix acid ratio (BAR) the choice of cultivar could be ‘Mamirio’ > ‘Tori’ > ‘TY VIP’ > ‘Arya’. Apart from flavor intensity, ‘Mamirio’ and ‘Tori’ also revealed the highest content of ascorbic acid while ‘Mamirio’ and ‘Arya’ had the highest carotenoids (lycopene and β -carotene) accumulation. On the other hand, the highest total phenolics content was recorded from ‘TY VIP’ and ‘Arya’. Moreover, the highest total flavonoids and GABA contents were recorded from ‘TY VIP’. Nevertheless, the antioxidant activity of ‘TY VIP’ was the lowest of all tested cultivars while the highest was recorded from ‘Mamirio’. Taken together, the findings of the present study could suggest that the consumers’ requirements could be better fulfilled by choosing cultivars for the specific target functional compounds. From the tested cultivars, if the target is ascorbic acid, carotenoids, and antioxidant activity then ‘Mamirio’ is the best choice. On the other hand, if the target is total phenolics, flavonoids, and GABA then ‘TY VIP’ is the best choice. One could also label ‘Mamirio’ as an ‘antioxidant tomato’ and ‘TY VIP’ as the ‘GABA tomato’.

Keywords: GABA; β -carotene; lycopene; flavonoids; phenolics; vitamin C

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a vegetable crop belonging to the Solanaceae (nightshade) family and it is native to the Andean region [1]. It is one of the most popular vegetable crops in the world in terms of production and consumption. According to the Food and Agriculture Organization’s corporate statistical database (FAOSTAT) [2], the world production of this crop in particular is 180.77 million tons from 5.03 million ha and

Asia holds 60.1% of the production share. The production in the Republic of Korea in 2019 was 420,573 tons from 6460 ha of land [2].

Tomatoes are one of the most widely consumed horticultural crops in a diversified fresh or processed form. The tomato water content is very high (approximately 93–95%) and solid matter content varies from 5.5–9.5% [3]. The crop contributes to overall health benefit [4]. It is rich in antioxidants such as vitamin C and E, lycopene, phenolics, flavonoids, and β -carotene [5], which may protect cells from the damage caused by free radicals [6]. Daily intake of tomatoes and their derivatives has the potential to lower the risk of many forms of cancer and cardiovascular diseases because of the protective effect of the antioxidants [3,7].

γ -Aminobutyric acid (GABA) is a four-carbon non-proteinogenic amino acid that is widely found in plants, animals, and bacteria [8]. GABA has received much attention as a health-promoting functional compound due to its role as an inhibitory neurotransmitter in the central nervous system [9] and the observation that it reduces blood pressure, induces relaxation, and improves immunity when administered orally [10]. In plants, GABA is primarily metabolized through the GABA shunt pathway which is involved in many physiological responses such as defense against insects, protection from oxidative stresses, and response to diverse stimuli including heat shock and phytohormones [10]. A high level of GABA was found to accumulate in tomato fruit before the breaker stage and catabolized rapidly thereafter [8]. Hence, screening the tomato cultivars that possess higher secondary metabolites, which are responsible for providing their antioxidant activity and cultivars that maintain GABA after the breaker stage, could assist in improving the nutritional quality of tomatoes.

In addition to the above-stated antioxidant properties and GABA content, the acceptance of tomato fruit by consumers is highly dependent on physicochemical characteristics such as firmness, color, total soluble solids (TSS), titratable acidity (TA), and Brix acid ratio (BAR) [11]. The physical properties such as color, shape, firmness, size, and absence of defects are the important components that determine the appearance. Color transformation, flavor changes, and loss of firmness are changes that take place during the maturation and ripening process [12,13]. The postharvest quality of tomatoes develops during the growth of the product and is dependent on several factors such as growing environment, agronomic practices, fertilization, and many other pre-harvest and post-harvest factors [11,14,15]. Moreover, research results have revealed the variation among tomato cultivars in their antioxidant properties and physicochemical properties [15–17]. Hence, the external and internal quality attributes during growing could be achieved by the selection of genotypes that must satisfy the consumers' requirements. Therefore, this study was initiated to evaluate the antioxidant properties, amino acid content (mainly GABA), and physicochemical characteristics of four tomato cultivars that are commonly grown in the Republic of Korea.

2. Materials and Methods

2.1. Plant Material

Four tomato cultivars ('Tori', 'TY VIP', 'Mamirio', and 'Arya'), which are commonly grown by the surrounding farmers, were selected and grown in the same climate-controlled greenhouse in Gangwon province, Republic of Korea, in spring 2019. The fertigation solution was prepared with EC 2.0–2.2 dS m⁻¹ and pH 5.5–5.8 depending on the growth stages. Harvest maturity was attained after 72 days from transplanting. The fruits were harvested from the third cluster of each plant and the fruits at the edge of the cluster have not been included in the experiment. Fruits free from any physical defects and of uniform size were at the pink stage using the United States Department of Agriculture (USDA) tomato ripeness color classification chart [18]. Sorting of the pink stage was conducted again in the laboratory using the color chart developed by USDA [18]. After selection, the physicochemical data were taken immediately and samples for the analysis of important secondary metabolites (lycopene, β -carotene, ascorbic acid, polyphenols, and

flavonoids) and antioxidant activity were frozen by liquid nitrogen and placed in a deep freezer (-80°C) for about three weeks until analysis. Samples for GABA analysis were freeze dried, grounded to fine powder, filtered with $40\text{-}\mu\text{m}$ mesh, and then stored at -20°C until extraction.

2.2. Firmness

The firmness of tomato fruit was determined using the methodology described by Tilahun et al. [16]. Measurements were made at the equator of the fruit using a Rheometer (Sun Scientific Co. Ltd., Tokyo, Japan) fitted with a 3 mm diameter round stainless-steel probe having a flat end and the results were expressed in N.

2.3. Total Soluble Solids (TSS), Titratable Acidity (TA), and Brix Acid Ratio (BAR)

TSS and TA were determined based on Tilahun et al. [19]. The tomato fruit was cut into two halves, squeezed by hand, and then filtered with gauze (Daehan Medical Supply Co., Ltd., Seoul, Korea). The TSS was measured from five sample fruits at 20°C using a Atago DR-A1 digital refractometer (Atago Co. Ltd., Tokyo, Japan) and expressed in degree Brix ($^{\circ}\text{Bx}$). TA was determined after titrating diluted tomato juice (1 mL juice: 19 mL distilled water) with 0.1 N NaOH until pH of 8.1 using a DL22 Food and Beverage Analyzer (Mettler Toledo Ltd., Zurich, Switzerland). The citric acid ($\text{mg } 100\text{ g}^{-1}$) of fresh tomato weight was used to express the TA result. BAR was determined by dividing the TSS with TA [20].

2.4. Color Changes

The color of the tomato fruit was determined using the Hunter a^* (redness), b^* (yellowness), and L^* (brightness) values [21] by a CR-400 Chroma meter (Minolta, Tokyo, Japan). Color variables were measured three times from each tomato close to the equatorial section and the average value was calculated.

2.5. Lycopene and β -Carotene Content

Measurements of lycopene and β -carotene content were performed with a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The lycopene content was measured from three tomato samples based on the Fish et al. [22] method with some adjustments as stated by Tilahun et al. [23]. The β -carotene content was also measured from three tomato samples as described by Tilahun et al. [23]. Quantification of lycopene content was then expressed as mg kg^{-1} of fresh weight, according to the method reported by Fish et al. [22], and the β -carotene content was quantified and expressed as mg kg^{-1} by comparing the sample readings with the standard curve.

2.6. Ascorbic Acid

A tomato fruit sample (1 g) was mixed with 10 mL of 5% metaphosphoric acid and homogenized for 1 min. The homogenized sample was centrifuged ($7828\times g$ for 10 min), the liquid layer of extracts was membrane-filtered ($0.22\text{ }\mu\text{m}$) (Advantec, Tokyo, Japan), and analyzed as described by Kim et al. [24]. Analysis was performed by using ZORBAX Eclipse XDB-C18 ($4.6\times 250\text{ mm}$, $5\text{ }\mu\text{m}$, Agilent, Santa Clara, CA, USA) column and detector (UV-2075, Jasco, Tokyo, Japan) at 265 nm. MeOH: 0.1 M KH_2PO_4 (1:9 ratio) was used as the mobile phase.

2.7. Total Phenolics and Flavonoids

A method described by Tilahun et al. [25] was used for the determination of total phenolics content. Briefly, 2 g of each tomato sample was extracted with 20 mL of 0.05% (v/v) aqueous HCl/methanol (10:90, v/v) using a homogenizer (Ultra Turrax T18 Basic, IKA, Staufen, Germany) at speed 5000 rpm for 1 min and the homogenate was membrane-filtered ($0.22\text{ }\mu\text{m}$) (Advantec, Tokyo, Japan). The sample extract (0.2 mL) was mixed with 2.6 mL of deionized water, 2 mL of 7% (w/v) Na_2CO_3 , and 0.2 mL of Folin-Ciocalteu's

phenol reagent. After incubating at 23 °C for 90 min, a spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) was used to measure the absorbance of the reaction mixture at 750 nm against a blank sample containing the same mixture solution without the sample extract. The total phenolics content was expressed as mg of gallic acid equivalents (GAE) kg⁻¹ fresh weight of the sample.

The total flavonoid content was determined using a method described by Zhishen et al. [26] with some modifications. Sample solution (0.5 mL) containing 5 mg mL⁻¹ of the freeze-dried extract was mixed with 1.5 mL of ethanol, 0.1 mL of 10% aluminum nitrite solution, 0.1 mL of 1 M potassium acetate solution, and 2.8 mL distilled water. The mixture was stirred and permitted to react for 30 min. The absorbance was then measured at 415 nm using a microplate reader (Spectramax i3, Molecular Devices, Sunnyvale, CA, USA). The measurements were compared to a calibration curve of rutin and the results were expressed as milligrams of rutin equivalents (RE) per kg⁻¹ fresh weight of the sample.

2.8. Amino Acids Mainly γ -Aminobutyric Acid (GABA)

Freeze dried fruit samples were extracted by 75% EtOH with ultrasonic extraction for 1 h followed by extraction at room temperature for 24 h. Analysis was made after filtering the extract with 0.2 μ m filter. The amino acids content including GABA content was measured by Dionex Ultimate 3000 HPLC using the method described by Henderson et al. [27].

2.9. Antioxidant Activity

The DPPH spectrophotometric assay (ThermoFisher Scientific, Madison, WI, USA) was used, as described by Pataro et al. [28], to determine the antioxidant activity from the same extract used for total phenolic content.

2.10. Statistical Analysis

The experiment was conducted in a completely randomized design. The data were subjected to analysis of variance (ANOVA) in order to determine the significance of differences between cultivars at $p < 0.05$ using SAS statistical software (SAS/STAT[®] 9.1; SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was performed to observe differences between the treatment means. Heat map analysis was used to visualize the differences in amino acids content between the cultivars.

3. Results and Discussion

3.1. Firmness

Firmness is one of the main aspects that consumers use to evaluate the quality of fresh tomato fruit [29]. It is also an important characteristic for resistance against mechanical injury during harvest or postharvest operations [15]. A statistically significant difference ($p < 0.05$) in firmness was observed among the four tomato cultivars in the present study (Table 1). The highest value (11.88 N) and the lowest value (8.31 N) of firmness were recorded from 'Mamirio' and 'TY VIP' cultivars, respectively, although the difference between 'Mamirio', 'Tori', and 'Arya' was not significant. Several research results have also found significant differences among different cultivars showing the dependence of firmness on cultivars [15,16,19,30,31]. However, the firmness result from all the four cultivars was above the recommended limits for home consumption (1.28 N) and retailers (1.46 N) [32]. This means that all the tested cultivars were very firm, which makes them the best choice for postharvest distribution of fresh market tomatoes as they resist impacts during harvesting and postharvest operations.

Table 1. Firmness, TSS, TA, and BAR of ‘Tori’, ‘TY VIP’, ‘Mamirio’, and ‘Arya’ tomato cultivars at the commercial pink ripening stage.

Varieties	Firmness (N)	TSS (°Bx)	TA (mg 100 g ⁻¹)	BAR
Tori	10.54 ± 0.64 a	4.86 ± 0.05 b	0.38 ± 0.01 ab	12.79 ± 0.16 b
TY VIP	8.31 ± 0.63 b	5.34 ± 0.08 a	0.47 ± 0.03 ab	11.36 ± 1.17 b
Mamirio	11.88 ± 0.47 a	4.84 ± 0.12 b	0.26 ± 0.06 b	18.61 ± 1.72 a
Arya	11.45 ± 0.48 a	4.46 ± 0.06 b	0.59 ± 0.04 a	7.56 ± 0.79 c

For each value, the mean ± standard error was determined in five replicates; values with different letters within the same column are significantly different ($p < 0.05$).

3.2. TSS, TA, and BAR

The main components of soluble solids are soluble sugars and organic acids, which affects the sweetness, sourness, and flavor intensity of tomato [33]. The current study result has shown the effect of cultivars on the content of total soluble solids. The cultivar ‘TY VIP’ had significantly higher TSS (5.34 °Bx) content while ‘Tori’, ‘Mamirio’, and ‘Arya’ are statistically similar to one another (Table 1). In agreement with this result, Tilahun et al. [25] have found a significant difference among two cultivars in TSS ranging from 5.16 to 5.62%.

Even though citric acid, malic acid, and glutamic acid are the contributors to TA, citric acid is the major one and low TA is assumed to be due to loss of citric acid during maturity or over maturity [34]. Similar to TSS, TA of the fruit also contributes to the flavor of the tomato [35]. The statistical analysis of the current study has shown a significant difference between the cultivars in TA content. ‘Mamirio’ was found to be the cultivar with the lowest (0.26) acidity, while ‘Arya’ possessed the highest (0.59) acidity (Table 1). Similarly, Tigist et al. [36] have found a significant difference in TSS and TA among different tomato cultivars.

In addition to the contribution to the sweetness and sourness of tomatoes, since both TSS and TA are the major factors in overall flavor intensity, BAR data could be improved in the estimation of flavor intensity of the fruits [20]. Hence, increasing BAR could contribute to improving the flavor of tomatoes because the lack of flavor is the common complaint about fresh market tomatoes. In the present study, BAR was inversely related to TA. ‘Mamirio’ had the highest (18.61) BAR, while ‘Arya’ had the lowest (7.56) (Table 1). Hence, the choice of cultivar could be ‘Mamirio’ > ‘Tori’ > ‘TY VIP’ > ‘Arya’ based on BAR.

3.3. Color Values

Color, which is derived from natural pigments, is the most commonly used criteria that determine the fruit’s appearance and affects consumers’ preference [34]. In the present study, harvesting at the commercial pink stage was preferred based on its relevance on functional substances (mainly to balance the contents of antioxidant properties and changes in GABA content since GABA catabolizes rapidly after breaker stage) and suitability for the postharvest distribution of fresh market tomatoes since the pink stage resists impact during harvesting and postharvest operations. Moreover, it was based on the growers’ experience to fulfill the consumers’ requirements. Objective color measurements were made by a Chroma meter and evaluated using Hunter a*, b*, and L* color values. The result of the present study revealed that the hunter a*, b*, and L* values were significantly different ($p < 0.05$) among all cultivars. The highest hunter a* value (+22.04) was recorded from the ‘Mamirio’ cultivar and this indicated more redness of the fruit while ‘Tori’, ‘TY VIP’, and ‘Arya’ showed statistically similar hunter a* values (Figure 1). The distinctive red color in tomato fruit is due to the accumulation of lycopene, which constitutes 98% of its carotenoid [37]. The accumulation takes place during ripening from the breakdown of chlorophyll, which is a genetically programmed process [38]. The highest hunter b* values (+24.74) and (+24.65) were recorded from ‘Mamirio’ and ‘Arya’, respectively, demonstrating more yellowness due to the higher content of β -carotene [23], while the lowest (+16.46) were recorded from ‘TY VIP’. Regarding hunter L*, ‘Mamirio’ was the cultivar that possessed

more bright fruit among the four cultivars (Figure 1). Similar to this study, Kaur et al. [39] and Tilahun et al. [19] have found a significant color difference between tomato cultivars.

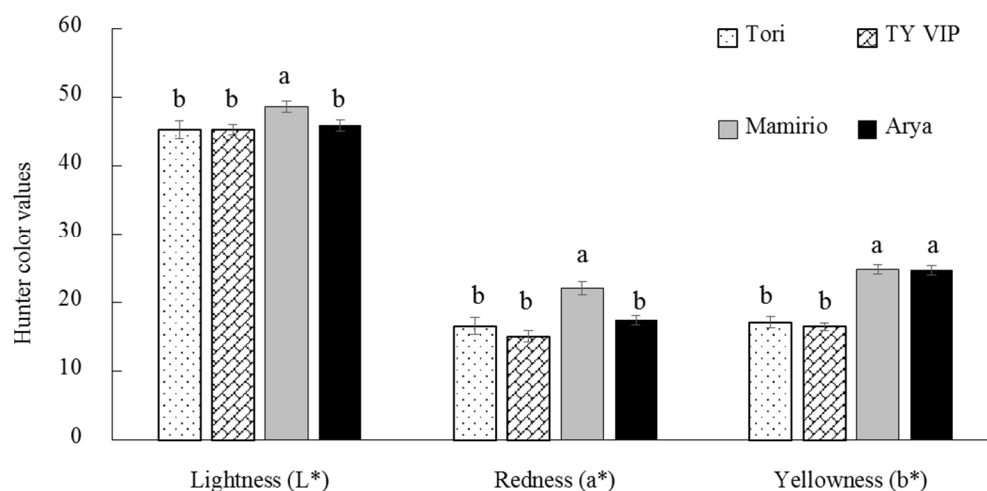


Figure 1. Hunter's L*, a*, and b* color values of 'Tori', 'TY VIP', 'Mamirio', and 'Arya' tomato cultivars at the commercial pink ripening stage. The bars with different letters indicate a significant difference ($p < 0.05$) between cultivars. The vertical bars represent the standard error of the means ($n = 5$).

3.4. Lycopene and β -Carotene Content

The transformation of chloroplasts to chromoplasts results in the characteristic pigmentation of tomato fruit due to the synthesis of carotenoids [38]. As the ripe tomato fruit accumulates lycopene (red linear carotene), it also accumulates its orange cyclization pro-vitamin A product (β -carotene) [40,41]. Lycopene is one of the most active antioxidants among dietary carotenoids, which is responsible for the redness of tomato fruit [42] and responsible for the biosynthesis of many carotenoids such as β -carotene [43]. The present study has demonstrated that there are significant variations among cultivars in lycopene content. 'Mamirio' was the highest (42.84 mg kg^{-1}) in lycopene content and was followed by 'Arya' (35.30 mg kg^{-1}). 'Tori' and 'TY VIP' were statistically the same in lycopene content. The lowest (24.86 mg kg^{-1}) was recorded from 'TY VIP' (Figure 2). Similar results were recorded from different studies supporting the variation of lycopene content among cultivars. Its amount in fresh tomato and tomato products shows variation depending on the cultivars and other factors [11]. According to Dominguez et al. [44], the amount of lycopene could range from 19 to 65 mg kg^{-1} fresh weight in ripened tomato fruits. Kaur et al. [39] have also found significantly different lycopene content among seven tomato cultivars ranging from 79.7 to 142.8 mg kg^{-1} . Similarly, Dobrin et al. [45] and Thompson et al. [46] have also found different lycopene content among tomato cultivars ranging from 47.4 to 66.4 mg kg^{-1} and 41.54 to 55.60 mg kg^{-1} , respectively.

In the present study, a significant variation among cultivars in β -carotene was also observed. 'Arya' was the highest (27.17 mg kg^{-1}) in β -carotene content, while 'TY VIP' exhibited the lowest (11.97 mg kg^{-1}). The range of β -carotene (11.97 – 27.17 mg kg^{-1}) in the present study is higher than in previous reports (Figure 2). Baranska et al. [47] reported β -carotene content ranging from 2.3 to 7.8 mg kg^{-1} from six fresh tomato samples. Similarly, Tilahun et al. [23] reported β -carotene content ranging from 0.24 to 8.72 mg kg^{-1} from '244' tomato cultivar that was harvested at different maturity stages. Overall, although this study was conducted at the commercial pink stage, the lycopene contents of all four cultivars were in the range of previous reports and their β -carotene contents were higher than previous reports. The results of the present study also confirm that higher hunter a* and b* values are corresponded to higher contents of lycopene and β -carotene contents (Figures 1 and 2).

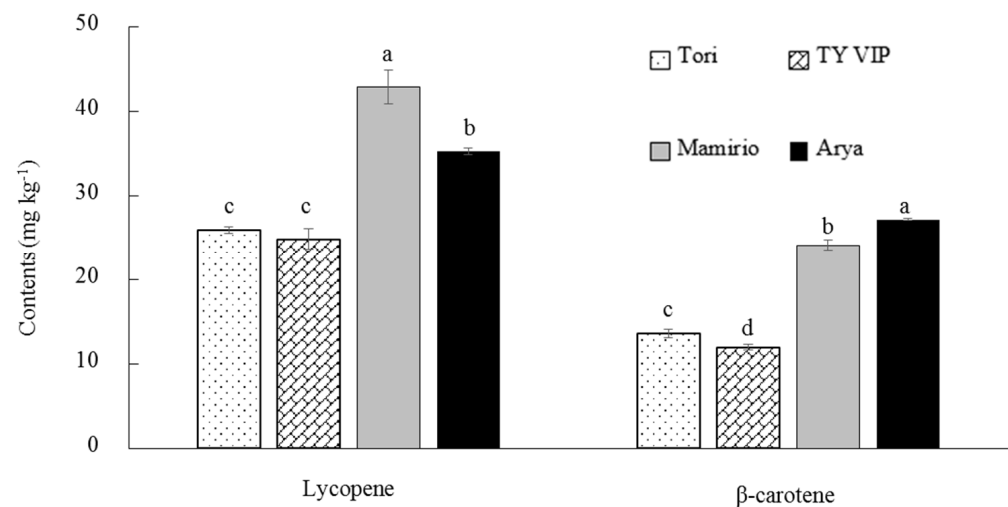


Figure 2. Lycopene and β-carotene contents of ‘Tori’, ‘TY VIP’, ‘Mamirio’, and ‘Arya’ tomato cultivars at the commercial pink ripening stage. The bars with different letters indicate a significant difference ($p < 0.05$) between cultivars. The vertical bars represent the standard error of the means ($n = 5$).

3.5. Ascorbic Acid

Tomatoes are rich in ascorbic acid (vitamin C) [48], which is easily absorbed by the body. It has a lot of health benefits such as preventing low-density lipoprotein oxidation, fighting scurvy, maintaining collagen, and ameliorate neurodegenerative disease [44,49]. As observed from this study, the content of vitamin C in tomato fruit is dependent on cultivars. Vitamin C has significantly varied among the four studied cultivars with the highest content ($65.63 \text{ mg } 100 \text{ g}^{-1}$) recorded in ‘Mamirio’ and lowest ($42.65 \text{ mg } 100 \text{ g}^{-1}$) in ‘TY VIP’ (Figure 3). Similar to the current study, Tilahun et al. [31] reported cultivar dependent ascorbic acid content ranging from 20.01 to 31.14 and from 20.97 to 30.16 $\text{mg } 100 \text{ g}^{-1}$ for ‘TY megaton’ and ‘Yureka’ tomato cultivars, respectively. Violeta et al. [50] have also reported a significant difference among ten tomato cultivars in their ascorbic acid content ranging from 9.58 to 32.97 $\text{mg } 100 \text{ g}^{-1}$. However, the range of ascorbic acid ($42.65\text{--}65.63 \text{ mg } 100 \text{ g}^{-1}$) in the present study is higher than those reported by Tilahun et al. [31] and Violeta et al. [50], indicating a higher ascorbic acid content of all the tested cultivars in the order of ‘Mamirio’ > ‘Tori’ > ‘Arya’ > ‘TY VIP’ (Figure 3).

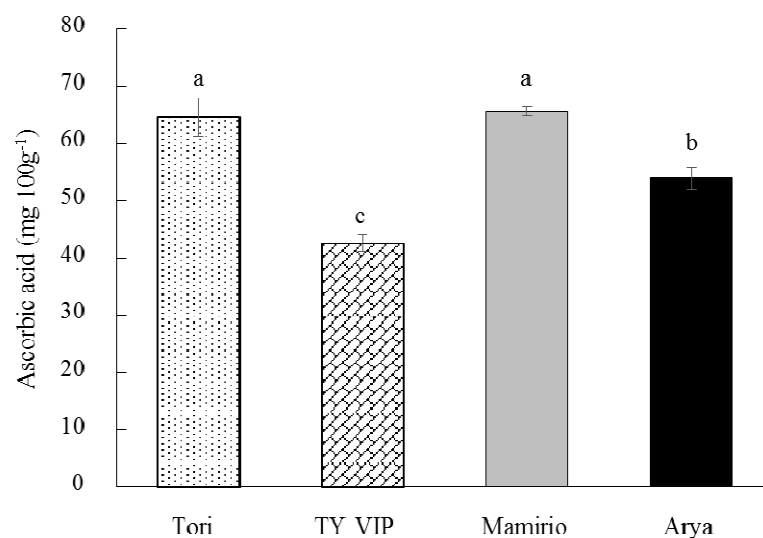


Figure 3. Ascorbic acid content of ‘Tori’, ‘TY VIP’, ‘Mamirio’, and ‘Arya’ tomato cultivars at the commercial pink ripening stage. The bars with different letters indicate a significant difference ($p < 0.05$) between cultivars. The vertical bars represent the standard error of the means ($n = 5$).

3.6. Total Phenolics and Flavonoids

Phenolics are a large group of molecules [51] that function as natural antioxidants in plants [52]. They can prevent chronic diseases related to excess free radicals [53] by reducing oxidative stress [48]. In this study, the tested four cultivars have shown significantly different contents of total phenolics. The ‘TY VIP’ and ‘Arya’ cultivars had statistically similar highest results of total phenolics (736.52 and 730.15 mg kg⁻¹, respectively), while the lowest (182.38 mg kg⁻¹) was recorded from ‘Tori’ (Figure 4). Several studies have shown the difference between tomato cultivars in total phenolics content and the results of the current study are within the range of previous reports. Violeta et al. [50] have reported the total phenolics ranging from 312.2 to 557.8 mg GAE kg⁻¹ among ten cultivars. Periago et al. [54] have also reported significant variation ranging from 259.15 to 498.60 mg kg⁻¹ among nine varieties of fresh tomato cultivars.

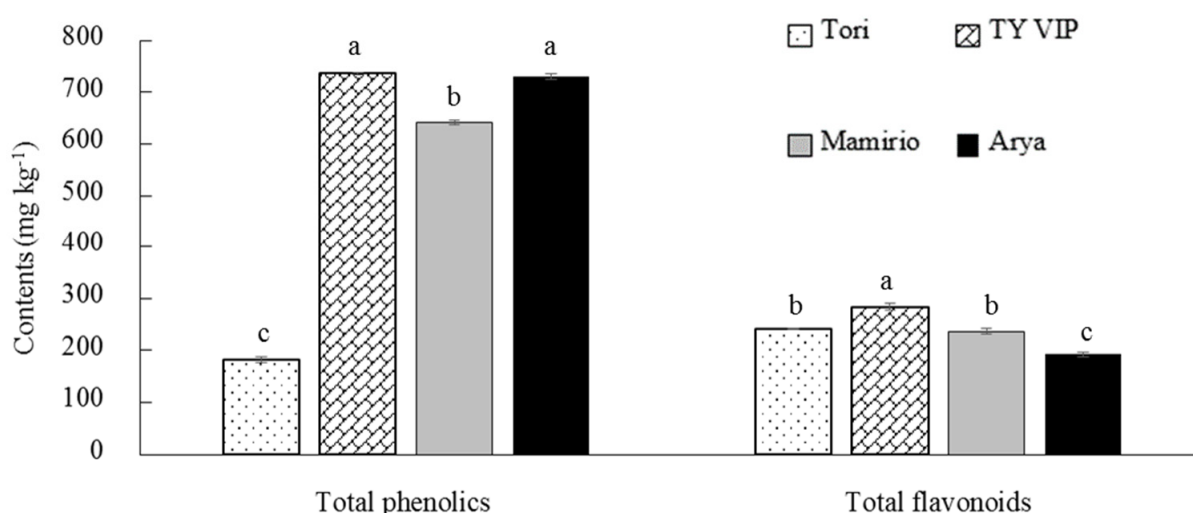


Figure 4. Total phenolics and flavonoids content of ‘Tori’, ‘TY VIP’, ‘Mamirio’, and ‘Arya’ tomato cultivars at the commercial pink ripening stage. The bars with different letters indicate a significant difference ($p < 0.05$) between cultivars. The vertical bars represent the standard error of the means ($n = 5$).

Flavonoids are the main component of total phenolics [55]. They have high antioxidant power that significantly contributes to the health benefit [56]. Hence, efforts have been made to elevate flavonoids concentration in different tomato cultivars [57,58]. The flavonoid content in the present study has shown significant variation among the four cultivars ranging from 192.22 mg kg⁻¹ in ‘Arya’ to 284.01 mg kg⁻¹ in ‘TY VIP’ (Figure 4). Previous results ranging from 40 to 260 mg kg⁻¹ were also reported by Slimestad et al. [57] for samples of different types of tomatoes observed. Taken together, all the tested cultivars in the present study have shown higher contents of total phenolics and flavonoids as compared to the previous reports. Moreover, ‘TY VIP’ and ‘Mamirio’ could be preferred in terms of total phenolics and flavonoids from the tested cultivars as indicated in Figure 4.

3.7. Free Amino Acids and γ -Aminobutyric Acid (GABA)

The content of free amino acids was significantly ($p < 0.05$) different among the four cultivars. From the tested cultivars, the total free amino acid content of ‘TY VIP’ was the highest (57.31 g kg⁻¹) followed by ‘Mamirio’ (41.08 g kg⁻¹), ‘Tori’ (39.76 g kg⁻¹), and ‘Arya’ (38.81 g kg⁻¹), respectively (Table 2). In agreement with the current results, Ahn [59] tested ‘Rafito’, ‘Momotaro’, and ‘Medison’ tomato cultivars and reported that the total free amino acid content ranged from 38.10 to 45.94 g kg⁻¹ dry weight. Of all free amino acids, glutamic acid (glutamate) was the most abundant free amino acid in all the four cultivars and ranged from 17.93 g kg⁻¹ for ‘TY VIP’ to 15.17 g kg⁻¹ for ‘Arya’. Glutamine was the second most abundant free amino acid in all the four tested cultivars. The contents ranged from 17.98 g kg⁻¹ in ‘TY VIP’ to 11.43 g kg⁻¹ in ‘Mamirio’. Sorrequieta et al. [60]

also reported glutamine and glutamate as the most abundant free amino acid during the ripening of tomato fruit. The relative proportions of aspartic acid, GABA, and asparagine were also higher than the other amino acids in all tested cultivars (Table 2 and Figure S1). Hence, glutamic acid, glutamine, aspartic acid, GABA, and asparagine were the abundant amino acids in all the four cultivars, comprising 80.65 to 89.98% of total free amino acids (Table 2). Ahn [59] also reported glutamic acid, glutamine, and aspartic acid as the primary amino acids that comprise 82.19 to 84.14% of total free amino acids in ‘Rafito’, ‘Momotaro’, and ‘Medison’ tomato cultivars. Similarly, Pratta et al. [61] reported a significant variation of free amino acids among different tomato genotypes and higher relative content of glutamine, glutamate, asparagine, aspartate, and GABA in all tested genotypes.

Table 2. Free amino acid content of ‘Tori’, ‘TY VIP’, ‘Mamirio’, and ‘Arya’ tomato cultivars at commercial pink ripening stage.

Amino Acids	Tori		TY VIP		Mamirio		Arya	
	mg kg ^{−1} (Dry Weight)	% of Total Amino Acids	mg kg ^{−1} (Dry Weight)	% of Total Amino Acids	mg kg ^{−1} (Dry Weight)	% of Total Amino Acids	mg kg ^{−1} (Dry Weight)	% of Total Amino Acids
Aspartic acid	3062.49 d	7.70	3781.79 a	6.60	3322.94 b	8.09	3309.36 c	8.53
Glutamic acid	15,587.17 c	39.20	17,926.04 a	31.28	17,293.17 b	42.10	15,166.22 d	39.07
Asparagine	1870.43 c	4.70	2481.90 a	4.33	1596.54 d	3.89	1945.60 b	5.01
Serine	525.38 c	1.32	1448.11 a	2.53	573.54 b	1.40	427.40 d	1.10
Glutamine	12,146.58 b	30.55	17,983.99 a	31.38	11,429.39 d	27.82	12,097.73 c	31.17
Histidine (EAA)	300.06 c	0.75	383.90 a	0.67	296.97 d	0.72	353.42 b	0.91
Glycine	69.14 b	0.17	303.31 a	0.53	61.64 c	0.15	46.66 d	0.12
Threonine (EAA)	503.59 c	1.27	1210.25 a	2.11	500.60 d	1.22	569.08 b	1.47
Arginine	137.17 d	0.34	571.49 a	1.00	196.80 c	0.48	208.68 b	0.54
Alanine	405.48 c	1.02	784.19 a	1.37	423.08 b	1.03	242.64 d	0.63
GABA	3112.77 b	7.83	4048.98 a	7.06	2977.96 c	7.25	2198.60 d	5.66
Tyrosine	199.67 c	0.50	758.07 a	1.32	165.02 d	0.40	288.26 b	0.74
Valine (EAA)	110.40 c	0.28	464.75 a	0.81	110.99 b	0.27	105.90 d	0.27
Methionine (EAA)	73.35 b	0.18	259.57 a	0.45	55.90 c	0.14	48.18 d	0.12
Tryptophane (EAA)	142.95 d	0.36	335.22 a	0.58	146.62 c	0.36	191.47 b	0.49
Phenylalanine (EAA)	745.75 d	1.88	2046.38 a	3.57	906.40 b	2.21	819.14 c	2.11
Isoleucine (EAA)	195.06 d	0.49	593.39 a	1.04	213.93 c	0.52	256.37 b	0.66
Leucine (EAA)	199.57 d	0.50	932.48 a	1.63	215.72 c	0.53	261.92 b	0.67
Lysine (EAA)	150.95 d	0.38	614.10 a	1.07	161.15 c	0.39	182.34 b	0.47
Proline	226.81 c	0.57	387.54 b	0.68	430.26 a	1.05	94.60 d	0.24
Total EAA	2421.68 d	6.09	6840.03 a	11.93	2608.28 c	6.35	2787.82 b	7.18
Total amino acids	39,764.76 c		57,315.44 a		41,078.61 b		38,813.57 d	

EAA: Essential amino acid, mean values ($n = 3$) in the same row with different letters are significantly different at $p < 0.05$.

3.8. Antioxidant Activity

Of the total free amino acids, the essential amino acid content ranged from 2.42 g kg^{−1} (6.09%) in ‘Tori’ to 6.84 g kg^{−1} (11.93%) in ‘TY VIP’. There was also a significant difference ($p < 0.05$) in the contents of essential amino acids of the four cultivars. The levels of nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) can be used to measure the quality of the protein in a foodstuff [59]. All the four cultivars demonstrated a good balance of all the essential amino acids in the following order: ‘TY VIP’ > ‘Arya’ > ‘Mamirio’ > ‘Tori’.

A high level of GABA was found to accumulate in tomato fruit before the breaker stage and catabolized rapidly thereafter [8]. In the present study, ‘TY VIP’ significantly dominates the other three cultivars in terms of GABA content at commercial pink stage (Table 2 and Figure S2). Sorrequieta et al. [60] emphasized that the GABA content reduced

significantly during the ripening transition. Hence, screening the tomato cultivars that maintain GABA after the breaker stage could assist in improving the nutritional quality of tomatoes. In this study, the highest GABA content (4.05 g kg^{-1}) was recorded from 'TY VIP', while the lowest (2.20 g kg^{-1}) was recorded from 'Arya' at the pink stage (Table 2 and Figure S2). Therefore, the order of preference in terms of GABA content could be 'TY VIP' > 'Tori' and 'Mamirio' > 'Arya'. Similar to the present findings, Akihiro et al. [8] reported differences among cultivars in terms of GABA content and labeled 'DG03-9' as GABA rich cultivar.

The nutritional quality of tomato fruit is strongly dependent on antioxidants [56]. The antioxidant activities of the four cultivars in the present study were significantly different ranging from 89.04% in 'TY VIP' to 99.16% in 'Mamirio' (Figure 5). The methanol extracts of the tested cultivars in the present study showed excellent scavenging effects similar to the results reported by Chang et al. [62], who reported antioxidant activity ranging from 87.7 to 99.7% for methanol extracts from fresh and various processed tomatoes. In agreement with the current study, Tilahun et al. [19] reported significant differences between 'TY Megaton' and 'Yureka' tomato cultivars in antioxidant activity. As observed in Figure 5 the order of preference in terms of antioxidant activity could be 'Mamirio' > 'Tori' > 'Arya' > 'TY VIP'.

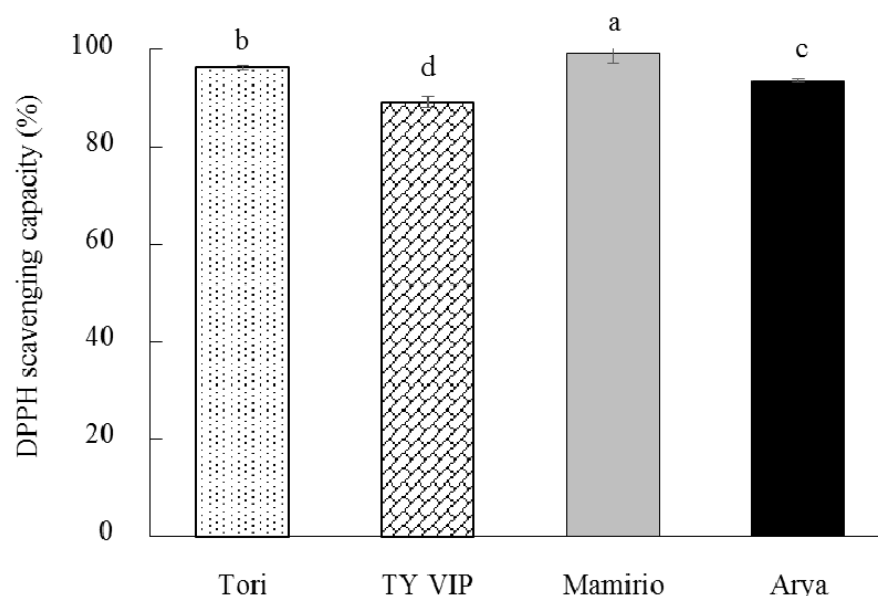


Figure 5. Antioxidant activity of 'Tori', 'TY VIP', 'Mamirio', and 'Arya' tomato cultivars at commercial pink ripening stage. The bars with different letters indicate a significant difference ($p < 0.05$) between cultivars. The vertical bars represent the standard error of the means ($n = 5$).

4. Conclusions

The results of the present study revealed that the tested 'Tori', 'TY VIP', 'Mamirio', and 'Arya' tomato cultivars were very firm which renders them the best choice for postharvest distribution of fresh market tomatoes as they resist impacts during harvesting and postharvest operations. Furthermore, their color meets consumers' preferences as the fruits were harvested at the commercial pink stage and in the range of previous reports. However, based on TSS, TA, and BAR for overall flavor intensity, the choice of cultivar from the four tested cultivars could be 'Mamirio' > 'Tori' > 'TY VIP' > 'Arya'. Apart from flavor intensity, 'Mamirio' and 'Tori' also revealed the highest content of ascorbic acid, while 'Mamirio' and 'Arya' had the highest carotenoids (lycopene and β -carotene) accumulation. On the other hand, the highest total phenolics content was recorded from 'TY VIP' and 'Arya'. Moreover, the highest total flavonoids and GABA contents were recorded from 'TY VIP'. Nevertheless, the antioxidant activity of 'TY VIP' was the lowest of all tested cultivars while the highest was recorded from 'Mamirio'. The findings of the present study could

suggest that the cumulative effect of all functional substances could affect the antioxidant activity of tomato fruit. In order to fulfill the consumers' requirements, it would be better to choose cultivar/s based on the specific functional compound. For instance, if the target is ascorbic acid, carotenoids, or antioxidant activity then 'Mamirio' is the best choice and one could label it as 'antioxidant tomato'. On the other hand, if the target is total phenolics, flavonoids, or GABA then 'TY VIP' is the best choice and it may be labeled 'GABA tomato'.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/agronomy-1212644/s1>, Figure S1: Heat map of the amino acids in 'Arya', 'Tori', 'Mamirio', and 'TY VIP' tomato cultivars. Three replicates were analyzed using three fruit for each replicate. The data were standardized for heat map. Figure S2: GABA content of 'Tori', 'TY VIP', 'Mamirio', and 'Arya' tomato cultivars at commercial pink ripening stage.

Author Contributions: Conceptualization, S.T., H.-R.C. and C.-S.J.; methodology, S.T. and H.-R.C. execution of experiment, S.T., M.-W.B., L.-H.C. and H.-R.C.; software, S.T. and T.S.; formal analysis, T.S. and H.-R.C.; resources, K.-W.K. and C.-S.J.; original draft preparation, T.S. and H.-R.C.; review and editing S.T., D.-S.P. and T.S.; supervision, C.-S.J.; project administration, M.-W.B. and K.-W.K.; funding acquisition, C.-S.J. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation (NRF), Korea, under BK21 FOUR under project.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data sets are available upon reasonable request from the corresponding author.

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

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