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Determination of bioactive components in different tomato lines: Physicochemical properties and antioxidant activity

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Abstract

Tomato, one of the most produced vegetables in the world, is experiencing continuous global increase in both production and consumption. Fruit quality traits are important for fresh market tomatoes as well as for the processing industry. Despite the growing demand for both fresh and processed tomatoes, consumers are not satisfied with the quality of available fruits. The main objectives of the present work were to determine the physicochemical characteristics [pH, total soluble solids (TSS), total titratable acids (TTA), TSS/TTA ratio, DMC, lycopene, β-carotene, vitamin C, and total phenolic content], as well as the antioxidant activity of 13 different tomato lines, and to identify the most promising ones in terms of fruit taste and quality. Antioxidant activity was determined using the ABTS and DPPH methods with Trolox used as the standard compound. PCA analysis was conducted to identify group patterns. The results of PCA analysis indicated a specific genotypic response in all investigated physicochemical traits. Genotypes 2, 10, and 13 were identified as the best for fresh consumption, as they exhibited the highest levels of compounds crucial for good taste, nutrition, and human health benefits. The most promising genotype related to fruit quality attributes was genotype 10 with the best TSS and TAA content and TSS/TAA ratio, which is important for overall taste perception. On the other hand, genotype 9 showed promise for industrial purposes due to its ideal pH value in the juice and good soluble solid content. High antioxidant activity was characteristic of genotypes 1 and 2, and their consumption as fresh tomatoes can be beneficial to human health. They also should be considered for further evaluation as potentially interesting genotypes for abiotic stress research and selection programs which can lead to the development of both superior fruit quality and stress tolerant genotypes.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables with world production around 186.8 Mt in 2020 (FAO, 2020). Despite the large production, consumers worldwide are still not satisfied with the taste of modern commercial tomatoes. Breeding programs in the past responded well to the demands of the producers (high yields, fruit firmness, long shell life, transportability, disease resistance, *etc*.). Still, modern cultivars failed to respond to consumer preferences in terms of quality, especially flavour, in comparison to the traditional genotypes (Tieman *et al*., 2017).

The quality of tomato fruit is determined by various parameters. Among the morphological characteristics, the key factors include the fruit's shape, size, and firmness. However, modern consumers are particularly interested in the sensory traits of tomatoes. Literature indicates a strong correlation between sensory traits and the physicochemical composition of tomato fruit (Sinesio *et al*., 2021). The overall tomato flavour is the result of interactions between taste and olfaction. The perception of tomato taste is primarily determined by the ratio of soluble solids to organic acids. Sweetness is determined by the soluble sugar content, while sourness is linked to the concentration of organic

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acids. Additionally, the sensory perception of tomato taste places importance on the amino acid composition, particularly the concentration of glutamic acid. Conversely, the fruit olfaction contributes to the overall perception of tomato fruit quality due to the complex interplay of various volatile organic compounds (Tieman *et al*., 2012).

The quality of tomatoes is further determined by health-promoting phytonutrients, and various secondary metabolites with antioxidant activity, which is highly genotype-specific (Nour *et al*., 2014). Among these, the most prevalent include carotenoids, phenolic compounds, and vitamin C. Lycopene stands out as one of the most characteristic carotenoids, playing a crucial role in determining the nutritional quality of tomatoes. Additionally, β-carotene (provitamin A), while nutritionally less significant than lycopene, remains a noteworthy contributor to the major antioxidants found in tomatoes (Rosales *et al*., 2006). Tomatoes also contain bioactive compounds like polyphenols and vitamin C, known for their high antioxidant activity. They play a vital role in scavenging reactive oxygen species (ROS), and providing protection against various environmental abiotic stresses (Wai *et al*., 2020). All these compounds have significant health-related benefits for consumers, including antihypertensive, anticancer, and cardioprotective effects (Khan *et al*., 2021).

One of the biggest challenges in tomato production is maintaining high yield, and at the same time responding to consumer preferences for better fruit quality (Natalini *et al*., 2021). Different studies have explored the potential of tomato lines for high yields and high fruit quality attributes related to primary and secondary metabolites (Oluk *et al*., 2019; Avdikos, 2021). Understanding the specific characteristics of different genotypes is important not just for assessing their market potential, but also for using them as parental lines in developing new hybrids. Current trends in breeding programs highlight the need for quality evaluation of recently developed genotypes in order to meet market demands and consumers' preferences (Felföldi *et al*., 2022). Given the high heritability of different quality traits (lycopene, soluble sugars, organic acids, fruit diameter, and weight) (Zörb *et al*., 2020), evaluating the quality of parental lines is paramount for successful breeding efforts.

The aim of the present work was, therefore, to evaluate different tomato lines in order to identify the most promising ones in terms of fruit taste and quality. These selections could be recommended for human fresh consumption or for industrial purpose, such as processing. Additionally, this evaluation could indicate their potential suitability for breeding programs.

Materials and methods

Sample cultivation and collection

A total of 13 tomato genotypes (G1 - G13) were grown under field conditions, in the Institute for Vegetable Crops, Smederevska Palanka, Serbia. The genotypes were chosen for their positive fruit characteristics that had been previously observed (size, shape, fruit firmness, early ripening, high yield, *etc*.). The experiment was carried out in spring/summer 2021. Seedlings were produced in a greenhouse. The seeds were sown in the first week of April. After preparing the soil and placing the mulch film, the tomato seedlings were planted in the field. The distance between the rows was 50 cm, and the distance between the plants was 35 cm. During the growing season, the plants were regularly irrigated, fertilised, and protected against diseases and insects. The fruits were harvested at the red ripe stage, and stored in a freezer (-20°C) until further analyses. Three replicates were used for the analysis of each genotype.

Physicochemical characteristics

The quality of the fruits was assessed based on physicochemical characteristics including pH, total soluble solids (TSS), total titratable acids (TTA), TSS/TTA ratio, lycopene, β-carotene, vitamin C, and total phenolic content. Fresh tomato juice was used for both pH and TSS analyses. The pH was measured using a benchtop pH-meter (Mettler-Toledo Five Easy Plus, LLC, USA). The TSS determination was conducted using the HI96801 refractometer (Hanna Instruments, USA), and the results were expressed in % Brix. The TTA was determined following the AOAC official method 942.15 (AOAC, 2000): tomato juice was diluted in distilled water (1:40, v/v), and titrated with 0.1 N sodium hydroxide (NaOH) to pH 8.1. The TTA was presented in grams of citric acid/g FW of the fruits.

The extraction of lycopene was carried out using a mixture of hexane, methanol, and acetone in a 2:1:1 ratio with the addition of BHT (butylated hydroxytoluene). The suspension was centrifuged at

8,000 rpm for 15 min at 4°C (2-16K, Sigma, Germany). Throughout the procedure, the samples were kept in a dark chamber. Absorbance of the upper hexane layer was measured at 505 nm (Spectro UV-VIS RS, 1166, Lambomed, Inc. USA), with hexane serving as the blank control. The results were expressed as lycopene content in mg/kg FW (Kuti and Konuru, 2005).

β-carotene was determined using the spectrophotometry method (Nagata and Yamashita, 1992). For extraction, a solution mixture of acetone and hexane (4:6) (1:16, v/w) was used. The suspension was shaken on ice for 15 min (F350 shaking stirrer, Falc, Italy), and centrifuged at 9,000 rpm for 15 min at 4°C (2-16K, Sigma, Germany). Throughout the procedure, the samples were kept in a dark chamber. Absorbance was read at 453, 505, 645, and 663 nm (Spectro UV-VIS RS, 1166, Lambomed, Inc. USA). The results were expressed as β-carotene content in mg/kg FW.

The extraction and analysis of vitamin C were carried out following the protocol outlined by Stevens *et al*. (2006). Fruit powder (500 mg) was mixed with 600 µL of 6% TCA (trichloroacetic acid). The mixture was vortexed and centrifuged (15 min, 4°C, 13,200 rpm), then the supernatant was used for vitamin C analysis. The analysis was conducted in a 96-micro-well plate. In the first three columns of the micro-well plate, vitamin C standards were added (20 µL of 0, 0.02, 0.05, 0.10, 0.15, 0.20, 0.30, and 0.40 mg/mL). The samples (20 µL) were added to the rest of the wells. Both the standards and samples were treated with 20 µL of DTT to obtain the total vitamin C content. DTT activity was halted after incubation (20 min, 37°C) by adding N-ethylmaleimide. A colouring reagent based on $FeCl_3$ was added to each well. After incubating for 60 min at 37°C, absorbance was read at 550 nm (Tecan microplate reader, Switzerland). Results were reported as mg of ascorbic acid/100 g FW.

The total phenolic content (TPC) of tomato extracts was determined using the Folin-Ciocalteu spectrophotometric method as described by Singleton and Rossi (1965). Tomato fruits were homogenised, and the extraction was performed using the 80% ethanol extraction solution (1:10, w/v). The extracts were transferred to 15 mL centrifuge tubes, and subsequently centrifuged at 6,000 *g* for 15 min (Hettich Mikro 22R centrifuge). The supernatants were collected, and placed in 1.5 mL Eppendorf tubes. Then, 0.1 mL of the supernatant, 0.2 mL of Folin-Ciocalteu, and 2 mL of distilled water were mixed. Next, 1 mL of sodium carbonate was added, and the mixture was incubated at 45°C for 15 min. Absorbance was then read at 765 nm using a Jenway 6850 UV/Vis Spectrophotometer. Gallic acid served as the standard. The results were expressed as mg/kg FW.

Antioxidant activity

To determine the total antioxidant activity, two methods were employed (ABTS and DPPH). The ABTS radical cation assay was conducted in accordance with the protocol established by Re *et al*. (1999). Tomato fruits were homogenised in a high-speed analysis grinder (A11 IKA, IKA®-Werke GmbH & Co. KG, Germany). Extraction was carried out with an 80% ethanol extraction solution (1:10, w/v) under agitation for 15 min. The homogenate was subsequently centrifuged at 10,000 rpm for 10 min, and the supernatant was stored at -20°C until analysis. A solution of 7 mM ABTS radical cation in 5 mM phosphate buffer (PBS), pH 7.4, was prepared by oxidising ABTS with manganese dioxide. Absorbance of the solution was adjusted to 0.7 at 734 nm using 5 mM phosphate buffer (PBS), pH 7.4. Prior to use, the ABTS radical cation was stabilised for 2 h at room temperature. Absorbance was then measured at 734 nm after 2 min of reaction. On the other hand, antioxidant activity was determined using DPPH as a free radical following the method outlined by Brand-Williams *et al*. (1995). A solution of 0.5 mM DPPH was prepared in 96% ethanol. Absorbance of the solution was adjusted to 0.650 at 517 nm using 96% ethanol, and left in the dark for the next 16 h to ensure stability. Subsequently, 200 µL of the sample was mixed with 1,800 µL of DPPH solution, and incubated for 30 min in the dark. Absorbance was measured at 517 nm (UV-1650PC, Shimadzu). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich) was used as antioxidant standard. A 2.5 mM Trolox solution prepared in a 5 mM phosphate buffer at pH 7.4 (for ABTS) and in 96% ethanol (for DPPH) served as the primary standard solution. A series of standard solutions (0, 20, 40, 60, 80, and 100 µM) was prepared immediately before use by diluting the primary standard solution. Results were expressed in µmol TU/g FW.

Statistical analysis

The statistical analyses and radar plot were carried out using SigmaPlot Software 14.0 (Systat Software Inc., USA). The data were statistically analysed using a One-way analysis of variance (ANOVA), and expressed as mean ± SE (n = 3). All results were calculated at a significance level of α of 0.05, and values followed by the same letter were not significantly different at the 0.05% level of probability based on Tukey's test. Principal component analysis (PCA) was performed using Statistica 8 software (StatSoft Inc., Tulsa, Oklahoma).

Results and discussion

Physicochemical characteristics

The quality attributes of tomato fruits vary among genotypes, especially in terms of pH value, which determines the fruit ripeness stage but also the postharvest quality of the fruit. Our findings revealed that most of the analysed fruits exhibited a pH within the range of 4 to 4.5 (Table 1), consistent with existing literature (Raiola *et al*., 2018). Tomato juice pH values were the lowest in genotype G11 (3.91), while the highest values were observed in genotypes G5 and G6 (4.53). The pH value can affect the subjective perception of the tomato taste, with higher pH value being negatively correlated with the perception of sour and grassy flavours (Maul *et al*., 2000). Ripe tomato fruits with higher pH tend to receive better rating for sweetness and odour in sensory analysis. In the present work, genotypes 4, 5, 6, 10, and 13 exhibited elevated pH values (4.52, 4.53, 4.53, 4.47, and 4.48, respectively), suggesting their suitability for fresh consumption. However, a high pH value is less desirable in tomatoes used for processing, as low acid foods with a pH > 4.6 require more rigorous heat treatments to prevent spoilage and ensure the safety of tomato products. The optimal pH range of tomatoes for processing falls between 4.2 and 4.3 (Anthon *et al*., 2011), which aligns with the characteristics of our genotypes 8 and 9. Additionally, genotype 9 had high soluble solids content, indicating its great potential as an excellent candidate for processing into tomato products (Young *et al*., 1993).

Table 1. Primary metabolic parameters [pH, total soluble solids (TSS), total titratable acidity (TTA), and TSS/TTA ratio] and dry matter content (DMC) in 13 analysed tomato genotypes (mean ± SD; n = 3).

Genotype	pH	TSS (% Brix)	TTA (g citric acid/100 g FW)	TSS/TTA	DMC (%)
G1	4.32 ± 0.02^{abc}	5.97 ± 0.03^{ab}	1.73 ± 0.14^{a}	3.60 ± 0.23^{f}	7.27 ± 0.32^{ab}
G2	4.06 ± 0.08^{bc}	5.83 ± 0.28^{ab}	1.07 ± 0.03^{de}	5.49 ± 0.16ab	7.70 ± 0.13^{ab}
G3	4.16 ± 0.15^{abc}	6.10 ± 0.30^{ab}	1.38 ± 0.03^{bc}	4.43 ± 0.17^{cdef}	7.24 ± 0.16^{ab}
G4	4.52 ± 0.11^{a}	6.03 ± 0.12^{ab}	1.30 ± 0.07b^{cd}	4.75 ± 0.29^{bcd}	7.75 ± 0.35a
G5	4.53 ± 0.08^{a}	5.13 ± 0.52^{ab}	1.05 ± 0.03^{e}	4.94 ± 0.30^{bc}	6.78 ± 0.02^{bc}
G6	4.53 ± 0.07^{a}	5.67 ± 0.30^{ab}	1.40 ± 0.03^{b}	4.07 ± 0.10^{cdef}	7.21 ± 0.25^{abc}
G7	4.15 ± 0.09^{abc}	6.27 ± 0.28^{ab}	1.68 ± 0.05^{a}	3.75 ± 0.13^{ef}	7.97 ± 0.36^{a}
G8	4.30 ± 0.05^{abc}	5.40 ± 0.11^{ab}	1.14 ± 0.04^{cde}	4.79 ± 0.20^{bcd}	7.55 ± 0.18^{ab}
G9	4.22 ± 0.04^{abc}	6.13 ± 0.34^{ab}	1.33 ± 0.02^{bc}	4.64 ± 0.13^{bcde}	7.83 ± 0.24^{a}
G10	4.47 ± 0.13^{ab}	6.47 ± 0.14^{a}	1.15 ± 0.02^{bcde}	5.61 ± 0.13^{a}	7.47 ± 0.15^{ab}
G11	3.91 ± 0.03^{c}	5.50 ± 0.51^{ab}	1.38 ± 0.06^{bc}	4.03 ± 0.24^{def}	7.50 ± 0.16^{ab}
G12	4.14 ± 0.05^{abc}	4.77 ± 0.30^{b}	1.24 ± 0.01b^{cd}	3.86 ± 0.14^{def}	5.95 ± 0.03^{c}
G13	4.48 ± 0.03^{ab}	6.10 ± 0.32^{ab}	0.95 ± 0.02^{e}	6.42 ± 0.20^{a}	8.05 ± 046^{a}

The most important taste traits in fresh fruits are soluble solids (mainly fructose, glucose, and citric acid), as well as TSS/TTA ratio and volatile compounds (Bertin and Genard, 2018). In cultivated tomatoes, predominant sugars are hexoses - glucose and fructose, while the most abundant organic acids are citric and malic acids (Grierson and Kader, 1986). The total soluble solid content is usually positively correlated with the perception of sweetness and sourness in the taste of tomatoes (Maul *et al*., 2000). In the present work, the highest total soluble solids (TSS) were observed in genotype 10 (6.47% Brix),

while genotype 12 had the lowest (4.77% Brix). Measurements of dry matter content revealed that the highest fruit DMC was detected in genotypes 7 and 13 (8.05%). These genotypes also displayed high total soluble solid content. Conversely, genotypes 5 and 12 were characterised by low DMC content and soluble solids (Table 1). These results aligned with literature data (Beckles, 2012), as dry matter content is primarily correlated with soluble solid content. Soluble solids also determine the quality of tomatoes for processing. Tomatoes intended for industrial purposes typically have TSS levels of at least 5°Brix (Peixoto *et al.*, 2018). In this context, only genotype 2 fell short as a candidate for industrial use due to its low TSS content.

Tomato taste remains the critical parameter, and most of the consumers in European countries prefer sweeter tomatoes (Oltman *et al*., 2014), which have higher soluble solids (therefore higher sugar content) and moderate acidity. The total titratable acid (TTA) values ranged from 0.95 g citric acid/100 g FW (G13) to 1.73 g citric acid/100 g FW (G1) (Table 1). Genotype 13 exhibited the highest TSS/TAA value due to its elevated TSS and low organic acid level. This suggested that consumers may perceive its taste as bland rather than tasty. A certain level of acidity is essential for the sensory perception of good tomato taste, so excessively high TSS and low acid levels can render the tomato's taste undesirable, and also impact its aroma perception. The most promising TSS/TAA ratio was noticed in genotype 10, which had high soluble solids and optimal organic acid content. On the other hand, genotypes with the least favourable taste characteristics were 1 (3.60), 7 (3.75), and 12 (3.86). Their fruits tasted sour and tart due to their high organic acid content, and could be classified as acidic tomatoes (Felföldi *et al*., 2021).

Bioactive components and antioxidant activity

In addition to tomato taste, two other important properties that determine consumers' preference are colour, health benefits, and characteristic of nutritionally rich tomatoes (Causse *et al*., 2010). Tomato fruit colour is determined by carotenoid content, mainly lycopene and β-carotene, which is also characterised by the antioxidant properties to scavenge ROS (Jomova and Valko, 2013). For human health, lycopene is especially important with 2.9 times stronger antioxidant activity than vitamin C, and 1.16 times stronger antioxidant activity than β-carotene (Arnao *et al*., 2001).

The testing of different tomato accessions selected for their high nutritional properties based on the content of bioactive components such as lycopene and β-carotene showed great variability (Adalid *et al*., 2010). This broad genotypic variation was also observed in the present work (Table 2), as lycopene content ranged from 129.02 mg/kg FW (G1) to 382.65 mg/kg FW (G12), while β-carotene levels spanned from 56.42 mg/kg FW (G11) to 86.96 mg/kg FW (G7). When evaluating fruit quality, special attention is paid to high-pigment genotypes and their potential for biofortification and making new lines with superior fruit quality (Ilahy *et al*., 2018). Our results showed that genotypes 10 and 12 had the highest lycopene content, while genotypes 2, 7, and 9 had high concentration of β-carotene. Given the high degree of genetic heritability observed in both carotenoids (Kumari *et al*., 2020), these results also pointed out the potential of these genotypes for future tomato breeding programs.

Among antioxidants, non-enzymatic components also play a crucial role in ROS elimination. Some of them that significantly contribute to antioxidant activity are phenols and vitamin C. The most abundant phenols in tomato (quercetin, rutin, and chlorogenic acid) have antimicrobial and antiviral activities, act as cardioprotective and hepatoprotective agents, and have anticarcinogenic and anti-inflammatory properties (Patel *et al*., 2018). Our results showed that genotypes 2, 10, and 11 (Table 2) displayed the highest content of phenolic compounds (1.72, 1.71, and 1.65 mg/g FW, respectively), while genotypes 4 and 12 the lowest (0.74 and 0.90 mg/g FW). The concentration of vitamin C ranged from the lowest in genotype 3 (20.84 mg/100 g FW) to the highest in genotype 8 (30.26 mg/100 g FW), aligning with existing literature data (Laayouni *et al*., 2022).

Various studies have indicated specific genotypic differences concerning the content of bioactive components in tomato fruits, especially in antioxidant components such as carotenoids, vitamin C, and polyphenols (Tudor-Radu *et al*., 2016; River *et al*., 2022). Our antioxidant analysis revealed that high antioxidant activity in genotype G1 was followed by high levels of vitamin C, while in genotype G2 it was related to high phenol and β-carotene content (Table 2). This implied distinct

Table 2. Secondary metabolic parameters (lycopene, vitamin C, phenols, and β-carotene content) and antioxidant activity in 13 analysed genotypes (mean ± SD; *n* = 3).

Genotype	Lycopene (mg/kg FW)	β-carotene (mg/kg FW)	Vitamin C (mg/100 g FW)	Phenols (mg/g FW)	ABTS (μmol TU/g FW)	DPPH (μmol TU/g FW)
G1	129.02 ± 6.74^{e}	64.21 ± 1.79^{def}	28.64 ± 1.32^{ab}	0.96 ± 0.01^{cd}	2824.5890.30^{a}	3459.17 ± 223.76^{ab}
G2	256.89 ± 13.11^{bc}	86.29 ± 3.36^{ab}	25.69 ± 0.81^{abc}	1.72 ± 0.17^{a}	2454.21 ± 39.02^{ab}	3517.50 ± 436.85^{a}
G3	224.74 ± 18.47^{cd}	71.56 ± 2.19^{cde}	20.84 ± 0.72^{c}	1.12 ± 0.06^{bcd}	2068.69 ± 103.04^{bc}	1559.17 ± 270.16^{bcd}
G4	211.44 ± 15.12^{cde}	66.70 ± 3.85^{def}	24.30 ± 0.50^{abc}	0.74 ± 0.01^{d}	2309.43 ± 237.64^{ab}	1355.00 ± 37.50^{bcde}
G5	216.80 ± 3.55^{cd}	64.58 ± 2.32^{def}	21.84 ± 1.29^{bc}	1.51 ± 0.15a^{bc}	1262.50 ± 111.17^{de}	1284.17 ± 235.11^{bcde}
G6	152.42 ± 2.18^{de}	65.40 ± 0.86^{def}	23.23 ± 2.44^{abc}	1.26 ± 0.13a^{bcd}	945.79 ± 155.23^{e}	1234.17 ± 311.69^{bcde}
G7	218.89 ± 30.55^{cd}	86.96 ± 5.39^{a}	27.54 ± 1.33^{abc}	1.22 ± 0.02^{abcd}	1486.19 ± 231.41^{cde}	1659.17 ± 36.32^{bc}
G8	254.28 ± 22.86^{bc}	61.87 ± 1.91^{def}	30.26 ± 0.83^{a}	1.20 ± 0.06^{abcd}	1378.45 ± 78.19^{de}	1709.17 ± 748.10^{b}
G9	222.88 ± 5.78^{cd}	86.62 ± 1.83^{abc}	21.20 ± 2.89^{bc}	1.12 ± 0.04^{bcd}	1521.55 ± 160.86^{cde}	1075.83 ± 217.15^{bcde}
G10	302.71 ± 8.22^{b}	72.53 ± 2.56^{bcde}	27.10 ± 0.19^{abc}	1.71 ± 0.14^{a}	1861.61 ± 185.60^{bcd}	1542.50 ± 499.37^{bcde}
G11	189.48 ± 16.37^{cde}	56.42 ± 2.26^{f}	22.05 ± 0.90^{bc}	1.65 ± 0.25^{ab}	946.53 ± 107.21^{e}	334.17 ± 104.42^{e}
G12	382.55 ± 0.03^{a}	59.61 ± 1.60^{ef}	23.39 ± 2.32a^{bc}	0.90 ± 0.04^{d}	1285.79 ± 51.01^{de}	409.17 ± 102.40^{de}
G13	170.52 ± 18.28^{de}	75.45 ± 4.78^{abcd}	26.43 ± 1.78^{abc}	1.11 ± 0.09^{bcd}	1803.10 ± 143.63^{bcd}	892.50 ± 325.32^{bcde}

genotypic variations in components that contribute to antioxidant activity (Laayouni *et al*., 2022). Furthermore, genotype 11, which exhibited the lowest antioxidant activity in both assays, was characterised by low levels of lycopene and β-carotene. This underscores the significance of these carotenoids in the overall antioxidant defence system. Some studies suggest that the ABTS assay is superior to the DPPH assay for measuring antioxidant activity in fruits with high carotenoid content such as tomatoes (Floegel *et al.*, 2011; Kaur *et al.*, 2013). Our results indicated that genotypes G1 and G2 displayed the highest total antioxidant activity, suggesting that their consumption as fresh tomatoes could be beneficial to human health. The evaluation of different genotypes in terms of antioxidant activity and its components in the present work provided valuable starting point for further investigations, especially considering the significance of antioxidant defence in mitigating the negative effects of abiotic stress, particularly drought (Ji *et al*., 2022).

PCA analysis

Principal component analysis (PCA) was conducted to determine the underlying correlations and group patterns (Figures 1A and 1B).

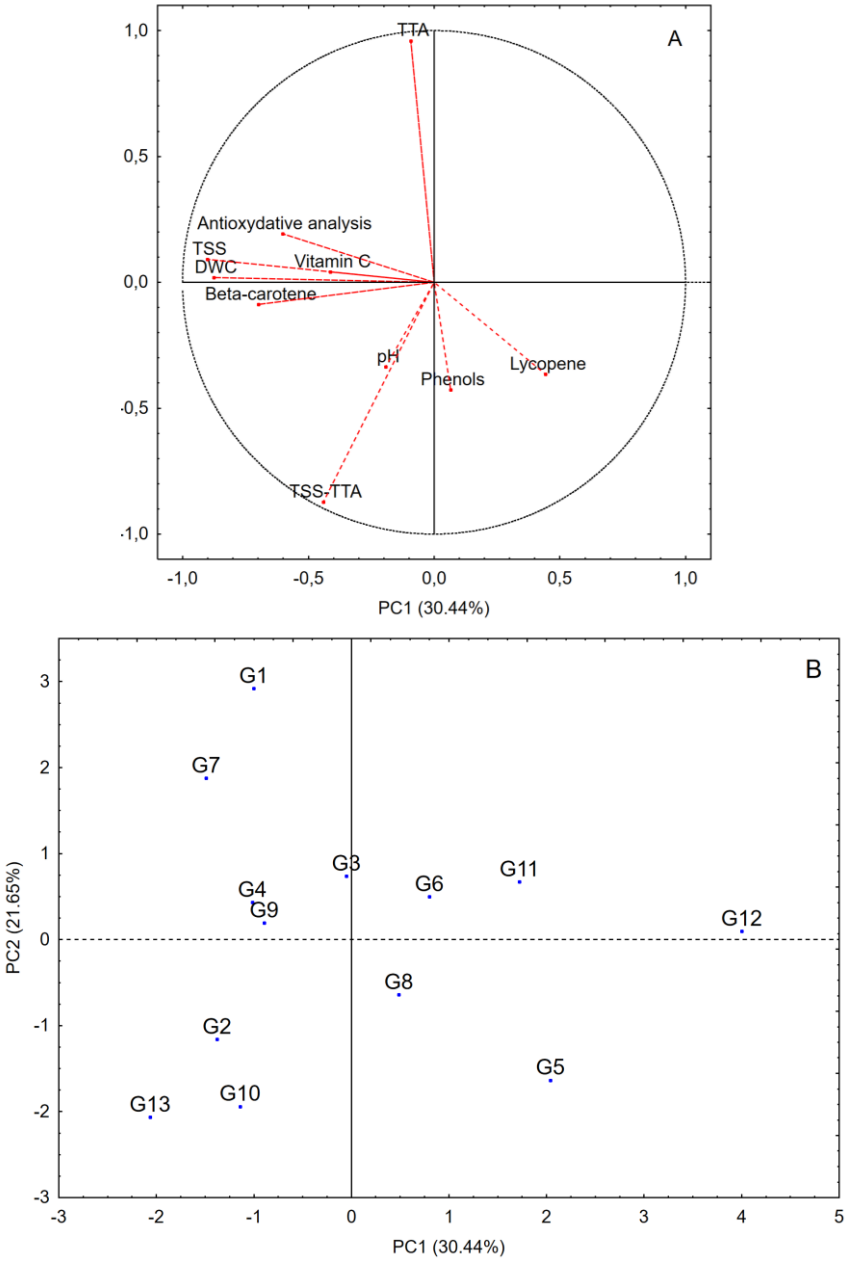

Figure 1. 2D scatter diagram of PCA-relationships for physicochemical properties **(A)**, and analysed tomato genotypes **(B)**.

The first two components accounted for 52.09% of the total variance (30.44% of variance for PC1 and 21.65% of variance for PC2). The first principal component was associated with factors such as antioxidant activity, β-carotene content, DMC, and TSS. It exhibited negative correlation with these four traits, indicating their tendency to vary together. The first principal component also divided the analysed tomato genotypes into five distinct clusters (1: genotypes 1, 2, 4, 7, 9, 10, and 13; 2: genotype 3; 3: genotypes 6 and 8; 4: genotypes 5 and 11; and 5: genotype 12). On the positive side of PC1, where eight genotypes were situated, TSS contributed most significantly. Five genotypes in the top-left quadrant were characterised by high TTA, low TSS/TTA, and lycopene values. Genotypes 2, 10, and 13 located in the lower left quadrant demonstrated high antioxidant activity, low TTA, and high TSS/TTA ratio, indicating their suitability for fresh consumption due to their elevated levels of compounds important for flavour, as well as nutritional and health benefits for humans. On the positive side of PC1, genotype 12 exhibited high values for all examined traits except for lycopene. PCA revealed that genotypes 5 and 11 from cluster 4 had high phenol content, but low in the following five traits: antioxidant activity, β-carotene, lycopene, TSS, and vitamin C. Genotypes 6 and 8 from cluster 3 had high pH values, and low antioxidant activity, β-carotene, and TSS. The second principal component was associated with TTA content and TSS-TAA ratio. Based on the data provided by PC1 and PC2, the greatest variance in results was related to TSS, TTA, TTS/TTA ratio, and DMC, with antioxidant activity and β-carotene contributing to a lesser extent.

Based on the combined results of the PCA and the analysis of physicochemical characteristics and antioxidant activity, three of the most promising genotypes were chosen from cluster 1 in the bottom-left quadrant (G2, G10, and G13) for better visualisation. The radar plot illustrated that these genotypes were characterised by high content of primary (TSS) and secondary metabolites (phenols and vitamin C), as well as notable antioxidative activity and high dry matter content (Figure 2). Among them, G10 stood out in terms of fruit quality attributes, displaying high values across nearly all measured parameters, except for organic acids.

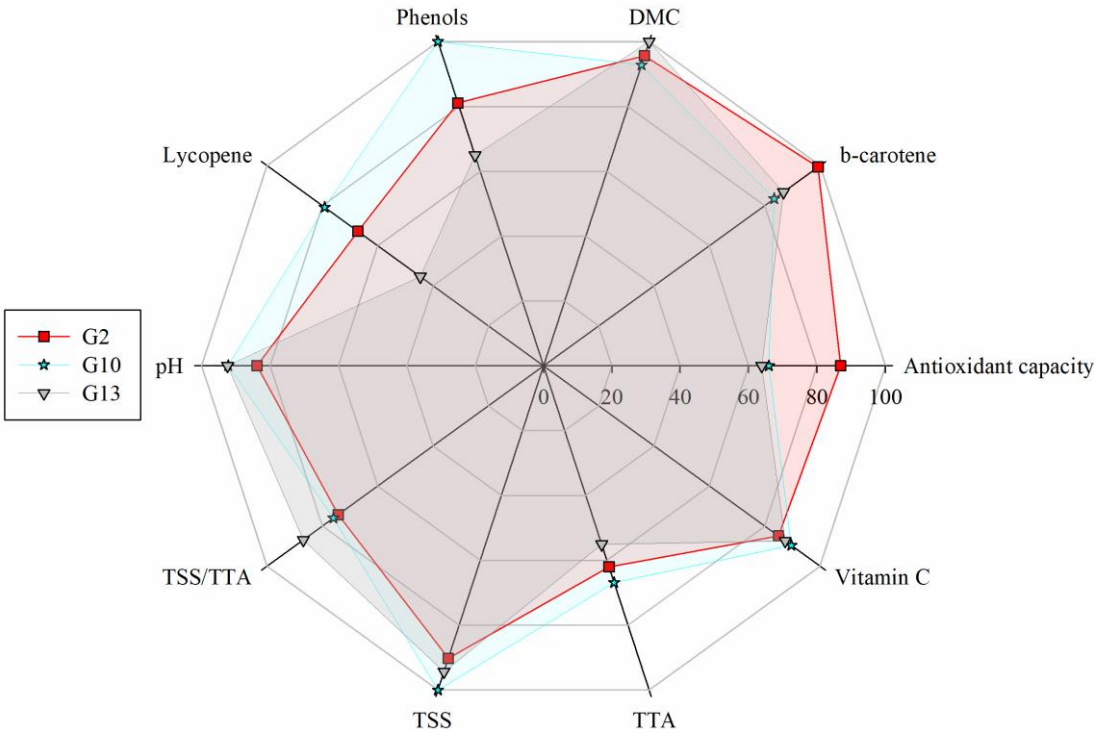

Figure 2. Radar plot of fruit quality parameters of three most promising tomato genotypes.

Conclusion

Fruit colour, health-promoting components, and nutritional value are among the most important quality characteristics of tomato fruit. Our results highlighted distinct genotypic responses regarding all fruit quality components, encompassing both primary and secondary metabolites. PCA revealed several clusters of genotypes based on the analysed physicochemical traits. One such cluster, comprising genotypes G2, G10, and G13 was determined as the best for fresh consumption due to their elevated levels of compounds essential for flavour, nutrition, and health. The most promising genotype related to fruit quality attributes was genotype G10 with the best TSS and TAA contents, and TSS/TAA ratio, which is important for overall taste perception. Conversely, genotype G9 showed the greatest potential for the processing industry, presenting an ideal pH value for juice and commendable soluble solids content. While components like carotenoids, vitamin C, and phenols contribute to health-promoting features, they also play a role in mitigating various abiotic stress factors. Notably, genotypes G1 and G2 exhibited elevated antioxidant activity, rendering them potentially beneficial for human health when consumed fresh. Future research should focus on evaluating genotypes with a strong antioxidant system under diverse abiotic stress conditions, providing insight into the maintenance or improvement of tomato fruit quality traits.

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