biology and life sciences forum

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Proceedings Paper

Chemical Characterization of Different Colored Tomatoes: Application of Biochemical and Spectroscopic Tools †

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† Presented at the 1st International Electronic Conference on Horticulturae, 16–30 April 2022; Available online: https://iecho2022.sciforum.net/.

Abstract: Traditional crop varieties are a useful source of desirable characteristics for developing a new cultivars with improved nutritive and sensory attributes. The aim of this study was to evaluate the fruit quality parameters in three traditional tomato genotypes: pink, yellow and dark colors. The results showed that yellow colored tomato had the highest TSS/TTA ratio and antioxidative activity, but the lowest content of lycopene and β-carotene. Genotypic differences in the carotenoid components are also confirmed by Raman spectroscopy. The advantage of the yellow tomato genotype related to fruit quality compounds compared to others genotypes indicated its potential in a breeding program.

Keywords: colored fruit tomatoes; Raman spectroscopy; fruit quality; carotenoids

Citation: Petrović, I.; Marjanović, M.; Pećinar, I.; Savić, S.; Jovanović, Z.; Stikić, R. Chemical Characterization of Different Colored Tomatoes: Application of Biochemical and Spectroscopic Tools. *Biol. Life Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: 16 April 2022

1. Introduction

Tomato fruit quality is determined by a combination of different organoleptic and nutritional characteristics. Soluble solids, organic acid, TSS/TTA ratio (TSS—Total Soluble Solids; TTA—Total Titratable Acidity) and pH, are crucial for the tomato taste [1], while carotenoids and vitamin C, as non-enzymatic antioxidants, have great importance for human health [2]. Due to high demands for the products with improved nutritive and sensory attributes, traditional tomato genotypes and wild relatives can be an important source of traits related to the consumer perception that could be used in a breeding program [3,4]. Thus, it is important to evaluate traditional accessions in terms of their quality, nutritive and health-promoting characteristic. Beside traditional morphological and biochemical analysis, novel techniques can be used for phenotypic characterization of tomato genotypes. One of the most promising tools is Raman spectroscopy coupled with chemometrics, which has great potential for evaluation of the nutritional attributes of plants [5,6]. The aim of this study was to evaluate the fruit quality of three traditional tomato genotypes from the Balkan with different colors using Raman spectroscopy, morphological and biochemical methods in order to detect the most important quality traits and provide important information for their potential to use in breeding programs.

2. Materials and Methods

In this study were used three traditional genotypes from the Balkan with different colors: pink tomato "Pirotski rozni", yellow and dark tomato (Figure 1). Tomatoes were grown in the open-field conditions. Morphological measurements (fruit weight, width and height and fruit shape index) and biochemical analyses were performed in red-ripe phase. The soluble solids were determined via refractometry, organic acids by the titratable acidity measurement as well as pH of tomato juice. Spectrophotometric methods were used for the determination of vitamin C [7], lycopene [8] and antioxidant activity [9]. The statistical analyses were performed with SigmaPlot software (version 14.0). The data were statistically analyzed using a one-way analysis of variance (ANOVA) and expressed as mean ± SE (n = 6). The significance of differences between the mean values was determinate using Tukey`s test for significance level $p \leq 0.05$. Correlations among the parameters were determinate by correlation-regression analysis and Pearson`s correlation coefficients.

Figure 1. Three traditional tomato genotypes (pink tomato "Pirotski rozni", yellow and dark tomato).

Raman spectroscopy was used to examine tomato samples in different regions of the fruit pericarp. Spectra were recorded using an XploRA Horiba Jobin Yvon. Raman scattering was excited by a laser at a 532 nm with grating at 1200 lines/mm. Spectra were acquired by applying exposure time 5 s and scanning the sample 5 times. Principal component analysis (PCA) was carried out on data normalized by the highest intensity band and using spectral region from 200 to 1800 cm^{-1}. The spectra preprocessing was realized using the Spectragryph software, version 1.2.14 [10], while PCA was performed using the PAST software [11].

3. Results

3.1. Morphological and Biochemical Parameters

Results of morphological parameters showed that pink genotype had the biggest fresh weight per fruit (365.89 g/fruit) as well as highest fruit shape index compared to others. Among the smaller fruit genotypes (yellow and dark tomatoes), there were no statistically significant differences in fresh weight and fruit width. However, the dark tomato genotype had the lowest fruit weight (74.11 g/fruit) and fruit height (43.92 cm) (Table 1).

Table 1. Morphological parameters of investigated tomato genotypes.

Morphological Parameters	Pink Tomato "Pirotski Rozni"	Yellow Tomato	Dark Tomato
fresh weight (g/fruit)	365.89 ± 21.70 a	93.89 ± 2.4 b	74.11 ± 2.91 b
fruit width (cm)	63.93 ± 2.45 a	57.07 ± 1.33 ab	50.48 ± 2.16 b
fruit height (cm)	66.30 ± 2.95 a	51.15 ± 1.19 b	43.92 ± 1.05 c
fruit shape index	1.04 ± 0.04 a	0.90 ± 0.03 b	0.88 ± 0.04 b

Biochemical analysis indicated statistically significant differences in tomato fruit quality parameters between genotypes (Table 2). Yellow genotype had the highest content of total soluble solids (TTS), TSS/TTA ratio, pH of fruit juice and total antioxidative activity compared to other genotypes. The high concentration of vitamin C was found in yellow and dark genotypes and statistically differed compared to pink genotype (17.83 mg/100 g). Analysis of carotenoid`s content showed statistical significant genotypic differences in lycopene with the highest value recorded in a pink tomato (247.13 mg/kg).

Dark genotype had the highest β–carotene content (6.41 mg/100 g), significantly differed then pink and yellow genotypes. On the contrary, the lowest content of lycopene and β–carotene was found in yellow tomato genotype.

Table 2. Biochemical parameters of analyzed tomato genotypes.

Biochemical Parameters	Pink Tomato "Pirotski Rozni"	Yellow Tomato	Dark Tomato
pH	3.9 ± 0.01 c	4.1 ± 0.03 a	4.0 ± 0.03 b
TSS (% Brix)	6.2 ± 0.00 b	8.6 ± 0.51 a	6.4 ± 0.07 b
TTA (% of Citric acid)	0.58 ± 6.78 × 10^{-3} a	0.54 ± 9.69 × 10^{-3} b	0.46 ± 0.01 c
TSS/TTA	10.57 ± 0.11 b	14.95 ± 0.10 a	13.90 ± 0.39 a
Antioxidative activity (µmol TU-Trolox units/g)	1504.06 ± 76.35 c	2448.24 ± 174.80 a	1976.69 ± 115.74 b
Vitamin C (mg/100 g)	17.83 ± 0.74 b	31.37 ± 0.97 a	33.37 ± 0.10 a
Lycopene (mg/kg)	247.13 ± 13.00 a	30.81 ± 2.18 c	128.75 ± 17.11 b
β–carotene (mg/100 g)	2.59 ± 0.22 b	1.63 ± 0.05 b	6.41 ± 0.53 a

3.2. Raman Signature of Tomato Pericarp and PCA

Analysis of the Raman spectroscopy showed that tomato genotypes differed for characteristic peaks in spectral regions at 1505–1515, ~1148 and 997–1001 cm^{-1} in all pericarp regions (exo-, meso- and endocarp) (Figure 2). The pink and dark tomato had bands around 284, 365, 419, 590, 640, 762, 1569, and 1628 cm^{-1}, that were not present in the yellow one, while yellow tomato had bands (391, 430, 490, 568, 665, 816, 914, 1059, 1187, 1423, 1582, 1670 cm^{-1}) specific only for this genotype (Figure 2B). Similarity in the bands detected in pink and dark tomatoes was founded also within different pericarp regions (Figure 2A,C), while the characteristic difference was observed in yellow tomato in all pericarp regions with the band at 1516 cm^{-1} (Figure 2B).

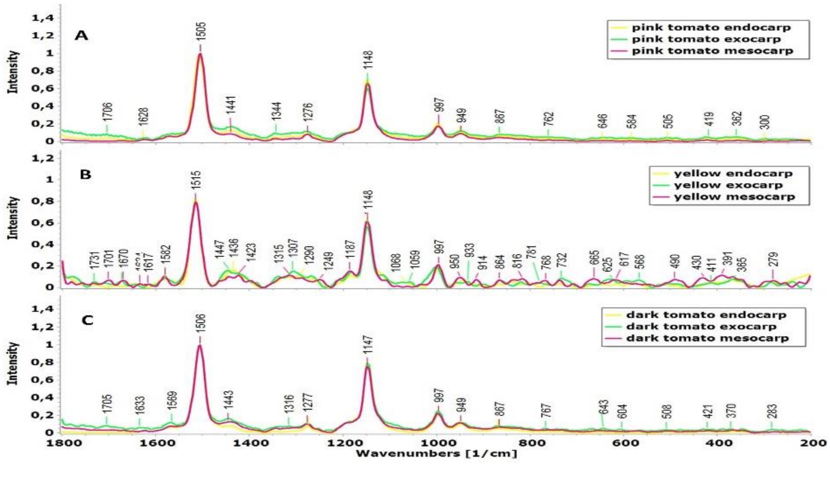

Figure 2. Raman spectra of three tomato genotypes at different pericarp regions, (**A**)-pink, (**B**)-yellow, (**C**)-dark tomato.

Multivariate analysis of Raman spectra (the score plot of PC1 versus PC2) showed a separation between the sample and described 76.65% of data variance. The score plot (Figure 3A) suggested the presence of two clusters along PC1 axis related to differences between genotypes. The loading plot of PC1 axis showed the variables with the highest contribution corresponded to the signals at 1521 and 1501 cm^{-1} with the highest negative effects (Figure 3B). The signals at 1498 cm^{-1} with the highest positive impact and 1521 cm^{-1} along PC2 axis (Figure 3C), indicated the separation of the exocarp of dark, pink and yellow tomato from endocarp and mesocarp of yellow tomato.

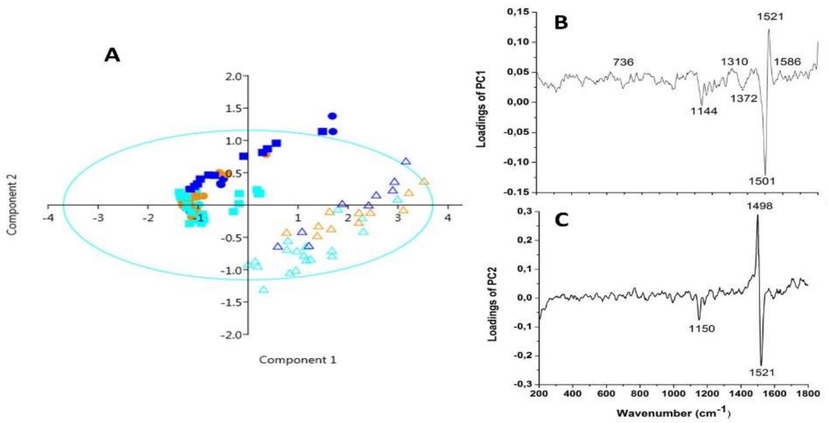

Figure 3. PCA of different pericarp regions in investigated tomato genotypes: (**A**) score plot, (**B**,**C**) loading plots. Circle-dark, square-pink, triangle-yellow tomato, endocarp-light blue, exocarp-royal blue, mesocarp-orange color.

4. Discussion

Morphological characteristics of fruits are a very important factor in the consumer`s evaluation of the product. Regarding fruit height, pink tomato is classified as a large class (above 60 mm), while others tomatoes—yellow and dark belong to the medium size tomatoes (50–60 mm) [4]. Literature data showed that the most appreciated fruit shape by consumers is slightly flattened, which was the case with tomato genotypes used in this study. Also, the majority of consumers prefer large fruits (like pink tomato), while only 7% of consumers prefer medium size [12].

Tomato fruit quality is determined by a combination of different attributes related to nutritional value and health characteristics that are highly genotypic depend. Among selected genotypes, yellow tomato had the highest TSS content compared to others, followed by a high TTA percent, which indicated good fruit taste, appointed to mild flavor fruits which is desirable characteristic [13,14]. Also, due to highest TSS and pH of fruit juice this genotype showed the best characteristics regarding potential for industrial processing [15].

Antioxidative activity is an important health-promoting characteristic in tomato fruits. The yellow tomato genotype was characterized with the highest value of antioxidative acitivity as well as high vitamin C content. According to Vela-Hinojosa et al. [16] genotypes with high vitamin C content could be candidates in improving tomato breeding lines, which is the case with our yellow and dark tomato. The higher antioxidative activity in yellow than in dark tomato could be related to other components, like lutein and phenolic compounds, since yellow tomato genotypes had higher total phenolic content compared to other colored tomato [17,18]. Characteristic biochemical differences in the carotenoid content between genotypes was noticed, the highest value lycopene was found in pink tomato, whereas β–carotene in dark tomato. On the contrary, yellow tomato genotype had the lowest content of both carotenoids which is in correspondence with literature, where yellow and orange tomatoes have less lycopene, phytoene and phytofluene compared to pink and red tomatoes [18–20].

All tomato fruit Raman profiles showed common three bands (~997, 1148 and ~1510 cm^{-1}) associated with C-CH_3 in-plane-rocking, C-C and C=C stretching, respectively [21–24]. However, Raman spectra showed a shift at ~1510 cm^{-1} that could be related to the length of the conjugated polyene chain or other constituents bonded to carotenoids that affect on the wavenumber of the vibrational band [23]. The bands with lower intensity in the pink and dark tomato were related to carotenoids, like lycopene (at 646 cm^{-1}) and β-carotene (at 1276 cm^{-1}) and some phenolic compounds (at 1569 and 1628 cm^{-1}) [6,25–27], but were not present in the yellow one, indicating a genotypic difference in antioxidant components. On another side, except strong carotenoids, in the mesocarp and endocarp of yellow tomato, some bands related to sugars were noticed, such as glucose at 430 and 768–781 cm^{-1} and fructose at 625 cm^{-1} [27]. In exocarp on cuticular waxes indicated bands at 1307 cm^{-1}, 1059 cm^{-1}, 1336 cm^{-1}, and 1731 cm^{-1}, while bands at 1447 and 1670 cm^{-1} were related to lipids and phenolic compound, respectively [25,26].

The analyses of the loading spectrum of PC1 showed that the positive signals at 1521 cm^{-1} (high intensity, assigned as C=C vibration of carotenes), at 1310 cm^{-1} (the lower intensity, directed to β-carotene), and at 1586 cm^{-1} arise from C-C vibration of phenolic compounds [26] were most responsible for the difference of yellow tomato from others. The negative signal at 1501 cm^{-1} (mainly depending on phenolic compounds) together with band at 1144 cm^{-1} indicated a difference among the genotypes [25]. According to PC2, the signals at 1499 and 1521, 1150 cm^{-1} were mostly responsible for the differentiation among the exocarp of all genotypes from endo- and mesocarp in phenolic compounds and carotenoids, respectively [25,26].

4. Conclusion

Investigation of morphological and biochemical characteristic in different colored tomatoes pointed out specific genotypic differences. Biochemical analysis indicated that the yellow colored tomato had better quality attributes related to the TSS/TTA ratio, pH and antioxidative activity compared to others. Genotypic differences in biochemical components like carotenoids were confirmed by Raman spectroscopy. Raman spectral signatures in different pericarp regions showed that carotene and carbohydrates (hemicellulose and fructose) were present in all regions, while cuticular wax, lipids and phenolic compounds were detected only in the exocarp. PCA results indicated that yellow tomato mostly differed in the carotenoids in relation to others, whereas pink and dark differed from yellow in phenolic compounds, and highlighted the difference in antioxidative components, which were obtained by biochemical analysis. Also, the exocarp of pink, dark and yellow tomato differed in phenolic compounds, while mesocarp and endocarp of yellow tomatoes differed in the carotenoids. These results also indicate the advantage of using Raman spectroscopy for analysis and understanding of the distribution of biochemical and nutritional components in tomato fruit.

Author Contributions: Conceptualization, I.P. (Ivana Petrović), M.M., I.P. (Ilinka Poćinar); methodology, I.P. (Ivana Petrović), M.M., I.P. (Ilinka Poćinar), S.S.; Raman spectroscopy analysis and Raman data interpretation, I.P. (Ilinka Poćinar); writing-original draft preparation, I.P. (Ivana Petrović), M.M. and I.P. (Ilinka Poćinar); visualization, M.M.; writing-review and editing, Z.J. and R.S.; supervision, Z.J.; All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. 451-03-68/2022-14/200116 and 451-03-68/2022-14/200216).

Institutional Review Board Statement:

Informed Consent Statement: Not applicable.

Data Availability Statement:

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

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