Carotenoid and flavonoid levels, antioxidant activity and antimicrobial properties of tomato grown in Serbia

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Summary

This work provides an insight into the nutritional quality of Hector-F1 tomato (*Solanum lycopersicum* L.) fruit grown in Serbia and the juice prepared from it regarding the contents of carotenoids, flavonoids and polyphenols as compounds beneficial for a healthy diet. Initially, ultra-high performance liquid chromatography with electrospray ionization mass spectrometry (UHPLC-ESI-MS) was employed for both polar and non-polar tomato extracts to identify compounds present in the samples. Then, lycopene, β-carotene, total polyphenols and flavonoids contents were determined by means of ultraviolet-visible (UV-Vis) spectrophotometry. To evaluate the antioxidant activity of the samples, four methods were applied, namely, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, the ferric ion reducing antioxidant power (FRAP) method and the cupric ion reducing antioxidant capacity (CUPRAC) method. In addition, the antimicrobial potential was tested against gram-positive bacteria, gram-negative bacteria and the yeast *Candida albicans*. The thermal treatment in all cases modified the chemical composition of tomato as demonstrated by a decrease in values of all measured parameters. This comprehensive analysis provided data applicable to profiling and comparing cultivars grown in different geographical regions.

Keywords

tomato; lycopene; β-carotene; flavonoid; antioxidant activity; antimicrobial potential

Fruits of tomato (*Solanum lycopersicum* L.) and tomato-based products bring multiple benefits to a health-promoting human diet as a source of natural polyphenols, vitamins A, C, D and E, together with carotenoids such as lycopene or β-carotene [1]. Carotenoids are a class of pigments of yellow to red colour, which are widely distributed in vegetables and fruits. Human body cannot synthesize them. More than 750 carotenoids have been isolated from various plant sources and their role as mediators in biochemical reactions of the human metabolism is well recognized [2].

Lycopene is one of the most potent naturally occurring antioxidant and antimicrobial agents. It is frequently found in tomatoes, pink grapefruit, apricots, red oranges, watermelon, and guava giving them a distinctive red colour. Lycopene is a symmetrical tetraterpene composed of eight isoprene units. It can effectively quench singlet oxygen and interact with free radicals reducing the risk of degenerative diseases such as cancer of the lungs, bladder, cervix, prostate, breast or skin, atherosclerosis and associated coronary artery disease [3]. Therefore, its presence in nutrition is of the utmost significance. β-Carotene is another lipid-soluble natural carotenoid often found in plants, fruits and fungi. It is a yellow-orange tetraterpenoid polyene containing 11 conjugated carbon-carbon double bonds and beta-rings at both ends of the molecule. Extensive conjugation

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of double bonds is responsible for its intense absorption of visible light and its health-promoting antioxidant properties. In addition to lycopene and β-carotene, tomato contains numerous phenolic compounds such as flavonoids quercetin, naringenin, rutin or chlorogenic acid, which can contribute to a health-promoting diet as well. Flavonoids are regarded as potentially beneficial compounds that can help the body fight inflammation, cardiovascular diseases and cancer.

Several factors can affect the composition and concentration of biomolecules in tomato and tomato-based products. These include genetic diversity, cultivation technology, climate, geographic site of production, fruit ripeness, techniques of harvesting and processing, as well as storage of the final products [4]. It is obvious that during ripening, processing and storage, most of the compounds are likely to undergo chemical changes including formation of various isomers or derivatives with specific properties. This is the reason for analysing the chemical composition of tomato and tomato-based products. A variety of methods have been employed to determine the content of various carotenoids in fruits and vegetables including high-performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), optothermal and photothermal methods, Raman, infrared and near-infrared spectroscopy, and UV-Vis spectrophotometric methods. Although HPLC with various detectors is a time-consuming method with the necessary sample pre-treatment step, it is still the most reliable and the most accurate method available for this purpose [5].

In this study, samples of tomato and tomato juice from Serbia were analysed with the aim to determine contents of lycopene, β-carotene, total polyphenols and flavonoids as compounds, which are important in the context of a health-promoting diet. In addition, the antimicrobial and antioxidant activities of the samples were tested to allow comparison to tomatoes and tomato-based products reported in the literature.

MATERIAL AND METHODS

Extraction procedures

Mature tomato fruits of cultivar Hector-F1 were purchased in September of 2021 at the local market in Niš, Serbia. Initially, fruits were washed with deionized water, then sliced by a knife and homogenized in a blender with a rotating metallic blade. The seeds were removed manually. Tomato juice was prepared according to a traditional recipe. Briefly, the milled tomato was heated to its boiling point, stirred for additional 5 min, salted, and put into glass flasks for later use.

Sample preparation for lycopene and β-carotene determination was performed by the method described by Perkins-Veazie et al. [6]. A known quantity of the pre-treated tomato or tomato juice sample was added to a mixture consisting of organic solvents (*n*-hexane ≥ 99 %, acetone ≥ 99 %, ethanol 96 % from Sigma Aldrich, St. Louis, Missouri, USA in volumes ratio of 2 : 1 : 1) and 50 mg·l^{-1} butylated hydroxytoluene (Merck, Darmstadt, Germany). The mixture was stoppered and mixed on an orbital shaker in a water bath at 3 Hz for 15 min at 5 °C. Then, 75 ml of cold deionized water for every 10 g of the starting sample was added to the mixture and it was agitated for another 5 min. The suspension was transferred to a separation funnel to separate the upper (non-polar) phase from the lower (polar) phase during 10 min at laboratory temperature (25 °C).

Polar extracts of the tomato samples were obtained by homogenizing a precisely determined amount of the sample and mixing it with three times the amount of ethanol (70% v/v). Then, this mixture was stirred on a magnetic stirrer for 10 min at 5 °C in a water bath and then centrifuged at 8 000 ×*g* for 10 min. The supernatant was decanted and the remainder of the sample was re-extracted by the method described above, with twice the volume of ethanol. The supernatants obtained were combined and made up to a known volume with 70% (v/v) ethanol. Analysis of this extract was performed by ultra-high performance liquid chromatography (UHPLC) in negative and positive ion mode.

UV-Vis analysis

The non-polar phase was subjected to UV-Vis spectrophotometric analysis. Determination of the lycopene, β-carotene, total polyphenols and flavonoids content along with antioxidant activity was performed by spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan). To determine lycopene and β-carotene concentration in the samples, the absorbance of the non-polar layer was measured in a 1 cm path length glass cuvette at 450 nm and 503 nm versus a blank of the above-mentioned solvent mixture. The content of each carotenoid was obtained from the system of linear equations:

$$A_{450} = \varepsilon_{L450} C_L + \varepsilon_{\beta 450} C_\beta \quad (1)$$

$$A_{503} = \varepsilon_{L503} C_L + \varepsilon_{\beta 503} C_\beta \quad (2)$$

where A_{450} and A_{503} are absorbances at 450 nm

and 503 nm, respectively, ε_{L450} and ε_{L503} are molar absorption coefficients of lycopene at 450 nm and 503 nm, respectively, $\varepsilon_{\beta 450}$ and $\varepsilon_{\beta 503}$ are molar absorption coefficients of β-carotene at 450 nm and 503 nm, respectively. Here, C_L and C_β stand for concentrations of lycopene and β-carotene, respectively (expressed in moles per litre). This unit refers to the working solution used in UV-Vis method, while lycopene and β-carotene content was calculated and expressed in milligrams per kilogram.

UHPLC-ESI-MS analysis

Both polar and non-polar extracts were subjected to qualitative UHPLC with electrospray ionization and mass spectrometer (ESI-MS). UHPLC-ESI-MS were performed on Hypersil gold C18 column (50 mm × 2.1 mm, 1.9 μm particle size) at 25 °C using a Dionex Ultimate 3000 UHPLC+ system equipped with a diode array (DAD) detector and LCQ Fleet Ion Trap Mass Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The method described by Abdul-Hammed et al. [7] was applied. In the case of the non-polar extract, an isocratic method with methanol and acetonitrile at 1 : 1 (v/v) at 0.25 ml·min^{-1} flow rate. The injection sample volume was 4 μl.

For the polar extract, the flow of the mobile phase was set to 0.250 ml·min^{-1}, while the volume of the sample was 8 μl. Mass spectrometric analysis was performed using a 3D-ion trap with electrospray ionization (ESI) in positive ion mode for non-polar extracts, while negative and positive ion mode were used for polar extracts. Mass spectra were acquired by full range acquisition of *m*/*z* 100–700, with a tandem mass spectrometry analysis performed by a data-dependent scan - the collision-induced dissociation of detected molecular ions peaks ($[M\text{-}H]^-$/$[M+H]^+$) tuned at 30 eV for both ionization modes. For both modes, the capillary temperature was 350 °C and nitrogen sheath and auxiliary gas flow were 32 and 8 arbitrary units, respectively. For instrument control, data acquisition and data analysis, Xcalibur software version 2.1 (Thermo Fisher Scientific) was used.

The qualitative analysis was based on comparison of their retention times and MS spectra with the corresponding molecular ion peaks as well as the characteristic ion fragmentation of selected peaks (MS/MS) from corresponding UHPLC chromatograms, and comparison with mass spectral database available online (MassBank, MassBank consortium) and available literature. Full identification was provided by using reference standards for some compounds (citric acid, chlorogenic acid and rutin dihydrate, all from Merck). Methanol, acetone, and water (LC-MS purity) from Thermo Fisher Scientific were used in the mobile phase along with formic acid of HPLC purity obtained from Carlo Erba (Emmendingen, Germany).

Total polyphenols content

The content of total polyphenols in tomato and tomato juice samples was determined by the Folin-Ciocalteu method according to Huang et al. [8]. Briefly, 0.4 ml of previously prepared and defatted sample was mixed with 0.5 ml of Folin-Ciocalteu reagent and 2 ml of 20% Na_2CO_3 solution in a volumetric flask of 10 ml. The flask was filled with deionized water to the line incubated for 2 h at 20 °C and absorbance was measured at 760 nm, relative to deionized water as a reference solution. The obtained calibration line was linear in the concentration range from 1 mg·l^{-1} to 9 mg·l^{-1}. The content of polyphenolic compounds in the tested samples was determined by the equation of the calibration line and expressed as a milligrams of gallic acid equivalents (GAE) per kilogram of the sample. The equation of the calibration line is given as:

$$A = 0.10262c_x + 0.05719\ (r^2 = 0.999469) \quad (3)$$

where A represents absorbance, c_x is analyte concentration and r^2 is the coefficient of determination.

Total flavonoids content

The method for the determination of total flavonoids content was performed as described by de Souza et al. [9]. The reaction mixture was prepared by mixing 0.25 ml of the sample, 3 ml of deionized water and 0.3 ml of 5% $NaNO_2$. After 5 min, 1.5 ml of $AlCl_3$ was added to this mixture and then, after another 5 min, 2 ml of 1 mol·l^{-1} NaOH and deionized water was added up to 10 ml. The absorbance of the solution was measured at 510 nm in relation to deionized water as a reference solution in a UV-Vis spectrophotometer. Blank solution contained all substances without the real sample. A series of working solutions were prepared from the solution of (+)-catechin to construct a calibration line. It was linear in the concentration range from 5 mg·l^{-1} to 40 mg·l^{-1}. Based on the obtained equation of the calibration line (Eq. 4), the content of total flavonoids was calculated and expressed as milligrams of catechin equivalents (CE) per kilogram of the sample.

$$A = 0.03612c_x + 0.0091\ (r^2 = 0.9993) \quad (4)$$

Antioxidant activity by ABTS method

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method [10] is based on decolorization of a blue-green ABTS radical cation formed by chemical or enzymatic oxidation of ABTS solution. In this method, 0.1 ml of the tomato sample was mixed with 2 ml of ABTS radical cation working solution. After keeping the solution in the dark for 6 min, absorbance was measured at 734 nm relative to methanol as the reference solution. The absorbance values of a series of standard solutions were subtracted from the absorbance of the blank. Graphic dependence is given by Eq. 5:

$$\Delta A = A_0 - A_S = f(c_x) \quad (5)$$

where A_0 is the absorbance of the blank and A_S is the mean value of three samples of the standard solutions, which have given concentrations. The calibration plot was linear in the range of concentrations from 0.5 μmol·l^{-1} to 2 μmol·l^{-1} and it is given as:

$$\Delta A = 0.0316c_x + 0.0068 \ (r^2 = 0.9998) \quad (6)$$

where c_x is ABTS radical cation concentration expressed in micromoles per litre. Based on the obtained equation, the antioxidant activity was calculated and expressed as millimoles of Trolox equivalents (TE) per kilogram of the sample.

Antioxidant activity by DPPH method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [11] method is based on similar mechanism as the ABTS method. A solution of DPPH radicals, which is purple, reacts with antioxidants in the tested samples. These radicals are reduced to yellow DPPH form, which is followed by a decrease in absorbance at 515 nm. In this method, 0.1 ml of the sample was mixed with 5 ml of DPPH radical working solution in a volumetric flask and made up to 10 ml with methanol. After standing at 20 °C for 6 min, the absorbance of the resulting solution was measured at 734 nm, relative to methanol as the reference solution. A series of standard solutions were prepared by adding 5 ml of DPPH to a certain volume of Trolox and making up to 10 ml with methanol. Absorbance was measured after 30 min and was given as the mean of three measurements. The absorbance values of series of standard solutions were subtracted from the absorbance of the blank. The calibration plot was linear in the range of concentrations from 0.5 μmol·l^{-1} to 5 μmol·l^{-1} and had the form:

$$\Delta A = 0.02449c_x + 0.00913 \ (r^2 = 0.9988) \quad (7)$$

where c_x is DPPH radical concentration expressed in micromoles per litre. Based on the obtained equation, the antioxidant activity was calculated and expressed as millimoles of TE per kilogram of the sample.

Antioxidant activity by FRAP method

The ferric ion reducing antioxidant power (FRAP) method [12] for determination of antioxidant activity is based on the formation of *o*-phenanthroline-Fe^{2+} complex and its degradation in the presence of chelating agents. Herein, the test solution (20 μl) was diluted with 0.38 ml of deionized water and 3 ml of FRAP reagent (mixture of acetate buffer, 2,4,6-tripyridyl-S-triazine (TPTZ) and $FeCl_3$ in a ratio of 10 : 1 : 1 (v/v/v) was added. The mixture was incubated for 5 min at 37 °C and absorbance was measured at 595 nm in relation to the blank, which contained the solvent instead of the sample. A series of standard solutions of $FeSO_4 \cdot 7H_2O$ was used to obtain the calibration line, which was linear in the concentration range from 1.39 μmol·l^{-1} to 13.9 μmol·l^{-1}. Based on the obtained equation of the line, antioxidant (FRAP) activity was calculated and expressed as micromoles of Fe^{2+} equivalent per kilogram of the sample. The calibration line was defined as:

$$\Delta A = 0.077c_x + 0.0286 \ (r^2 = 0.9999) \quad (8)$$

where c_x is concentration of Fe^{2+} ions expressed in millimoles per litre.

Antioxidant activity by CUPRAC method

The cupric ion reducing antioxidant capacity (CUPRAC) method described by Apak et al. [13] is based on formation of copper(I)-neocuproine complex, which shows maximum absorption at 450 nm. In our work, a series of standard solutions of Trolox was used to obtain the calibration line, which was linear in the concentration range from 3 μmol·l^{-1} to 18 μmol·l^{-1}. Based on the obtained equation of the line, antioxidant (CUPRAC) activity was calculated and expressed as micromoles of TE per kilogram of the sample. The calibration line was defined as:

$$\Delta A = 0.0606c_x + 0.0449 \ (r^2 = 0.9993) \quad (9)$$

where c_x is concentration of Trolox expressed in millimoles per litre.

Antimicrobial activity

Testing of antimicrobial activity of the samples was performed against strains of microorganisms from the laboratory collection. Gram-positive bacteria, namely, *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 19433), and *Bacillus cereus* (ATCC 11778) were used for the

tests. Gram-negative bacteria were *Escherichia coli* (ATCC 25922), *Salmonella* Enteritidis (ATCC 13076), *Enterobacter aerogenes* (ATCC 13048) and *Pseudomonas aeruginosa* (ATCC 9027). Yeast *Candida albicans* (ATCC 24433) was used for testing the antimicrobial activity, as well. Overnight culture on oblique Mueller-Hinton agar (Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia) was prepared for bacterial analysis, while oblique Sabouraud dextrose agar (Institute of Virology, Vaccines and Sera "Torlak") was used for the yeast.

The microdilution method was used to examine the antimicrobial activity of the extracts. Overnight cultures of selected strains of microorganisms were used to make suspensions of 0.5 McFarland turbidity corresponding to a density of 1×10^8 CFU·ml^{-1}. Two types of sample solutions were prepared from the tomato and tomato juice extracts. The first type of extract was obtained by using a mixture of solvents (250 ml of hexane, 125 ml of acetone, 62.5 ml of ethanol, and 62.5 ml of 50 mg·l^{-1} butylated hydroxytoluene) and dry residues were dissolved in 100% dimethyl sulfoxide (DMSO; Merck). The second type of extract was prepared by using 70% ethanol as solvent. Volumes of 160 μl of inoculated Mueller-Hinton broth were introduced into the microtiter plate with 96 wells and 40 μl of the initial sample and a series of binary dilutions were pipetted. The final volume in each well was 100 μl with density of microorganisms of 10^6 CFU·ml^{-1}. Cultivation of all tested microorganisms was carried out at 37 °C for 18 h according to the recommended Clinical and Laboratory Standards Institute procedure [14]. For *C. albicans*, this testing time was enough because the test was not meant to determine the exact number of cells (viable counts) but only to observe whether culture growth (inhibitory activity of the extract) or cell death (microbicidal activity) occured.

The minimum inhibitory concentration (*MIC*) is the concentration of the sample in which there is no visible growth of microorganisms. It was determined using 5 g·l^{-1} aqueous solution of triphenyltetrazolium chloride (Sigma Aldrich). The minimum microbicidal concentration (*MMC*) is defined as the sample concentration at which 99.9% of microbial cells are killed. It was determined by transferring the contents of wells with no visible growth to Petri dishes with Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for yeasts, incubating them and counting colonies. All tests were performed in triplicate and the obtained results were processed by analysis of variance (ANOVA) with 95% confidence ($p \leq 0.05$).

RESULTS AND DISCUSSION

Identification of compounds by chromatographic analysis

Two carotenoid compounds (lycopene and β-carotene) were detected in the non-polar extracts of tomato as shown in the chromatogram (Fig. 1). Lycopene and β-carotene are compounds with the same molecular mass, which leads to the corresponding molecular ion peaks detectable at the same *m/z* values (in the positive ESI-MS $[M+H]^+$ at *m/z* 537, Tab. 1) with similar MS/MS spectra. Position of the peaks in the corresponding chromatogram defined assignation of the peaks – first to be lycopene and second β-carotene, as is the elution order on C18 column used for separation. UHPLC chromatograms for the polar extract with MS detection in negative mode is given in Fig. 2.

From the DAD signal at 300 nm, typical UHPLC-DAD chromatogram was recorded (Fig. 3). In Tab. 1, the full list of detected and identified compounds in the extracts of tomato and tomato juice is given. More than twenty compounds in two main classes were detected and identified in the polar extracts, namely, phenolic acids and flavonoids. From the class of phenolic acids and their derivatives, several compounds are listed, such as caffeic acid, caffeic acid hexosides (tentatively galactoside and glucoside), chlorogenic acid, homovanillic acid hexoside, 4-*O*-caffeoyl-quinic acid and caffeic acid (unknown derivative). From other acids, quinic and citric acids were also identified in the samples. It is noticeable that the chemical composition was almost the same for the two samples. Carotenoids lycopene or β-carotene were found as the 22nd peak. This peak most probably originated from lycopene, bearing in mind that it is a major compound in tomatoes and the corresponding products. In the ESI-MS/MS spectrum of the compound No. 22 (Tab. 1) assigned to lycopene with molecular ion peak $[M+H]^+$ detected at *m/z* 537, abundant ion detected at *m/z* 457, corresponding to loss of 80 units, i.e. $[M+H-C_6H_8]^+$.

For the corresponding spectrum of the first compound in the table, the presence of additional ions at *m/z* 85 and 127 was indicating quinic acid [15]. For the identification of citric acid, the corresponding reference standard was used. An ion with *m/z* 179 was related to caffeic acid [16]. The presence of *m/z* 343 fragment revealed homovanillic acid hexoside with *m/z* 181 fragment as a result of sugar cleavage and *m/z* 137 fragment from the loss of CO_2 [17]. The 4-*O*-caffeoyl-quinic acid derivatives, from the molecular ion peak $[M-H]^-$ at *m/z* 353, have characteristic peaks

Tab. 1. Compounds detected by UHPLC-DAD-MS/MS in tomato and tomato juice.

Peak	tR [min]	λmax [nm]	Molecular ion $[M-H]^-$ *m/z*	MS/MS fragment ions *m/z*	Assignment	Presence in the sample	
						Tomato	Tomato juice
1	0.75	–	191	173, 127, 111, 85 (100 %)	Quinic acid [11]	+	+
2	0.92	–	191	173, 111 (100 %)	Citric acid (standard)	+	+
3	1.50	–	179	161, 143, 131, 119, 113, 101, 89 (100 %), 71	Caffeic acid [16]	+	+
4	3.14	320 293sh	341	281, 179 (100 %), 135	Caffeic acid hexoside tentatively galactoside	+	+
5	5.14	326 315sh	341	281, 251, 221, 179 (100 %), 135	Caffeic acid hexoside tentatively glucoside	+	+
6	5.30	325 303sh	353	191 (100 %), 179, 173	Chlorogenic acid (standard)	±	+
7	5.40	318 298sh	343	181 (100 %), 137	Homovanilic acid hexoside [17]	+	+
8	5.50	–	477	431 (100 %)	Not identified	+	+
9	5.76	325 300sh	353	191, 179, 173 (100 %), 135	4-*O*-caffeoyl-quinic acid	+	+
10	6.23	–	457	411 (100 %)	Not identified	+	+
11	6.63	–	457	411 (100 %), 341	Not identified	+	+
12	7.04	291 305sh	427	381 (100 %), 249, 161	Not identified	+	+
13	7.90	–	629	585 (100 %)	Not identified	+	–
14	7.98	359 257	499	453 (100 %)	Not identified flavonoide	+	+
15	8.18	354 262	471	425 (100 %), 263	Not identified flavonoide	+	±
16	8.99	351 289	597	487, 387, 357 (100 %)	Phloretin-*C*-diglycoside [17]	+	±
17	8.99	355 258	609	301 (100 %) 299, 271	Rutin (quercetin-3-*O*-rutinoside) (standard)	+	+
18	9.26	321 293	579	533, 459 (100 %), 357, 313, 271, 235	Not identified	+	±
19	9.48	324 295	503	323 (100 %), 264, 179	Not identified	+	+
20	9.77	–	517	323 (100 %), 223, 221, 161	Not identified	+	±
21	10.90	–	537#	457 (100 %)	Lycopene or β-carotene [37]	+	+
22	11.20	326	425	361, 263, 179 (100 %), 135	Caffeic acid derivative (tentative)	+	+
23	11.40	291	271	227, 177, 151 (100 %), 107, 93	Naringenin [11, 12]	+	+
24	13.47	343	386#	201 (100 %)	Not identified	+	–
25	14.25	358	274#	256 (100 %), 230, 106, 102, 88	Not identified	+	+

Value 100 % in the brackets in the column of MS/MS fragment ions mean ion abundance of the base ion peak in the corresponding MS/MS spectrum of the compound.
sh – shoulder, # – electrospray ionisation mass spectrometry (ESI-MS) data are corresponding to positive mode ($[M+H]^+$).
Presence in sample: (–) – compound is not present, (+) – compound is present, (±) – compound is present but less than in other samples.

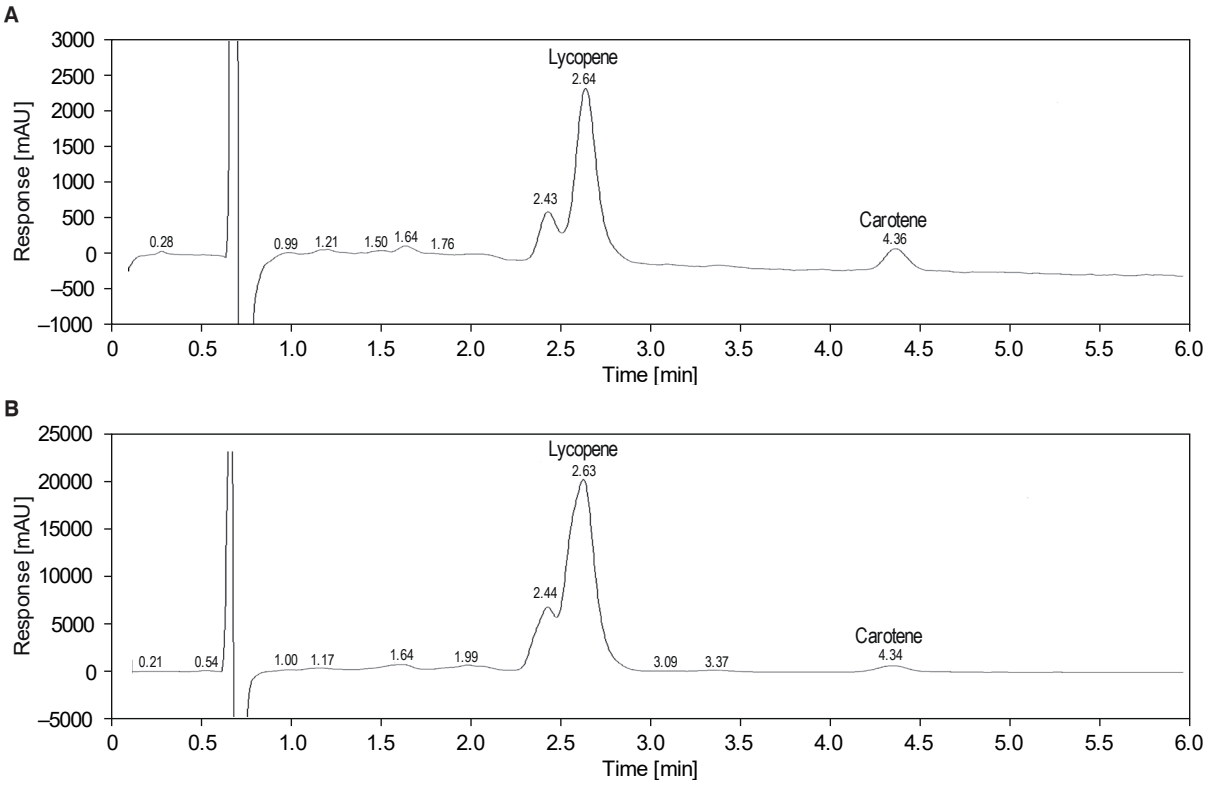

Fig. 1. UHPLC-DAD chromatograms with detection at 472 nm for the non-polar extract.

A –tomato, B – tomato juice.

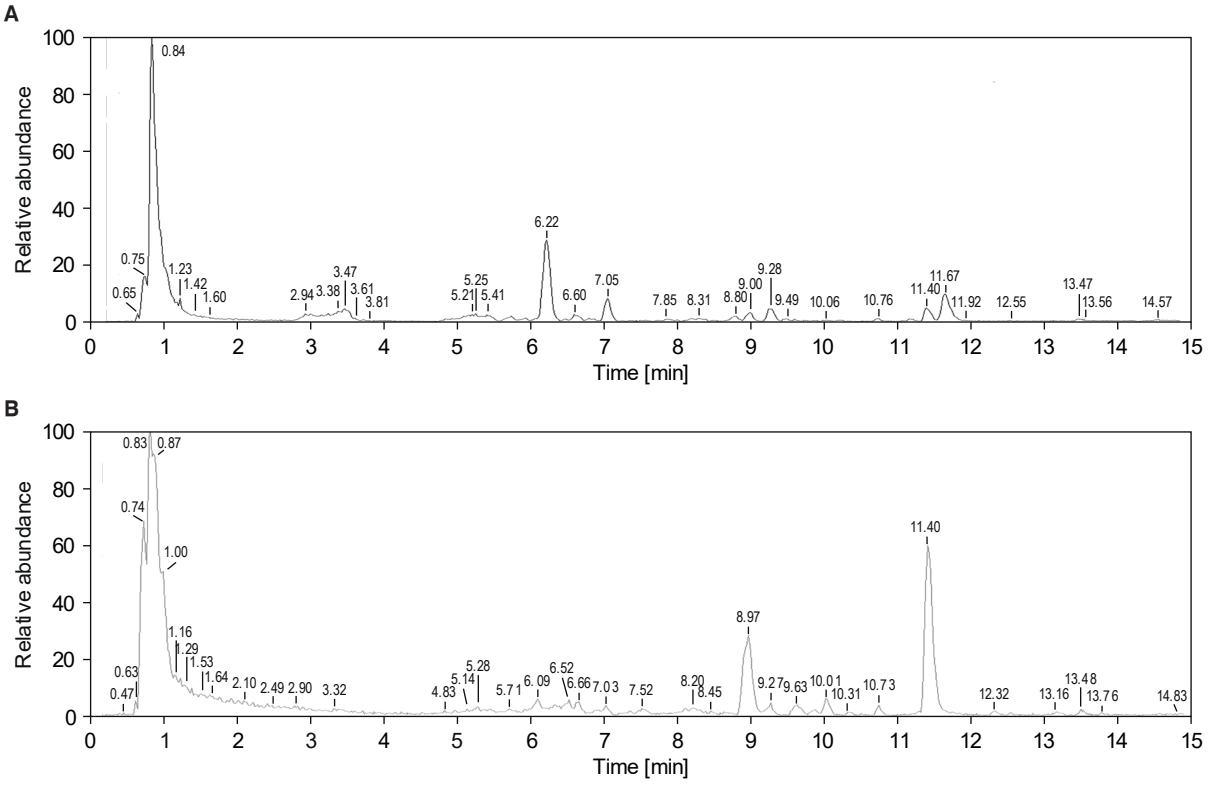

Fig. 2. UHPLC chromatograms for the polar extract with MS detection in negative mode.

A – tomato, B – tomato juice.

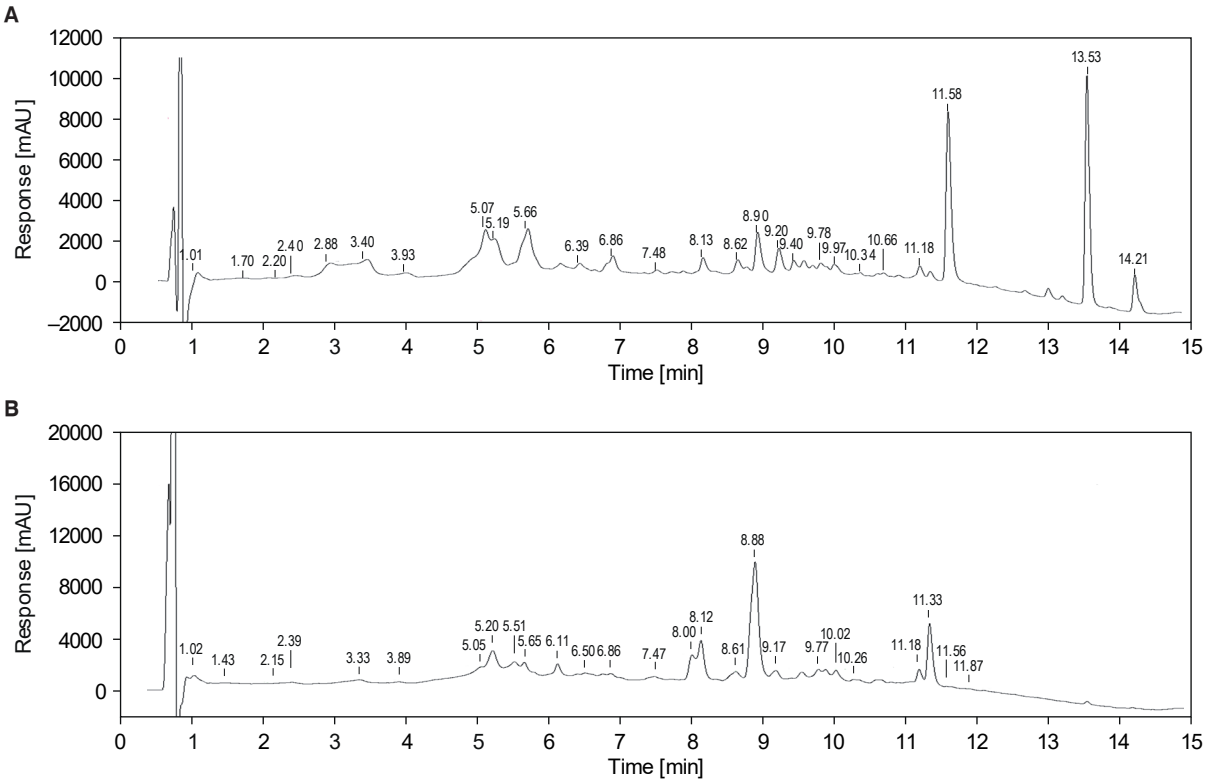

Fig. 3. UHPLC-DAD chromatograms for the polar extract.

A – tomato, B – tomato juice.

at *m/z* 191 (base peak) and 173 (base peak). In all tomato samples, derivative product phloretin-*C*-diglycoside (*m/z* 597) was identified tentatively [17]. For identification of rutin, standard of rutin dihydrate was used. The fragment ions at *m/z* 271 and *m/z* 151 indicated naringenin.

Rutin, naringenin and quercetin were flavonoids that were also present in 40 % (v/v) methanol-water extract of tomato [18]. In addition, NICOLETTI et al. [19] extracted tomato fruit with 70% (v/v) methanol-water mixture and, by using HPLC with photo diode array detector, they identified several components also found in our report, namely, chlorogenic acid, caffeic acid, rutin, quercetin, and naringenin. SUAREZ et al. [20] analysed organic acids in tomato cultivars grown in Spain. Citric acid was identified in 80% (v/v) ethanol extract with the aid of HPLC-DAD, which corresponds well with the fact that citric acid was also present in our samples in this study. Similar findings were also reported by PAREDES et al. [21] who employed HPLC with inductively coupled plasma atomic emission spectroscopy to assay a water extract of tomato. So far, the most detailed analysis of the chemical composition of tomato was published by MIKLAVČIČ VIŠNJEVEC et al. [22]. These authors analysed aqueous extracts of tomato by HPLC-DAD interfaced with a electrospray ionization-quadrupole time-of-flight. Common components identified in these two works are caffeic acid hexoside, homovanillic acid, chlorogenic acid, caffeic acid, rutin, naringenin, phloretin-*C*-diglycoside and quercetin. Conclusively, it might be stated that the compounds listed in Tab. 1 are typical for tomato-based samples. One may come to the opinion that the content of these compounds differs among various samples, which are influenced by their unique contents in fruits of individual cultivars.

Total lycopene and β-carotene contents

Total lycopene and β-carotene contents are given in Tab. 2. It can be seen that thermal treatment basically caused an increase in the content of both analysed compounds in the final product. According to available literature, there is a huge variation in lycopene and β-carotene content in tomatoes. This is reasonable since different cultivars were used and also, there is a difference between cherry and high-pigment tomatoes in terms of chemical compositionIn the study of LENUCCI et al. [1], 14 cultivars of cherry tomato and 4 cul-

Tab. 2. Total lycopene and β-carotene contents as determined by UV-Vis spectrophotometry.

Compound	Tomato		Tomato juice	
	Content	*RSD* [%]	Content	*RSD* [%]
Lycopene [mg·kg^{-1}]	34.2 ± 0.6	1.8	151.4 ± 0.3	0.2
β-carotene [mg·kg^{-1}]	18.6 ± 1.6	4.8	35.5 ± 0.6	1.7

Content is expressed per kilogram of fresh weight.
RSD – relative standard deviation.

tivars of high-pigment tomato were analysed. So, 120 mg·kg^{-1} (expresed as fresh weight (FW)) was the highest content of lycopene in cherry tomato cv. LS203, while the lowest lycopene content was in cv. Rubino Top (43 mg·kg^{-1} FW). Among the high-pigment tomatoes, cv. Kalvert had the highest content of lycopene (253 mg·kg^{-1} FW) and β-carotene 5 mg·kg^{-1} FW, while cv. HLY13 had the lowest lycopene (175 mg·kg^{-1} FW) and β-carotene (20 mg·kg^{-1} FW) content [1].

Tomatoes cultivated in Hungary also showed variation in lycopene content ranging from 500 mg·kg^{-1} to 1100 mg·kg^{-1}. For tomatoes grown in India, lycopene content was determined to be from 20 mg·kg^{-1} to 70 mg·kg^{-1} [23]. In cultivars grown in the North American continent, cherry tomatoes showed to be richer in lycopene (field-grown mean value 91.9 mg·kg^{-1}; greenhouse-grown mean value 56.1 mg·kg^{-1}) than cluster and round tomatoes (field-grown mean value 25.2 mg·kg^{-1}, greenhouse-grown mean value 30.3 mg·kg^{-1}) [24]. It is known that during ripening, the content of lycopene is increasing, while air temperatures exceeding 30 °C during the harvest period result in the reduction of lycopene content [25].

An extraction procedure similar to that used in our work is described in a paper by Agarwal et al. [26], where the lycopene content in tomato and tomato juice were determined to be 125.4 mg·kg^{-1} FW and 101.6 mg·kg^{-1} FW, respectively. Lycopene content in tomato juice was not affected by processing and was stable for up to 12 months when kept at ambient conditions. As in our work, the tendency of increasing lycopene content was reported for all-trans-lycopene after processing of tomato [27].

Lycopene content in tomato juice made from cultivar Rumba grown in Poland was reported to be 125.2 mg·kg^{-1} in organic and 154.3 mg·kg^{-1} in non-organic conditions [28]. The values for lycopene and β-carotene contents in the samples in this work are comparable and they correspond better to cases with lower carotenoid content. This fact could be influenced by the cultivar Hector-F1, which probably has a lower content of lycopene and β-carotene in general.

Bioavailability of lycopene from tomatoes is increasing with processing and serum lycopene levels are increasing only when the processed tomato is getting consumed. Principally, the content of lycopene and β-carotene is affected by various factors such as agronomic factors, climatic conditions or geographical location [29]. Right correlation between all of these factors and imperative for health-promoting food is of paramount importance in reaching benefits in production of tomato and tomato juice.

Total polyphenols and flavonoids contents

Antioxidant activity of plants is often correlated with the content of their phenolic compounds [30]. Determination of polyphenols and flavonoids content stands right in the context of analysing the health-promoting potential of the food. Results from this study are given in Tab. 3. In this case, thermal treatment caused a decrease in the total contents of both polyphenols and flavonoids.

Tomatoes belong to the richest sources of

Tab. 3. Total polyphenols and flavonoids contents as determined by UV-Vis spectrophotometry.

	Tomato		Tomato juice	
	Content	*RSD* [%]	Content	*RSD* [%]
Total polyphenols [mg·kg^{-1}]	298.0 ± 7	2.3	213.2 ± 5	2.4
Total flavonoids [mg·kg^{-1}]	216.4 ± 15	7.0	36.1 ± 3	8.6

Content of total polyphenols is expressed as milligrams of gallic acid equivalents per kilogram fresh weight of the sample.
Content of total flavonoids is expressed as milligrams of catechin equivalents (CE) per kilogram fresh weight of the sample.
RSD – relative standard deviation.

polyphenols in the human diet. According to LENUCCI et al. [1], the average content of polyphenols in cherry tomatoes is 1 345 mg·kg^{-1} and 1 265 mg·kg^{-1} in high-pigment cultivars (expressed as GAE). On the other hand, regular tomato cultivars contain phenolic compounds in the range from 259 mg·kg^{-1} to 498 mg·kg^{-1}. It was reported that organic tomato juice contains 517.4 mg·kg^{-1}, while non-organic has 453.5 mg·kg^{-1} [28]. From this, as previously underlined, it is obvious that the method of cultivation along with cultivar are responsible for the polyphenols content. In the case of the present paper, the content of total polyphenols in tomato was in the range of previously published values for tomato samples. An interesting point here was the lower concentration of polyphenols in tomato juice in comparison to the fresh fruit.

Flavonoids are the most represented components in the total phenolic content. In the work of LENUCCI et al. [1], flavonoids accounted for 72.6 % of tomato and 16.9 % of tomato juice among all polyphenols. Flavonoids were in the range from 134 mg·kg^{-1} FW to 622 mg·kg^{-1} FW (expressed as rutin equivalents) and this corresponds well with the findings reported in our work. Again, a much lower content found in the case of tomato juice could be ascribed to the heating step that they underwent, which might have led to some sort of decomposition of the compounds. The measured value was similar to values reported for organic tomato juice, which contained 28.1 mg·kg^{-1} and for non-organic tomato juice, which contained 25.7 mg·kg^{-1} [28].

Antioxidant activity

The antioxidant activity is defined as the ability to delay an oxidation process [23]. To give a detailed profile of tomato cv. Hector-F1 from the perspective of choosing it for consumption either fresh or processed, the antioxidant activity was determined by using ABTS, DPPH, FRAP and CUPRAC methods. Results are displayed in Tab. 4.

Antioxidant activity differed among tomato samples. A proper comparison might be a useful tool for establishing relations between cultivar characteristics and their significance in the diet. For instance, antioxidant activity evaluated by FRAP for high-pigment and cherry tomatoes was determined to be in the range from 2.16 mol·kg^{-1} to 4.53 mol·kg^{-1} [1]. Depending on the solvents and the assay method used, the antioxidant activity of tomato extracts varied. Even two methods based on the same property (ABTS and DPPH) can rank tomato extracts differently because of the different solvents used. The total antioxidant activity of tomato fruits is commonly classified into two types. The first is conferred mainly by soluble phenolic compounds and vitamin C, showing a significant impact on total antioxidant activity (accounts for 83 %). The second is conferred by carotenoids, vitamin E and lipophilic phenols (accounts for 17 %) [31]. It can also be expected that agronomical, geographical and environmental factors affect the total antioxidants content of fresh vegetables [1]. The results for antioxidant activity of tomato juice are rare in literature, but the lower values of antioxidant activity reported in our work are in agreement with ANESE et al. [32] who noticed that heating (2–4 h) during the preparation of tomato juice decreased its antioxidant potential.

Conclusively, it is obvious that tomato cultivar Hector-F1 exerts strong antioxidant activity as measured by the four assays. Activity decreased in the case of tomato juice probably due to the thermal treatment during preparation, which is prob-

Tab. 4. Antioxidant activity of the analysed tomato and tomato juice samples.

	Tomato		Tomato juice	
	Antioxidant activity	*RSD* [%]	Antioxidant activity	*RSD* [%]
ABTS [mmol·kg^{-1}]	2.57 ± 0.01	0.3	1.88 ± 0.04	1.9
DPPH [mmol·kg^{-1}]	2.36 ± 0.02	0.9	1.27 ± 0.04	3.4
FRAP [μmol·kg^{-1}]	4.51 ± 0.08	1.7	2.74 ± 0.02	0.9
CUPRAC [mmol·kg^{-1}]	16.56 ± 0.06	0.4	15.03 ± 0.04	0.3

ABTS – antioxidant activity determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method (expressed as millimoles of Trolox equivalents per kilogram of the sample), DPPH – antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl method (expressed as millimoles of Trolox equivalents per kilogram of the sample), FRAP – antioxidant activity determined by ferric reducing antioxidant power method (expressed as micromoles of Fe^{2+} equivalents per kilogram of the sample), CUPRAC – antioxidant activity determined by cupric ion reducing antioxidant capacity method (expressed as millimoles of Trolox equivalents per kilogram of the sample).
RSD – relative standard deviation.

Tab. 5. Antimicrobial activity of the extracts.

	Tomato		Tomato juice	
	MIC [mg·ml^{-1}]	*MMC* [mg·ml^{-1}]	*MIC* [mg·ml^{-1}]	*MMC* [mg·ml^{-1}]
G$^+$ bacteria				
Staphylococcus aureus	2.0	> 2.0	1.0	> 2.0
Enterococcus faecalis	2.0	> 2.0	2.0	> 2.0
Bacillus cereus	2.0	2.0	1.0	1.0
G$^-$ bacteria				
Escherichia coli	2.0	> 2.0	1.0	> 2.0
Salmonella Enteritidis	2.0	> 2.0	2.0	> 2.0
Enterobacter aerogenes	2.0	> 2.0	1.0	> 2.0
Pseudomonas aeruginosa	2.0	> 2.0	2.0	> 2.0
Yeasts				
Candida albicans	1.0	1.0	1.0	1.0

MIC – minimum inhibitory concentration, *MMC* – minimum microbicidal concentration.

ably responsible for partial decomposition of compounds with antioxidant activity.

Antimicrobial activity

Examination of antimicrobial activity is relevant regarding nutrition quality. The bacterial strains in this study were selected as the most common pathogenic microorganisms of the gastrointestinal tract or as species causing food spoilage. The results on the antimicrobial activity of tomato fruit and tomato juice are given in Tab. 5. The results show that the antimicrobial activity of tomato and tomato juice samples in this study was notable but lower than that previously reported in several studies [33–36].

CONCLUSIONS

Identification of the chemical profile of Hector-F1 tomato and tomato juice samples carried out by UHPLC provided helpful evidence for comparison with other cultivars or the same cultivar grown in other geographical regions. Lycopene and β-carotene contents were found to be in the range typical for regular tomato cultivars and slightly lower than for cherry and high-pigment tomatoes. The same was worth for total polyphenols and flavonoids contents with the remark that heating treatment in the case of tomato juice led to a certain decrease in the quantity probably due to decomposition. Four methods used for estimating the antioxidant activity of the analysed samples proved their notable potential in neutralization of free radicals and confirmed their significance for a health-promoting diet. Antimicrobial activity was determined to be modest in comparison to previous studies of other authors. Conclusively, it was shown that Hector-F1 cultivar grown on the territory of the Serbia has a specific chemical composition as examined by UHPLC, carotenoids and polyphenols levels typical for regular tomato cultivars and pronounced antioxidant activity. So, this paper provided a functional insight in the analytical methods for determining several types of compounds in tomato which are important for the science of healthy food.

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