TITLE: Tomato (*Solanum lycopersicum L.*) processing main product (juice) and by-product (pomace) bioactivity potential measured as antioxidant activity and angiotensin-converting enzyme inhibition

AUTHORS: Miona M. Belović, Amadeo Gironés-Vilaplana, Diego A. Moreno, Ivan LJ. Milovanović, Aleksandra R. Novaković, Maja A. Karaman, Nebojša M. Ilić

Tomato (*Solanum lycopersicum* L.) processing main product (juice) and by-product (pomace) bioactivity potential measured as antioxidant activity and angiotensin-converting enzyme inhibition

Short title: Tomato antioxidant and ACE inhibitory activity

Miona M. Belović[a]*, Amadeo Gironés-Vilaplana[b], Diego A. Moreno[b], Ivan Lj. Milovanović[a], Aleksandra R. Novaković[a], Maja A. Karaman[c], Nebojša M. Ilić[a]

[a]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

[b]Department of Food Science and Technology, Phytochemistry Lab, CEBAS-CSIC, Campus Universitario de Espinardo – Edificio 25, Espinardo, E-30100 Murcia, Spain

[c]Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 3, 21000 Novi Sad, Serbia

*Corresponding author (Miona M. Belović): Telephone: +381 21 485 3779; e-mail: miona.belovic@fins.uns.ac.rs

Abstract

Bioactivity potential of tomato (*Solanum lycopersicum* L.) juice and pomace was explored by performing four different antioxidant *in vitro* assays and angiotensin-converting enzyme (ACE) inhibition assay on their lipophilic and hydrophilic extracts. The content of major phytochemicals responsible for bioactivity was determined using HPLC-DAD and HPLC/UV-PAD/ESI-MS^n. Rutin was found in higher quantity in pomace, while juice was richer in caffeoyl-hexosides. Ferric ion reducing ability (FRAP) of both pomace extracts was significantly higher than those of juice, while juice hydrophilic extract showed higher nitric oxide radical scavenging capacity. Tomato carotenoids were shown to be more efficient superoxide anion scavengers than phenolic compounds which, in turn, showed higher activity in FRAP assay. ACE inhibitory activity of pomace hydrophilic extract was significantly higher than that of juice at extract concentration of 10 mg/mL. This gives the tomato pomace a new possible utilization for economic and eco-friendly tomato juice and paste production.

Keywords: *tomato; antioxidant activity; ACE inhibitory activity; carotenoids; phenolic compounds.*

Practical Applications

During industrial processing of tomato into juice, a by-product – tomato pomace is formed, consisting mainly of skin and seeds. Tomato pomace could be extracted consecutively with hexane and ethanol as environmentally preferable solvents, giving extracts which contain carotenoids and phenolic compounds. These extracts possess bioactivity potential and could be used as ingredients for value added food products.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops consumed worldwide either raw or after processing (Capanoglu *et al.* 2008; Gómez-Romero *et al.* 2010). During industrial processing of tomato into juice, ketchup or sauce, a by-product, tomato pomace is formed: a solid biomass that consists mainly of skin, seeds, and vascular tissue, having high moisture content (Al-Wandawi *et al.* 1985). Pomace is usually considered a waste product and discarded; however, in some cases it is used for the extraction of lycopene by supercritical CO_2 (Saldaña *et al.* 2010), bioethanol production (Lenucci *et al.* 2013) or as an ingredient in animal feed (Knoblich *et al.* 2005).

Among all phytochemicals present in tomato, most of the research has been focused on lycopene – a major carotenoid in tomato fruits, which is one of the most potent antioxidants and the predominant carotenoid found in human plasma after ingestion of tomato or tomato products (Agarwal and Rao, 2000). Besides lycopene, tomatoes also contain moderate amounts of α- and β-carotene and lutein (Grassi *et al.* 2013), valuable both for their provitamin A and antioxidant activity (Capanoglu *et al.* 2008; Chanforan *et al.* 2012). Tomato also contains different phenolic compounds, mostly flavonoids and hydroxycinnamic acid derivatives (Gómez-Romero *et al.* 2010; Kalogeropoulos *et al.* 2012). It has been demonstrated that phenolic compounds have protective role against several chronic diseases, including cancer, cardiovascular disease, diabetes and Alzheimer's disease (Soto-Vaca *et al.* 2012).

During the last decades, oxidative stress has been considered as one of the factors associated with an increased risk of chronic diseases, such as various tumours, atherosclerosis, and neurological diseases (Ferreres *et al.* 2010; Wang *et al.* 2011). Major radicals found in biological systems are superoxide anion ($O2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical

(ROO$^{\bullet}$) and nitric oxide (NO$^{\bullet}$) (Gülçin 2012). Superoxide anion and nitric oxide can form peroxynitrite (ONOO$^{\bullet-}$), which is proven to be a highly cytotoxic agent (Bor *et al.* 2006; Ferreres *et al.* 2010). On the other hand, cardiovascular disease (CVD) is the leading cause of death in developed countries, and high blood pressure is one of the major risk factors for CVD (Erdmann *et al.* 2008). Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is one of the main regulators of blood pressure (Hernández-Ledesma *et al.* 2011).

To the best of our knowledge, there are no studies that deal with ACE inhibitory activity of tomato juice and pomace extracts. Furthermore, the effect of tomato lipophilic and hydrophilic extracts on free radicals formed in biological systems, such as nitric oxide and superoxide anion, has not been sufficiently investigated. Therefore the aim of the present research was to determine the antioxidant activity of both lipophilic and hydrophilic fraction of tomato juice and pomace using different *in vitro* antioxidant assays and to explore whether hydrophilic extracts of tomato juice and pomace possess ACE inhibitory activity.

Materials and Methods

Material

The tomato variety "Knjaz", produced commercially for industrial processing in Kać (Serbia), was used for this research. Tomato fruits were harvested at the red stage of ripeness according to USDA standard (USDA, 1991) and immediately transported to laboratory. Tomato juice was prepared using kitchen juicer (Gorenje, Velenje, Slovenia), and tomato residues were collected in a separate container. The residues were further processed in the juicer until pomace consisting mainly of skin and seed was obtained. The yield of tomato juice was 70.3%, while the moisture content of juice and pomace fractions was 96.5% and 85.45%,

respectively, as determined by AOAC method 925.10 (AOAC, ~~2010~~2000). Tomato juice and pomace were lyophilized for 72 h (Martin Christ GmbH, Osterode am Harz, Germany) at temperature gradient from -30°C to +30°C. The process was controlled in such a way that when pressure in the system reached its minimum value (0.05 mbar), temperature was increased by 5°C. When temperature reached +30°C, tomato samples were left at that temperature until the end of process (72 h).

Reagents and standards

Lycopene, β-carotene, rutin (quercetin-3-rutinoside), chlorogenic acid (5-caffeoylquinic acid), Folin-Ciolcateu (FC) reagent, sodium carbonate anhydrous, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), iron(III) chloride anhydrous, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), disodium hydrogen phosphate, thiazolyl blue tetrazolium bromide, phenazine methosulfate (PMS), β-nicotinamide adenine dinucleotide (NADH), angiotensin converting enzyme (ACE) from rabbit lung (EC 3.4.15.1), and hippuryl-histidyl-leucine (HHL) were purchased from Sigma-Aldrich (Steinheim, Germany). Ascorbic acid, potassium dihydrogen phosphate, sodium nitroprusside dihidrate (SNP), naphthylethylenediamine dihydrochloride (NEDA), and sulfanilamide (SA) were from Lach-ner (Neratovice, Czech Republic).

Preparation of lipophilic and hydrophilic extracts

Lyophilized tomato juice and pomace were ground in coffee grinder (Gorenje, Velenje, Slovenia) to obtain powder (particle mean diameter = 132 μm as determined by rotational sieving machine (Bühler, Switzerland)). Four grams of ~~lyophilizate~~ lyophilized powder was extracted with n-hexane (8 x 20 mL) in an ultrasound bath (ATM40-3LCD, Aplicationes

Técnicas de Ultrasonidos, S.L.L, Valencia, Spain) for 2 minutes at room temperature to obtain lipophilic fraction. Ultrasound frequency and power were 40±2% kHz and 100 W, respectively. The final cycle of extraction was defined as the cycle in which colourless extract was obtained. The extract was filtered through filter paper and evaporated under vacuum at 37°C, using rotary evaporator. The residue was dried under nitrogen flow, and re-extracted with ethanol (40 mL) for 24 hours on a shaker (Memmert, Schwabach, Germany) at room temperature. The obtained hydrophilic extract was filtered and evaporated under vacuum at 37°C. The samples were stored in a refrigerator (+4°C) until analysis.

Analysis of carotenoids using HPLC-DAD

Analysis of carotenoids using HPLC-DAD was performed according to method by Kevrešan *et al.* (2013) with some modifications. Solutions of dried hexane extract (1 mg/mL) in mobile phase B (acetone-methanol 75:25, v/v) were filtered through 0.45 µm regenerated cellulose membrane filters (Agilent, Paolo Alto, CA, USA) before injection of 10 µl into the HPLC system. HPLC analysis was performed by a liquid chromatograph (Agilent Infinity 1260 series, Paolo Alto, CA, USA), equipped with a diode array detector (DAD), on Zorbax® C18, 3 µm, 3 mm x 250 mm column. The mobile phase A consisted of acetone-water (75:25, v/v) ~~and the mobile phase B consisted of acetone-methanol (75:25, v/v)~~. The gradient used at a flow rate of 1.500 mL/min was 50% A and 50% B at initial time; 0% A, 100% B at 15 min; and back to 50% A, 50% B at 20 min, with post-time of 5 minutes. The spectra were acquired in the range of 250–600 nm and chromatograms plotted at 460 nm.

Carotenoids were identified by matching the retention time and their spectral characteristics against those of standards, and the external standard method was used for quantification. For each compound, a stock solution (concentration of 1 mg/ml) was made by dissolution of

accurately weighed commercial standard in mobile phase B. Solutions used for generation of calibration curve were prepared by dilution of the stock solutions. Peak areas from chromatograms were plotted against known concentrations of standards and linear regression equations were used to calculate the concentrations of carotenoids in samples.

Total phenolic content

Total phenolic content in the hydrophilic extract of tomato juice and pomace was determined according to the method of Singleton *et al.* (1999), adapted for detection on plate reader (Multiskan Ascent, Thermo Electron Corporation, USA) as described in Novaković *et al.* (2015). Standard curve was prepared for gallic acid, and total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g tomato juice or pomace dry weight (DW).

Analysis of phenolic compounds profile using HPLC/UV-PAD/ESI-MS^n

The procedure used for extraction, identification and quantification of individual phenolic compounds was described previously by Sánchez-Rodríguez *et al.* (2011, 2012). Briefly, 0.1 g of lyophilized sample was extracted with 1 mL of water/methanol (1:1) by sonication for 1 h, followed by overnight maceration at 4 °C and another sonication period (1 h). The resulting extract was centrifuged (10000 g, 15 min) and filtered through a 0.45 µm PVDF membrane. Chromatografic analyses were carried out on a Phenomenex reverse-phase column (250 x 4.6 mm, Luna 5 µm C18 (2) 100A). Water: formic acid (99:1, v/v) and acetonitrile were used as mobile phases A and B, respectively, with a flow rate of 800 µL/min. The linear gradient started with 1% of solvent B, reaching 17% of solvent B at 15 min up to 17 min, 25% at 22 min, 35% at 30 min, and 50% at 35 min, which was maintained up to 45 min. The injection

volume was 6 µL. Spectral data from all peaks were acquired in the range of 200–400 nm, and chromatograms were recorded at 280, 320, and 360 nm. The HPLC/UV-PAD/ESI-MS^n analyses were carried out with an Agilent HPLC 1200 series equipped with a PAD (photodiode array detector) and mass spectrometer in series.

The mass detector was a Bruker ion trap spectrometer (model HCT Ultra) equipped with an electrospray ionization interface and was controlled by LCMSD software (Agilent, version 6.1). The ionization conditions were adjusted at 350°C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 60 p.s.i. and 11 L/min, respectively. The full-scan mass covered the range from m/z 100 to 1200. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Mass spectrometry data were acquired in the negative ionization mode. MS^n was carried out on the most abundant fragment ion observed in the first-generation mass spectrum.

The identification of the peaks was obtained by analysing the extracted-ion chromatograms of the ion current at m/z values corresponding to the $[M-H]^-$ ions of the individual investigated compounds as well as their fragmentation. Quantification of the identified compounds was performed by HPLC-PAD detection using the external standard method with calibration graphs as a function of concentration based on peak area. Flavonoids were quantified as quercetin 3-rutinoside at 360nm, and cinnamic acids as 5-caffeoylquinic acid at 320 nm.

In vitro antioxidant activity assays

FRAP test was performed according to procedure of Benzie and Strain (1999), with ascorbic acid used to construct the standard curve. Results were expressed as mg ascorbic acid equivalents (AAE)/100 g tomato juice or pomace dry weight (DW). Spectrophotometric

determination of free radical scavenging activity was based on the monitoring of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical transformation in the presence of antioxidants according to Espin *et al.* (2000). Nitric oxide radical scavenging capacity (RSC) was determined according to the procedure by Green *et al.* (1982). Superoxide anion RSC of extracts was determined by measuring their ability to neutralize superoxide anion radicals generated during aerobic reduction of nitro blue tetrazolium (NBT) by β-nicotinamide adenine dinucleotide (NADH) mediated by phenazine methosulfate (PMS) (Nishikimi *et al.* 1972). All *in vitro* antioxidant activity assays were modified for detection on plate reader as described by Novaković *et al.* (2015).

Angiotensin-converting enzyme (ACE) inhibitory activity

The ACE inhibitory activity was measured by the method of Cushman and Cheung (1971) with some modifications. A sample solution (50 µl) was incubated at 37°C for 10 min with 50 µl of ACE solution (100 m~~u~~Units/mL). After the addition of 150 µl of substrate (8.3 mM HHL in 50 mM sodium borate buffer and 500 mM NaCl, pH adjusted to 8.3 using 1 M HCl), the reaction mixture was incubated for 80 min at 37°C. The reaction was terminated by the addition of 250 µl of 1 M HCl. The resulting hippuric acid was extracted with 3 x 500 µl of ethyl acetate and centrifuged at 800 g for 15 min. 750 µl of the upper layer was transferred into test tube and evaporated in the air flow at 37°C. The hippuric acid was dissolved in 1 mL of distilled water, and the absorbance was measured at 228 nm using UV/Vis spectrophotometer (Cintra 303, GBC Scientific Equipment, Australia). 100% of the enzyme activity was defined with 50 µl of buffer instead of sample. The reaction blank (0% enzyme activity) was prepared by adding the HCl before adding the enzyme. The sample blank was prepared in the same way that the reaction blank was prepared, replacing the volume of buffer

by the sample evaluated. In order to eliminate the interferences in the analysis, ACE inhibition was calculated according to Hernández-Ledesma *et al.* (2003):

$$\% \text{ ACE inhibition} = 100 \cdot [(A - B) - (C - D)]/(A - B) \quad (1)$$

where A represents absorbance in the presence of ACE, B absorbance of the reaction blank, C absorbance in the presence of ACE and inhibitor, and D absorbance of the sample blank.

Statistical analysis

Experiments were performed twice in triplicates. Results were expressed as mean ± SEM (standard error of the mean). Five different concentrations of extracts were used in antiradical assays in order to calculate IC_{50} or IC_{25}values (concentration that scavenged 50% or 25% of radicals, respectively). Statistical analysis was performed using software system STATISTICA (StatSoft, Inc. (2013), version 12.0 (www.statsoft.com)). Analysis of variance (ANOVA) and Duncan's Multiple Range Test were applied to compare means at 5% significance level. Pearson correlation coefficients were calculated between content of antioxidant compounds in extracts and their antioxidant activity in different assays.

Results and Discussion

Content of carotenoids and phenolic compounds

Lycopene and β-carotene were identified as the main carotenoids in lipophilic extracts, with significantly higher content of both compounds in tomato pomace, which also contained

significantly higher amount of phenolic compounds (Table 1). This was expected because it is well known that tomato skin is richer in lycopene than tomato pulp; in addition, skin and seeds (major fractions in tomato pomace) have been previously reported to possess significantly higher content of hydrophilic phenolics than pulp (Al-Wandawi *et al.* 1985; George *et al.* 2004; Toor and Savage 2005).

HPLC/UV-PAD chromatograms of tomato juice and pomace phenolic compounds recorded at 330 nm are presented in Figure 1. When the lists of identified phenolic compounds are compared, it can be observed that tomato pomace contains a broader spectrum of phenolic compounds (Tables 2 and 3). Hydroxycinnamic acids were present both in the juice and the pomace, which is in accordance with previous findings (Long *et al.* 2006; Moco *et al.* 2006; Slimestad and Verheul 2009). Caffeoyl-hexoside and its isomers are the main hydroxycinnamic acid derivatives found in tomato juice, with higher total content in comparison with pomace. Rutin was the predominant flavonoid in tomato pomace and the only flavonoid detected in tomato juice. In previous studies, flavonoids were detected only in the skin tissue or found in traces in other tomato tissues (Long *et al.* 2006; Moco *et al.* 2006; Slimestad and Verheul 2009). Other flavonoids, detected only in tomato pomace, were quercetin-3-apiosyl-rutinoside, chalconaringenin-hexoside, and chalconaringenin. Quercetin glycosides, naringenin chalcone and chlorogenic acid are compounds of intermediate hydrophobicity previously shown to contribute significantly to the antioxidant activity of tomato fruit (Capanoglu *et al.* 2008).

Antioxidant activity of lipophilic and hydrophilic extracts

Four different antioxidant assays were used in order to compare the antioxidant activity of lipophilic and hydrophilic extracts of tomato juice and pomace. Hexane and ethanol were

chosen for the consecutive extraction since it has been shown that alkanes and simple alcohols are environmentally preferable solvents (Capello *et al.* 2007). In addition, ethanol is widely used in the food industry and was previously shown to be safe and efficient solvent for the extraction of phenolic compounds from tomato (Li *et al.* 2012). The results obtained are presented in Table 1 along with major antioxidant compounds present in the extracts. Considering lipophilic extracts, tomato pomace extract had significantly higher FRAP values than juice extract. FRAP test was previously shown to detect only water-soluble antioxidants, and that carotenoids do not possess ferric reducing ability (Apak *et al.* 2007). However, the results of research by Ilahy *et al.* (2011) showed that there is significant correlation between FRAP assay and total carotenoid content. Scavenging capacity of both analysed lipophilic extracts tested against DPPH radical expressed concentration dependent antioxidant activity, and their IC_{50} values did not differ significantly. Both lipophilic extracts also showed concentration dependent scavenging activity against nitric oxide and superoxide anion radical; however, in concentration range used in these tests (2.5-40 mg/mL, final concentrations in reaction mixture were 0.1-1.6 mg/mL), none of them had activity high enough to calculate IC_{50} values so IC_{25} values were calculated. Higher concentrations of hexane extracts could not be tested due to their low solubility in polar media used in these tests. Obtained IC_{25} values did not differ significantly for tomato juice and pomace lipophilic extract. Therefore, correlation analysis (Table 4) was performed in order to find out how antioxidant activity of extracts is related to their lycopene and β-carotene content, and significant correlation ($r = 0.975$ for both lycopene and β-carotene at $p \leq 0.05$) was found between their content and ferric reducing antioxidant power. Significant correlation between the results of FRAP assay and lycopene content was also demonstrated by Ilahy *et al.* (2011).

FRAP values (calculated as ascorbic acid equivalents) obtained for tomato juice and pomace hydrophilic extracts were statistically different, with values similar to those obtained

for other fruits (Gil *et al.* 2002). However, these extracts did not differ significantly in terms of DPPH radical scavenging, with IC_{50} values similar to those obtained for the whole tomato (80% methanol extracts) in study conducted by Choi *et al.* (2011). Considering nitric oxide and superoxide anion radical scavenging, tomato juice ethanol extract had lower IC_{25} values for both radicals with statistically significant difference observed for nitric oxide radical. Concentration dependent nitric oxide and superoxide anion radical scavenging effect was also observed in tomato seed aqueous extract, with similar IC_{25} values (Ferreres *et al.* 2010). In addition, correlation analysis (Table 4) was performed between antioxidant activity of extracts and their total phenolic content, as well as content of groups of phenolic compounds (hydroxycinnamates, flavonols, and dihydrochalcones) detected in extracts in order to provide a better insight into their relation. FRAP values showed significant linear correlation ($r = 0.887$, $p \leq 0.05$) with total phenolic content, suggesting that phenolic compounds in general are responsible for ferric ion reduction. Regarding groups of phenolic compounds, significant negative correlation was found between hydroxycinnamic acid content and IC_{25} values for nitric oxide radical ($r = –0.950$, $p \leq 0.05$) . Flavonols and dihydrochalcones showed significant correlation only with FRAP assay ($r = 0.894$ and $r = 0.905$, respectively, when $p \leq 0.05$). Only weak and moderate correlations (from $r = –0.006$ to $r = –0.677$) were found between the results of DPPH and superoxide anion assay and content of phenolic compounds, both total and by groups. This implies that residual carotenoids present in hydrophilic extracts could also contribute to the antioxidant activity of these extracts.

By comparing the antioxidant activity of lipophilic and hydrophilic extracts, we can observe that FRAP values are significantly lower for lipophilic extracts, implying that phenolic compounds are more efficient in reduction of ferric ions (Table 1), similarly to the results of Ilahy et al. (2011). Considering scavenging capacity against DPPH radical, hexane and ethanol extracts did not differ significantly by IC_{50} values. This is in accordance with

results by Elbadrawy and Sello (2011) who found that RSC of predominantly lipophilic tomato extract (petroleum ether) and predominantly hydrophilic extract (methanol) did not differ significantly when extract concentration of 200 µg/mL was used. Nitric oxide RSC of tomato juice hydrophilic extract was significantly higher than those of lipophilic extract (lower IC_{25} value); on the other hand, tomato pomace lipophilic extract showed higher scavenging capacity against nitric oxide than hydrophilic extract. Regarding superoxide anion radical scavenging activity, both lipophilic extracts had significantly ($p < 0.05$) lower IC_{25} values than hydrophilic extracts, suggesting that carotenoids are stronger antioxidant agents against superoxide anion than phenolic compounds.

ACE inhibitory activity of hydrophilic extracts

Vegetables of the Solanaceae family such as eggplant, pepper and tomato have high phenolic content (Kwon *et al.* 2008). Since tomato hydrophilic extract is rich in phenolic compounds, especially quercetin and caffeic acid derivatives, it is presumed that these phenolic compounds could exert some angiotensin-converting enzyme (ACE) inhibitory activity (Actis-Goretta *et al.* 2006; Al Shukor *et al.* 2013). For that purpose, a percentage of ACE inhibition was determined for two concentrations (1 mg/mL and 10 mg/mL, final concentrations in reaction mixture were 0.2 mg/mL and 2 mg/mL, respectively) of tomato juice and pomace extracts. According to the results presented in Table 1 it could be concluded that lower concentrations of both extracts showed no inhibitory activity. However, higher concentrations showed inhibitory effect which was significantly stronger for tomato pomace extract. This might be explained by the higher total phenolic content in tomato pomace, especially by higher content of rutin and presence of kaempferol and other quercetin glycosides which were absent in juice extract. Although ethanol was shown to be efficient

agent for the extraction of phenolic compounds present in tomato, ACE inhibitory activity of ethanol extract could be underestimated in this *in vitro* assay due to low solubility of certain phenolics in this medium (Al Shukor *et al.* 2013). Further experiments should be carried out in order to verify the contribution of individual phenolic compounds to ACE inhibitory activity of tomato ethanol extract.

Conclusions

Antioxidant activity of lipophilic and hydrophilic tomato pomace extracts was similar to or higher than those of juice extracts, except for the activity of hydrophilic extract against nitric oxide radical. The results of FRAP assay were significantly positively correlated with total phenolic content and content of flavonols, dihydrochalcones, lycopene and β-carotene. Hydroxycinnamic acid content showed a significant negative correlation with IC_{25} values for nitric oxide radical scavenging. Tomato carotenoids were shown to be more efficient superoxide anion scavengers than phenolic compounds which, in turn, had superior ferric reducing ability. Angiotensin-converting enzyme inhibitory activity of pomace hydrophilic extract was significantly higher at concentration of 10 mg/mL. These results could give a new possible utilization for pomace, enabling an economic and eco-friendly tomato juice and paste production.

Acknowledgements

This paper is a result of the research within the project No. III 46001, financed by the Ministry of Education, Science and Technological Development, Republic of Serbia. Part of this work was also funded by the project "Group of Excellence" (04486/GERM/06) from the Regional Agency for Science and Technology of Murcia (Fundación Séneca), and was carried out with support of the CYTED Program (Ref. 112RT0460) CORNUCOPIA Thematic Network (URL: redcornucopia.org). AGV ~~also~~ thanks the CSIC and the European Social Funds for the JAE Predoctoral Grant.

The authors would like to thank Milena Rašeta and Saša Bijalković for their expert and technical assistance.

References

1. ACTIS-GORETTA, L., OTTAVIANI, J.I. and FRAGA, C.G. 2006. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J. Agric. Food Chem. *54*, 229-234.
2. AGARWAL, S. and RAO, A.V. 2000. Tomato lycopene and its role in human health and chronic diseases. Can. Med. Assoc. J. *163*, 739-744.
3. AL SHUKOR, N., VAN CAMP, J., GONZALES, G.B., STALJANSSENS, D., STRUIJS, K., ZOTTI, M.J., RAES K. and SMAGGHE, G. 2013. Angiotensin-converting enzyme inhibitory effects by plant phenolic compounds: A study of structure activity relationships. J. Agric. Food Chem. *61*, 11832-11839.
4. AL-WANDAWI, H., ABDUL-RAHMAN, M. and AL-SHAIKHLY, K. 1985. Tomato processing wastes as essential raw materials source. J. Agric. Food Chem. *33,* 804-807.
5. AOAC INTERNATIONAL. 2000. Official Methods of Analysis of Association of Official Analytical Chemists, (17th ed.). Arlington, Virginia, USA.
6. APAK, R., GÜÇLÜ, K., DEMIRATA, B., ÖZYÜREK, M., ÇELIK, S. E., BEKTAŞOĞLU, B., BERKER, K. I. and ÖZYURT, D. 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules *12*, 1496-1547.
7. BENZIE, I.F. and STRAIN, J.J. 1999. [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. *299,* 15-27.

8. BOR, J.Y., CHEN, H.Y. and YEN, G.C. 2006. Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. J. Agric. Food Chem. *54*, 1680-1686.
9. CAPANOGLU, E., BEEKWILDER, J., BOYACIOGLU, D., HALL, R. and DE VOS, R. 2008. Changes in antioxidant and metabolite profiles during production of tomato paste. J. Agric. Food Chem. *56,* 964-973.
10. CAPELLO, C., FISCHER, U. and HUNGERBÜHLER, K. 2007. What is a green solvent? A comprehensive framework for the environmental assessment of solvents. Green Chem. *9*, 927-934.
11. CHANFORAN, C., LOONIS, M., MORA, N., CARIS-VEYRAT, C. and DUFOUR, C. 2012. The impact of industrial processing on health-beneficial tomato microconstituents. Food Chem., *134,* 1786-1795.
12. CHOI, S.H., KIM, H.R., KIM, H.J., LEE, I.S., KOZUKUE, N., LEVIN, C.E. and FRIEDMAN, M. 2011. Free amino acid and phenolic contents and antioxidative and cancer cell-inhibiting activities of extracts of 11 greenhouse-grown tomato varieties and 13 tomato-based foods. J. Agric. Food Chem. *59,* 12801-12814.
13. CUSHMAN, D.W. and CHEUNG, H.S. 1971. Spectrometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. *20*, 1637-1648.
14. ELBADRAWY, E. and SELLO, A. 2011. Evaluation of nutritional value and antioxidant activity of tomato peel extracts. Arabian J. Chem., *in press*, doi:10.1016/j.arabjc.2011.11.011
15. ERDMANN K., CHEUNG B.W.Y. and SCHRÖDER H. 2008. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J Nutr Biochem *19*, 643–654.

16. ESPÍN, J.C., SOLER-RIVAS, C. and WICHERS, H.J. 2000. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2, 2-diphenyl-1-picrylhydrazyl radical. J. Agric. Food Chem. *48,* 648-656.

17. FERRERES, F., TAVEIRA, M., PEREIRA, D.M., VALENTÃO, P. and ANDRADE, P.B. 2010. Tomato (*Lycopersicon esculentum*) seeds: new flavonols and cytotoxic effect. J. Agric. Food Chem. *58,* 2854-2861.

18. GEORGE, B., KAUR, C., KHURDIYA, D.S. and KAPOOR, H.C. 2004. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chem. *84,* 45-51.

19. GIL, M.I., TOMÁS-BARBERÁN, F.A., HESS-PIERCE, B. and KADER, A.A. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. J. Agric. Food Chem. *50*, 4976-4982.

20. GÓMEZ-ROMERO, M., SEGURA-CARRETERO, A. and FERNÁNDEZ-GUTIÉRREZ, A. 2010. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry *71,* 1848-1864.

21. GRASSI, S., PIRO, G., LEE, J.M., ZHENG, Y., FEI, Z., DALESSANDRO, G., GIOVANNONI J.J., LENUCCI, M.S. 2013. Comparative genomics reveals candidate carotenoid pathway regulators of ripening watermelon fruit. BMC Genomics *14*, 781.

22. GREEN, L.C., WAGNER, D.A., GLOGOWSKI, J., SKIPPER, P.L., WISHNOK, J.S. and TANNENBAUM, S.R. 1982. Analysis of nitrate, nitrite, and [^{15}N] nitrate in biological fluids. Anal. Biochem. *126,* 131-138.

23. GÜLÇIN, İ. 2012. Antioxidant activity of food constituents: an overview. Arch. Toxicol. *86,* 345-391.

24. HERNÁNDEZ-LEDESMA B., CONTRERAS M.M. and RECIO I. 2011. Antihypertensive peptides: Production, bioavailability and incorporation into foods. Adv. Colloid Interface Sci. *165*, 23-35.

25. HERNÁNDEZ-LEDESMA, B., MARTÍN-ÁLVAREZ, P.J. and PUEYO, E. 2003. Assessment of the spectrophotometric method for determination of angiotensin-converting enzyme activity: influence of the inhibition type. J. Agric. Food Chem. *51*, 4175-4179.

26. ILAHY, R., HDIDER, C., LENUCCI, M.S., TLILI, I. and DALESSANDRO, G. 2011. Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. J. Food Compos. Anal. *24*, 588-595.

27. KALOGEROPOULOS, N., CHIOU, A., PYRIOCHOU, V., PERISTERAKI, A. and KARATHANOS, V.T. 2012. Bioactive phytochemicals in industrial tomatoes and their processing byproducts. LWT - Food Sci. Technol. *49,* 213-216.

28. KEVREŠAN, Ž., MASTILOVIĆ, J., MANDIĆ, A. and TORBICA A. 2013. Effect of different ripening conditions on pigments of pepper for paprika production at green stage of maturity. J. Agric. Food Chem. *61,* 9125−9130.

29. KNOBLICH, M., ANDERSON, B. and LATSHAW, D. 2005. Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. J. Sci. Food Agric. *85,* 1166-1170.

30. KWON, Y.-I., APOSTOLIDIS, E. and SHETTY K. 2008. *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour. Technol. *99*, 2981–2988.

31. LENUCCI, M.S., DURANTE, M., ANNA, M., DALESSANDRO, G., and PIRO, G. 2013. Possible use of the carbohydrates present in tomato pomace and in byproducts

of the supercritical carbon dioxide lycopene extraction process as biomass for bioethanol production. J. Agric. Food Chem. *61*, 3683-3692.

32. LI, H., DENG, Z., WU, T., LIU, R., LOEWEN, S. and TSAO, R. 2012. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. Food Chem. *130,* 928-936.
33. LONG, M., MILLAR, D.J., KIMURA, Y., DONOVAN, G., REES, J., FRASER, P.D., BRAMLEY, P.M. and BOLWELL, G.P. 2006. Metabolite profiling of carotenoid and phenolic pathways in mutant and transgenic lines of tomato: Identification of a high antioxidant fruit line. Phytochemistry *67,* 1750–1757.
34. MOCO, S., BINO, R.J., VORST, O., VERHOEVEN, H.A., DE GROOT, J., VAN BEEK, T.A., VERVOORT, J. and DE VOS, C.H.R. (2006). A liquid chromatography-mass spectrometry-based metabolome database for tomato. Plant Physiol. *141,* 1205-1218.
35. NISHIKIMI, M., APPAJI RAO, N. and YAGI, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem. Biophys. Res. Commun. *46,* 849-854.
36. NOVAKOVIĆ, A.R., KARAMAN, M.A., MILOVANOVIĆ, I.LJ., BELOVIĆ, M.M., RAŠETA, M.J., RADUSIN, T.I. and ILIĆ, N.M. 2015. Edible mycorrhizal species *Lactarius controversus* Pers. 1800 as a source of antioxidant and cytotoxic agents. Hem. ind. *OnLine-First* (00):17-17, doi:10.2298/HEMIND141229017N
37. SALDAÑA, M.D.A., TEMELLI, F., GUIGARD, S.E., TOMBERLI, B. and GRAY C.G. 2010. Apparent solubility of lycopene and β-carotene in supercritical CO_2, CO_2 + ethanol and CO_2 + canola oil using dynamic extraction of tomatoes. J. Food Eng. *99*, 1-8.

38. SÁNCHEZ-RODRÍGUEZ, E., MORENO, D.A., FERRERES, F., RUBIO-WILHELMI, M.M. and RUIZ, J.M. 2011. Differential responses of five cherry tomato varieties to water stress: Changes on phenolic metabolites and related enzymes. Phytochemistry *72,* 723–729.

39. SÁNCHEZ-RODRÍGUEZ, E., RUIZ, J.M., FERRERES, F., and MORENO, D.A. 2012. Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. Food Chem. *134*, 775-782.

40. SINGLETON, V.L., ORTHOFER, R. and LAMUELA-RAVENTOS, R.M. 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. *299,* 152-178.

41. SLIMESTAD, R. and VERHEUL, M. 2009. Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* Mill.) cultivars. J. Sci. Food Agric. *89,* 1255–1270.

42. SOTO-VACA, A., GUTIERREZ, A., LOSSO, J.N., XU, Z. and FINLEY, J.W. 2012. Evolution of phenolic compounds from color and flavor problems to health benefits. J. Agric. Food Chem. *60,* 6658-6677.

43. TOOR, R.K. and SAVAGE, G.P. 2005. Antioxidant activity in different fractions of tomatoes. Food Res. Inter. *38,* 487-494.

44. USDA. 1991. United States Standards for Grades of Fresh Tomatoes. United States Department of Agriculture, Agricultural Marketing Service, pp. 4.

45. WANG, S., MELNYK, J.P., TSAO, R. and MARCONE, M.F. 2011. How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. Food Res. Inter. *44,* 14–22.

TABLE 1.
CONTENT OF BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY AND ACE INHIBITORY ACTIVITY OF TOMATO JUICE AND POMACE EXTRACTS

Extract	Antioxidant content and activity	Tomato juice	Tomato pomace
Lipophilic	Lycopene (mg/100 g DW)	19.54 ± 0.04^{b}	31.49 ± 0.04^{a}
	β-carotene (mg/100 g DW)	6.71 ± 0.02^{b}	21.46 ± 0.06^{a}
	FRAP (mg AAE/100 g)	9.90 ± 0.72^{Bb}	144 ± 11.0^{Ba}
	DPPH IC_{50} (µg/mL)	146 ± 1.33^{Aa}	175 ± 9.66^{Aa}
	Nitric oxide IC_{25} (µg/mL)	741 ± 40.2^{Aa}	719 ± 98.6^{Ba}
	Superoxide anion IC_{25} (µg/mL)	8.78 ± 0.77^{Ba}	7.79 ± 0.79^{Ba}
Hydrophilic	Total phenolics (mg GAE/100 g DW)	784 ± 61.7^{b}	1315 ± 68.1^{a}
	FRAP (mg AAE/100 g)	364 ± 26.3^{Ab}	740 ± 62.9^{Aa}
	DPPH IC_{50} (µg/mL)	164 ± 10.0^{Aa}	164 ± 22.8^{Aa}
	Nitric oxide IC_{25} (µg/mL)	240 ± 58.5^{Bb}	1259 ± 104^{Aa}
	Superoxide anion IC_{25} (µg/mL)	49.2 ± 3.11^{Aa}	133 ± 30.3^{Aa}
	% IACE (1 mg/mL)	-3.4 ± 2.7^{a}	-2.0 ± 2.8^{a}
	% IACE (10 mg/mL)	7.7 ± 0.5^{b}	13.5 ± 0.5^{a}

Data are expressed as means ± SEM (n = 6).
Values in the rows followed by different lowercase letters and in the columns (antioxidant activity) followed by different uppercase letters are significantly different ($p < 0.05$).

TABLE 2.
CHROMATOGRAPHIC (HPLC) AND SPECTRAL (UV AND HIGH-RESOLUTION MS) DATA RECORDED FOR TOMATO JUICE EXTRACT

Compounds Peak no.	t_R, min	UV_{max}, nm (online HPLC)	$[M-H]^-$ *m/z*	MS/MS Fragments *m/z* (%)	Putative ID	Content (µg/g lyophilizate)
1	21.4	290, 318	341	179, 135	Caffeoyl-hexoside	0.815±0.012
						0.037±0.002
2-4	21.6-23.3	290, 312-318	341	179	Caffeoyl-hexoside isomers	0.173±0.007
						0.184±0.002
5	24.1	292, 318	353	191, 179, 173	4-Caffeoylquinic acid	0.166±0.010
6	27.0	296, 320	337	191, 173	5-*p*-Coumaroylquinic acid	0.032±0.001
7	29.4	254, 270, 356	609	301	Quercetin-3-rutinoside (Rutin)	0.058±0.003
8	31.1	298	223	179	Synapoyl derivative	0.093±0.004
9	36.0	298, 328	425	179,135	Caffeic acid derivative	0.105±0.004

Data are expressed as means ± SEM (n = 3).

TABLE 3.
CHROMATOGRAPHIC (HPLC) AND SPECTRAL (UV AND HIGH-RESOLUTION MS) DATA RECORDED FOR TOMATO POMACE EXTRACT

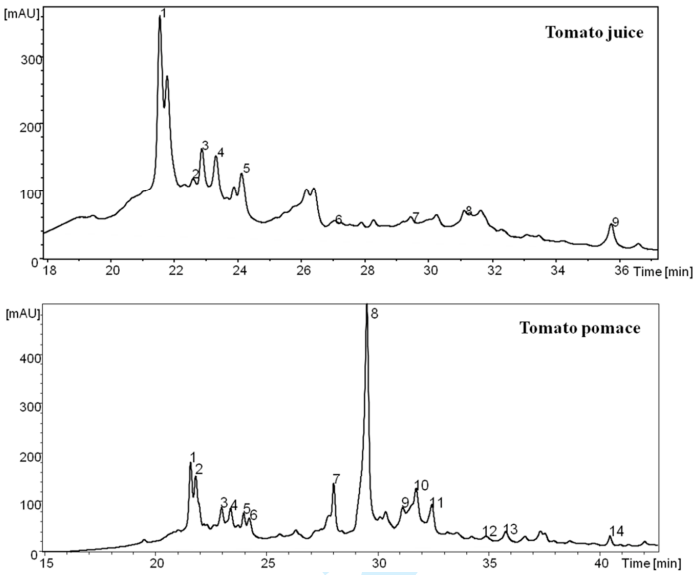
Compounds Peak no.	t_R, min	UV_{max}, nm (online HPLC)	$[M-H]^-$ *m/z*	MS/MS Fragments *m/z* (%)	Putative ID	Content (µg/g lyophilizate)
1	21.3	290, 318	341	179, 135	Caffeoyl-hexoside	0.369±0.006 0.307±0.014
2-4	21.5-23.1	290, 312-318	341	179	Caffeoyl-hexoside isomers	0.112±0.007 0.126±0.002
5	23.8	300sh, 330	353	191,179	3-Caffeoylquinic acid	0.082±0.002
6	24.0	~290, ~316	341	179	Caffeoyl-hexoside isomer	0.087±0.002
7	27.8	256, 268sh, 300sh, 352	741	609, 301	Quercetin-3-apiosyl-rutinoside	0.878±0.003
8	29.2	254, 298, 356	609	301	Quercetin-3-rutinoside (Rutin)	4.420±0.041
9	30.8	267, 326	593	285	Kaempferol-3-rutinoside	0.129±0.001
10	31.4	314sh, 328	515	353	di-Caffeoylquinic acid	0.237±0.011
11	32.2	314sh, 328	515	353	di-Caffeoylquinic acid isomer	0.182±0.015
12	35.0	308sh, 366	433	271	Chalconaringenin-hexoside	0.093±0.005
13	35.5	298, 328	425	179,135	Caffeic acid derivative	0.092±0.003
14	40.2	308sh, 366	271		Chalconaringenin	0.190±0.003

Data are expressed as means ± SEM (n = 3).

TABLE 4.
CORRELATION COEFFICIENTS BETWEEN CONTENT OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF TOMATO JUICE AND POMACE EXTRACTS

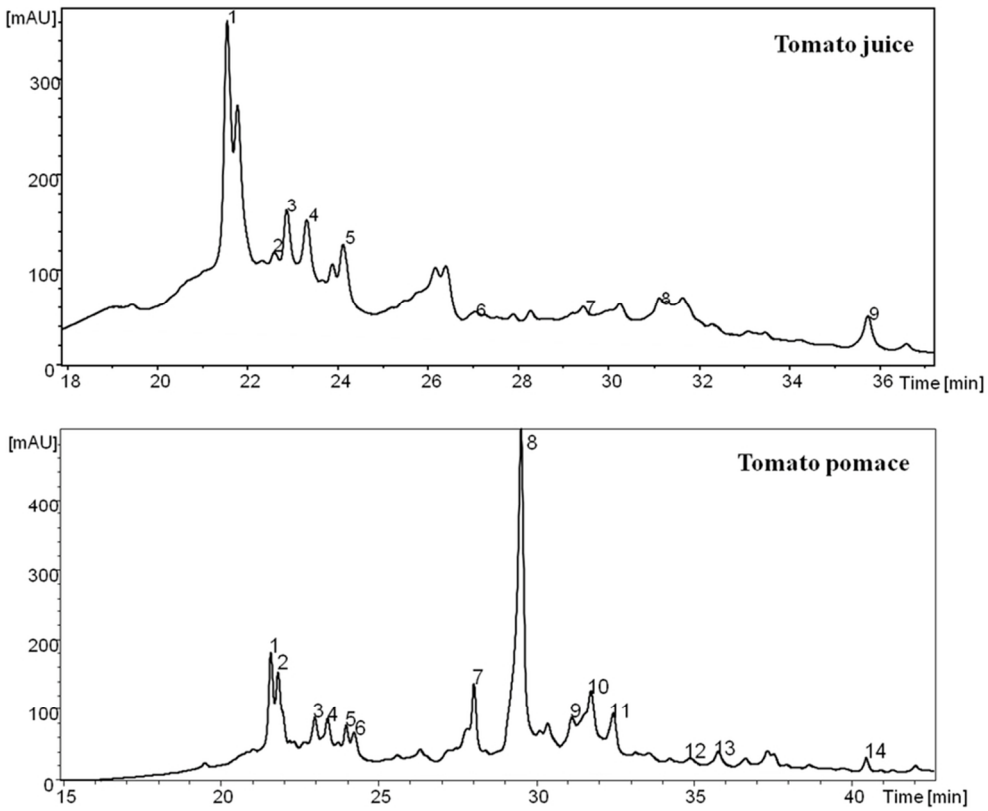
Bioactive compounds	**FRAP**	**DPPH IC_{50}**	**Nitric oxide IC_{50}**	**Superoxide anion IC_{50}**
Lycopene	0.975*	0.726	-0.081	-0.302
β-carotene	0.975*	0.727	-0.080	-0.303
Total phenolics	0.887*	0.020	0.947*	0.663
Hydroxycinnamic acids	-0.904*	-0.008	-0.950*	-0.677
Flavanols	0.894*	-0.006	0.953*	0.687
Dihydrochalcones	0.905*	-0.009	0.960*	0.662

*Correlations are significant at $p<0.05$.



~~FIGURE 1.~~
~~CHROMATOGRAMS OF TOMATO JUICE AND POMACE PHENOLIC COMPOUNDS RECORDED AT 330 NM (PEAK NUMBERS REFER TO TABLE 2 FOR TOMATO JUICE AND TABLE 3 FOR TOMATO POMACE)~~

Formatted: Font: Times New Roman, 12 pt



CHROMATOGRAMS OF TOMATO JUICE AND POMACE PHENOLIC COMPOUNDS RECORDED AT 330 NM (PEAK NUMBERS REFER TO TABLE 2 FOR TOMATO JUICE AND TABLE 3 FOR TOMATO POMACE)
78x63mm (300 x 300 DPI)