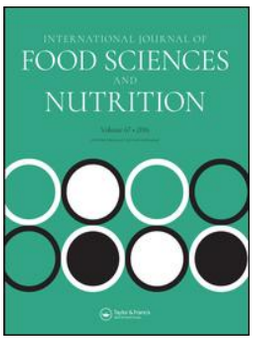

International Journal of Food Sciences and Nutrition

ISSN: 0963-7486 (Print) 1465-3478 (Online) Journal homepage: https://www.tandfonline.com/loi/iijf20

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To cite this article: Sanja Vlaisavljević, Mariel Colmán Martínez, Anamarija Stojanović, Miriam Martínez-Huélamo, Bjørn Grung & Rosa María Lamuela Raventós (2019): Characterisation of bioactive compounds and assessment of antioxidant activity of different traditional *Lycopersicum esculentum* L. varieties: chemometric analysis, International Journal of Food Sciences and Nutrition, DOI: 10.1080/09637486.2019.1587742

To link to this article: https://doi.org/10.1080/09637486.2019.1587742

Published online: 10 Apr 2019.

INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION
https://doi.org/10.1080/09637486.2019.1587742

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RESEARCH ARTICLE

Characterisation of bioactive compounds and assessment of antioxidant activity of different traditional *Lycopersicum esculentum* L. varieties: chemometric analysis

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ABSTRACT

Eight different Serbian genotypes were analysed for their polyphenol, carotenoid, vitamin C content and evaluated for their antioxidant properties. The highest content of biologically important carotenoids such as lutein (4.58 mg/10 g), lycopene (160.64 mg/10 g) and β-carotene (189.64 mg/10 g) were detected in the genotype S606. Rutin was the most abundant phenolic compound in all tastes samples, but its content is highest in the genotype S615 (1424.30 μg/100 g dw). All tomato samples were the great source of vitamin C, where the sample S615 stood out (68.54 mg AA g^{-1} of dw). Their content of antioxidant compounds suggested that genotypes S606 and S615 showed the best antioxidant potential. Principal component analysis (PCA) and Partial least squares (PLS) were applied to analyse results. The results obtained in the present study could be of considerable interest for breeding programmes wishing to select tomato genotypes with high biological and nutritional properties.

ARTICLE HISTORY
Received 19 December 2018
Revised 20 February 2019
Accepted 23 February 2019

KEYWORDS
Lycopersicum esculentum L.; genotype; polyphenols; carotenoids; antioxidant potential; chemometric

Introduction

The nutritional quality and many sensorial properties of fruits and vegetables are conditioned by their content of vitamins, minerals, dietary fibre, carbohydrates, proteins and antioxidant phytochemicals, which have been associated with the prevention and decreased risk of different diseases (Villa-Rodriguez et al. 2015). The analysis of biomolecules in plant food, whose levels depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental) factors (Carbonell-Capella et al. 2014), is therefore very important for an assessment of both sensory qualities and health benefits for the consumer.

Although tomato (*Lycopersicum esculentum* L.) is a seasonal fruit, it can be found in markets and supermarkets throughout the year, because its production is carried out in greenhouses. It is one of the most consumed fruits in Europe, especially in the Mediterranean area, both fresh and in tomato-based products (Ilahy et al. 2011). Tomato contains a high amount of bioactive compounds, notably phenolics, carotenoids and other antioxidants (Raffo et al. 2006; Georgé et al. 2011), the content depending on the variety, growing conditions, ripeness stage, among other factors (Akbudak et al. 2009; Svanberg and Kamal-Eldin 2009; Domínguez et al. 2012). Tomato fruit ripening is a complex process characterised by various physiological, biochemical and molecular transformations, which determine the nutritional quality and antioxidant potential at each stage.

Consumers tend to choose tomatoes on the basis of their visual and functional properties, and may consider fruit with an attractive appearance to be healthier. In the commercialisation of tomato production, the most profitable and high-yielding cultivars and hybrids are given predominance, while other cultivars, which might have a higher nutritional value, and be an important source of genes for breeding, are disregarded. In order to increase the nutritional value and the content of bioactive compounds, today a range of

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cultivars and varieties are being produced. There are several studies related to the phytochemical analysis and antioxidant properties of tomato from different origins (Kotíková et al. 2011; García-Valverde et al. 2013; Kaur et al. 2013; Martins and de Rosso 2016), but to date no reports have been published on Serbian varieties.

In Serbia there is a strong consumer demand for traditional fruits and vegetables. The aim of Serbian farmers and breeders is to obtain cultivars with a high content of nutrients and bioactive compounds, and provide food that is both tasty and healthy. For this study, eight tomato cultivars with different colour, morphology and sensorial characteristics that are attractive for consumers were selected. The phytochemical profile as well as antioxidant properties of these eight Serbian tomato varieties was determined for the first time.

Materials and methods

Tomato samples

For the purposes of existing breeding programmes and the preservation of traditional cultivars, the Institute of Field and Vegetable Crops in Novi Sad, Serbia (IFVCNS) has established a collection of over 400 different tomato accessions. Eight accessions significantly different in morphological and organoleptic characteristics were chosen for the quantification of polyphenols and carotenoids, determination of vitamin C content as well as evaluation of antioxidant capacity (Table 1 and Figure 1). The trial was conducted in Rimski Šančevi (45°39′58.02″N 19°04′51.16″E), Serbia in 2015. Tomatoes at the fruit maturity stage were hand-harvested, from an average of 10 plants of each of the eight genotypes.

Preparation of plant material

Sampled fruits of each genotype were cut into small pieces and sequentially homogenised in a domestic blender for 2 min. The homogenised fruits were introduced into jars and then lyophilised (Christ Alpha 1-2 LD Freeze Dryer, Switzerland) for 48 h at ice condenser temperature −55 °C. The samples were kept at room temperature in a dark and dry place.

Extraction

Tomatoes were extracted by liquid-liquid extraction for the analysis of both polyphenols and carotenoids, and analysed by UHPLC-MS/MS in the case of polyphenols (Di Lecce et al. 2013) and HPLC-UV for carotenoids (Colmán-Martínez et al. 2016). Extractions were performed in triplicate and quantified with the corresponding commercial standards. When standards were not available, the compounds were quantified based on the free form of the corresponding metabolite.

For evaluation of antioxidant activity and determination of total phenol and vitamin C content, 0.5 g of lyophilised tomato samples were weighed, homogenised with 5 mL of 80% ethanol (v/v) and then added to the flask and sonicated continuously for 15 min on an ultrasonicator. The extraction was repeated twice.

HPLC-UV separation of carotenoids

Chromatographic separation was carried out in an HP 1100HPLC system (Hewlett-Packard, Waldbronn, Germany), consisting of a quaternary pump and an auto-sampler coupled to a diode array detector DAD G1315B according to the methods by Colmán Martínez (2016). Twenty microlitres of the samples were injected in the HPLC-UV system.

UHPLC-MS/MS separation of polyphenols

The UHPLC analysis was performed using an Acquity UHPLC chromatograph equipped with a Waters binary pump system (Milford, MA) according to the method by Di Lecce et al. (2013).

The phenolic compounds present in tomatoes were detected and quantified by using the multiple reaction

Table 1. Morphological characteristics and description of eight Serbian tomato varieties.

Accession N°	Predominant fruit shape	Fruit size	Exterior colour of mature fruit	Intensity of exterior colour	Fruit blossom end shape	Taste	Uses
S 364	Plum-shaped	5–8 cm	Red	Dark	Indented	Not sweet	Processing
S 590	Slightly flattened	8–10 cm	Pink	Light	Flat	Sweet	Fresh/Processing
S 606	Plum-shaped	3–5 cm	Orange	Dark	Flat and pointed	Sweet	Fresh
S 607	Plum-shaped	3–5 cm	Red	Intermediate	Flat	Very sweet	Fresh
S 608	High rounded	<3 cm	Yellow	Intermediate	Flat	Very sweet	Fresh
S 612	Heart-shaped	>10 cm	Pink	Light	Pointed	Very sweet	Fresh
S 615	Rounded	8–10 cm	Yellow	Intermediate	Flat	Very sweet	Fresh
S 616	Long-oblong	5–8 cm	Red	Dark	Indented	Not sweet	Processing

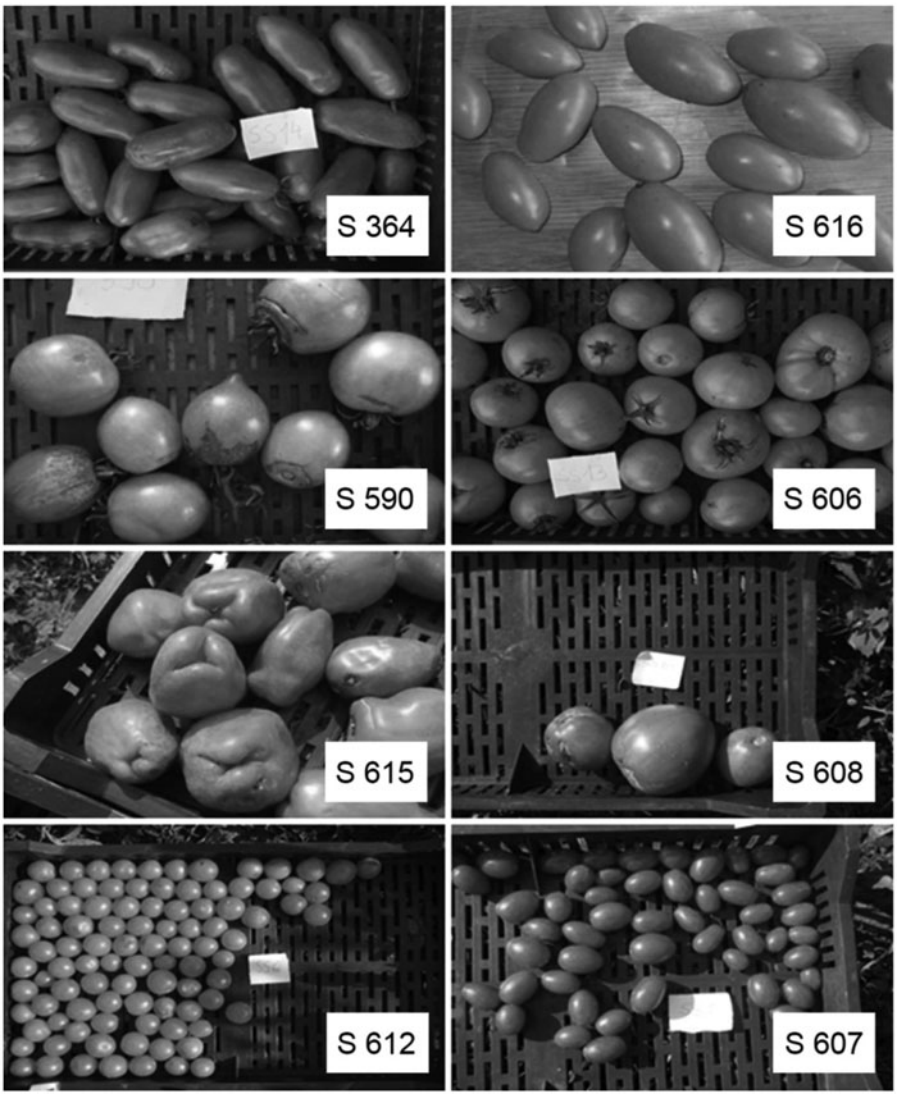

Figure 1. Morphological characteristics of eight Serbian tomato varieties.

monitoring mode (MRM), tracking the transition of the parent and product ion specific to each compound. The system was controlled by Analyst v.1.4.2 software supplied by Sciex (Foster City, CA).

Total phenol content

The total phenolic content was determined according to a previously reported method (Fukumoto and Mazza 2000), customised for 96-well microplates. The phenolic concentration was determined by comparison with the standard calibration curve of Gallic acid, and results were presented as a mean value of triplicate tests. The total phenol value was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dry weight (dw) calculated according to the standard calibration curve (linear regression).

Ascorbic acid (vitamin C) content

Ascorbic acid was determined according to the method by Klein and Perry (1982) modified for 96-well microplates. All measurements were performed in triplicate and the results were expressed as mg of ascorbic acid (AA) per g of dry weight (dw).

Reduction of the DPPH radical

Tomato extracts were tested for their scavenging effect on the DPPH radical according to the method by Vlaisavljević et al. (2018). Results were expressed as milligrams of Trolox equivalents (TE) per gram of dw of extract calculated according to the standard calibration curve.

ABTS assay

The ABTS assay was performed by a modified previously described procedure (Arnao et al. 2001). The results were expressed as Trolox equivalents per g of dw (TEAC/g dw).

Statistical analysis

For linear regression analysis, Origin software version 8.0 was used. All the results were expressed as the mean ± SD of three different trials. A comparison of the group means and the significance between the groups were verified by one-way ANOVA followed by Tukey's *post hoc* test. Statistical significance was set at $p < .05$.

Multivariate analysis was carried out using Sirius 9.0 (PRS AS, Bergen, Norway). Principal component analysis (PCA) (Joliffe 2002) and partial least squares (PLS) regression (Manne 1987) were used for exploratory analysis.

Results and discussion

Quantitative determination of carotenoids

Carotenoids protect plants against photooxidative processes, cell damage, and have an important role in the photosynthesis. They are responsible for the colour of many fruits and vegetables and their concentration depends on the activity of different enzymes of the pathway and also genetic expression (Cooperstone et al. 2016). Thus, the colour of tomato could be modified by regulation of carotenoids biosynthesis, cloning and genetic modifications (Hirschberg 2001). A greater content in carotenoids concentration could be obtained by the transgenic expression of the enzymes (Kachanovsky et al. 2012). They are used in industry as colourants such as human food and feed additives. Several epidemiological studies have provided evidence for the protective effect of carotenoids from tomato, such as a decreasing risk of different degenerative diseases and some types of cancer (Del Giudice et al. 2015; Marques et al. 2015). The carotenoids content of eight Serbian tomato varieties is shown in Table 2.

These data demonstrate that the concentration of carotenoids can vary considerably according to the genotype, which may affect their biosynthesis (Kaur et al. 2013).

The most abundant carotenoid in almost all the varieties of tomato samples was lycopene. Tomato is considered the best dietary source of lycopene, which

Table 2. Quantification of carotenoids compounds in eight Serbian tomato varieties.

	Content of carotenoids in eight tomato genotypes (mg/100g dw)							
Compound	S364	S616	S590	S606	S615	S608	S612	S607
Lutein	$^{\S}1.19 \pm 0.06^{e}$	1.71 ± 0.04^{c}	1.29 ± 0.02^{e}	4.58 ± 0.03^{a}	1.46 ± 0.04^{d}	1.07 ± 0.03^{f}	2.01 ± 0.02^{b}	0.86 ± 0.04^{g}
Zeaxanthin	nd	nd	nd	$0.13 \pm .2.01^{b}$	1.30 ± 1.13^{a}	nd	nd	nd
Trans-β-Apo-8'-carotenal	nd	nd	0.85 ± 3.03^{b}	7.20 ± 1.97^{a}	nd	nd	nd	nd
Criptoxanthin	nd	nd	1.36 ± 0.14^{b}	13.92 ± 1.74^{a}	1.37 ± 0.14^{b}	nd	nd	nd
15cis-β-Carotene	nd	4.88 ± 1.58^{b}	0.99 ± 0.12^{d}	nd	0.91 ± 0.08^{d}	nd	10.15 ± 0.32^{a}	1.57 ± 1.44^{c}
13 Cis-β-Carotene	nd	nd	1.68 ± 1.14^{c}	5.33 ± 0.22^{b}	nd	0.57 ± 2.23^{d}	11.36 ± 0.11^{a}	1.62 ± 0.01^{c}
α-Carotene	nd	7.79 ± 3.11^{a}	nd	nd	nd	nd	nd	nd
β-Carotene	nd	18.73 ± 0.04^{c}	8.26 ± 0.07^{d}	189.64 ± 0.48^{a}	4.56 ± 0.16^{d}	6.71 ± 0.21^{d}	88.17 ± 0.06^{b}	4.87 ± 0.22^{d}
9-Cis-β-Carotene	nd	5.58 ± 0.13^{d}	nd	8.56 ± 0.16^{b}	2.00 ± 0.15^{f}	4.74 ± 0.11^{e}	13.25 ± 0.16^{a}	7.05 ± 0.08^{c}
All-trans-Lycopene	nd	207.42 ± 0.32^{b}	95.20 ± 0.41^{d}	160.64 ± 0.22^{c}	1.08 ± 0.03^{f}	nd	339.53 ± 2.00^{a}	91.62 ± 0.30^{e}

nd: not detected; dw: dry weight.
[§]Data are means ± SD of three measurements ($n = 3$).
Values with different letters in the same row are significantly different ($p < .05$).

is one of the most beneficial carotenoids for human health (Agarwal and Rao 2000). Lycopene usually occurs in red coloured fruit. Interestingly, this was not the case with tomato samples tasted in this study. It is probably the result of some mutation occurred in genes responsible for synthesis of the enzymes involved in lycopene synthesis and phenotype of tomato. The highest level of lycopene was detected in the varieties S606, S616 and S612, which are of variable colour and shape. Lycopene was not found in S364 and S608.

Trans-lycopene was not detected in S364 and S608 samples. However the *cis* form was present in both of them, being the major compounds in the sample S608.

Numerous studies have reported that dietary intake of foods rich in lycopene results in a decreased incidence of certain cancers, including prostate, lung and colon cancers, as well as coronary heart diseases and macular degeneration (Dillingham 2009).

The health benefits of tomato are due to its antioxidant and anti-inflammatory activity, as well as the improvement of the plasma lipid profile, which are associated with the intake of lycopene, β-carotene and other carotenoids (Stahl and Sies 2005). Lycopene occurs in various geometrical configurations, being mainly all *trans* in tomato fruits. Thermal processing causes some loss of lycopene in tomato and tomato-based foods. Therefore, dehydrated tomatoes have poor lycopene stability (Gomez-Romero et al. 2010).

An isomer of lycopene, 5-Z-lycopene, was detected in all the tested samples, with the highest level in S606 and S615.The other dominant carotenoid detected was β-carotene, which is nutritionally important because of its provitamin A activity (Domínguez et al. 2012). In this study, concentrations of β-carotene in tomato (mg/100g dw) were in the range of 4.57–189.64. S606 and S612 contained the highest amount of this compound, and much lower in the other samples. The sample S612 had the highest content of the β-carotene isomers 15-Z-β-carotene and 13-Z-β-carotene. Interestingly, α-carotene was only detected in the "roller" tomato variety S606. Criptoxanthin was found only in S590, S606 and S615, with the highest content in S606 (13.92 mg/100g dw).

Lutein absorbs blue light, thus it appears as yellow pigment. It was found in all varieties, with the highest level detected in S606 (4.58 mg/100g dw) and similar amounts in the rest. As can be observed in Table 2, varieties S606, S616 and S612 had the highest level of total carotenoids, which is in accordance with their morphological and sensorial characteristics. The variety S616 is especially interesting because of its intense orange colour with an unusual elongated shape. Therefore, it could be very attractive for both growers selecting high nutrient cultivars, and for consumers interested in a healthy diet. Further, it can be observed that the colour of the tomato may not be affected by the content of carotenoids. This could be result of some mutation in genes that encode synthesis of enzymes which may become disrupted in early biosynthetic stage. This mutation was probably occurred in genes that direct the accumulation of the lycopene in tomato samples (Kachanovsky et al. 2012). The values of the most important carotenoids found in the present study were difficult to be compared with those reported on cultivars from different origin such as Taiwan (Chang et al. 2006), Tunisia (Ilahy et al. 2011), India (Siddiqui et al. 2014) and Spain (García-Valverde et al. 2013), due the data in reported studies were expressed on a fresh weight (FW) basis.

Quantification of selected phenols in tomato samples

Dietary phenols are potentially beneficial for health because they may protect the body against major degenerative diseases, aging and some cancers (Neagu et al. 2015). Phenolic content in fruits and vegetables depends primarily on genetic control, as well as environmental factors (Barros et al. 2012). All the samples tested in the present study were found to be a good source of flavonoids. Predominant phenolic compounds were selected to investigate the differences between the eight varieties of tomato, as shown in Table 3.

The differences in content of hydroxycinnamoylquinic acid derivatives and flavonoids and their derivatives were quite pronounced among the eight varieties. Rutin was the most abundant polyphenol in almost all samples, ranging from 359.98 to 1424.30 µg/100g dw, with the highest level detected in S615. All the varieties are a good source of phenolic acids (µg/100g dw) and the major acids determined were caffeic acid, ranging from 57.63 to 688.54 µg/100g dw, followed by chlorogenic acid, *p*-coumaric acid and ferulic acid. The other phenolic acid derivatives were detected in similar amounts to phenolic acids in all the samples. Protocatechuic acid was found in lower levels and was similar in all the varieties. The samples with the highest levels of individual phenolic acids were S615, S590 and S364.

Table 3. Quantification of individual phenolic compounds in eight Serbian tomato varieties.

	§Content of selected phenolics (μg/100g dw)							
Compound	S 364	S 616	S 590	S 606	S 615	S 608	S 612	S 607
Caffeic acid	482.90 ± 0.1^{b}	315.36 ± 0.36^{e}	339.07 ± 0.59^{c}	329.74 ± 0.25^{d}	688.54 ± 6.31^{a}	57.63 ± 0.33^{h}	166.09 ± 0.61^{g}	215.07 ± 0.80^{f}
Caffeic acid hexoside I	15.18 ± 0.36^{f}	32.05 ± 0.09^{d}	69.41±.0.10^{b}	30.99 ± 0.06^{e}	71.67 ± 0.012^{a}	n.d.*	6.78 ± 0.12^{g}	40.68 ± 0.01^{c}
Chlorogenic acid	114.44 ± 0.43^{c}	94.79 ± 0.01^{f}	562.06 ± 1.13^{a}	97.47 ± 0.230.11^{e}	140.59 ± 0.62^{b}	57.90 ± 0.12^{g}	48.81 ± 0.03^{h}	110.80 ± 0.05^{d}
Coumaric acid hexoside I	34.24 ± 0.22^{b}	24.46 ± 0.29^{c}	34.44 ± 0.03^{b}	20.44 ± 0.30^{d}	61.44 ± 0.31^{a}	n.d.	13.03 ± 0.09^{e}	5.37 ± 0.24^{f}
Coumaric acid hexoside II	96.62 ± 0.39^{b}	70.55 ± 0.26^{c}	96.75 ± 0.15^{b}	69.41 ± 0.35^{d}	103.99 ± 0.58^{a}	22.18 ± 0.50^{g}	55.28 ± 0.50^{e}	52.93 ± 0.81^{f}
Criptochlorogenic acid	59.46 ± 0.37^{d}	142.58 ± 0.35^{a}	56.48 ± 0.26^{e}	115.48 ± 0.16^{c}	119.55 ± 0.23^{b}	10.22 ± 0.14^{g}	45.44 ± 0.10^{f}	46.13 ± 0.30^{f}
Dicaffeoylquinic acid	25.64 ± 0.40^{d}	16.66 ± 0.22^{f}	36.60 ± 0.31^{b}	21.95 ± 0.08^{e}	62.30 ± 0.32^{a}	5.52 ± 0.24^{h}	10.83 ± 0.15^{g}	26.69 ± 0.22^{c}
Ferulic acid	292.26 ± 0.37^{c}	269.18 ± 0.50^{f}	391.21 ± 0.16^{a}	276.93 ± 0.36^{e}	326.32 ± 0.62^{b}	54.38 ± 0.10^{h}	287.74 ± 1.53^{d}	129.07 ± 0.25^{g}
Ferulic acid hexoside	226.32 ± 0.35^{f}	592.67 ± 0.27^{a}	565.22 ± 2.04^{b}	498.23 ± 0.89^{c}	253.28 ± 0.38^{e}	282.58 ± 0.73^{d}	155.86 ± 0.20^{h}	223.66 ± 20^{g}
Naringenin	10.19 ± 0.18^{f}	49.85 ± 0.26^{e}	8.95 ± 0.13^{g}	60.46 ± 0.17^{d}	153.54 ± 0.23^{c}	182.17 ± 0.31^{a}	7.49 ± 0.29^{h}	154.49 ± 0.27^{b}
Naringenin glucoside	5.83 ± 0.07^{h}	47.95 ± 0.10^{e}	6.82 ± 0.21^{f}	54.19 ± 0.43^{d}	116.89 ± 0.11^{c}	284.23 ± 0.17^{a}	2.70 ± 0.28^{g}	134.91 ± 0.10^{b}
Neochlorogenic acid	5.84 ± 0.9^{c}	n.d.	5.92 ± 0.07^{c}	3.49 ± 0.06^{d}	6.41 ± 0.20^{b}	1.42 ± 0.07^{e}	n.d	7.26 ± 0.17^{a}
p-Coumaric acid	646.50 ± 2.05^{a}	187.87 ± 0.14^{d}	74.04 ± 1.15^{f}	188.01 ± 0.08^{d}	464.25 ± 0.16^{b}	73.70 ± 0.39^{f}	255.21 ± 0.15^{c}	115.38 ± 0.23^{e}
Protocatechuic acid	16.60 ± 0.32^{a}	11.16 ± 0.08^{d}	15.27 ± 0.06^{b}	10.76 ± 0.12^{e}	7.54 ± 0.33^{f}	2.38 ± 0.22^{h}	13.08 ± 0.09^{c}	3.70 ± 0.21^{g}
Quercetin	29.41 ± 0.21^{d}	22.30 ± 0.15^{f}	31.16 ± 0.19^{c}	24.80 ± 0.10	30.96 ± 0.02^{c}	37.40 ± 0.07^{b}	65.06 ± 0.10^{a}	28.39 ± 0.40^{e}
Rutin	619.36 ± 0.4^{f}	751.46 ± 0.24^{d}	359.98 ± 0.58^{g}	861.34 ± 0.34^{b}	1424.30 ± 0.72^{a}	766.28 ± 0.13^{c}	183.44 ± 0.10^{h}	718.13 ± 0.20^{e}

nd: not detected; dw: dry weight.
§Data are means ± SD of three measurements ($n = 3$).
Values with different letters (a, b, c, d, e, f, g, h) in the same row are significantly different ($p < .05$).

Table 4. Total phenolics and vitamin C content and antioxidant capacity of eight Serbian tomato varieties.

	S364	S616	S590	S606	S615	S608	S612	S607
Total phenolics (mg GAEg^{-1} of dw)	¥7.18 ± 1.56^{c}	10.02 ± 0.97^{c}	6.30 ± 0.48^{c}	14.73 ± 1.53^{b}	36.19 ± 5.35^{a}	11.80 ± 1.30^{c}	4.91 ± 2.36^{c}	9.80 ± 0.84^{c}
Vitamin C (mg AA g^{-1} of dw)	14.45 ± 0.37^{e}	21.81 ± 0.58^{d}	31.85 ± 0.79^{c}	68.54 ± 2.18^{a}	43.40 ± 0.36^{b}	29.44 ± 0.38^{c}	1.52 ± 0.71^{f}	20.52 ± 0.42^{d}
DPPH$^{\bullet}$ (mg TEAC g^{-1}of dw)	9.00 ± 0.01^{c}	5.78 ± 0.22^{e}	2.66 ± 0.18^{f}	48.57 ± 0.50^{a}	15.82 ± 0.29^{b}	6.08 ± 0.073^{e}	7.55 ± 0.12^{d}	5.17 ± 0.04^{e}
ABTS$^{\bullet}$ (mg TEAC g^{-1}of dw)	15.13 ± 1.36^{c}	8.50 ± 0.92^{f}	5.97 ± 2.11^{h}	126.52 ± 0.47^{a}	23.41 ± 1.88^{b}	11.02 ± 2.01^{e}	14.11 ± 0.75^{d}	6.55 ± 0.21^{g}
Reducing power (mg EAA g^{-1}of dw)	17.82 ± 0.11^{f}	20.39 ± 1.07^{c}	17.16 ± 0.53^{f}	84.37 ± 0.21^{a}	23.87 ± 0.11^{b}	22.91 ± 0.03^{b}	10.42 ± 0.04^{g}	19.10 ± 0.02^{e}

¥Data are means ± of three measurements.
In each row different letters indicate mean significant differences ($p < .05$).
dw: dry weight; GAE: gallic acid equivalent; EAA: equivalents of ascorbic acid.

The greatest content of the flavanone naringenin was found in sample S608 (182.17 µg/100 g dw). Similar levels of naringenin glucoside were also detected. The content of the flavonol quercetin was markedly higher in S612 (65.06 µg/100g dw) in comparison with the other samples such as those reported by Kaur et al. (2013).

It is difficult to compare the results of polyphenols quantification in this study with those reported in the literature, considering that many factors can affect the phenolic content including genetic variability, country of origin, environmental conditions and storage methods. Most of the studies indicate that the phenolic acids and their derivatives, and the flavonoid rutin are the most abundant polyphenols in tomato. The content of phenolic acids and flavonoids determined here was higher than those reported in other studies (Barros et al. 2012; García-Valverde et al. 2013). Also, some authors have reported higher levels of polyphenols compared with this study (Gomez-Romero et al. 2010; Vallverdú-Queralt et al. 2011; Meng et al. 2012).

Total phenolic content

A high total phenolic content was observed in all samples (mg GAE/g dw), but the highest level was found in S615 (36.19) (Table 4). The values found in the present study are higher than those previously reported by Chang and Liu (2007), Ilahy et al. (2011), Dávila-Aviña et al. (2014) and García-Valverde et al. (2013), but some values are very similar to those obtained by Li et al. (2012). These results were in accordance with the phenolics contents quantified by UPLC-MS/MS technique, which were highest in S606, S615 and S608.

Vitamin C content and antioxidative activity

As a strong antioxidant, ascorbic acid is one of the most important bioactive molecules in tomato fruits, playing an important role in disease prevention (Kaur et al. 2013). The content of ascorbic acid in the samples (1.52–68.54 mg AA/g dw) was similar to previously reported results (2.20–85.00 mg AA/100 g dw)

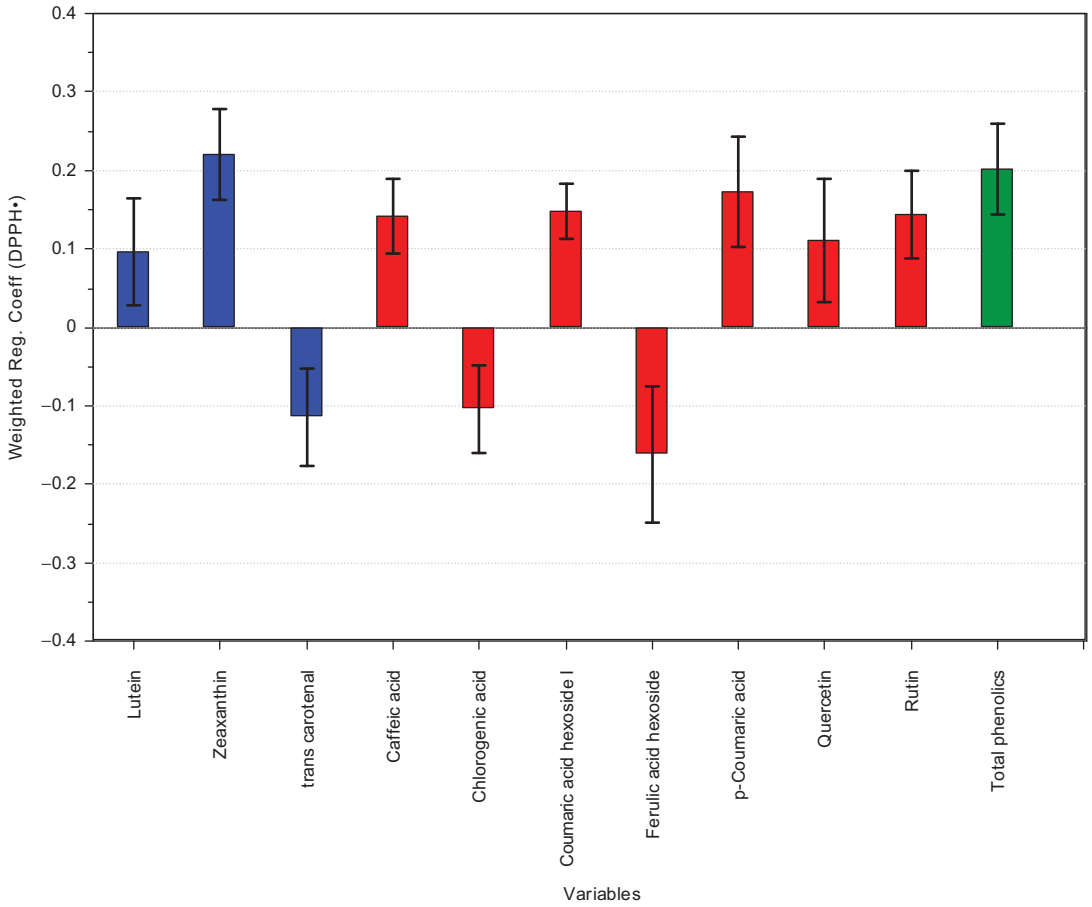

Figure 2. Bar graph of the regression coefficients for the DPPH model.

(Pinela et al. 2012; García-Valverde et al. 2013; Kotíková et al. 2011; Toor and Savage 2005). All the samples tested in the present study are a rich source of vitamin C, especially S606, which may be useful data for the promotion of these fruit products in terms of nutrition (Table 4).

In accordance with the data presented earlier, the tomato varieties showed a high antioxidant potential measured by radical scavenging capacity against DPPH and ABTS radicals as well as reducing power (FRAP).The samples with the highest antioxidant capacity were S606 and S615, which could be linked with the higher content of carotenoids, flavonoids and vitamin C found in these varieties. These bioactive compounds, which may act independently or synergistically, are responsible for the health benefits of tomato fruits.

Multivariate analysis

The data in Tables 2–4 were collected in a data matrix subjected to multivariate analysis. Each row represented a tomato, and each variable corresponded to the content of the various compounds in each tomato. The analysis was done to highlight similarities and differences among the tomatoes taking into account simultaneously all the variables measured. Also, this strategy illuminates correlation patterns among the variables. As can be seen in Tables 2 and 3, the contents of the various phenolics and carotenoids, differ to a large extent. The individual variables were thus standardised (divided by their standard deviation) prior to the multivariate analysis.

Initial analysis flagged S606 as a potential outlier. This tomato deviates to such a large extent from the other tomatoes investigated that its inclusion in the multivariate analysis would mask any systematic trends among the other tomatoes. It was therefore excluded from any further analysis.

Three separate PLS regression models were built to try to predict the three responses related to antioxidative properties (ABTS, DPPH and reduction power). The models were pruned until all variables having insignificant regression coefficients were deleted. Bar graphs of the regression coefficients for the three models are shown in Figures 2–4. Upwards pointing bars indicate a positive influence, whereas downwards pointing indicates a negative influence.

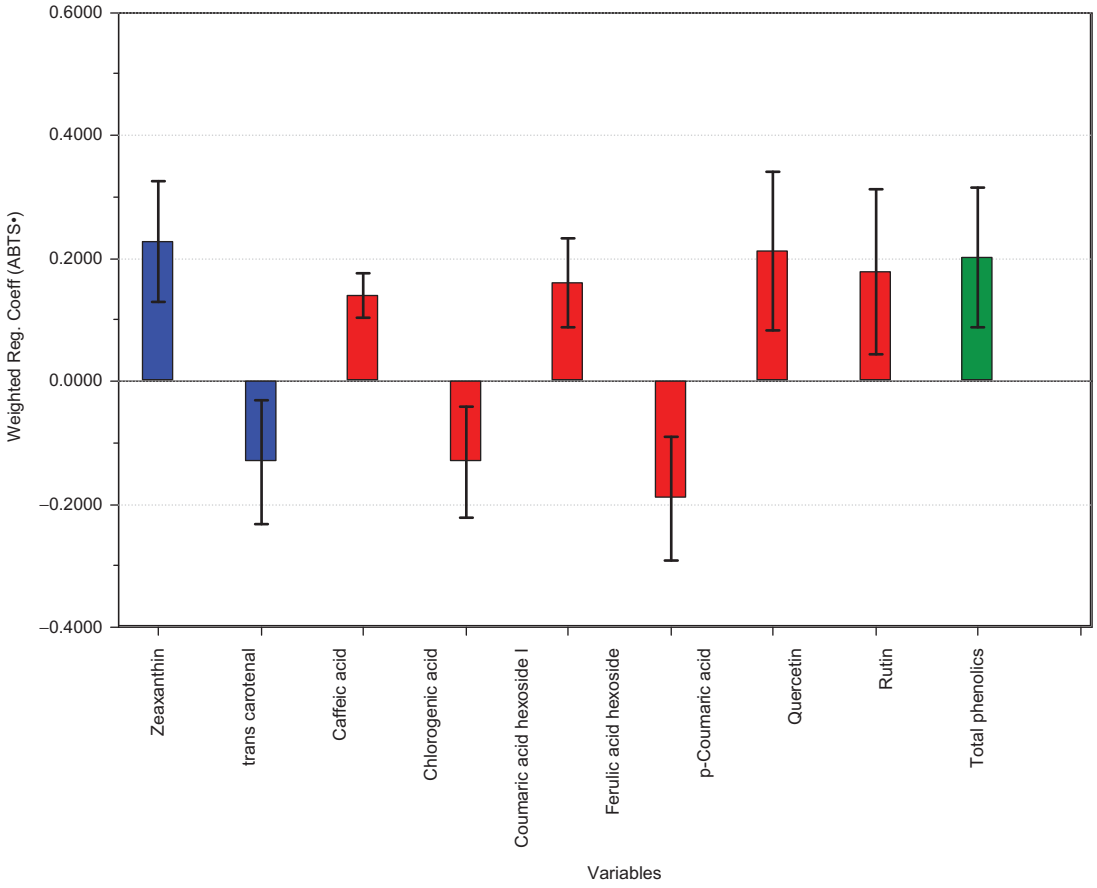

Figure 3. Bar graph of the regression coefficients for the ABTS.

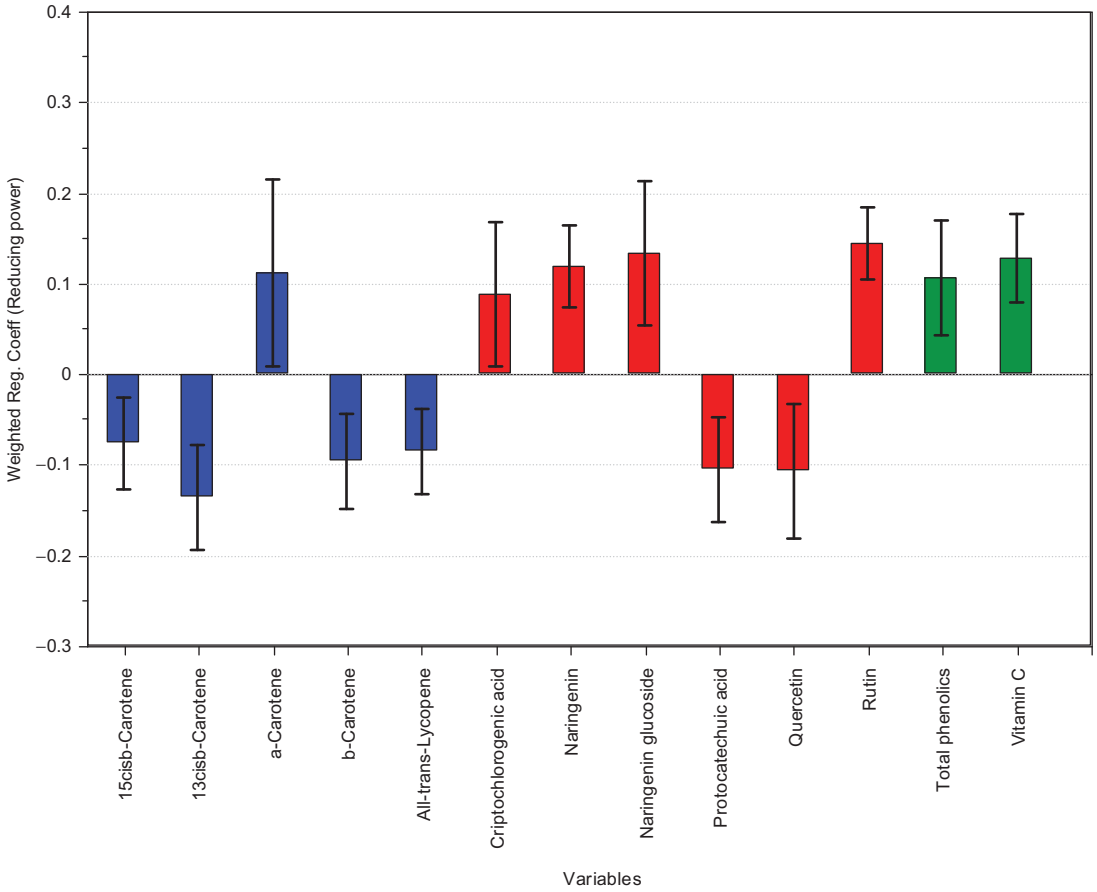

Figure 4. Bar graph of the regression coefficients for the reducing power model.

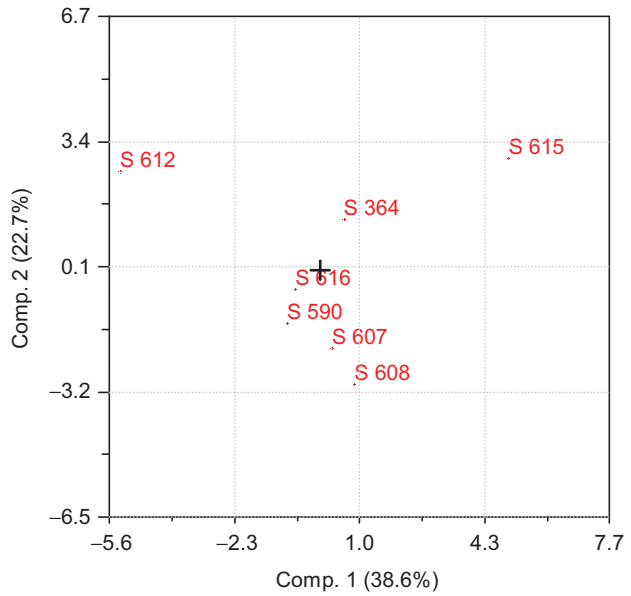

Figure 5. Scoreplot after PCA on a matrix consisting of the variables identified in the PLS analysis.

For the DPPH model (Figure 2), the important variables are lutein, zeaxanthin, trans-carotenal, caffeic acid, chlorogenic acid, coumaric acid hexoside I, ferulic acid hexoside, p-coumaric acid, quercetin, rutin and total phenolics. The ABTS model (Figure 3) contains nine of these 11 variables. The ones missing are lutein and rutin. This correspondence strongly indicates that these two tests effectively measure the same underlying feature. The reduction power model (Figure 4) is more complex than the two previous ones. The important variables seen in Figure 4 are from left to right: 15-cis-β-carotene, 13-cis-β-carotene, α-carotene, β-carotene, All-trans-lycopene, 5-cis-lycopene, chlorogenic acid, naringenin, naringenin glucoside, protocatechuic acid, quercetin, rutin, total phenolics and vitamin C.

A final data matrix was created by taking all the variables with significant regression coefficients from the analysis above, and subjecting this matrix to PCA. Figures 5 and6 show the resulting score plot and loading plot. The loading plot (Figure 6) indicates that variables to the right are responsible for high reducing power, as well as a high result for the DPPH and ABTS tests. Again, the plot shows that DPPH and ABTS more or less measure the same underlying phenomenon. The loading plots explains the positioning of the tomatoes in the score plot (Figure 5). This means that S615 have significantly better properties

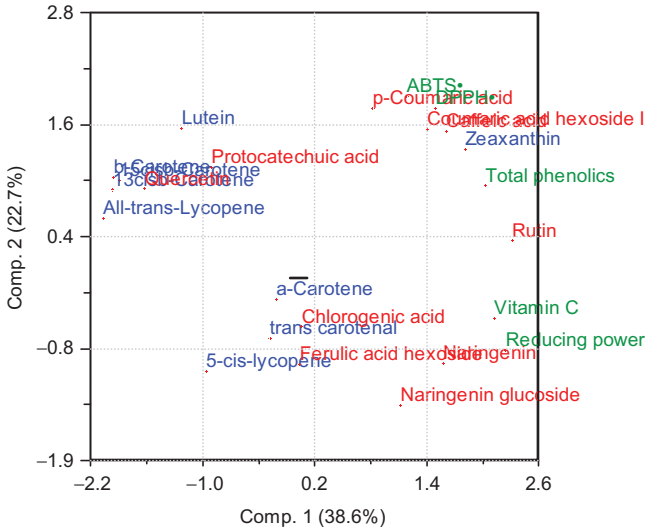

Figure 6. Loading plot after PCA on a matrix consisting of the variables identified in the PLS analysis.

than S612. The remaining five tomatoes lie somewhere between these two extremes. It is also worth noting that in general, the phenolics seem much more important with regards to antioxidative behaviour compared to the carotenoids. The plots also show the importance of vitamin C when it comes to reducing power.

Conclusions

Antioxidant content and activity varied significantly among samples. The varieties S606, S615 and S608 are of particular interest for tomato breeding, as their genotypes could be a source of increased polyphenol, carotenoid and vitamin C content. Although with completely different morphological and organoleptic characteristics, these three varieties showed similarities in the levels of bioactive molecules and therefore antioxidant potential. Interesting data were obtained for S364, since only two of twelve carotenoids were quantified in this sample, yet it showed a good antioxidant capacity, due to a high level of polyphenols and vitamin C. Overall, the results obtained in the present study can serve as the basis for increasing the breeding, cultivation and marketing of nutritionally superior varieties of tomato as a healthy alternative for consumers worldwide, not only in Serbia.

Acknowledgements

This work was benefited from the networking activities within the European funded COST Action FA1106 QualityFruit.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Serbia [Grant No. 172058] and in part by CICYT [AGL2013-49083-C3-1-R] and the Instituto de Salud Carlos III, ISCIII (CIBEROBN) from the Ministerio de Economía y Competitividad (MEC) and Generalitat de Catalunya (GC) 2014 SGR 773.

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