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The contribution of lipophilic, hydrophilic and dietary fibre fractions to total antioxidant activity of tomato waste

Die Beteiligung von lipophilen, hydrophilen und Nahrungsfaser-Fraktionen an der antioxidativen Aktivität von Tomatenreststoffen

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Summary In this study waste of five tomato genotypes was used to prepare lipophilic, hydrophilic and dietary fibre fractions. Depending on the tomato genotype, waste contained different quantities of insoluble dietary fibre (23.67–36.20 g/100 g DW) and soluble dietary fibre (0.07–7.39 g/100 g DW). Total antioxidant activity of tomato waste, presented as Trolox equivalent antioxidant capacity (TEAC), ranged from 1143.58 µmol TEAC/100 g DW for the Rutgers waste to 1822.83 µmol TEAC/100 g DW for the Knjaz waste. Our results showed that tomato waste is a rich source of bioactive compounds and may be used as a functional food ingredient.

> **Keywords:** tomato pomace, tomato fractions, dietary fibre content, antioxidant capacity

Zusammenfassung In dieser Studie wurden die Reststoffe von fünf verschiedenen Tomaten-Genotypen verwendet, um lipophile, hydrophile und Nahrungsfasern zu gewinnen. Abhängig vom Genotyp der Tomaten enthielten die Reststoffe unterschiedliche Mengen an unlöslichen und löslichen Nahrungsfaser-Fraktionen (23.67–36.20 g/100 g TG und 0.07–7.39 g/100 g TG). Die gesamte antioxidative Aktivität des Tomatenreststoffe, als Trolox Equivalent Antioxidative Capacity (TEAC) dargestellt, betrug von 1,143.58 µmol TEAC/100 g TG (Rutgers-Genotyp) bis 1,822.83 µmol TEAC/100 g TG (Knjaz-Genotyp). Die Ergebnisse zeigen, dass Tomatenreststoffe eine reiche Quelle an bioaktiven Stoffen beinhalten und als funktionelle Zutat in Lebensmittel verwendet werden können.

> **Schlüsselwörter:** Tomatenreststoffe, Tomatenfraktionen, Nahrungsfasern, antioxidative Kapazität

Introduction

The food industry produces a huge amount of waste and by-products and most of them are disposed or used on a low technological and economical level. Nowadays, food processing waste valorisation has become important issue in food industry and modern life because waste is promising source of valuable compounds such as dietary fibres, essential fatty acids, antioxidants, antimicrobials, and minerals. These substances may be used because of their favourable technological, nutritional and functional properties (Laufenberg et al., 2003; Schieber et al., 2001; Tangirala et al., 2012).

The dietary fibre and bioactive compounds are widely used as functional ingredients in processed foods. There is a trend to find new sources of functional components that have traditionally been undervalued (Rodríguez et al., 2006). Numerous studies have pointed out that fruit and vegetable waste materials could be promising sources of dietary fibres and functional compounds (Esposito et al., 2005; García Herrera et al., 2010; Grigelmo-Miguel and Martin-Belloso, 1999). Therefore, the composition, functional properties, and physiological effects of many waste materials have been investigated with the aim to explore their potential applications (García Herrera et al., 2010).

Tomato (*Lycopersicon esculentum* Mill., Solanaceae) is one of the most popular and widely consumed vegetables all over the world. Besides being consumed fresh, the tomato can be processed into many products, such as ketchup, sauces, pasta dishes and juices (Navarro-González et al., 2011). Tomato, both in the fresh and processed forms, possess a high nutritional value due to its content of carotenoids, polyphenolics, dietary fibres, vitamins C and E, etc. (Capanoglu et al., 2008; Ilahy et al., 2011; Slimestad and Verheul, 2009). Carotenoids are essential for evaluating the nutritional quality of tomato, given their antioxidant and antiproliferative activity (Kotake-Nara et al., 2001; Shi and Qu, 2004). Lycopene is the major carotenoid present in tomato, accounting for >80 % of the total tomato carotenoids in fully red-ripe fruits, where it is responsible for their characteristic colour (Lenucci et al., 2006). Tomato contains a range of polyphenols, such as flavonoids (quercetin, kaempferol, naringenin and their derivatives) and phenolic acids (caffeic, chlorogenic, *p*-coumaric and ferulic acid) (Slimestad and Verheul, 2009). Polyphenols are one of the phytochemicals whose 'protective' properties include antioxidant, antimicrobial, anticancer and cardiovascular-protective activities (Bendini et al., 2006; Hertog et al., 1995). Also, tomato represents a relevant dietary source of soluble and insoluble dietary fibres, constituted by pectins, hemicelluloses and cellulose (Claye et al., 1996; Frusciante et al., 2007). The main physiological effect of insoluble fibre is the improvement of gut peristalsis, while soluble fibre represents a good substrate for some lactic bacteria and Bifidobacteria strains (prebiotic action), it is also able to control glycemic index, and it reduces plasmatic cholesterol (Esposito et al., 2005).

A significant amount of waste (10–30 % of their weight), consisting of skin, seeds and a part of the pulp, is generated during the industrial processing of tomato (Rahmatnejad et al., 2009). The by-product that remains after processing can still contain considerable amount of nutrients and phytochemicals. Depending on tomato product and step during industrial processing of tomato, formed tomato waste differs in chemical composition (Knoblich et al., 2005; Del Valle et al., 2006). In addition to dietary fibres, which are the main compounds $({\sim}60\%)$ of tomato waste, carotenoids and phenols are present in significant quantities (Del Valle et al., 2006; Peschel et al., 2006). In the study of Knoblich et al. (2005), the content of carotenoids in peel and seed byproducts of commercial tomato canning were determined. The lycopene content of peel by-product was about 5 times higher than in seed by-product. In addition to lycopene, other carotenoids including lutein, ß-carotene, and cis-ßcarotene were present in significant amounts. Other carotenoids were approximately two to three times more concentrated in the peel by-product than in the seed byproduct. Peschel et al. (2006) determined the total phenolic content in five different solvent extracts of tomato waste from canning factory; the highest total phenolic content was reported in ethanolic and acetone extracts, 42.00 and 49.61 mgGAE/g dry extract, respectively. According to the fact that tomato waste consist of lot of nutrients, it can be a promising source for food supplementation. Previously was reported that incorporation of tomato waste into foods could improve the beneficial or functional properties of food products such as pasta and snacks (Karthika et al., 2016; Padalino et al., 2017).

This paper deals with characterisation of tomato waste obtained during juice processing from different tomato genotypes (Bačka, Knjaz, Novosadski niski, Rutgers and Saint Pierre) in terms of total antioxidant activity. So the aim of our research was: (a) to determine total, soluble and insoluble dietary fibre content of insoluble residues (IR), (b) to evaluate their antioxidant activity by DPPH test and (c) to investigate contribution of lipophilic, hydrophilic and dietary fibre fractions to the total antioxidant activity of tomato wastes.

Materials and methods

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) and trolox (6 hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97 %) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals and solvents were of the highest analytical grade.

Waste preparation

Tomato genotypes (Bačka, Knjaz, Novosadski niski, Rutgers and Saint Pierre) grown in the fields of the Institute of Field and Vegetable Crops, Novi Sad, Serbia were taken for testing. The dried tomato waste of different genotypes was prepared as reported in our previous paper (Ćetković et al., 2012).

Preparation of tomato waste fractions

Sample of dried tomato waste (10 g) was successively extracted with hexane to obtain lipophilic fractions (LF), then extracted with 80% ethanol to obtain hydrophilic fractions (HF). The extraction of each sample was performed in triplicate as reported previously (Cetkovic´ et al., 2012; Savatović et al., 2012; Stajčić et al., 2015).

The insoluble residue (IR) after extractions, contained dietary fibre, were dried at temperature below 50 °C. The weights of IR were: Bačka, $m = 5.18 \pm 0.25$ g; Knjaz, $m =$ 5.39 ± 0.08 g; Novosadski niski, m = 5.00 ± 0.24 g; Rutgers, m = 4.31 ± 0.15 g; Saint Pierre, m = 3.78 ± 0.18 g.

Dietary fibre content

The content of dietary fibers in IR was determined according to AOAC Method 991.43 (AOAC, 2007) and AACC Method 32-07 (AACC, 2000), expressed in g per 100 g DW of tomato waste. Briefly, the dried samples (duplicate) were subjected to sequential enzymatic digestion for determination of total as well as for determination of insoluble dietary fibre. For determination of total dietary fibre (TDF), fibre was precipitated with ethanol and residue was then filtered, dried, and weighed. For determination of insoluble dietary fibre (IDF), IDF was filtered, and then the residue was washed with warm distilled water, dried and weighed. One residue from each type of fibre was analysed for protein, and the second residue of the duplicate was analysed for ash. Both TDF and IDF residues were corrected for protein and ash for the final calculation of TDF and IDF values. The soluble dietary fibre (SDF) was determined as the difference between TDF and IDF (Segura-Campos et al., 2014). The analysis was done using the Megazyme TDFR 06/01 Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland).

Antioxidant activity of dietary fibre fractions

The antioxidant activity of dietary fibre fractions was determined spectrophotometrically using the DPPH method of Serpen et al. (2007), slightly modified. The decrease in the absorbance was determined at 515 nm. Briefly, a 0.5 ml of solution containing from 1 to 20 mg of dietary fibre fractions in distilled water or 0.5 ml of distilled water (control) were mixed with 1.5 ml of 90 µM DPPH solution and 3 ml of methanol. The mixture was vortexed for 3 min at 0, 15, and 25 min to facilitate the surface reaction between the insoluble matter and the DPPH reagent. Following centrifugation at 1209.6 g for 2 min, the absorbance of the optically clear supernatant was measured at 515 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan) against blank that had been prepared in a similar manner as control, by replacing the DPPH solution with methanol. All measurements were performed at exactly 30 min after mixing the dietary fibre fractions with the DPPH reagent.

The DPPH radical scavenging activity (SA_{DPPH}) was calculated using the following equation:

$$
SA_{\text{DPPH}}\left(\% \right) = 100 \times (A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}} \tag{1}
$$

where A_{Control} is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of the extract.

The inhibitory concentration (IC_{50}) , defined as the concentration of extract required for 50 % scavenging of DPPH radicals under experimental conditions employed, was used to measure the free radical scavenging activity (Cuvelier et al., 1992).

Statistical analysis

The results of determination of the antioxidant activity and dietary fibres in tomato waste fractions were carried out in triplicate, and presented as mean \pm SD. IC₅₀ values were calculated using Microsoft Office Excel 2010. A comparison of the group means and the significance between the groups were verified by one-way ANOVA using OriginPro 8 SRO (OriginLab Corporation, Northampton, USA). Statistical significance was set at $p < 0.05$.

Results and discussion

In this paper, the waste obtained from old (Rutgers and Saint Pierre) and new (Bačka, Knjaz and Novosadski niski) tomato genotypes were examined. According to previously reported principal component analysis (Glogovac et al., 2012; Gvozdenović-Varga et al., 2016), selected tomato genotypes had a high content of dry matter. Tomatoes with high dry matter content are highly desirable in processing as they significantly increase the quality of the processed product (De Pascale et al., 2001). Determined dry matter values ranged from 5.67 % for Bačka to 7.63 % for Novosadski niski genotype. Selected tomato genotypes were very divergent in lycopene content since it ranged from 62.78 to 1135.9 mg/100 g DW. The highest amount of lycopene was established for Rutgers genotype, while the least amount of lycopene was measured for Novosadski niski genotype. Varieties rich in lycopene are very important for industrial processing and for fresh consumption as well, since consumers are demanding food with high nutraceutical values. It is known that high content of ascorbic acid in tomato fruit determinates its high biological value. However, ascorbic acid content is highly unstable and varies greatly depending on environmental conditions. In the selected tomato genotypes, ascorbic acid content ranged from 557.5 for Bačka genotype to 954.1 mg/100 g DW for Rutgers genotype.

Selected tomato genotypes, Bačka, Knjaz, Novosadski niski, Rutgers and Saint Pierre were used to prepare tomato waste, a by-product in juice processing. All of these were used to produce lipophilic (LF), hydrophilic (HF) and insoluble residue (IR) fractions. The yields of tomato waste fractions are shown in Table 1.

The highest yields of LF and HF were obtained from the Rutgers and Saint Pierre tomato waste, amounting to 8.29 % and 44.35 %, respectively. IR of the Knjaz tomato waste was obtained in the highest yield (53.92 %).

Carotenoids, lycopene and ß-carotene, were present in LF, as reported previously in our study (Stajčić et al., 2015). The content of lycopene in waste of investigated tomato genotypes ranged from 13.40 mg/100 g DW for the Knjaz waste to 81.54 mg/100 g DW for the Rutgers waste, while the content of ß-carotene was slightly lower and varied from 8.64 mg/100 g DW for the Knjaz waste to 50.14 mg/100 g DW for the Rutgers waste. In the study of Knoblich et al. (2005), amounts of lycopene and ß-carotene in tomato skin alone were 73.4 and 2.93 mg/100 g, while lycopene and ßcarotene in seed were 13.0 and 1.44 mg/100 g of dry byproduct, respectively. Kalogeropoulos et al. (2012) found that lycopene and ß-carotene content of tomato waste was 413.7 mg/kg and 149.8 mg/kg, respectively. The results of research conducted on tomato waste by Chérif et al. (2010) reported that lycopene was present in an average of 22.76 mg/100 g.

Phenolic acids (caffeic, chlorogenic, *p*-coumaric, ferulic and rosmarinic acid, and rosmarinic acid derivatives), flavonols (quercetin, rutin and rutin derivative) and flavanone (naringenin-derivative), as well as ascorbic acid were found in HF (Ćetković et al., 2012; Savatović et al., 2012). The investigated waste samples contain different quantities of listed phenolic compounds depending on the tomato genotype. Total phenolic content ranged from 69.83 to 177.94 mg/100 g DW. Among investigated waste samples, the Novosadski niski had the highest total phenolic content and contained flavonols (quercetin, rutin and its derivative;

TABLE 1: *The yields of tomato waste fractions*

1 : expressed in % per DW of tomato waste; ² : LF – lipophilic fraction, HF – hydrophilic fraction and IR – insoluble residue; $a-d$ – means with different letters within each column differ significantly $(p<0.05)$.

TABLE 2: *The content of insoluble dietary fibre (IDF), soluble dietary fibre (SDF) and total dietary fibre (TDF) in tomato wastes*

Genotype	IDF ¹	SDF ¹	TDF1
Bačka	36.20 ± 1.36 ^a	0.07 ± 0.00 ^a	36.27 ± 1.36 ^a
Knjaz	28.30 ± 1.28 ^b	$7.39 \pm 0.26^{\circ}$	35.69 ± 1.55 ^a
Novosadski niski	28.23 ± 1.13^b	6.90 ± 0.32^b	35.13 ± 1.45 ^a
Rutgers	23.67 ± 0.91 ^c	5.99 ± 0.18 ^c	$29.66 \pm 1.09^{\circ}$
Saint Pierre	26.79 ± 1.08 _{b,c}	0.38 ± 0.02 ^a	27.17 ± 1.10^b
\cdots	$\mathbf{a} = \mathbf{a} \cdot \mathbf{a}$		$1.1 \t B100 \t 1.1 \t 1.1$ A.L.A.

 \cdot : expressed in g per 100 g DW of tomato waste; ac – means with different letters within each column differ significantly (p<0.05).

TABLE 3: *IC₅₀ values of dietary fibre tomato waste fractions*

Genotype	IC_{50} (mg/ml)	
Bačka	3.01 ± 0.12 ^a	
Knjaz	$1.82 \pm 0.09^{\circ}$	
Novosadski niski	1.48 ± 0.05 ^c	
Rutgers	2.15 ± 0.10^d	
Saint Pierre	0.85 ± 0.03^e	

 $a-e$ – means with different letters within each column differ significantly (p<0.05).

52.50 mg/100 g DW) and flavanone (naringenin-derivative; 80.87 mg/100 g DW) as the major phenolic compounds. Valdez-Morales et al. (2014) determined the phenolic composition and content in the peel and seeds of different tomato types (grape, cherry, bola and saladette type). Phenolic acids (caffeic, vanillic, ferulic, sinapic, chlorogenic, *p-*coumaric, *trans*-cinnamic acid and gallic acid) and flavonoids (quercetin-3-O-ß-glucoside, rutin, isorhamne-

tin, kaempferol, naringenin, quercetin, apigenin and myricetin) were identified and quantified. The highest total phenolic content was determined for grape tomato type (120.7 mg/100 g DW in the peel and 22.5 mg/100 g DW in the seeds).

Insoluble dietary fibre (IDF), soluble dietary fibre (SDF) and total dietary fibre (TDF) were determined in IRs (Table 2.).

Depending on the tomato genotype, waste samples contain different quantities of dietary fibres. The waste of the Bačka tomato showed the highest TDF content (36.27 g/100 g DW), while the sample from the Saint Pierre had the lowest (27.17 g/100 g DW). TDF contents of investigated tomato waste samples are in agreement with the contents of dietary fibre in some tomato waste in several other studies, which are in the range from 250.4 to 537.9 g/kg dry matter (Del Valle et al., 2006; Abaza et al., 1987; Knoblich et al., 2005). It was observed that IDF was the major group of fibres in all investigated tomato waste samples (23.67–36.20 g/100 g DW). In regard to SDF, the Bačka and Saint Pierre samples, with the lowest content (0.07 and 0.38 $g/100 g$ DW, respectively), were significantly different from the other three genotypes. The presented results are in agreement with research by García Herrera et al. (2010) who reported that the insoluble fibre content is much higher than soluble fibre in "tomato fibre" samples, obtained from tomato peels (after tomato processing). Insoluble fibres were also found to be the predominant fibre fraction in many other fruit and vegetable byproducts (Grigelmo-Miguel and Martin-Belloso, 1999). Since the insoluble fibre-rich tomato waste is available in large quantities, it could be a good source of IDF whose application could increase firmness of the products and provide higher fat absorption capacity (Elleuch et al., 2011; Navarro-González et al., 2011).

The assessment of antioxidant activity of food products has become a very popular objective for food science research because antioxidant compounds may contribute to the health potential of food products. Antioxidant activity is one of the most considered features in the definition of food nutritional quality (Gökmen et al., 2009). Measurement of antioxidant activity is mainly focused on the soluble components of food products although it is clear that many of them contain insoluble components that cannot be solubilized without altering their structure by chemical or enzymatic treatments. However, some research indicated that many food insoluble components have a significant antioxidant activity (Serpen et al., 2007).

The antioxidant properties of IRs were evaluated by DPPH radical scavenging assay. DPPH, a stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of different food products or specific compound (Seal, 2012). The DPPH radical scavenging activity of IRs increased with an increasing concentration (Fig. 1.).

The 50 % inhibition of DPPH radical (IC_{50}) by the different IRs of tomato waste was determined (Table 3.); a lower value reflects greater antioxidant activity. In the present study the highest radical scavenging activity was shown by IR of the Saint Pierre tomato waste ($IC_{50} = 0.85$) \pm 0.03 mg/ml), whereas IR of the Bačka tomato waste showed the lowest activity (IC₅₀ = 3.01 \pm 0.12 mg/ml).

The measurement of total antioxidant activity of food products can provide a variety of information, such as

FIGURE 1: DPPH radical scavenging activity (SA_{DPPH}) of dietary fibre *tomato waste fractions.*

 $a-d$ – means with different letters within each column differ significantly (p<0.05).

resistance to oxidation, the quantitative contribution of antioxidant substances and antioxidant activity when they are in the organism. Numerous in vitro studies have been conducted to evaluate the total antioxidant activity of food products. However, there is no official standardised method, and the Trolox equivalent antioxidant capacity (TEAC) is one of the most popular (Gökmen et al., 2009). The TEAC assay is based on scavenging of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation or DPPH radical by the antioxidants present in a sample (Capanoglu et al., 2008; Gökmen et al., 2009; Serpen et al., 2007). TEAC value can be assigned to various food components or products by comparing their scavenging capacity to that of Trolox, a water soluble vitamin E analogue.

In our investigations, TEAC (µmol TEAC/100 g DW of tomato waste) value of IRs as well as TEAC value of LF and HF was determined on the basis of their DPPH radical scavenging activity (Table 4.) (Savatović et al., 2012; Stajčić et al., 2015).

TEAC value of HF ranged from 793.82 ± 34.27 µmol TEAC/100 g DW for the Novosadski niski waste to 1467.95 \pm 67.31 µmol TEAC/100 g DW for the Knjaz waste, while TEAC value of LF ranged from 88.77 ± 3.09 µmol TEAC/100 g DW for the Novosadski niski waste to 193.54 \pm 6.12 µmol TEAC/100 g DW for the Saint Pierre waste (Table 4.). These results are in agreement with Capanoglu et al. (2008), who reported that TEAC value of hydrophilic and lipophilic 'seed and skin' extracts taken after the pulp separation, during the production of tomato paste, was 879.4 ± 184.6 and 132.6 ± 10.2 µmol TEAC/100 g DW, respectively.

In the study of Serpen et al. (2007), the possibility that antioxidant functional groups bound to the insoluble components of different food items, to exert an antioxidant action was examined using a new procedure based on two well-known colored radicals: ABTS and DPPH. It was hypothesized that when free functional groups on the surface of insoluble solid particles came into contact with the radicals, they were able to quench them. TEAC values of insoluble matters of tomato (obtained after extensive washing procedure), based on ABTS and DPPH radical scavenging capacity assays, were 0.36 ± 0.06 and 0.42 ± 0.00 mmol Trolox/kg insoluble matter (which correspond 36 and 42 µmol Trolox/100 g insoluble matter). Navarro-González et al. (2011) reported that antioxidant activity of tomato peel fiber, based on ABTS radical scavenging capacity assays, was 3.944 µmol TEAC/g (which corresponds 394.4 µmol TEAC/100 g). In our investigation, TEAC value of IR ranged from 137.19 ± 5.35 µmol

TEAC/100 g DW for the Bačka sample to $354.80 \pm$ 13.12 µmol TEAC/100 g DW for the Saint Pierre sample.

Total antioxidant activity (TAA) of tomato waste samples is presented as the sum of TEAC values of LF, HF and IR (Table 4.). TAA of investigated tomato waste ranged from 1143.58 ± 50.68 µmol TEAC/100 g DW for the Rutgers waste to 1822.83 ± 82.81 µmol TEAC/100 g DW for the Knjaz waste. The distribution of antioxidant activity of tomato waste fractions is shown in Figure 2. The highest contribution to TAA had antioxidant activity of HFs, and these ranged from 68.87 % for Novosadski

niski to 81.27 % for Bačka. Antioxidant activity of LFs and IRs contributed to the lesser extent to TAA, and ranged from 6.54–12.63 % and 9.42–23.43 %, respectively.

Ilahy et al. (2011) reported that hydrophilic antioxidant activity was higher than lipophilic antioxidant activity of high-lycopene tomato cultivars, and Arnao et al. (2001) showed that hydrophilic antioxidant activity was also much higher than lipophilic antioxidant activity of tomato soup. These findings are in accordance with results obtained in the present study.

Conclusion

This study indicated that selected tomato waste samples have significant total antioxidant activity (presented as the sum of TEAC values of lipophilic, hydrophilic and dietary fibre fractions). Among investigated waste fractions of different tomato genotypes, hydrophilic tomato waste fractions showed the highest contribution to the total antioxidant activity. From the obtained results it can be concluded that the tomato waste could be used for the application of food ingredients with potential healthpromoting effects.

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FIGURE 2: *Distribution of antioxidant activity of tomato waste fractions.*

Conflict of interest

The authors declare that there is no conflict of interest.

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