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UTILISATION OF TOMATO WASTE AS A SOURCE OF POLYPHENOLIC ANTIOXIDANTS

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This study is concerned with the effects of two extraction procedures (using ultrasonic bath and high performance homogenizer) on the extraction efficiency of polyphenolics present in the tomato waste. The isolation of flavonoid fraction of obtained extracts was performed by solid-phase extraction. The antioxidant activity of flavonoid fractions was determined using different spectrophotometric tests, including reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. The content of total polyphenolics and flavonoids in extract obtained employing homogenizer (E2) was higher than in the extract obtained employing ultrasonic bath (E1), and it was 14.33 mg/g and 7.70 mg/g, respectively. The flavonoid fraction (EF2) of extract E2 showed higher antioxidant activity than flavonoid fraction (EF1) of extract E1. The DPPH free radical scavenging activity of fractions EF1 and EF2, expressed as EC_{50} value, were 0.78 mg/ml and 0.45 mg/ml, respectively. The obtained results show that tomato wastes can be used as an easily accessible source of antioxidant polyphenolics.

KEYWORDS: Tomato wastes, polyphenolics, flavonoids, antioxidant activity

INRODUCTION

By-products of fruits and vegetables processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used for various purposes in the food, pharmaceutical and cosmetic industries (1).

Tomato (*Lycopersicon esculentum*) is, after potato, the second most consumed vegetable in the world and approximately 30% is consumed as processed products. Both fresh and processed tomato possesses a high nutritional value, due to its content of different types of micronutrients: vitamins (C and E), folates, carotenoids (lycopene and β-carotene) and polyphenolic compounds (flavonoids - quercetin, kaempferol and narangenin, and phenolic acid - caffeic, chlorogenic, ferulic and *p*-coumaric acids). The skin and seeds of tomatoes have been found to be richer sources of polyphenolic compounds than the pulp (2). George et al. (3) studied 12 genotypes of tomatoes, and found that the free polyphenolic content (expressed as mg catechin/100 g, fresh weight) in pulps ranged from

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9.2 to 27.0 mg/100 g, compared to 10.4 to 40.0 mg/100 g in skin, and also that for each genotype, the polyphenolic content in skin was higher than in pulp. A similar observation has been made by Toor and Savage (4), who reported that the total polyphenolic content (expressed as mg gallic acid equivalents/100 g) of skin and seeds of tomatoes were, respectively, 29.1 and 22.0, compared to 12.7 mg/100 g in the pulp. However, when tomatoes are processed into products like ketchup, sauces or juice, 3-7% of their weight become waste (1, 5). Tomato waste, since it contains a significant amount of skin and seeds, is a potential source of natural antioxidants.

The extraction and purification of phytochemicals from natural sources is desirable, since these bioactive substances are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients, food additives, pharmaceuticals and cosmetic products (6). The purpose of the extraction of phytochemicals from their plant sources is to liberate these compounds from the vacuolar structures where they are found, either through rupturing plant tissue or through a process of diffusion (7). Extraction yield is dependent on the solvent and method of extraction (8). Water, aqueous mixtures of ethanol, methanol and acetone are commonly used as solvents (9). The chosen extraction method should enable complete extraction of the compounds of interest and avoid their chemical transformation (10). The application of ultrasound as a laboratory-based technique for assisting extraction from plant material is widely published (11). Among the several types of sonicator systems currently available, bath and probe-type sonicators are used. The homogenization is also a process which is commonly used for assisting extraction (12).

This study has been carried out with the aim to investigate the influence of applied extraction procedures, ultrasonic bath and high performance homogenizer for solid-liquid extraction on the contents of polyphenolics in the tomato waste extracts. The isolation of flavonoid fraction of obtained extracts was performed using solid-phase extraction (SPE) using CHROMABOND® PA column. The antioxidant activity of flavonoid fraction of tomato waste extracts was determined by different tests, including the reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays.

EXPERIMENTAL

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, trichloroacetic acid, chlorogenic acid and rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These chemicals were of analytical reagent grade. Other chemicals and solvents used were of the highest analytical grade, obtained from „Zorka“ Šabac (Serbia).

Waste preparation

Tomato (genotype Zora) harvested in Serbia in 2008, was obtained from the Institute of Field and Vegetable Crops, Novi Sad. Tomatoes (1 kg) were cleaned, cut in pieces and tomato juice was prepared using the juice extractor Neo, SK-400. The obtained tomato waste (yield 8.10 ± 0.39%) was used for the experiment.

Extraction procedures

Sample of tomato waste (10 g) was extracted at room temperature, using an ultrasonic bath Sonic 12GT (Vims elektrik, Loznica, Serbia). The extraction was performed three times with different amounts of 80% ethanol: 160 ml in 30 min, 80 ml in 30 min, 80 ml in 15 min at room temperature. The total extraction time was 75 min. The three extracts were combined and evaporated to dryness under reduced pressure. The weight of polyphenolic extract obtained using a bath-type sonicator (E1) was m = 0.56 ± 0.02 g.

In the second procedure, sample of tomato waste (10 g) was extracted at room temperature, using a high performance homogenizer, Heidolph DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed with different amounts of 80% ethanol: 160 ml in 30 min, 80 ml in 30 min, 80 ml in 15 min at room temperature. The total extraction time was 75 min. The three extracts were combined and evaporated to dryness under reduced pressure. The weight of polyphenolic extract obtained using the high performance homogenizer (E2) was m = 0.58 ± 0.02 g.

SPE procedure

The isolation of flavonoid fraction was performed according to the method, MACHEREY-NAGEL Appl. No. 300150 SPE (13). Tomato waste extract (0.4 g) was dissolved in 4 ml of distilled water and passed under vacuum through conditioned (with 6 ml methanol, followed by 20 ml bidistilled water) CHROMABOND® PA column (500 mg; J.T. Baker, Phillipsburg, NJ, USA). For SPE, a vacuum manifold processor (system spe-12G; J.T. Baker, Großgerau, Germany) was used. The column was washed with 8 ml of distilled water and flavonoids were eluted with 6 ml of methanol. The flavonoid fraction was evapored to dryness under reduced pressure. The weights of flavonoid fractions EF1 and EF2 obtained from extracts E1 and E2 were: EF1, m=0.0179 g and EF2, m=0.0137 g.

Total polyphenolic content

The amount of total soluble polyphenolics in the tomato waste extracts E1, E2, EF1 and EF2 was determined spectrophotometrically by the Folin-Ciocalteu method (14). The total polyphenolic content was expressed as mg of chlorogenic acid equivalents per g of extract.

Total flavonoid content

Total flavonoids were measured in the tomato waste extracts E1, E2, EF1 and EF2 by the aluminum chloride spectrophotometric assay (15). Total flavonoid content was expressed as mg of rutin equivalents per g of extract.

Free radical scavenging activity

The free radical scavenging activity of the flavonoid fractions EF1 and EF2 was determined spectrophotometrically using the DPPH method (16), modified for this assay.

Briefly, a 0.5 ml of solution containing from 0.025 to 2 mg of EF1/EF2 in methanol or 0.5 ml of methanol (control) were mixed with 1.5 ml of 90 μM DPPH solution and 3 ml of methanol. The mixture was vortexed and left at room temperature for 60 min, than the absorbance was read against a blank at 515 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The blank probe contained all components except the radicals. The capability to scavenge the DPPH radicals, DPPH scavenging activity (SA_{DPPH}), was calculated using the following equation:

$$SA_{DPPH}(\%) = (A_{Control} - A_{Sample})/A_{Control} \times 100$$

where $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the extract) and A_{Sample} is the absorbance in the presence of the extract. The values of scavenging activity were calculated for the various concentrations of extract.

Reducing power

The reducing power of the flavonoid fractions EF1 and EF2 was determined by the method of Oyaizu (17). For this purpose, suspension of EF1/EF2 (0.025 - 2.5 mg) in 1 ml of distilled water or 1 ml of distilled water (control) was mixed with 1 ml of phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide, $K_3[Fe(CN)_6]$. The mixture was incubated at 50°C for 20 min, 1 ml of trichloroacetic acid (10%) was added and the mixture was then centrifuged at 3000 rpm for 10 min. A 2 ml aliquot of the upper layer was mixed with 2 ml of distilled water and 0.4 ml of 0.1% $FeCl_3$ and the absorbance of the mixture was measured at 700 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Increased absorbance of the reaction mixture indicates increased reduction capability.

RESULTS AND DISCUSSION

Total Polyphenolic and Flavonoid Content

Two procedures were applied for the efficient extraction of polyphenolics from tomato waste. The extractions were carried out using 80% aqueous ethanol solvent system. The contents of total polyphenolics and flavonoids in the polyphenolic extracts (E1 and E2) and in its flavonoid fractions (EF1 and EF2), as well as the ratio of total flavonoids/polyphenolics are presented in Table 1. The total polyphenolic contents in extracts and flavonoid fractions were determined from the regression equation of chlorogenic acid calibration curve, and expressed as mg of chlorogenic acid equivalents per g of dry extract. Similarly, total flavonoids in extracts and flavonoid fractions were determined from the regression equation of rutin calibration curve, and expressed as mg of rutin equivalents per g of dry extract. The content of total polyphenolics and flavonoids in E2 extract was higher than in E1 extract and it was 14.33 mg/g and 7.70 mg/g, respectively. Also, the content of total polyphenolics and flavonoids in EF2 fraction was higher than in EF1 fraction, and it was 73.22 mg/g and 69.82 mg/g, respectively. It is evident from Table 1 that the high

performance homogenization (E2 and EF2) showed better results for the extraction of polyphenolics and flavonoids than the ultrasonic assisted extraction (E1 and EF1).

Table 1. Total polyphenolics and flavonoids in tomato waste extracts and in its flavonoid fractions

Extracts	Polyhenolics (mg/g)	Flavonids (mg/g)	Flavonoids/polyphenolics
E1	11.41	4.03	0.35
E2	14.33	7.70	0.54
EF1	71.03	64.32	0.91
EF2	73.22	69.82	0.95

Based on spectrophotometric determination, it can be observed that the ratio total flavonoids/polyphenolics in flavonoid fractions obtained after SPE procedure, EF1 (0.91) and EF2 (0.95), was very high (Table 1). The applied SPE showed good results for concentrating flavonoids from the extracts.

Antioxidant activity

The antioxidant activity of the EF1 and EF2 fractions of the tomato waste extracts was determined using different tests, including reducing power and DPPH free radical scavenging assays. These extracts were chosen, since the content of flavonoids was higher than in the extracts E1 and E2. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activity in a relatively short time compared with other methods (18). The hydrogen atom or electron donation ability of the extract was measured from the bleaching of a purple-coloured methanol solution of stable DPPH radical. Figure 1 shows the dose response curve for the DPPH radical scavenging activity (SA_{DPPH}) of the EF1 and EF2 fractions. The DPPH radical scavenging activities of the EF1 and EF2 fractions were concentration dependent. The high correlation coefficients (R^2>0.90), calculated from logarithmic regression analysis, indicated that there is a significant positive correlation between the concentration and DPPH radical scavenging activity.

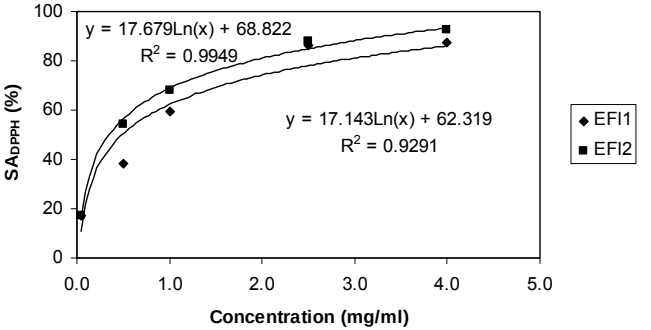

Figure 1. DPPH radical scavenging activity (SA_{DPPH}) of EF1 and EF2 fractions

The EC_{50} value, defined as the concentration of the extract required for 50% scavenging of DPPH radicals under experimental condition employed, is a parameter widely used

to measure the free radical scavenging activity (19); a smaller EC_{50} value corresponds to a higher antioxidant activity. The higher DPPH free radical scavenging activity, expressed as EC_{50} value, showed the fraction EF2 (0.45 mg/ml) than the fraction EF1 (0.78 mg/ml).

For the measurements of the reducing power, the Fe^{3+} - Fe^{2+} transformation was investigated in the presence of the EF1 and EF2 fractions using the method of Oyaizu (17). In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of the antioxidant in the extracts. The capacity of the extracts to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at 700 nm (20). Figure 2 shows the reducing powers of the EF1 and EF2 fractions. Like the DPPH radical scavenging activity, the reducing power of the EF1 and EF2 fractions increased with increasing concentration. All of the applied concentrations of EF2 flavonoid fraction showed higher reducing powers than the EF1 fraction.

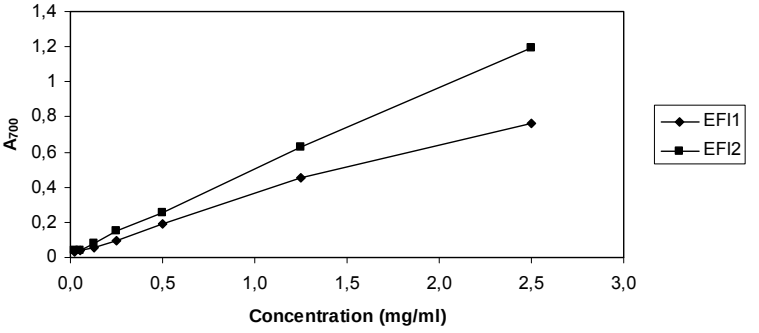

Figure 2. Reducing power of different concentrations of EF1 and EF2 fractions

The obtained results show that the tomato waste should be regarded as a valuable product and has potential as a value-added ingredient which can be used because of their favourable technological or nutritional properties for functional foods.

CONCLUSION

The analysis of the polyphenolic and flavonoid content in the tomato waste extracts show that the high performance homogenization gave better results for the extraction of polyphenolics than the bath-type sonication.

The ratio total flavonoids/polyphenolics in flavonoid fractions obtained after SPE procedure was very high (>0.90), and this fact indicates that SPE is a good method for concentrating flavonoids from polyphenolic extracts.

The antioxidant activity of the EF1 and EF2 flavonoid fractions increased with increasing concentration. The higher reducing power and DPPH free radical scavenging activity showed flavonoid fraction EF2 than the EF1. The EC_{50} value of the fraction EF2, determined based on DPPH radical scavenging activity, was 0.45 mg/ml.

Also, the obtained results show that the tomato waste can be used as an easily accessible source of antioxidant polyphenolics and flavonoids.

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REFERENCES

1. A. Schieber, F.C. Stintzing and R. Carle: By-products of plant food processing as a source of functional compounds – recent developments. Trends Food Sci. Tech. **12** (2001) 401-413.
2. I. Martínez-Valverde, M.J. Periago, G. Provan and A. Chesson: Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersium esculentum*). J. Sci. Food Agric. **82** (2002) 323-330.
3. B. George, C. Kaur, D.S. Khurdiya and H.C. Kapoor: Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chem. **84** (2004) 45-51.
4. R.K. Toor and G.P. Savage: Antioxidant activity in different fractions of tomatoes. Food Res. Int. **38** (2005) 487-494.
5. V. Oreopoulou and C. Tzia: Utilization of Plant By-Products for the Recovery of Proteins, Dietary Fibers, Antioxidants, and Colorants in Utilization of By-Products and Treatment of Waste in the Food Industry. Eds. V. Oreopoulou and W. Russ, Springer, US (2007) pp. 209-232.
6. U.D. Chavan, F. Shahidi and M. Naczk: Extraction of condensed tannins from beach pea (*Luthyrus maritimus* L.) as affected by different solvents. Food Chem. **75** (2001) 509-512.
7. J. Tabart, C. Kevers, A. Sipel, J. Pincemail, J.O. Defraigne and J. Dommes: Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem. **105** (2007) 1268-1275.
8. A.H. Goli, M. Barzegar and M.A. Sahari: Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chem. **92** (2005) 521-525.
9. T. Sun and C. Ho: Antioxidant activities of buckwheat extracts. Food Chem. **90** (2005) 743-749.
10. Y. Zuo, H. Chen and Y. Deng: Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pureh teas using HPLC with a photodiode array detector. Talanta **57** (2002) 307-316.
11. K. Vilkhu, R. Mawson, L. Simons and D. Bates: Applications and opportunities for ultrasound assisted extraction in the food industry - A review. Innovat. Food Sci. Emerg. Tech. **9** (2008) 161-169.
12. N. Castillo-Muñoz, M. Fernández-González, S. Gómez-Alonso, E. García-Romero and I. Hermosín-Gutiérrez: Red-Color Related Phenolic Composition of Garnacha Tintorera (*Vitis vinifera* L.) Grapes and Red Wines. J. Agric. Food Chem. **57** (2009) 7883-7891.
13. http://www.mn-net.com/DesktopModules/TabID/7647/defauld.aspx

14. V.L. Singleton, R. Orthfer and R.M. Lamuela-Raventos: Analysis of total phenols and others oxidation substrates and oxidant by means of Folin-Ciocalteu reagent. Meth. Enzymol. **299** (1999) 152-178.
15. J. Zhishen, T. Mengcheng and W. Jianming: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. **64** (1999) 555-559.
16. W. Brand-Williams, M.E. Cuvelier and C. Berset: Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology **28** (1995) 25-30.
17. M. Oyaizu: Studies on product of browning reaction prepared from glucose amine. Japan. J. Nutr. **44** (1986) 307-315.
18. D. Villãno, M.S., Fernández-Pachón, M.L. Moyá, A.M. Troncoso and M.C. García-Parrilla: Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta **71** (2007) 230-235.
19. M.E. Cuvelier, H. Richard and C. Berset: Comparison of the antioxidative activity of some acid phenols: Structure-activity relationship. Biosci. Biotechnol. Biochem. **56** (1992) 324-325.
20. Y.C. Chung, C.T. Chang, W.W. Chao, C.F. Lin and S.T. Chou: Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. J. Agric. Food Chem. **50** (2002) 2454-2458.

ИСКОРИШЋЕЊЕ ОТПАТКА ПАРАДАЈЗА КАО ИЗВОРА ПОЛИФЕНОЛНИХ АНТИОКСИДАНАТА

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У овом раду је испитан утицај ултразвучног купатила и хомогенизатора на ефикасност екстракције полифенолних једињења из отпатка парадајза. Након екстракције, из добијених екстраката су издвојени флавоноиди екстракцијом на чврстој фази. Антиоксидативна активност фракција флавоноида испитана је спектрофотометријски 2,2-дифенил-1-пикрилхидразил (DPPH) тестом, а такође је испитана и њихова редукциона способност. Садржај укупних полифенолних једињења и флавоноида у екстракту добијеном применом хомогенизатора (E2) је већи у односу на садржај у екстракту добијеном применом ултразвучног купатила (E1) и износи 14,33 mg/g, односно 7,70 mg/g. Фракција флавоноида (EF2) екстракта E2 показала је већу антиоксидативну активност од фракције флавоноида (EF1) екстракта E1. Скевинџер активност на DPPH радикале фракција EF1 и EF2 изражена као EC_{50} износи 0,78 mg/ml, односно 0,45 mg/ml. Добијени резултати показују да отпадак парадајза представља значајан извор полифенолних једињења.

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