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Analytical Methods

Tomato waste: Carotenoids content, antioxidant and cell growth activities

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ABSTRACT

The carotenoid content, antioxidant and cell growth activities of tomato waste extracts, obtained from five different tomato genotypes, was investigated. High performance liquid chromatography was used to identify and quantify the main carotenoids present in tomato waste extracts. The antioxidant activity of tomato waste extracts was tested using spectrophotometric methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power assay. The highest DPPH scavenging activity (IC_{50} = 0.057 mg/ml) was obtained for Bačka extract. The Knjaz extract showed the best reducing power (IC_{50} = 2.12 mg/ml). Cell growth effects were determined in HeLa, MCF7 and MRC-5 cell lines by sulforhodamine B test. Anti-proliferative effects were observed in all cell lines at higher concentrations ($\geqslant$0.125 mg/ml). The carotenoid contents exhibited a strong correlation with antioxidant and anti-proliferation activity. The results obtained indicated that tomato waste should be regarded as potential nutraceutic resource and may be used as a functional food ingredient.

1. Introduction

Tomatoes are one of the most widely used and versatile fruit crops. They are consumed fresh and processed into a wide range of manufactured products (De Sousa, Borges, Magalhães, Ricardo, & Azevedo, 2008). Epidemiologic studies suggest that consumption of tomato and tomato-based products reduces the risk of chronic diseases such as cardiovascular disease and cancer (Giovannucci, 1999; Willcox, Catignani, & Lazarus, 2003). In particular, intake of tomato and tomato-based products has been relatively consistently associated with a lower risk of cancers of the prostate, lung and stomach (Hwang & Bowen, 2005; Palozza, Simone, Catalano, & Mele, 2011; Yang, Yang, Wang, Wang, & Song, 2013). Typically, this protective action is attributed to antioxidant components like carotenoids (in particular, lycopene and β-carotene), ascorbic acid, flavonoids and tocopherols, and synergistic interactions among them (Martínez-Valverde, Periago, Provan, & Chesson, 2002; Podsędek, Sosnowska, & Anders, 2003; Raffo, La Malfa, Fogliano, Maiani, & Quaglia, 2006).

Lycopene is the major carotenoid present in tomatoes, accounting for >80% of the total tomato carotenoids in fully red-ripe fruits, where it is responsible for their characteristic colour (Lenucci, Cadinu, Taurino, Piro, & Dalessandro, 2006; Leonardi, Ambrosino, Esposito, & Fogliano, 2000). Tomatoes also contain moderate amounts of α- and β-carotene and lutein (George, Kaur, Khurdiya, & Kapoor, 2004). The antioxidant activities of lycopene and other carotenoids are related to their abilities to quench singlet oxygen ($O_2^{\cdot-}$) and to trap peroxyl radicals ($ROO^{\cdot}$) (Stahl & Sies, 2003). There are a number of investigations demonstrating that lycopene is a more potent ROS (reactive oxygen species) scavenger than many other dietary carotenoids and other antioxidants, including vitamin E, and the rate constant for lycopene quenching of singlet oxygen is almost double that of β-carotene (Shi & Qu, 2004). Generally, it is believed that more conjugated double bonds and opening of the β-ionone ring increase the quenching ability of lycpoene towards singlet oxygen compared with the other carotenoids (Shi & Qu, 2004). Moreover, carotenoids have been found to inhibit the growth of several cancer cell lines including, prostate cancer cells, lung, mammary, two human colon cancer cell lines and leukemia cancer cells (Giovannucci, 1999; Palozza et al., 2011; Yang et al., 2013). In addition to these properties, lycopene has also been shown to induce cell-to-cell communications and

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modulate hormones, the immune system and other metabolic pathways, while β-carotene is known for its pro-vitamin A activity (George et al., 2004).

The skin and seed fractions of tomatoes have been found to be a rich source of antioxidant compounds (Knoblich, Anderson, & Latshaw, 2005; Toor & Savage, 2005). Thus, removal of skin and seeds of tomato during processing results in a significant loss of these antioxidants and their potential health benefits (Toor & Savage, 2005; Ćetković et al., 2012). The quantity of the wastes generated during tomato processing, combined with the potentially beneficial characteristics of components of the wastes, justifies the interest of researchers and manufacturers in extracting carotenoids from tomato waste and, specifically, the wastes generated by agro-industrial sites due to their geographical concentration (Riggi & Avola, 2008).

In the present study, waste from tomato juice processing (obtained from different tomato genotypes – Bačka, Knjaz, Novosadski niski, Rutgers and Saint Pierre) was used as a potential source of bioactive carotenoids. Carotenoids (lycopene and β-carotene) in tomato waste extracts were determined by high performance liquid chromatography (HPLC/DAD). Antioxidant activity of extracts were evaluated using spectrophotometric methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (SA) and reducing power (RP) assay. Cell growth activity was tested on human HeLa (cervix epithelioid carcinoma), MCF7 (breast adenocarcinoma) and MRC-5 (fetal lungs) cell lines. Also, the possible correlation of carotenoid content with antioxidant and/or antiproliferation activity was investigated.

2. Materials and methods

2.1. Chemicals

Trichloroacetic acid, DPPH, 2-*tert*-butyl-4-hydroxyanisole (BHA), ferric chloride, β-carotene and lycopene were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Potassium ferricyanide, sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate were purchased from Lach-Ner (Brno, Czech Republic). Doxorubicin (Doxorubicin-Teva®) was purchased from Pharmachemie B.V. (Haarlem, Netherlands) and gemcitabine (Gemzar®) from Lilly France S.A. (Fegersheim, France). All other chemicals and solvents were of the highest analytical grade.

2.2. Waste preparation

Tomato genotypes (Bačka, Knjaz, Novosadski niski, Rutgers and Saint Pierre) were grown in the fields of the Institute of Field and Vegetable Crops Novi Sad (Serbia). Dried tomato waste from different genotypes was prepared as previously described (Ćetković et al., 2012).

2.3. Extraction procedure

Samples of dried tomato waste (10 g) were extracted with hexane at room temperature, using a high performance homogenizer, Heidolph DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed three times with 160 ml hexane for 10 min at room temperature. The total extraction time was 30 min. The extracts were combined and evaporated to dryness under reduced pressure.

Weights of extracts (average of triplicate analysis) were: Bačka $m = 0.121 \pm 0.005$ g; Knjaz $m = 0.085 \pm 0.004$ g; Novosadski niski $m = 0.706 \pm 0.034$ g; Rutgers $m = 0.828 \pm 0.041$ g; Saint Pierre $m = 0.461 \pm 0.023$ g.

2.4. Determination of carotenoids (lycopene and β-carotene) by HPLC/DAD

The dry hexane extracts (10 mg) were dissolved in 1 ml of solvent system consisting of acetone/methanol (75:25, v/v) and ultrasonicated for 1 min. Solutions were filtered through Iso-Disc™ Filters, PTFE 25-4, 25 mm × 0.45 μm (SUPLECO, Bellefonte, PA, USA) before injection into the HPLC/DAD system.

HPLC analysis was performed using Agilent 1200 series (Paolo Alto, CA, USA) equipped with a diode array detector (DAD), on an Agilent, ZORBAX® SB-C18, 5 μm, 3.0 × 250 mm column, using an isocratic solvent system acetone/methanol (75:25, v/v) at a flow rate of 1.5 ml/min. The total analysis time was only 5 min. The column was operated at 26 °C. Using autosampler, 10 μl of samples were injected into the system. The spectra were acquired in the range 350–600 nm and chromatograms plotted at 473/10 nm with a reference wavelength at 360/1 nm.

The carotenoids in samples were identified by matching the retention time and their spectral characteristics against those of standards. Peak purity was determined using the option in ChemStationsoftware package, which controls Agilent liquid chromatography system and is used for analyses of chromatogram data. The external standard method was used for quantification. A stock solution (concentration of 1 mg/ml) was made up using a commercially sourced standard dissolved in acetone/methanol (75:25, v/v). Working solutions used for calibration were prepared prior to analyses by dilution of the stock solutions. Peak areas from chromatograms were plotted against known concentrations of standards and equations, generated via linear regression, were used to determine the concentrations of samples.

2.5. Antioxidant assays

2.5.1. DPPH radical scavenging activity

The DPPH radical scavenging activity (SA_{DPPH}) of tomato waste extracts was determined spectrophotometrically using the DPPH method of Espin, Soler-Rivas, and Wichers (2000), modified for this assay. The decrease in absorbance was determined at 580 nm because of some carotenoids interfered at 515 nm (Jiménez-Escrig, Jiménez-Jiménez, Sánchez-Moreno, & Saura-Calixto, 2000). Briefly, a 0.5 ml of solution containing from 0.05 to 5 mg of extract in acetone/methanol (1/1, v/v) or 0.5 ml of acetone/methanol (1/1, v/v) (control) were mixed with 1.5 ml of 90 μmol/l DPPH solution and 3 ml of methanol. The mixture was shaken vigorously and left at room temperature, and the absorbance was read at 580 nm, after 30 min, against a blank that had been prepared in a similar manner but the DPPH solution replaced with methanol. The capability to scavenge the DPPH radicals, SA_{DPPH} was calculated using the following equation:

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

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 condition employed, was used to measure the free radical scavenging activity (Cuvelier, Richard, & Berset, 1992). BHA was used as control compound.

2.5.2. Reducing power

The reducing power (RP) of the extracts was determined using the method of Oyaizu (1986). For this purpose, extracts (0.25–12.5 mg) in 1 ml of acetone/methanol (1/1, v/v) or 1 ml of acetone/methanol (1/1, v/v) (blank) were 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 and