TITLE: Tomato waste: Carotenoids content, antioxidant and cell growth activities

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Highlights

• Waste from tomato juice processing was evaluated as a potential source of carotenoids. • Lycopene and β-carotene were identified and quantified by HPLC. • Tomato waste extracts rich in carotenoids exhibited good antioxidant properties. • Cell growth of tomato waste extracts rich in carotenoids depend on cell lines and dose. • Tomato waste should be regarded as a good source of carotenoids.

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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 (⩾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

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then rapidly cooled. Following this, 1 ml of trichloroacetic acid (10%) was added and the mixture was then centrifuged at 3000 rpm for 10 min. An aliquot (2 ml) of the upper layer mixed with 2 ml of distilled water and 0.4 ml of 0.1% $FeCl_3$ was left to stand for 10 min. The absorbance of the mixture was measured at 700 nm against blank (acetone/methanol; 1/1, v/v).

The inhibitory concentration (IC_{50}), defined as concentration of extract that produces 0.5 absorbance unit at λ_{700nm}, was used to define reduction capability. BHA was used as control compound.

2.6. Cell growth activity

2.6.1. Grow and culture of the cell lines

Human cell lines HeLa (cervix epithelioid carcinoma), MCF7 (breast adenocarcinoma) and MRC-5 (fetal lung) were used to estimate effects on cell growth. Cell lines were grown in Dulbecco's modified Eagle's medium (DMEM; PAA Laboratories GmbH, Pashing, Austria) with 4.5% glucose and L-glutamine supplemented with 10% heat inactivated fetal calf serum (FCS; NIVNS, Serbia), 100 IU/ml of penicillin and 100 μg/ml of streptomycin (Galenika, Belgrade, Serbia). All the cell lines grow attached to the surface. Cells were cultured in 25 ml flasks (Corning, New York, USA) at 37 °C in atmosphere of 5% CO_2 and 100% humidity, sub-cultured twice a week and a single cell suspension was obtained using 0.1% trypsin (Serva, UK) with 0.04% EDTA.

2.6.2. Samples used in cell growth assay

Extracts were dissolved in 80% acetone to obtain 20 mg/ml stock solution for the evaluation of cytotoxic activity. Stock solutions were then diluted in equal volumes of 0.9% NaCl, filtered through a 0.22 μm micro filters (Sartorius, Germany) and used for the preparation of four additional serial working solutions, again using 0.9% NaCl. Final concentrations of extracts were in the range of 0.002–1 mg/ml.

2.6.3. Sulforhodamine B (SRB) assay

Cell lines were harvested and plated on to 96-well microtiter plates (Sarstedt, Newton, USA) at seeding density of 3×10^3 cells per well (Četojević-Simin et al., 2012), in a volume of 180 μl, and pre-incubated in complete medium supplemented with 5% FCS, at 37 °C for 24 h. Serial dilutions and solvent were added (20 μl/well) to achieve required final concentrations and controls. The microplates were then incubated at 37 °C for a further 48 h. Cell growth was evaluated using the colorimetric SRB assay according to Skehan et al. (1990). Cells were fixed with 50% TCA (1 h, +4 °C), washed with distilled water (Wellwash 4, Labsystems; Helsinki, Finland) and stained with 0.4% SRB (30 min, room temperature). The plates were then washed with 1% acetic acid to remove unbound dye. Protein-bound dye was extracted with 10 mmol/l TRIS base. Absorbance was measured on a microplate reader (Multiscan Ascent, Labsystems; Helsinki, Finland) at 540/620 nm. Effects on cell growth were expressed as a percentage of the control and calculated as:

$$\%\ \text{Control} = (\text{At}/\text{Ac}) \times 100$$

where At is the absorbance of the test sample and Ac is the absorbance of the control.

The concentration–cell growth (dose effect) curves were drawn for each treatment and IC_{50} values defined as concentration of extract that inhibits 50% of cell growth were calculated. BHA, β-carotene and two well known cytotoxic drugs (doxorubicin and gemcitabine) were used as control compounds.

2.7. Determination of bioactivity index

The bioactivity index (BI) for tomato waste extracts was determined according to Sun, Chu, Wu, and Liu (2002) with modifications, using the following equation:

$$\text{BI} = (\text{Score of antioxidant activity} + \text{Score of anti-proliferation activity on cancer cells})/2,$$

where,

$$\text{Score of antioxidant activity} = \left(\sum_{i=1}^{n} \text{smallest } IC_{50} \text{ value}/IC_{50,i} \text{ value}\right) \Big/ n$$

$$\text{Score of anti-proliferation activity on cancer cells} = \left(\sum_{i=1}^{n} \text{smallest } IC_{50} \text{ value}/IC_{50,i} \text{ value}\right) \Big/ n$$

n – number of tests for antioxidant or anti-proliferation activity on cancer cells.

2.8. Statistical analysis

Carotenoid determinations in tomato waste extracts by HPLC/DAD and antioxidant activity were carried out in triplicate, and presented as mean ± SD. Cell growth activity were expressed as mean ± SD of two independent experiments, each performed in quadruplicate. IC_{50} values were calculated using Calcusyn for Windows (Version 1.1.0.0.; Biosoft). Linear regression analysis was carried out using Origin 7.0 (OriginLab Corporation, Northampton, USA, 1991–2002).

3. Results and discussion

Tomato waste extracts were prepared from Bačka, Knjaz, Novosadski niski, Rutgers and Saint Pierre. The carotenoids (lycopene and β-carotene) present in the extracts were quantified by HPLC/DAD analysis. Fig. 1 shows a typical HPLC chromatograms from one of the samples (Knjaz). Amounts of lycopene and β-carotene measured in the extracts are shown in Table 1.

The highest content of lycopene was found in Knjaz (15.69 mg/g), while Bačka contained the most β-carotene (11.95 mg/g). Lycopene (13.40–81.54 mg/100 g) and β-carotene (8.64–50.14 mg/100 g) content were in the accordance with the previous research (Chérif, Jémai, Ben Rahal, Jrad, & Trabelsi-Ayadi, 2010; Kalogeropoulos, Chiou, Pyriochou, Peristeraki, & Karathanos, 2012; Knoblich et al., 2005). In the study of Knoblich et al. (2005), amounts of lycopene and β-carotene in tomato skin alone were 73.4 and 2.93 mg/100 g, while lycopene and β-carotene in seed were 13.0 and 1.44 mg/100 g of dry by-product, respectively. Kalogeropoulos et al. (2012) found that lycopene and β-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.

The antioxidant activity of food, plant extracts and beverages has been tested using a wide variety of assays. Schlesier, Harwat, Bohm, and Bitsch (2002) strongly suggested the application of at least two methods for determination of antioxidant activity, due to differences between the test systems. One part of our research was to investigate the antioxidant activity of the tomato waste extracts using two assays, DPPH radical scavenging activity and reducing power.

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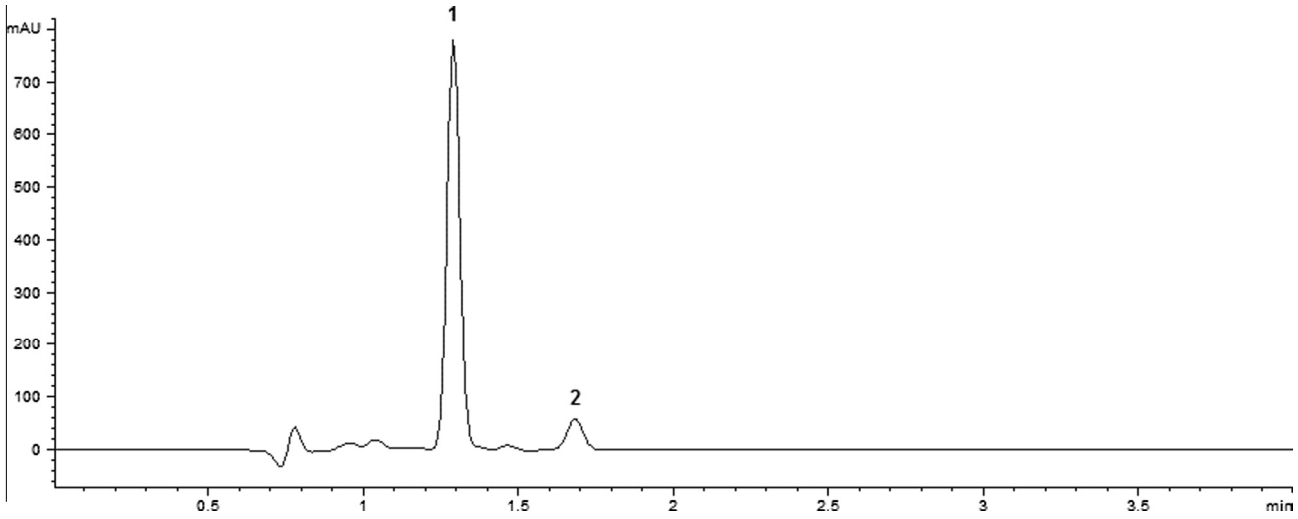

Fig. 1. HPLC chromatogram of carotenoids in Knjaz waste extract; peaks: (1) lycopene and (2) β-carotene.

Table 1
The content of lycopene and β-carotene in tomato waste extracts.

Genotype	Lycopene (mg/g)	β-Carotene (mg/g)
Bačka	13.63 ± 0.11	11.95 ± 0.25
Knjaz	15.69 ± 0.11	10.12 ± 0.43
Novosadski niski	6.34 ± 0.03	2.73 ± 0.29
Rutgers	9.84 ± 0.47	6.05 ± 0.26
Saint Pierre	9.34 ± 0.08	5.02 ± 0.17

The scavenging activity for DPPH radicals assay is widely used, easy to perform and highly reproducible method for testing the antiradical activity of a large variety of compounds and natural extracts (Villãno, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla, 2007). The DPPH radicals assay is a model system for testing the impact of lipophilic antioxidants, and the results provide information about the protective effects of compounds during oxidation of lipids. Fig. 2A shows that SA_{DPPH} of the extracts was depended on genotype and concentration. The decoloration of the solution of DPPH radicals was almost complete with addition 0.1 mg/ml of Bačka extract, 0.2 mg/ml of Knjaz and 0.5 mg/ml of Saint Pierre.

The IC_{50} values of tomato waste extracts, determined on the basis of DPPH radical scavenging activity, are presented in Table 2. Lower IC_{50} indicates a higher DPPH free radical scavenging activity. The DPPH free radical scavenging effect of extracts and BHA, based on the IC_{50} values, decreased in the order of BHA > Knjaz > Bačka > Saint Pierre > Rutgers > Novosadski niski, which were 0.0025, 0.057, 0.071, 0.190, 0.457 and 0.633 mg/ml, respectively. The study conducted by Cherif et al. showed that tomato waste had a good DPPH free radical scavenging activity, which was slightly lower than the concentrate and higher than juice.

Different studies have indicated that the electron donation capacity, reflecting the reducing power, of bioactive compounds is associated with antioxidant activity (Arabshahi-Delouee & Urooj, 2007; Chang, Lin, Chang, & Liu, 2006). The presence of compounds such as antioxidants reduces the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Perl's Prussian blue (Chung, Chang, Chao, Lin, & Chou, 2002). The method of Oyaizu (1986) was used to measure the reducing power of the tomato waste extracts. Fig. 2B shows RP of the tomato waste extracts as a function of their genotype and concentration. At the concentration range of 1.25–12.5 mg/ml, Bačka extract showed the highest increase in absorbance i.e. reducing power. A desirable outcome of reducing reactions is to terminate free radical chain reactions that may otherwise damage cell structure and function.

The IC_{50} values, determined on the basis of reducing power of tomato waste extracts and BHA, are shown in Table 2. The synthetic antioxidant BHA had a significantly higher reducing power (IC_{50} = 0.0288 mg/ml) than any of the tomato wastes. Bačka had the highest reducing power (IC_{50} = 2.12 mg/ml) among investigated extracts.

The cell growth activity of tomato waste extracts and standard compound (β-carotene) was evaluated *in vitro* in a panel of three human cell lines: HeLa, MCF7 and MRC-5. The capacity of investigated cell lines to take up lipophilic compounds was confirmed by using β-carotene standard solution that induced significant cell growth inhibition of all cells after 48 h exposition, reaching extremely low IC_{50} values in the range from 10.65 to 17.33 × 10^{-3} mg/ml (Table 2).

Anti-proliferation effects ($p < 0.01$) of extracts were observed only at higher investigated concentrations (⩾0.125 mg/ml) and were more pronounced in MRC-5 cell line than in HeLa and MCF7 cell line (Fig. 3). At concentration range of 0.002–0.06 mg/ml, all extracts induced proliferation of cell lines (data not shown). IC_{50} values were in the range 0.43–0.75 mg/ml, 0.60–1.00 mg/ml and 0.51–3.08 mg/ml for MRC-5, MCF7 and HeLa cell line, respectively (Table 2). This activity range was comparable to the activity of carotenoid-rich saffron extract (IC_5 = 0.4–4 mg/ml) in the prostate cancer cell lines obtained D'Alessandro et al. (2013) and to the activity of carotenoids separated from marine green algae (IC_{50} = 0.04 mg/ml) in human colon cancer cells reported by Cha, Koo, and Lee (2008). In all cell lines, the most pronounced effects, i.e. the lowest IC_{50} values, were achieved with Bačka and Knjaz extracts that possess the highest concentration of lycopene and β-carotene, suggesting their role in the observed effects.

These results are consistent with studies by different authors who have found that tomato, its products and, in particularly, lycopene possess anti-proliferative effect *in vitro* in various malignant cell lines. Hwang and Bowen (2005) reported that the hexane extract of tomato paste has ability of deceleration of the proliferation cells, cell cycle progression and apoptosis in LNCaP human prostate cancer cells. The findings in the work of Salman, Bergman, Djaldetti, and Bessler (2007) indicate that lycopene inhibited the proliferation capacity of human colon carcinoma, myeloid leukemia and lymphoma cell lines in a dose-dependent manner. Teodoro et al. (2012) showed the capacity of lycopene to inhibit cell proliferation, arrest cell cycle in different phases

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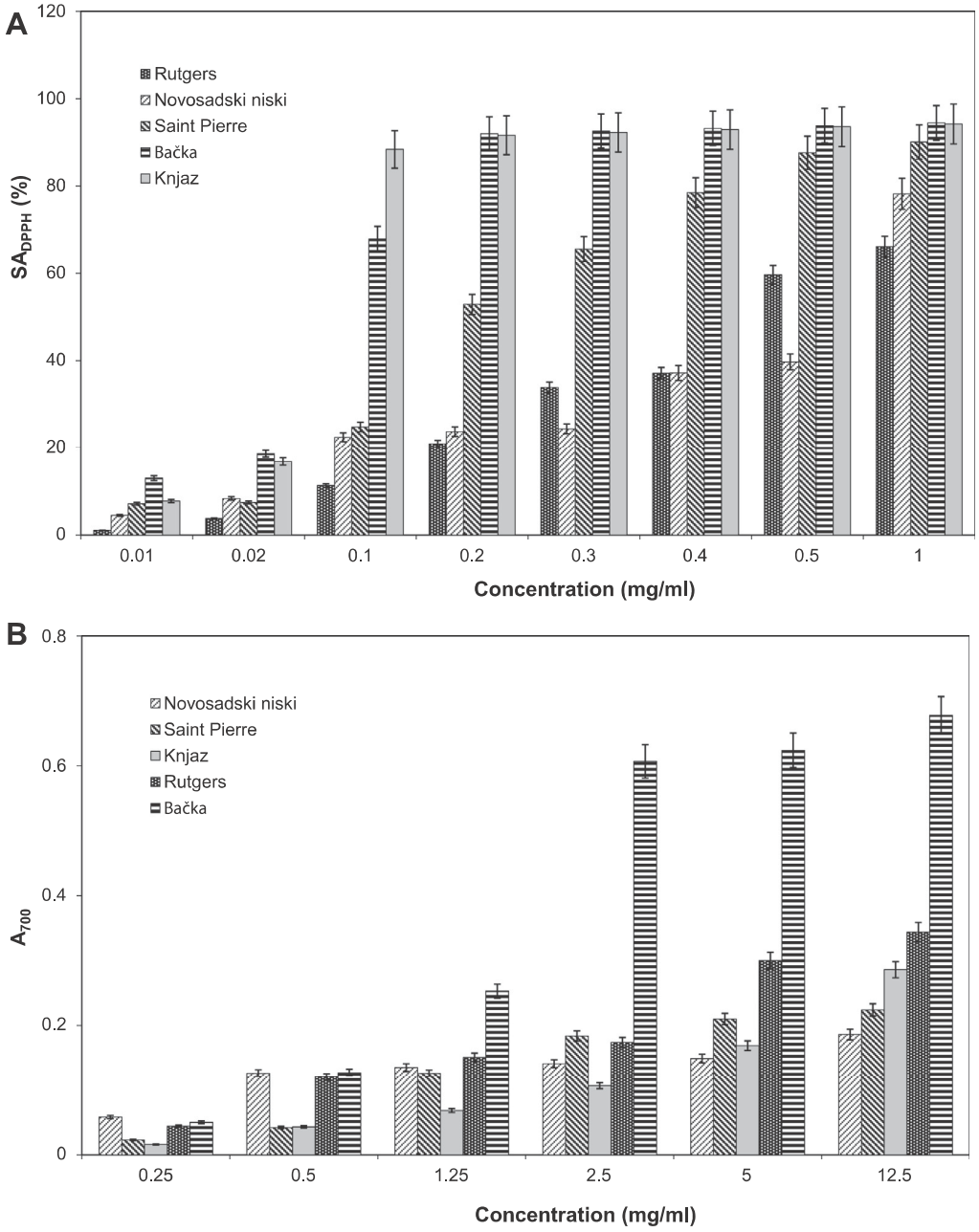

Fig. 2. DPPH radical scavenging activity (A) and reducing power (B) of tomato waste extracts.

Table 2
IC_{50} (mg/ml) values of tomato waste extracts and control compounds in the antioxidant/anti-proliferation activity evaluation assays.

Extracts and controls	DPPH	RP	HeLa	MCF7	MRC-5
Bačka	0.071 ± 0.003	2.12 ± 0.09	0.51 ± 0.02	0.60 ± 0.02	0.47 ± 0.01
Knjaz	0.057 ± 0.002	>12.5	0.57 ± 0.03	0.68 ± 0.02	0.43 ± 0.02
Novosadski niski	0.633 ± 0.022	>12.5	3.08 ± 0.13	1.00 ± 0.04	0.75 ± 0.03
Rutgers	0.457 ± 0.018	>12.5	1.20 ± 0.06	0.88 ± 0.03	0.47 ± 0.02
Saint Pierre	0.190 ± 0.006	>12.5	0.81 ± 0.02	0.85 ± 0.04	0.53 ± 0.02
BHA	2.5×10^{-3}	28.8×10^{-3}	7.87×10^{-3}	8.23×10^{-3}	10.94×10^{-3}
β-Carotene	–	–	13.83×10^{-3}	10.65×10^{-3}	17.33×10^{-3}
Doksorubicin	–	–	0.339×10^{-3}	0.414×10^{-3}	1.046×10^{-3}
Gemcitabine	–	–	0.388×10^{-3}	0.473×10^{-3}	7.804×10^{-3}

and increase apoptosis, mainly in breast, colon and prostate lines. The researches listed suggest that lycopene exert inhibition of carcinogenesis by several mechanisms i.e. scavenging of reactive oxygen species, enhancement of detoxification systems, suppression of cell cycle progression, as well as modulation of signal transduction pathways. Also, these data indicated that the

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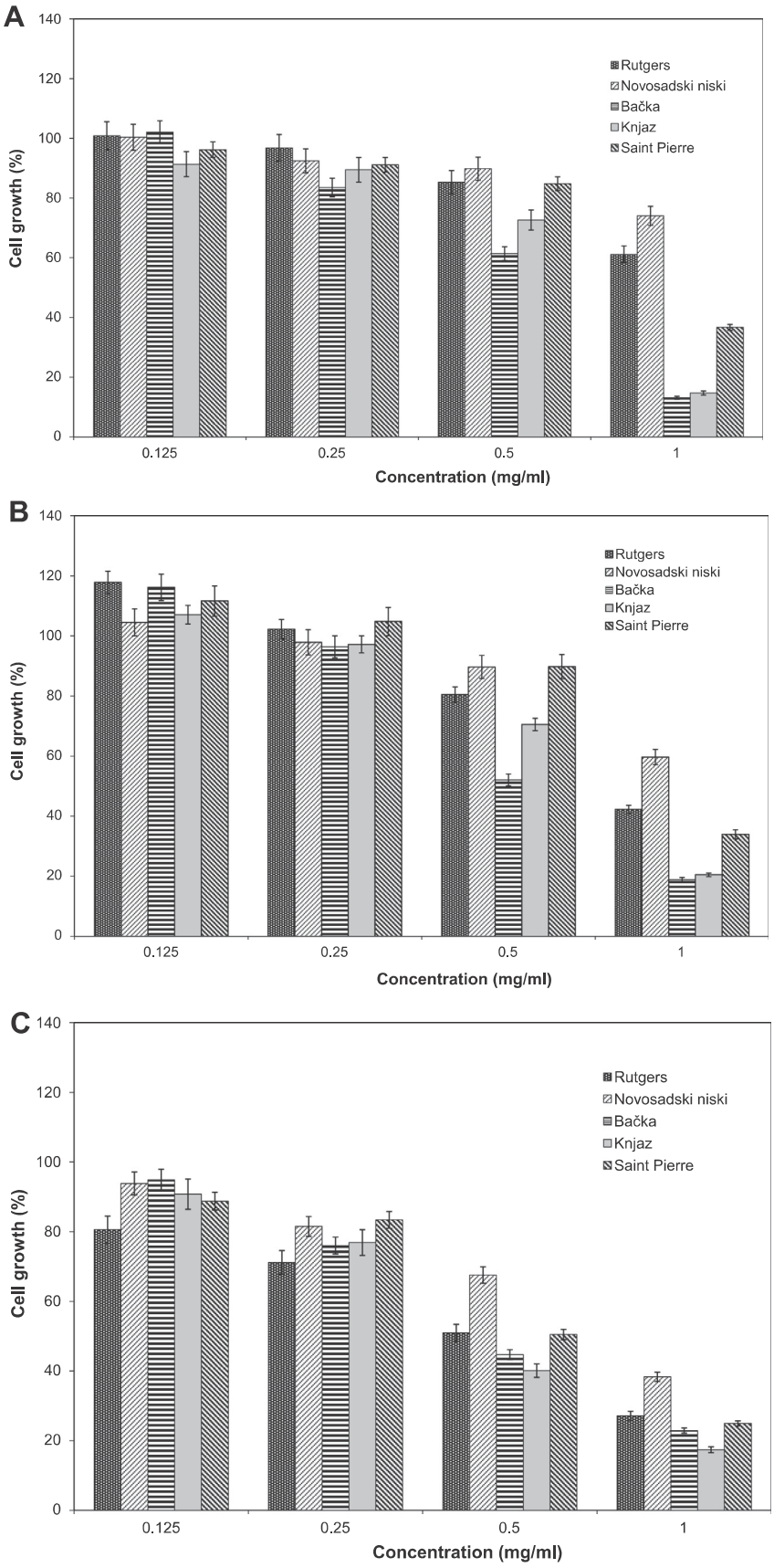

Fig. 3. Cell growth activity of different tomato waste extracts in HeLa (A), MCF7 (B) and MRC-5 (C) cell line. Results are expressed as mean ± SD of two independent experiments, each performed in quadruplicate.

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Table 3
Correlation coefficients between IC_{50} values of antioxidant/anti-proliferation activity and carotenoid (lycopene and β-carotene) content in tomato pomace extracts.

	DPPH	HeLa	MCF7	MRC-5
Lycopene	−0.88	−0.82	−0.94	−0.84
β-Carotene	−0.84	−0.78	−0.99	−0.78

anti-proliferative effect of lycopene was cellular type, time and dose-dependent.

The anti-proliferation effects of extracts were compared with the effects of compounds with known cytotoxic activity, specifically Doxorubicin® and Gemcitabine®. All the extracts examined had lower activities (higher IC_{50} values) than these pharmaceutical products (Table 2).

Based on IC_{50} values, the bioactivity index (BI), an alternative biomarker for classification of extracts was calculated. It could be used by consumers as criteria for choosing fruits and vegetables on the basis of their beneficial activities or as a characteristic for ranking data in epidemiological studies (Sun et al., 2002). The BI is a more useful measure of extract activity than either total antioxidant activity or anti-proliferation activity alone. Knjaz (BI = 0.95) and Bačka (BI = 0.90) had the highest BI values among the extracts, followed by Saint Pierre (BI = 0.49) > Rutgers (BI = 0.34) > Novosadski niski (BI = 0.24). Reducing power was not taken into account for the calculation of BI because the IC_{50} values for all the extracts could not be determined. However, this model was relatively simple, and further research is needed to determine the real value of BI in epidemiological studies.

Many authors have studied relationship between content of bioactive compounds and antioxidant/anti-proliferation properties of numerous extracts (Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004; Rodriguez-Amaya, 2010). Rodriguez-Amaya (2010) stated the contribution of carotenoids to (overall) antioxidant activity has been found in some studies while, in other studies, no association between carotenoids and antioxidant activity has been confirmed. In the study of Olsson et al. (2004), inverse correlation was found for total content of lutein and β-carotene and the proliferation of MCF7 cells. The correlation coefficient of lutein content and proliferation of MCF7 cells was −0.72.

Linear correlation analysis was used to ascertain relationships between individual parameters and to establish their relative importance in determining antioxidant/anti-proliferation status of tomato waste. Considering our data, a good degree of correlation existed between the content of lycopene and β-carotene and IC_{50}^{DPPH} ($r = -0.88$ and $r = -0.84$) (Table 3). Cell growth activity of the extracts was correlated with content of lycopene ($r = -0.82$ for HeLa, $r = -0.94$ for MCF7 and $r = -0.84$ for MRC-5 cells), as well as with content of β-carotene ($r = -0.78$ for HeLa, $r = -0.99$ for MCF7 and $r = -0.78$ for MRC-5 cells). These suggest that a lycopene and β-carotene was responsible for DPPH radical scavenging and cell growth activities.

4. Conclusion

Tomato juice processing generates of large amounts of waste, mainly consisting of peel and seeds. If these waste materials remain unused, they not only create a disposal problem, but also aggravate environmental pollution. According to our results, tomato waste extracts contained considerable amounts of carotenoids (lycopene and β-carotene), and exhibit good antioxidant and anti-proliferation activities. Therefore, because of their low costs and bio-renewable nature, tomato waste could be alternative source of valuable bioactive compounds. Based on the significant antioxidant and anti-proliferation activity, tomato waste should be regarded as a good source of carotenoids for improving human nutrient supply and reducing the risks of diseases caused by oxidative damage, such as cancer. More research is needed to establish bioavailability and real benefits of these extracts obtained from tomato residues *in vivo*.

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