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CORRELATION OF PHENOLIC COMPONENTS IN RED AND PURPLE TOMATOES

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Abstract

Different parts of plants (roots, leaves, flowers, fruit, stem, bark) have been successfully used to treat numerous diseases. Tomato is known for its medicinal properties. The components that affect its activity are different phenolic compounds.
In this paper, we compared the content of phenolic compounds between the Russian Black Prince variety, type were tested, which is with high content of anthocyanins with hybrid Sidra F_1 selections Institute of Vegetable S.Palanka. In the phase of technological maturity, the selection of sample produce for the purpose of chemical analysis has been performed. The object of the paper has been to define and establish the correlation between the total phenolic compounds and their antioxidant activity in the ethanol extracts of tomato .

Key words: phenolic components, antimicrobial properties, tomato, extract.

Introduction

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity (Vaquero et al., 2010). Antimicrobial activity of herbs has been known and described for several centuries (Begamboula et al., 2003). Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against bacteria and fungi (Deans and Ritchie, 1987; Janssen et al., 1985). Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage (Cantore et al., 2009). Human organism is exposed to a large number of foreign chemicals everyday . The most of which are man-made and our inability to properly metabolize them negatively affects our health by the generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells (Carmen and Florin, 2009; Ghaseme et al.,2009). The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumour (prostate and colon cancers) and coronary heart diseases (Duh and Yet, 1997). Antioxidants can significantly delay or prevent the oxidation of easily oxidizable substances (Cao et al., 1997). Natural antioxidants are classified according to their mechanism of action as chain-breaking antioxidants which scavenge free radicals or inhibit the initiation step or interrupt the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions but do not convert free radicals. However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis. There is growing interest toward natural antioxidants from herbal sources.

Materials and methods

Determination of total phenolic content

Total phenols were estimated according to the Folin-Ciocalteu method. The extract was diluted to the concentration of 1 mg/ml, and aliquots of 0.5 ml were mixed with 2.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 ml of $NaHCO_3$ (7.5 %). After 15 min at 45 ˚C, the absorbance was measured at 765 nm using a spectrophotometer against a blank sample. Total phenols were determined as gallic acid equivalents (mg GAE/g extract), and the values are presented as means of triplicate analyses.

Determination of flavonoid content

Total flavonoids were determined according to (Brighente et al., 2007). A total of 0.5 ml of 2 % aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of methanol solution of plant extract. After 1 hour of staying at room temperature, the absorbance was measured at 415 nm in a spectrophotometer against the blank sample. Total flavonoids were determined as rutin equivalents (mg RE/g dry extract), and the values are presented as means of triplicate analyses.

Determination of total antioxidant capacity

The total antioxidant activity of the vegetable extracts was evaluated by the phosphomolybdenum method (Satyajit et al., 2007). The assay is based on the reduction of Mo (VI) – Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A total of 0.3 ml of sample extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using spectrophotometer against the blank after cooling to room temperature. Methanol (0.3 ml) in place of extract was used as the blank. Ascorbic acid (AA) was used as the standard and total antioxidant capacity was expressed as milligrams of ascorbic acid per gram of dry extract.

HPLC analysis

HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies, USA), a binary pump, an online vacuum degasser, an autosampler and a thermostated column compartment, on an Agilent, Zorbax Eclipse Plus-C18, 1.8 µm, 600 bar, 2.1×50 mm column, at a fow-rate of 0.8 mL/min. Gradient elution was performed by varying the proportion of solvent A (methanol) to solvent B (1% formic acid in water (v/v)) as follows: initial 0-2 min, 100% B; 2-4 min, 100-98% B; 4-6 min, 98-95% B; 6-7 min, 95-73% B; 7-10 min, 75-48% B; 10-12 min 48% B; 12-20 min, 48-40% B. The total running time and post-running time were 21 and 5 min, respectively. The column temperature was 30ºC. The injected volume of samples and standards was 5 µL and it was done automatically using autosampler. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280, 330 and 350 nm with a bandwidth of 4 nm, and with reference wavelength/bandwidth of 500/100 nm.
All standards for HPLC analysis were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA) and Alfa Aesar (Karlsruhe, Germany). Acetonitrile and phosphoric acid were of HPLC grade (Tedia Company, USA). Ethanol was of analytical grade (Aldrich Chemical Co., Steinheim, Germany). Spectrophotometric measurements were performed using a UV-VIS spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia).Plant sample (10.0 g) were extracted by concentration $70\%_{vol}$ ethanol or ethanol (100.0 ml) as a solvent. The extraction process

was carried out using ultrasonic bath (Brason and Smith-Kline Company, B-220, (Smith-KlineCompany, USA)) at the room temperature for 1 hours. After filtration, 5 ml of liquid extract was used for extraction yield determination. Solvent was removed by rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenija) under vacuum, and was dried at 60°C to the constant mass. Dry extracts were stored in the glass bottles at 4°C to prevent oxidative damage until analysis.

Results and discussion

Fruits and vegetables contain antioxidant compounds broadly called polyphenols that are known to reduce oxidative stress and prevent chronic diseases (Ames et al., 1993; Diaz et al., 1997). The antioxidant properties of these compounds are responsible for their anticancer, antiviral and antiinflammatory properties (Ames et al., 1995). They can also prevent capillary fragility and platelet aggregation (Benavente- Garcia et al., 1997).

Determination of antioxidant compounds

As a chemical structure of phenolic compounds is responsible for their antioxidant activity, so measurement of total phenolics content could be related to antioxidant properties of investigated material. Total phenolics content, total flavonoids content and total flavonoids/total phenolics ratio (TF/TP) are presentand in the Table 1. In all tested samples content of total phenolics was higher than the content of total flavonoids. T evaluated by the Folin-Ciocalteu, has been determined by means of spectrophotometric method. Results show that the total phenolic content was higher in the Black Prince (E_1) (0.0877 ± 0.0001 g GAE/100g sample) than in Sidra (E_2) (0.0711 ± 0.0001 g GAE/100g sample)

Table 1. Total phenolics, flavonoids, total flavonoid and total phenolics content ratio (TF/TP) and total antioxidant capacity types of vegetable extracts

Type of extract	Total phenolics (mg GAE g^{-1} d. e.)	Flavonoids (mg RE g^{-1} of d.e.)	$\frac{TF}{TP}100$ [%]	Total antioxidant capacity (μg AA g^{-1} d. e.)
Black Prince(E_1)	87.71±0.0265*	23.18±0.5437	45.99	48.50±1.1872
Sidra(E_2)	71.19±0.6583	17.43±0.3898	52.93	72.20±0.7255

*Results are mean values ± SD from three experiment

Content of plant phenolics in vegetables extracts, expressed as mg/g of dry extracts are show in Table 2 and 3. In crude extracts, the following compounds were identified and quantified: gallic acid, protocatehuic acid, caffeic acid, vanillic acid, chlorogenic acid, rosmarinic acid, ferulic acid, sinapic acid, siringic acid, naringenin, miricetin, rutin and quercetin. The dominant components of cultivar Black Prince extracts were chlorogenic acid and rosmarinic acid. In the extract of cultivar Black Prince were less common acids: protocatehuic acid, sinapic acid, vanillic acid. Content of gallic acid, caffeic acid and ferulic acid are lower than 0.1 mg/ g of dry extracts. In cultivar Sidra extract dominant components were gallic (0.369 mg/g) and caffeic acid (0.545 mg/g).

Table 2. Quantitative and qualitative contents of phenolic components in Black Prince

Sample	Component	Content mgg^{-1}
Black Prince	Gallic acid	0.086
	Protocatehuic acid	0.343
	Caffeic acid	0.064
	Vanillic acid	0.146
	Chlorogenic acid	0.799
	Ferulic acid	0.072
	Rosmarinic acid	0.648
	Sinapic acid	0.250

Table 3. Quantitative and qualitative contents of phenolic components in Sidra

Sample	Component	content mgg^{-1}
Sidra	Gallic acid	0.369
	Caffeic acid	0.545
	Rutin	0.089
	Rosmarinic acid	0.060
	Naringenin	0.044
	Siringic acid	0.045

Conclusions

Insensitive research for new, unexplored, natural antioxidant and antimicrobial source is very significant and can bring new natural products in pharmaceutical and food industry for their every day battle with reactive oxygen species. Discovering a natural source of antioxidants could be significant and for artificial toxic antioxidants replacement in food industry.The results of this study clearly indicated that vegetable extracts be use as antioxidant and antimicrobial products. Also, they all possess reductive capabilities. They all are adequate source of phenolic and flavoniods compounds, compounds well known as an antioxidants with high antioxidant activity. This study demonstrate good antioxidant and antimicrobial properties of all investigated vegetables extracts prepared of tomato cultivar Black Prince and cultivar Sidra.

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