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The polyphenolics and carbohydrates as indicators of botanical and geographical origin of Serbian autochthonous clones of red spice paprika

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Spice peppers (*Capsicum annuum* L.) var. Lemeška and Lakošnička paprika were investigated to evaluate their polyphenolic and carbohydrate profiles and antioxidant activity. A total of forty-nine polyphenolics were identified using ultrahigh-performance liquid chromatography (UHPLC) coupled to LTQ OrbiTrap mass analyzer. Twenty-five of them were quantified using available standards, while the other compounds were confirmed by exact mass search of their deprotonated molecule $[M-H]^-$ and its MS^4 fragmentation. Thirteen carbohydrates were quantified using high-performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). Radical scavenging activity (RSA) ranged from 17.32 to 48.34 mmol TE (Trolox equivalent)/kg DW (dry weight) and total phenolics content (TPC) was ranged between 7.03 and 14.92 g GAE (gallic acid equivalents)/kg DW. To our best knowledge, five polyphenolic compounds were for the first time tentatively identified in paprika: 5-*O*-*p*-coumaroylquinic acid, luteolin 7-*O*-(2″-*O*-pentosyl-4″-*O*-hexosyl)hexoside, quercetin 3-*O*-(2″-*O*-hexosyl)rhamnoside, isorhamnetin 3-*O*-[6″-*O*-(5-hydroxyferuloyl)hexoside]-7-*O*-rhamnoside, and luteolin 7-*O*-[2″-*O*-(5‴-*O*-sinapoyl)pentosyl-6″-*O*-malonyl]hexoside.

1. Introduction

Mediterranean countries, Asia and Africa are the main breeders of peppers (Silva, Azevedo, Pereiara, Valentão, & Andrade, 2013; Loizzo, Pugliese, Bonesi, Menichini, & Tundis, 2015). Pepper fruits are popular vegetables in human nutrition because of their high nutritional value, bioactive compounds and antioxidant properties (Jeong et al., 2011; Morales-Soto, Gómez-Caravaca, García-Salas, Segura-Carretero, & Fernández-Gutiérrez, 2013). They also can be used as a food colorant providing redness and as spice due to its specific aroma (Maji & Banerji, 2016; Materska, Perucka, Stochmal, Piacente, & Oleszek, 2003). Beside different size and shape the pepper fruits also have distinctive flavor and pungency. According to its pungency properties they are designated as hot, semi-hot and sweet pepper (Palacios-Morillo, Jurado, Alcázar, & Pablos, 2014). Pepper fruits contain essential nutrients such as carbohydrates, vitamins C and E, minerals and polyphenolic compounds (Morales-Soto et al., 2013; Yang, Wang, & Li, 2013). These compounds prevent diseases improving immune system and protect brain cells by preventing oxidation of essential fatty acids (Silva et al., 2013). Antioxidants are compounds of interest due to their role in human body, protection against free radicals and reactive oxygen species, and prevention from cancer, cardiovascular and degenerative diseases (Campos, Gomez, Ordonez, & Ancona, 2013; Jeong et al., 2011). Among them, the most important are vitamins and phenolics, i.e. phenolic acids and flavonoids (Skupien & Oszmanski, 2004). Phenolic compounds are widely distributed in plants, many being essential metabolites, and contribute to the sensory properties associated with food quality such as color and aroma (Macheix, Fleuriet, & Billot, 1990). Phenolic compounds are known to have antidiabetic, antioxidant, antimutagenic, antitumor, and anti-inflammatory properties (Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011). It is well-known that a content of bioactive compounds and flavor characteristics may differ due to plant varieties, ripening stage, agronomic condition and pre- and postharvest treatment (Domínguez-Martínez, Meza-Márquez, Osorio-Revilla, Proal-Nájera, & Gallardo-

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Velázquez, 2014). Mostly, they exist in plants as esters, glycosides and complexes, very rarely in free forms (Nardini & Ghiselli, 2004). Research of phenolic compounds is important, as it provides information of their impact on food quality and human health-promoting effects (Sapozhnikova, 2014).

Previous studies reported that the main phenolic compounds found in paprika are vanillic, caffeic, ferulic, *p*-coumaric, and *p*-hydroxybenzoic acids (Chen & Kang, 2013). Ferulic acid has strong antiradical properties and vanillic acid is mainly used as flavoring enhancer (Karunaratne & Zhu, 2016). Flavonoids are considered as one of the main polyphenolic compounds abounded in sweet, hot and green paprika (Bae, Jayaprakasha, Jifon, & Patil, 2012).

As bioactive compounds the carbohydrates provide energy to living beings and they can be attached with proteins, lipids and other biomolecules as flavonoids (Xiong et al., 2015). Also, a taste of vegetables and fruits depends of carbohydrates amount (Beaulieu & Baldwin, 2002). Literature survey showed that the most investigated sugars in pepper fruits are glucose, sucrose and fructose (Lopez, Fenoll, Hellín, & Flores, 2014; Perla et al., 2016).

The aim of this work was to fully characterize polyphenolic compounds and carbohydrates in two Serbian autochthonous clones of red spice paprika in order to better understand their quality and phytochemical composition. In this study, we determined the quantity of twenty-five polyphenolics and thirteen carbohydrates in Lemeška and Lakošnička paprika samples, both of them widely used in traditional cuisine.

The main goal of this study was to evaluate possibility of verifying the regional and botanical origin of Serbian autochthonous clones of red spice paprika using multivariate statistical methods. Considering importance of particular phytochemicals for detailed characterization and classification of paprika, similarities and differences between two varieties will be examined by comparing their polyphenolic and carbohydrate content using principal component analysis (PCA).

2. Materials and methods

2.1. Plant material

Among main growing regions of red paprika being used for processing and home usage, we have selected regions of village Svetozar Miletić (west Bačka, north-west Serbia) which provide Lemeška paprika samples and village Donja Lakošnica (Leskovac's valley, south Serbia) which provide Lakošnička paprika samples (Table 1). The studied varieties of Lemeška paprika are exclusively used for homemade sausages such as kulen, known for its specific taste and quality. Second clone was sweet Lakošnička paprika, one of the most famous products from South Serbia (Leskovac area), well-known because of specific drying method (exclusively in the sun). Fruits from selected fields and clones were harvested in two different years in order to follow up its polyphenolic profile and antioxidant activity in the stage of full physiological maturity. After process of natural drying, fruits were collected and kept in plastic tubes in dark on room temperature until the analysis.

2.2. Reagents and standards

Acetonitrile and acetic acid (both MS grade), methanol (HPLC grade), Folin-Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) and Trolox were purchased from Merck (Darmstadt, Germany).

Gallic, protocatehuic, 5-*O*-caffeoylquinic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, caffeic, vanillic, syringic, *p*-coumaric, sinapic, cinnamic, and ferulic acid were supplied by Sigma Aldrich (Steinheim, Germany), while aesculin, rutin, vitexin, cynaroside, hyperoside, vanillin, umbelliferone, coniferyl aldehyde, naringenin, apiin, luteolin, kaempferol, quercetin, and apigenin were purchased from Fluka AG (Buchs, Switzerland).

Trehalose, fructose, succrose, maltose, glucose, arabinose, ribose and raffinose were purchased from Tokyo Chemical Industry, TCI, (Europe, Belgium), while galactitol, rhamnose and sorbitol were purchased from Sigma-Aldrich (Steinheim, Germany). Xylose, mannose, sodium hydroxide, and sodium acetate trihydrate were from Merck (Darmstad, Germany).

Ultrapure water (ThermoFisher TKA MicroPure water purification system, 0.055 μS/cm) was used to prepare standard solutions and blanks. Syringe filters (13 mm, PTFE membrane 0.45 μm) were purchased from Supelco (Bellefonte, PA). Filter paper (Whatman No. 1) was supplied by Merck (Darmstadt, Germany).

2.3. Preparation of standard solutions

A 1000 mg/L stock solution of a mixture of all phenolic standards was prepared in methanol. Dilution of the stock solution with ultrapure water yielded the working solution of concentrations 0.025, 0.050, 0.100, 0.250, 0.500, 0.750, and 1.000 mg/L. Calibration curves were obtained by plotting the peak areas of the standards against their concentration.

The evaluation of the carbohydrate content of the paprika samples was obtained from calibration curves of pure compounds. The calibration was performed with standard solutions of sugars and sugar alcohols dissolved in ultrapure water. Working concentrations of carbohydrate standards were prepared in concentration range to match their concentrations in paprika samples.

2.4. Preparation of paprika extracts

The damaged pepper fruits were discarded from the samples. Fruits were washed and dry on air. Lemeška paprika has a unique way of preparation, grinding all fruit without stems. Lakošnička were prepared by grinding without seeds and stems. Pericarp was ground to powder with kitchen blender. Five grams of homogeneous samples were extracted with 10 mL of 80% methanol in ultrasonic bath for 20 min at room temperature. The extract was centrifuged at 3000 rpm for 15 min. Supernatant was collected and extraction repeated two more times with pellet. The final extract was collected and evaporated under vacuum at a temperature of 40 °C. Residue was then dissolved in methanol/water (3:2) up to 25 mL. The solution was filtrated (0.45 μm PTFE membrane filter) and stored at −20 °C until analysis. This extract was used for determination of total phenolic content (TPC), radical scavenging activity (RSA), and LC-MS analysis.

Carbohydrates from pepper fruits were extracted according to method proposed by Hubbard and Pharr (1992), with slight modification. Paprika samples were extracted three times with 10 mL (80%) ethanol in ultrasonic bath for one hour. After extraction solution was centrifuged for 15 min at 8000 rpm. The final extract was collected and evaporated under vacuum at a temperature of 40 °C. Residue was then dissolved in water up to 25 mL. The solution was filtrated (0.45 μm PTFE membrane filter) and stored at −20 °C until analysis.

2.5. Determination of Total Phenolic Content (TPC)

Folin-Ciocalteu reagent (1 M) was used for determination of total phenolic content (Singleton, Orthofer, & Lamuela-Raventos, 1999). Beside total phenolics it measures non-phenolic compounds (ascorbic acid, sugar, etc.) present in the sample, so the amount of total phenolics could be overestimated (Bae et al., 2014). The 600 μL of filtered extract was mixed with 1 mL of Folin-Ciocalteu