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Anthocyanins profile and antioxidant capacity of red cabbages are influenced by genotype and vegetation period

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ABSTRACT

The profile and content of anthocyanins in five red cabbage varieties were analyzed using HPLC–DAD–MS/MS method. The antioxidant capacity of red cabbages was evaluated using five *in vitro* assays. Red cabbages contained twenty different anthocyanins with the main structure of cyanidin-3-diglucoside-5-glucosides. The presence of nonacylated, monoacylated and diacylated form was identified. Nonacylated and diacylated with sinapic acid derivatives of cyanidin-3-diglucoside-5-glucoside were predominant. The content of anthocyanins varied significantly in plants grown in two different years ($P < 0.05$). Moreover, the vegetation period length was demonstrated to affect the anthocyanins profile. The extract of red cabbages scavenged radicals and their antiradical potential differed significantly across the varieties ($P < 0.05$). In addition, red cabbage antioxidant capacity was positively and significantly correlated with anthocyanins content ($P < 0.05$). This study demonstrates that red cabbage varieties possess own anthocyanins fingerprint and specific antioxidant capacity, which are also under the influence of vegetation period.

1. Introduction

Anthocyanins are the water-soluble, naturally occurring colorants which are probably the most important food pigments besides chlorophyll. It is generally accepted that anthocyanins found in foods do not have any toxic, teratogenic and mutagenic properties, even at high doses of these compounds (Clifford, 2000; Thounaojam, Jadeja, Sankhari, Devkar, & Ramachandran, 2011). This property in correlation with the evidences of positive physiological functions of anthocyanins cause an unremitting interest in these food constituents (Duthie, Duthie, & Kyle, 2000; Lazze et al., 2003; Norberto et al., 2013). Moreover, a consumer's rejection of artificial pigments stimulates a demand for natural food colorants. Therefore, the popularity of anthocyanins as natural pigments has been constantly developing. In addition, the colors of anthocyanins increase the esthetic value of food. Depending on pH of food products, anthocyanins are the source of red, orange, blue and purple colour, what usually makes foods more attractive for consumption.

Among foods, besides berries, colorized vegetables are a rich source of anthocyanins. These compounds are analyzed in radish, curly kale, black carrot, purple sweet potato, eggplant, red bean, violet cauliflower, red lettuce, red onion, and red cabbage (Wu et al., 2006). The profile and content of anthocyanins in berries have been widely investigated (Bakowska-Barczak, 2005; Wu, Gu, Prior, & McKay, 2004), however, knowledge related to the profile of anthocyanins,

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especially acylated anthocyanins, and to the concentration of anthocyanins in colorized vegetables still needs to broadened.

The red cabbage is a rich and important source of anthocyanins in human diet. Belonging to the family of *Brassicaceae* this plant was originated from Asia and is now widespread throughout the world. A number of studies indicate a strong influence of *Brassica* species consumption on human health as it plays a crucial role in the prevention of cardiovascular diseases and some types of cancer (Fowke, Chung, Jin, Qi, & Conaway, 2003; Komatsu, Miura, & Yagasaki, 1998; Manchali, Murthy, & Patil, 2012). Among the compounds that seem to be responsible for those properties are anthocyanins (Nielsen et al., 2005; Wang & Stoner, 2008). These substances are characterized by complex patterns of hydroxylation, methoxylation, glycosylation and acylation (Clifford, 2000; Wu & Prior, 2005). These factors are linked to plant species and form a characteristic pattern of anthocyanins. Red cabbage also has its own characteristic anthocyanin pattern (Wiczkowski, Szawara-Nowak, & Topolska, 2013). However, many varieties of red cabbage cultivated in recent years may have their individual and unique profile of anthocyanins and the unidentified compounds may possess unexplored biological activity. Generally, there has been no systematic information about anthocyanins profile, their content in different varieties of red cabbage and their antioxidant properties. Moreover, no data have been obtained in relation to the influence of different vegetation period on the red cabbage anthocyanins content, as well as the effect the length of vegetation period exert on the red cabbage profile. Therefore, taking an insight into the profile of anthocyanins in different varieties of red cabbage and its correlation with their antioxidant properties may provide a new perspective on the use of this plant. Taking into account the above information, the aim of the present study was to determine the anthocyanins profile, content and antioxidant activity of red cabbage varieties. Moreover, the influence of two consecutive vegetation period and the length of vegetation period on the red cabbage anthocyanins content and profile were studied.

2. Material and methods

2.1. Reagents

2,2′-Azobis(2-amidopropane) hydrochloride (AAPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium fluorescein was obtained from Fluka (Buchs, Switzerland). ACW (hydrophilic condition) and ACL (lipophilic condition) kits (model No. 400.801) for the photochemiluminescence (PCL) assay were received from Analytik Jena AG (Jena, Germany). Cyanidin aglycone was obtained from Extrasynthese (Genay, France). All other reagents of gradient-grade including acetonitrile, methanol, trifluoroacetic and formic acid were purchased from Merck KGaA (Dramstad, Germany). Water was purified with a Mili-Q system (Millipore, Bedford, MA, USA).

2.2. Plants material

Bulbs of five varieties of red cabbage (*Brassica oleracea* L. var. *capitata* L. f. *rubra*) grown in central Poland (Plantico, Zielonki, Poland) were selected as experimental materials. Four of the varieties were characterized by the bulbs of a spherical shape ("Langedijker Dauer 2", "Kissendrup", "Koda", and "Langedijker Polona") while the one variety had a conical shape (Kalibos). What is more, the varieties studied had a diversified length of the vegetation period: early varieties with 80–90 days of vegetation ("Koda"), medium-late varieties with 110–120 days of vegetation ("Kissendrup" and "Kalibos"), and late varieties with 140–150 days of vegetation ("Langedijker Dauer 2" and "Langedijker Polana"). The plants harvested had been planted in 2008 and 2009. The bulbs obtained (7 bulbs for each variety) were purified from the dried outer leaves and subsequently chopped into eight equal parts. Two opposite pieces from each bulb (a total of 14 pieces) were frozen together with liquid nitrogen. After lyophilization, the samples obtained were pulverized and stored at −80 °C until chromatographic analysis.

2.3. Extraction and chromatographic analysis

Extraction and analysis of anthocyanins in red cabbage products were carried out as described previously by Wiczkowski et al. (2013). Briefly, freeze-dried and pulverized red cabbage tissues were extracted 5 times with mixture of methanol/water/trifluoroacetic acid (0.58:0.38:0.04, v/v/v) by sonication and vortexing. After each extraction the sample was centrifuged. Supernatant was collected in 5 mL flask. The extraction was carried out in triplicate for each variety. Finally, before HPLC analysis the extracts were centrifuged (20 min, 13,000×*g*). Chromatographic determinations of the extracts were performed on HPLC with diode array detection (DAD) at 520 nm (Shimadzu, Kyoto, Japan) at 45 °C with the flow rate of 0.2 mL/min on a 150 × 2.1 mm i.d. XBridge C18 3.5 μm column (Waters, Milford, MA, USA). The anthocyanins were eluted in a gradient system composed of water/formic acid (phase A: 99.4:0.6, v/v) and acetonitrile/formic acid (phase B: 99.4:0.6, v/v). Gradient was as follows: 3–17% B (0–77 min), 17–80% B (77–80 min), 80–3% B (80–84 min), and 3% B (84–105 min). Confirmation of the compound identity was performed on the mass spectrometer (QTRAP 5500, AB SCIEX, Vaughan, ON, Canada) with the following conditions, curtain gas: 20 L/min, collision gas: 9 L/min, ionspray voltage: 5300 V, temperature: 550 °C, 1 ion source gas: 55 L/min, 2 ion source gas: 45 L/min, declustering potential: 60–180 V, entrance potential: 10 V, collision energy: 20–40 eV, collision cell exit potential: 10–15 V, and ionization of positive mode. Anthocyanins were identified basing on the comparison of their retention time, UV–visible spectrum and MS/MS fragmentation spectrum (*m*/*z* values) with the previously published data (Charron, Clevidence, Britz, & Novotny, 2007; Wiczkowski et al., 2013; Wu & Prior, 2005). Anthocyanins quantity was calculated from HPLC–DAD peak area at 520 nm against cyanidin as the external standard. The calibration curve (the range of 0.2–90 μM) was linear with a correlation coefficient of 0.997.

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