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Characterization of phenolic compounds and antioxidant and anti-inflammatory properties of red cabbage and purple carrot extracts

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ABSTRACT

The qualitative and quantitative evaluation of phenolic compounds contained in extracts derived from red cabbage and purple carrot were performed. Their antioxidant and anti-inflammatory properties were determined. In purple carrot and red cabbage extracts, 7 and 21 anthocyanins were identified, respectively, of which 83 and 88% were acylated. The total anthocyanin content of purple carrot and red cabbage extracts was 154.0 mg cyanidin 3-O-glucoside equivalents (Cy 3-glcE) per g dry matter (DM) and 175.1 mg Cy 3-glcE/g DM, respectively. The content of phenolic acids in the purple carrot extract was 133.7 mg 5-O-caffeoylquinic acid equivalents per g DM. In red cabbage extract, 21 hydroxycinnamic acid derivatives (HCAs) were identified for the first time. These compounds mainly include residues of *p*-coumaric, ferulic and sinapic acids or their hydrated forms. Purple carrot extract showed a superior ability to inhibit COX-2 (44%) compared to red cabbage (24%).

1. Introduction

Anthocyanins are natural pigments that belong to the group of flavonoids and are responsible for the attractive colors of fruits, vegetables, and flowers. Anthocyanins are compounds having multidirectional biological activity. Numerous scientific studies have confirmed the antioxidant, anti-inflammatory and antitumor activities (Hou, Fujii, Terahara, & Yoshimoto, 2004; Wang & Stoner, 2008). The antioxidant properties of anthocyanins are related to their ability to reduce or prevent the harmful effects of free radicals on the human body, and their protective role in the inflammatory process consists in reducing the pro-inflammatory enzymes, e.g. COX-2, and activating the synthesis of prostacyclin (PGI_2) (Bowen-Forbes, Zhang, & Nair, 2010). During inflammation, there occurs formation of large amounts of reactive oxygen species (ROS), e.g. superoxide ($O_2^{\bullet-}$) and hydroperoxyl ($HO_2^{\bullet}$), as well as reactive nitrogen species (RNS) consisting of nitrogen dioxide (NO_2), nitric oxide ($^{\bullet}NO$) and peroxynitrite (ONO_2^-), which leads to a distortion of

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cellular redox balance. This results in increased activity of pro-inflammatory agents such as inducible nitric oxide synthase (iNOS), inducible cyclooxygenase-2 (COX2), as well as cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). As demonstrated, anthocyanins have the ability to inhibit the inducible form of nitric oxide synthase (iNOS), thereby reducing the synthesis and release of the active, in the processes of oxidation, nitric oxide ($^{\bullet}$NO) (Joseph, Edirisinghe, & Burton-Freeman, 2014; Lau, Joseph, McDonald, & Kalt, 2009). Therefore, the addition of anthocyanin to products makes it possible to not only improve the color but also to enrich them in bioactive compounds. Acylated anthocyanins have the greatest potential to be used as natural dyes, because they exhibit greater stability at higher pH values under the action of heat and radiation as compared to non-acylated anthocyanins. They can, therefore, be used for coloring products of neutral or slightly alkaline pH, such as powder desserts, dairy products, and also for dyeing batches of fruit or vegetable beverages (Bąkowska-Barczak, 2005; de Pascual-Teresa & Sánchez-Ballesta, 2008; Giusti, Rodríguez-Saona, & Wrolstad, 1999; Giusti & Wrolstad, 2003). A good source of acylated anthocyanins is red vegetables. High concentrations of anthocyanins and availability of raw materials makes red cabbage and purple carrot interesting in terms of obtaining the dye extracts.

Red cabbage (*Brassica oleracea* L. var. *capitata* L. f. *rubra*) is one of the most important vegetables grown around the world for consumption. For many years, it has also been used for therapeutic purposes (Wiczkowski, Szawara-Nowak, & Topolska, 2013). Recent studies by Zielińska et al. (2015) show the possibility of applying red cabbage extract as a dietary supplement in the therapy of inflammatory bowel disease. In addition to the acylated anthocyanins, also glucosinolates, carotenoids and tocopherols are responsible for its health benefits (Podsędek, 2007; Volden et al., 2008). Many authors have noted (Ahmadiani, Robbins, Collins, & Giusti, 2014; Podsędek, 2007) that, due to the prevalence, intense coloration and high content of anthocyanins (40–188 mg Cy 3-glcE/100 g FW), red cabbage is an excellent vegetable suitable for obtaining natural dyes for food products.

Purple carrot (*Daucus carota* subsp. *sativus* var. *atrorubens* Alef) is grown in Middle Asia, Far East and in Europe (Kammerer, Carle, & Schieber, 2004a, 2004b; Türkyılmaz, Yemiş, & Özkan, 2012). Acylated anthocyanins contained therein are an excellent source of natural dyes and are used among other things for coloring nectars and soft drinks, jellies and sugar confectionery (Türkyılmaz et al., 2012). This material, in addition to anthocyanins (1.5–126.4 mg Cy 3-glcE/100 g FW) (Algarra et al., 2014; Montilla, Arzaba, Hillebrand, & Winterhalter, 2011) also contains phenolic acids and their derivatives in the amount of 74.64 mg in 100 g FW (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001).

As seen from the short outline above, both vegetables are rich sources of acylated anthocyanins, but only extracts from purple carrot are commonly used in food industry as colorants. Thus, the question arises, whether extracts from red cabbage could find similar use. Therefore, in this paper, we decided to perform comparison of extracts from the two vegetables.

Red vegetables, besides anthocyanins, also contain other phenolic compounds. They are present in significantly smaller amounts than in fruits, but during preparation of the red dyes, after removal of sugars, aliphatic carboxylic acids and other ingredients, their concentration in the extracts is increased. The presence of these compounds in the anthocyanin extracts can favorably influence the color (due to intermolecular co-pigmentation effect) and enhance their biological value. In purple carrot extracts, phenolic compounds have been identified (Alasalvar et al., 2001; Kammerer et al., 2004a), but there is no information on the content of phenolic compounds, other than anthocyanins, in red cabbage. There is also no information on the anti-inflammatory activity of extracts from red cabbage and purple carrot. Therefore, the aim of this study was to perform qualitative and quantitative comparison of phenolic compounds, contained in red cabbage and purple carrot extracts, and to determine their antioxidant and anti-inflammatory activity.

2. Materials and methods

2.1. Chemicals

The following were acquired from Sigma-Aldrich (Steinheim, Germany): 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)- S-triazine (TPTZ), dimethyl sulphoxide (DMSO), $FeCl_3$, acetonitrile, formic acid, TMPD (*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine), cyclooxygenase 1 from sheep (C0733-5000UN), cyclooxygenase 2 human (C0858-1000UN), arachidonic acid from porcine liver and hematin porcine. Tris(hydroxymethyl)-aminomethane (TRIS), acetic acid, acetone and $NaHSO_3$ were obtained from Chempur (Piekary Śląskie, Poland). Acetonitrile for LC–MS was purchased from POCh (Gliwice, Poland). Cyanidin 3-*O*-glucoside (C 3-glc) and 5-*O*-caffeoylquinic acid (5-CQA), 3-*O*-caffeoylquinic acid (3-CQA), 4-*O*-caffeoylquinic acid (4-CQA), rosmarinic acid (RA), *o*-, *m*- and *p*-coumaric (*o*-, *m*-, *p*-CuA) acid, caffeic acid (CA), ferulic acid (FA), isoferulic acid (iFA), sinapic acid (SA), protocatechuic acid (PA) and syringic acid (SgA) were purchased from Extrasynthese (Genay, France). Cyanidin 3-*O*-coumaroyl-sambubioside-5-*O*-glucoside (Cy 3-coumsamb-5-glc) was purchased from Polyphenols (Sandnes, Norway). All reagents were of analytical grade.

2.2. Plant material

Research material consisted of commercially available heads of red cabbage (*Brassica oleracea* L. var. capitata L. f. rubra) of the 'Garance' variety. The roots of the purple carrot variety 'Deep Purple' (*Daucus carota* subsp. *sativus* var. *atrorubens* Alef) came from a private farm. Seeds were from the company Bejo Zaden (Ożarów Mazowiecki, Poland).

2.3. Extraction procedure

Heads of red cabbage were shredded in Thermomix (Wuppertal, Vorwerk, Germany). The resulting material (1.0 kg) was extracted with acetone with the addition of acetic acid (2.0 L)