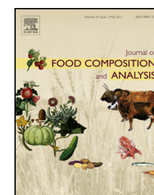
Journal of Food Composition and Analysis 31 (2013) 173–179

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca



Original Research Article

Profiling antioxidant activity of two primocane fruiting red raspberry cultivars (*Autumn bliss* and *Polka*)

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ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form 11 April 2013
Accepted 20 May 2013

Keywords:
Antioxidative capacity
Flavonoids
Food analysis
Food composition
HPLC
Isoelectric focusing
Peroxidase
Phenolic acids
Polyphenol oxidase
Primocane raspberries
Rubus idaeus L.

ABSTRACT

Fruit extracts of two raspberry (*Rubus idaeus* L.) cultivars (*Autumn Bliss* and *Polka*) were analysed for total phenolic (TPC) and anthocyanin (TACY) content. Correlation of TPC with total antioxidant capacity (TAC) showed higher free-radical scavenging properties of *Autumn Bliss* ($r^2 = 0.9999$) compared to *Polka* ($r^2 = 0.8972$). Correlation coefficient between TACY and TAC were higher in *Autumn Bliss* ($r^2 = 0.9939$) compared to *Polka* fruits ($r^2 = 0.8419$). Although total protein concentrations were similar in both cultivars (~0.35 mg mL^{-1}), activities of peroxidases and polyphenol oxidases were much higher in *Polka*, which were confirmed with isoelectric focusing in cationic (p*I* 9.3) and anionic (p*I* 3.6) range. HPLC detection showed that among detected flavonoids (catechin, epicatechin, epicatechin gallate, rutin, myricetin, resveratrol, quercetin and kaempferol) epicatechin appears to be the most abundant compound. Chlorogenic, caffeic and *p*-coumaric acid were also detected. The results indicate that the changes in enzymes activities related to content of substrates play an important role in nutrient quality definition of raspberry fruits.

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1. Introduction

Reactive oxygen species (ROS), such as superoxide anion ($^{\bullet}O_2^-$) and hydroxyl radical ($^{\bullet}OH$), singlet oxygen and hydrogen peroxide (H_2O_2), are produced during normal growth and metabolism of plants (Walz et al., 2002). At low concentrations, ROS fulfil crucial physiological functions within the plant (cell wall biosynthesis, antipathogen defence, etc.) (Elstner, 1987). Environmental and biotic stresses, are known to cause an increase in ROS production in plant cells which can exert detrimental effects by damaging membranes, proteins, chlorophyll, and nucleic acids (Scandalios, 1997). To prevent injuries, plants have developed different mechanisms to convert ROS in less toxic products. These defence mechanisms are based on metabolic compounds (phenolics, ascorbate), as well as enzymatic antioxidant system that consists of several different proteins including peroxidases (POD) and polyphenol oxidases (PPO).

Several previous studies have shown that berries are a good source of natural antioxidants (Heinonen et al., 1998; Wang et al., 1996). These phytochemicals, responsible for the antioxidant capacity are capable of quenching free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (Chun et al., 2003; Heim et al., 2002). Red raspberry (*Rubus idaeus* L.) belongs to plants with lignifying organs where the role of phenolic metabolism in the defence response was documented (Kozlowska et al., 2001). Phenolics are important bioactive compounds that fulfil a broad range of physiological functions *in planta* such as pollinator and bird attraction, UV and light protection, metal chelation, herbivore deterrence, and defence against pathogens (Halbwirth et al., 2009). Phenolic compounds are ubiquitously present in plants and therefore also in human food and they can be divided into four classes based on their chemical structures: simple phenols, phenolic acids, phenylpropanoids and flavonoids (Takahama and Oniki, 2000). Flavonoids are further subdivided into classes: flavones and flavonols,

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; AsA, ascorbic acid; IEF, isoelectric focusing; FW, fresh weight; GA, gallic acid; HPLC, high performance liquid chromatography; POD, peroxidases; PPO, polyphenol oxidases; TAC, total antioxidant capacity; TACY, total anthocyanin content; TPC, total phenolic content.

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0889-1575/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jfca.2013.05.008

catechins and anthocyanins. Epicatechin together with catechin and their esters with gallic acid are the main representatives of catechins. Catechins display a number of biological activities including antioxidant and free radical scavenger activity (Plumb et al., 1998), antibacterial and angioprotective properties (Vennat et al., 1988), and ability to induce metabolic enzymes (Bu-Abbas et al., 1995).

Phenolic acids have been associated with colour, sensory qualities, organoleptic characteristics (flavour, astringency, and hardness) and, in this regard, with nutritional and antioxidant properties of foods (Robbins, 2003). The nutritional relevance of flavonoids is demonstrated by an impressive spectrum of health-related effects (de Pascual-Teresa and Sanchez-Ballesta, 2008). One of the most versatile classes of flavonoids, the anthocyanins, are natural pigments belonging to the flavonoid family. They are responsible for the colour of many fruits and vegetables and they are interesting for two reasons: their impact on the organoleptic characteristics of food which may influence their technological behaviour during food processing and their implication in human health through different pathways. Unlike the traditional vitamins, they are not essential for short-term well-being, but there is increasing evidence that modest long-term intakes may exhibit a potential for modulating human metabolism in a manner favourable for the prevention or reduction in the risk of degenerative diseases such as cardiovascular diseases, diabetes, obesity, and cancer (Jaganath and Crozier, 2010). Therefore, there is a growing interest in the availability of fruit crops showing an optimal composition and concentration of different phenolic compounds for specific purposes.

Phenolics, especially flavonols, hydroxycinnamic acids and anthocyanins, can be oxidised by peroxidase (POD, EC 1.11.1.7), which belong to a large family of enzymes able to oxidise a wide variety of organic and inorganic substrates in the presence of H_2O_2. The oxidation of phenolic compounds results in the formation of yellow-brown products. Such browning could serve as an evidence of a battle between plants and infectious microorganisms and/or is the result of healing of the damaged tissues, as well as ageing process, by producing antimicrobial components and reactive oxygen species (Takahama, 2004). Besides polyphenols oxidation, POD redox activity allows them to promote polymerisation and oxidative cross-linking of cell wall polymers (Bradley et al., 1992).

Because PPO (EC 1.10.3.l) are typically present in the majority of plant tissues they have received much attention from researchers in the field of plant physiology and food science. PPO-catalysed browning reactions are of significant importance in the fruit and vegetable industry. Enzymatic browning occurs as a result of the oxidation of phenolic compounds to quinones and their eventual (nonenzyme-catalysed) polymerisation to melanin pigments (Yoruk and Marshall, 2003). A large number of monophenolic and/or diphenolic compounds catalysed by PPO may in turn form a variety of products (quinones and condensation products) (Eskin, 1990; Jimenez and Garcia-Carmona, 1999; Whitaker, 1995). Even though oxidation of phenols and formation of melanins are normal physiological processes of PPO in plants, the significance of the enzyme activity in living intact plant tissues is not fully understood.

Considering that phytochemicals in plant tissues responsible for the antioxidant capacity vary among species and cultivars (Prior et al., 1998) the present investigation evaluated their content in named raspberry cultivars. Moreover, the aim of this study was to exam the reaction properties, biochemical characteristics and potential physiological roles of POD and PPO in raspberry fruits.

2. Material and methods

2.1. Chemicals

All standard and solutions were prepared with p.a. chemicals and 18 MΩ redistilled and deionised water (Millipore, Bedford, MA, USA). Two phenolic acid standards (gallic and *p*-coumaric acids), six flavonoids (epicatechin, catechin, resveratrol, kaempferol, quercetin and rutin), Folin–Ciocalteu reagent, sodium carbonate, ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), hydrogen peroxide, horse radish peroxidase, polyvinylpyrrolidone (PVP), Triton X 100, bovine serum albumin, guaiacol, pyrocatechol, ampholite solution, 4-chloro-1-naphthol, L-DOPA, ascorbic acid and formic acid, as well as compounds used for buffer preparations were purchased from Sigma–Aldrich Chemical Company (Sigma Co., St. Louis, MO, USA). HPLC-grade solvents (methanol and acetonitrile) and analytical grade methanol used for extraction were purchased from J.T. Baker (Deventer, Netherlands). Potassium chloride, sodium acetate, and Bradford reagents were provided by Merck KGaA (Darmstadt, Germany).

2.2. Fruit sample handling and extract preparation

Two primocane fruiting red raspberry cultivars (*R. idaeus* L.) were used in our experiment, *Autumn Bliss* and *Polka*. Fruit samples were collected in triplicate from the experimental raspberry orchard of the Faculty of Agriculture, Belgrade University in the period of 2008–2009. Only commercial mature fruits were harvested from full sunny side of the hedgerow and selected for uniform development of red colour. Although primocane raspberries bear fruit twice, samples consisting of 30 fruits per repetition (90 fruits per cultivar) were picked only once in late July. Afterwards, fruits from each repetition were separately pooled to obtain a composite sample for analysing biochemical composition and then stored briefly at $-20\,^{\circ}C$ before use, in order to minimise the influence of post-harvest factors.

Extraction of phenolic compounds was carried out in extraction solution containing methanol/water/hydrochloric acid at a ratio of 70:30:5 by volume. The homogenates were centrifuged at $10{,}000 \times g$ for 20 min and supernatants were used for further analyses. Three extracts were prepared for each sample analysed.

2.3. Determination of total phenolic content (TPC)

The TPC in extracts was determined according to the Folin–Ciocalteu's spectrophotometric (2501 PC Shimadzu, Kyoto, Japan) procedure (Singleton and Rossi, 1965) using gallic acid (GA) as a standard for the calibration curve. The linear reading of the curve was from 0 to 350 μg of GA mL^{-1}. Samples were mixed with 0.25 N Folin–Ciocalteu reagent and after 3 min 0.2 M sodium carbonate solution were added and incubating for 60 min. Results were read at 724 nm and expressed as milligrams of GA equivalent per gram of fresh weight (mg GA equiv. g^{-1} FW).

2.4. Determination of total anthocyanin content (TACY)

The TACY was measured with the modified pH differential absorbance method (Cheng and Breen, 1991). Briefly, absorbance of the extract was measured at 510 and 700 nm (Multiscan® Spectrum, Thermo electron corporation, Vantaa, Finland) in 0.025 M potassium chloride buffer adjusted at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5. Anthocyanin content was calculated using a molar extinction coefficient of 29,600 (cyanidin-3-glucoside) and absorbance of $A = [(A_{510} - A_{700})$ pH $1.0 - (A_{510} - A_{700})$ pH 4.5]. Results were expressed as mg

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