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Raspberry marc extracts increase antioxidative potential, ellagic acid, ellagitannin and anthocyanin concentrations in fruit purees

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ABSTRACT

Two types of fruit puree consisting of (1) pears/apples/yellow cherry plums and (2) apples/black currants were enriched with raspberry marc extract containing valuable antioxidants. The addition of 2% marc extract increased total phenolic content (TPC) from 108.8 to 345.8 and from 176.1 to 396.2 mg/100 g in the puree (1) and (2), respectively. The total anthocyanin content (TAC), ellagic acid and ellagitannin concentrations as well as antioxidant capacity of purees also increased with increasing concentration of marc extract additive. The increase of free ellagic acid (up to 44%) in the purees with extract additives after preparation was related to a partial hydrolysis of ellagitannins. Higher extract concentrations resulted in the increased bitterness and astringency of the products; therefore the concentration of raspberry marc additives in purees of up to 1.6% may be recommended.

1. Introduction

Enrichment of foods with healthy constituents is the main way in developing functional foods. Healthy phytochemicals are available in raw and processed edible plants, or may be obtained from agricultural processing by-products. Berries are the richest sources of polyphenolics; they are consumed fresh or processed, due to a limited shelf life. Pressing of berry juices results in high amounts of by-products (the pulp/peels and the seeds), which are rich sources of health-beneficial compounds (Viskelis et al., 2009). For instance, after juice separation the yield of raspberry marc may account for up to 34% of the original fruit mass (Četojević-Simin et al., 2015). Considering that the global production of raspberries is increasing (>0.55 million tons in 2011) (FAO, 2013) development of the effective processing methods of raspberry by-products may provide remarkable amounts of high value functional ingredients.

Strawberry achenes were reported to contain remarkably higher concentrations of ellagitannins and free ellagic acid than their flesh (Aaby, Wrolstad, Ekeberg, & Skrede, 2007). High ellagic acid and ellagitannins contents were also reported in red raspberry marc (Bobinaitė, Viškelis, Šarkinas, & Venskutonis, 2013). Ellagitannins and ellagic acid exhibit a wide range of biological effects such as antioxidant, antimutagenic, anticarcinogenic, antibacterial, and antiviral, suggesting that they could have beneficial effects on human health (Landete, 2011). Therefore, it may be expected that adding raspberry marc constituents to foods might increase the levels of important phytonutrients in human diet.

Although official daily requirements for many phytonutrients have not been established it is suggested that dietary recommendations and regulations for foods enriched with polyphenols should limit their consumption to dietary reference levels and avoid mega-doses (Bohn, 2014; Williamson & Holst, 2008). The cases of severe toxicity associated with consumption of phenolic phytonutrients are very scarce; for instance, grade 4 toxicity reactions associated with consumption of green tea polyphenols and quercetin were reported (Thomasset et al., 2007). However, a number of dietary intervention studies have not revealed any negative effects for the studied polyphenols (Bhatt, Thoman, & Nanjan, 2012; Perumalla & Hettiarachchy, 2011; Thomasset et al., 2007). The more recent study demonstrated that three ellagitannin-rich pomegranate extract capsules per day (each containing 1000 mg polyphenol extract) to men with recurred

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prostate cancer did not show any adverse effect (Paller et al., 2013).

Functional ingredients may be used in foods and nutraceuticals. In our study, a wide range of raspberry marc extract concentrations was used for the enrichment of fruit purees, assuming that the addition of up to 250 mg of polyphenols, expressed in gallic acid equivalents (GAE), to 100 g puree would be potentially useful in supplementing the diet with antioxidants, and at the same time safe. Especially, considering the fact that individuals consuming recommended five portions of fruit and vegetables a day could be exposed to more than 500 mg of polyphenols (Williamson & Holst, 2008), while consumption of certain foods (cocoa, tea, coffee) could naturally increase this dose by 500–1000 mg (Bohn, 2014; Williamson & Holst, 2008).

The objectives of this study were to evaluate how much of the raspberry marc extract phenolic antioxidants are retained after being added into a food system, such as fruit puree, and what effect the extracts may have on the content of the main bioactive constituents, antiradical activity and sensory properties of purees.

2. Materials and methods

2.1. Chemicals and fruit raw material

Ethanol was from distillery AB Stumbras (Kaunas, Lithuania). HPLC grade acetonitrile and methanol, gallic acid, NaOH, anhydrous Na_2CO_3, ellagic acid and 2,2-diphenyl-1-picrylhydrazyl hydrate stable radical (DPPH$^•$, 95%) were from Sigma–Aldrich (Steinheim, Germany). Folin-Ciocalteu phenol reagent and analytical grade methanol were from Fluka Chemie (Buchs, Switzerland), concentrated HCl and formic acid (98–100%) from Merck (Darmstadt, Germany).

The fruits and berries were grown in the Institute of Horticulture of Lithuanian Research Centre for Agriculture and Forestry (LRCAF IH). Raspberry marc was obtained from fresh berries of different cultivars using de-stoning/straining machine EP1000 (Voran Maschinen, Pichl bei Wels, Austria), with changeable screen (aperture size 1 mm).

2.2. Preparation of raspberry marc extract and fruit purees

Raspberry marc was dried in a convection oven (Memmert GmbH, Schwabach, Germany) at 50 °C and ground in a laboratory mill Retsch ZM 200 (Retsch GmbH, Haan, Germany) using 0.5 mm sieve. Three-step batch extraction (5 h each) was carried out at 50 °C with aqueous ethanol (80% EtOH) at 5:1 (I), 3:1 (II), and 3:1 (III) solvent to marc ratios. The extracts were combined, filtered, pre-concentrated in a rotary evaporator Rotavapor R-250 (Büchi Labortechnik AG, Flawil, Switzerland), freeze-dried (ilShin Europe B.V., Ede, The Netherlands) and kept in the hermetically sealed container at −20 ± 2 °C.

Fresh fruits and berries were blanched; their edible parts were separated and pureed using a de-stoning/straining machine EP1000. All purees were prepared using standardized formulae developed in LRCAF IH. Puree of pears, apples and yellow cherry plums (PACP) was prepared by blending 20% pear, 10% apple and 20% yellow cherry plum purees and adding 50% sucrose syrup (30 °Brix). Puree of apples and black currants (ABCP) was prepared by blending 50% apple and 25% black currant purees and by adding 25% sucrose syrup (30 °Brix). These recipes were selected as the most successful products, which have been commercialised and gained the popularity among the consumers. Raspberry marc extract was added to the blended purees at the concentrations of 0.0 (control), 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2%; then they were heated up to 85 ± 5 °C by stirring, poured to 500 mL glass jars, pasteurized for 20 min at 85 ± 2 °C and air-tight sealed with metal covers.

2.3. Determination of total phenolic content (TPC) and total anthocyanin content (TAC)

The TPC of the samples was determined with Folin-Ciocalteu's phenol reagent (Slinkard & Singleton, 1977) as described in detail elsewhere (Bobinaitė, Viškelis, & Venskutonis, 2012). For TPC measurement in purees, 5 g were extracted with 50 mL methanol at ambient temperature for 1 h under shaking; the solution was filtered, and the residue was repeatedly extracted under the same conditions. The combined extracts were diluted 1:3 (v/v) with methanol.

For TPC measurement in dry extract, 200 mg of extract was dissolved in 1 mL water and 99 mL methanol and diluted 1:3 (v/v) with methanol.

The TAC was determined using the pH differential method (Giusti & Wrolstad, 2001) in the dried extract or the liquid part of the centrifuged purees and expressed in mg of cyanidin-3-glucoside in 100 g of puree or 1 g of dry extract.

2.4. Analysis of ellagic acid (EA) and ellagitannins (ETs) hydrolysis products

ETs were determined as EA equivalents after acidic hydrolysis (Koponen, Happonen, Mattila, & Törrönen, 2007). Two quantitatively major ETs hydrolysis products, namely methylsanguisorboate and ellagic acid were measured.

For ETs analysis in purees, 5 g were mixed with 50 mL of acidified methanol (8.3 mL of conc. HCl in 50 mL methanol) and refluxed for 20 h at 85 °C. After hydrolysis the samples were cooled, filtered and made up to 50 mL with methanol.

For ETs analysis in dry extract, 200 mg were dissolved in 1 mL water and 49 mL acidified methanol and hydrolysed under the same conditions as described above. The sample was cooled, filtered and made up to 100 mL with methanol.

Free EA was analysed in the samples without hydrolysis. For purees 5 g were extracted with 25 mL of 90% methanol at room temperature for 30 min under shaking. The suspension was filtered and the residue was re-extracted under the same conditions. The supernatants were combined and made up to 50 mL with methanol.

For free EA analysis in dry extract, 500 mg were dissolved in 20 mL of 99% methanol.

The extracts prepared for ETs and free EA determination were filtered and transferred into LC vials. The HPLC system consisted of a Shimadzu HPLC (Model LC-10Avp with two pumps and DGU-14A Degasser) equipped with a UV–Vis detector SPD-10AV$_{vp}$ (Shimadzu, Kyoto, Japan). The separation was performed on a LiChroCART LiChrospher 100 RP-18 column, 5 μm, 125 × 4 mm (Merck, Darmstadt, Germany) as reported previously (Bobinaitė et al., 2012).

2.5. Evaluation of radical scavenging capacity (RSC)

The extracts of purees that were prepared for free EA determination were used for the measurement of RSC against stable DPPH$^•$ (Brand-Williams, Cuvelier, & Berset, 1995). Briefly, DPPH$^•$ methanolic solution (2 mL, 6 × 10^{-5} M) was mixed with 20 μL of prepared extract and the absorbance was measured after 30 min on a spectrometer Genesys-10 UV/Vis (Thermo Spectronic, Rochester, USA). Antiradical activity was expressed in trolox equivalents (TE) i.e., trolox quantity (μmol), which at the equal conditions possesses the same antioxidant capacity as 1 g of puree.

2.6. Colour measurement, determination of total soluble solids and pH

The CIELab values of purees were measured with a