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Phenolic profiles and metal ions analyses of pulp and peel of fruits and seeds of quince (*Cydonia oblonga* Mill.)

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ABSTRACT

Six hydroxycinnamic acids were identified and determined quantitatively in methanol and acetone extracts from quince peel and pulp, namely 3-*O*-caffeoylquinic acid (3-CQA), 4-*p*-coumaroylquinic acid (HC1), 4-*O*-caffeoylquinic acid (4-CQA), 5-*O*-caffeoylquinic acid (5-CQA), derivative of *p*-coumaroylquinic acid (HC2) and 3,5-dicaffeoylquinic acid (3,5-diCQA). The most abundant hydroxycinnamic acid was 5-CQA (neochlorogenic acid) with 259.12–481.4 mg/kg f.w. in peel and 97.33–217.36 mg/kg in quince pulp. Six flavonols were determined in the extracts from quince, quercetin-3-galactoside (Q-Ga), quercetin-3-rutinoside (Q-Ru), quercetin-3-glucoside (Q-Glu), kaempferol-3-rutinoside (K-Ru), kaempferol-3-glucoside (K-Glu) and derivative of quercetin produced in the reaction between quercetin-glucoside and *p*-coumaric acid (Q-Glu-*p*-CouA). Elemental analysis of quince seeds has not been performed previously. Also, using principal component and cluster analyses, we determined a strong negative relationship between total phenols and flavonoids, and Ni and Pb, specifically higher concentrations of these compounds were associated with lower concentrations of these metals.

1. Introduction

Quince (*Cydonia oblonga* Mill.) is a species in genus *Cydonia*, which belongs to family *Rosaceae,* subfamily *Pomoideae*. Apple (*Malus domestica* Borkh.), pear (*Pyrus* spp.), Japanese medlar (*Eriobotrya japonica*) and common medlar also belong to the subfamily *Pomoideae*. Quinces rarely grow outside of a Mediterranean climate, and are sometimes mistaken for the related species, Japanese quince (*Chanomeles japonica*) or Chinese quince (*Pseudocydonia sinensis*).

Quince is an important source of compounds with positive impacts on health, due to their antioxidant, anti-microbial and anti-ulcer properties (Fattouch et al., 2007; Fiorentino et al., 2008; Oliveira, Pintado, Domingos, & Almeida, 2012; Silva, Valentao, Seabra, & Andrade, 2008). In a previous study, total phenols were reported in the range 11.7–268.3 mg/kg in pulp and between 243 and 1738 mg/kg in quince peel. 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glycoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin-glucoside acetylated with *p*-coumaric acid and kaempferol glycoside acetylated with *p*-coumaric acid have been determined in quince pulp and peel. Cyanidin-3-glucoside and cyanidin-3,5-diglucoside are the main anthocyanins detected in quince fruits with red pigments in the peel. Rutin and 5-*O*-caffeoylquinic acid are the main phenols in peel, and 3-*O*-caffeoylquinic and 5-*O*-caffeoylquinic acid are the main phenols in the pulp (Silva et al., 2008).

There are only few reports on the nutrient and bioactive compound content of quince, and none on quince seeds. The aims of this work were to determine various natural compounds, among them total phenols and total flavonoids, and examine the role of solvents in the yield obtained.

2. Materials and methods

2.1. Fruit material

Quince fruits (Leskovačka) were selected randomly (10 pieces of different shapes and sizes) from producers from Southeast Serbia who proved the geographical origin of the selected fruits.

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2.2. Chemicals and reagents

The following reagents were used: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium-persulfate, methanol, ethanol, acetone, ethyl acetate, acetonitrile, formic and acetic acids (J.T. Baker, Deventer, Netherlands). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,4,6-tri (2-pyridinyl)-1,3,5-triazine (TPTZ) were purchased from Acros Organics (Geel, Belgium). Folin-Ciocalteu reagent (FC), dimethyl sulfoxide (DMSO), 2,9-dimethyl-1,10-phenanthroline (neocuproine), Na_2CO_3, $NaNO_2$, NaOH, $FeSO_4{\cdot}7H_2O$, HCl, CCl_4, Na_2HPO_4, NaH_2PO_4, NaCl, $K_3[Fe(CN)_6]$, trichloroacetic acid (TCA) and $AlCl_3$ were obtained from Merck (Darmstadt, Germany). The following certification standards were also used: gallic acid (Carl Roth, Karlsruhe, Germany); catechin (Sigma-Aldrich, Steinheim, Germany), ferulic and caffeic acids (Sigma-Aldrich, Steinheim, Germany), quercetin-rutinoside, quercetin-glucoside, kaempferol-glucoside, *p*-coumaric acid, chlorogenic acid (Merck, Darmstadt, Germany), and quercetin-galactoside (Extrasynthese, Genay, France). For 4-*O*-coumaroylquinic acid (HC1) *p*-coumaric acid was used as the equivalent; for 4-*O*-caffeoylquinic acid (4-CQA, cryptochlorogenic acid) chlorogenic acid was used as the equivalent; for 5-*O*-caffeoylquinic acid (5-CQA, neochlorogenic acid) chlorogenic acid was used as the equivalent; for derivatives of *p*-coumaroylquinic acid (HC2) *p*-coumaric acid was used as the equivalent; for 3,5-dicaffeoylquinic acid (3,5-diCQA) chlorogenic acid was used as the equivalent; for kaempferol rutinoside kaempferol-3-glucoside was used as the equivalent; for Q-Glu-*p*-CouA quercetin-3-glucoside was used as the equivalent.

All chemicals and solvents were *pro analysis* or HPLC purity. Flasks were washed with ethanolic potassium hydroxide, HCl (1:1; v/v) and then tap, distilled and deionized water before use. Solutions were prepared using deionized water (specific conductivity 0.05 μS/cm).

2.3. Samples preparation

Samples for analysis were prepared using extraction or mineralization processes, depending whether phenolic or metal ions were to be determined. Fruits were separated into peel, pulp and seeds, packed into plastic bags and stored in the freezer (−20 °C) until use.

2.3.1. Extraction procedure of samples for HPLC and UV–Vis analysis

A precisely measured mass of previously homogenized fruit material using an electrical blender (Gorenje, Slovenia) (ca. 10.0 g) was transferred to an Erlenmeyer flask with 20 ml of solvent. After 1 h in an ultrasound bath (Bandelin SONOREX Digital 10 P, Sigma, USA), the solvent was decanted, fresh solvent added and the process repeated five times. The extracts were combined, filtered using Whatman Filter Paper 3 Qualitative (pore size 6 μm) and made up to 50 ml with the solvent. Extracts were kept in the dark and in a cool place until analysis (Borowska, Mazur, Kopciuch, & Buszewski, 2009; Katalinic, Motina, Sktroza, & Generalic, 2010).

The following solvents were used: 60% and 80% methanol, and 60% and 80% acetone for quince peel and pulp.

2.3.2. Mineralization procedure

Samples (ca. 10 g) of previously homogenized fresh fruit material were transferred to a porcelain crucible and heated from 50 °C to over 8 h in an electric oven. The temperature was kept at a constant 450 °C for a further 12 h, and then the samples were cooled and dissolved in 2.5 ml HNO_3 (1:1, v/v), filtered (filter paper with 6 μm pore size – Whatman, USA) and made up to 50 ml using deionized water (Radojevic & Bashkin, 1999).

2.4. Determination of total phenols in fruit samples

Total phenols in the fruit extracts were determined using Folin-Ciocalteu method (Singleton & Rossi, 1965). The reaction mixture was prepared by mixing the extract (0.1 ml), 7.9 ml distilled water, 0.5 ml Folin-Ciocalteu reagent and 1.5 ml 20% Na_2CO_3. Identical blank samples were prepared using 0.1 ml of distilled water to replace the sample. After 2 h at room temperature, the absorbance was measured at 760 nm. The procedure was repeated trice for each sample.

Based on the absorbance of standard solutions of gallic acid, concentrations (μg/ml) of polyphenolic compounds were determined using the equation $A = 0.0855\ [GA] + 0.0065$ ($n = 5$, $r^2 = 0.9998$). Total phenols in the samples are expressed as mg of equivalent of gallic acid in 100 g of fresh fruit weight ± standard deviation (mg GAE/100 g f.w. ±SD).

2.5. Determination of total flavonoids in fruit samples

The method is based on the fact flavonoids complex with metal cations (such as Al^{3+}), which moves the UV and VIS absorption bands 50 nm towards higher wavelengths, *i.e.* solution colour changes from yellow to an intense yellow or yellow-green (Veljkovic et al., 2013).

The reaction mixture was prepared by mixing the sample with 4 ml of deionized water and 0.3 ml 5% $NaNO_2$. After 5 min, 1.5 ml 2% $AlCl_3$ were added, followed 5 min later by 2 ml 1 mol/l NaOH, and deionized water up to 10 ml. Absorbances were measured at $\lambda = 415$ nm against deionized water (blank).

Based on absorbances of a standard catechin solution, concentrations (μg/ ml) of flavonoids were determined using the equation $A = 0.0249\ [C] + 0.005$ ($n = 7$, $r^2 = 0.9994$). Flavonoid content is expressed as mg of catechin equivalent in 100 g of fresh fruit weight ± standard deviation (mg CE/100 g ± SD).

2.6. Determination of antioxidant activity using DPPH test

Antioxidant capacity was determined spectrophotometrically using $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) (Brand-Williams, Cuvelier, & Berset, 1995), where the solution colour changes from violet to yellow.

A previously determined volume of samples was added into 2.5 ml of a working solution of $DPPH^{\cdot}$ radical, and methanol added up to 5 ml. The mixture was stirred well and, after 30 min at 25 °C, the absorbance was measured at 515 nm, with methanol as a reference. All analyses were performed in triplicate. A blank solution was obtained by replacing the sample with 2.5 ml of methanol.

Based on differences in absorbance, using a standard solution of trolox, total antioxidant activity (μmol/ml) was determined using the equation $\Delta A = -0.01 + 0.0313\ [T]$, ($r^2 = 0.9998$, where [T] is the concentration of trolox in μg/ml), and expressed as mmol equivalent of trolox per 100 g of fresh weight of the sample (mmol TE/100 g f.w.).

2.7. Determination of antioxidant activity using ABTS test

ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation test was performed according to Ozgen, Resse, Tulio, Scheerens, and Miller (2006). $ABTS^{\cdot+}$ was obtained by incubation of 7 mM aqueous solution of ABTS (Sigma Aldrich, Germany) with 2.45 mM $K_2S_2O_8$ for 16 h in the dark at room temperature. Samples (0.1 ml) were mixed with 3.9 ml working solution of the $ABTS^{\cdot+}$ radical, and left to stand for 6 min in the dark. Absorbances