Journal of Food Composition and Analysis 26 (2012) 128–135

Contents lists available at SciVerse ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

Identification of apples rich in health-promoting flavan-3-ols and phenolic acids by measuring the polyphenol profile

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ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 19 December 2011
Accepted 27 December 2011

Keywords:
Apple cultivar screening
Flavan-3-ols
Phenolic acids
Polyphenol profile
UHPLC–MS
Folin-Ciocalteu
Antioxidant potential
TEAC
FRAP
Food analysis
Food composition

ABSTRACT

It has been reported that polyphenolic compounds from various plant foods produce physiological effects beneficial to health. Nevertheless, comprehensive evaluations of the polyphenol content and profile of different apple cultivars are scarce. This study examined 104 European apple cultivars for 12 polyphenols by UHPLC–MS, total polyphenol content (TPC) by Folin-Ciocalteu and antioxidative potential by Trolox® equivalent antioxidative capacity (TEAC) and ferric reducing antioxidant power (FRAP). The highest concentrations of individual polyphenols were found for epicatechin, procyanidin B2 and chlorogenic acid. Individual apple polyphenols ranged from below the limit of detection (LOD) to 70 mg/100 g FM, and varied strongly between the different cultivars. The TPC varied from 49 mg to 377 mg catechin equivalents (CE) per 100 g, and was much higher than the sum of the 12 individual polyphenols. TPC as well as the antioxidative potential correlated well with the sum of individual polyphenols as quantified by UHPLC–MS. Based on this polyphenol profiling, apples can be divided into flavan-3-ol predominant or phenolic acid predominant cultivars. Both classes of polyphenols are reported in the literature as having physiological effects beneficial to health.

1. Introduction

Apples are the most popular fruits in Europe and are grown all over the world. From 2003 to 2005 the average per capita apple consumption in Europe was 61 g per day, which is twice as high as per capita consumption worldwide and represents one-quarter of total European fruit consumption. Price, convenience and a positive "health image" are all reasons for this popularity (Harker et al., 2003). The beneficial health effects of apples have been attributed to the polyphenolic compounds, a group of secondary plant metabolites, of which several thousand different compounds have been identified (Sies, 2010). The four polyphenol classes which predominate in apples are the flavan-3-ols, phenolic acids, dihydrochalcones and flavonols. These compounds are present in the monomeric forms, but occur mostly as high molecular weight polymers. Only low molecular weight, mainly monomeric polyphenols are reported to be absorbed from polyphenol-containing foods (Manach et al., 2004, 2005), whereas breakdown products from high molecular weight polyphenols may be absorbed after degradation by the microflora in the colon (Deprez et al., 2000; Monagas et al., 2010). The potential health benefits of polyphenols have been reviewed by Scalbert et al. (2005). The major apple polyphenols, flavan-3-ols and chlorogenic acid have been investigated for their health effects in other foodstuffs. The flavan-3-ols from cocoa have been reported to have a vasodilatory effect which improves blood flow (Faridi et al., 2008). Chlorogenic acid, which is present in high amounts in coffee, is reported to have beneficial effect on cardiovascular disease (Bonita et al., 2007) and to decrease the risk of type II diabetes (Bidel et al., 2008; Johnston et al., 2003).

A detailed knowledge of the polyphenol profile and content in different apple cultivars is necessary in order to evaluate their potential beneficial health effects. At present, there are literature reports on the contents of selected polyphenols in a small number of cultivars (Imeh and Khokhar, 2002; Lee et al., 2003; Neveu et al., 2010; Podsedek et al., 2000; Vrhovsek et al., 2004; Wojdylo et al., 2008) and studies reporting the influence of pre- and post-harvest factors (D'Abrosca et al., 2007; Mari et al., 2010; McGhie et al., 2005) or farming methods (Chinnici et al., 2004; Hecke et al., 2006; Lamperi et al., 2008; Valavanidis et al., 2009) on their levels, but there is no comparison of individual low molecular weight polyphenols in a comprehensive range of different apple cultivars. Polyphenols in apples have usually been measured in the flesh after separation of peel (Lata et al., 2005; Tsao et al., 2003; van der

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doi:10.1016/j.jfca.2011.12.002

Sluis et al., 2001), after processing into a puree (Dragovic-Uzelac et al., 2005; Oszmianski et al., 2008), or more frequently as a juice from cider apples (Alonso-Salces et al., 2004; Kahle et al., 2005; Mangas et al., 1999; Oszmianski et al., 2007; Wu et al., 2007). The aim of this study was to quantify potentially absorbable low molecular weight polyphenols, with potential health benefits, in 104 different apple cultivars grown in Switzerland. The polyphenols were measured by UHPLC–MS in the combined peel and flesh so as to be more representative of normal eating habits. For comparison, total polyphenol content (TPC) and antioxidative potential were measured by simpler colorimetric methods.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Liquid nitrogen for sample preparation was purchased from Messer Schweiz AG (Lenzburg, Switzerland). Methanol and formic acid (analytical grade) for extraction of polyphenols were obtained from Acros Organics (Chemie Brunschwig, Basel, Switzerland) and from Merck (Darmstadt, Germany), respectively.

Methanol and formic acid (99%) for preparing the mobile phase were acquired from Biosolve (Valkenswaard, The Netherlands). Water was distilled and filtered through a 0.2 μm nylon filter (WICOM, Heppenheim, Germany). Folin-Ciocalteu reagent and anhydrous sodium carbonate from Merck (Darmstadt, Germany) were used for total polyphenol determination.

For measuring the antioxidative potential diammonium 2,2′-azinobis[3-ethylenbenzothiazoline]-6-sulfonic acid (ABTS, >99%), potassium persulfate (>99%) and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Fluka (Buchs, Switzerland). Sodium acetate and acetic acid (100%, water free) from Merck (Darmstadt, Germany) and ferric chloride hexahydrate (Riedel de Haën, Seelze, Germany) were used for the Ferric Reducing Antioxidant Power (FRAP) assay. Potassium phosphate (Sigma–Aldrich, Buchs, Switzerland) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Sigma, Steinheim, Germany) were used for the Trolox® Equivalent Antioxidative Capacity (TEAC) assay.

Catechin (C), epicatechin (E), chlorogenic acid (CA), quercetin rhamnoside (QR), quercetin glucoside, quercetin galactoside and rutin (R) were purchased from Fluka (Buchs, Switzerland), phloridzin (P) from Sigma (St. Louis, USA) and procyanidin B1 (PCB1) and B2 (PCB2) from Extrasynthèse (Genay, France). Coumaroylquinic acid (CQA) was quantified as chlorogenic acid and phloretin-xyloglucoside (PXG) as phloridzin.

2.1.2. Apple cultivars

The 104 apple cultivars analyzed (Table 1) were harvested at optimal maturity in 2008, 2009 and 2010 from different locations in Switzerland, except for cultivar PRI 159 and Red Delicious, which were a gift from Plant Research International (Wageningen, The Netherlands) and the Research Centre for Agriculture and Forestry Laimburg (Italy), respectively. The tested cultivars included 15 cider cultivars and 89 dessert apples, of which 46 were new cultivars.

2.2. Methods

2.2.1. Sample preparation

Sampling and sample treatment was done as described by Ceymann et al. (2011). Briefly, at harvest, 20 fruits out of the whole production were randomly chosen and pooled together to one sample. The 20 fruits were sliced with an apple divider (DIVISOREX, Famos-Westmark GmbH, Lennestadt-Elspe, Germany) into 10 pieces and the core area. The core area was discarded and two opposite cuts of each fruit were randomly chosen and immediately frozen in liquid nitrogen and all 40 slices were pooled as representative sample. The frozen apple pieces were ground to fine powder with a dry ice mill (Meidinger AG, Kaiseraugst, Switzerland) and a cutter (La Moulinette DPA 1, Moulinex, Germany). Afterwards, the samples were stored at −20 °C until extraction.

2.2.2. Extraction

Extraction was carried out as described earlier (Ceymann et al., 2011). Briefly, within three months after harvest aliquots (2.50 g) of the frozen powder were mixed with 50 mL of methanol containing 1% formic acid (v/v) and homogenized. For TPC, TEAC and FRAP the supernatants were used directly. For UHPLC–MS analysis the supernatants were filtered through 0.7 μm glass fibre filters (OPTI – Flow®, WICOM, Heppenheim, Germany), diluted 1:1 (v/v) with distilled water and filtered a second time through 0.2 μm nylon filters (OPTI – Flow®, WICOM, Heppenheim, Germany) directly in UHPLC vials. All extractions were done in duplicate and analyzed twice.

2.2.3. Polyphenol analysis by UHPLC–MS

Immediately after extraction, UHPLC–MS analysis was carried out as described earlier (Ceymann et al., 2011) by using an ACQUITY Ultra Performance LC™ system (UPLC™) with binary solvent manager and single quadrupole micromass ZQ Mass Detector (Waters Corporation, Milford, USA) equipped with an electrospray ionization (ESI) source operating in negative mode. For instrument control, data acquisition and processing MassLynx™ software (Version 4.1) was used.

A reversed phase column (BEHC$_{18}$ 1.7 μm, 2.1 mm × 50 mm with a BEH C$_{18}$ 1.7 μm VanGuard™ Pre-Column, 2.1 mm × 5 mm, Waters Corporation, Milford, USA) at 40 °C was used for separation of individual polyphenols. Elution was completed in 10 min with a sequence of linear water–methanol gradient (Ceymann et al., 2011) and a flow rate of 0.3 mL/min. The software QuantLynx™ (Waters Corporation, Milford, USA, Version 4.1) was used for integration and calculations based on external standards. Samples were analyzed twice and mean values were used for calculation.

2.2.4. TPC by Folin-Ciocalteu

The analysis of TPC was carried out as described earlier (Ceymann et al., 2011). Ten microliters of Folin-Ciocalteu reagent and 100 μL of distilled water were automatically pipetted to 10 μL of methanolic extract in a 300 μL cuvette. After 1 min, 40 μL sodium carbonate solution (200 g/L) and 40 μL distilled water were added, thoroughly mixed and incubated for 30 min at 37 °C. Absorption was measured automatically at 700 nm. Total polyphenol content was calculated by means of an external standard calibration with methanolic catechin standards and expressed as mg catechin equivalents/100 g FM.

2.2.5. Antioxidative potential by TEAC

TEAC was measured by using a Konelab ARENA 20XT analyzer (Thermo Fisher Scientific OY, Vantaa, Finland) with a method adapted from Re et al. (1999). To measure TEAC, 200 μL of a mixture of ABTS (7 mM) and potassium persulfate (2.45 mM) in a saline phosphate buffer (prepared the day before, pH 6.7), 4 μL of extract and 4 μL of distilled water were pipetted into a 300 μL cuvette, mixed and incubated for 15 min at 37 °C. The absorbance at 700 nm was recorded automatically. Quantification was achieved with an external Trolox® standard calibration and results were expressed as mg Trolox® equivalents (TE)/100 g FM.