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Improved analysis of anthocyanins and vitamin C in blue-purple potato cultivars

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ABSTRACT

Methods were optimized for extraction and quantification of anthocyanins (ACY) and vitamin C in potatoes. Acidified aqueous methanol (70%) was the optimal extraction solvent and freeze-drying significantly improved the extraction yield of ACY. The content of ACY varied widely in five potato cultivars from 0.42 to 3.18 mg/g dry weight, with the latter being the highest value found in the Finnish cultivar 'Synkeä Sakari'.

Compared with dithiothreitol (DTT), tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was more efficient in reducing dehydroascorbic acid (DHA) to ascorbic acid (AA) and for quantifying the content of total ascorbic acid (TAA). For raw potatoes, quantification of TAA after treatment with TCEP was more reliable than a direct analysis of AA, whereas AA can be analyzed directly in steam-cooked samples. The TAA contents in the three potato cultivars were around 0.30–0.35 mg/g dry weight. The loss of AA in steam cooking was 24%.

1. Introduction

Globally, potatoes (*Solanum tuberosum* L.) are the fourth largest food crop after wheat, rice, and corn. In developing countries, potatoes provide a daily dietary energy source of 130 kcal per person, whereas in developed countries they provide only 41 kcal (Ezekiel, Singh, Sharma, & Kaur, 2013). Potatoes are a source of carbohydrates, proteins, vitamins (B_6, B_3 and C), minerals (potassium, phosphorous and magnesium), and are rich in antioxidants (Andre et al., 2007).

Anthocyanins are the major components responsible for the antioxidative activities of purple-red potatoes (Kaspar et al., 2011). Potato anthocyanins are derived from delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin. Most of the anthocyanins in potatoes are acylated with hydroxycinnamic acids, such as coumaric, ferulic and caffeic acids (Giusti, Polit, Ayvaz, Tay, & Manrique, 2014; Hillebrand, Naumann, Kitzinski, Köhler, & Winterhalter, 2009).

Anthocyanins are unstable in the presence of oxygen, high temperatures, light, high pH, and enzyme activities, and they are easily degraded in food and in the body. However, acylation of anthocyanins improve their stability through intramolecular and intermolecular copigmentation and self-association reactions (Giusti & Wrolstad, 2003).

Anthocyanins may enhance human health due to their antioxidative, antimicrobial (Bontempo et al., 2013), hypotensive (Bell & Gochenaur, 2006; Vinson, Demkosky, Navarre, & Smyda, 2012), neuroprotective (Prior & Wu, 2006; Zafra-Stone et al., 2007), antiobesity (Prior & Wu, 2006), anti-inflammatory (DeFuria et al., 2009; Zafra-Stone et al., 2007), antidiabetic (DeFuria et al., 2009), and even anticarcinogenic properties (Bontempo et al., 2013; Madiwale, Reddivari, Stone, Holm, & Vanamala, 2012). As a staple food, purple-red potatoes have great potential to promote public health by increasing the dietary intake of these compounds.

To better assess the intake and significance of the health effects of potato anthocyanins, it is important to obtain accurate information on the stability and availability after cooking. Some authors have reported higher contents of anthocyanins in cooked potatoes than in raw potatoes (Lachman et al., 2012; Lemos, Aliyu, & Hungerford, 2015) whereas other authors have reported no significant impact caused by cooking (Burgos et al., 2013). A high content of anthocyanins in cooked samples are in contradiction with the fact that anthocyanins are unstable at higher temperatures. Furthermore, the loss of anthocyanins may occur due to enzymatic oxidation during the cutting and extraction process in raw potatoes (Giusti et al., 2014). In addition, the extraction yield and accuracy of quantification of anthocyanins are often compromised by insufficient sample milling.

Humans need to obtain vitamin C from their diet due to the lack of L-gulono-γ-lactone oxidase involved in ascorbic acid (vitamin C) synthesis (Lachapelle & Drouin, 2011). As a staple food, potatoes are a good source of Vitamin C (11–40 mg/100 g fresh weight) (Navarre, Shakya, Holden, & Kumar, 2010).

The quantification of total ascorbic acid is challenging due to the

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high sensitivity of these compounds to temperature, high pH, oxygen, light, and the presence of oxidizing metals and enzymatic degradation. AA is enzymatically oxidized to DHA, which is irreversibly oxidized to diketogulonic acid (Phillips et al., 2010). Due to the high content of starch, the high activity of oxidative enzymes, and difficulties in sample milling, it is important to optimize the procedure for sample preparation and extraction in order to achieve accurate quantification of the vitamin C content in potatoes.

Total ascorbic acid is determined by reducing dehydroascorbic acid to ascorbic acid (TAA = AA + DHA). Different reducing agents have been applied to reduce DHA to AA. Dithiotreitol (DTT) has been widely used in the determination of DHA in plants and in human plasma. DTT acts best in neutral or weak alkaline conditions (Lykkesfeldt, 2000). However, in the last decade tris(2-carboxyethyl)phosphine hydrochloride (TCEP) has shown to be more efficient in reducing DHA to AA at low pH.

In the present study, we evaluated the effect of sample treatment (fresh vs. freeze-dried) and the efficiency of different reducing agents (TCEP and DTT) at varying concentrations.

The aim of this study was to improve the extraction efficiency and minimize the effect of external factors in the determination of anthocyanins and ascorbic acid in raw and cooked blue-purple potatoes, as well as to assess the effect of cooking on the retention of these compounds. Potato samples of several cultivars were included in the study in order to evaluate the performance of the methods with different cultivars.

2. Materials and methods

2.1. Samples and sample pre-treatment

For anthocyanin determination five potato cultivars were studied. A cultivar commonly known as 'Lomito Negro' bought in a market in Peru was used for the method development. The method was validated for four cultivars of purple potatoes: one purple cultivar bought in a market in Cuzco-Peru, two purple cultivars ('Synkeä Sakari' and 'Blue Congo') harvested in Muhos (Finland) in 2014, and one Chinese purple cultivar ('Black Beauty') grown in a greenhouse in Finland in 2014. Additional information concerning the cultivars is given in the Supplementary material (Supplementary Table 1). For the recovery assays, the reference compound cyanidin-3-*O*-glucoside chloride was spiked to a white cultivar bought in a market in Finland ('Rikea'). Commercial black currant juice was also used to evaluate the recovery of anthocyanins in potato samples.

For the ascorbic acid determination the three cultivars studied were 'Synkeä Sakari', 'Blue Congo' and a yellow cultivar 'Van Gogh'.

To improve the method for the determination of anthocyanins and ascorbic acid, five tubers were included in the sample of each cultivar. The tubers were cut into two halves. One half was used to quantify anthocyanins and ascorbic acid in raw samples with and without freeze-drying, and the other half was steamed and used for the quantification of these compounds with and without freeze-drying.

2.1.1. Raw samples

Raw samples were immediately immersed in liquid nitrogen after cutting and stored at −80 °C. Half of the cut samples were freeze-dried for three days. After freeze-drying, the samples were ground and stored at −80 °C until analysis. All samples were ground in a mortar with liquid nitrogen

2.1.2. Steamed samples

Unpeeled samples of five half-tubers of each cultivar were steam-cooked for 7–10 min depending on the cultivar. Cooking was stopped when a needle could easily penetrate the tubers. After cooking, the samples were cooled in an ice bath for 10 min and immersed in liquid nitrogen. One part was stored as such at −80 °C, and the rest were freeze-dried for three days and stored at −80 °C. Before analysis all samples were ground to powder in a mortar with liquid nitrogen

2.2. Chemicals

Methanol and acetonitrile of HPLC grade, certified ACS-grade HCl, and formic acid were purchased from VWR Chemicals (Fontenay-sous-Bois, France). Metaphosphoric acid (MPA) was purchased from Sigma Aldrich (Steinheim, Germany) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was obtained from Sigma Aldrich (Switzerland). Dithiotreitol (DTT) was purchased from Thermo Scientific (Lithuania). EDTA was acquired from J.T. Baker (Holland). Ultrapure water was obtained from a PureLab Ultra system ELGA LabWater (United Kingdom). Cyanidin-3-*O*-glucoside was used as an external standard for quantification of anthocyanins, and it was purchased from Extrasynthese (Genay, France). Ascorbic acid was purchased from Sigma Aldrich (China).

2.3. Extraction of anthocyanins

2.3.1. Extraction solvents

Three extraction solvents were used including acidified (0.122 M HCl) methanol and acidified aqueous 50% and 70% methanol.

2.3.2. Extraction procedure

An aliquot of a potato sample (2 ± 0.001 g of frozen ground sample or 0.2 ± 0.005 g of freeze-dried powdered sample) was weighed into a 15 mL tube. The extraction solvent (5 mL) was added and homogenized for 1 min (Ultra-Turrax IKA T 25, Staufen, Germany). The mixture was centrifuged at 1500 × *g* for 5 min (Sorvall TC-6, Rotor H-400, Du Pont, Newtown, CT, U.S.A.). The supernatant was collected and stored at −80 °C. For the second extraction, 5 mL of the extraction solvent was added to the residue, mixed vortically for 1 min and centrifuged at 1500 × *g* for 5 min. The upper layer was removed and stored at −80 °C. The third, fourth, fifth and sixth extractions were performed in the same way as the second extraction. Finally, all the six extracts were collected separately for analysis.

2.3.3. Recovery and repeatability of anthocyanin analysis

A stock solution of 0.1 mg/mL of cyanidin-3-*O*-glucoside chloride was used for the recovery assay because the compound does not exist in potatoes. A freeze-dried sample of raw white potatoes (0.2 ± 0.005 g of the cultivar 'Rikea') was spiked with 100 μL and 300 μL of the stock solution of cyanidin-3-*O*-glucoside chloride. Additionally, a black-currant juice concentrate with a known concentration of anthocyanins was used to spike the white potato samples in order to test the recovery. A sample of freeze-dried powder was weighed (0.2 ± 0.005 g) and the black currant juice concentrate was added at two different levels (40 or 80 μL). For quantitative purposes the addition of juice was also weighed accurately. After 15 min storage in the dark at room temperature, the extraction procedure continued as described in Section 2.3.2. Four extracts were collected in a 20 mL graduated flask, which was filled up to the mark. An aliquot was filtered and analyzed by HPLC-DAD. All the analyses were performed by triplicate.

2.4. Quantification of anthocyanins with HPLC-DAD

The HPLC-DAD system consisted of a GT-154 vacuum degasser, two LC-10AT pumps, a SIL-10A autosampler, a CTO-10A column oven, and an SPD-M10AVP diode array detector linked to an SCL-M10AVP data handling station (Shimadzu Corporation, Kyoto, Japan). The system was operated using the LC-Solution Workstation software. HPLC separation was carried out on a Kinetex C18 column (100 × 4.60 mm, 2.6 μm, U.S.A.) with a guard column (AJO-8946, U.S.A.). The chromatographic conditions were: flow rate, 1 mL/min; injection volume, 10 μL; mobile phase A, 5% formic acid in water; mobile phase B,