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Anthocyanin, phenolics and antioxidant activity changes in purple waxy corn as affected by traditional cooking

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ABSTRACT

Antioxidant components, including anthocyanins and phenolic compounds, antioxidant activity, and their changes during traditional cooking of fresh purple waxy corn were investigated. As compared to the raw corn, thermal treatment caused significant ($p \leqslant 0.05$) decreases in each antioxidant compound and antioxidant activity. Steam cooking preserved more antioxidant compounds than boiling. Boiling caused a significant loss of anthocyanin and phenolic compounds into the cooking water. This cooking water is a valuable co-product because it is a good source of purple pigment. By comparing levels of antioxidant compounds in raw and cooked corn, we determined that degradation results in greater loss than leaching or diffusion into cooking water. Additionally, separation of kernels from the cob prior to cooking caused increased loss of antioxidant compounds.

1. Introduction

Free radicals are known to be a major contributor to degenerative diseases of aging (Atoui, Mansouri, Boskou, & Kefalas, 2005). Dietary antioxidants might confer health-protective benefits by alleviating oxidative stress by preventing free radicals from damaging proteins, DNA and lipids (Huang, Ou, & Prior, 2005). Corn is a good source of natural antioxidants such as vitamins, carotenoids, flavonoids, and other phenolic compounds (Lopez-Martinez, Oliart-Ros, Valerio-Alfaro, Lee, & Parkin, 2009; Montilla, Hillebrand, Antezana, & Winterhalter, 2011). Accumulated evidence suggests that anthocyanin pigments in corn are responsible for its high antioxidant activities and have been shown to potentially reduce the risk of colon cancer (Hagiwara et al., 2001), prevent heart ischemia–reperfusion injury and hyperlipidemia (Toufektsian et al., 2008), anti-inflammatory effects (He & Giusti, 2010) and prevent diabetes and obesity (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003).

Waxy corn (*Zea mays* L. var. *ceratina*) is increasingly consumed in China, Korea, Vietnam, Taiwan, Laos, Myanmar and Thailand, and is harvested while immature and consumed on the cob as fresh food similar to sweet corn. Normally, this type of corn is cooked by boiling or steaming. It is known that cooking induces changes in physiological and chemical composition, influencing the concentration and bioavailability of bioactive compounds in food (Turkmen, Sari, & Velioglu, 2005). However, there are conflicting results on the effects of conventional cooking methods on dietary antioxidant levels obtained by consumers. To understand better the effects of cooking on antioxidant levels in food, it is necessary to test real cooking conditions, because the behavior of any food cannot be predicted (Oliveira, Amaro, Pinho, & Ferreira, 2010). The potential changes in anthocyanins, phenolic acids and antioxidant activity of waxy corn during thermal treatment have not been investigated yet. The aim of this work was to evaluate the effects of different domestic cooking conditions namely boiling and steaming, on kernels on or off the cob on anthocyanins, phenolics and antioxidant activity of purple waxy corn. Moreover, we determined these antioxidant compounds in residuals after cooking such as cob and water, to understand the potential for developing value-added co-products and utilizing of waste. This information may have a significant impact on consumers' selection of cooking methods and allow them to better preserve the nutritional value of their food.

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2. Materials and methods

2.1. Chemicals and reagents

Authentic anthocyanin standards: cyanidin-3-*O*-glucoside; pelargonidin-3-*O*-glucoside; peonidin-3-*O*-glucoside, Folin–Ciocalteu's phenol reagent, 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), ferulic, protocatechuic, *p*-coumaric, vanillic, caffeic, *p*-hydroxybenzoic, syringic, gallic, chlorogenic acids and Trolox (6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma–Aldrich (USA). 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was from Fluka (Switzerland). HPLC-grade methanol, acetonitrile and reagents were purchased from Labscan (Poland). All of the chemicals and reagents used in the experiments were of analytical grade.

2.2. Plant material and sample preparation

For this study, the purple waxy corn variety ''Khao Niew Dum'', developed by the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand was used. Corn was grown during October to December 2011 and recommended practices for commercial production of corn were followed. Ears were picked by hand at the milk stage (20 days after pollination; DAP). For the analyses, only physiologically undamaged ears with the mass 200–220 g were used. For raw corn, a length of 3 cm from the terminal tip end was removed from 10 waxy corn ears to reduce the kernel maturity variation. Then, kernels were manually cut from cob and dipped into liquid nitrogen to stop enzymatic activity, corn kernels and cobs were freeze-dried and finely ground with a sample mill, sieved through a 60-mesh screen, thoroughly mixed and stored at −20 °C until analysis.

Boiling and steaming were used because these are common methods of cooking fresh corn. Cooking conditions were optimized by preliminary experiments carried out for each treatment. For all cooking treatments, the minimum cooking time to reach tenderness for adequate palatability and taste was used. For cooking, the following methods were used:

(a) 10 corn ears (~2 kg) were boiled in 4L tap water in a covered stainless-steel pot and cooked for 19 min in boiling water. After cooking, boiled corn kernels were cut from the cob with a knife.
(b) A single layer 10 corn ears (~2 kg) were steamed in a covered stainless-steel steamer (45 cm diameter) for 26 min. The water in steamer was maintained boiling throughout the process to generate steam. After cooking, steamed corn kernels were separated from the cob by using a knife.
(c) Fresh cut corn kernels from 20 corn ears (2 kg) were boiled in 2L tap water in a covered stainless-steel pot and cooked for 7 min to boiling water.
(d) Fresh cut corn kernels from 20 corn ears (2 kg) were steamed in a covered stainless-steel steamer (45 cm diameter) for 12 min. The water in steamer was maintained boiling throughout the process to generate steam.

Each method was carried out in triplicate. After all cooking experiments, samples were cooled rapidly on ice. Then, specimens were frozen and freeze–dried similarly to raw corn. Cooking water was thoroughly mixed with a homogenizer prior to antioxidant analyses.

2.3. Extraction of anthocyanin determination

The anthocyanins in ground waxy corn kernels were extracted according to the method described by Rodriguez-Saona and Wrolstad (2001) and Jing, Noriega, Schwartz, and Giusti (2007) with slight modifications. Approximately 2 g of each sample were added to a flask containing 25 mL of 70% aqueous acetone acidified by the addition of HCl to 0.01% and mixed well. The flasks were shaken on a platform shaker (LabScientific Inc, USA) at 200 rpm and room temperature for 2 h. Each sample was filtered through Whatman # 1 filter paper under vacuum using a Büchner funnel, and the slurry was washed with 10 mL of acidified 70% acetone. The filtrate was transferred to a separatory funnel, and 15 mL of chloroform were added. The mixture was gently mixed by turning the funnel upside down a few times. The samples were stored overnight at 4 °C or until a clear partition between the two phases was obtained. The solution was transferred to a centrifuge tube and centrifuged at 11,538×g and 4 °C for 10 min. The upper aqueous layer containing the acetone/water mixture was collected, and the chloroform/acetone layer was carefully discarded. The residual acetone and chloroform were removed from the anthocyanin extract using a rotary evaporator at 40 °C under vacuum. The volume of the extracts was increased to 25 mL in a volumetric flask by the addition of 0.01% HCl-acidified methanol.

2.4. Determination of monomeric anthocyanin content

Monomeric anthocyanin content was measured by the pH differential method, as described by Giusti and Wrolstad (2001). A UV–vis spectrophotometer (GENESYS 10S, Thermo Scientific, USA) was used to measure the absorbance at 510 and 700 nm. Anthocyanin levels were expressed as μg of cyanidin-3-glucoside equivalents per g of dry weight (μg CGE/g DW), using the reported molar extinction coefficient of 26,900 M^{-1} cm^{-1} and a molecular weight of 449.2 g/mol.

2.5. Quantification of specific anthocyanins

Reversed-phase HPLC analysis of anthocyanins was performed using a Shimadzu system (Shimadzu, Japan) equipped with a binary pump (LC-20AC pump) and a diode array detector (SPD-M20A). Chromatographic separations were performed on an Xselect CHS C-18 column (4.6 × 250 mm, i.d. 5 μm) (Waters, USA). The composition of solvents and the gradient elution conditions used were those described by Kim et al. (2007), with slight modifications. The mobile phases used were 0.1% hydrochloric acid in methanol (15:85 v/v) (phase A) and 8% formic acid (phase B), at a flow rate of 1 mL/min. Gradient elution was performed as follows: 0–0.5 min, 0–80% phase B; 0.5–9.5 min, 80–10% phase B; 9.5–10 min, 10–15% phase B; 10–15 min, 15–5% phase B; 15–20 min, 5–80% phase B; and a re-equilibration period of 1 min with 80% phase B used between individual runs. Operating conditions were as follows: column temperature 30 °C, injection volume 20 μL, and a detection wavelength of 250–600 nm (a representative wavelength of 520 nm). Solutions were injected after being filtered through a 0.20 μm nylon membrane filter. Anthocyanins in samples were identified by comparing their relative retention times and UV spectra with those of standards and were detected using an external standard method. The results for the anthocyanins were expressed as μg per g of dry weight (μg/g DW).

2.6. Extraction of phenolic compounds and antioxidant activity determination

Free phenolic compounds in waxy corn kernels and cobs were extracted according to the method described by Adom and Liu (2002), with slight modifications. Approximately 2 g of each sample were added to a flask containing 25 mL of 80% chilled ethanol. The flask was shaken on a platform shaker (LabScientific Inc, USA) at 200 rpm at room temperature for 10 min. After centrifugation at