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Anthocyanins and antioxidant activity in coloured waxy corn at different maturation stages

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ABSTRACT

The anthocyanin content and antioxidant activity of kernels from 12 genotypes of waxy corn at two maturation stages (milk and mature) were investigated. The individual anthocyanins contained in coloured waxy corn were identified and quantified by HPLC-DAD-ESI/MS analysis. Cyanidin-3-glucoside and its derivatives were detected as being most dominant. Furthermore, acylated anthocyanins constituted 67.1–88.2% and 46.2–83.6% of the total contents at the milk and mature stages, respectively. The concentration of monomeric anthocyanin increased throughout the development of each genotype of corn. The antioxidant activity, which was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, increased with ripening. However, the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) revealed decreases in some genotypes during ripening. The kernels of a purplish black waxy corn genotype (KKU-WX111031) exhibited the greatest antioxidant activity and contained the highest level of anthocyanins among the genotypes tested at both maturation stages.

1. Introduction

Epidemiological studies have confirmed that a high dietary intake of fruits, vegetables and whole-grains is strongly associated with reduced risk of chronic diseases, such as cancer and cardiovascular disease (CVD), which are the top two causes of death in the United States and in most developing countries (Isabelle et al., 2010; Liu, 2004). These health benefits are attributed to the antioxidant compounds present in edible plants. Among these compounds, anthocyanins are naturally occurring polyphenol pigments found in many fruits, vegetables, and crops (He & Giusti, 2010). There is evidence supporting a positive association between their intake and healthy biological effects displayed *in vivo*, including anticancer, anti-inflammatory, and antioxidant characteristics (Norberto et al., 2013). Purple corn is a special cultivar of corn that is rich in anthocyanins and other functional phytochemicals, and has been regarded as a health-promoting food that is widely consumed in Peru and other Andean countries (Aoki, Kuze, & Kato,

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2002; Jones, 2005). The health benefits of anthocyanins in purple corn have been attributed to their high antioxidant activities and to other mechanisms, such as the presence of components that have been shown to potentially reduce the risk of colon cancer by inhibiting the proliferation of human colon cancer cells *in vitro* (Fukamachi, Imada, Ohshima, Xu, & Tsuda, 2001; Hagiwara et al., 2001). These compounds have also been shown to help prevent heart ischaemia-reperfusion injury, cerebrovascular diseases (Toufektsian et al., 2008), diabetes and obesity (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003).

The cultivation of waxy corn (*Zea mays* L. var. *ceratina*), which produces grain predominantly comprised of amylopectin starch, has increased in many Asian countries and has led to the development of new hybrid varieties with improved eating quality (Lertrat & Thongnarin, 2008; Perera, Lu, Sell, & Jane, 2001). This type of corn is eaten directly on the cob after cooking by boiling or steaming, similar to sweet corn. Waxy corn is harvested and consumed prior to maturity to garner the original components and to enhance its palatability. Waxy corn should ideally be harvested at various maturities according to the requirements of different foods (Hu & Xu, 2011). The phytochemical compositions and concentrations vary significantly depending on the kernel colour. Specifically, waxy corns have various kernel colours, including white, yellow, purple and black. Moreover, the pigmented corn contains more antioxidants and exhibits higher antioxidant activity than non-pigmented corn (Lopez-Martinez, Oliart-Ros, Valerio-Alfaro, Lee, & Parkin, 2009). Therefore, the interest in coloured waxy corn has increased in recent years due to consumer awareness of its diverse health benefits. Corn breeders have focused on new waxy corn hybrids containing various kernel colours to enhance the functional and antioxidant compounds found in the corn (Ji, Lee, & Yamakawa, 2010). In contrast to a dietary source with a different phytochemical composition, the corn-based phytochemicals are more easily accepted among malnourished and low-income consumers, particularly in the rural areas of developing countries (Chander, Meng, Zhang, Yan, & Li, 2008).

The individual anthocyanins of purple corn have been characterized, and these include cyanidin-3-glucoside, cyanidin-3-(6″-malonylglucoside), cyanidin-3-(3″, 6″-dimalonylglucoside), pelargonidin-3-glucoside, peonidin-3-glucoside and their malonated counterparts as the major anthocyanins (Abdel-Aal, Young, & Rabalski, 2006; Aoki et al., 2002). Furthermore, a similar anthocyanin profile was also found in corn leaves, cobs and husks (Fossen, Slimestad, & Andersen, 2001; Li et al., 2008; Zhao et al., 2008). Although data on the anthocyanins and antioxidant activity of purple corn are available, these parameters have not yet been examined in waxy corn (Mahan, Murray, Rooney, & Crosby, 2013). Hence, the anthocyanins found in coloured waxy corn were determined for the first time in this study. The main purpose of the present study was to determine the antioxidant activity exhibited by 12 genotypes of waxy corn at two maturation stages through the use of three commonly used spectrophotometric methods, namely the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, ferric ion-reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC). Moreover, the individual anthocyanins were characterized by HPLC-electrospray ionization-mass spectrometry coupled with diode array detection. The results reported in this study will be valuable to consumers, corn breeding researchers, and food and nutrition researchers.

2. Materials and methods

2.1. Chemicals and reagents

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), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma (St. Louis, MO, USA). All of the chemicals and reagents used in the experiments were of analytical grade.

2.2. Samples

In the present investigation, 12 waxy corn genotypes were selected based on the kernel colour, total anthocyanin concentration, and total phenolic concentration, which were determined in a preliminary study (Harakotr, B., Suriharn, B., Scott, M. P., & Lertrat, K., 2014). The Vegetable Corn Improvement Project of the Plant Breeding Research Center for Sustainable Agriculture at Khon Kaen University in Thailand (Fig. 1 and Table 1) collected these genotypes, which included commercial and landrace cultivars from various countries of Asia. These waxy corns were grown at the Vegetable Research Farm at Khon Kaen University during the dry season of 2011 (November 2011 through January 2012). Each genotype was planted in three replicates in a randomized complete block design. The recommended practices for the commercial production of corn were followed. Ears were harvested by hand at the milk stage (20 days after pollination; DAP) and the mature stage (35 DAP). For the analyses, only physiologically undamaged ears with mass between 200 and 220 g were used. At the milk stage, a length of 3 cm from the terminal tip end was removed from 10 waxy corn ears to reduce the kernel maturity variation. The kernels were then manually cut from the cob, frozen in liquid nitrogen to stop the enzymatic activity, and freeze-dried. The kernels from five mature ears were manually separated from the cob and then dried at 40 °C (moisture content ≤ 13%). All of the samples were finely ground in a sample mill, sieved through a 60-mesh screen, thoroughly mixed and stored at −20 °C until analysis.

2.3. Extraction

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., Livingston, NJ, 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