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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

11,538×g and 4 °C for 10 min, the supernatant was removed; extraction was repeated two more times. Supernatants were pooled and then evaporated at 40 °C under vacuum. The residue from vacuum evaporation was re-dissolved in 25 mL of 80% ethanol. Extracts were stored at −20 °C until analysis.

### 2.7. Determination of phenolic contents

The phenolic contents were determined using the Folin–Ciocalteu (F–C) method as described by Hu and Xu (2011). Briefly, the appropriate dilutions of the extracts were oxidized with F–C reagent for 90 min, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 765 nm, and the phenolic content was expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

### 2.8. Quantification of phenolic compounds

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). Butsat, Weerapreeyakul, and Siriamornpun (2009) described the composition of solvents and the gradient elution conditions used with slight modifications. The mobile phase consisted of deionized water with acetic acid (pH 2.74) (phase A) and acetonitrile (phase B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: 0–5 min, 5–9% phase B; 5–15 min, 9% phase B; 15–22 min, 9–11% phase B; 22–38 min, 11–18% phase B; 38–43 min, 18–23% phase B; 43–44 min, 23–90% phase B; 44–45 min, 90–80% phase B; 45–55 min, isocratic at 80% phase B; 55–60 min, 80–5% phase B; and a re-equilibration period of 5 min with 5% phase B used between individual runs. Operating conditions were as follows: column temperature 38 °C, injection volume 20 μL, and UV-diode array detection at 280 nm (hydroxybenzoic acids) and 325 nm (hydroxycinnamic acids). Spectra were recorded from 200 to 600 nm. Phenolic compounds 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 phenolics were expressed as μg per g of dry weight (μg/g DW).

### 2.9. Determination of antioxidant activities

The reducing ability was determined using the ferric reducing antioxidant power (FRAP) assay, which was performed according to the method described by Hu and Xu (2011). The FRAP values are expressed as μmol of Fe (II) per g of dry weight (μmol Fe (II)/g DW). The linear range of the calibration curve was 10–100 μM.

The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of radical cations of ABTS by antioxidants, was conducted as described by Lopez-Martinez, Oliart-Ros, Valerio-Alfaro, Lee, and Parkin (2009). Trolox was used as the reference compound. The results are expressed in μmol of Trolox equivalents per g of dry weight (μmol TE/g DW). The linear range of the calibration curve was 100–1000 μM.

### 2.10. Statistical analysis

All data were reported as means ± standard deviation. Data were subjected to two-way analysis of variance (ANOVA), Duncan's multiple range test (DMRT) ($p \leqslant 0.05$) was used to identify significant differences between group means and the calculated effects for the $2^2$ factorial design were obtained by using JMP Pro software (version 10.0, SAS institute Inc., Chicago, IL, USA). The Pearson correlation test was conducted to determine the correlation between variables.

## 3. Results and discussion

### 3.1. Effects of cooking conditions on monomeric anthocyanin and phenolic contents

Concentration of anthocyanin may vary among foods produced by a given plant species due to different external and internal factors, such as genetic and agronomic factors, intensity and type of light, temperature, processing and storage (de Pascual-Teresa & Sanchez-Ballesta, 2008). Raw corncob exhibited a monomeric anthocyanin content (2534.1 μg CGE/g DW) higher than that of raw corn kernels (754.0 μg CGE/g DW) (Table 1). However, the anthocyanin content in waxy corn kernels was found to be substantially higher than those reported for pigmented corn from Bolivia (19–717 μg CGE/g DW) (Montilla et al., 2011), Mexico (721 μg CGE/g DW), and the United States (307 μg CGE/g DW) (del Pozo-Insfran, Brences, Sena, Saldivar, & Talcott, 2006). The cooking process had a significant ($p \leqslant 0.05$) impact on the retention of monomeric anthocyanin content. Cooking cut-kernels by boiling resulted in the greatest decreases (60.7%), followed by boiled whole-ears (31.7%), steamed cut-kernels (19.2%), and steamed whole-ears (3.5%). The preservation of natural pigments after thermal processing is a major quality parameter. In a study of the effect of a cooking on anthocyanins in sweet potato, steaming reduced anthocyanin content by nearly half of original amount (Kim et al., 2012). Therefore, the various results indicated the importance of cooking method on nutrient retention.

The lost anthocyanin content in corn could be due to degradation or decomposition of anthocyanin upon thermal treatments (Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012). Beginning with the monomeric anthocyanin content in raw corn, it was possible calculated the percentage of loss to the cooking water and to degradation in the different cooking conditions. In the case of the whole-ear corns, boiling resulted in a higher percentage of anthocyanin degradation than steaming by around 4 times. Boiling resulted in a higher percentage of anthocyanin being degraded than was retained in the kernels. The percentage of anthocyanin in cooking water was not significant ($p \leqslant 0.05$). These suggest the decrease of anthocyanin during boiling was predominantly caused by breakdown of anthocyanins rather than their release to the cooking water. Cut-kernels showed similar result to whole-ear cooking, however, boiling resulted in a greater percentage loss of anthocyanin to cooking water than steaming up to 900-folds (Fig. 1). The stability of anthocyanin and other food pigments decreased with increasing temperature (Xu & Chang, 2008). Jing and Giusti (2007) observed a consistent decrease of protein at 100 °C in purple corn water extracts indicating a possible protein denaturation at high temperatures, which could result in anthocyanin complexation and precipitation leading to a decline in anthocyanin content.

Data on phenolics in the literature about cooked waxy corn are very limited. Total phenolic content of corn revealed a trend similar to that described for anthocyanin contents. Significant differences ($p \leqslant 0.05$) in phenolic content were found among treatments (Table 1). In the present study, boiled cut-kernels resulted in the greatest decreases (47.8%), followed by the boiled whole-ears (30.4%), steamed cut-kernels (27.5%), and steamed whole-ears (10.1%). These results are in good agreement with those reported by Turkmen et al. (2005), who found that thermal treatments decreased the total phenolics in squash, peas and leek. As compared to the boiling treatments, steaming treatments retained greater phenolic contents. These significant losses could be

**Table 1**
Anthocyanins, phenolic compounds and antioxidant activities in raw and cooked purple waxy corn kernel under different processing and cooking conditions[a].

| Treatments/Compounds | Raw kernel | Whole-ear corn | | Cut-kernel | |
|---|---|---|---|---|---|
| | | Boiling[b] | Steaming[b] | Boiling[b] | Steaming[b] |
| *Anthocyanins* | | | | | |
| MAC (μg CGE/g) | 754.0 ± 62.8 | 514.7 ± 6.3c | 727.9 ± 32.2a | 298.1 ± 36.0d | 609.5 ± 11.3b |
| Cy-3-Glu (μg/g) | 121.6 ± 6.8 | 61.8 ± 1.9c | 82.9 ± 3.1a | 40.3 + 1.3d | 72.0 ± 2.5b |
| Pg-3-Glu (μg/g) | 46.7 ± 1.8 | 35.7 ± 1.3b | 40.7 ± 1.9a | 15.2 + 1.3c | 37.6 ± 0.9ab |
| Pn-3-Glu (μg/g) | 39.0 ± 1.8 | 23.4 ± 0.5c | 28.9 ± 0.8a | 11.2 + 0.1d | 24.3 ± 0.3b |
| *Phenolic compounds* | | | | | |
| TPC (mg GAE/g) | 6.9 ± 0.4 | 4.8 ± 0.2b | 6.2 ± 0.2a | 3.6 ± 0.1c | 5.0 ± 0.2b |
| PCCA (μg/g) | 9.9 ± 0.3 | 7.0 ± 0.2c | 14.1 ± 0.3a | 4.8 ± 0.2d | 12.0 ± 0.1b |
| *p*-OH (μg/g) | 1.8 ± 0.1 | 0.4 ± 0c | 1.5 ± 0a | 0.3 ± 0.1d | 1.1 ± 0.1b |
| VA (μg/g) | 9.8 ± 0.9 | 6.2 ± 0.4b | 8.6 ± 0.5a | 2.3 ± 0.5c | 6.8 ± 0.3b |
| CFA (μg/g) | 2.9 ± 0.1 | 1.5 ± 0.1b | 2.3 ± 0.2a | 1.1 ± 0c | 1.4 ± 0.1bc |
| *p*-CA (μg/g) | 11.0 ± 1.4 | 7.5 ± 0.3b | 9.4 ± 0.2a | 5.8 ± 0.6c | 8.0 ± 0.4b |
| FA (μg/g) | 23.1 ± 0.6 | 17.8 ± 0.8b | 20.4 ± 0.7a | 9.0 ± 0.9d | 15.1 ± 0.6c |
| *Antioxidant activities* | | | | | |
| FRAP (μmol Fe(II)/g) | 102.3 ± 6.5 | 72.2 ± 1.1b | 81.8 ± 0.8a | 43.6 ± 1.9d | 54.9 ± 3.4c |
| TEAC (μmol TE/g) | 94.6 ± 2.5 | 67.4 ± 5.5b | 90.6 ± 2.5a | 45.6 ± 3.7c | 62.7 ± 3.8b |

[a] Values are means ± SD. MAC, monomeric anthocyanin content; Cy-3-Glu, cyanidin-3-glucoside; Pg-3-Glu, pelargonidin-3-glucoside; Pn-3-Glu; peonidin-3-glucoside, TPC, total phenolic content; PCCA, protocatechuic acid; *p*-OH, *p*-hydroxybenzoic acid; VA, vanillic acid; CFA, caffeic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity.
[b] Means in the same lines with different letters are significant ($p \leqslant 0.05$) determined by Duncan's multiple range test (DMRT).

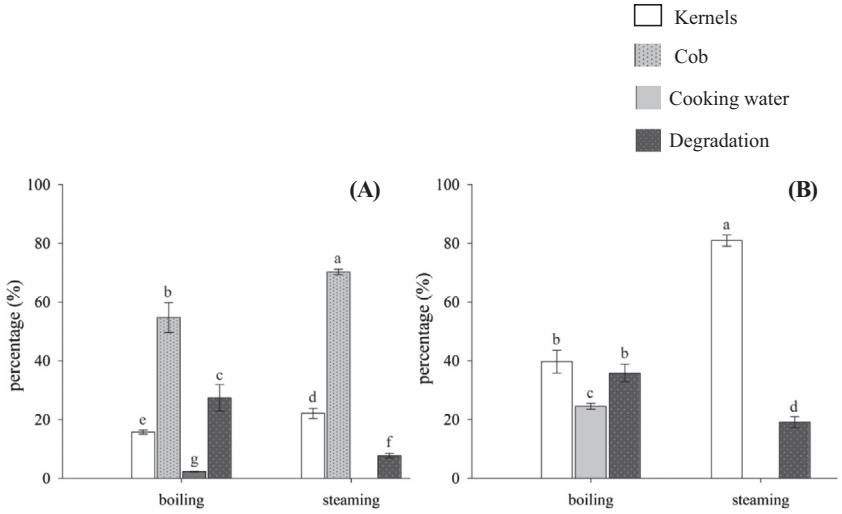


**Fig. 1.** Percentage of total monomeric anthocyanin content in cooked, cooking water and degradation of whole-ear corns (**A**) and cut-kernels (**B**) cooking as effect of different cooking conditions. Values are expressed as the mean ± SD based on the initial contents in raw corn in dried weight.

attributed to water soluble phenolics leaching into cooking water as well as breakdown of phenolics during thermal processing. Similar to whole-ear anthocyanins, boiling showed a higher percentage of phenolic degradation than steaming around 3 times, and the percentage of phenolics in cooking water was not significant ($p \leqslant 0.05$). Additionally, the percentage of phenolics retained in kernels after boiling was less than the percentage lost due to degradation. In the case of cut-kernels, steaming resulted in greater retention of phenolics in the kernels (73.0%) than boiling (53.0%). These results indicated that phenolics were reduced by boiling by nearly half of original content. Phenolic content degradation showed a trend similar to that described for whole-ear cooked corn. However, boiling caused phenolics to leach into cooking water nearly 7 folds more than steaming (Fig. 2). These results support the hypothesis that separation of kernels from the cob caused loss of phenolics. Thermal treatment does not always result as the destruction of bioactive compounds. In some cases, cooking can induce the formation of novel compounds and improve antioxidant properties (Xu & Chang, 2008). Cooking was found to give rise to an increase in the level of phenolic compounds in sweet corn (Dewanto, Wu, & Liu, 2002). Various effects can be attributed easily to the diversity of food products used and lack of the standardization of domestic cooking methods (Irina & Mohamed, 2012).

### 3.2. Effects of cooking conditions on anthocyanin compositions

The concentration of cyanidin-3-glucoside decreased significantly across all cooking methods. Boiled cut-kernels resulted in the greatest decreases (66.9%), followed by the boiled whole-ears (49.2%), steamed cut-kernels (40.8%), and steamed whole-ears (31.8%) (Table 1). Pelargonidin-3-glucoside and peonidin-3-glucoside were also decreased in the same manner; however, total losses were lower than those of cyanidin-3-glucoside (except peonidin-3-glucoside in boiled cut-kernels). In contrast, peonidin 3-glucoside was lost to the greatest degree among all anthocyanins in black soybean during thermal processing (Xu & Chang, 2008).

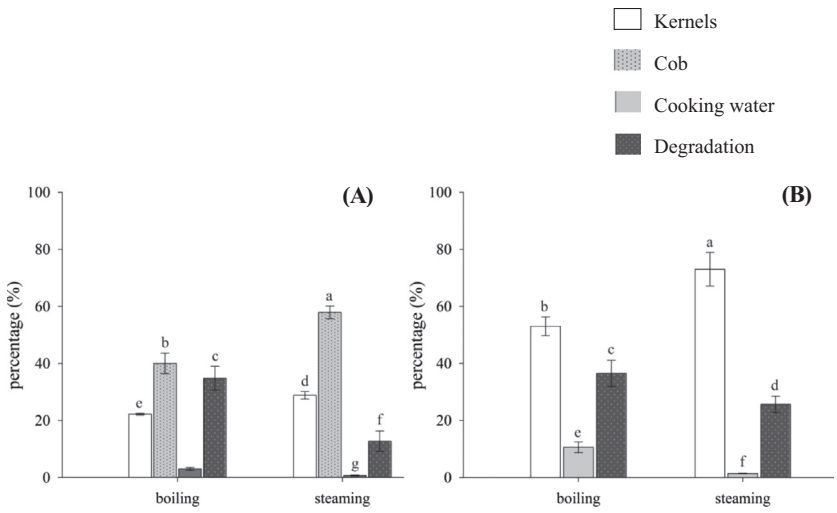


**Fig. 2.** Percentage of total phenolic content in cooked, cooking water and degradation of whole-ear corns (**A**) and cut-kernels (**B**) cooking as effect of different cooking conditions. Values are expressed as the mean ± SD based on the initial contents in raw corn in dried weight.

This study observed that pelargonidin-3-glucoside had the greatest thermal stability of all anthocyanins, while, Sadilova, Stintzing, and Carle (2006) suggested that additional hydroxylation apparently did not affect pigment stability when pelargonidin-3-glucoside was compared to cyanidin-3-glucoside.

It is well known that anthocyanins are readily degraded when exposed to heat, resulting in dramatic impact on color and their health-promoting properties. Degradation starts with opening of the central ring followed by hydrolysis of the molecule, establishing colorless products. In the case of whole-ear corns, steaming retained individual anthocyanin concentration better than boiling. As regards content and percentage of anthocyanin degradation similarly to retain of their content in kernels; however, most of anthocyanin content was preserved in the cob (Table 2). Additionally, pelargonidin-3-glucoside does not show degradation, in fact the concentration of pelargonidin-3-glucoside in the cob slightly increased after cooking with either cooking method. This could be because the cob absorbs this compound from the cooking water. Bunea et al. (2008) suggested that the increase in concentrations of certain bioactive compounds after thermal treatment may be explained either by their better release from the food matrix as a result of the breakdown of supramolecular structures containing functional groups or their thermal stability. In the case of the cut-kernels, similar to whole ears, it was found that steam cooking resulted in retention of more anthocyanins than boiling. Additionally, boiling resulted in a greater percentage of anthocyanin degradation than the percentage retained in the kernels (Table 3). Steam cooking showed a lower percentage of pelargonidin-3-glucoside and peonidin-3-glucoside degradation than those of boiling. However, cyanidin-3-glucoside degradation was not significant different between steaming and boiling ($p \leqslant 0.05$), but total of anthocyanin degradation together with cooking water was 2 times less than that of boiling. Either anthocyanin was not detected by HPLC in steaming water from whole-ears or cut-kernels, mostly the leaching effect on these compounds might not occur. Additionally, after cooking whole-ears retained anthocyanin content better than cut-kernels. This is possibly because when kernels were removed from the cob, the pericarp was ruptured, which may reduce the barrier to migration into the cooking water as well as increase the surface area exposed to the cooking medium. Processing such as peeling, trimming, chopping, slicing, crushing, pressing, and sieving was expected to affect content, activity, and availability of antioxidant composition (Ioannou et al., 2012). The resulting pigmented boiling water is a potentially valuable co-product and can be simmered and mixed with pineapple, sugar, juices and spices for cooked beverages such as "Chicha Morada" (Aoki, Kuze, & Yashiaki, 2002). Moreover, pigmented boiling water may be used to cook rice or soak glutinous rice before cooking to make purple rice, and therefore, may be a suitable replacement for others, more expensive source of purple color such as black rice or Asian pigeon wing (*Clitorea ternatea*), a traditional source of purple/blue colorant in Asia.

### 3.3. Effects of cooking conditions on phenolic compounds

This work focused on free phenolic acids because it has been shown that the bioavailability of soluble phenolic acids is

**Table 2**
Distribution in percentage of anthocyanins and phenolic compounds in cooked, cooking water and degradation of whole-ear cooking in two cooking conditions[a].

| Compounds | Boiling | | | | Steaming | | | |
|---|---|---|---|---|---|---|---|---|
| | Kernel | Cob | Water | Degradation | Kernel | Cob | Water | Degradation |
| Cy-3-Glu | 12.0 ± 0.5e | 61.6 ± 0.9b | 5.0 ± 0.4f | 21.4 ± 1.2c | 16.2 ± 1.0d | 79.2 ± 2.9a | – | 4.6 ± 2.1f |
| Pg-3-Glu | 20.8 ± 0.3d | 77.7 ± 2.2b | 5.3 ± 0.4e | – | 23.8 ± 1.9c | 86.5 ± 2.6a | – | |
| Pn-3-Glu | 10.5 ± 0.7f | 54.0 ± 1.0b | 2.9 ± 0.8g | 32.6 ± 2.2g | 12.9 ± 0.3e | 71.3 ± 0.7a | – | 15.8 ± 0.8d |
| PCCA | 28.5 ± 1.8d | 31.2 ± 5.7cd | 3.2 ± 0.4e | 37.1 ± 3.9bc | 54.1 ± 1.5a | 42.6 ± 4.4b | 0.7 ± 0.1e | 3.4 ± 2.7e |
| *p*-OH | 9.7 ± 1.3e | 28.5 ± 1.0c | 24.7 ± 2.3d | 37.1 ± 2.4a | 34.0 ± 0.7b | 37.4 ± 1.2a | 2.3 ± 0.3f | 26.3 ± 0.8cd |
| VA | 22.3 ± 1.6d | 38.9 ± 1.6b | 5.0 ± 1.8ef | 33.7 ± 1.4c | 31.2 ± 2.1c | 60.5 ± 3.1a | 0.7 ± 0.1f | 7.6 ± 4.7e |
| CFA | 18.6 ± 0.6d | 41.5 ± 3.7b | 9.4 ± 0.7e | 30.5 ± 2.8c | 28.1 ± 4.2c | 51.0 ± 6.5a | 1.3 ± 0.1f | 19.7 ± 4.3d |
| *p*-CA | 23.0 ± 0.6e | 39.5 ± 1.3b | 1.4 ± 0.2g | 36.2 ± 0.6c | 28.7 ± 1.3d | 53.2 ± 1.9a | 0.5 ± 0.1g | 17.6 ± 2.9f |
| FA | 29.6 ± 0.3d | 47.0 ± 0.7b | 11.2 ± 1.1e | 12.2 ± 1.6e | 33.9 ± 1.4c | 59.4 ± 2.4a | 1.2 ± 0.1g | 5.4 ± 2.1f |

[a] Values are means ± SD. Means in the same lines with different letters are significant ($p \leqslant 0.05$) determined by Duncan's multiple range test (DMRT). The abbreviations in the column of compounds correspond to the same compounds represented in Table 1. Values were calculated based on the initial contents in raw corn in dried weight.

**Table 3**
Distribution in percentage of anthocyanins and phenolic compounds in cooked, cooking water, and degradation of cut-kernel cooking in two different cooking conditions[a].

| Compounds | Boiling | | | Steaming | | |
|---|---|---|---|---|---|---|
| | Kernel | Water | Degradation | Kernel | Water | Degradation |
| Cy-3-Glu | 33.2 ± 1.9bc | 26.4 ± 2.6c | 40.4 ± 3.8b | 59.5 ± 6.0a | – | 40.5 ± 6.0b |
| Pg-3-Glu | 32.8 ± 5.7bc | 28.6 ± 4.3c | 38.7 ± 4.1b | 80.6 ± 3.9a | – | 19.4 ± 3.2d |
| Pn-3-Glu | 28.6 ± 1.8d | 27.3 ± 5.5d | 44.1 ± 6.9b | 63.8 ± 0.5a | – | 36.2 ± 0.5c |
| PCCA | 48.3 ± 1.2b | 10.5 ± 1.1d | 41.2 ± 0.2c | 60.0 ± 3.6a | – | – |
| *p*-OH | 15.3 ± 4.0c | 62.0 ± 2.5a | 22.7 ± 1.9c | 64.3 ± 5.8a | – | 34.6 ± 5.8b |
| VA | 24.7 ± 4.4c | 30.1 ± 5.9c | 45.2 ± 9.6b | 71.2 ± 0.9a | 1.1 ± 0.3d | 27.7 ± 1.2c |
| CFA | 38.2 ± 3.0b | 35.5 ± 5.5b | 26.3 ± 3.6c | 47.8 ± 0.4a | – | 52.0 ± 0.4a |
| *p*-CA | 52.7 ± 5.7b | 6.3 ± 0.2d | 41.0 ± 5.5b | 73.5 ± 9.3a | – | 26.4 ± 9.3c |
| FA | 38.8 ± 4.2b | 16.6 ± 2.1d | 44.5 ± 6.2a | 65.5 ± 5.3a | 4.6 ± 1.0e | 29.9 ± 4.4c |

[a] values are means ± SD. Means in the same lines with different letters are significant ($p \leqslant 0.05$) determined by Duncan's multiple range test (DMRT). The abbreviations in the column of compounds correspond to the same compounds represented in Table 1. Values were calculated based on the initial contents in raw corn in dried weight.

considerably higher than that of the insoluble phenolic acids (Zhao, Egashira, & Sanada, 2004). To the best of our knowledge, phenolic acid profiles in processed waxy corn have not been systematically investigated. Three benzoic type phenolic acids (protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid) and three cinnamic type phenolic acids (caffeic acid, *p*-coumaric acid and ferulic acid) were detected in both raw and cooked waxy corn. Gallic acid, syringic acid and chlorogenic acid were not detected in either kernels or cob (data not shown). The predominant phenolic acids in both raw and cooked purple waxy corn were ferulic, followed by *p*-coumaric and protocatechuic acids. In comparison to original raw corn, both thermal treatments caused significant ($p \leqslant 0.05$) decreases in all measured phenolic compounds (except protocatechuic acid cooked by steaming) (Table 1). The thermal treatment resulted in a 16.7–83.3% loss of *p*-hydroxybenzoic acid, 12.2–76.5% of vanillic acid, 20.7–62.1% caffeic acid, 14.5–47.2% of *p*-coumaric acid, and 11.7–61.0% of ferulic acid compared to uncooked corn. The steamed whole-ear retained the greatest proportion of phenolic acids, followed by steamed cut-kernels, boiled whole-ears and boiled cut-kernels, respectively. However, steamed cut-kernels and boiled whole-ears were not different in vanillic, caffeic and *p*-coumaric acid but were lower in ferulic acid. Boiling had a more detrimental effect on phenolic acids than steaming. The boiling treatment causes of disruption cellular components with the consequent release of these molecules into the cooking water (Migilo, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). Moreover, the steaming treatment could cause matrix softening and increased extractability of antioxidant components from the raw materials (Huang, Chang, & Shao, 2006). The protocatechuic acid slightly increased during steam cooking of cut-kernels, indicating thermal treatment could cause changes in phenolic substances in the corn by liberating the bound phenolic compounds into free form (Xu & Chang, 2009). Hiemori, Koh, and Mitchell (2009) found that this compound was generated with concomitant cyanidin-3-glucoside degradation during thermal processing.

With its solubility in water, free phenolic acid can undergo large losses during corn processing due to considerable leaching to the water used in cooking. This study found a high content of phenolics in the water, especially in the boiling treatment, which produced higher levels of phenolic compounds than steaming in both whole-ear corns and cut-kernels (Tables 2 and 3). This result could be attributed to the losses of phenolic compositions in cooking water. However, the total of phenolic compounds in cooked samples and cooking water was similar to the amount of phenolics in raw samples. Differences in phenolic content could be due to breakdown of phenolics, since the sum of phenolics in cooked samples and cooking water consistently differed from their content in raw samples. Degradation of phenolics in corn may be a more serious problem than leaching. The percentage of these substances undergoing degradation was higher than the percentage in cooking water in either whole-ear corns or cut-kernels, except *p*-hydroxybenzoic and caffeic acid in boiled cut-kernels. Moreover, protocatechuic, *p*-hydroxybenzoic, caffeic and *p*-coumaric were not found in the water used to steamed cut-kernels (Tables 2 and 3). These results allow us to conclude that degradation of phenolic acids was greater than losses due to leaching into cooking water. On the other hand, the different results could be attributed to several factors including the type of material used, cultivars, cooking medium and vessels, cooking time, pressure, and energy (Ioannou, Hafsa, Hamdi, Charbonel, & Ghoul, 2012). Some authors have indicated that pressure-cooking enhanced the antioxidant compositions and palatability of vegetables (Xu & Chang, 2009). However, higher power also could result in greater degradation (Hiemori et al., 2009). Further studies on pressure-cooking of antioxidant compositions in corn of this type need to be conducted.

### 3.4. Effects of cooking conditions on antioxidant activity

Thermal treatments are generally regarded as being destructive to antioxidants. Antioxidant activities of raw and cooked corn, including FRAP and TEAC assays are presented in Table 1. Significant differences ($p \leqslant 0.05$) in FRAP values were found among most treatments. Boiled cut-kernels had the greatest decrease (57.4%), followed by boiled whole-ears (46.3%), steamed cut-kernels (29.4%), and steamed whole-ears (20.0%). The TEAC values were similar to FRAP, however, the boiled whole-ear was not differed with steamed cut-kernels. These results allow us to conclude steam cooking preserved greater antioxidant content as compared to the boiling in all treatments by all assays. Other studies have suggested that boiling is generally regarded as being destructive to antioxidant components (Krishnaswamy & Raghuramulu, 1998). On the contrary, the antioxidant activities were increased for several vegetables such as carrots, spinach, mushroom, asparagus, broccoli and cabbage after thermal treatment (Halvorsen et al., 2006). The loss in antioxidants from cooked corn can be attributed to synergistic combinations or interactions of several types of chemical reactions, diffusion of water soluble compounds, and the formation or breakdown of them. Therefore, we investigated the antioxidant activity of cooking water. Boiling water had high antioxidant values, especially in water from boiled cut-kernels, followed by whole-ears; whereas, steaming water had low values of both antioxidant activity assays (Tables 2 and 3). However, the sum of antioxidant activity of the cooked corn and cooking water is different from antioxidant activity of raw materials. These results may suggest losses in their antioxidants are due to breakdown of antioxidant compounds.

**Table 4**
Correlations between antioxidant activities, three specific anthocyanins and phenolic compounds found in waxy corn[a].

| Correlation coefficient ($r$) | FRAP | TEAC |
|---|---|---|
| Monomeric anthocyanin content | 0.77** | 0.90** |
| Cyanidin-3-glucoside | 0.77** | 0.89** |
| Pelargonidin-3-glucoside | 0.80** | 0.82** |
| Peonidin-3-glucoside | 0.83** | 0.89** |
| Total phenolic content | 0.83** | 0.95** |
| Protocatechuic acid | 0.60* | 0.79** |
| *p*-Hydroxybenzoic acid | 0.58* | 0.77** |
| Vanillic acid | 0.83** | 0.90** |
| Caffeic acid | 0.85** | 0.91** |
| *p*-Coumaric acid | 0.80** | 0.87** |
| Ferulic acid | 0.94** | 0.92** |

[a] Values were from triplicate determinations on raw corn and the corn cooked by two cooking methods. FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity.
*,** The correlation is significant at $p \leqslant 0.05$ and 0.01, respectively.

### 3.5. Correlations of anthocyanin, phenolic compounds and antioxidant activity

The linear correlation coefficients between selected predominant anthocyanins, phenolic compounds and overall antioxidant activities of corn are presented in Table 4. In the case of anthocyanin, the three specific anthocyanins (cyanindin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside), exhibited significant ($p \leqslant 0.01$) and high correlation with antioxidant activities (0.77**–0.89**). In the case of phenolic compounds, most of the hydroxybenzoic types (protocatechuic, *p*-hydroxybenzoic and vanillic acid) and hydroxycinnamic types (caffeic, *p*-coumaric and ferulic acid), exhibited significant ($p \leqslant 0.01$) and moderate to high correlation with the antioxidant activities. The TEAC value correlated with the phenolic compounds in the same manner. These results suggest that hydroxycinnamic phenolics are more effective antioxidant than hydroxybenzoic phenolics. The electron-withdrawing property of the carboxylate group in benzoic acids has a negative influence on the H-donating abilities of the hydroxyl benzoates which is reflected in the FRAP and TEAC assays (Rice-Evans, Miller, & Paganga, 1996). In contrast, Xu and Chang (2009) found that benzoic and cinnamic acids were not different in correlation with antioxidant activity in black beans.

## 4. Conclusions

Boiling and steaming significantly affected the total anthocyanin content, individual anthocyanins, total phenolic content, individual phenolic compounds and antioxidant activities of cooked corn. The extents of the changes depend upon the processing and cooking methods. A greater quantity of antioxidant compounds and antioxidant activity could be recovered by consuming steamed corn, as compared with corn prepared by boiling. Furthermore, kernels that were removed from the cob prior cooking had grater losses of antioxidants and their activity than kernels cooked on the ear. Cooking water was a source of abundant antioxidants and may be a valuable cooking co-product. We can conclude that by careful selection of cooking practices, it is possible to preserve the content of nutrients in corn.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.05.069.

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