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Effects of domestic cooking on enzyme activities, bioactives and antioxidant capacities in mini-ear supersweet corn

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Abstract

Sweet corn is a rich source of bioactives. Naulthong (*Zea mays* L. var *saccharata* Bailey) is new hybrid bicolor mini-ear supersweet corn. The Naulthong corn was prepared in whole-ear and cut-piece forms, boiled (ca. 100°C), atmospheric-steamed (ca. 100°C), and pressure-steamed (ca. 15 lb/in2, 121°C) for 4-20 min, and subsequently determined for lipoxygenase (LOX) and peroxidase (POD) activities. It was found that both enzyme activities were inactivated more effectively in kernels than in cobs. It was worth noting that LOX activity was completely inactivated by the three cooking methods at 8 min, regardless of serving form, while POD in cobs needed longer times of 12 and 16 min to inactivate for cut-piece and whole-ear corn, respectively. The corn on the cob of both serving styles was cooked at the selected cooking times using the three cooking methods, then, the edible part (corn kernels) was investigated for physical and chemical attributes. It was found that all cooked kernels had visually brighter color with higher redness (a^*) and yellowness (b^*, $p \leq 0.05$). There was no significant difference in lightness ($p > 0.05$). Boiling caused more effective heat transfer and leaching, resulting in the lowest firmness and total soluble solids ($p \leq 0.05$). The two steaming methods showed no significantly different color and firmness. In both serving forms, atmospheric-steamed kernels had higher lutein, zeaxanthin, ferulic acid, total phenolic contents, and antioxidant capacities than those of pressure-steamed and boiled kernels. Boiling resulted in reduction of antioxidant capacities by means of DPPH and ABTS assays, but not for FRAP assay. Steaming at normal pressure, rather than at high pressure, is endorsed for corn cooking in order to maximize health benefits from the delivery of bioactives.

Introduction

Sweet corn (*Zea mays* L. var. *saccharata*) is a rich source of bioactive compounds, i.e. lutein, zeaxanthin, ferulic acid and phenolic compounds, and also has high dietary fiber content (Lui, 2007; Hu and Xu, 2011). These provide health benefits by preventing non-communicable diseases such as cardiovascular diseases, diabetes and cancers (Thompson, 1994; Meyer *et al*., 2000; Jacobs *et al*., 2001; Lui, 2007). Lutein and zeaxanthin, the key carotenoids found in corn, play an important role in macula lutea, and their unique presence has been proposed to protect the eyes from free radicals and near-UV blue light (Stahl and Sies, 2002b; Wenzel *et al*., 2003). Lutein and zeaxanthin could reduce the risk of cataracts and age-related macular degeneration, which are the leading causes of blindness among the elderly (Brown *et al*., 1999; Chasan-Taber *et al*., 1999). Furthermore, phenolic compounds and ferulic acid could prevent the risk of Alzheimer's disease, diabetes, liver and colon cancers, and muscular fatigue, as well as allow improvement in hypertension, cataracts and age-related functional decline (Slavin *et al*., 2000; Temple, 2000). However, these bioactives are sensitive to oxidation, resulting in degradation due to both enzymic and non-enzymic reactions.

Raw or unblanched vegetables are deteriorated by peroxidase, lipoxygenase, lipase, catalase, protease, etc. (Kaur *et al*., 1999). Peroxidase (POD) and lipoxygenase (LOX) are oxidoreductases, commonly found in fruits and vegetables, and are key enzymes for blanching. POD and LOX cause deteriorations of eating quality of corn, such as off-flavors and odors, after harvesting and processing (Lee and Hammes, 1979; Rodiguez-Saona *et al*., 1995; Morales-Blancas *et al*., 2002). POD is the most heat-tolerant enzyme and is used as an index for efficiency of blanching. However, LOX has been suggested as a key enzyme for blanching in many fruits and

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vegetables due to its catalysis of essential fatty acids, off-flavor development, and carotene and chlorophyll bleaching (Chen and Whitaker, 1986; Williams *et al.*, 1986; Lim *et al*., 1989; Sheu and Chen, 1991; Theerakulkait *et al.*, 1995). LOX is more sensitive to heat than POD, resulting in shorter heating time. The enzyme activities are dependent on cultivar, part of plant, maturity, cultivation area, etc.

Corn on the cob is generally cooked by boiling or steaming prior to eating. Qualities of food are affected by cooking methods in different ways, including enzyme activities, depending on temperature, time, size, etc. (Kaur *et al*., 1999). Barrett and Theerakulkait (1995) reported that LOX and POD in supersweet corn were inactivated at 93°C at 6-9 and 18-20 min, respectively. Food processing not only prolongs shelf life but also proposes new value-added products from agricultural produce. Changes in bioactives and antioxidant capacities after food processing depended on cooking methods (Olivera *et al.*, 2008; Ruiz-Rodriguez *et al*., 2008; Yuan *et al*., 2009). Processed sweet corn had higher contents of lutein, zeaxanthin, ferulic acid, total phenolics and total antioxidant capacities than those of fresh corn (Dewanto *et al*., 2002, Scott and Eldridge, 2005). Thermal processing may release more bound bioactive compounds due to the breakdown of cellular constituents. Disruption of cell walls also releases oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables. However, thermal processing will inactivate these enzymes, thus avoiding the loss of phenolic acids (Chism and Haard, 1996; Dewanto *et al*., 2002). This study was aimed to investigate the impact of cooking methods on the inactivation of LOX and POD activities in both kernels and cobs, and also determine bioactives and antioxidant capacities of cooked mini-ear supersweet corn, Naulthong *var*., in serving styles of whole-ear and cut-piece corn on the cob. The information would be useful to nutritionists, food manufacturers and consumers for considering cooking methods to inactivate the enzymes that cause deterioration in corn during storage, and preserve bioactives and quality of cooked corn.

Materials and Methods

Plant materials and sample preparation

Mini-ear supersweet corn, Naulthong *var.*, was obtained from the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University. The corn ears were harvested at 18 days after pollination (DAP), and then immediately handled at the food pilot plant, Faculty of Technology, Khon Kaen University for preparation, cooking and analysis. All chemicals used were of analytical grade.

The mini-ear corn was dehusked, desilked and brushed-cleaned. The corn was prepared in two serving styles of whole-ear (10-12 cm in length, 3-4 cm in diameter) and cut-piece corn (5 ± 0.2 cm in thickness). Both serving styles of corn were cooked in boiling water (ca. 100°C, corn-water ratio of 1:2), atmospheric steaming (ca. 100°C), and pressure steaming (ca. 15 lb/in2, 121°C) for 4, 8, 12, 16 and 20 min, then the corn was cooled in tap water. The heating water remained boiling throughout the cooking periods. All untreated (fresh) and cooked samples were vacuum-packed, cryogenically frozen by immersing into liquid nitrogen, and kept at -18°C as means of sample stabilization. The samples were thawed at 4°C for 12 h prior to chemical analysis. The LOX and POD activities were determined.

Determination of enzyme activity

Ground corn kernels or cobs (2 g) were weighed, transferred into centrifuge tubes, added with 20 mL of 0.1 M Tris-HCl buffer at pH 8.0, and then mixed for 5 min. The suspension was centrifuged at 10,000 rpm (Legend-Mach 1.6R, Thermo Fisher Scientific, Germany) for 30 min at 4°C, and the supernatant was collected as crude extract prior to analysis.

POD activity was determined by the spectrophotometric method with slight modification from Boyes *et al.* (1997). The substrate solution was prepared by mixing guaiacol (560 µl) and 30% H_2O_2 (195 µl), and increasing the volume to 100 mL using 0.2 M sodium phosphate buffer at pH 6.5. The cocktail (3.6 mL) was transferred to a 1 cm-path cuvette and the POD reaction was started by adding 0.15 mL of crude enzyme extract. POD activity was measured for the increase in absorbance at 420 nm using the LAMBDA 25 Spectrophotometer (PerkinElmer, USA).

LOX activity was performed spectrophotometrically following Tangwongchai *et al*. (2000) with slight modification. The substrate solution was prepared by mixing 7.1 mL of 1% linoleic acid in absolute ethanol, 0.2 mL of Tween-20, and 10 mL of 1% absolute ethanol. The mixture was rotary-vacuum evaporated until dry at 40oC for 30 min. Then, the residue was reconstituted with 150 mL of 0.05 M Na_2HPO_4 and adjusted to pH 6.5 with HCl. The substrate solution (3.6 mL) was transferred into a cuvette and the measurement was started by adding 0.15 mL of crude enzyme extract. LOX activity was monitored for the increase in absorbance at 234 nm for 15 min using LAMBDA 25 Spectrophotometer (PerkinElmer, USA). One unit of enzyme activity

was defined as an absorbance change of 1.0 per min. The results were expressed as units of activity per gram of fresh weight of tissue (unit/g fresh weight (fw)).

Then the mini-ear supersweet corn were cooked to inactivate LOX and POD activities by boiling, atmospheric steaming and pressure steaming, for 16 min (whole-ear corn) and 12 min (cut-piece corn). The uncooked and cooked kernels were detached from cobs after treatments, and then investigated for color, texture, total soluble solid (TSS), bioactive contents and antioxidant capacities.

Color, texture and total soluble solid

Color parameters (CIELAB: L^*, a^* and b^*) was measured using the Hunter LabScan (UltraScan XE, Hunter Associates Laboratory, Inc., USA). Texture of corn kernels was determined by puncture tests using the TA.XT2 Plus texture analyzer with P/2 cylinder probe (Stable Micro Systems, Surrey, England). The single kernels were 50% strain compressed individually. The average maximum force (g) was reported as firmness. Total soluble solid (TSS) measurement was performed using the 0-32% Brix hand refractometer (ATAGO, Japan).

Determination of lutein and zeaxanthin contents

The bioactive extraction was done as per Galicia *et al.* (2009). Ground corn kernels of 500 mg underwent 5 min ethanol precipitation (100 mL of ethanol containing 1% butylated hydroxytoluene (BHT)) at 85°C before saponification with 120 µL of potassium hydroxide (1 g of KOH dissolved in 1 mL of H_2O) for 6 min. They were then immediately placed in an ice bath and 4 mL of cold deionized distilled H_2O were added. Each sample was added with a 3 mL mixture of petroleum ether (PE) and diethyl ether (DE) (at 2:1 ratio), mixed and centrifuged for 10 min at 1400 g. The upper layer was pitted into a separate test tube, and the pellet was re-extracted twice more using the same solvent mixture. Each PE-DE fraction was accumulated and centrifuged for 10 min. It was then dried using a rotary evaporator, and the extract was reconstituted with 3 mL of HPLC mobile-phase mixture (acetonitrile-dichloromethane-methanol ratio of 70:20:10) prior to injection into the HPLC (Waters, USA).

Lutein and zeaxanthin contents were quantified by the HPLC method which was slightly modified from Kurilich and Juvik (1999). Briefly, the corn extracts were diluted with the mobile-phase mixture prior to analysis. The separation was carried on the YMC carotenoid C30 column (4.6 x 250 mm, 5 µm) with the isocratic elution of the mobile phase at flow rate of 1 mL/min using the Waters 484 UV-visible detector at 450 nm. Detector signals were acquired and integrated by the Waters Millennium 32 software (Waters, USA). Peak identification of lutein and zeaxathin in sample extracts was based on retention time and chromatography of authentic lutein and zeaxanthin standards (Chromadex, USA). The stock solutions of standard lutein and zeaxanthin were prepared at concentration of 50 mg/L in the mobile-phase mixture. Calibrated standard solutions were prepared in the range of 5–50 mg/L. The relation of the peak areas of the analytes against the lutein and zeaxanthin concentration was used as calibration curve.

Determination of ferulic acid contents

Extraction of ferulic acid was done as per Adom and Liu (2002) with slight modifications. Ground corn kernels (2 g) were extracted with 40 mL of 1 M NaOH was added and sonicated for 3 h. Samples were filtered through the Whatman No. 40 filter paper. The filtered samples were normalized with 20 mL of 2 M HCl. Then, the samples were added with 30 mL of ethylacetate, shaken, and allowed to be separated into two layers. The top layer was isolated and centrifuged at 5,000 g for 5 min. The ethylacetated supernatant was separated from the residue. Then, the residue was collected together with the bottom layer and re-extracted four times. The accumulated supernatant was dried in a rotary evaporator at 40°C and 250 mbar for 30 min. The extract was frozen at -30°C until analysis. It was then reconstituted in a 3 mL mixture of deionized water and methanol (at 1:1 ratio) prior to injection into the HPLC. The content of ferulic acid was determined by the HPLC method following Butsat *et al*. (2009) with slight modification. Briefly, the extract was added a 3 mL mixture of deionized water and methanol (at 1:1 ratio) prior to analysis. Ferulic acid was isolated on a symmetry reversed-phase C18 column (3.9 x 150 mm, 5 µm). The mobile phase consisted of 1.0% acetic acid (A) and acetonitrile (B) using a gradient program of 3% B at 0-15 min, 35-100% B at 16-39 min and 3% B at 40-50 min with flow rate of 1 mL/min. A 484 UV-visible detector (Waters, USA) at a wavelength of 280 nm was used to monitor the separation of ferulic acid. Detector signals were acquired and integrated by the Waters Millennium 32 software (Waters, USA). Peak identification of ferulic acid extract was based on retention time and chromatography of authentic ferulic acid standard (Sigma-Aldrich, USA). The stock solution of standard ferulic acid was prepared at concentration of 100 mg/L in deionized water and methanol (at 1:1 ratio). Calibrated standard solutions

were in the range of 10–100 mg/L. The relation of the peak areas of the analytes against the concentration of ferulic acid was used as calibration curve.

Determination of total phenolics contents and antioxidant capacities

The corn kernels were extracted in 80% methanol following the procedure of Dewanto *et al.* (2002) before the total phenolic and antioxidant activity analyses. Ground corn kernels (200 mg) were weighed into centrifuge tubes, mixed with 20 mL of 80% aqueous methanol for 30 min, then centrifuged using the refrigerated centrifuge for 20 min. The supernatant was collected and used for total phenolic content and antioxidant capacities by ABTS, DPPH and FRAP assay.

The total phenolic was determined using the Folin–Ciocalteu reagent according to Kahkonen *et al.* (1999). The Na_2CO_3 (1.6 µL) was transferred into a test tube and allowed to react to 2 mL of Folin–Ciocalteu reagent for 10 min in darkness at room temperature. Then, the extract (77 µL) was transferred into a test tube, mixed and allowed to react for 20 min in darkness at room temperature. Absorbance was measured at 725 nm using a spectrophotometer and the results were expressed in gallic acid equivalents (mg GAE/g fw) calculated from a gallic acid (20-100 mg/L) standard curve. Additional dilution was needed to achieve absorbance over the linear range of the standard curve; then, the dilution factor was taken into account for the quantification.

The ABTS radical-scavenging activity assay was followed the method of Re *et al.* (1999) with slight modification. The ABTS stock aqueous solution (7 mM) was prepared. ABTS radical cations ($ABTS^{\bullet+}$) were produced by reacting the ABTS stock solution with 2.45 mM of potassium persulfate at a ratio of 2:1, and left in darkness at room temperature for 12–16 h before use. Then, the $ABTS^{\bullet+}$ solution was diluted with phosphate-buffered saline (PBS) at pH 7.4 to have absorbance of 0.70 ± 0.02 at 734 nm, and then left in darkness again for 2 h. The $ABTS^{\bullet+}$ solution was freshly prepared for each day of the assay. The extract (10 µL) was added to 3 mL of $ABTS^{\bullet+}$ solution, immediately mixed, and monitored for absorbance at 734 nm and measured for free radical scavenging reactions. The standard curve was linear between 50 and 500 µM of Trolox solution. Results are expressed in µmolTrolox equivalents (TE)/ g fw.

DPPH radical-scavenging activity assay was performed according to Brand-Williams *et al.* (1995), with slight modification. The DPPH free radical ($DPPH^{\bullet}$) stock solution of 6x10-5 mol/L was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The extract (77 µL) was added to 3 mL of $DPPH^{\bullet}$ stock solution and allowed react for 15 min in darkness. Absorbance was determined at 515 nm. The standard curve was linear between 50 and 500 µM of Trolox solution. Results are expressed in µmol Trolox equivalents (TE)/ g fw.

Ferric Reducing Antioxidant Power (FRAP) assay was determined according to Benzie and Strain (1996) with slight modification. The FRAP stock solution included 300 mM of sodium acetate buffer at pH 3.6, 10 mM of TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM of HCl, and 20 mM of $FeCl_3{}^{\bullet}6H_2O$ solution. The working solution was freshly prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3{\bullet}6H_2O$ solution. The extract (200 µL) was allowed to react with 3.8 mL of FRAP solution for 30 min in darkness at room temperature. Readings of the colored product (ferrous-tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 50 and 400 µM of Trolox solution. Results are expressed in µmolTrolox/g fw.

Statistical analysis

The data were analyzed with ANOVA using SPSS version 17 (SPSS, USA). Significant differences between means were estimated by Duncan's new multiple range test (DMRT) at significance level of 0.05.

Results and Discussions

LOX and POD activities in the new breed of mini-ear corn, Naulthong *var.*, varied depending on the location on the corn ear. It was found that LOX had higher activities in kernels while POD activities were elevated in cobs. LOX activities in kernels and cobs of uncooked corn were 63.71 ± 0.21 and 5.32 ± 0.23 unit/g fw, respectively, while POD activities of uncooked corn were 187.99 ± 0.85 and 1,170.27 ± 5.47 unit/g fw in kernels and cobs, respectively. This agreed with Boyes *et al.* (1997) which reported that the key enzyme causing off-flavor in corn kernels was LOX whereas POD was rich in cobs.

Cooking methods caused not only changes in eating quality but also in bioactives and enzyme activities. The new breed of mini-ear supersweet corn, Naulthong *var.*, was cooked in serving styles of whole-ear and cut-piece corn by boiling and steaming (under both atmospheric and high-pressure conditions) for different periods of time. The percentages of LOX and POD inactivation in kernels and cobs of cooked whole-ear and cut-piece serving styles of corn are shown in Tables 1 and 2, respectively. Cooking method and time had effects

Table 1. Inactivation of LOX and POD activities in kernels and cobs of cooked whole-ear corn on the cob, Naulthong *var.*

Cooking method	Time (min)	% Inactivation of LOX activity		% Inactivation of POD activity	
		Kernels	Cobs	Kernels	Cobs
Boiling	4	68.46 ± 0.12^{e}	52.00 ± 0.37^{c}	30.42 ± 0.35^{g}	16.99 ± 0.25^{i}
	8	99.76 ± 0.02^{b}	99.64 ± 0.02^{ab}	71.53 ± 0.03^{e}	56.24 ± 0.10^{g}
	12	99.97 ± 0.00^{a}	99.68 ± 0.03^{ab}	95.73 ± 0.04^{b}	71.79 ± 0.12^{d}
	16	99.99 ± 0.00^{a}	99.76 ± 0.00^{a}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
	20	99.99 ± 0.00^{a}	99.77 ± 0.03^{a}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
Atmospheric steaming	4	63.94 ± 0.23^{g}	47.50 ± 0.04^{e}	29.00 ± 0.01^{i}	8.08 ± 0.13^{k}
	8	98.24 ± 0.06^{d}	99.54 ± 0.02^{b}	67.21 ± 0.11^{f}	46.45 ± 0.78^{h}
	12	99.93 ± 0.00^{a}	99.53 ± 0.00^{b}	94.26 ± 0.07^{c}	70.76 ± 0.06^{e}
	16	99.97 ± 0.00^{a}	99.70 ± 0.01^{ab}	99.99 ± 0.00^{a}	98.42 ± 0.07^{b}
	20	99.99 ± 0.00^{a}	99.69 ±0.02^{ab}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
Pressure steaming	4	64.87 ± 0.04^{f}	48.62 ± 0.16^{d}	29.96 ± 0.10^{h}	8.33 ± 0.23^{j}
	8	99.50 ± 0.02^{c}	99.68 ± 0.01^{ab}	73.04 ± 0.09^{d}	58.62 ± 0.16^{f}
	12	99.90 ± 0.00^{a}	99.55 ± 0.02^{b}	95.65 ± 0.07^{b}	97.85 ± 0.02^{c}
	16	99.97 ± 0.00^{a}	99.57 ± 0.31^{b}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
	20	99.99 ± 0.00^{a}	99.56 ± 0.01^{b}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}

Data are expressed as mean ± standard deviation from six determinations.
Means in the same column with different superscript letters are significantly different ($p \leq 0.05$) as determined by Duncan's new multiple range test.

Table 2. Inactivation of LOX and POD activities in kernels and cobs of cooked cut-piece corn on the cob, Naulthong *var.*

Cooking method	Time (min)	% Inactivation of LOX activity		% Inactivation of POD activity	
		Kernels	Cobs	Kernels	Cobs
Boiling	4	69.23 ± 0.09^{c}	53.08 ± 0.06^{h}	37.02 ± 0.32^{g}	18.33 ± 0.20^{e}
	8	99.97 ± 0.00^{a}	99.14 ± 0.01^{g}	75.96 ± 0.15^{d}	59.55 ± 0.64^{c}
	12	99.97 ± 0.00^{a}	99.86 ± 0.00^{c}	98.66 ± 0.02^{b}	98.39 ± 0.01^{b}
	16	99.99 ± 0.00^{a}	99.90 ± 0.00^{b}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
	20	99.99 ± 0.00^{a}	99.98 ± 0.00^{a}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
Atmospheric steaming	4	64.10 ± 0.03^{d}	48.62 ± 0.13^{j}	32.74 ± 0.15^{i}	9.40 ± 0.26^{f}
	8	99.90 ± 0.00^{b}	99.30 ± 0.03^{f}	66.44 ± 0.10^{f}	57.17 ± 1.55^{d}
	12	99.97 ± 0.00^{a}	99.81 ± 0.02^{d}	98.00 ± 0.01^{c}	97.76 ± 0.00^{b}
	16	99.97 ± 0.00^{a}	99.93 ± 0.03^{b}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
	20	99.99 ± 0.00^{a}	99.97 ± 0.00^{a}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
Pressure steaming	4	65.46 ± 0.02^{e}	48.82 ± 0.01^{i}	36.51 ± 0.04^{h}	8.24 ± 0.76^{g}
	8	99.92 ± 0.00^{b}	99.12 ± 0.04^{g}	75.35 ± 0.40^{e}	57.46 ± 0.58^{d}
	12	99.97 ± 0.00^{a}	99.62 ± 0.01^{e}	98.59 ± 0.00^{b}	98.13 ± 0.00^{b}
	16	99.98 ± 0.00^{a}	99.79 ± 0.01^{d}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}
	20	99.99 ± 0.00^{a}	99.97 ± 0.00^{a}	99.99 ± 0.00^{a}	99.99 ± 0.00^{a}

Data are expressed as mean ± standard deviation from six determinations.
Means in the same column with different superscript letters are significantly different ($p \leq 0.05$) as determined by Duncan's new multiple range test.

on the inactivation of LOX and POD in both serving styles of the mini-ear supersweet corn ($p \leq 0.05$).

LOX activity in kernels and cobs of cooked whole-ear corn was inactivated by 64-69% and 48-52%, respectively, for all cooking methods at 4 min. On the other hand, LOX activity in kernels and cobs of cut-piece corn cooked in the same cooking conditions was reduced by 64-69% and 49-53%, respectively. For both serving styles, LOX activity in both kernels and cobs was inactivated by more than 99% under all cooking methods at 8 min ($p \leq 0.05$; Tables 1 and 2), except for atmospheric steaming which inhibited 98% of LOX activity in kernels of whole-ear corn (Table 1). All cooking methods at 8 min had no significant difference in LOX inhibition in corn kernels and cobs. Boiling and pressure steaming had more effects on LOX inactivation than atmospheric steaming ($p \leq 0.05$; Tables 1 and 2).

POD in mini-ear supersweet corn was more heat-resistant than LOX. At cooking time of 12 min, POD activity in kernels was inactivated by 96% in whole-ear corn and 98-99% in cut-piece corn for boiling and pressure steaming ($p > 0.05$), but by 94% in whole-ear corn for atmospheric steaming ($p \leq 0.05$). POD activity in cobs needed longer time to be fully inhibited. It took 16 min to achieve complete inactivation of POD in both kernels and cobs. However, atmospheric steaming for 16 min inactivated 98% of POD activity in cobs ($p \leq 0.05$;

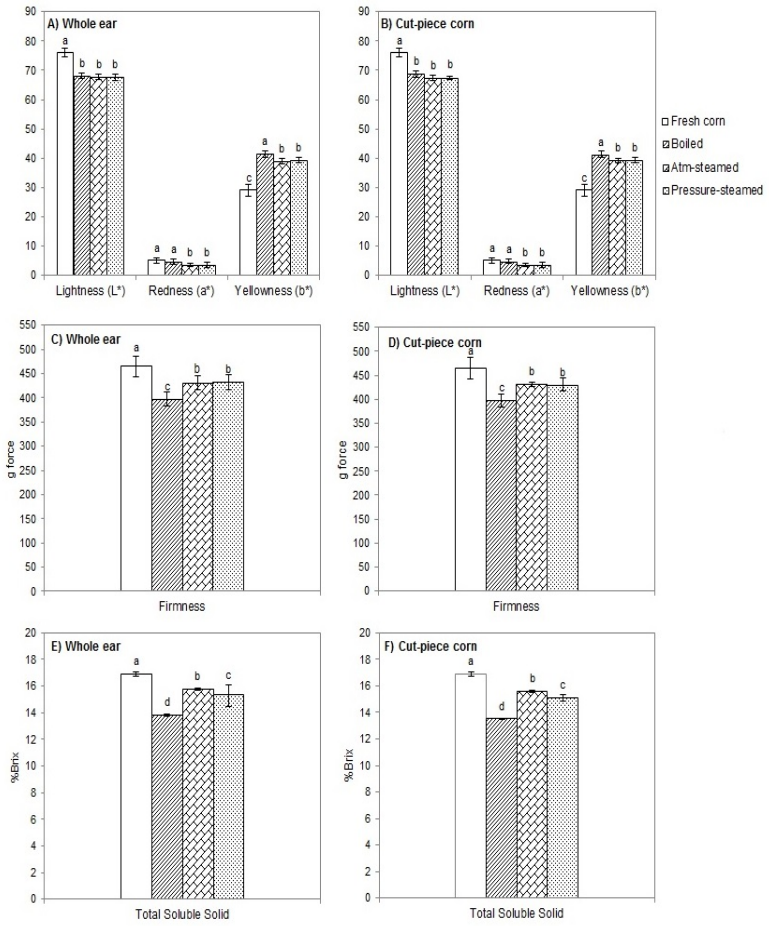

Figure 1. Physical properties: A), B) Color parameters of cooked whole ear and cut-piece corn, respectively; C), D) firmness of cooked whole ear and cut-piece corn, respectively; and E), F) Total Soluble Solid (TSS) of cooked whole-ear and cut-piece corn, respectively. The cooking time for whole-ear and cut-piece were 16 and 12 min, respectively, for all cooking methods. Different letters indicates significant differences between treatments ($p \leq 0.05$) as determined by Duncan's new multiple range test

Table 1). Boiling and high-pressure steaming were more effective in POD inactivation than atmospheric steaming in both serving styles of corn, especially in cobs ($p \leq 0.05$; Tables 1 and 2). Pressure steaming for 12 min seemed to be more effective in inactivating POD in cobs of whole-ear corn than boiling for the same cooking time (Table 1).

It was clear that LOX and POD were inactivated at different cooking conditions and their activities decreased with longer cooking times in both serving styles of corn ($p \leq 0.05$). According to Chombodin (1998), the cultivar may vary widely in initial enzyme activities and quality. The fraction of enzymes may vary depending on type or location of tissue, cultivation, and maturity (Morales-Blancas *et al*., 2002). Enzymes normally have both heat-labile and heat-stable isozymes. Efficient enzyme inactivation depended on fraction of enzymes (Lee and Hammes, 1979). It was reported that LOX was more sensitive to heat than POD, resulting in reduction of blanching or cooking time of corn kernels (Williams *et al*., 1986). The result was in the same line as that of Barrett *et al*. (2000) which investigated LOX and POD inactivation in three cultivars of sweet corn; and reported that LOX inactivation by steaming at 100°C required a period of 6 min for sugary A and sugary B varieties, and 4 min for supersweet corn variety, while POD inactivation required a period of 8 min for these three cultivars of sweet corn. Lee and Hammes (1979) reported that the percentages of POD activity residue in the outer cobs and the center cobs of whole-ear corn after steam blanching at 99°C for 15 min were 13% and 45%, respectively. Inactivation of POD was very quick in kernels and slow in the center of cobs due to slow heat penetration from the outer to the inner parts. Rodriguez-Sauna *et al.* (1995) reported that whole-ear corn of two cultivars (Jubilee and GH-2684) was steam-blanched at 100oC for 9 min, leading to inactivation of LOX activity, whereas inactivation of POD required a longer heating time of 15 min. Furthermore, Neveh *et al.* (1982) found that blanching of whole-ear corn in boiling water

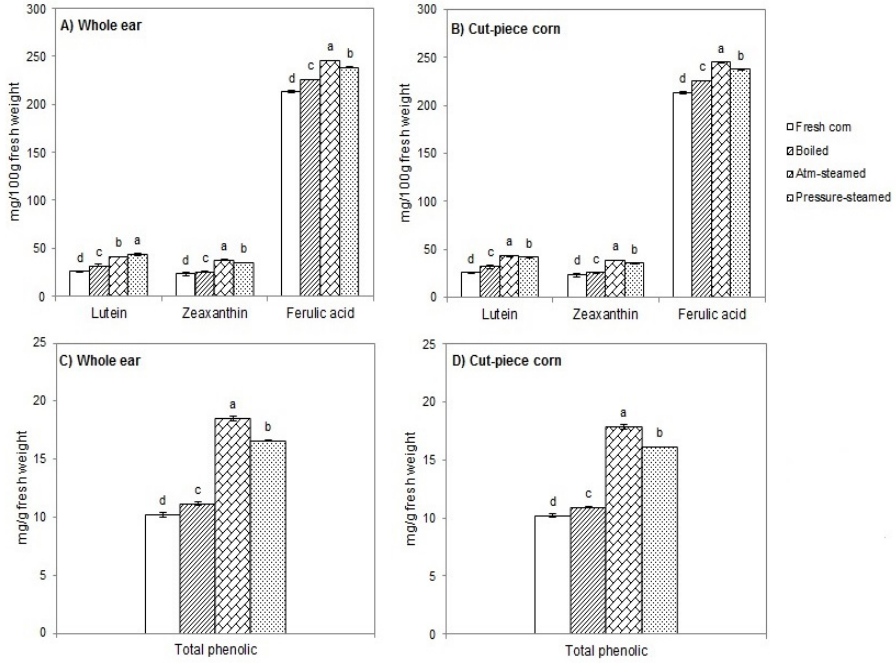

Figure 2. Bioactive compounds: A), B) Lutein, Zeaxanthin and Ferulic acid of cooked whole ear and cut-piece corn, respectively; and C), D) Total phenolic of cooked whole ear and cut-piece corn, respectively. The cooking time for whole-ear and cut-piece were 16 and 12 min, respectively, for all cooking methods. Different letters indicates significant differences between treatments ($p \leq 0.05$) as determined by Duncan's new multiple range test

(ca. 100°C) for 10 min resulted in 1% residual POD activity. Barrett and Theerakulkait (1995) found that whole-ear LOX inactivation in supersweet corn at 93°C was achieved in 6 to 9 min, while whole-ear POD inactivation under the same conditions required 18 to 20 min. Kernel LOX inactivation in supersweet corn at 93°C took 40 seconds while kernel POD inactivation under the same conditions required 60 seconds.

These present results showed that cut-piece corn took a shorter time (12 min) to inactivate POD in cobs than that of whole-ear corn (16 min), regardless of cooking method. This was due to a quicker heat-penetration rate in cut-piece corn than that of whole-ear corn. The rate of heat penetration depended on thermal conditions, pH, size of food, cooking medium, and heat resistance of microbes and enzymes in food. Water transfers heat more efficiently than air because it has a higher specific heat (Brown, 2011). Normally, boiling offers a convection heat transfer, resulting in a faster heat penetration compared to steaming which offers a conduction heat transfer. Thus, boiling (100°C) was more efficient enzyme inactivation in cob than atmospheric steaming (100°C) (Lee and Hammes, 1979; Heldman and Hartel, 1997). However, it was found that the enzyme inactivation in cob under a pressure steaming (ca. 15 lb/in2, 121°C for 12 min) was as effective as boiling (100°C for the same period of time). This was due to heat transfer under pressure condition was more efficient than atmospheric pressure. It was agreed with Nurhuda *et al*. (2013) reported that steam blanching for 5 min using autoclaving (100°C) caused a more reduction in POD and PPO activities of rambutan peel compared to water blanching (100°C).

LOX activity in kernels and cobs of both serving styles of the mini-ear supersweet corn, Naulthong *var*., was completely inactivated at cooking time of 8 min for all three cooking methods. POD needed longer times of 12 and 16 min for complete inactivation in cut-piece and whole-ear corn, respectively. However, POD was more effectively inactivated in cut-piece corn than in whole-ear corn. Therefore, in order to reduce cooking time and delay quality changes due to enzymes during storage, cut-piece corn was suggested for cooking rather than whole-ear corn.

After cooking in order to inactivate the LOX and POD, both serving styles of cooked mini-ear supersweet corn were determined for CIELAB and firmness. The L^*, a^* and b^* of uncooked kernels were 75.96 ± 1.38, 5.08 ± 0.91 and 29.07 ± 1.95, respectively. Fresh firmness was 464.57 ± 21.76 gforce. All cooked corn kernels, regardless of serving form, had lower lightness (L^*) and redness (a^*), but higher yellowness (b^*) than those of fresh kernels ($p \leq 0.05$; Figure 1A, B). Regardless of serving form, cooking methods did not show significant difference in lightness ($p > 0.05$). Boiled kernels had higher

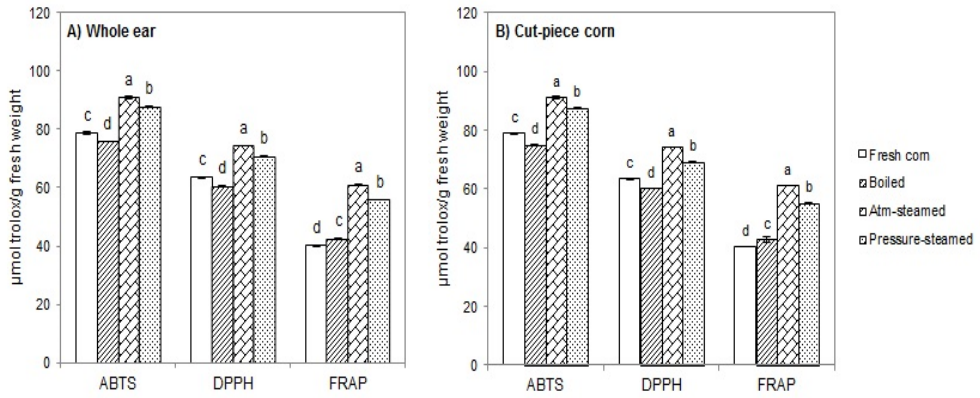

Figure 3. Antioxidant capacities by ABTS, DPPH and FRAP assays of A) cooked whole ear and B) cut-piece corn. The cooking time for whole-ear and cut-piece were 16 and 12 min, respectively, for all cooking methods. Different letters indicates significant differences between treatments ($p \leq 0.05$) as determined by Duncan's new multiple range test

redness and yellowness than those of steamed kernels ($p \leq 0.05$). Barrett *et al*. (2000) reported that steam-blanched corn kernels had smaller hue angle (more yellowness) than that of unblanched kernels. Longer heating time led to more yellowness of kernels. Carotenoids in sweet corn were isomerized from trans- to cis-form due to heat, causing an increase in b-value. This was because blanching removed gases from tissue, leading to a decrease in lightness *(L^*)* but an increased value of yellowness *(b^*)* (Chombordin, 1998). All cooking methods reduced firmness and TSS when compared to those of uncooked kernels ($p \leq 0.05$). Boiling caused more reduction in firmness than steaming ($p \leq 0.05$). Both steaming methods caused no significant difference in CIELAB and firmness ($p > 0.05$; Figure 1C, D). Barrett *et al*. (2000) reported that heat resulted in texture changes of fruits and vegetables. The long period of heating process caused a decrease in firmness of sweet corn due to turgor pressure loss. Furthermore, the gelatinization of starch in sweet corn and perhaps the changes in pectic substance resulted in a softened texture of kernels (Chombordin, 1998; Van Buggenhout *et al*. 2009). The results agreed with those of Dutta *et al*. (2009) which stated that heating pumpkins to 85°C and 95°C affected their tissue by the removal of intercellular air, resulting in the softening of cell walls. It was found that boiled kernels had lower TSS than that of steam-cooked corn. This was probably due to the leaching of TSS into water medium (Rickman *et al.*, 2007). This was on the same line as that of Martinez *et al.* (2013) which reported that TSS in fresh turnips decreased after blanching in water. Thus, longer blanching time led to higher loss of TSS. Heat treatments could cause changes in structural properties of tissue, leading to losses of soluble solids (Grzeszczuk *et al.*, 2007). Boiling caused more solid loss than steaming due to leached water-soluble composition content (phenolic, fiber and starch, etc.). Xu and Chang (2008) reported that atmospheric-boiling, pressure-boiling and steaming caused 11-14%, 4.4-11.8% and 1-7% of solid loss in legumes. However, the significant solid losses by boiling and steaming could be attributed to the diffusion of water-soluble composition into cooking water and steam condensate. Furthermore, interaction of food composition or structural changes during cooking, such as sugar interaction, starch gelatinization and protein aggregation or precipitation, could resulting in a reduction of TSS. According to Guzel and Sayer (2012) reported that the total soluble solids loss between 2 and 19% of the dry legumes seed during soaking and cooking, depending on the water temperature, type of seed, and physiochemical on seed.

It was well-known that corn is a significant vegetable source of the carotenoids lutein and zeaxanthin (Holden *et al*., 1999; Rodriguez-Amaya and Kimura, 2004; Scott and Eldridge, 2005; Kuhnen *et al*., 2009; Kao *et al.*, 2012). The contents of lutein, zeaxanthin, ferulic acid and total phenolics in uncooked kernels were 25.60 ± 1.09, 23.24 ± 1.43, 213.24 ± 1.69 mg/100 g fw and 10.18 ± 0.21 mgGAE/g fw, respectively (Figure 2A-D). All cooked samples of both serving forms had higher contents of the investigated bioactives than those of uncooked ones ($p \leq 0.05$). Regardless of serving form, atmospheric-steamed kernels had higher bioactive contents than those of pressure-steamed and boiled kernels ($p \leq 0.05$). Except for lutein content of whole-ear serving form, pressure-steamed kernels had higher content than that of atmospheric-steamed and boiled kernels, respectively ($p \leq 0.05$). Thermal processing could disrupt cell membranes and cell

walls, leading to a release of bound bioactives from the tissue structure of sweet corn. After boiling, some bioactives were leaching into the boiling medium. (Dewanto *et al.*, 2002; Ahmed and Ali, 2013). However, pressure-steamed kernels had lower bioactives than atmospheric-steamed kernels which may be due to higher cooking temperature, resulting in a further degradation of the bioactives. Regardless to the cooking methods, retention of bioactives decreased with longer cooking time and higher cooking temperature (Ruiz-Rodriguez *et al*., 2008).

After cooking, corn kernels of both serving forms had increased lutein, zeaxanthin, ferulic acid and total phenolics contents by 1.25-1.70, 1.10-1.64, 1.05-1.15 and 1.07-1.82 times, respectively, when compared with those of uncooked corn. Scott and Eldridge (2005) reported that there was no significant increase in lutein and zeaxanthin (xanthophylls carotenoids) between those of fresh and canned corn of two varieties of corn kernels. Xanthophylls were sensitive to heat, light, oxygen and pH. Although canning would minimize degradation caused by light, oxygen and pH, the high temperature used in canning was adequate to induce carotenoid isomerization from trans- to cis-form (Nguyen *et al*., 2001; Updike and Schwartz, 2003; Rodriguez-Amaya and Kimura, 2004). The results agreed with those of Janpatiw et al. (2013) in that lutein and zeaxanthin contents in sweet corn were higher after boiling and steaming than those of uncooked ones. The effects of cooking methods were dependent on corn cultivars. Related results were reported by Dutta *et al*. (2009) in that pumpkins blanched at 55-95°C for 3-10 min initiated an increase in total carotenoids when compared to those of fresh pumpkins. Processed carrots and spinach had higher total carotenoid contents than those of fresh ones (Rock *et al.*, 1998). This was similar to that of Stahl and Sies (1996a) who reported that thermally processed tomatoes and carrots had increases in total carotenoid contents. This was probably due to tissue breakdown, better accessibility to solvent extraction, and disruption of carotenoid-protein complexes. Heat treatments provided potential for increasing bioavailability of total carotenoids in vegetables (Van Het Hof *et al*., 1998). Furthermore, Dewanto *et al*. (2002) reported that sweet corn thermally processed at 115°C for 25 min had significantly elevated ferulic acid, total phenolics and antioxidant activities. Roy *et al.* (2009) reported that steaming broccoli for 5-10 min led to increase total phenolic contents when compared with those of fresh broccoli. Dini *et al.* (2013) reported that steamed pumpkin pulp had higher total phenolic contents than those of boiled and fresh pumpkin pulp. This was probably due to the disruption of cell membranes caused by heat which provided opportunity for the bound phytochemical compounds to be released into the medium (Howard *et al*., 1999). Esterified and insoluble bound forms of phytochemicals were released from the breakdown of cell walls and also oxidative and hydrolytic enzymes, leading to antioxidant destruction. However, the amount of enzymes inactivated by thermal processing were dependent on both heating temperature and time used (Chism and Haard, 1996; Dewanto *et al*., 2002). Nevertheless, our results were contrary to those of Gayathri *et al.* (2003) which reported that pressure cooking for 10 min led to greater losses of β-carotene in carrots and pumpkins than those of open-pan boiling. They also disagreed with those of Mazzeo *et al.* (2011) in that boiling resulted in losses of total carotenoids and phenolics in carrots and spinach due to leaching of carotenoids into heating media. Heating boosted diffusion of cellular fluids which contained phytochemical compounds from plant cells into water medium (Leong and Oey, 2012). The compounds released during heating process could degrade due to long exposure duration (Heldman and Hartel, 1997).

The antioxidant capacities of fresh kernels were 63.29 ± 0.26, 78.80 ± 0.14 and 40.19 ± 0.18 μmol Trolox/g fw for DPPH, ABTS and FRAP assays, respectively (Figure 3A, B). For both serving forms, the antioxidant capacities of kernels were affected by cooking in different ways. When compared to uncooked kernels, boiling decreased radical scavenging activities by 0.95 times for DPPH and ABTS assays, whereas there was an increase in reducing power of cooked kernels by 1.06 times ($p \leq 0.05$) for FRAP assay. It was worth noting that this mini-ear corn cultivar had various antioxidants. The water-soluble antioxidants, mainly free radical scavenging antioxidants, leached into the boiling water. Atmospheric and pressure steaming resulted in higher antioxidant capacities of cooked kernels by 1.15-1.52 and 1.09-1.39 times, respectively ($p \leq 0.05$). It seemed that serving forms of corn did not affect the extent of both bioactives and antioxidant capacities. Thermal processing enhanced antioxidant activities in sweet corn due to increases in lutein, zeaxanthin, ferulic acid and total phenolics (Eberthardt *et al.*, 2000; Dewanto *et al.*, 2002; Leong and Oey, 2012). Moreover, Dewanto *et al.* (2002) reported that sweet corn thermally processed at 115°C for 10-50 min had significantly elevated total antioxidant activities. Dini *et al*. (2013) stated that steamed pumpkins had higher antioxidant activities in FRAP and DPPH assays than those of boiled and fresh pumpkins. The results agreed with those of Mazzeo *et al.* (2011)

in which steamed spinach had higher TEAC and FRAP than that of uncooked and boiled spinach, respectively. Nonetheless, Turkmen *et al.* (2005) stated that changes in total antioxidant activities were reliant on types of vegetables, not cooking methods.

Conclusion

Boiling and steaming produced effects on enzyme activities, bioactive compounds and antioxidant capacities of sweet corn. LOX was more sensitive to heat than POD. The enzymes in kernels were more effectively inactivated than those in cobs. Boiling and pressure steaming were more effective in enzyme inactivation than atmospheric steaming, regardless of serving pattern of corn. Enzyme inactivation seemed to be more effective in cut-piece corn than in whole-ear corn as an outcome of less cooking time. In order to process corn on the cob, it was necessary to completely inactivate POD with cooking times of 12 and 16 min for cut-piece and whole-ear corn, respectively. The kernels were more yellow after cooking. Boiled kernels had lower firmness and total soluble solids than those of steamed kernels. The results revealed that cooked kernels had higher contents of lutein, zeaxanthin, ferulic acid, total phenolic compounds and antioxidant capacities than those of uncooked kernels. Although boiling increased bioactives in corn kernels, it could promote leaching in the released bioactives. Pressure steaming provided high temperature conditions, leading to thermal degradation of bioactives.Therefore, atmospheric steaming was the most appropriate cooking method for improving the bioavailability of bioactives and, consequently, increasing antioxidant capacities. A shorter cooking time is preferred in order to retain high contents of bioactives in corn, thus, cut-piece corn is recommended over whole-ear corn.

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