



# Effect of high-pressure processing on colour, phytochemical contents and antioxidant activities of purple waxy corn (*Zea mays* L. var. *ceratina*) kernels



Kawinchaya Saikaew<sup>a</sup>, Kamol Lertrat<sup>b,c</sup>, Mutita Meenune<sup>d</sup>, Ratchada Tangwongchai<sup>a,\*</sup>

<sup>a</sup> Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand

<sup>b</sup> Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

<sup>c</sup> Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

<sup>d</sup> Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Songkla 90112, Thailand

## ARTICLE INFO

### Keywords:

High-pressure  
Anthocyanins  
Bioactive compounds  
Antioxidant capacity  
Purple corn  
Functional foods

## ABSTRACT

High-pressure processing (HPP) at 250–700 MPa for 30–45 min affects the colour parameters, phytochemical contents and antioxidant activities of purple waxy corn kernels ( $p < 0.05$ ). The higher pressure-level, the lower  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $h^o$  ( $p < 0.05$ ). However, pressure-treated kernels at 700 MPa showed a similar colour profile to steam-treated kernels. HPP caused a loss in the total phenolic, flavonoid, and anthocyanin contents and antioxidant activities. The pressure-treated kernels had a higher phytochemical content than the steam-treated kernels. The phytochemicals and antioxidant activities decreased as the pressure increased from 250 to 550 MPa, but the levels recovered at 700 MPa. The longer holding-time, the greater loss of the compounds and antioxidant activities ( $p < 0.05$ ). Pressure treatment at 700 MPa yielded the highest total phenolic and anthocyanin contents ( $p < 0.05$ ). Water-soluble compounds can leach from food materials due to cell rupture. Nevertheless, HPP is a potential process to preserve the phytochemicals in food.

## 1. Introduction

Corn is a source of carbohydrates and is rich in phytochemicals, which have health benefits. Waxy corn (*Zea mays* L. var. *ceratina*) is a vegetative corn variety that is widely consumed in Asia, i.e., China, Korea, Vietnam, Taiwan, Laos, Myanmar and Thailand (Harakotr, Suriharn, Tangwongchai, Scott, & Lertrat, 2014), because of its sticky, chewy texture, taste and nutrition. Recently, purple waxy corn, another variety of waxy corn, was grown to improve the anthocyanin content in corn. Anthocyanins are natural pigments with strong antioxidant properties, anti-inflammatory and anticarcinogenic activities. They can help prevent cardiovascular disease, control obesity and alleviation diabetes (He & Giusti, 2010). However, they are susceptible to the processing temperature, the presence of enzymes, co-pigmentation and the pH level (Cavalcanti, Santos, & Meireles, 2011). Hence, when food is thermally processed, its composition, bioactive content, and colour are likely to change. For example, heat treatment causes a reduction in the anthocyanin content in Korean purple-fleshed sweet potatoes (Kim et al., 2012) and purple waxy corn (Harakotr et al., 2014) and an increase in coloured-flesh potatoes (Lachman et al., 2012), which affects consumer acceptance of a product with a dramatic colour change.

To overcome the effect of the heat treatment, high-pressure

processing (HPP), a non-thermal process, is an alternative to preserve foods containing heat-sensitive compounds. HPP causes a partial unfolding of proteins during the process and upon the release of the pressure, which leads to the inactivation of microorganisms and enzyme mechanisms. However, HPP creates healthy and fresh-like food products since low molecular-weight compounds with covalent bonds, i.e., flavours, pigments, vitamins and antioxidants, are not affected by high pressures of 100–1000 MPa (Oey, Lille, Loey, & Hendrickx, 2008). For consumers looking for healthy, convenient, ready-to-eat (RTE), natural foods, HPP is a promising technology to satisfy their needs. The technology has been applied to several commercial products on the premium food market, such as cooked meats, seafood, fish, vegetables and fruit juices (Rastogi, Raghavarao, Balasubramaniam, Niranjana, & Knorr, 2007). Though many studies have shown that the HPP retains higher anthocyanin contents in wild berry pulps (Liu et al., 2016), strawberry pulps (Cao et al., 2011), and strawberry and blackberry purées (Patras, Brunton, Pieve, & Butler, 2009) compared to the high-temperature treatment, the effectiveness of the method depends on the processing conditions (pressure, hold time, pH and temperature) and food forms (whole, pieces, puree or juice) (Vega-Gálvez et al., 2014). For example, high-pressure treatment at 0.1–700 MPa combined with an elevated temperature from 95 to 130 °C caused anthocyanin

\* Corresponding author at: Department of Food Technology, Faculty of Technology, Khon Kaen University, 123 Mittapap Rd., Nai-Muang, Muang District, Khon Kaen 40002, Thailand.  
E-mail addresses: [kawinchaya@gmail.com](mailto:kawinchaya@gmail.com) (K. Saikaew), [kamol9@gmail.com](mailto:kamol9@gmail.com) (K. Lertrat), [mutita.m@psu.ac.th](mailto:mutita.m@psu.ac.th) (M. Meenune), [ratchada@kku.ac.th](mailto:ratchada@kku.ac.th) (R. Tangwongchai).

degradation in strawberry paste (Verbeyst, Oey, Van der Plancken, Hendrickx, & Van Loey, 2010). Anthocyanin degradation resulted in the loss of red colour in plum purée (García-Parra, González-Cebrino, Cava, & Ramírez, 2014) and Cape gooseberry pulp (Vega-Gálvez et al., 2014). This study aims to investigate the changes in colour, phytochemicals and antioxidant activity in purple waxy corn kernels compared to untreated or steamed kernels after applying a HPP at different pressure levels and holding times. The obtained information is beneficial for using purple waxy corn cut kernels for RTE foods.

## 2. Materials and methods

### 2.1. Chemicals

Gallic acid, catechin, Trolox (6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid), fluorescein, AAPH (2,2'-azobis-2-amidinopropane-dihydrochloride), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) and ABTS (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonate) diammonium salt) were purchased from Sigma-Aldrich (USA). Folin-Ciocalteu's reagent was obtained from Carlo Erba (France). All other chemicals and reagents used in the experiments were of analytical grade.

### 2.2. Plant material

Purple waxy corn (*Zea mays* L. var. *ceratina*), "KGW1" variety, was cultivated and harvested in a demonstration field by the Faculty of Agriculture at Khon Kaen University. The corn ear was harvested at the eating stage (20 days after pollination; DAP), husked and separated from the corn silks. To minimize the variation in the kernel size and the maturity of the corn kernels for further treatments, the corn ears were cut, and approximately 3 cm from the corn tip and the other end were discarded. Whole kernels were separated from the cob in a column along the ear using a cutter with an acute-angle blade. The kernels were washed twice in tap water to separate the silk and chaffs prior to treatment.

### 2.3. HPP treatments

Fresh purple waxy corn kernels (100 g) were packed in a flexible Nylon/LLDPE pouch (30 mm diameter and 200 mm length), airtight sealed with minimal headspace, and placed into a high-pressure rig (Food-Lab Model S-FL-850-9-W, Stansted Fluid Power Ltd., UK). A mixture of castor oil and ethanol (1:4 v/v) was used as the pressure-transmitting medium. The samples were subjected to pressures of 250, 400, 550 and 700 MPa for 30 and 45 min at room temperature (ca. 30 °C) and then depressurized to atmospheric pressure. Temperature within the pressure chamber was increased from 30 to approximately 39, 41, 45 and 45 °C when the pressure was increased to 250, 400, 550 and 700 MPa, respectively. The colour parameters of all the samples were measured after the pressurization. The pressurized kernels were freeze-dried (Alpha 2–4 LD plus, Martin Christ, Germany) to minimize changes, pulverized using a pin mill (Lab mill 3100, Perten Instruments, Sweden) and sieved through a screen (50 mesh for phytochemicals and antioxidant activity and 100 mesh for DSC to determine the gelatinization percentage). The powder was vacuum-packed in an aluminium foil, laminated plastic bag and kept at –18 °C until analysis (no longer than a month). The samples were thawed at ambient temperature (ca. 35 ± 2 °C) for 2–3 h prior to the analysis.

The conventional steaming, fresh kernels (800 g) were steamed in a domestic stainless-steel steamer under heated water for 15 min. Samples were then immediately cooled to room temperature in an ice-water bath. Untreated and steam-cooked kernels (95–100 °C, 15 min) were used as the control and reference samples, respectively.

### 2.4. Colour measurement of the purple waxy corn kernels

The colour parameters,  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness), of the untreated and treated corn kernels were measured using a HunterLab colorimeter (MiniScan XE Plus, Hunter Associates Laboratory, Inc., USA) under a D-65 diffused illumination 10° observer. The standard light trap and white tile of  $L = 100$  were used as references to calibrate the colorimeter. In addition, the parameters  $L^*$ ,  $a^*$  and  $b^*$  were converted into  $C^*$  (chroma),  $h^\circ$  (hue angle) and  $\Delta E$  (total colour change) using the following equations:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h^\circ = \arctangent(b^*/a^*)$$

$$\Delta E^* = \sqrt{(L^* - L_{untreated}^*)^2 + (a^* - a_{untreated}^*)^2 + (b^* - b_{untreated}^*)^2}$$

### 2.5. Extraction of phytochemicals from purple waxy corn kernels

The sample extraction was performed using a slight modification of the method of Hosseini, Li, and Beta (2008). Briefly, 30 mL of solvent (95% methanol acidified with 0.5 N HCl in a ratio of 85:15 v/v) was added to 2.000 ± 0.005 g of the powdered sample, and the solution was mixed and shaken in the dark at room temperature for 60 min and centrifuged at 10,000g for 10 min at 25 °C (Sorvall Legend Mach 1.6 R, Thermo Fisher Scientific, Germany). The residue was re-extracted again. The supernatant was combined and filtered through Whatman No. 1 filter paper. The filtrate was collected and rotary evaporated at 45 °C (Rotavapor R-124, Buchi Labortechnik AG, Switzerland). The matrix was reconstituted in 7 mL of the extracting solvent and filtered through both a Whatman No. 1 filter and a 0.45 µm syringe filter. Finally, the extract was adjusted to a volume of 10 mL using the extracting solvent and stored at –30 °C in the dark for no longer than 2 days prior to the analysis of the phytochemical contents and antioxidant activities.

### 2.6. Determination of the phytochemical content

#### 2.6.1. Total phenolic content (TPC)

The TPC was colourimetrically determined using a Folin-Ciocalteu assay following the method of Liu et al. (2011). The extract was diluted 5 times with the extracting solvent before analysis. The mixture contained 0.2 mL of the diluted extract, 0.5 mL of diluted Folin-Ciocalteu's reagent (ratio of 1:2 with deionized water) and 4 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> (w/v) and was left in the dark for 30 min at the ambient temperature to allow the reaction to take place. The absorbance of the mixture was spectrophotometrically measured at 750 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Japan). The extracting solvent was used as the blank instead of the extract. TPC was determined using the standard calibration curve of gallic acid (concentration from 50 to 200 mg/L) and is expressed as mg of gallic acid equivalents per 100 g of dry matter (mg GAE/100 g DM).

#### 2.6.2. Total flavonoid content (TFC)

TFC was colorimetrically performed following the method of Shen, Jin, Xiao, Lu, and Bao (2009) with minor modifications. Briefly, the extracts (0.5 mL) were mixed with deionized water (2 mL) and 5% NaNO<sub>2</sub> (0.15 mL), and the reaction was allowed to occur for 5 min. Then, 10% AlCl<sub>3</sub>·6H<sub>2</sub>O (0.15 mL) was added, and the mixture was allowed to stand for another 5 min. The mixture was added with 1 M NaOH (1 mL), mixed and kept in the dark for 15 min. The absorbance was determined at 510 nm. For the blank, the extracting solvent was used instead of the extract. A standard curve of 25–200 mg/L catechin was used to estimate the TFC, which is expressed as mg of catechin equivalents per 100 g of dry matter (mg CE/100 g DM).

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