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Original Research Article

Effects of roasting conditions on bioactive compound profile of purple maize (*Zea mays* L.) using response surface methodology

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ABSTRACT

The objective of this study was to optimize the roasting temperature and time for caffeine-free beverage production from purple maize kernels. Response surface methodology (RSM) was used to optimize the temperature and time. Purple maize kernels were roasted at different temperatures (100, 120, 140, 160 and 180 °C) and different times (10, 15, 20, 25 and 30 min), subsequently extracted with ethanol. The condition used for extraction process was purple maize/ethanol ratio (1:10) which was controlled in the water bath for 4 hours at 32 °C. Total phenolic content, total anthocyanin content, antioxidant activity values EC_{50} determined by 2,2–diphenyl–1–picrylhydrazyl (DPPH) and free sugar were analyzed. The levels of total phenolic content, anthocyanin content, DPPH radical scavenging activity and free sugar were 183.99-907.81 mg gallic acid equivalent/g crude extract, 3.27-258.57 mg of cyanidin-3-glucoside equivalent/100g dry seed, 10.80-210.73 g flour/g DPPH and 0-0.076 g/100 ml, respectively. Considering the results of the total phenolic content and anthocyanin content, it was found that they increase gradually when temperature rising from 100-180 °C and reached highest at 160 °C. Meanwhile antioxidant activity values EC_{50} and free sugar content were found lowest at 160 °C. According to bioactive compound analysis, antioxidant efficiency was increased at the higher levels of total phenolic content and anthocyanin content. Therefore, the roasting temperature was the important factor effecting the bioactive compounds. The contour plots indicated that the optimum roasting temperature and time were 160 °C and 25 min, respectively.

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INTRODUCTION

Purple maize *(Zea mays* L.*)* is a kind of cereal belonging to the *Poaceae* family. The varieties of purple maize have been developed from waxy maize (Singh *et al*., 2011) which composes of carbohydrate, protein and vitamin etc. Furthermore, the purple maize kernels are rich in phenolic compounds and anthocyanins with well-established antioxidant and bioactive properties (Adom and Liu, 2002). Bioactive compounds are presented in vegetables and fruits including germ fraction of cereals. However bioactive compounds in cereals have not received as much attention as in vegetables and fruits.

The roasting process causes changes in the chemical composition and biological characteristics of the coffee bean, allowing other antioxidant compounds to be formed (Wang *et al*.,2011). Previous studied have been carried out to determine the effects of roasting for bioactive compounds in soybean (Lee and Lee, 2009), wheat (Krings *et al*., 2000), barley (Sharma and Gujral, 2011), pistachio nuts (Yazdanpanah *et al*., 2005), cocoa beans (Redgwell *et al*., 2003), coffee beans (Vignoli *et al*., 2013) and wattle seeds (Ee *et al*., 2011). To improve the roasting effects, the roasting conditions can be optimized using response surface methodology (RSM), which is a statistical method that can be used to describe the changes in the physiochemical quality indicators during roasting (Thomson, 1982). Therefore, the purpose of this study was to investigate the effects of roasting temperature and time on the bioactive compound of purple maize, in order for this to be used as a resource for nutraceutical products or food industry in the future.

MATERIALS AND METHODS

Materials

Purple maize *(Zea mays* L.*)* kernels from Talaad Thai in Patumthani, Thailand were used for this study. The purple maize kernels were separated from the cob, they were then tray dried until moisture content reached about 7%.

Preparation of purple maize kernels for roasting

The purple maize kernels (200 g) were roasted using a rotary roaster under the conditions for each experiment. The kernels were ground and screened using a sieve of size 20 mesh. After that, the purple maize kernels were extracted using 95% ethanol ratio (1:10), controlled in the water bath for 4 hours at 32 ° C and kept at 4 ° C until use.

Experimental design and statistical analysis

A statistical experimental design based on central composite design (CCD) and RSM were planned. The independent variables were temperature (X_1) and time (X_2), varied from 100-180 ° C and 10-30 min, respectively. The three levels of temperature and time given the best response values were selected. The responses were related to the independent variables using the equation.: $Y = ß_0 + ß_1X_1 + ß_2X_2 + ß_{12}X_1X_2 + ß_1{}^2X_1{}^2 + ß_2{}^2X_2{}^2$.

Determination of the total phenolic content (TPC)

The TPC was evaluated using the Folin-Ciocalteau colorimetry following Marinova *et al*., 2005. In short, 0.1 ml of purple maize extract, 0.5 ml of Folin-Ciocalteau reagent, 1.5 ml of 7% Na_2CO_3 and 7.9 ml distill water were comprised for the reaction mixture. Absorbance was measured at 765 nm. Results were reported as g of gallic acid/100 g crude extracts.

Determination of the total anthocyanin content (TAC)

The TAC was measured by the pH differential method (Yang and Zhai, 2010). Absorbance was measure at 510 nm and 700 nm. The absorbance was calculated by Abs = $(A_{510}–A_{700})$ pH 1.0 – $(A_{510}–A_{700})$ pH 4.5. TAC was calculated using the following equation: TAC (mg/100 g) = [(A/*eL*) x MW x D x (*V/G*) x 100)] where A is absorbance, e is cyanidin-3-glucoside molar absorbance (26900), L is the cell path length (1 cm), MW is the molecular weight of anthocyanin (449.2 Da), D is the dilution factor, V is the final volume (ml), and G is the dry weight (mg), expressed as milligrams of cyanidin-3-glucoside equivalents/100 g dry seeds.

Determination of the free sugar content

Free sugars were determined using HPLC according to the method of Youn and Chung (2012). The purple maize extracts were passed through Sep-Pak C_{18} cartridges and 0.45 µm membrane filters. The mobile phase were acetonitrile and water (69 : 31) at a flow rate of 4 mL/min and used Evaporative Light Scattering (ELS) detector. Sucrose, glucose and fructose standard solutions were used for calibration.

Antioxidant activity determination

Radical scavenging activity of the purple maize extracts was determined using DPPH assay (Leong and Shui, 2002). Briefly, a 3 x 10^{-5} M DPPH solution in methanol was prepared. An aliquot (50 µl) of each sample was added to 950 µl of methanol DPPH solution. Absorbance was measured at 517 nm after holding for 30 minutes at the room temperature. The radical scavenging activity was calculated by the following formula: percentage inactivation = [1 – (absorbance of sample/absorbance of DPPH) x 100], reported by EC_{50}.

RESULTS AND DISCUSSION

Statistical analysis

The effects of two independent variables; temperature (X_1) 100-180 ° C and time (X_2) 10-30 min on 4 response variables; TPC, TAC, EC_{50} and free sugar evaluation were studied using Response Surface Methodology (RSM). Results of CCD were 13 treatments as shown in Table 1. The analysis of variance for purple maize quality and regression coefficient were shown in Table 2 and 3, respectively. The significance of the equation parameter was estimated by F value at probability (Prop > F) less than 0.05.

Effect of roasting temperature and time

The total phenolic content of purple maize extract increased with increasing roasting temperature and time. The highest levels of total phenolic compounds were obtained at roasting temperature and time; 162.60 ° C and 25.26 min, respectively (Figure 1). These results indicated that roasting may have partially destroyed the cell structure in the seeds, resulting in the release of some phenolic compound from the cell wall (Ee *et al*., 2011). In addition, the roasting is likely to induce Maillard reaction (Manzocco *et al*., 2001)

Table 1 Central Composite Design arrangement for independent variables Temperature (X_1) and Time (X_2) and their response; total phenolic content, total anthocyanin content, DPPH scavenging activity and free sugar.

Exp. no.	Coded variable		Actual variable		TPC	TAC	EC_{50}	Free sugar
	Temp. (° C)	Time (min)	Temp. (° C)	Time (min)				
1	0.00	-1.41	160.00	25.00	907.81	258.57	10.80	0.0013
2	0.00	0.00	140.00	25.00	183.99	183.99	204.65	0.0020
3	-1.41	0.00	160.00	25.00	907.81	258.57	10.80	0.0013
4	1.00	-1.00	180.00	30.00	194.57	3.27	49.64	0.0000
5	0.00	0.00	140.00	30.00	417.76	27.35	107.47	0.0000
6	-1.00	-1.00	180.00	20.00	221.74	9.59	114.54	0.0056
7	0.00	-1.00	180.00	25.00	327.03	3.74	113.40	0.0023
8	1.00	1.00	160.00	30.00	555.56	161.31	80.33	0.0043
9	0.00	0.00	160.00	25.00	907.81	258.57	10.80	0.0013
10	0.00	0.00	160.00	25.00	907.81	258.57	10.80	0.0013
11	0.00	1.41	160.00	20.00	784.57	216.95	114.54	0.0128
12	1.41	0.00	140.00	20.00	256.19	158.27	210.73	0.0760
13	-1.00	1.00	160.00	25.00	907.81	258.57	10.80	0.0013

TPC = total phenolic content (mgGAE/100g crude extract), TAC = total anthocyanin content (mg/100g), EC_{50} = half maximal effective concentration (g flour/g DPPH), free sugar (g/100g).

Table 2 Analysis of variance for qualities of the purple maize.

Source	Degree of freedom	Sum of square			
		TPC	TAC	EC_{50}	Free sugar
Model	5	995800*	123400*	55008.6*	0.002947*
Linear	2	10607.82	28690.85	11155.36	0.001708
Quadratic	2	976300*	90833.22*	43485.31*	0.0004532
Residual	7	133500	18154.65	7703.24	0.002138
Lack of fit	3	133500	18154.65	7703.24	0.002138
Pure error	4	0.000	0.000	0.000	0.000
R^2		0.8818	0.8718	0.8772	0.6000

* Significant at $p < 0.05$

Table 3 Regression coefficients for TPC, TAC, EC_{50} and free sugar response of temperature and time.

Coefficient	TPC	TAC	EC_{50}	Free sugar
$ß_0$	907.81*	258.57*	10.80*	0.008423*
$ß_1$	-27.66	-53.46*	-35.38*	-0.008747
$ß_2$	-23.68	-26.99	-11.94	-0.0012
$ß_{12}$	-47.19	31.15	9.59	0.018*
$ß_1^2$	-351.71*	-105.32*	77.55*	
$ß_2^2$	-173.76*	-57.69*	25.37	

$ß_0$ = constant, $ß_1$ = temperature, $ß_2$ = time; $ß_1^2$ = temperature^2, $ß_2^2$= time^2 , $ß_{12}$=coefficient of interaction between temperature and time; *Significant at *P < 0.05*

The total anthocyanin content increased with increasing roasting temperature and time. The highest levels of total anthocyanin content were obtained at roasting temperature and time; 159.26 ° C and 24.24 min, respectively and then decreased with increasing roasting temperature and time (Figure. 1). The stability of anthocyanin can be destroyed by heat which caused sugar hydrolysis, leading to the changes of blue color to red one as reported by Roobha *et al*. (2011) that the high temperature could destroy the anthocyanin and copigment structure.

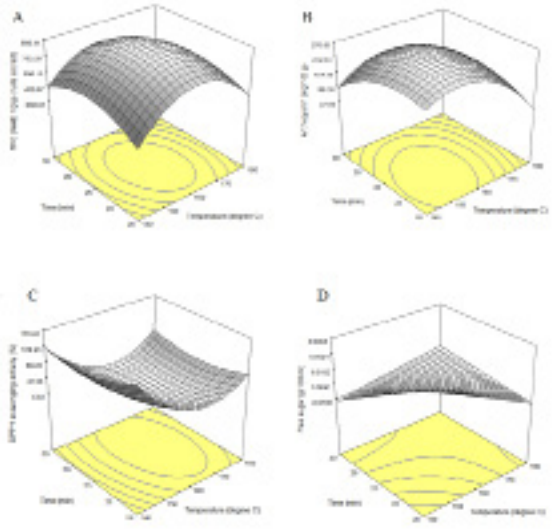

Figure 1 Response surface of total phenolic content (A), total anthocyanin content (B), DPPH radical scavenging activity (C) and free sugar content (D)

The free sugar contents were measured in different amounts depending on the substrate concentration of non-enzymatic browning reaction (Yaylayan and Kaminsky, 1998) The result showed that the lowest levels of free sugar content were obtained at roasting temperature and time; 157.43 ° C and 24.33 min, respectively. Free sugar content was decreased to undetectable level as roasting temperature and time are increased, this may be related to the increased generation of Maillard reaction. The Maillard reaction is a chemical reaction between an amino acid and a reducing sugar, usually requiring the addition of heat. High-temperature of roasting speeds up the Maillard reaction because heat both increases the rate of chemical reactions and accelerates the evaporation of water. When the food dries, the concentration of reactant compounds increases and the temperature climbs more rapidly. Therefore, the high temperature should not be used which may cause degradation of many nutrients including carbonyl and amino compounds (Yaylayan and Kaminsky, 1998). However the temperature of 180-200 ° C does not cause formation of acrylamide compounds (Brathen and Knutsen, 2005).

The DPPH scavenging activity reported as EC_{50} decreased with increasing roasting temperature and time. The lowest levels of EC_{50} were obtained at roasting temperature and time; 159.26 ° C and 24.24 min, respectively. However the lower EC_{50} value represents more efficiency of antioxidants because EC_{50} value indicates the amount of antioxidants that cause concentration of DPPH radical remaining 50%.

CONCLUSION

Purple maize kernels prepared using specified roasting temperature and time, and extracted with 95% ethanol were studied for total phenolic content, total anthocyanin content, EC_{50} and free sugar by using experimental design based on CCD and RSM. The results were significantly influenced by roasting temperature and time which optimum condition was 160 ° C for 25 min. Furthermore EC_{50} have related with phenolic content because structure of phenolic compound is a hydroxyl group which can combine to free radical, leading to the increase of antioxidant activity same as occurred with anthocyanin. The optimum roasting temperature and time may be useful for food industry such as beverage manufacturing or food additive in order to enhance flavor.

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