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Optimization of Anthocyanin and Effects of Acidulants on Phytochemicals and Antioxidant Activities in Purple Waxy Corn Cookies

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Abstract

The objectives of this study were: 1) to investigate the optimal condition of purple waxy corn cookies (PWCCs) process on the maximal anthocyanin content using response surface methodology (RSM) 2) to evaluate the effects of adding organic acids of PWCCs on the total anthocyanin content (TAC), total flavoniod content (TFN), total phenolic content (TPN), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging ability (ABTS-RSA), ferrous chelating ability (FCA) and 3) to investigate the effects of storage temperatures of PWCCs on TAC and total plate counts (TPC). The optimum condition for maximal TAC (221.47 µg CE/g) was: 1.08% guar gum content and 24.5% water content (flour base basic). The acidification of PWCCs with four organic acids including citric acid, fumaric acid, glucono-delta-lactone (GDL) and lactic acid at 0, 1, 2, 3, 4, 5, or 6% (flour weight basis) could reduce TAC, TFN, TPN, ABTS-RSA and FCA degradations. In addition, PWCCs exhibited a significant decrease in phytochemical and antioxidant degradations as increasing of acid concentrations. However, acidified PWCC with 3% citric acid showed the highest of TAC (269.2 µg CE/g), TFN (398.9 mg QE/g), TPN (688.6 mg GE/g), ABTS-RSA (3.8 mg TE/g), and FCA (544.6 µg EDTA/g). Lightness and chroma values of PWCCs were significantly increased as acid concentrations increased meanwhile hue value was decreased. Storage condition of PWCCs at 4 ^{o}C could reduce the anthocyanin degradation and the growth of microbes higher than storage at room temperature. This study confirmed that RSM was appropriately used to maximize the anthocyanin contents in PWCCs and adding organic acids could reduce the phytochemical and antioxidant degradations as well as microbial growth.

Keywords : *Purple waxy corn, response surface methodology, phytochemical, antioxidant*

1. Introduction

Anthocyanins are natural pigments and responsible for the red, purple and blue colors of many fruits and cereal grains.[1] They are types of flavonoid, one of the largest classes of phenolic compounds in plants.[2] Currently, anthocyanins are receiving interest due to the various health benefits and nutraceutical effects because of their antioxidant and bioactive properties.[1]

Purple waxy corn (Zea mays L.) is widly cultivated in Thailand. This plant is continually developed and improved the genotype to increase the production and phytochemical compounds.[3] Anthocyanins are associated with the aleurone layer of the endosperm and affect the visible color of the grain.[1] Generally, kernels of small to medium size produce more intense purple coloration since they possess a higher proportion of aleurone layer and are less diluted by the starchy endosperm.[4] Cyanidin-3-glucoside is the major anthocyanin presented in these kernels almost 75% of total anthocyanins. However, the anthocyanin contents in purple corn genotype are difference due to environment, season, and geographical growing conditions.[5] Purple waxy corn is the main source of low priced anthocyanins compared with other phytochemical plants.[6] Therefore, the development of purple waxy corn products for lifestyle of the modern consumer, who expect the health benefit and modern products, can rapidly increase the market share.[3]

However, anthocyanins are rapidly degraded from the food processing and storage. The important factors affect to anthocyanin degradation such as oxygen, light, pH, sugar, and high temperature.[7] In addition, many studies reported that water activity (a_w) or amount of water also affected on anthocyanin degradation,[8] since antocyanins were hydrolyzed by water at high temperature condition. Anthocyanins are significantly degraded as increasing of a_w at 100 ^oC.[7] Moreover, Del Pozo-Insfran et al[4] revealed that adding organic acids in products could reduce the anthocyanin and antioxidant degradations.

Cookies are favorite bakery products.[9] Therefore, using the purple waxy corn flour substitute wheat flour in cookie product can be the antioxidant product delivery to the consumer. However, gluten in wheat flour is the main structure of protein that enhances the dough forming and v iscoelastic properties of food products.[10] Some food additives such as starches, gums, hydrocolloids or dairy products should be added to the gluten-free food products to obtain the desired quality.[11]

Guar gum is polysaccharide (complex carbohydrate) with long chain molecule and high molecular weight. Guar gum is mostly added to the food products mainly for their thickening, gelling and water retention properties.[12] Guar gum is used to improve mouth feel and to change the viscosity of solutions due to their high polymeric nature and the interactions between polymer chains when It is dissolved or dispersed.[13] In addtion, guar gum is highly stable in pH range of 4.0-10.5 and high temperature.[14] The regulation of adding guar gum in food products is around 0.1-0.3%.[12]

This research primary focused at the optimum PWCC process to maximize the total anthocyanin content (TAC) using response surface methodology (RSM). RSM, which is a collection of statistical and mathematical techniques, is a useful tool for development, improvement, and optimization of processes.[15] It is used to examine the relationship between one or more response variables and a set of quantitative experimental variables or factors.[16]

2. Materials and methods

This research was conducted as an experimental study to determine the maximum of TAC of PWCC using central composite design (CCD) and effects of acidulants on phytochemical and antioxidant contents of PWCCs was performed using the completed randomized design (CRD).

2.1 Purple waxy corn flour preparation

Dried purple waxy corn kernels (PWCKs) in harvest maturity were used in this study. These corns were obtained from Tambol Khamtao, Muang, Nakhon Phanom. PWCKs were milled into whole-grain purple waxy corn flour by a disk mill (DISK MILL, AK-45, FFC-45), followed by sifting through 80 mesh screens. Purple waxy corn flour (PWCF) was used to prepare the purple waxy corn cookie.

2.2 Purple waxy corn cookie process

Purple waxy corn cookie (PWCC) recipe composed of the blended flours and guar gum. The total flour was a blend of whole-grain purple waxy corn flour and wheat flour. The final blend was set at 80:20 (w/w) corn/wheat.[9] Guar gum was added to the cookie recipe to increase the water retention capacity.[14] A cookie recipe containing 100% total flour, 72.5% sugar, 28.4% shortening, 0.9% salt and 1.1% sodium bicarbonate (all percentages are given on flour weight basis) was used in the experiments. Guar gum and water content were variable at 0.5-1.5 and 18.5-24.5 % (flour weight basis), respectively.[9] Preparation of cookie, firstly, dry ingredients (total flour, sodium bicarbonate, salt and gum) were mixed thoroughly. In a separate cup, sugar and shortening were mixed for 5 min at 125 rpm by using a mixer (Kitchen Aid, 5K45SS, USA). Then, dry ingredient mix and water were added simultaneously to this mixture and mixed for 1 min at 85 rpm. The cookie dough was rolled out to 0.5 cm thick, and then cut out the cookies into circle shape (4 cm diameter). The cookie was baked at 185 °C for 15 min.[6] PWCC Samples were placed in re-sealable plastic bags after cooling down and immediately stored at -18 °C.

2.3 Effects of acidulants on phytochemical and antioxidant properties

Organic acids including citric acid, fumaric acid, glucono-delta-lactone (GDL), and lactic acid were used to study the stability of cookie on the phytochemical and antioxidant contents as well as color values. All organic acids were used in the food industry in order to improve the functional and nutritional properties of food preparations.[4] Each acid was added to the optimal PWCC recipe obtained from previously step at 0, 1, 2, 3, 4, 5, or 6% (flour weight basis).

2.4 Effects of temperature storages on anthocyanins and total plate counts

Acidified PWCCs were stored at room temperature and 4 °C for 4 weeks to study the effect of storage temperature on anthocyanin content and total plate counts (TPC). The samples were analyzed the anthocyanin contents and total plate counts in every week.

2.5 Analytical methods

2.5.1 The pH measurments

The pH measurements on the cookie were analyzed according to method of Li et al.[9] A 10 g sample of cookie was crushed and dispersed in

100 mL of distilled water and allowed to settle for 30 min. After that the pH was taken with pH meter (pH211, Hanna Instrument Ltd., USA.).

2.5.2 Phytochemical extraction

Phytochemicals were extracted following the method of Abdel-Aal and Hucl.[6] A sample (3 g) was weighed in a 50 mL centrifuge tube, and 24 mL of acidified ethanol (ethanol and HCl 1.0 N, 85:15, v/v) was added. The mixture was shaken for 30 min and centrifuged at 27,200 x *g* for 15 min (Centrifuge refrigerator, AVANTI ™ J25, Beckman Ltd., USA.). The supernatant or phytochemical extract was dried by using rotary evaporator (BUCHI R-114, BUCHI Corp., USA.) at 50 °C. The phytochemical extract was kept at -18 °C to use for the next experiment.

2.5.3 Total anthocyanin content

Total anthocyanin content (TAC) was determined following the pH differential method and quantified as cyanidin-3-glucoside equivalents.[17] Absorbance was measured with a UV-visible spectrophotometer (LAMBDA 25, Perkin Elmer, Inc., Germany) simultaneously at λ =420 and λ = 700 nm in buffers at pH 1.0 and 4.5, and the formula, A= (A420-A700) pH 1.0 - (A420-A700) pH 4.5, was used. TAC was calculated as follow: TAC = (A x MW x DF x 1000)/(ε x l) where, DF was dilution factor, ε was a molar absorption of 26,900 L/cm/mol, MW was molecular weight of 449.2 g/mol. TAC was expressed as µg CE/g dry sample.

2.5.4 Total flavonoid content

Total flavonoid content (TFN) was conducted by aluminium chloride colorimetric assay described by Nurhanan and Wan Roli.[18] In this test, 0.25 mL of the extract (1000 µg/ml) was added into a bottle followed by addition of 75 µL of sodium nitrate (5% w/v). The mixture was reacted for 6 min after which 150 µL of aluminum chloride (10% w/v) was added. The mixture was left to react for another 5 minbefore added with 0.5 mL of NaOH (1 M). The solution was raised to 2 mL with distilled water. The absorbance of the resultant sample was measured at 510 nm by using UV-vis spectrophotometer. Quercetin was used as a standard and TFN was expressed as mg QE/g dry sample.

2.5.5 Total phenolic content

The total phenolic content (TPN) was determined with Folin-Ciocalteau reagent assay decribed by Inglett et al.[19] One mL of the diluted extract and 0.5 mL of Folin-Ciocalteau reagent (Folin-Ciocalteau reagent diluted with water, 1:1, v/v) were added into a 10 mL volumetric flask. The solution was swirled and added with 1.5 mL of sodium bicarbonate (20% w/v) and raised with distilled water. The solution was left to stand at room temperature for 2 h in the dark. The absorbance was recorded at 765 nm by using UV-vis spectrophotometer against blank. TPN was compared with gallic acid standard curve and expressed as mg GAE/g dry sample.

2.5.6 ABTS radical scavenging ability

ABTS radical scavenging ability (ABTS-RSA) was determined according to the method of Yin et al.[20] Two mM 2,2’-azono-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) was prepared using 0.1 M phosphate buffer containing 0.818% NaCl and 0.0015% KCl, pH 7.4. ABTS solution was mixed with an equal volume of 70 mM $K_2S_4O_8$ to form $ABTS^{\bullet+}$. Five mL PWWC extract, 0.3 mL $ABTS^{\bullet+}$ solution was added and uniformly mixed. The resultant samples were incubated at room temperature for 6 min in the dark and then the absorbance at 734 nm was measured by using UV-vis spectrophotometer. ABTS-RSA was determined by standard curve of Trolox (6-hydroxy-2.5.7.8 tetramethyl-chloroman -2-carboxylic acid) concentration versus ABTS-RSA (%) and expressed as mg TE/g dry sample.

2.5.7 Ferrous chelating ability

Ferrous chelating ability (FCA) was performed according to the method of Yin et al.[20] One mL PWCC extract, 3.7 mL ddH_2O and 0.1 mL 2 mM $FeCl_2$ $4H_2O$ were added and then 0.1 mL 5 mM Ferrozine (3-(2-Pyridyl)-5, 6-diphenyl-1,2,4-triazine-4',4"-disulfonic acid monosodium salt) was added. After 10 min of reaction, the resultant samples were measured the absorbance at 562 nm by using UV-vis spectrophotometer. The lower the absorbance indicated higher ferrous chelating ability was obtained. FCA was determined by standard curve of EDTA (Ethylenediminetetraacetic acid) concentration versus FCA (%) and expressed as µg EDTA/g dry sample.

2.5.8 Color measurments

Color characteristics of ground cookie were measured by Hunter CIE color (CIE, 2004).[21] Lightness (L^*), redness (a^*), and yellowness (b^*) values were measured using a chromameter (Minolta CR-300 series, Japan). Chroma, hue, and E values were calculated using three equations: Chroma = $(a^{*2} + b^{*2})^{1/2}$; Hue =tan-1 (b^*/a^*); and E value = $(\Delta L^{*2} + a^{*2} + b^{*2})^{1/2}$.

2.5.9 Total plate count

Total plate count (TPC) was performed by pour plate method [22] using plate count agar (PCA). The amount of TPC was reported as CFU/g.

2.6 Statistical analysis

All tests were performed in duplicate and data were averaged. CCD was conducted using design expert software version 5.08 (Stat-Ease, Inc., Minneapolis, MN, USA.). Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined using Duncan's multiple range test ($P \leq 0.05$). Statistical analysis was carried out using SPSS statistic program (Version 17) for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Experimental design

RSM was used to optimize the PWCC process conditions to maximize the total anthocyanin content (TAC; Y), with CCD.[23] The main variables, which were chosen for CCD including the guar gum content (0.5-1.5% (w/w); x_1), water content (18.5-24.5% (w/w); x_2). Symbols and coded variable levels for the variables (x_1 and x_2) are given in Table 1 (response surface were obtained using $\pm|\alpha|= 1.41$). A total of 13 experiments were designed (Table 2). The second-order model was obtained from CCD. The model proposed for the response (Y) was:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad (1)$$

where Y is the response variable, x_1 and x_2 are the coded process variables and β_0, β_1, β_2, β_{11}, β_{22}, and β_{12} are the regression coefficients.[24]

Table 1. Independent variables and levels of CCD applied to Purple waxy corn cookie process.

Independent variables		Levels				
Code	Real	-α	+1	0	+1	+α
x_1	Guar gun content (%, w/w)	0.3	0.5	1.0	1.5	1.7
x_2	Water content (%, w/w)	17.3	18.5	21.5	24.5	25.7

Table 2. Response values for total anthocyanin content *(Y)* with different combinations of guar gum content (x_1) and water content (x_2) in CCD.

Experiment	Code level				Response values
	guar gum content (x_1) (%, w/w)		water content (x_2) (%, w/w)		Total anthocyanin content *(Y)* (µg CE/g)
1	0	(1.0)	-1	(21.5)	224.5
2	0	(1.0)	0	(21.5)	198.2
3	0	(1.0)	0	(21.5)	224.1
4	+α	(1.7)	0	(21.5)	201.8
5	-α	(0.3)	0	(21.5)	183.1
6	+1	(1.5)	+1	(24.5)	184.9
7	0	(1.0)	-α	(17.3)	193.3
8	-1	(0.5)	-1	(18.5)	165.8
9	0	(1.0)	+α	(25.7)	174.5
10	-1	(0.5)	+1	(24.5)	152.8
11	+1	(1.5)	-1	(18.5)	157.5
13	0	(1.0)	0	(21.5)	226.3
12	0	(1.0)	0	(21.5)	231.8

Yetilmezsoy et al [25] reported that the analysis of variance was essential for significance of the model. The developed model could adequately represent the real relationship among the chosen variables when the *P*-value of the regression model significance is less than 0.05. The coefficient of determination (R^2) is used to determine a degree of fit of model. For a suitable fit of a model, R^2 should be at least 0.80. In addition, the lack-of-fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. The insignificant value of lack of fit (P>0.05) revealed that the quadratic model was statistically significant for the response; therefore, it could be used for further study. Finally, the three-dimensional response and contour plot for the measured responses were formed based on the second-order model as a function of two variables, maintaining the other variables at fixed levels.

All 12 designed experiments were conducted for optimizing the two variables (guar gum content and water content) in CCD. The response values (*Y*) with different combinations of guar gum content (x_1) and water content (x_2) were shown in Table 2. Total anthocyanin content *(Y)* in CCD varied from 152.8 to 231.8 µg CE/g. The regression modelfor these variables did not show lack of fit (*P=0.2033*) and were statistically significant ($P \leq 0.05$) with R^2=0.8784, indicating that the adjustment of the model to the experimental data was appropriate. The second-order model was presented in Eq. (2).

$$Y = 220.98 - 20.00x_1^2 - 24.27x_2^2 \quad (2)$$

Eq. (1) indicated that x_1 and x_2 displayed quadratic effects. Contour plot and the predicted values of *Y* based on the range of x_1 and x_2 in RSM were estimated as illustrated in Figure 1. TAC increased as these two variables decreased, maximum TAC (221.47 µg CE/g) was obtained at 1.08% guar gum content and 24.5 % water content and then followed a decline as guar gum content and water content further increased.

Li et al [9] demonstrated that the TAC in bakery products was less degraded as water content of 21.5% and guar gum content of 1% (flour weight basis). Water activity (a_w) in bakery products could reduce the anthocyanin degradation at high temperature condition. According to the research of Gradinaru et al[26] revealed that decreasing a_w from 0.99 to 0.34 was the main effect to increase the total anthocyanin degradation around 4 times. In higher temperature and lower a_w conditions increased the total anthocyanin degradation almost 70 times.[8] Moreover, Jiménez et al [27] studied the effect of difference a_w on the total anthocyanin degradation of blackberry product at above 100 °C. The resulted showed that the maillard reaction, which condensed between reducing sugar and amino acids, was significantly factor in the total anthocyanin degradation.[28] The intermediete a_w (0.5-0.8) condition demonstrated the highest non-enzymatic browning reaction however reaction was declined as increasing of a_w value. Decreasing of reaction might effect of the reactor in solvent that diluted with water following law of mass action.[26] Therefore, the non-enzymatic browning reactions directly effect to the anthocyanin degradation.[27] However, Stauffer [29] revealed that adding guar gum in food recipe enhanced the water retention capacity in product since adding guar gum could decrease the anthocyanin degradation from high temperature.[9]

To confirm the validity of the statistical experimental strategies and to gain a better understanding of TAC, three additional verification experiments were conducted. The chosen conditions for guar gum content and water content determined with the statistical model, and the experimental results of the *Y* are listed in Table 3. All observation values were close to the predicted values using RSM showing % error less than $\pm$5%. These results indicated that the model was adequate and useful for reduction of anthocyanin degradation in PWCCs.

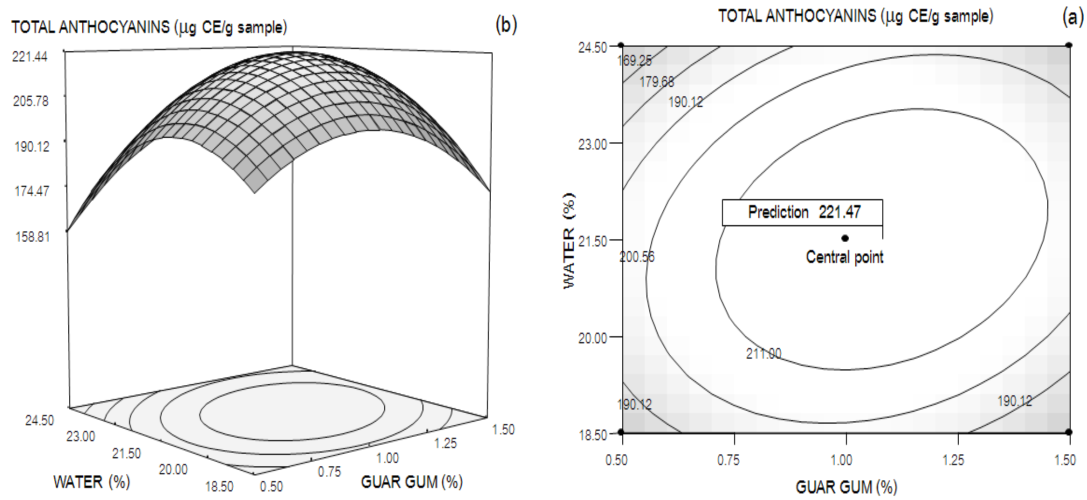

Figure 1. Contour plots (a) and 3-D response surface curves (b) showing effects of guar gum content (% w/w) and water content (% w/w) on total anthocyanin content (µg CE/g sample) of

Table 3. Verification experiments of the purple waxy corn cookies.

Experient	Conditions		Response values
	Guar gum content (x_1) (%, w/w)	Water content (x_2) (%, w/w)	Total anthocyanin content (Y_1) (µg CE/g)
1*	75	9.8	166.7
1**	75	9.8	169.7±0.2
	% Error		1.8
2*	67	6.5	221.0
2**	67	6.5	225.7±0.5
	% Error		2.1
3*	54	5.5	196.7
3**	54	5.5	188.9±0.8
	% Error		-4.1

* Predicted value, ** Observed value.

3.2 Effects of acidulants on phytochemical and antioxidant properties

The acidified PWCCs with citric acid, fumaric acid, glucono-delta-lactone (GDL) and lactic acid showed higher TAC, TFN, and TPN than nonacidified PWCC. Moreover, TAC, TFN, and TPN markly increased up to 3% organic acids concentrations and slightly decreased after that (Table 4). However, adding 3% citric in PWCC showed the highest TAC, TFN, and TPN (269.2 µg CE/g, 398.9 mg QE/g, and 688.6 mg GE/g) followed fumaric acid (265.8 µg CE/g, 387.6 mg QE/g, and 449.8 mg GE/g), GDL (262.0 µg CE/g, 379.1 mg QE/g, and 265.6 mg GE/g), and lactic acid (240.1 µg CE/g, 321.3 mg QE/g, and 339.9 mg GE/g) respectively, (Table 4). The results suggested that adding organic acids in PWCC recipes could reduce TAC, TFN, and TPN degradations during baking processs. According to the study of Li et al[10] revealed that adding organic acids in cookie recipe to decrease the pH values could significantly increase TAC in cookie. Del Pozo-Insfran et al [4] indicated that acidified dough decreased TPN, TFN, and TAC degradations of tortilla product since organic acids enhanced the stability of phytochemical compounds.

ABTS-RSA and FCA increased as acid concentrations were increased. Acidified PWCC with 3% organic acids showed the highest ABTS-RSA and FCA. However, acidified PWCCs with 3% citric acid demonstrated the higest the ABTS-RSA and FCA (3.8 mg TE/g, and 544.6 µg EDTA/g) followed fumaric acid (3.3 mg TE/g and 432.6 µg EDTA/g), GDL (3.0 mg TE/g, and 343.9 µg EDTA/g), and lactic acid (3.3 mg TE/g, and 333.5 µg EDTA/g), respectively. The results showed that antioxidant activites of PWCCs depended on amount of phytochemical content. According to the research of Chang et al [30] exhibited that antioxidant properties of purple corn depened on phenolic content especially feruric acid and *p*-coumaric acid. Moreover, Bily et al [31] revealed that the anthocyanin or phenolic compounds affected to the higher antioxidant capacities of purple waxy corn product. These phytochemical compounds are reducing, donating hydrogen, singlet oxygen scavenging agents meanwhile flavonoids compound is composed of hydroxyl group that exhibited the free radical scavenging and ferrous chelating capacities.[4] The results concluded that adding organic acids in purple waxy corn products affected to the antioxidant properties due to the higher reducing property.[9]

Table 4. Effects of adding organic acids (flour weight basis) on pH, total anthocyanin content (TAC), total flavonoid content (TFN), total phenolic content (TPN), and ABTS radical scavenging activity (ABTS-RSA), and ferrous chelating ability (FCA) of purple waxy corn cookies[1].

Acids	Conc. (% w/w)	pH	TAC [2] (µg CE/g)	TFN[2] (mg QE/g)	TPN[2] (mg GE/g)	ABTS-RSA[2] (mg TE/g)	FCA[2] (µg EDTA/g)
Control	0	7.2	145.7±4.5^{g}	157.5±3.9^{l}	187.3±6.2^{h}	2.6±0.1^{k}	221.5±8.1^{l}
Citric acid	1	6.1	179.9±7.4^{f}	295.0±7.1^{g}	424.3±5.1^{d}	3.4±0.1^{d}	357.2±7.0^{g}
	2	5.2	229.5±4.5^{d}	356.8±11.1^{e}	601.0±4.6^{c}	3.6±0.1^{c}	478.0±8.0^{d}
	3	4.5	269.2±10.9^{a}	398.9±7.1^{a}	688.6±12.0^{a}	3.8±0.1^{a}	544.6±7.2^{a}
	4	4.1	266.6±5.7^{a}	395.1±10.1^{a}	685.4±10.7^{a}	3.7±0.1^{b}	538.7±8.2^{a}
	5	3.9	265.2±4.3^{a}	382.8±9.5^{b}	654.7±11.7^{b}	3.7±0.1^{b}	526.9±9.6^{b}
	6	3.7	260.9±7.8^{ab}	376.4±12.0^{c}	647.2±9.3^{b}	3.6±0.1^{c}	515.1±10.6^{c}
Fumaric	1	5.2	160.7±8.6^{g}	244.4±9.9^{j}	266.8±7.3^{f}	2.8±0.1^{j}	322.4±9.6^{i}
acid	2	4.5	233.3±3.8^{cd}	370.9±8.6^{cd}	382.4±6.4^{d}	3.0±0.1^{h}	393.1±10.3^{f}
	3	3.9	265.8±5.5^{a}	387.6±8.7^{b}	449.8±5.5^{d}	3.3±0.1^{d}	432.6±7.4^{e}

Table 4. Effects of adding organic acids (flour weight basis) on pH, total anthocyanin content (TAC), total flavonoid content (TFN), total phenolic content (TPN), and ABTS radical scavenging activity (ABTS-RSA), and ferrous chelating ability (FCA) of purple waxy corn cookies[1] (Continue).

Acids	Conc. (% w/w)	pH	TAC [2] (µg CE/g)	TFN[2] (mg QE/g)	TPN[2] (mg GE/g)	ABTS-RSA[2] (mg TE/g)	FCA[2] (µg EDTA/g)
	4	3.7	261.1±9.0^{ab}	371.5±9.9^{c}	477.6±5.2^{d}	3.2±0.1^{f}	422.1±8.9^{e}
	5	3.5	259.2±7.7^{b}	365.8±8.3^{d}	469.7±4.7^{d}	3.2±0.1^{f}	420.9±7.9^{e}
	6	3.4	258.8±7.1^{b}	371.4±9.5^{cd}	457.4±9.4^{d}	3.1±0.1^{g}	405.3±10.0^{f}
Glucono-delta-lactone (GDL)	1	6.4	179.9±7.4^{f}	164.6±5.5^{k}	194.9±5.5^{h}	2.6±0.1^{k}	262.5±6.1^{k}
	2	6.3	235.9±4.5^{c}	260.3±5.8^{i}	238.2±7.3^{g}	2.8±0.1^{j}	294.0±7.6^{j}
	3	5.9	262.0±8.9^{a}	379.1±9.1^{c}	265.5±5.6^{f}	3.0±0.1^{h}	343.4±9.4^{g}
	4	5.3	264.0±7.8^{a}	368.2±6.2^{d}	258.3±5.4^{e}	2.9±0.1^{i}	323.9±10.1^{h}
	5	5.2	263.5±4.2^{a}	359.4±9.2^{e}	256.2±6.2^{e}	2.8±0.1^{j}	327.7±11.9^{h}
	6	4.9	258.4±9.3^{b}	365.8±8.6^{d}	245.9±5.2^{g}	2.8±0.1^{j}	330.0±9.1^{h}
Lactic acid	1	6.2	187.3±6.2^{e}	241.2±5.3^{j}	254.6±6.0^{f}	2.8±0.1^{j}	262.1±4.0^{k}
	2	5.4	231.0±4.5^{d}	271.3±6.4^{h}	274.4±1.7^{f}	3.0±0.1^{h}	297.0±8.2^{j}
	3	5.0	240.1±6.7^{c}	321.3±9.5^{f}	339.9±5.4^{e}	3.3±0.1^{e}	333.5±6.3^{h}
	4	4.3	239.3±5.6^{c}	323.0±7.1^{f}	345.1±6.5^{e}	3.2±0.1^{f}	318.5±8.5^{i}
	5	4.0	235.7±4.7^{c}	298.3±7.1^{g}	339.6±5.0^{e}	3.2±0.1^{f}	320.6±5.8l^{i}
	6	3.8	230.6±3.5^{d}	301.1±7.9^{g}	325.4±5.7^{e}	3.1±0.3^{g}	323.3±8.6l^{i}

[1] Mean ± SD obtained from duplicated experiments.

[2] Values followed by different letters within columns are significantly different (Duncan, $P < 0.05$).

3.3 Effects of acidulants on color values

Results from instrumental color evaluations (Table 5) were parallel to changes in TAC retention. The formation of brown pigments from the maillard reaction is a common explanation for the colorimetric changes that take place during high temperature process.[27] The acidified PWCCs with 4 organic acids presented significantly higher the *E* value than nonacidified PWCCs (P<0.5). Moreover, lightness value of all PWCCs were increased as acid concentrations increased (Table 5). According to the study of Poei-Langston and Wrolstad et al [32] revealed that acidification induced increasing of product brightness because of hydrogen peroxide which produced from acid oxidation or condensation between acids and anthocyanin compound.

Hue values of acidified PWCCs were significantly lower than nonacidified PWCCs. Acidification of PWCCs decreased the hue value, which suggested that the lower pH favored a purple hue over the burgundy-brown. Increasing of acid concentration of PWCCs presented a higher decline in hue values (Table 5). From the hue value, nonacidified PWCCs may appear to the human eye as deeper purple color. However, baking process resulted in significant changes in hue value of PWCCs, suggesting the destruction of anthocyanins due to high processing temperatures. For all PWCCs, acidification resulted in significantly lower hue angles, which can be interpreted as a better purple color (purple not burgundy). Based on hue angles alone, acidified PWCCs resulted in a better appearance. The lower pH induced the anthocyanin oxidation affected to bleaching of purple color.[33] Poei-Langston and Wrolstad [32] revealed that the red color of product was increased with 4% ascorbic and anthocyanin compound.

Chroma values increased when increasing of acid concentration (Table 5), indicating a decrease in the opaqueness of the PWCC surface, possibly caused by changes in the visual characteristics of starch and cell walls due to maillard reaction. Maillard reaction produced the furfural products, which are an organic and darker brown compound occurred in high carbohydrate food.[34] Moreover, furfural and its derivatives can form the colorless and dark compounds in solutions containing anthocyanins.[34] Organic acids significantly affected anthocyanin degradation by a direct condensation mechanism, and enhanced polymeric pigment formation and increased browning in the oxygen environment.[30] Therefore, adding organic acids could enhance the maillard reaction as well as red or purple of anthocyanins were changed to brown pigment in PWCCs.[35] In our study, adding 3% organic acids in PWCCs demonstrated the highest TAC, TFN, and TPN as well as producing the good color appearance of PWCCs.

Table 5. Effects of adding organic acids (flour weight basis) on color values of purple waxy corn cookies.

Acids	Conc. (% w/w)	Lighness (L^*)[1]	Redness (a^*)[1]	Yellowness (b^*)[1]	Hue value[1,2]	Chroma value[1,3]	*E* value[1,4]
Control	0	50.3[e]	4.6[h]	9.6[c]	1.1[a]	10.6[h]	51.4[h]
Citric acid	1	50.5[e]	7.4[g]	10.6[a]	1.0[b]	12.9[c]	52.1[g]
	2	51.1[d]	8.2[e]	10.4[b]	0.9[c]	13.2[b]	52.7[f]
	3	51.5[d]	8.4[e]	10.3[b]	0.9[c]	13.3[b]	53.1[e]
	4	51.6[d]	9.2[d]	10.2[b]	0.8[d]	13.7[a]	53.3[e]
	5	51.9[d]	9.5[c]	10.1[b]	0.8[d]	13.8[a]	53.7[d]
	6	52.2[c]	10.2[b]	9.6[c]	0.8[d]	13.9[a]	54.0[c]
Fumaric	1	50.9[e]	7.1[g]	9.2[d]	0.9[c]	11.6[f]	52.1[g]
acid	2	51.7[d]	7.9[f]	8.8[e]	0.8[d]	11.8[f]	53.0[e]
	3	52.6[c]	8.5[e]	8.5[e]	0.8[d]	12.0[f]	53.9[d]
	4	52.8[c]	10.2[b]	7.4[h]	0.6[f]	12.5[d]	54.2[c]
	5	53.1[b]	10.7[a]	6.7[i]	0.6[f]	12.6[d]	54.5[b]
	6	54.0[a]	10.8[a]	6.9[i]	0.6[f]	12.7[d]	55.4[a]
Glucono-	1	50.9[e]	7.0[g]	10.1[b]	1.0[b]	12.2[e]	52.3[g]
delta-	2	51.8[d]	7.1[g]	8.9[e]	0.9[c]	11.3[g]	53.0[e]
lactone	3	51.9[d]	7.5[f]	8.1[f]	0.8[d]	11.0[g]	53.0[e]
(GDL)	4	52.4[c]	8.0[e]	7.7[g]	0.8[d]	11.0[g]	53.5[d]
	5	52.6[c]	8.4[e]	7.6[g]	0.7[e]	11.3[g]	53.7[d]
	6	53.5[b]	8.5[e]	7.3[h]	0.7[e]	11.1[g]	54.6[b]

Table 5. Effects of adding organic acids (flour weight basis) on color values of purple waxy corn cookies (continue)

Acids	Conc. (% w/w)	Lighness (L*)[1]	Redness (a*)[1]	Yellowness (b*)[1]	Hue value[1,2]	Chroma value[1,3]	E value[1,4]
Lactic acid	1	51.9[d]	7.1[g]	9.8[c]	0.9[c]	12.0[e]	53.2[e]
	2	52.2[c]	8.0[f]	9.5[c]	0.9[c]	12.3[e]	53.6[d]
	3	52.5[c]	8.3[e]	8.5[e]	0.8[d]	11.8[f]	53.8[d]
	4	53.2[b]	8.5[e]	8.1[f]	0.8[d]	11.7[f]	54.4[c]
	5	53.5[b]	9.6[c]	7.9[f]	0.7[e]	11.4[g]	54.9[b]
	6	54.0[a]	9.8[c]	7.5[g]	0.7[e]	11.3[g]	55.3[a]

[1] Values followed by different letters within columns are significantly different (Duncan, $P < 0.05$).
[2] Hue = TAC^{-1} (b*/a*)
[3] Chroma = $(a^{*2} + b^{*2})^{1/2}$
[4] E value = $(L^{*2} + a^{*2} + b^{*2})^{1/2}$

3.4 Effects of temperature storages on anthocyanin and total plate counts

Acidified PWCCs with 3% organic acids exhibited the highest phytochemical and antioxidant contents were stored at room temperature (25 °C) and 4 °C for 4 weeks. The storage of products at 4 °C showed higher total anthocyanin content than room temperature (P<0.05). The results indicated that storage of products at 4 °C could reduce the anthocyanin degradation (Figure 2a). According to the study of Yuan et al[36] revealed that anthocyanin content in dried berry insignificantly reduced during storage at 4 °C or -20 °C for 2 weeks. Nohynek et al [37] studied the stability of phenolics and anthocyanins in berry. They found that storage berry at 4°C demonstrated higher stability of phytochemical compounds than storage at room temperature.

Moreover, storage the acidified PWCCs with 3% organic acids at 4°C for 4 weeks demonstrated lower the TPC than room temperature (P<0.05) (Figure 2b). The results showed that the storage of products at 4°C could significantly inhibit the growth of microbes. As the same results of Nohynek et al[37] revealed that antimicrobial activity of berry was increased as storage at -20 °C. According to the research of Tajkarimi and Ibrahim[38] presented that organic acids significantly affected in antimicromial activity. Moreover, lower pH condition enhanced the antimicromial activity. Friedman and Jürgens [39] demonstrated that antimicromial activity of phenolic compound in fruit juice depended on pH values. Decreasing pH of corn products to 5.5-5.8 with fumaric acid enhanced the effectiveness of antimicrobial agents and extended the shelf life of products up to 60 days.[5]

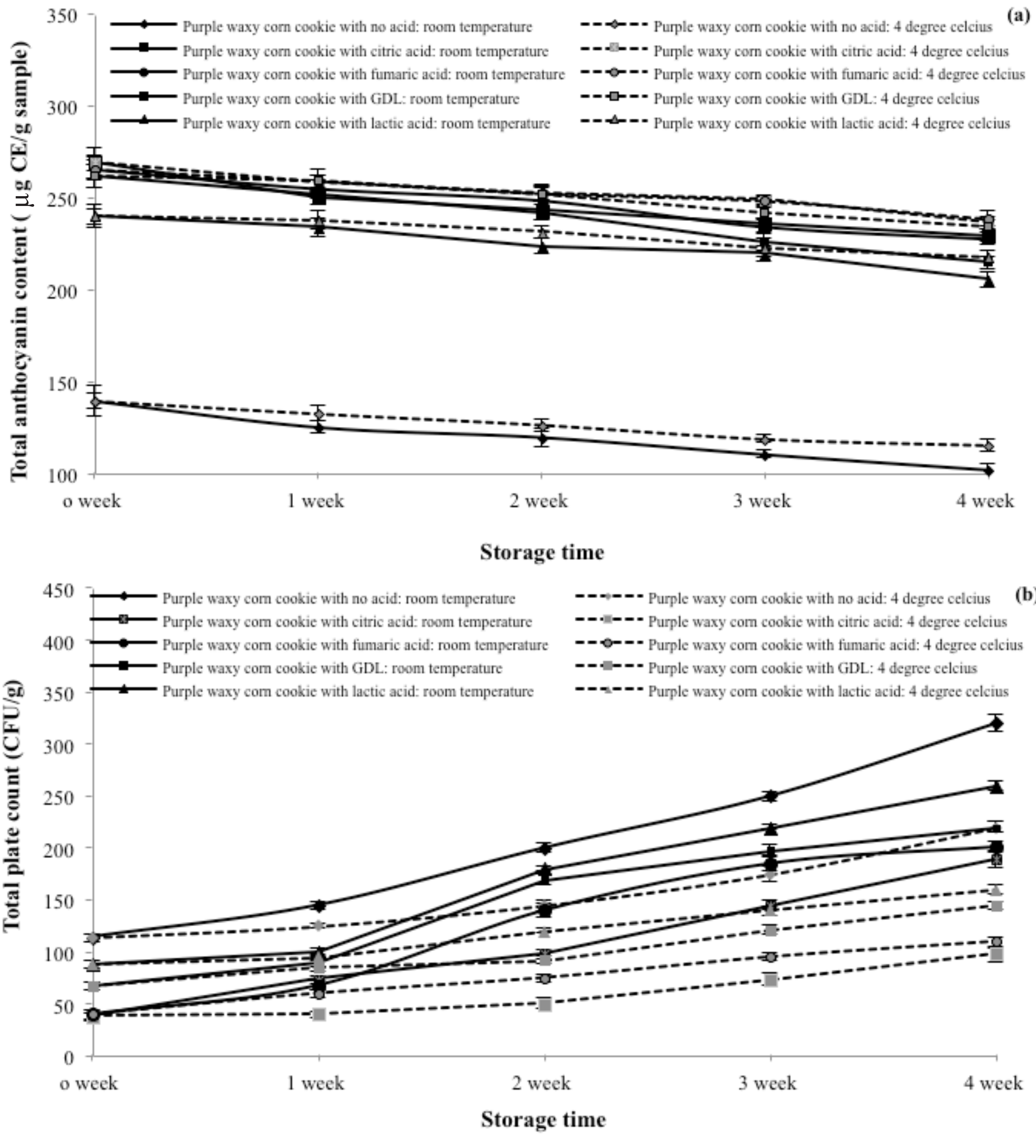

Figure 2. Total anthocyanin content (mg CE/g sample) (a) and total plate counts (CFU/g) (b) of acidified purple waxy corn cookies with 3% organic acids stored at room temperature and 4^{o}C for 4 weeks.

4. Conclusion

Central composite design was employed to optimize the variables of PWCC process. Optimum conditions for maximal total anthocyanin content (221.47 µg CE/g) was: 1.08% (w/w) guar gum content and 24.5 % (w/w) water content. Acidified PWCC with 3% citric acid reduced the highest phytochemical and antioxidant degradation. Storage of acidified PWCCs at 4 ^{o}C reduced the anthocyanin degradation and inhibited the growth of microbes more than storage at room temperature. This study would be alternative way for sustainable management of agricultural materials and added values with high nutrition and health benefit to purple waxy corn products.

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