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Optimization of Ingredients using Response Surface Methodology and Effects of Organic Acids on Phytochemicals and Antioxidant Activities in Extruded Purple Corn Noodle

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ABSTRACT

Response surface methodology (RSM) was performed to optimize the extruded purple corn noodles (EPCNs) for maximizing total anthocyanin content (TAC), total phenolic content (TPC), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging ability (ABTS-RSA), ferrous chelating ability (FCA) and tensile strength (TS). Optimal EPCN conditions were obtained with a ratio of purple corn flour to rice flour, guar gum content and water content of 66.0:34.0, 2.1 and 50.0 (% total weight flour basis), respectively. These conditions showed maximal TAC, TPC, TFC, ABTS-RSA, FRAB, FCA and TS were 128.9 µg C3GE/g, 1,018.8 µg GE/g, 1,640.7 µg TE/g, 478.4 µg TE/g and TS 18.6 g, respectively. Acidified EPCNs with citric acid, fumaric acid and ascorbic acid at 0, 0.2, 0.4, 0.6, 0.8, and 1.0% (% total flour weight basis) could enhance reduction of phytochemical and antioxidant degradations, improving tensile strength, cooking loss, color qualities and inhibiting total microbial counts as the concentration of acid was increased. These results confirmed that RSM was an efficient method for maximizing the phytochemicals and antioxidant activities and adding organic acids could improve the qualities of EPCNs. Therefore, this study would be an alternative approach in value-addition of agricultural materials for functional foods preparation with high nutraceutical values.

Keywords: Extruded purple corn noodles; Response surface methodology; Phytochemical; Antioxidant activity; Organic acid

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1. Introduction

Traditionally, rice noodles producing high energy and nutrition values are widely consumed in Asian countries. Rice noodles usually are made from rice grains with high amylose content [1]. Extrusion technology has been extensively used to obtain various food products. Extruded products are generally produced from starchy cereal grains such as corn, sorghum and rice [2]. Therefore, the extrusion process has been efficiently applied to be an alternative method for producing noodles. Purple corn (*Zea mays* L.) grain is a rich source of beneficial phytochemicals such as phenolics, flavonoids and anthocyanins. Especially, anthocyanins are the most important phytochemical compounds that influence a visible purple color and appear in the aleurone layer of endosperm [3]. Recently, anthocyanins have been increasingly focused on as being nutraceuticals exhibiting physiological functions such as antioxidant, anticancer antimicrobial, anti-inflammatory activities and reducing hypertension [4]. Rice flour contains high amylose content widely used to produce acceptable rice noodles. Amylose in rice starch plays a crucial role in creating the gel network and setting of noodle structures [5]. Hormdok and Noomhorm [6] reported that rice noodle qualities significantly correlated with swelling power, paste viscosities and gel texture of starch in rice flour. Purple waxy corn grain contains a minimal amount of amylose [7] therefore substitution of rice flour with purple corn flour in rice noodles might reduce qualities of rice noodles. Guar gum, which has a long chain molecule structure and high molecular weight, commonly was used as a thickening agent for improving mouth feel and viscosity of rice noodles [1]. Kaur et al. [8] demonstrated that addition of gums in corn starch noodles could increase cooking time and decrease cooking loss, firmness and cohesiveness. Moreover, guar gum significantly enhanced rehydration and shape of extruded rice products [9].

Furthermore, enrichments of acidic food additives in food products importantly improve the degree of gelatinization, water absorption index and rehydration ratio [10] as well as reduction of phytochemical and antioxidant degradations [11]. Water content of noodle dough is a critical factor and influences color, brightness and textural qualities of cooked noodles [12]. A low water addition in noodles causes unsuitable dough development and problem in dough stretching. Meanwhile, a high water addition causes problems during the noodle mixing and sheeting processes [13]. There have been only a few studies on optimal substitution of purple corn flour for rice flour to enhance phytochemicals and antioxidant activities of extruded noodles. Therefore, this research is focused on the optimal ingredients for maximal phytochemicals and antioxidant activities using response surface methodology (RSM). Furthermore, effects of acidic food additive enrichment on phytochemicals, antioxidant activities and physical properties of extruded purple corn noodles were also evaluated.

2. Materials and Methods

2.1 Purple corn flour preparation

Purple corn (*Zea mays* L.) cultured in Nakhon Phanom Province, Thailand, harvested after 60 days, with high contents of TAC and TPC (285 μg C3GE/g and 2,800 μg GE/g) was used to prepare purple corn flour. Briefly, purple corn kernels were dried at 60 °C for 24 h [4] using a hot air oven (U30, Memmert, German) and then milled with a disk mill (Disk Mill FFC-45, Qingdao King Lion Imp & Exp Co. Ltd, China). Subsequently, the milled purple corn was sieved through 70 mesh screens. The flour was stored in a polyethylene bag at 4 ^{o}C in the dark to inhibit microbial growth and phytochemical degradation.

2.2 Extruded purple corn noodle preparation

A single-screw extruder (Polydrive with Rheomex R 252 HAAKE, Germany) was used to prepare the extruded purple corn noodles (EPCNs). Experimental samples of extruded noodles were prepared by blending the purple corn flour (PCF) and rice flour (RF) according to the method described by Fari et al. [5]. Blended flour of PCF and RF (25:75-75:25) was thoroughly mixed with guar gum (1-3% total flour weight basis), lecithin (3% total flour weight basis) and hydrated with distilled water (30-50% total flour weight basis) to form a dough for 10 min in a mixer (Kitchen Aid, Model MK45SSWH, St. Joseph, Michigan, USA). The mixture was refrigerated at 48 ^{o}C for 12 h to obtain better hydration of starch particles and kept in the dark to avoid the phytochemical degradation [14]. EPCN conditions were set according to the method of Sobowale et al. [14] including the barrel temperatures of 100 ^{o}C, screw speed of 6.3 m/s and die opening diameter of 2 mm. Meanwhile, a constant feeding rate was kept throughout the experiments to avoid accumulation of feeding mixture in the hopper. Obtained EPCNs were cooled at 25 ^{o}C for 1 h and then dried to moisture content less than 9% (wet basis) by hot air oven at 60 ^{o}C for 1 h. Dried EPCNs were sealed in polyethylene bags and kept at 5 ^{o}C in a dark place.

2.3 Acidic food additive enrichment

Acidic food additives, including citric acid, fumaric acid and ascorbic acid, were used to improve the qualities of EPCNs. Fumaric acid exhibits the strongest organic food acid. Meanwhile, citric and ascorbic acids are widely used in the food industry in order to improve the functional and nutritional properties of food products. The selected acidic food additives were added to the optimal noodle formula with variation of concentration at 0, 0.2, 0.4, 0.6, 0.8, and 1 (% total flour weight basis). Our previous study showed that addition of 3% organic acid in purple corn cake exhibited the highest qualities of purple corn cake; however, addition of acid concentrations greater than 1% exhibited stronger sourness [11]. Therefore, the suitable concentrations of less than 1% organic acid were chosen in this study. Acidified EPCNs were measured for the reduction of phytochemical, antioxidant degradations, improvement of tensile strength, cooking loss, color values and total plate count reductions.

2.4 Analytical methods

2.4.1 Phytochemical extraction

Phytochemicals were extracted according to the modified method of Dasgupta and De [15]. Briefly, the ratio between ground EPCNs and methanol was 1:25. The mixture was thoroughly shaken using a shaker (Nine, Wizard, Thailand) for 30 min then centrifuged at 12,000 x g for 15 min. (Centrifuge refrigerator, Avanti ^{TM} J25, Beckman Ltd., USA.). The supernatant was filtered through a filter paper (Whatman No. 1) and collected for analyzing phytochemicals and antioxidant activities in vitro.

2.4.2 Anthocyanin assay

Total anthocyanin content (TAC) was determined following the pH differential method [16]. The absorbance of reaction mixture was measured by a UV-visible spectrophotometer (Lambda 25, Perkin Elmer, Inc., Germany) simultaneously at 420 and 700 nm in buffers at pH 1.0 and 4.5 and then A was generated as follows: A = $(A_{420} - A_{700})$ pH 1.0 - $(A_{420} - A_{700})$ pH 4.5. TAC was calculated as cyanidin-3-glucoside equivalents as follows: 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, and MW was molecular weight of 449.2 g/mol. Meanwhile, TAC was exhibited as µg C3GE/g dry sample.

2.4.3 Phenolic assay

Total phenolic content (TPC) was investigated using the Folin-Ciocalteau reagent assay [17]. Briefly, a 100 µL extract and 0.5 mL Folin-Ciocalteau reagent (Folin-Ciocalteau reagent diluted with water, 1:1, v/v) were added into a test tube and then thoroughly shaken. The reaction mixture had 0.8 mL sodium bicarbonate (7% w/v) added to it and was left to stand in the dark at room temperature for 2 h. The absorbance of the reaction mixture was measured at 765 nm with a UV-vis spectrophotometer. Lastly, TPC was compared with gallic acid standard curve and expressed as µg GE/g dry sample.

2.4.4 Flavonoid assay

Total flavonoid content (TFC) was performed by aluminium chloride colorimetric assay [18]. Briefly, 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 5 min before adding 0.5 mL of NaOH (1 M) and then raising the mixture level to 2 mL with distilled water. The absorbance of the reaction solution was measured at 510 nm by using UV-Vis spectrophotometer. Quercetin was used as a standard and TFC was expressed as µg QE/g dry sample.

2.4.5 ABTS radical scavenging activity assay

ABTS radical scavenging activity (ABTS-RSA) was analyzed according to the modified method of Re et al. [19]. Firstly, ABTS stock solutions of 7 mM with 2.45 mM $K_2S_4O_8$ in 1:0.5 (v/v) were prepared and left for 12-16 h in the dark. The solution was then diluted in 0.1 M phosphate buffer containing 0.818% NaCl and 0.0015% KCl, pH 7.4 to give an absorbance of 0.70 $\pm$ 0.2 at 734 nm. A 100 µL extract was added to a 3 mL $ABTS^{\bullet+}$ solution and completely mixed. The mixture was incubated in the dark at room temperature for 6 min. The mixture absorbance was determined at 734 nm with a UV-vis spectrophotometer. The standard curve of Trolox (6-hydroxy-2.5.7.8 tetramethyl-chloroman-2-carboxylic acid) concentration versus ABTS-RSA (%) was generated to determine ABTS-RSA and then expressed as µg TE/g dry sample.

2.4.6 Ferrous chelating ability assay

Ferrous chelating ability (FCA) was investigated following the modified method of Yin et al. [20]. A 200 µL diluted extract was uniformly mixed with 3.7 mL methanol and 0.1 mL of 2 mM $FeCl_2.4H_2O$. The mixture was then added to 100 µL of 5 mM ferrozine and left at room temperature for 10 min. The absorbance was determined at 562 nm with UV-vis spectrophotometer. The Standard curve of FCA (%) and ethylenediminetetraacetic acid (EDTA) concentration was constructed to determine FCA as µg EDTA/g dry sample.

2.4.7 Ferric reducing/antioxidant power assay

Ferric reducing/antioxidant power (FRAP) was assayed following the Benzie and Strain [21] method with modification. Firstly, FRAP reagent was prepared from 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 20 mM $FeCl_3$. $6H_2O$ and 0.3 mM acetate buffer pH 3.6 (molar ratio of 10:1:1). A 100 µL extract was mixed with 3 mL FRAP reagent and then incubated at 37 ^{o}C for 30 min. The mixture was monitored for absorbance at 593 nm using UV-vis spectrophotometer. The standard curve of Trolox concentration versus FRAB (%) was constructed to determine the FRAB value and expressed as µg TE/g dry sample.

2.4.8 Tensile strength measurement

Cooked EPCNs were prepared in accordance with the method of Shiau et al. [22]. About 5 g EPCNs were boiled in 200

mL distilled water on a hot plate for 5 min and then drained for 5 min. The tensile strength (TS) of the cooked EPCNs was measured using a texture analyzer (TA-XT2, Stable Micro Systems, Surrey, UK). To test the TS, five strands of cooked noodles were fixed to the arms of tensile grips. TS at the breaking point was measured at a speed of 1.0 mm/s.
Each sample was measured five times.

2.4.9 Cooking loss measurement

Cooking loss (CL) was evaluated following the method of Shiau et al. [22] with modification. About 5 g EPCNs were boiled in 200 mL distilled water on a hot plate for 5 min. Cooked EPCNs were washed with distilled water, drained for 5 min and weighed immediately. Cooking water was recorded and dried at 105 ^{o}C to a constant weight. Reduction of cooking loss was expressed by CL reduction (%) = (1-w_2/w_1) x 100 where, w_1 was weight of dried noodle before cooking and w_2 was weight of dried solids in cooking water.

2.4.10 Color measurement

Hunter CIE color [23] in Lightness (L^*), redness (a^*) and yellowness (b^*) values was performed to estimate the color characteristics of EPCNs using a chroma meter (Minolta CR-300 series, Minolta Co. Ltd., Japan). The hue (H^*), and chroma (C^*) values were determined according to H^* = tan-1 (b^*/a^*), and $C^* = (a^{*2} + b^{*2})^{1/2}$.

2.4.11 pH measurements

Acidified EPCNs were measured for pH following the method of Li et al. [24]. Briefly, EPCNs (10 g) were ground and dispersed in 100 mL of distilled water. The mixture was left at room temperature for 30 min and then pH was determined using a pH meter (pH211, Hanna Instrument Ltd., USA.).

2.4.12 Total microbial count

Total microbial count (TMC) was performed by the pour plate method [25] using plate count agar (PCA). The amount of TMC was reported as CFU/g.

2.5 Experimental design

Experimental design was generated through central composite design (CCD) for three variables and five levels each with 6 center point combinations. Selected independent variables, including the ratio of PCF to RF (x_1: 25.0-75.0%), guar gum content (x_2: 0.5-1.5%) and water content (x_3: 30.0-50.0%) (% total flour weight basis), significantly affecting the qualities of extruded noodles were investigated to optimize TAC (Y_1), TPC (Y_2), ABTS-RSA (Y_3), FCA (Y_4) and TS (Y_5) of EPCNs. Response surface was obtained using three factors at five levels (-1.68, -1, 0, +1, +1.68). Symbols and coded variable levels for x_1, x_2 and x_3 of this study are shown in Table 1. CCD created 20 designed experiments (Table 2). In addition, response data was achieved from experiment replications. A quadratic equation was used (Y) as follows: $Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{123}x_1x_2x_3$, where Y is the response variable, x_1, x_2 and x_3 are the coded process variables and β_0, β_1, β_2, β_3, β_{11}, β_{22}, β_{33} and β_{123} are the regression coefficients.

2.6 Statistical analysis

Design expert software version 7.0 (Stat-Ease, Inc., Minneapolis, MN, USA.) was used to conduct CCD. ANOVA was used to analyze the variance. In addition, Duncan's multiple range tests were used to determine the significant differences between means ($p<0.05$). Statistical analysis of data was conducted using the SPSS statistic program (Version 19) for Windows (SPSS Inc., Chicago, IL, USA). Means were accepted with significantly different at a 95% confidence interval [26].

3. Results and Discussion

3.1 Optimization of EPCNs

3.1.1 Statistical analysis and model fitting

Second-order polynomial equations and coefficient of determinations (R^2) after using multiple regression analysis. R^2 of Y_1, Y_2, Y_3, Y_4 and Y_5 were 0.8895, 0.8421, 0.8311, 0.8672 and 0.8750, respectively. Meanwhile, p-values of lack of fit in Y_1, Y_2, Y_3, Y_4, and Y_5 regression model (0.9957, 0.5224, 0.3671, 0.1676 and 0.1353, respectively) were not significant (Table 3). The results indicated that these models were suitable for optimizing the EPCN conditions. The positive linear and quadratic effects of x_1 and x_3 significantly influenced Y_1, Y_2, Y_3, and Y_4 ($p<0.05$). It was concluded that the ratio of PCF to RF and water content had more influence on phytochemicals and antioxidant activities than guar gum content. However, the ratio of PCF to RF had negative linear and quadratic effects while guar gum had positive linear and quadratic effects on TS. Li et al. [23] reported that blue corn cookies containing above 21% of water content (flour weight basis) could reduce degradation of phytochemicals and antioxidant activities. In addition, Turabi et al. [1] demonstrated that the fortification of guar gum could improve qualities of rice noodle structures.

3.1.2 Response surface analysis

Three-dimensional response surface plots were established with dependent variables (Y_1, Y_2, Y_3, Y_4, and Y_5) related to three independent variables (X_1, X_2, and X_3) shown in Fig. 1a, b, c, d, e. At fixing of X_2 and X_3, Y_1, Y_2, Y_3, and Y_4 clearly increased with increase in X_1. Similarly, Y_1, Y_2, Y_3, and Y_4 quickly increased initially and then slightly decreased with further increase in x_3 at a fixed x_1 and x_2 (Fig. 1a, b, c, d). Variation of Y_5 with x_1, x_2 and x_3 is presented in Fig. 1e. It was evident that at constant x_2 and x_3, Y_5 decreased with increasing x_1. At fixed x_1 and x_3, Y_5 increased with increase of x_2 in an initial experiment and then insignificantly decreased. The response surface plots indicate that Y_1, Y_2, Y_3, and Y_4 significantly increased with the increase of x_1 and x_3. However, increasing x_1 had negative effects on Y_5. Meanwhile, increasing x_2 satisfyingly improved Y_5 of EPCNs. Kokkaew et al. [4] reported that increase of water content in purple cookies could reduce anthocyanin and phenolic degradations at high temperature conditions. Zazueta-Morales et al. [27] found that higher temperature and lower aw conditions significantly affected the increase of phytochemical degradation. Our results indicate that ABTS-RSA and FCA of EPCNs have been increased as TAC and TPC increase. According to Bily et al. [28], anthocyanin or phenolic retentions in purple corn products influenced higher antioxidant capacities. Del Pozo-Insfran et al. [29] found that polyphenol compounds potently exhibit reducing, donating hydrogen and singlet oxygen scavenging properties, while flavonoids composed of the hydroxyl group demonstrate free radical scavenging and ferrous chelating capacities. Yalcin and Basman [30] reported that addition of xanthan gum in noodle sample improved cooking and sensory properties. Kaur et al. [8] reported that gums could increase noodle cooking time and decrease cooking loss, firmness and cohesiveness of noodle-making properties of potato, corn and mung bean starches.

Table 1. Independent variables and levels of CCD applied to extruded purple corn noodle ingredients.

Independent variables		Levels				
Code	**Real**	**-α**	**-1**	**0**	**+1**	**+α**
x_1	Ratio of purple corn flour to rice flour (% total weight flour basis)	8.0:92.0	25.0:75.0	50.0:50.0	75.0:25.0	92.0:8.0
x_2	Guar gum content (% total weight flour basis)	0.3	1.0	2.0	3.0	3.7
x_3	Water content (% total weight flour basis)	23.2	30.0	40.0	50.0	56.8

Table 2. Response values for Y_1, Y_2, Y_3, Y_4, and Y_5 with different combinations of x_1, x_1, and x_3 in CCD.

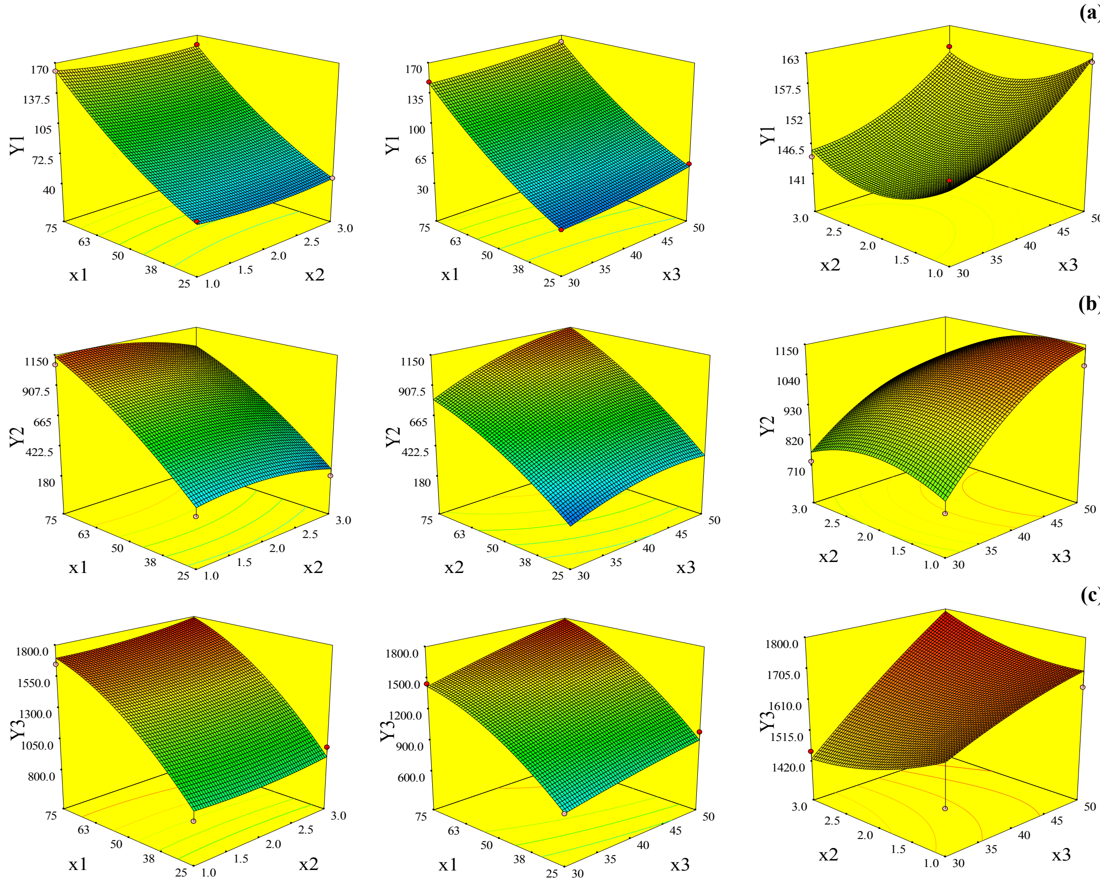
No.	Code level of variables[1]			Response values[2]				
	x_1 (%)	x_2 (%)	x_3 (%)	Y_1 (µg C3GE/g)	Y_2 (µg GE/g)	Y_3 (µg TE/g)	Y_4 (µg EDTA/g)	Y_5 (g)
1	25.0:75.0	3.0	30.0	35.5	150.1	686.9	213.8	40.8
2	50.0:50.0	2.0	23.2	75.1	445.1	1,142.8	318.9	30.6
3	50.0:50.0	2.0	40.0	89.5	666.6	1,342.8	383.2	25
4	92.0:8.0	2.0	40.0	202.2	1225.3	1,802.8	564.9	4.6
5	50.0:50.0	2.0	40.0	64.1	815.5	1,386.9	413.5	26.5
6	50.0:50.0	2.0	40.0	81.9	717.3	1,342.8	384.1	29.5
7	50.0:50.0	2.0	56.8	98.9	871.2	1,542.8	433.3	26.8
8	50.0:50.0	2.0	40.0	81.9	880.5	1,186.9	359	28.5
9	75.0:25.0	1.0	30.0	148.4	717.8	1,433.7	409.5	6.8
10	25.0:75.0	1.0	30.0	34.9	103.2	986.9	243.4	35.2
11	75.0:25.0	1.0	50.0	161.4	1074.6	1,650.8	464.6	7.9
12	50.0:50.0	3.7	40.0	90.5	622.3	1,375.8	367.4	24.3
13	25.0:75.0	3.0	50.0	46.1	180.6	986.9	225.9	37.7
14	50.0:50.0	2.0	40.0	88.7	616.9	1,542.8	399.4	30.2
15	25.0:75.0	1.0	50.0	53.3	259.1	808.5	209.4	30.4
16	8.0:92.0	2.0	40.0	11.2	60.1	268.0	99.6	45.5
17	75.0:25.0	3.0	50.0	158.8	913.2	1,647.7	488.1	7.1
18	50.0:50.0	0.3	40.0	93.7	689.8	1,542.8	361.8	15.1
19	75.0:25.0	3.0	30.0	144.2	723.5	1,450.8	434.2	5.3
20	50.0:50.0	2.0	40.0	75.2	875.2	1,242.8	362.8	28.4

Note: [1] x_1: ratio of purple corn flour to rice flour (%), x_2: gaur gum content (%), x_3: water content (%).
[2] Y_1: total anthocyanin content (µg C3GE/g), Y_2: total phenolic content (µg GE/g), Y_3: ABTS radical scavenging activity (µg TE/g), Y_4: ferrous chelating ability (µg EDTA/g), Y_5: tensile strength (g).

Table 3. Second order polynomial equations, coefficients of determinations and lack of fits of response variables.

Responses	Second order polynomial equations	R^2	Lack of fits
Y_1	$= 80.2 + 55.96x_1 + 7.1x_3 + 9.2x_1^2 + 4.6x_2^2$	0.8895	0. 9957
Y_2	$= 766.2 + 387.7x_1 + 88.4x_3 – 69.7x_1^2 – 65.0x_2^2$	0.8421	0.5224
Y_3	$= 1,343.1 + 343.8x_1 + 106.1x_3 – 122.5x_1^2$	0.8311	0.3671
Y_4	$= 384.3 + 123.5x_1 + 20.5x_3 – 22.4x_1^2$	0.8672	0.1676
Y_5	$= 28.1 – 13.6x_1 – 1.9x_2 – 1.7x_1^2 – 3.6x_2^2$	0.8750	0.1358

(a)



(d)

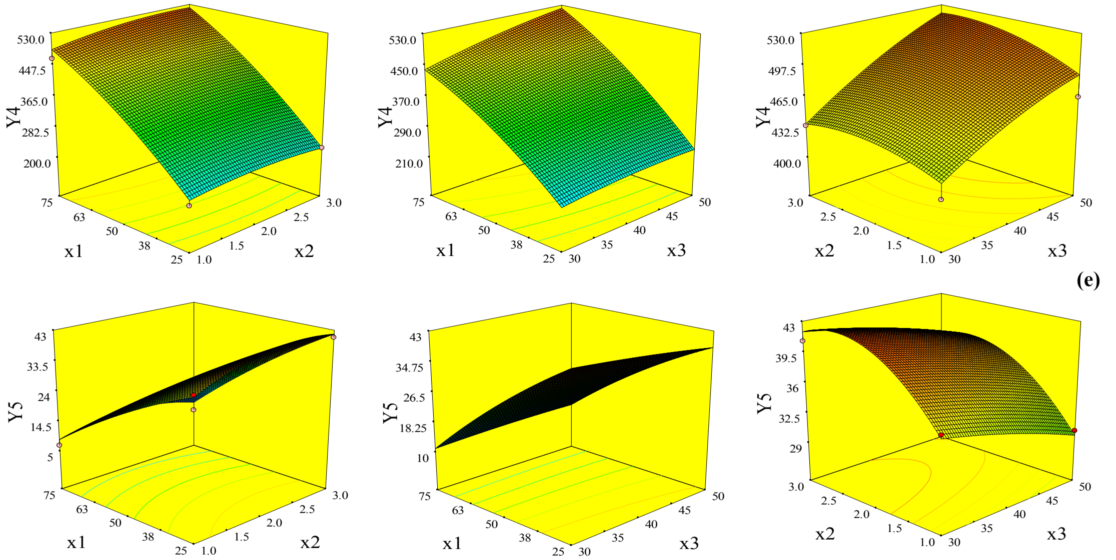


Fig. 1. Response surface plots for the effect of independent variables (x_1, ratio of purple corn flour to rice flour; x_2, guar gum content; x_3, water content) on response variables: (a) total anthocyanin content (Y_1); (b) total phenolic content (Y_2); (c) ABTS radical scavenging activity (Y_3); (d) ferrous chelating ability (Y_4); (e) tensile strength (Y_5).

3.1.4 Optimization and verification of model

Response surface regression analysis predicted the optimal ingredients of EPCNs to obtain maximal responses were a ratio of PCF to RF, guar gum content and water content of 66.0:34.0, 2.1 and 50 (% total weight flour basis), respectively. At these levels, TAC (128.9 µg C3GE/g), TPC (1,018.8 µg GE/g), ABTS-RSA (1,640.7 µg TE/g), FCA (478.4 µg EDTA/g) and TS (18.6 g) were predicted. In addition, triplicate experiments of optimal conditions were generated to verify availability and accuracy of equation models for maximal responses. Results indicated that observed values of TAC, TPC, ABTS-RSA, FCA and TS (129.9 µg C3GE/g, 1,027.9 µg GE/g, 1,636.4 µg TE/g, 473.2 µg EDTA/g and 18.0 g, respectively) were in good agreement with predicted values (data not shown). Therefore, statistical models were satisfied and adequate for optimization of EPCNs.

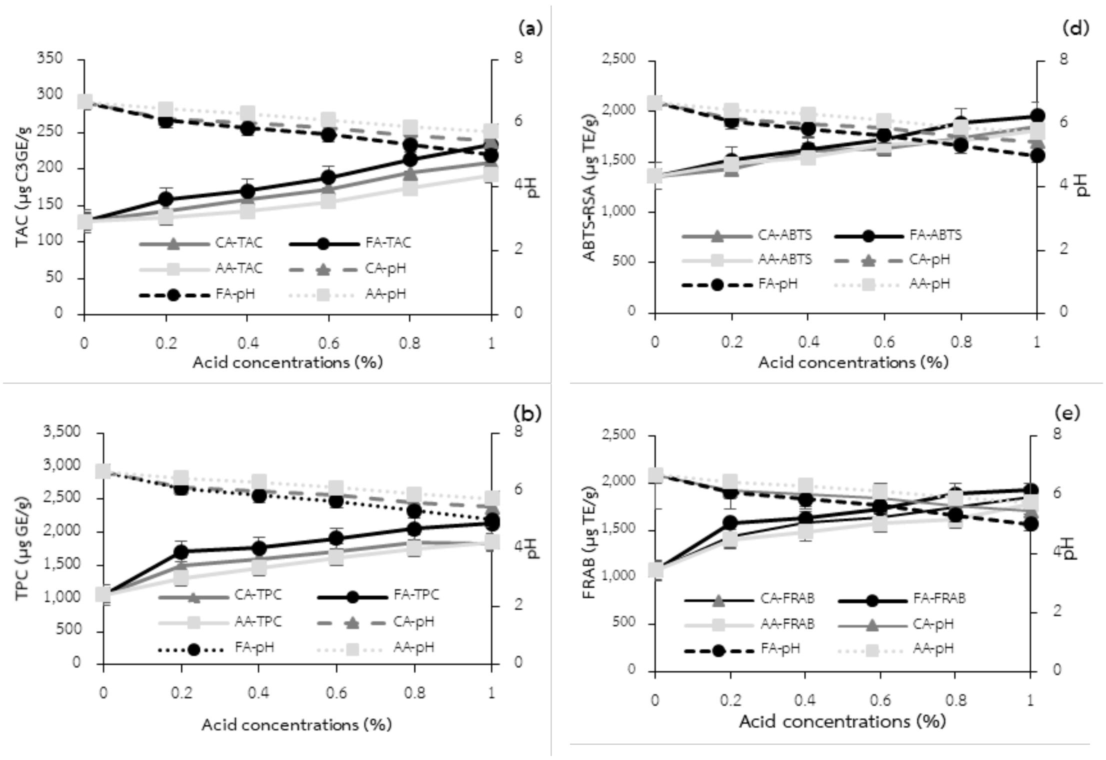
3.2 Effects of acidic food additive enrichment on extruded noodle qualities

3.2.1 Phytochemicals and antioxidant activities

Fig. 2a, b, c, d, e, f shows that acidified EPCNs with citric acid, fumaric acid, and ascorbic acid have higher TAC, TPC, TFC, ABTS-RSA, FRAB and FCA and lower total plate counts than non-acidified EPCNs. Results show that addition of acidic food additive in EPCN formulas could decrease phytochemical and antioxidant degradations during drying processes. Moreover, TAC, TPC, TFC, ABTS-RSA, FRAB and FCA dramatically increased with addition of each acid from 0.2 to 0.6% and then insignificantly increased. However, adding 1% fumaric in EPCNs showed the highest TAC, TPC, TFC, ABTS-RSA, FRAB and FCA (233.6 µg C3GE/g, 2,136.7 µg GE/g, 1,742.5 µg QE/g, 1,957.6 µg TE/g, 1,927.6 µg TE/g and 772.7 µg EDTA/g) followed by 1% citric acid (209.7 µg C3GE/g, 1,835.7 µg GE/g, 1,715.2 µg QE/g, 1,849.28 µg TE/g, 1,849.28 µg TE/g and 704.2 µg EDTA/g) and 1% ascorbic acid (191.8 µg C3GE/g, 1,855.3 µg GE/g, 1,556.4

μg QE/g, 1,814.3 μg TE/g, 1,774.3 μg TE/g and 653.1 μg EDTA/g), respectively.

Adding fumaric acid to the EPCN formula exhibited greater reduced degradations of phytochemicals and antioxidant activities than citric acid and ascorbic acid during extrusion and drying processes. Citric acid, fumaric acid, ascorbic acid are weak organic acids. Their degree or intensity of sourness varies in decreasing order of fumaric acid, citric, and ascorbic acid, respectively, at equal concentration [31]. Fumaric acid is the strongest organic food acid and has the highest degree or intensity of sourness [11]. Sour taste response imparted to a food is attributed to hydrogen (H^+) or hydronium (H_3O^+) ions [32]. Francis [33] found that more free anions of acids are associated with more phytochemical retention in lower pH condition. The study of Li et al. [24] revealed that adding organic acids to decrease pH values of purple corn cookies could reduce phytochemical and antioxidant degradations due to their reducing activity. Del Pozo-Insfran et al. [29] indicated that acidification of white and blue corn tortilla dough enhanced the stabilities of phytochemicals and antioxidant activities. In our previous study, addition of 3% fumaric acid in purple corn cake showed the highest phytochemicals and antioxidant activities; however, this concentration was not suitable in noodles because of exhibiting the strong sourness [4].



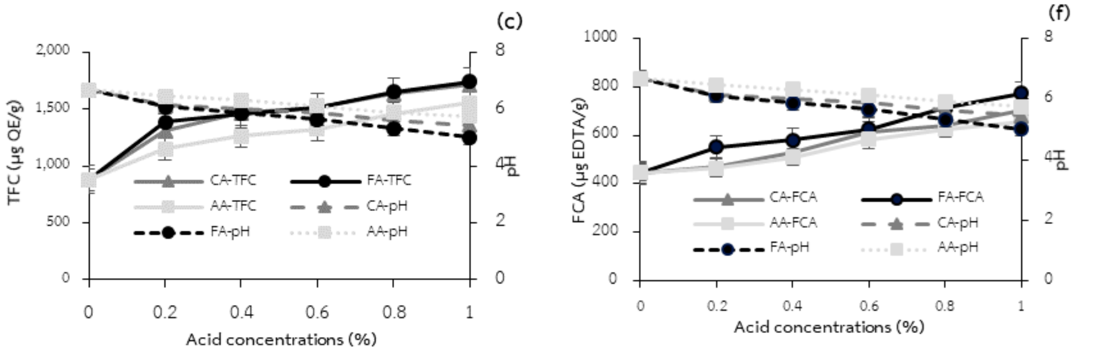
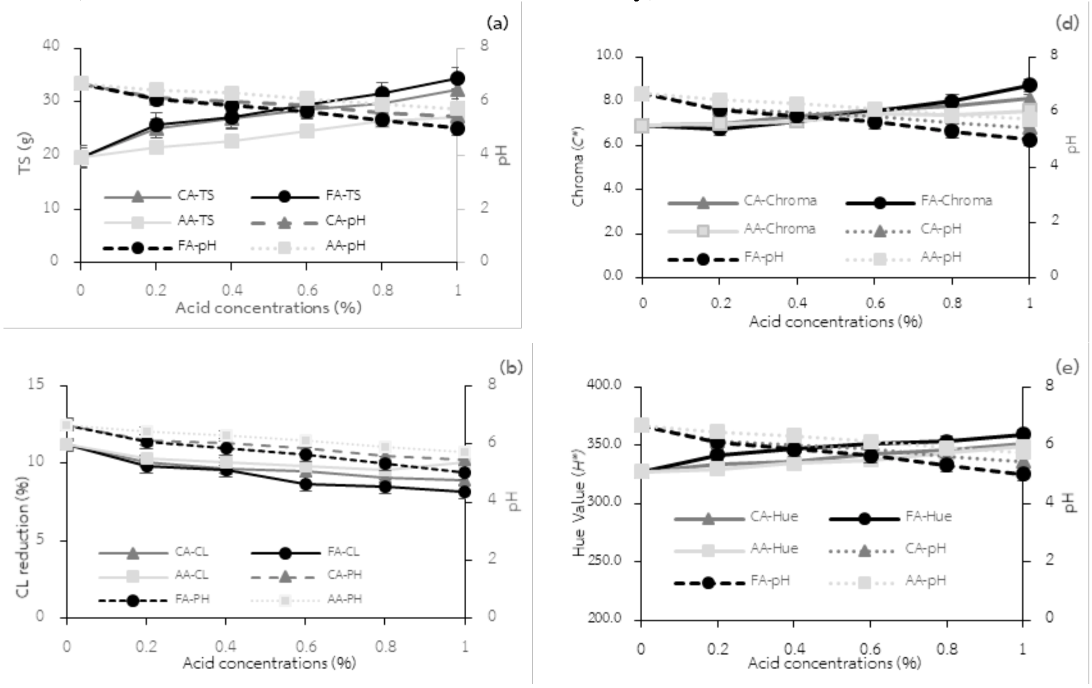


Fig. 2. Effect of various levels of citric acid (CA), fumaric acid (FA) and ascorbic acid (AA) on total anthocyanin content (TAC) and pH (a), total phenolic content (TPC) and pH (b), total flavonoid content (TPC) and pH (c), ABTS radical scavenging activity (ABTS-RSA) and pH (d), ferric reducing/ antioxidant power (FRAP) and pH (e) and ferrous chelating ability (FCA) (f) and pH in extruded purple corn noodles.

3.2.2 Tensile strength, cooking loss color values and total microbial counts

Addition of organic acids in EPCNs with citric acid, fumaric acid, and ascorbic acid demonstrated higher qualities of tensile strength (TS), cooking loss (CL) color values, and total microbial counts than non-acidified EPCNs (Fig. 3a, b, c, d, e, f). Adding 1% fumaric in EPCNs showed the highest tensile strength and cooking loss (34.4 g and 8.2%) followed by citric acid (32.4 g and 8.9%) and ascorbic acid (27.1 g and 10.1%), respectively (Fig. 3a, b). In our study,



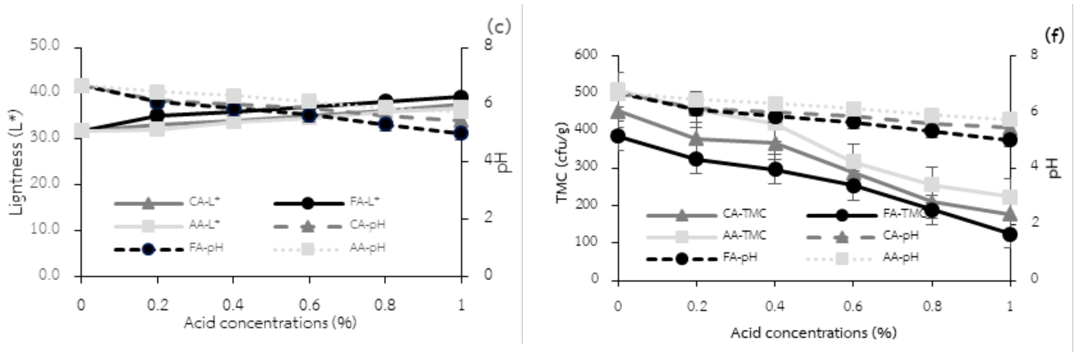


Fig. 3. Effect of various levels of citric acid (CA), fumaric acid (FA) and ascorbic acid (AA) on tensile strength (TS;g) and pH (a), cooking loss (CL;%) and pH (b), lightness (*L**) and pH (c), chroma (*C**) and pH (d), hue value (*H**) and pH (e) and total microbial counts (TMC) and pH (f) in extruded purple corn noodles.

color value evaluation was compared to the changes in the TAC retention. Acidified EPCNs exhibited *L** of 31.8-39.1, *C** of 7.0-8.7 and *H** of 327.4-359.1. At these *H** values indicated that acidified EPCNs with organic acids presented purple-red color. Fig. 3c, d, e show that the acidified EPCNs with 1% fumaric acid was significantly higher values of *L*, C** and *H** (39.1, 8.7 and 359.1 respectively) than 1% citric acid (38.4, 7.9 and 350.2) and 1% ascorbic acid (36.5, 7.2 and 343.0) respectively) ($p<0.05$). Meanwhile, addition of 1% fumaric in EPCNs showed the lowest total microbial counts (125 cfu/g) followed by citric acid TPC (178 cfu/g) and ascorbic acid (224 cfu/g) respectively (Fig. 3f).

Higher concentration of organic acids exhibited higher TS and lower CL. Acidified EPCNs became remarkably softer than the non-acidified EPCNs (Fig. 3a, b). These organic acids could affect to the functional properties of starch before and after gelatinization [34]. Addition of ascorbic acid in wheat starch gel caused the reductions of temperature and enthalpy of starch gelatinization influenced on softer, less cohesive, elastic and gummy of starch gel [35]. In addition, Mua and Jackson [36] reported that the molecular starch degradation in the presence of organic acids affected the increase of water solubility, decrease of water absorption, changes of morphological granule structure, decrease of temperature and enthalpy of gelatinization as well as decrease of starch gel hardness. As results concluded that the addition of organic acids in EPCN formula resulted in increase of TS improved elastic and gummy properties as well as decreasing CL.

The higher *L** values indicated that the increase in the EPCN brightness had been influenced by the oxidization or condensation of organic acid with anthocyanins [11]. Meanwhile, the *C** value indicated that a decrease in the opaqueness of EPCN surface had been caused by changes in the visual characteristics of starch due to Maillard reaction [37]. Organic acids significantly affected the anthocyanin degradation by a direct condensation mechanism which enhanced the brown pigment formation in the oxygen environment [38]. In our study, the *H** values of acidified EPCNs (327.4-359.1) indicated acidified EPCNs favored purple hue. In addition, increase of organic concentrations in acidified EPCNs influenced on decrease of *H** value exhibited a purple hue at the lower prominently appeared over the burgundy-brown. The *H** value of nonacidified EPCNs may appear to the human eye as a deeper purple color. The lower pH induced the anthocyanin oxidation affected to bleaching of purple color [39]. Therefore, acidic food additives are reducing agents that can be used to improve the color quality of products.

Increases of organic acid concentrations demonstrated higher microbial reductions (Fig. 3f). According to the research of Tajkarimi and Ibrahim [39], organic acids significantly affect antimicrobial activity. Moreover, lower pH conditions enhanced the antimicrobial activity. Friedman and Jürgens [41] demonstrated that antimicrobial 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 [29].

4. Conclusion

Optimal ingredients of EPCNs were a ratio of PCF to RF, guar gum content and water content of 66.0:34.0, 2.1 and 50.0 (total weight flour basis), respectively, which exhibited maximal TAC, TPC, ABTS-RSA, FCA and TS. Addition of acidic food additives in EPCNs could enhance the reduction of phytochemical and antioxidant activity degradations, increase the TS, decrease the CL and improve the color appearance as well as inhibiting microbial growths. This study would be an alternative approach in value-addition of agricultural materials for functional foods preparation with high nutraceutical values.

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