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Optimisation of the dielectric barrier discharge to produce Riceberry rice flour retained with high activities of bioactive compounds using plasma technology

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Abstract

Riceberry rice is a hybrid rice that contains polyphenol compounds, anthocyanin, and high antioxidants. Plasma technology has been used to improve the quality of rice and rice flour. Some conditions of the plasma process can be altered to get the combination that can achieve maximum result. The present work aimed to identify the optimal combination of a plasma treatment condition by varying three variables: time (3 - 10 min), power (140 - 180 W), and oxygen flow rate (0.0 - 0.8 L/min) in improving the nutrient and antioxidant agent of Riceberry rice flour. The increase in time and power significantly increased the percenatge of the scavenging ability of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), peonidin 3-glucoside, cyanidin 3-glucoside, and total anthocyanin; while the decrease in oxygen significantly decreased all the parameters analysed. The optimal plasma conditions were 7.87 min, 166 W, and 0.64 L/min of oxygen flow rate. Under this condition, the Riceberry rice flour consisted of 65.05% scavenging ability of DPPH, peonidin 3-glucoside of 45.21 mg/100 g, cyanidin 3-glucoside of 179.6 mg/100 g, and total anthocyanin of 225.81 mg/100 g. Finally, the plasma technique can improve the antioxidant activity and anthocyanin of Riceberry rice flour.

Introduction

Plasma technology is a non-thermal process which can be achieved by subjecting a high frequency electric field in a gaseous state. When the kinetic energy is increased, the ionisation or free electrons rapidly increase. Plasma products like free electrons, ions, and radicals collide with atoms, thus resulting in an increased number of collisions (Chen *et al.,* 1998). Several researchers have reported about the plasma treatment in different flours such as wheat, rice, corn, and tapioca (Wongsagonsup *et al.,* 2014; Misra *et al.,* 2015a; Pal *et al.,* 2016; Bahrami *et al.,* 2016; Dong *et al.,* 2017; Scholtz *et al.,* 2019). Plasma treatment could also improve the flour and gel hydration properties of the parboiled rice (Sarangapani *et al.,* 2016). Moreover, an improvement in phenolic content and antioxidant properties of treated rice flour has also been reported. It was found that the low-pressure plasma improved the nutrition of germinated brown rice by enhancing the growth and GABA accumulation of germinated brown rice. For the effect of plasma process on antioxidant activity, most studies have focused on fruits like strawberry, kiwifruit, pomegranate, mango, naringin, and prickly pear cactus fruit (Kim *et al.,* 2014; 2019; Misra *et al.,* 2015b; Ramazzina *et al.,* 2015; Herceg *et al.,* 2016; Abidin *et al.,* 2018).

Riceberry rice is a deep purple grain (*Oryza sativa*), which is a crossbreed strain from the Khao Hom Nin and Khao Hom Mali 105 rice variety, and contains high antioxidants. In previous research, we found that Riceberry rice from Mueang Pan District, Lampang province (in the northern region of Thailand) had high antioxidant activities and anthocyanin content (Settapramote *et al.,* 2018). The dielectric barrier discharge (DBD) is plasma generated, and is characterized by the presence of insulating material between the electrodes and the discharge gap(s) (Kogelschatz and Eliasson, 2000). Consequently, the present work aimed to find an optimal process

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condition of plasma technology using DBD to increase bioactive properties (antioxidant activities and anthocyanin content) of Riceberry rice flour.

Materials and methods

Rice sample

Riceberry rice was harvested in 2016 from Mueang Pan District, Lampang province (in the northern part of Thailand). Briefly, Riceberry rice flour was prepared by milling with a hammer mill, and sieving through 70 mesh. Then, the sample was kept at 4°C in a vacuum pack for further analysis.

Conditions of plasma technology

The modification of Riceberry rice flour was carried out by the DBD (PMU 330, Plasmart Inc., Korea). Three variables of DBD in plasma technology were considered: time (3 - 10 min), power (140 - 180 W), and oxygen flow rate (0 - 0.8 L/min) by central composite design (CCD) with three centre points. Also, the fixed variable was 16 L/min of argon gas and 10 L/min of nitrogen gas. There were 17 treatments generated in this experiment, as shown in Table 1. Ten grams of sample was placed on the tray of the machine. After that, the sample was analysed for antioxidant activity and anthocyanin content of interest and relevant method.

Analysis of total phenolic content and antioxidation activities

The preparation of the sample was modified from the method of Sompong *et al.* (2011). The milled sample was extracted in 85% of the aqueous methanol solution (ratio 1:10), and agitated for 30 min. Then, the extracted sample was centrifuged at 5,000 rpm for 10 min, and passed through the filter paper. The supernatant was separated and stored at 4°C until further analysis.

The TPC was performed following the modification method of Sompong *et al.* (2011). Briefly, 200 µL of the extract were reacted with 1 mL

Table 1. The matrix of the central composite design (CCD) by plasma treatment and the responded variables of Riceberry rice flour.

No.	Independent variable			Dependent variable					
	X_1	X_2	X_3	Cyanidin 3-glucoside (mg/100 g)	Peonidin 3-glucoside (mg/100 g)	Total anthocyanins (mg/100 g)	Total phenolic contents (mg GAE/100 g)	DPPH (%)	ABTS (%)
1	140	3.0	0.16	172.8 ± 0.6	42.7 ± 0.5	215.4 ± 1.6	428.4 ± 1.9	62.8 ± 1.2	92.1 ± 1.3
2	180	3.0	0.16	171.6 ± 0.9	41.1 ± 0.4	211.7 ± 0.9	451.7 ± 1.4	61.2 ± 1.5	90.6 ± 1.0
3	140	10.0	0.16	171.5 ± 0.5	41.5 ± 0.2	211.9 ± 0.5	447.9 ± 1.2	61.7 ± 0.8	92.1 ± 1.5
4	180	10.0	0.16	167.2 ± 0.3	37.1 ± 0.5	204.2 ± 0.3	450.3 ± 1.0	57.9 ± 1.2	90.9 ± 2.5
5	140	3.0	0.64	170.2 ± 1.0	40.5 ± 0.2	211.7 ± 1.8	449.3 ± 1.3	60.8 ± 1.4	86.1 ± 0.8
6	180	3.0	0.64	174.1 ± 0.1	44.3 ± 0.1	218.4 ± 0.1	455.3 ± 1.2	64.2 ± 1.4	89.4 ± 1.3
7	140	10.0	0.64	177.3 ± 0.2	47.1 ± 0.1	224.4 ± 0.2	444.8 ± 1.5	67.1 ± 1.4	39.1 ± 2.9
8	180	10.0	0.64	174.5 ± 0.6	44.8 ± 0.7	218.3 ± 0.6	441.1 ± 1.2	64.1 ± 1.1	90.3 ± 2.5
9	126	6.5	0.40	172.6 ± 0.4	43.9 ± 0.3	216.5 ± 0.4	457.9 ± 1.5	62.5 ± 1.8	91.5 ± 1.5
10	194	6.5	0.40	175.4 ± 0.2	45.2 ± 0.5	220.6 ± 1.2	445.8 ± 2.1	65.8 ± 1.1	91.1 ± 1.3
11	160	0.6	0.40	170.5 ± 0.3	40.7 ± 0.2	212.2 ± 0.3	445.3 ± 0.8	60.1 ± 1.6	92.5 ± 7.5
12	160	12.4	0.40	171.6 ± 0.8	41.2 ± 0.4	211.8 ± 0.8	451.3 ± 1.7	61.1 ± 1.8	93.3 ± 2.1
13	160	6.5	0.00	172.9 ± 0.9	42.6 ± 0.1	215.5 ± 1.9	452.1 ± 1.9	62.1 ± 1.5	90.6 ± 2.3
14	160	6.5	0.80	173.1 ± 0.2	43.2 ± 0.3	217.3 ± 1.2	458.8 ± 1.7	63.8 ± 1.1	89.5 ± 1.7
15	160	6.5	0.40	175.2 ± 0.3	45.6 ± 0.2	220.8 ± 0.5	454.1 ± 1.6	65.5 ± 1.5	91.8 ± 1.5
16	160	6.5	0.40	175.7 ± 0.5	45.3 ± 0.3	221.1 ± 0.5	453.9 ± 1.8	66.5 ± 0.7	91.1 ± 0.8
17	160	6.5	0.40	175.8 ± 0.6	41.8 ± 0.1	221.5 ± 0.6	453.2 ± 1.3	65.8 ± 1.3	92.5 ± 1.8

Note: mean ± SD, X_1 = power (watt, W), X_2 = time (min), and X_3 = oxygen (L/min).

of 10% Folin-Ciocalteu's reagent and 2.8 mL of distilled water, and then kept for 5 min. After that, 1 mL of 7.5% (w/v) Na_2CO_3 was added into the reaction mixture. After incubation for 1 h in the dark at room temperature, the absorbance of the extract was measured using a spectrophotometer at 765 nm. The calibration curve was prepared with gallic acid at concentrations of 0 to 1 mg/mL, and was demonstrated as gallic acid equivalents (GAE).

The 2,2-diphenyl1-picrylhydrazyl (DPPH) radical scavenging was analysed using the method of Brand-Williams *et al.* (1995). The reaction mixture contained 2,940 µL of DPPH working solution (4.73 mg of DPPH in 100 mL ethanol solution) and 60 µL of rice extract. The mixture was shaken and incubated for 30 min in the dark at room temperature. The absorbance was read at 517 nm using a spectrophotometer. The DPPH radical scavenging was calculated by scavenging ability (%) = [(absorbance$_{517nm}$ of control - absorbance$_{517nm}$ of sample) / absorbance$_{517nm}$ of control] × 100.

The ABTS radical scavenging was assayed by the modified method of Wojdyło *et al.* (2007). Preparations of working ABTS+ solution includes 7 mM ABTS and 2.4 mM potassium persulfate which were mixed in equal quantities, and then placed to react for 12 h in the dark at room temperature. The mixed solution was diluted with water to make an absorbance of 0.95 ± 0.01 units at 734 nm. The extracted sample (60 µL) was reacted with 2,940 µL of the mixed solution. After 30 min of incubation in the dark at room temperature, the absorbance was spectrophotometrically measured at 734 nm. The formula of ABTS radical scavenging was calculated by scavenging ability (%) = [(absorbance$_{734nm}$ of control - absorbance$_{734nm}$ of sample) / absorbance$_{734nm}$ of control] × 100.

Determination of anthocyanin content

One gram of the milled rice was mixed with 10 mL of methanol acidified with 1% HCl (v/v), and then shaken at 200 rpm for 1 h. The mixture was centrifuged at 6,000 *g* for 20 min at 5°C, and the supernatant was then concentrated and filtered through a 0.45 µL syringe filter before being injected to HPLC. Cyanidin 3-glucoside and peonidin 3-glucoside, which are the main anthocyanins in Riceberry rice were then separated and quantified by HPLC (Agilent Technologies, Santa Clara, CA, USA) at 530 nm following the method of Laokuldilok *et al.* (2011). The total anthocyanin was a combination of cyanidin 3-glucoside and peonidin 3-gluocoside.

Statistical analysis

All data were collected in triplicate, and expressed as mean ± standard deviation (SD). The design expert program (Version 6.0.2, Stat-Ease, Inc., MN, USA) was used to analyse the response surface methodology.

Results and discussion

Effect of plasma condition on TPC and antioxidant activities

The results showed that the total phenolic content was 428.41 - 458.84 mg GAE/100 g, 57.94 - 67.07% of DPPH radical scavenging, and 86.18 - 93.34% of ABTS radical scavenging (Table 1). The regression model showed a significant percentage of DPPH radical scavenging ($p \leq 0.05$) (Table 2). This is similar to the anthocyanin results where percentage of DPPH radical scavenging was positively affected by power and time, and negatively affected by oxygen flow rate. The percentage of DPPH radical scavenging response surface is shown in Figure 1. Researchers reported that plasma treatment had a positive effect on DPPH radical scavenging; for instance, plasma-treated onion (400 W for 40 min) and plasma-treated mandarin peel (Won *et al.,* 2017; Kim *et al.,* 2017; Muhammad *et al.,* 2018). However, there was no significant regression model of total phenolic content and percentage of ABTS radical scavenging. Similarly, the effect of plasma treatment showed no significant difference in the antioxidant activity of kiwifruit (Ramazzina *et al.,* 2015). On the other hand, the blueberries treated with plasma for 1 min could be seen to increase the TPC (Sarangapani *et al.,* 2017). Hence, the plasma treatment affected the percentage of DPPH radical scavenging significantly; however, there was not significantly different in TPC and percentage of ABTS radical scavenging of plasma treated Riceberry rice flour.

Effect of plasma condition on anthocyanins content

The cyanidin 3-glucoside, peonidin 3-glucoside, and total anthocyanins of treated Riceberry rice flour were 167.16 - 177.27, 37.13 - 47.13, and 204.24 - 224.43 mg/100 g, respectively (Table 1). The results revealed that the highest degree of cyanidin 3-glucoside, peonidin 3-glucoside, and total anthocyanin content was found in treatment No. 14 (6.5 min, 160 W, and 0.8 L/min). The regression models with significant ($p \leq 0.05$) independent variables predicting different anthocyanins and relevance contents are shown in Table 2. Based on the ANOVA results, the R^2 of each response was more than 0.70. The response surface plot showed cyanidin

Table 2. Regression equations of significant responses from the plasma process ($p < 0.05$).

Response	Regression	*p*-value	R^2
Cyanidin 3-glucoside	125.70+0.51* POWER+3.85* TIME-23.47* OXYGEN-1.44*10^{-3}* POWER^2-0.13* TIME^2-16.02* OXYGEN^2-0.017* POWER * TIME +0.17* POWER * OXYGEN+1.96* TIME * OXYGEN	0.03	**0.84**
Peonidin 3-glucoside	9.74+0.35* POWER+3.63* TIME-23.71 * OXYGEN-1.06*10^{-3}* POWER^2-0.14* TIME^2-17.91* OXYGEN^2-0.01* POWER * TIME +0.19 * POWER * OXYGEN+1.83 * TIME * OXYGEN	0.02	**0.87**
Total anthocyanins	130.51+0.932* POWER+7.01* TIME-36.91* OXYGEN -2.74*10^{-3}* POWER^2-0.28* TIME^2-32.53* OXYGEN^2-0.03* POWER * TIME +0.31* POWER * OXYGEN+3.49* TIME * OXYGEN	0.04	**0.84**
DPPH (%)	14.70+0.51* POWER+3.77* TIME-16.14*OXYGEN-1.48*10^{-3}* POWER^2-0.15* TIME^2-18.35*OXYGEN^2-0.01* POWER * TIME +0.15* POWER * OXYGEN+1.60* TIME * OXYGEN	0.02	**0.86**

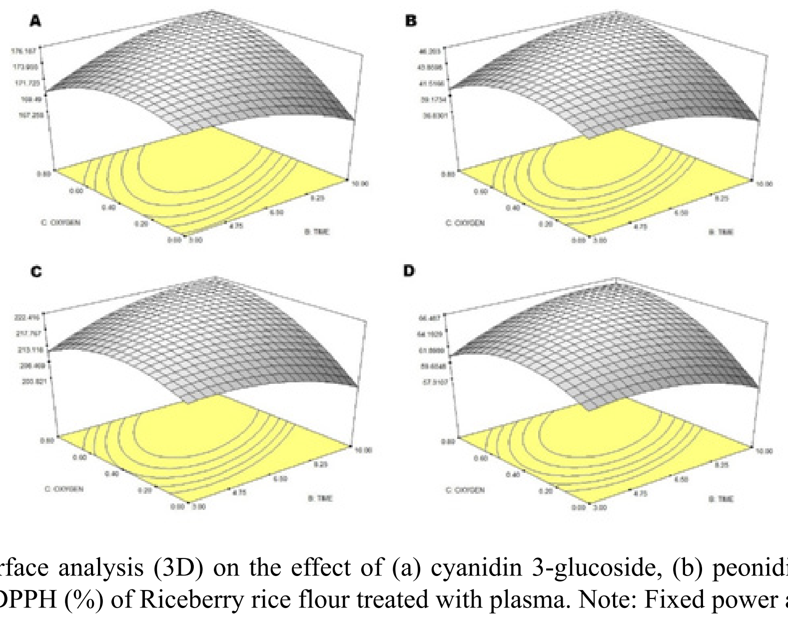

Figure 1. Response surface analysis (3D) on the effect of (a) cyanidin 3-glucoside, (b) peonidin 3-glucoside, (c) total anthocyanins, and (d) DPPH (%) of Riceberry rice flour treated with plasma. Note: Fixed power at 160 W.

3-glucoside, peonidin 3-glucoside, and total anthocyanins (Figure 1). The result showed that the power and time of the plasma method had a significantly positive effect. An increase in cyanidin 3-glucoside, peonidin 3-glucoside, and total anthocyanin contents may be a result of changing the chemical structure and corresponding stability. The plasma process may be gradual degradation for anthocyanin structures (Grzegorzewski *et al.,* 2010; Bursać Kovačević *et al.,* 2016). Another possible reason is that anthocyanins occurred with co-pigmentation, so anthocyanins were persistent. Moreover, it was found that the effect of gas-phase plasma treatment on the anthocyanin of sour cherry Marasca juice and its anthocyanins was higher than the pasteurised and untreated juices (Elez Garofulić *et al.,* 2015). Furthermore, it was noted that anthocyanin content in pomegranate juice increased with increasing plasma parameters (Bursać Kovačević *et al.,* 2016). In a different study, slight degradation of anthocyanin content in strawberries with plasma

Table 3. Comparison of response values of predicted and experimental data of optimal plasma treatment.

Dependent variable	Response value		
	Predicted	Experimental	% Error
Cyanidin 3-glucoside	176.19	179.60 ± 0.68	2.25
Peonidin 3-glucoside	46.24	45.21 ± 0.12	2.27
Total anthocyanins	222.46	225.81 ± 0.43	1.48
DPPH (%)	66.53	65.05 ± 1.22	2.27

Note: TIME = 7.87 min, POWER = 166 W, and OXYGEN = 0.64 L/min.

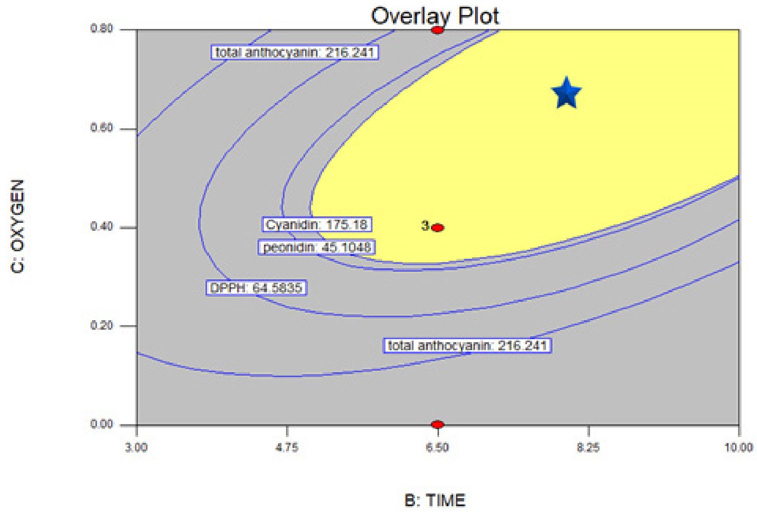

Figure 2. Response surface analysis (3D) on the optimal plasma treatment condition of Riceberry rice flour. = the optimal plasma treatment condition. Note: Fixed power at 160 W.

treatment was found (Misra *et al.,* 2015b). Unlike time and power, oxygen gas negatively affected the antioxidants of Riceberry rice flour because oxygen creates an oxidation reaction that destroys the anthocyanin (Dangles and Fenger, 2018). Therefore, this result showed that power and time have a positive effect, but oxygen has a negative effect on the anthocyanins in plasma-treated Riceberry rice flour.

The optimisation of the plasma condition

For analysis of the response surface plots, the optimal plasma process condition was 7.87 min, 166 W, and 0.64 L/min of oxygen flow rate. The overlay plot showed the optimal condition plasma technique to maximise cyanidin 3-glucoside, peonidin 3-glucoside, total anthocyanins, and the DPPH responses (Figure 2). An optimal formulation was reproduced using the optimum condition, and validated with the observed data. The agreement of observed and predicted values was measured. The absolute percentages of approximated error of cyanidin 3-glucoside, peonidin 3-glucoside, total anthocyanins, and the DPPH were below 10% (ranged from 1.48 - 2.27%) (Table 3) with the desirable agreement between the predicted and measured values (Wakeling, 2001).

Conclusion

Plasma treatment is a novel and an eco-friendly technology. The present work was carried out to find an optimal process condition of plasma technology that improves antioxidant activity and anthocyanin content of Riceberry rice flour. The obtained results revealed that the power and time of plasma method significantly increased cyanidin 3-glucoside, peonidin 3-glucoside, total anthocyanins, and the DPPH; whereas oxygen decreased these variables. The optimal plasma process condition was 7.87 min, 166 W, and 0.64 L/min of oxygen flow rate. The findings conclude that the use of plasma technology could increase antioxidant and anthocyanin content in Riceberry flour; therefore, this technology could also be applied to other coloured flour.

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