CHEMICAL ENGINEERING TRANSACTIONS

VOL. 114, 2024

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Petar S. Varbanov, Min Zeng, Yee Van Fan, Xuechao Wang

ISBN 979-12-81206-12-0; **ISSN** 2283-9216

DOI: 10.3303/CET24114188

Two-Step Optimization of Processing Conditions to Extract Bioactive Compounds from Eggplant Peels

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Optimization of processing conditions to extract bioactive compounds from plant sources has attracted great attention due to their high potential applications. In this study, Plackett-Burman design (PBD) was used for screening to select three significant factors among seven factors including ethanol content, extraction duration, solvent/solid ratio, extraction temperature, pH, and drying temperature for the ultrasound-assisted extraction of bioactive compounds from eggplant (*Solanum melongena*) peels. Subsequently, Box-Behnken design (BBD) was used to determine the optimal levels of the three significant factors to maximize the total phenolic content (TPC), anthocyanin content (AnCo) and antioxidant activity (AnAc) of the extract. The optimized conditions included the drying temperature of 53°C, ethanol content of 78% and the solvent/solid ratio of 44 mL/g, leading to the predicted optimal values for the TPC of 21.98 mg gallic acid equivalent (GAE)/g dry weight (DW), AnCo of 0.67 mg cyanidin-3-glucoside equivalent (CGE)/g DW and AnAc of 27.04 mg Trolox equivalent (TE)/g DW. In the validation, these values were 21.43±0.62 mg GAE/g DW, 0.64±0.02 mg CGE/g DW, and 25.51±0.38 mg TE/g DW, respectively, close with their predicted ones, confirming the adequacy of the models. In conclusion, eggplant peels could be a good source of bioactive compounds, and the combination of PBD and BBD could be effectively employed in screening and optimization for various extraction techniques.

1. Introduction

Eggplant, a traditional crop in Eastern regions, was domesticated between northeastern India and southern China. During its pulp processing (*e.g.* frozen grilled peeled eggplants), a significant amount of peels is discarded. Eggplant peel is known for its high level of bioactive compounds, such as phenolics and anthocyanins (Akhbari et al., 2019). Phenolics, having an aromatic ring with one or more hydroxyl groups and structures ranging from a simple phenolic molecule to a complex high-molecular-weight polymer, are a common group of compounds found in plants. They may have physiological actions such as anti-inflammatory, anti-infective, antiproliferative, and antioxidant (Tatipamula & Kukavica, 2021). Meanwhile, anthocyanins are pigments in plants that have a pleasing tint such as blue, crimson, or purple. Apart from the usage as natural colorants, they offer various health benefits to prevent chronic illnesses, such as cardiovascular disease (Mattioli et al., 2020).
Extraction is an essential stage in the recovery of bioactive substances. Conventional extraction methods include decoction technique and Soxhlet extraction. However, both traditional methods require extended heat treatment, so these procedures have a low efficiency because anthocyanin is vulnerable to high temperature (Ferarsa et al., 2018). Assisted methods have been widely employed to protect sensitive compounds. The key benefits of these approaches are great extraction efficiency, good end-product quality at lower temperatures, and minimal energy/solvent use (Barba et al., 2016). Among these, ultrasound-assisted extraction (UAE), which uses high intensity sound waves, could be a potential. Because of the physical forces created during acoustic cavitation, ultrasonic waves produce disturbance in plant tissue and aid in the release of extractable components in the solvent in a relatively short period of time by boosting mass transfer (Ashokkumar, 2015).
In the process of extracting bioactive compounds, there are numerous influencing factors (Akhbari et al., 2019). To determine the optimal conditions, a two-step procedure of screening and optimization could be effective for the identification of significant factors and finding of their optimal levels, respectively.

Paper Received: 15 July 2024; Revised: 21 October 2024; Accepted: 10 December 2024
Please cite this article as: Pham L.T., Le N.L., 2024, Two-Step Optimization of Processing Conditions to Extract Bioactive Compounds from Eggplant Peels, Chemical Engineering Transactions, 114, 1123-1128 DOI:10.3303/CET24114188

Plackett-Burman design, a type of two-level factorial design, could be an effective tool for screening because it can find significant main effects inexpensively while neglecting two-factor interactions. This design is applied in many studies, including the extraction of anthocyanins from blackcurrant marc (Li et al., 2016). Meanwhile, Box-Behnken design (BBD) has been demonstrated for its efficiency in optimization, which could build higher order response surfaces with fewer runs than a traditional factorial approach (Edwards & Mee, 2011). BBD also does not contain combinations in which all elements are at their peak or lowest values at the same time. As a result, the design is effective in avoiding trials done under severe circumstances, which may provide disappointing findings. Box-Behnken design was used in extraction of phenolics from other plants (Elboughdiri et al., 2020). Therefore, in this study, we aimed to integrate PBD and BBD in the experimental design to maximize the yields and activity of bioactive compounds from eggplant peels.

2. Materials and methods

2.1 Materials and chemicals

The fresh eggplants were bought from a local market in Ho Chi Minh City, Vietnam. The selected samples had even purple color, long and oval shapes, and without any defects on the peels. The chemicals of the analytical grade were purchased from local distributors.

2.2 Sample preparation and ultrasound-assisted extraction

The peels were cut into small pieces with a thickness of approximate 1 mm and put into a drying oven (UNE 700, Memmert, Germany) at a set temperature (40 to 80 ºC) until the moisture content of less than 10 % (wet basis). After that, the dried peels were grinded (A11 Basic, IKA, Germany) and sieved (AS 200 Basic, Retsch, Germany) to obtain intended sizes. The eggplant peel powder was stored in the dark at 4 °C for further steps.
The amounts of 1 g eggplant peel powder at distinctive particle sizes (125 µm and 355 µm) were weighed and mixed with the solvent mixture of ethanol and water at different ethanol contents (0% and 90%) and solvent/solid ratios (10 mL/g and 50mL/g). The mixtures were adjusted to a set pH (2 and 9) and placed into an ultrasonic bath (40kHz) (WUC-A10H, Daihan, South Korea) for the extraction at various conditions of temperature (30 °C and 70 °C) and duration (10 and 90 min). The samples were cooled down to room temperature and centrifuged (Z326K, Hermle, Germany) at 7000 rpm for 10 minutes. The supernatants were used for further analyses.

2.3 Factor screening and optimization

Placket-Burman design (PBD) was used to screen the significant effects of particle size (µm), extraction temperature (°C), extraction duration (min), solvent/solid ratio (mL/g), ethanol content (%), pH of the solvent, and drying temperature (°C). Table 1 lists the low (-1) and high (+1) levels of each factor in PBD. TPC was used as the response for the screening. Box-Behnken design (BBD) with three levels (coded as -1, 0 and +1) was applied for the three most significant variables from the screening to determine their optimal levels. The responses included TPC, AnCo and AnAc. Regression analysis of the data to fit a second-order polynomial equation (quadratic model) was conducted as follow:

$$Y = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 \sum \sum \beta_{ij} x_i x_j \quad (1)$$

where Y represents the response function; β_0 is a constant coefficient; β_i, β_{ii} and β_{ij} are the coefficients of the linear, quadratic, and interactive terms, respectively, and x_i and x_j represent the coded independent variables.

Table 1: The factor levels used in Plackett-Burman design

Factor	Symbol	Factor level	
		Low (-1)	High (+1)
Particle size (µm)	A	125	355
Ethanol content (%)	B	0	90
Extraction duration (min)	C	10	90
Solvent/solid ratio (mL/g)	D	10	50
Extraction temperature (°C)	E	30	70
pH	F	2	9
Drying temperature (°C)	G	40	80

2.4 Analytical methods

TPC was determined using the Folin-Ciocalteu assay (Akhbari et al., 2019) and expressed as mg gallic acid equivalent per gram dry weight of peels (mg GAE/g DW). AnCo was determined using the pH-differential method

(Lee et al., 2005) and expressed as cyanidin-3-glucoside equivalent per gram dry weight (mg C3G/g DW). AnAc was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Huang et al., 2005) and expressed as Trolox equivalent per gram dry weight (mg TE/g DW).

2.5 Statistical analysis

All the experiments were carried out in triplicate. Data were expressed as mean and standard deviation. Minitab 19 Statistical Software was used for design of experiments and analysis of variance (ANOVA).

3. Results and discussion

3.1 Screening by Plackett-Burman design (PBD)

Table 2 presents the run matrix of PBD and its results. With 7 factors, PBD required 12 samples and the TPC ranged from 1.21 to 15.81 mg GAE/g DW. Figure 1 illustrates the Pareto chart of factors that influenced the response, where the existence of a bar beyond the vertical line denoted the term relevance. The chart indicates that 3 factors of significant effects on TPC included G (drying temperature), B (ethanol content), and D (solvent/solid ratio). These results were relatively relevant to those from (Akhbari et al., 2019) which stated that the solvent/solid ratio and ethanol content significantly affected TPC. On the other hand, drying temperature was also confirmed for its remarkable effect on the extracted yield of phenolics from other plants (Stephenus et al., 2023). As the result, these three factors were used for optimization.

Table 2: Plackett-Burman design and response

Run Order	A (µm)	B (%)	C (min)	D (mL/g)	E (°C)	F	G (°C)	TPC (mg GAE/g DW)
1	355	90	10	50	70	2	80	7.24±0.14
2	125	90	10	10	30	9	80	1.26±0.05
3	125	90	90	10	70	2	40	10.73±0.12
4	125	90	90	50	30	9	80	5.77±0.07
5	355	0	90	50	30	9	40	10.79±0.03
6	125	0	90	50	70	2	80	4.35±0.25
7	355	90	10	50	30	2	40	15.81±0.13
8	125	0	10	50	70	9	40	7.12±0.02
9	355	90	90	10	70	9	40	10.99±0.11
10	125	0	10	10	30	2	40	1.23±0.08
11	135	0	10	10	70	9	80	1.21±0.24
12	355	0	90	10	30	2	80	1.32±0.02

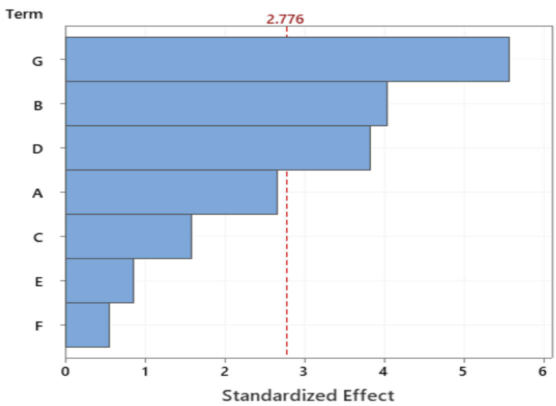

Figure 1: Pareto chart for the effects of factors on TPC

3.2 Optimization by Box-Behnken design (BBD) and validation

Table 3 lists the three levels of the three factors found in Section 3.1 and the matrix of BBD with the results of three responses (TPC, AnCo and AnAc). The data indicate that three responses achieved their lowest and highest values at different conditions, *e.g.* at Run No.14 (8.67 mg GAE/g DW) and No.4 (23.84 mg GAE/g DW), respectively for TPC, at Run No.7 (0.25 mg CGE/g DW) and No.2 (0.70 mg CGE/g DW), respectively for AnCo; and at Run No.15 (13.31 mg TE/g DW) and No.2 (27.24 mg TE/g DW), respectively for AnAc.

Table 3: Results of bioactive compounds by Box-Behnken design

Run Order	G (°C)	B (%)	D (mL/g)	TPC (mg GAE/g DW)	AnCo (mg CGE/g DW)	AnAc (mg TE/g DW)
1	60	90	30	14.85±0.27	0.35±0.02	17.43±0.11
2	50	80	50	18.08±0.42	0.70±0.01	27.24±0.44
3	50	90	40	14.85±0.30	0.47±0.09	24.19±0.07
4	60	80	40	23.84±0.28	0.56±0.07	23.99±0.12
5	70	70	40	14.22±0.21	0.41±0.01	16.18±1.38
6	50	70	40	16.87±0.16	0.63±0.02	21.97±0.15
7	70	80	30	13.36±0.05	0.25±0.02	18.09±0.75
8	60	70	50	19.77±0.19	0.61±0.01	19.88±0.76
9	60	80	40	23.01±0.38	0.58±0.06	24.68±0.22
10	70	80	50	14.99±0.43	0.48±0.02	20.33±0.01
11	50	80	30	16.86±0.31	0.57±0.01	24.25±0.69
12	60	90	50	14.92±1.43	0.50±0.02	16.42±0.38
13	60	80	40	22.63±0.33	0.57±0.01	24.80±0.53
14	70	90	40	8.67±0.88	0.29±0.09	17.89±0.17
15	60	70	30	16.81±0.28	0.41±0.01	13.31±0.20

Table 4 illustrates the analysis of variance of BBD for the three responses. All three models had $p < 0.001$, indicating that the models were significant. Furthermore, the high R^2 values of > 97% showed that the data were significantly close to the fitted regression line and there was a high reliability of a correlation. The p-values of the Lack-of-Fit were higher than 0.05, which confirmed the good fitting. Table 4 also indicates that most effects of factors on the responses were significant at their linear and quadratic levels ($p < 0.05$), except the effects of G^2 for AnAc and D^2 for AnCo ($p > 0.05$). Regards the interactions, the ones between B (ethanol content) and D (solvent/solid ratio) significantly influenced TPC and AnAc ($p < 0.05$) while the one between G (drying temperature) and B had a significant impact on TPC only ($p < 0.05$). Hence, the ethanol content was concluded to be the most important factor in extracting phenolics from eggplant peels. Similar findings were observed in the study of (Liao et al., 2022). The polynomial equations for three responses were computed as follow.

Table 4: Analysis of variance of Box-Behnken design for three responses (TPC, AnCo and AnAc)

Run Order	TPC		AnCo		AnAc	
	F-Value	p-Value	F-Value	p-Value	F-Value	p-Value
Model	87.35	0.000	27.09	0.001	69.15	0.000
G	101.54	0.000	117.93	0.000	213.96	0.000
B	88.40	0.001	26.19	0.000	7.09	0.045
D	14.79	0.012	66.96	0.004	39.31	0.002
G2	332.99	0.000	7.88	0.038	4.10	0.099
B2	241.43	0.000	22.41	0.005	257.05	0.000
D2	61.16	0.001	2.23	0.196	70.08	0.000
GB	10.65	0.022	0.41	0.550	0.17	0.695
GD	0.16	0.709	2.45	0.178	0.38	0.564
BD	7.10	0.045	0.56	0.486	38.75	0.002
Lack-of-Fit	0.62	0.666	17.11	0.056	2.62	0.288
R^2	98.89%		97.99%		99.20%	

$$TPC = 23.158 - 1.926G - 1.797B + 0.735D - 5.134G^2 - 4.317B^2 - 2.200D^2 - 0.882GB + 0.107GD - 0.720BD \quad (2)$$

$$AnCo = 0.5706 - 0.1191G - 0.0561B + 0.0897D - 0.0453G^2 - 0.0764B^2 - 0.0241D^2 + 0.0099GB + 0.0243GD - 0.0117BD \quad (3)$$

$$AnAc = 24.49 - 3.15G + 0.5727B + 1.35D + 0.6411G^2 - 5.08B^2 - 2.65D^2 - 0.125GB - 0.1877GD - 1.894BD \quad (4)$$

Figure 2 illustrates the 3D-images describing the effects of two factors on the responses, where the third factor was kept at its center level. Figures 2A-C indicate that all three factors expressed the parabolic curves of their effects on TPC. Increasing temperature from 50°C to 60°C could shorten the drying duration, preserving phenolics and hence enhancing TPC. However, exceeded temperatures could cause the degradation of phenolics or the collapse of plant cells, leading to difficulty of subsequent extraction (Jahangiri et al., 2011). Consistent results for the effects of drying temperature on TPC were reported previously (Mbondo et al., 2018). Similarly, there were peaks in the effect of ethanol content on TPC. The ethanol/water mixture has been demonstrated as effective solvent to extract phenolics. However, if the ethanol content is too high, protein denaturation might occur, resulting in a decline in the extraction efficiency (Yang et al., 2010). Meanwhile, the effect of solvent/solid ratio was consistent with a previous report (Liao et al., 2022). A high solid-to-solvent ratio could be beneficial in extraction, compatible with the mass transfer principle (Al-Farsi & Lee, 2008). Nevertheless, the lower TPC at the excess of solvent quantity could be due to the non-uniform distribution of ultrasound waves (Nayak et al., 2015). Meanwhile, Figures 2D-E-G-H express the different influence trends of drying temperature on AnCo and AnAc, where low temperatures were desirable to obtain higher anthocyanins and antioxidant capacity. Anthocyanins were reported for their heat sensitivity (Rodriguez-Amaya, 2019). Heat processing could lead anthocyanins undergoing a variety of processes such as glycosylation, nucleophilic attack of water, cleavage, and polymerization, resulting in the loss of this pigment and its decomposition.

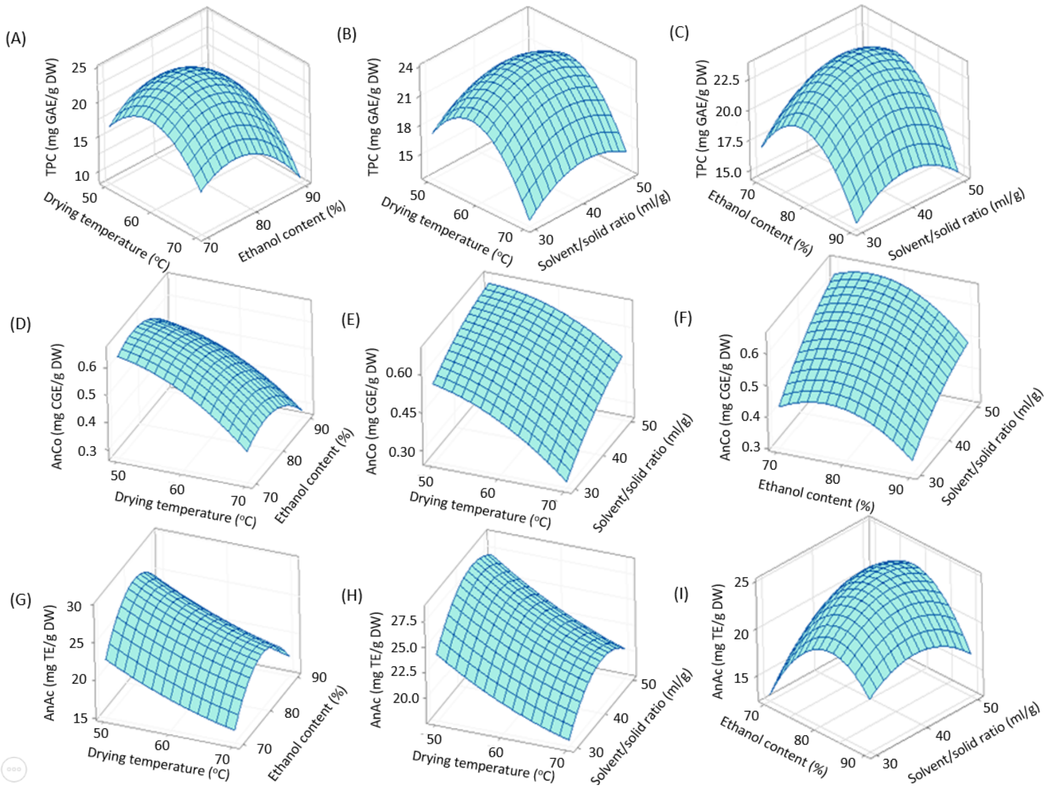

Figure 2: 3D-response surfaces for the effects of two factors on responses

Since the three factors influenced on the three responses in different manners, optimization could balance the trade-off phenomenon. By using the desirability ramp, the goal of simultaneously maximizing all responses could be achieved. With the desirability of 0.925 (close to 1), the optimal conditions included the drying temperature of 53°C, ethanol content of 78.4% and the solvent/solid ratio of 44.4 mL. The predicted values for the responses were 21.98 mg GAE/g DW for TPC, 0.67 mg CGE/g DW for AnCo, and 27.04 mg TE/g DW for AnAc. The experimental values in the validation test were close with their predicted ones, confirming the reliability of the models. The TPC and AnCo were comparable with those reported in previous research for eggplant peels (23-30 mg GAE/g DW and 0.93-1.4 mg CGE/g DW) (Akhbari et al., 2019; Liao et al., 2022). The difference could be due to the variation in raw materials used for experiment. Furthermore, the multi-response optimization in this study had the advantage of achieving high values for several targets (*i.e.* TPC, AnCo and AnAc) rather than only one (TPC or AnCo) in previous research.

4. Conclusion

This study demonstrated the efficiency of integrating PBD and BBD in screening and optimizing the processing condition to maximize the extraction yields of phenolics and anthocyanins from eggplant peels. PBD was the effective-cost tool to identify three most significant factors, including drying temperature, ethanol content, and solvent/solid ratio. BBD provide the optimal settings of these three factors, which included the drying temperature of 53°C, ethanol content of 78.4% and the solvent/solid ratio of 44.4 mL. The validation test confirmed the fitting of the models and the maximized values were 21.98 mg GAE/g DW for TPC, 0.67 mg

CGE/g DW for AnCo, and 27.04 mg TE/g DW for AnAc. Further investigation should focus on the identification of phenolic and anthocyanin profiles of the extract and their potential applications.

Acknowledgments

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.99-2019.346.

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