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Optimization of Oven Drying Process for High Yields of Flavonoids and Phenolics from Pandan Leaves (*Pandanus Amaryllifolius* Roxb) using Response Surface Methodology

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Pandan leaves are one of the most popular herbs from Southeast Asia. Scientifically, the herb is demonstrated to contain a variety of bioactive compounds that are beneficial for human health. For extraction and preservation purposes, oven drying is one of the critical pre-treatment steps. Therefore, in this study, response surface methodology was applied to optimize drying condition to maximize the contents of flavonoids and phenolics in the pandan leaf extract. A central composite design was utilized to investigate the effects of two main independent variables, namely drying temperature and drying time. Ultrasound-assisted extraction (UAE) with fixed parameters was employed to enhance the release of target compounds from the dried pandan leaves. The results revealed that the optimal oven-drying condition was 82.14 °C for 9.46 hours, yielding a predicted total flavonoid content of 2.47 mg QE/g DW and a total phenolic content of 2.22 mg GAE/g DW. The experimental results were compared and aligned with the predicted values, demonstrating the adequacy and suitability of the models. The leaf extract at this condition exhibited both antidiabetic and antioxidant activities with IC_{50} values of 1023.03 µg/mL and 1489.92 µg/mL, respectively for alpha-amylase inhibition and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activities. These findings suggest a promising approach for optimizing the oven drying process to maximize the recovery of bioactive compounds from pandan leaves for functional food and nutraceutical applications.

1. Introduction

Pandan (*Pandanus amaryllifolius* Roxb) is primarily cultivated in Southeast Asia, New Guinea and India where high humidity levels are prevalent. It is a tropical herbaceous plant with a distinctive aroma and a member of the screw pine group belonging to the family Pandanaceae. Traditionally, pandan leaves have been used as a natural food additive to improve the flavor, aroma and appearance due to the presence of 2-acetyl-1-pyrroline and a large amount of chlorophyll (Wang et al., 2024). In Vietnam and other Southeast Asian countries, pandan leaf extract is widely employed in traditional foods such as cakes, desserts, and beverages. Beyond culinary applications, pandan leaves have also been utilized in traditional medicine treatment of several diseases, including fever, diabetes and chronic diseases (Ningrum and Schreiner, 2014). Furthermore, pandan leaves have been reported to contain abundant phenolic compounds, including flavonoids (rutin, catechin, epicatechin, naringin, and kaempferol), and phenolic acids such as gallic acid, ferulic acid, cinnamic acid, and caffeic acid (Ghasemzadeh and Jaafar, 2013). These compounds are known to possess various health-promoting properties, including antioxidant and antidiabetic activities. Therefore, among various medicinal plants, pandan leaves have emerged as a notable candidate for diabetes management. According to Hasan et al. (2023), pandan leaves have been reported relatively high α-amylase inhibitory activity compared to several medicinal herbs, including *Cinnamomum verum*, *Tithonia diversifolia*, *Zingiber officinale* and *Syzygium polyanthum.*
Drying is an essential pre-treatment step prior to extraction of bioactive substances from plant materials. Among various drying methods, oven drying is commonly utilized because it is simple, affordable, and controllable. However, excessive heat exposure may lead to the degradation and loss of these valuable constituents, reducing the functional quality of the final product (Antony and Farid, 2022). A previous study also confirmed

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that drying temperature significantly influenced the drying kinetics of pandan leaves (Fitri et al., 2023). Although oven drying is widely used, the optimization studies defining precise oven drying conditions to maximize the retention of phenolic compounds in pandan leaves remain limited, particularly regarding the evaluation of its antidiabetic potential. As a result, this study aimed to apply Central Composite Design (CCD) with two factors (temperature and time) to optimize the oven drying process, maximizing the phenolic and flavonoid retention. Furthermore, the α-amylase inhibitory and antioxidant activities of the extract under the optimal condition were evaluated, providing new insights into the preservation of functional bioactivities and offering a practical baseline for the industrial processing of pandan leaves.

2. Materials and methods

2.1 Materials and chemicals

The fresh pandan leaves were obtained from a local market, Vietnam. The used chemicals included ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, sodium phosphate buffer, gallic acid, alpha-amylase, acarbose and quercetin.

2.2 Sample preparation and oven drying process

The leaves were carefully selected and washed thoroughly with water to remove dirt, dust and surface contaminants. The cleaned leaves were drained on trays to remove excess surface water and then cut into smaller segments (approximately 2-3 cm in length). After drying in an oven (ThermoStable OF 155, Daihan, Korea), the dried leaves were ground and passed through a sieve with a mesh size of 250 µm to obtain a uniform powder. Finally, the powder was extracted with water using an ultrasound bath (WUC A10H, Daihan, Korea) at 45 °C for 20 min with a solid-to-solvent ratio of 1:30 (w/v). The extraction conditions were selected based on our preliminary screening in the operation ranges suggested by Oreopoulou et al. (2019). The supernatant obtained after centrifugation (REF 1406-Hettich-Germany) at 7000 rpm for 15 min was used for determination of total phenolic content (TPC), total flavonoid content (TFC), antioxidant and antidiabetic activities.

2.3 Experimental design of optimization

A rotatable CCD was constructed by a total of 13 experimental runs, including 4 factorial points, 4 axial points, and 5 center points. The design contained two independent variables including drying temperature (X_1) of 66, 70, 80, 90 and 94 °C; and drying duration (X_2) of 7, 8, 10, 12 and 13 h. The selection of the levels was based on one-factor-at-a-time screening, where 80 °C and 10 h displayed the best results.
Two responses were TPC and TFC. Second-order polynomial models were constructed as below:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_i \sum_{j \neq i}^{k} \beta_{ij} X_i X_j \quad (1)$$

where: Y was the response; k was the factor number; X_i and X_j were the independent variables; β_0, β_i, β_{ii}, and β_{ij} were the regression coefficients.

2.4 Analytical methods

TPC was assessed using Folin–Ciocalteu reagent by following the method of Cheng et al. (2024) and displayed as mg gallic acid equivalent (GAE) per gram dry weight (DW). TFC was evaluated according to the method of (Quyen et al., 2020) and expressed as mg quercetin equivalent (QE)/g DW. DPPH was used to evaluate the antioxidant activity of the extract by adapting the method of Padhi et al. (2024). Alpha-amylase inhibition was assessed by using the dinitrosalicylic acid (DNS) method of Guzmán et al. (2022). Both antioxidant and α-amylase inhibitory activities were expressed as IC_{50} values. Ascorbic acid and acarbose were used as positive controls, respectively.

2.5 Statistical analysis

All experiments were conducted in triplicate and randomized to minimize bias. Experimental design and statistical analyses were performed using Minitab 20 and SPSS 26.

3. Results and discussion

3.1 Optimization by central composite design

Table 1 presents the CCD matrix with two independent variables of oven-drying process (temperature and time) and the experimental and predicted results for two responses (TFC and TPC). The results ranged from 0.52 to

2.64 mg QE/g DW for TFC and from 1.45 to 2.25 mg GAE/g DW for TPC. Run No.13 (80 ºC and 10 h) exhibited the highest results for both TFC and TPC with approximately 2.64 mg QE/g DW and 2.25 mg GAE/g DW, respectively.

Table 1: Results of bioactive compounds by central composite design

Run	Independent variables		TFC (mg QE/ g DW)		TPC (mg GAE/g DW)	
	X_1	X_2	Experimental	Predicted	Experimental	Predicted
1	70	8	1.11±0.03	1.09	1.80±0.08	1.76
2	70	12	1.55±0.03	1.44	1.85±0.16	1.83
3	90	8	2.06±0.01	2.10	2.08±0.08	2.06
4	90	12	1.67±0.03	1.63	1.95±0.08	1.95
5	66	10	0.52±0.05	0.61	1.45±0.06	1.48
6	94	10	1.48±0.05	1.46	1.78±0.04	1.78
7	80	13	1.96±0.14	2.05	2.16±0.08	2.16
8	80	7	2.17±0.08	2.14	2.15±0.13	2.19
9	80	10	2.42±0.06	2.44	2.23±0.09	2.21
10	80	10	2.33±0.03	2.44	2.20±0.12	2.21
11	80	10	2.26±0.04	2.44	2.18±0.06	2.21
12	80	10	2.54±0.06	2.44	2.20±0.06	2.21
13	80	10	2.64±0.02	2.44	2.25±0.11	2.21

Table 2 provides the analysis of variance results for the regression models of the drying process. The p-values for both TFC and TPC were below 0.01, indicating that the models were statistically significant ($p < 0.05$) and adequately fitted well with experimental data. In addition, the lack-of-fit values for both responses were not significant (0.72 and 0.24 respectively for TFC and TPC), confirming the suitability of the models.
Moreover, the high R^2 and adjusted R^2 values for TFC (97.12% and 95.06%) and TPC (98.98% and 98.26%) suggested a strong correlation between the experimental and predicted data.

Table 2: Analysis of variance of Central Composite design for two responses (TFC and TPC)

Source	TFC (mg QE/g DW)		TPC (mg GAE/g DW)	
	F-Value	p-Value	F-Value	p-Value
Model	47.15	<0.01	136.52	<0.01
Linear	19.95	<0.01	44.51	<0.01
X_1	39.46	<0.01	88.49	<0.01
X_2	0.43	0.53	0.53	0.49
Square	93.41	<0.01	292.77	<0.01
X_1^2	184.39	<0.01	582.78	<0.01
X_2^2	10.99	0.01	2.26	0.18
2-way interaction, X_1X_2	9.05	0.02	8.06	0.03
Lack-of-fit	0.47	0.72	2.11	0.24
CV (%)	2.22		4.37	
R^2	97.12%		98.98%	
Adjusted R^2	95.06%		98.26%	

Results also indicated that TFC was significantly affected ($p < 0.05$) by the linear effect of drying temperature (X_1, $p < 0.01$) and quadratic effects of drying temperature (X_1^2, $p < 0.01$) and drying time (X_2^2, $p < 0.01$) as well as the temperature–time interaction (X_1X_2, $p = 0.02$).
Similarly, TPC was significantly influenced by the linear effect of drying temperature (X_1, $p < 0.01$), its quadratic term (X_1^2, $p < 0.01$) and the temperature–time interaction (X_1X_2, $p = 0.03$), while the remaining coefficients were not statistically significant ($p > 0.05$) for both responses. As a result, the second-order polynomial models for TFC and TPC were constructed by using the actual values of the variables, as represented in the equations below, respectively.

$$TFC = -57.34 + 1.2581X_1 + 1.664X_2 - 0.007032X_1^2 - 0.0429X_2^2 - 0.01027X_1X_2 \quad (2)$$

$$TPC = -19.36 + 0.4960X_1 + 0.2655X_2 - 0.002894X_1^2 - 0.00450X_2^2 - 0.002244X_1X_2 \quad (3)$$

3.2 Effects of drying conditions on total flavonoid and total phenolic contents of pandan leaves and model validation

Figure 1 illustrates the interaction effects of drying temperature and drying time on TFC and TPC. As shown in Figures 1A and 1B, increasing temperature from approximately 70 °C to 80-85 °C and extending time to around 9-10 h significantly enhanced the TFC of pandan leaves. A similar trend was observed for TPC in Figures 1C and 1D, where moderate drying conditions promoted the preservation of phenolic compounds. This may be attributed to the fact that rational heat exposure during drying could facilitate cell wall disruption, improving the penetration of solvents into the matrix in the subsequent extraction to effectively recover targeted components (Mediani et al., 2013). However, further increases in temperature (> 85-90 °C) accompanied by prolonged drying time (12 h) caused gradual decreases in both TFC and TPC. This phenomenon was because of thermal degradation and possible oxidation of heat-sensitive compounds (Le Son et al., 2023; Nguyen et al., 2024). This trend was consistent with previous studies reporting that drying at around 80 °C provided better retention of phenolic and flavonoid compounds than drying at lower and higher temperatures in various plant materials (Nguyen et al., 2024; Ratseewo et al., 2025). Other research also suggested that appropriate selection of drying conditions could effectively preserve the stability of bioactive compounds (Hassan et al., 2022; Routray and Rayaguru, 2010).

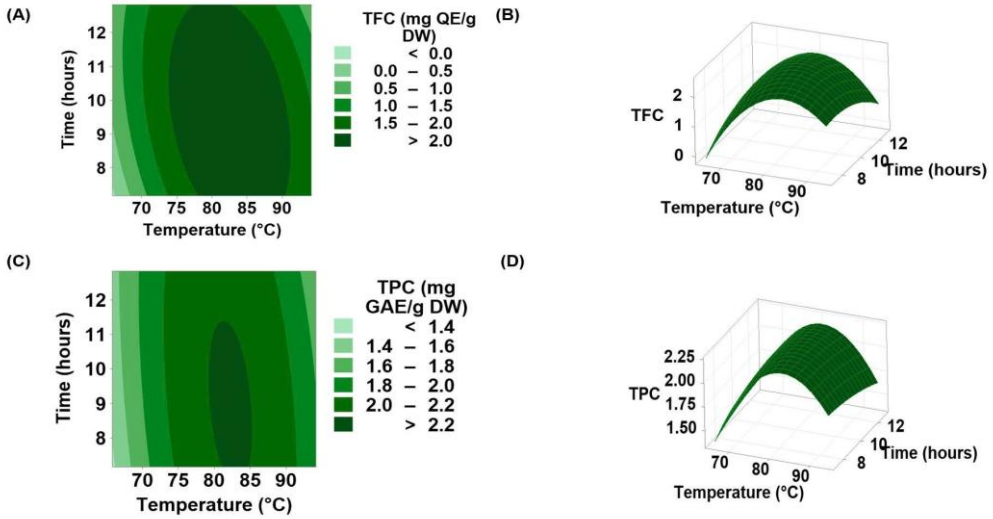

Figure 1: Contour plots (A and C) and response surface plots (B and D) for the effects of two factors on total flavonoid and total phenolic contents.

The desirability function was employed to find the optimal oven drying condition. It transforms multiple targets into a scale from 0 to 1 where 1 represents the ideal outcome. Based on the RSM optimization, an overall desirability of 0.95 was obtained, indicating a high degree of satisfaction for both TFC and TPC. The result included the drying temperature of 82.14 °C and the drying duration of 9.46 h, leading to the predicted outcomes of 2.47 mg QE/g DW for TFC and 2.22 mg GAE/g DW for TPC. The validation experiment obtained the TFC and TPC values of 2.51 ± 0.03 mg QE/g DW and 2.20 ± 0.04 mg GAE/g DW, respectively. These values were in close agreement with their predicted ones, verifying the adequacy of the models. The TFC value in our study paralleled with the findings of Dewi et al. (2019) from 1.73 to 4.73 mg QE/g DW. Meanwhile, the TPC value was higher than that reported by the findings of Zaki et al. (2020), approximately 0.98 - 1.56 mg GAE/g DW. These fluctuations among studies may be attributed to differences in drying methods, drying conditions, experimental conditions, equipment and the sources of materials.

3.3 Bioactivities of the pandan leaf extract

The bioactivities of the pandan extract at the optimal condition of the oven drying process were assessed through its antidiabetic and antioxidant activities. The pandan leaf extract showed moderate antidiabetic activity, with an IC_{50} value of approximately 1023.03 ±1.86 µg/mL for its alpha-amylase inhibition compared with 91.18 ± 0.97 µg/mL for acarbose. This suggests that optimal drying conditions may help preserve bioactive compounds

and their bioactivities. Antidiabetic activity has been reported to be mainly attributed to the presence of phenolic and flavonoid compounds through their hydrogen bonding and hydrophobic interactions with the active site of α-amylase (Bamigboye et al., 2020). The result was generally consistent with the findings of Nurdin et al. (2019), reporting that the inhibitory activity of plant extracts varied depending on plant species and composition, with IC_{50} values of approximately 700 to 1000 µg/mL. In contrast, Cheng et al. (2024) reported a much higher IC_{50} value of around 16000 µg/mL. Buddhakala and Yongkhamcha (2025) demonstrated that mature pandan leaves exhibited a stronger α-amylase inhibitory activity, with an IC_{50} value of 470 µg/mL. Such differences may be attributed to variations in raw materials, extraction methods, and experimental conditions. Therefore, an appropriate control of oven-drying parameters is essential to preserve bioactive compounds associated with enzyme inhibition (Nurdin et al., 2019). This finding was also consistent with Hassan et al. (2022) and Bamigboye et al. (2020), who reported that higher levels of bioactive compounds were generally associated with stronger biological activities, such as antioxidant and antidiabetic effects. In addition, the pandan leaf extract exhibited measurable antioxidant capacity, with an IC_{50} value of approximately 1489.92 ± 0.15 µg/mL in the DPPH radical scavenging assay. In comparison, *L*-ascorbic acid showed a stronger radical scavenging capacity, with a lower IC_{50} value of 8.54 ± 0.26 µg/mL. This is because the antioxidant activity of pandan leaf extract is mainly dependent on the specific composition of phenolic and flavonoid compounds rather than their total contents (Gulcin, 2025; Rice-Evans et al., 1996). Le Son et al. (2023) reported relatively comparable antioxidant activity for dried pandan leaves, with IC_{50} values ranging from 1430 to 2060 µg/mL for oven-dried samples and from 900 to 1080 µg/mL for shade-dried samples. Furthermore, the result of the present study was slightly similar to the investigation of Buddhakala and Yongkhamcha (2025), with an IC_{50} value of approximately 1780 µg/mL. Overall, the optimized oven-drying conditions reduced the moisture content of pandan leaves from 87.5% to 5.3% while retaining measurable antioxidant and alpha-amylase inhibitory activities. Although these activities were significantly weaker than those of the purified positive control, this was expected because crude plant extracts inherently exhibit lower biological potency than pure reference compounds. Furthermore, the obtained IC_{50} values were comparable to those reported in previous studies on pandan leaves, indicating that the optimized drying process effectively preserved the extract's bioactive potential. These findings highlight the importance of precise control over oven-drying parameters to minimize the degradation of bioactive compounds in pandan leaves.

4. Conclusion

Oven drying is a crucial pre-treatment step that directly affects the preservation of bioactive compounds in plant materials. CCD successfully optimized the oven drying conditions while maintaining the bioactive potential of pandan leaves. These findings confirm that properly dried pandan leaves represent a valuable source of functional bioactive compounds. To facilitate potential industrial scaling, future studies should focus on evaluating drying kinetics and energy consumption, as well as comparing these results with novel or hybrid drying technologies.

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