

Process Analysis of Antioxidant Biomass Extraction from Circular Pineapple Leaves for Functional Material Applications Using Response Surface Methodology

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Abstract

This study investigated the phytochemical composition of pineapple leaves, with a particular focus on flavonoids and polyphenols known for their strong antioxidant properties. The presence of allantoin, a compound recognized for its skin-repairing activity, was also examined. The primary objective was to optimize extraction conditions in order to maximize flavonoid content, total polyphenol content, and antioxidant capacity, thereby identifying the most effective parameters for antioxidant extraction. Powdered pineapple leaves were extracted using ethanol as the solvent. Initially, a one-factor-at-a-time approach was conducted to determine appropriate ranges for each extraction variable. Subsequently, Response Surface Methodology (RSM) was employed to optimize the extraction process, using extraction temperature (45°C, 50°C, and 55°C), extraction time (2, 3, and 4 h), and ethanol concentration (75%, 85%, and 95%) as independent variables. Under the optimized conditions, the maximum flavonoid content reached 5.20 mg rutin equivalents per gram, total polyphenol content was 63.53 mg gallic acid equivalents per gram, and DPPH radical scavenging activity achieved 92.72%. The ethanol extract exhibited a protein concentration of 9.21 µg/µL and a pH value of 5.96. Based on these results, the optimal extraction conditions were determined to be an extraction temperature of 55°C, an extraction time of 4 h, and an ethanol concentration of 85%.

Keywords

Extraction, Antioxidant, Functional Material, Pineapple Leaf, Response Surface Methodology

1. Introduction

Pineapple (*Ananas comosus*) is a perennial monocotyledonous plant primarily cultivated in Southeast Asia, South America, Africa, and Pacific islands. Major producing regions include Costa Rica in the Americas, as well as Indonesia, the Philippines, and Thailand in Southeast Asia [1]. Pineapple cultivation generates substantial agricultural residues, including roots, stems, peels, fruits, and leaves. Although often regarded as waste, these by-products are not entirely without value; different parts of the plant contain distinct bioactive compounds with considerable economic potential [2]. Notably, pineapple leaves are rich in phytochemicals, which significantly enhance their value. In addition to being used as a source of animal feed, pineapple leaves contain abundant polyphenols, flavonoids, and trace amounts of allantoin [3]. These compounds confer strong antioxidant properties and broaden their potential applications. Consequently, the reutilization of pineapple leaf residues not only contributes to sustainable agricultural practices but also establishes a promising resource-recycling model, providing new material sources for environmental protection and economic development.

Polyphenols are structurally diverse compounds derived from various plant metabolic pathways. They may exist as monomers or polymers and play essential physiological roles in plants, particularly in defense against pathogens, herbivores, and environmental stress such as nutrient deficiency. Over time, plants have evolved the ability to synthesize phenolic compounds to adapt to changing environmental challenges. Structurally, polyphenols consist primarily of aromatic rings bearing hydroxyl groups, and include phenolic acids, flavonoids, and anthocyanins, among others. These compounds are commonly used as indicators of antioxidant capacity. In the human diet, fruits, vegetables, and beverages are the main sources of phenolic compounds, contributing to enhanced antioxidant activity and associated health benefits. Plant-derived polyphenols exhibit antioxidant, anti-aging, anti-inflammatory, and cytoprotective effects in the human body. Consumption of polyphenol-rich foods may help regulate oxidative mechanisms and reduce the incidence of chronic diseases such as diabetes, cancer, and cardiovascular disorders. As essential secondary metabolites in plants, polyphenols play critical roles in defense mechanisms and, due to their antioxidant properties, represent one of the largest groups of phytochemicals, with more than 8000 phenolic compounds identified to date [4].

Flavonoids, derived from the Latin word *flavus* meaning “yellow”, are yellow pigments widely distributed among plant polyphenols [4]. They are commonly consumed through foods such as parsley, onions, berries, tea, citrus fruits, red wine, and dark chocolate. Chemically, flavonoids are characterized by a flavone nucleus and possess a wide range of pharmacological properties, including anti-cancer, antioxidant, anti-inflammatory, and antiviral activities. Numerous studies have demonstrated their effectiveness in the prevention and treatment of various diseases. Flavonoids play important roles not only in plant physiological processes but also in human health through dietary intake. The chemical properties of fla-

vonoids largely depend on the number and position of hydroxyl (-OH) groups attached to their phenolic rings. Variations in these functional groups give rise to multiple subclasses, including flavones, flavonols, flavanones, flavanols, isoflavones, and anthocyanins [4].

Allantoin is a compound found in plant extracts such as comfrey (*Symphytum officinale*) and is well known for its skin-soothing properties. Due to its moisturizing and keratolytic (skin-softening) effects, allantoin is widely used in skincare products. It promotes cell regeneration, thereby facilitating wound healing and skin repair. In addition, allantoin exhibits anti-inflammatory properties and helps soothe and protect the skin [5].

Many naturally occurring plant compounds possess antioxidant properties and free radical scavenging capabilities, which significantly impact human skin health [6]. The antioxidant activity and free radical scavenging capacity of plants are closely associated with flavonoids, polyphenols, tocopherols, carotenoids, and ascorbic acid [6]-[8]. Free radical scavengers neutralize free radicals by donating electrons or hydrogen atoms, thereby slowing or preventing damage to other molecules and inhibiting radical chain reactions [9]. Common free radical scavengers include vitamin C, vitamin E, polyphenolic compounds (such as flavonoids), and β -carotene. These natural or synthetic antioxidants are widely present in foods and contribute to cellular protection and the delay of aging processes by stabilizing free radicals.

Compared with liquid-liquid extraction, solid-phase extraction offers several advantages, one of which is the significantly reduced solvent requirement, typically only a few milliliters, whereas liquid-liquid extraction often requires tens of milliliters or more. This reduction in solvent usage not only lowers costs but also minimizes environmental impact. In addition, solid-phase extraction provides higher selectivity and reduced interference, thereby enhancing analyte concentration and recovery. Its advantages include purification, concentration, operational simplicity, low solvent consumption, rapid processing, simple equipment requirements, and the ability to process multiple samples simultaneously.

Physical extraction is a method used to separate target compounds from an aqueous phase into an organic phase based on differences in solubility, without involving chemical reactions. This technique operates on the principle of “like dissolves like”. When the polarity of the organic phase is low, polar solutes in the aqueous phase exhibit limited solubility, resulting in a low distribution coefficient. Conversely, increasing the polarity of the organic phase can enhance the distribution coefficient but may also increase mutual solubility between the organic and aqueous phases, leading to solvent loss [10]. Therefore, the selection of an appropriate organic phase must consider both the properties of the target compound and the aqueous solution.

Physical extraction is suitable when the target compound exhibits good solubility in the organic phase, eliminating the need for extractants. During the process, the target compound is co-extracted with water molecules into the organic phase

and subsequently recovered through post-treatment methods such as precipitation and crystallization [11]. In contrast, chemical extraction involves chemical reactions between components in the organic phase and the target compound, enabling its transfer from the aqueous phase into the organic phase. This approach allows effective extraction and controlled release of the target compound back into the aqueous phase when necessary. A major advantage of chemical extraction is the reversibility of the reaction, which facilitates controlled release and recovery of the target compound while offering enhanced selectivity. The organic phase plays a critical role in chemical extraction and typically consists of solvents with strong bonding capabilities and high affinity for the target compound. The selection of the organic phase depends on the chemical properties of the target compound and reaction conditions, allowing efficient extraction, separation, and adsorption of the desired substance.

2. Experiments

A one-factor-at-a-time (OFAT) experimental approach was employed to determine the appropriate ranges of key extraction parameters, including solvent concentration, extraction temperature, and extraction time. Parameters yielding higher antioxidant compound contents after extraction were selected for subsequent optimization experiments.

Under baseline conditions of room temperature (25°C), an extraction time of 1 h, and a solid-to-liquid ratio of 1:10, the effect of ethanol-water solvent concentration on flavonoid content was investigated. Extractions were conducted using ethanol concentrations of 95%, 85%, 75%, 65%, and 55%. After extraction, the mixtures were separated using vacuum filtration, and the filtrates collected from the bottom of the containers were analyzed for flavonoid content. Suitable solvent concentration ranges were then selected for further experiments.

Next, under conditions of a solid-to-liquid ratio of 1:10 and an extraction time of 1 h, the effect of extraction temperature using 95% ethanol was evaluated. Extractions were performed at temperatures of 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, and 65°C. After vacuum filtration, the extracts were analyzed for flavonoid content, and appropriate temperature ranges were selected.

Finally, under identical conditions of a solid-to-liquid ratio of 1:10 and room temperature (25°C), the effect of extraction time using 95% ethanol was investigated. Extraction times of 0.5, 1, 2, 3, 4, 5, and 6 h were examined. After extraction and vacuum filtration, the filtrates were analyzed for flavonoid content, and suitable time ranges were selected for subsequent optimization experiments.

Response Surface Methodology (RSM), first proposed by Box and Wilson in 1951, is an empirical statistical modeling technique used to evaluate the relationships between experimental parameters and response variables, including the interactions among factors, and to identify optimal conditions. After determining suitable parameter ranges through OFAT experiments, RSM was applied for optimization. The RSM procedure consisted of three main stages: 1) conducting sta-

tistically designed experiments to obtain experimental data, 2) constructing mathematical models to estimate response values, and 3) comparing experimental and predicted responses for model validation. Regression analysis was used to develop mathematical models describing the relationships among parameters and response variables and to explore optimal conditions.

3. Results and Discussion

Fourier-transform infrared (FT-IR) spectroscopy was used to investigate the structural features and functional groups present in pineapple leaf-derived materials. **Figure 1** shows the FT-IR spectra of four samples: Pineapple Leaf Powder (PALP), Pineapple Leaf Fiber (PALF), Pineapple Leaf Residue after mechanical blade fiber extraction (PALR), and Pineapple Leaf Residue after hydraulic fiber extraction (PALWJ). A broad absorption band observed in the 3300 - 3500 cm^{-1} region corresponds to N-H stretching vibrations associated with amino (-NH) and amide (-CONH) groups in allantoin. The absorption band in the 1690 - 1760 cm^{-1} region is attributed to C=O stretching vibrations of the amide group (-CONH) in allantoin molecules. Peaks observed in the 1180 - 1360 cm^{-1} region arise from C-N stretching vibrations, while absorption bands in the 3010 - 3095 cm^{-1} region correspond to C-H bending vibrations in allantoin molecules.

Among the four samples, PALP exhibited the strongest characteristic peaks, indicating a higher presence of allantoin-related functional groups. Therefore, PALP was selected as the primary raw material for subsequent experiments. Allantoin is known for its skin-soothing properties, including astringent, anti-inflammatory, and cell-regenerative effects, and it enhances nutrient absorption by the skin while alleviating roughness and sensitivity.

Following a series of extraction experiments, ethanol was selected as the extraction solvent due to its superior performance in yielding higher flavonoid content

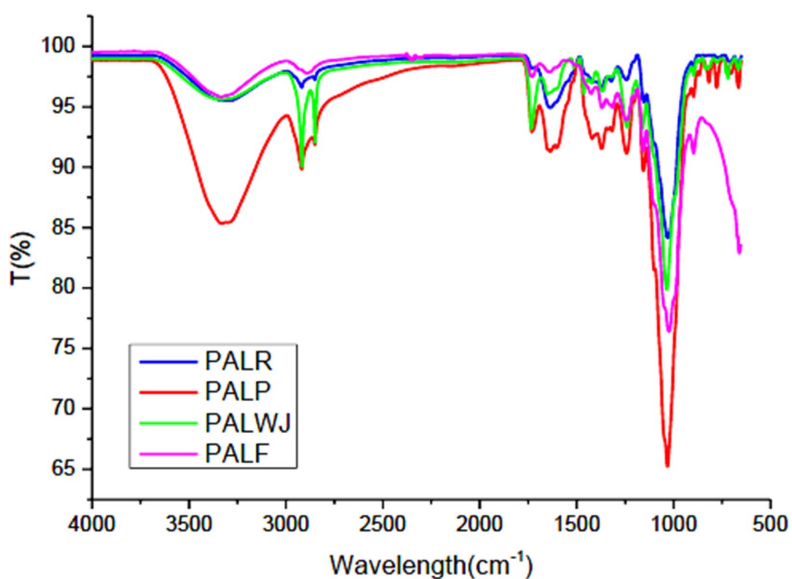


Figure 1. FT-IR spectra of PALR, PALP, PALWJ, and PALF.

and stronger DPPH free radical scavenging activity. Ethanol possesses moderate polarity, enabling effective dissolution and extraction of various plant compounds, particularly flavonoids. Its relatively low boiling point minimizes the thermal degradation of heat-sensitive compounds during extraction, preserving their integrity and bioactivity. In addition, ethanol-extracted flavonoids exhibited excellent antioxidant performance, demonstrating high DPPH radical scavenging activity. Compared to methanol, ethanol has lower toxicity, ensuring greater safety for researchers and higher purity of extracts.

To identify optimal parameter ranges for further optimization, the effects of temperature, extraction time, and ethanol concentration on flavonoid extraction efficiency were evaluated using OFAT experiments. After selecting ethanol as the extraction solvent, the parameter ranges determined from OFAT experiments were used for RSM analysis. Three operational parameters were selected: temperature (X_1), extraction time (X_2), and ethanol concentration (X_3). The parameter ranges and 15 experimental runs designed using the Box–Behnken model are presented in **Table 1**. The response variables included flavonoid content (Y_1), total polyphenol content (Y_2), and DPPH free radical scavenging activity (Y_3). Regression analysis was performed using statistical software to compare experimental data with model-predicted values.

Table 1. Results of 15 RSM experiments.

No.	Pattern	X1 (°C)	X2 (hr)	X3 (%)	Y1 mg rutin/g	Y2 mg GAE/g	Y3 (%)
1	-+0	45	4	85	4.00	53.39	81.27
2	+0+	55	3	95	4.83	36.25	92.72
3	000	50	3	85	4.24	59.86	88.75
4	000	50	3	85	4.25	59.99	88.32
5	-0+	45	3	95	4.87	34.20	85.49
6	000	50	3	85	4.27	59.91	87.87
7	+0-	55	3	75	3.45	63.53	86.34
8	0-+	50	2	95	4.73	33.68	89.98
9	0--	50	2	75	3.44	56.69	85.75
10	+ -0	55	2	85	4.14	50.57	85.49
11	-0-	45	3	75	3.44	61.84	83.23
12	0+-	50	4	75	3.55	56.69	85.49
13	--0	45	2	85	3.66	57.81	81.27
14	0++	50	4	95	5.17	42.81	86.35
15	++0	55	4	85	5.2	62.92	86.35

The results indicate that the highest flavonoid content (5.20 mg rutin/g) was obtained in experiment No. 15 at 55 °C, 4 h, and 85% ethanol. The maximum pol-

polyphenol content (63.53 mg GAE/g) occurred in experiment No. 7 at 55°C, 3 h, and 75% ethanol. The highest DPPH free radical scavenging activity (92.72%) was observed in experiment No. 2 at 55°C, 3 h, and 95% ethanol.

Given that DPPH scavenging activity is a representative indicator of antioxidant capacity, further optimization focused on response Y_3 . The regression equation derived from **Table 1** is shown below. Comparison between experimental and predicted values (**Figure 2**) yielded a coefficient of determination (R^2) of 0.96, indicating excellent agreement between the model predictions and experimental data and confirming the strong explanatory power of the model.

$$Y_3 = 88.3133 + 2.4925 \left[\frac{(X_1 - 50)}{5} \right] + (-0.3412)(X_2 - 3) + 1.71625 \left[\frac{(X_3 - 85)}{10} \right] + \left[\frac{(X_1 - 50)}{5} \right] \left[\frac{(X_2 - 3)}{0.29} \right] + \left[\frac{(X_1 - 50)}{5} \right] \left\{ \left[\frac{(X_3 - 85)}{10} \right] 1.03 \right\} + (X_2 - 3) \left\{ \left[\frac{(X_3 - 85)}{10} \right] (-0.8425) \right\} + \left[\frac{(X_1 - 50)}{5} \right] \left\{ \left[\frac{(X_1 - 50)}{5} \right] (-2.2954) \right\} + (X_2 - 3) * \left[\frac{(X_2 - 3)}{(-2.34791)} \right] + \left[\frac{(X_3 - 85)}{10} \right] \left\{ \left[\frac{(X_3 - 85)}{10} \right] 0.92708 \right\}$$

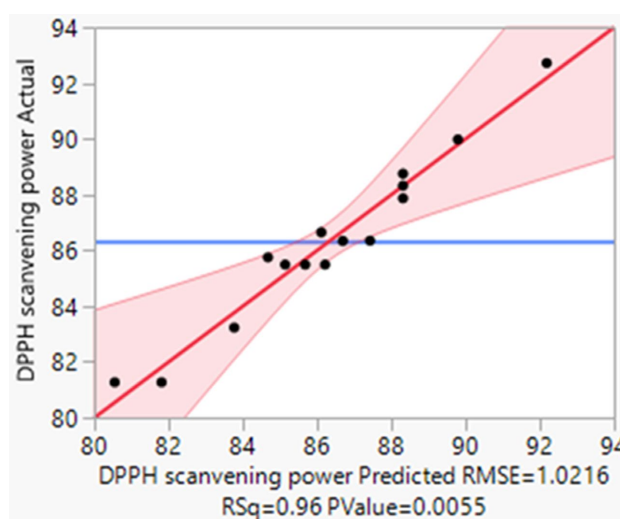


Figure 2. Regression analysis comparing predicted and experimental DPPH scavenging activity.

Based on the results in **Table 2**, temperature (X_1), ethanol concentration (X_3), the quadratic effect of temperature (X_1^2), and the quadratic effect of extraction time (X_2^2) exhibited p-values below 0.05, indicating significant effects on DPPH scavenging activity. These quadratic terms suggest complex nonlinear relationships between these parameters and antioxidant capacity. Other interaction terms exhibited p-values greater than 0.05, indicating no significant effects.

Considering potential commercial applications, experiment No. 15 was selected for further comparison between ethanol and water extraction. Comparative results for flavonoid content, polyphenol content, and DPPH scavenging activity are shown in **Table 3**. For commercial applications, water extraction may be considered as an alternative to ethanol extraction to eliminate the need for solvent removal.

Protein concentration analysis revealed that ethanol extraction yielded higher protein content than water extraction (**Table 4**), likely due to ethanol's ability to

disrupt cell membranes more effectively and dissolve certain protein fractions. Although water extraction resulted in lower protein concentrations, it still achieved acceptable yields from a sustainability perspective.

Table 2. Significance analysis of factors affecting DPPH scavenging activity.

Term	Estimate	Standard Error	t Ratio	P-value
Intercept	88.313333	0.58985	149.72	<0.0001*
X ₁	2.4925	0.361208	6.90	0.0010*
X ₂	-0.34125	0.361208	-0.94	0.3882
X ₃	1.71625	0.361208	4.75	0.0051*
X ₁ X ₂	0.29	0.510825	0.57	0.5948
X ₁ X ₃	1.03	0.510825	2.02	0.0998
X ₂ X ₃	-0.8425	0.510825	-1.65	0.1600
X ₁ X ₁	-2.295417	0.531683	-4.32	0.0076*
X ₂ X ₂	-2.347917	0.531683	-4.22	0.0069*
X ₃ X ₃	0.9270833	0.531683	1.74	0.1417

Table 3. Comparison between ethanol and water extraction.

Extraction Solvent	Flavonoid content mg rutin/g	Phenol content mg GAE/g	DPPH (%)
Ethanol	5.20	63.53	86.65
Water	2.56	42.77	72.22

Table 4. Comparison of protein concentration.

Extraction Solvent	Protein Concentration (µg/µl)
Ethanol	9.21
Water	8.01

4. Conclusions

This study investigated the ethanol extraction of antioxidant phytochemicals from pineapple leaves under various operating conditions. The target responses included flavonoid content (Y_1), polyphenol content (Y_2), and DPPH free radical scavenging activity (Y_3). Optimal extraction conditions were identified using RSM, and water extraction was evaluated as a reference for commercial feasibility.

Ethanol was found to be a more effective solvent than methanol and water for flavonoid extraction. Optimal conditions yielded a maximum flavonoid content of 5.20 mg rutin/g (55 °C, 4 h, 85% ethanol), a maximum polyphenol content of 63.53 mg GAE/g (55 °C, 3 h, 75% ethanol), and a maximum DPPH scavenging activity of 92.72% (55 °C, 3 h, 95% ethanol). Temperature and ethanol concentration were identified as the most significant factors influencing antioxidant activity.

Although water extraction produced lower overall yields than ethanol extraction, it offers advantages in process simplification and commercial scalability by eliminating solvent removal steps. Given the low cost and abundant availability of pineapple leaves, water extraction remains a viable option for industrial applications. These findings demonstrate that pineapple leaves are a valuable source of functional materials suitable for use in cosmetics, skincare, and cleaning products, supporting sustainable resource utilization and circular bioeconomy development.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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