Section A-Research paper
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Extraction and purification of ferulic acid- A bioactive compound from pineapple peel by alkaline hydrolysis

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Abstract

Ferulic acid also known as hydroxycinnamic acid, is abundant in pineapple peel. It was identified and quantified by using both a UV spectrophotometer and HPLC. The antioxidant capacities of pineapple peel extracts and the total phenolic content were determined using DPPH scavenging capacity and Folin – Ciocalteu reagent Method. The alkaline hydrolysis of pineapple peel was optimized using Response Surface Methodology (RSM) via Central Composite Design (CCD) to enhance ferulic acid extraction. The parameters involved, namely temperature (°C) and duration of extraction (min.). The result obtained was 3.34 mgGAE/g for TPC and 46.34% DPPH. The extraction yield of ferulic acid was 0.471% using HPLC. The optimum conditions obtained from this study for temperature and extraction time are (42˚C, and 200 min), respectively.

Keywords: Ferulic acid, Pineapple peel, Central Composite Design, High-performance liquid chromatography (HPLC)

Introduction

Pineapple (*Ananas cosmosus* L., family Bromeliaceae) is a fruit grown in the tropical and sub-tropical regions. Piňa a, Nanas and Ananas are often referred to as pineapple. It is the third most important tropical fruit produced globally, after bananas and mangos [1, 2]. India is one of the largest producers of pineapple, and the other leading producers are Thailand, the Philippines, Brazil, China, Nigeria, Mexico, Indonesia, Columbia, and the USA. Four major varieties of Pineapple available worldwide are Smooth Cayenne, Red Spanish, Queen, and Abacaxi [3, 4]. Pineapples are cultivated on more than a million hectares of soil, resulting in US$ 9 billion to the global economy annually [1, 2]. According to Roda et al. [5], about 75% of the original fruit is

converted into byproducts during the industrial processing of pineapple, where the peel is the largest proportion generated and it is not usually utilized for commercial purposes [6]. It is consumed as fresh fruit, juice, canned, jelly, and dried products worldwide. Therefore, with increasing pineapple production, pineapple waste increases proportionally. Waste disposal is a growing issue because it is usually prone to microbial spoilage and causes serious environmental problems. It directly represents the enormous challenge of pineapple waste disposal, which will further lead to environmental pollution if not successfully used [7].

Pineapple waste is a rich source of bioactive compounds present in core, leaves, stems, crown and peel. It is rich in many bioactive compounds such as ferulic acid, vitamin A and C as antioxidants, and contain alkaloids, flavonoids, saponins, tannins, cardiac glycoside, steroids, triterpenoids, and phytosterols that may provide a good source of several beneficial properties. Gallic acid, catechin, epicatechin, and ferulic acid were found to be the main polyphenolics in pineapple peels [8]. Ferulic acid has a wide range of medicinal applications, including antioxidant, anti-inflammatory, and antimicrobial characteristics [5, 9, 4]. It is most prevalent in hydroxycinnamic acid. Pineapple peel and crown leaves have a high content of ferulic acid and are commonly available in the local agriculture industry [10]. It is covalently connected by ester linkages to polysaccharides and ether or ester bonds to lignin. There are two approaches for FA planning. Chemical synthesis of vanillin condensation reaction with piperidine-catalyzed malonic acid and extraction from natural resources where FA is one of most abundant phenolic acid. It is widely used in the food and cosmetics industries. Ferulic acid, which may be isolated and utilized as an anticancer and antioxidant, is abundant in pineapple [11]. Ferulic acid has low toxicity and a wide range of biomedical benefits, including antioxidant, anti-inflammatory, antibacterial, anti-allergic, anti-carcinogenic, antithrombotic, antiviral, cardioprotective, and vasodilatory characteristics. It decreases cholesterol and improves the viability of sperm [12, 13, 14, 15]. The aim of this study was to utilize fruit waste byproducts and to obtain natural ferulic acid from pineapple peel which is a by-product. Alkali hydrolysis extraction was used for the obtainment of the ferulic acid. The effects of alkali hydrolysis extraction conditions such as temperature, and extraction time, on the recovery of ferulic acid was investigated.

Material and methods

Materials

The pineapples were purchased from the local market of Phagwara, Punjab. The pineapples were peeled out manually, washed, and dried at 50˚C in a tray dryer. The powder formed was sieved, and kept at room temperature for further study. The chemicals used were of analytical grade.

Alkaline hydrolysis extraction of ferulic acid

Pineapple peel powder was weighed 4 g and saponified with NaOH solution (2 M, 60 ml) at various temperatures and times on a rotary shaker at 180 rpm. Sodium hydrosulfite was added (2 mg) to the mixture to stop oxidation. The alkali extract was centrifuged, precipitate formed was discarded, and the supernatant was used for (TPC and antioxidant determination). Subsequently, to achieve pH < 2, the supernatant was acidified with dilute hydrochloric acid (2 M), and the released phenolic acids were extracted with ethyl acetate for liquid-liquid extraction (60 ml, three times). Using a rotary vacuum evaporator, the organic fractions were concentrated. 2 mL solution of acetonitrile and water (1:1) was used to dissolve the concentrated ferulic acid extract before quantitative analysis [16].

Ferulic Acid Purification

The brownish extract was mixed with 96% ethanol to get a final volume of 30% ferulic acid. The musky solution was then centrifuged for 20 minutes at 6000 rpm. To purify ferulic acid, the supernatant was evaporated in a rotary evaporator. For a less prevalently musky solution, add 6mL of anhydrous ethanol. Re-centrifuge for 20 minutes at 6000 rpm. High-performance liquid chromatography (HPLC) was used to analyze the ethanolic solution [17].

FT-IR spectroscopy

The ferulic acid in pineapple peel was analyzed by FT-IR spectrometer in the range of 400-4000 cm^{-1} using a KBr disc [18].

Quantitative analysis (HPLC)

An HPLC system (Agilent, HP-1200) equipped with a reverse-phase C18 column, a guard column (Eclipse plus, 3.5 m, 4.6 x 100 mm), and a 1260-quat pump UL was used to quantify the quantity of FA. The condition of the column was controlled at 30°C. A mobile phase

combination of 3% acetic acid in water for solvent A and acetic acid and acetonitrile: water was used to elute the FA in a gradient from 100% A to 65% A and 35% B to 55% A and 45% B to 30% A and 70% B in 50 minutes (3:50:47). The UV detector (280 nm) was used to monitor the peaks, and the injection volume was 20 µL. The total time duration was 50 min. Standard dilution with FA concentrations ranging from 1 mg/mL to 6 mg/mL was prepared [19].

Total phenolic content (TPC) estimation

Following the addition of 0.5 mL of Folin-Ciocalteu reagent, 0.1 mL of phenol extract was diluted with 7.9 mL of deionized (DI) water. Na_2CO_3 (20%) solution of 1.5 ml was added after 5 minutes. The final mixture was allowed to develop color for 2 hours and the absorbance at 765 nm was recorded using a Shimadzu UV spectrophotometer (UV-1800). The calibration curve for quantification was designed using gallic acid as a standard. The results were given in terms of mg total phenol content in gallic acid equivalents (GAE)/g sample [20].

Estimation of antioxidant activity

Briefly, 0.1 mL of the hydrolyzate was added to 1.5 mL of 0.1 mM DPPH in methanol. After vortexing, the mixed solution was incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a Shimadzu UV spectrophotometer (UV-1800) [21].

Scavenging activity (%) = [(A_{517} of control- A_{517} of sample)/ A_{517} of control] ×100

Experimental design

Using Design Expert version 8.0.6, Response Surface Methodology (RSM) was applied to the experimental data. Design Expert 8.0.6 software was used to build a set of three-dimensional response surfaces (Fig. 1 a, b, and c) to identify differences in responses with regard to factors. Temperature and time were chosen as the independent factors, while the yield of FA (mg FA/g PP) was chosen as the dependent variable. Table 1 contains the design in coded (x) form, which were coded as -1 +1 0 - + for the five levels of process variables. Total phenolic content and antioxidant activity were evaluated for their effects on extraction in response to various factors.

Table 1: Level of independent variable, code, and optimized value

Independent variable	Units	Symbols	Ranged and level				
			1.68179(-α)	-1	0	+1	1.68179(+α)
Time	Min	A	54	100	200	300	341
Temperature	˚C	B	29	34	46	58	63

Statistical analysis

Analysis of variance was used to determine statistical significance. If $p<0.05$, the results were considered significant. The model's adequacy was further assessed using the coefficient of determination (R^2). The ideal levels of independent variables were identified using both graphical and numerical optimizations.

Results and discussions

In order to assess the variables and any possible interactions between them, an ANOVA was used. The ANOVA for TPC and DPPH, of the pineapple peel extract model is quadratic for its significance value ($P<0.05$), demonstrating that the model's random error was less than 5% and that the lack of fit is not significant ($P>0.05$). The second-order quadratic model and Analysis of Variance (ANOVA) of the regression equation for TPC and DPPH, optimization are shown in Table 3.

The prediction equations of RSM were used to determine the optimum conditions for extracting phenolic compounds from pineapple peel depending on the factors examined. Table 2 displays the actual value and range of responses for total phenol and antioxidant activity. Total phenol levels were reported to vary between 2.90 and 3.62 mg GAE/g, antioxidant activity between 45.7 and 48.6044%. Table 2: The experimental values of the optimization of pineapple peel (PP) extract using a central composite design (CCD)

Experimental run	Source	Coded Variables		Experimental values	
		X_1	X_2	TPC (mg GAE/g)	DPPH (%)
1	PP	200(-1)	46(-1)	3.31	48.05
2	PP	300(1)	34(-1)	3.11	47.98

ل	PP	200(-1)	46(1)	3.38	48.50
4	PP	59(1)	46(1)	3.17	45.97
5	PP	200(1.41)	46(0)	3.31	48.42
6	PP	200(1.41)	46(0)	3.38	48.60
7	PP	100(0)	34(-1.41)	3.06	47.35
8	PP	341(0)	46(1.41)	3.22	47.95
9	PP	200(0)	46(0)	3.31	48.05
10	PP	100(0)	58(0)	3.28	45.78
11	PP	200(0)	63(0)	3.46	45.88
12	PP	300(0)	58(0)	3.62	45.83
13	PP	200(0)	29(0)	2.90	48.25

PP: Pineapple peel; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalent; TPC: total phenolic content. X1: Time (min), X2: temperature (˚C). Values are the average of three replicates.

Total Phenolic Content

The following is the RSM equation derived from the extraction process:

$$X = 1.83 + 0.022A + 0.075B + 0.037AB - 0.029\ A^2 - 0.035\ B^2 \quad \text{(Eq. 1)}$$

The variables X, A, B, and AB in Eq. 1 stand for the FA yield, time, temperature, and process interactions, respectively.

The response surface regression model on TPC showed excellent agreement with R2 = 0.924, Adj. R-Squared = 0.869, and Pred. R-Squared = 0.539 for pineapple peel extract. Figures 1(a) and (b) depict the Surface Response Graph of TPC and DPPH, between Time and Temperature of Ferulic Acid Extract of Pineapple Peel. The x-axis in Figure 1(a) depicts time, while the y-axis depicts temperature. The 3D figure, as illustrated, renders the geometry of the reaction surface produced by the interaction between these components more evident. The total phenol content of pineapple peel extract increased with increasing temperature and duration of extraction; and tended to decrease later on, according to the reaction surface's treatment value of extraction temperature and time. The analysis revealed that the temperature and time thresholds were, respectively, 58°C and 300 minutes. Total phenol was expected to reach 54.3919 mg/g at this point. Unfortunately, the high temperature during the extraction process caused the phenol concentration to decrease at a temperature over 58 °C. According to the study by [22], the greater temperature used in the extraction process causes a higher inactivation of the polyphenol oxidase enzyme, which results in reduced enzyme activity and less phenol damage. The quantity of total phenol measured will reach its highest peak and then remain steady and begin to decline. Nevertheless, the phenol content is further impeded by the rising extraction temperature. Also,

according to Sulaiman et al. [23], certain phenolic compounds are heat sensitive, therefore raising the temperature will result in a reduction in phenol. Yet, the phenolic compound will increase with increasing temperature and extraction time. Long extraction times can also cause phenolic oxidation and breakdown.

Antioxidant Activity Content

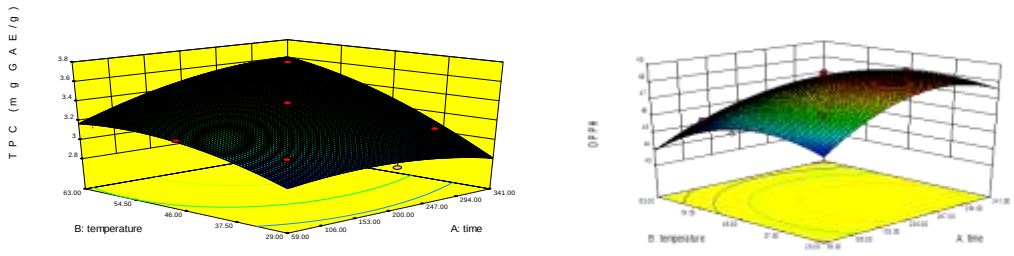
The following is the RSM equation derived from the extraction process:

$$Y = 6.95 + 0.044A - 0.091B - 0.109A^2 - 0.101B^2 \quad \text{(Eq. 2)}$$

In Eq. 2, Y stands for FA yield, A for time, B for temperature, and AB for process interactions.

The time factor is represented by the x-axis in Figure 1(b), and the temperature factor is represented by the y-axis. The 3D figure, as illustrated, makes the geometry of the reaction surface produced by the interaction between these components more obvious. The DPPH concentration of the pineapple extract declined as the temperature and duration of extraction rose, and later tended to increase at a certain point, then reduced again. This can be observed in the reaction surface, Figure 2, in the treatment value of extraction temperature and time. According to the research, the critical temperatures for time and temperature were 46°C and 200 minutes, respectively. The anticipated DPPH at that point was 48.6044%. Nevertheless, because of the high temperature during the extraction process, the DPPH concentration dropped at temperatures over 46 °C. According to Zhao et al. [24], increasing temperature and extraction time both increase and reduces antioxidant activity. This is due to the fact that heat damages the extracted plants' cell tissue, which causes an increase in the number of active components released. But, as the temperature and time increase, the component changes and the amount released decreases. Antiradical activity declines as the temperature rises [25]. It was noted that the radical scavenging activity of DPPH increased over time to a certain amount before plateauing despite additional increases throughout time. It was shown that even with subsequent increases over time, the radical scavenging action of DPPH remained stagnant after initial rising to some extent. The dielectric constant of the seed decreased at high temperatures, but not that of the pulp [26]. This suggests that the majority of the chemicals in the extract were heat-sensitive. Although increased temperature could have sped up the chemical breakdown of bioactive chemicals, it may also have decreased the solvent's dielectric constant, which might

have made it easier to extract less polar molecules [27]. The reports by Burci et al. [28], Shafiq et al. [29], and Zhu et al. [30] as well as the reports by Jagtap et al. [31] all confirm these findings.



(a) (b)

Fig. 1. The surface responses of TPC (a) and DPPH (b), demonstrated the influence of temperature and extraction time on ferulic acid of pineapple peel extract.

Table 3: Optimization of TPC and DPPH using Analysis of Variance (ANOVA) of Regression Equation

Source	**TPC(mg GAE/g)**				**DPPH (%)**			
	dF[a]	SS[b]	F value	p- Value	dF[a]	SS[b]	F value	p- Value
Model	5	0.029	17.02	0.0009	0.0009	**0.07599**	20.098	0.0005
A	1	2.00E-03	5.83	0.0464	0.0464	**0.00808**	10.6782	0.0137
B	1	0.023	65.96	< 0.0001	< 0.0001	**0.03317**	43.8574	0.0003
AB	1	1.38E-03	4.01	0.0852	0.0852	**0.00045**	0.58973	0.4676
A^2	1	1.48E-03	4.31	0.0764	0.0764	**0.02081**	27.5139	0.0012
B^2	1	2.12E-03	6.17	0.0419	0.0419	**0.01794**	23.7191	0.0018
Residual	7	2.40E-03				**0.00529**		
Lack of fit	3	1.95E-03	5.75	0.0621	0.0621	**0.00392**	3.79803	0.115
Pure Error	4	4.51E-04			4	**0.00138**		

Core	1	0.032		12	**0.081**
Total	2				**29**

dF[a]: degree of freedom; SS[b]: sum of square; TPC: total phenolic content; DPPH: antioxidant activity;

Identification of Ferulic acid

The calibration curve was used to quantify the ferulic acid content. The straight-line regression equation was y = 2E+08x so a concentration V/S peak area plot within the concentration range of 1 mg/mL to 6 mg/mL revealed a proportion with a correlation coefficient value of r2 = 0.99. The extraction yield of ferulic acid from PP was estimated by HPLC to be 0.471 mg/g. The HPLC chromatogram of standard ferulic acid and pineapple peel purified extract is shown in Figure 2.

(a) (b)

Figure 2: Ferulic acid HPLC chromatogram in C18 reverse phase chromatography at 280nm. Standard pineapple peel extract and alkali-treated pineapple peel extract (ferulic acid peak time: 17.180 min).

Pineapple peel hydrolyzed in alkaline solution yielded 0.471 mg/g ferulic acid. Some have reported finding up to 0.018 mg/g of pineapple peel [32], which was lower than our results. According to a study by RODRIGUEZ et al. [33], ferulic acid concentration varies on both duration and the concentration of NaOH. According to a study by Hossain et al. [34], methanol (21.50%) had the highest yield, followed by ethyl acetate (4.90%) and water extract (4.30%). Comparing pomegranate skins and seeds to solvents like ethyl acetate and water, Negi et al. [35] obtained the greatest yield of methanol extract.

FT-IR Spectroscopy

In the given graph, ferulic acid showed peaks at 3648.66cm $^{-1}$ is characteristic of OH group in phenolic compound, C-H stretching of aromatic ring on methyl group at 2977.34 cm $^{-1}$, Band at 1740.62 cm $^{-1}$ for C=O, while vibration for C=C on aromatic ring is at 877.01cm^{-1}. Similar results were reported by Aarabi *et a*l. [16]

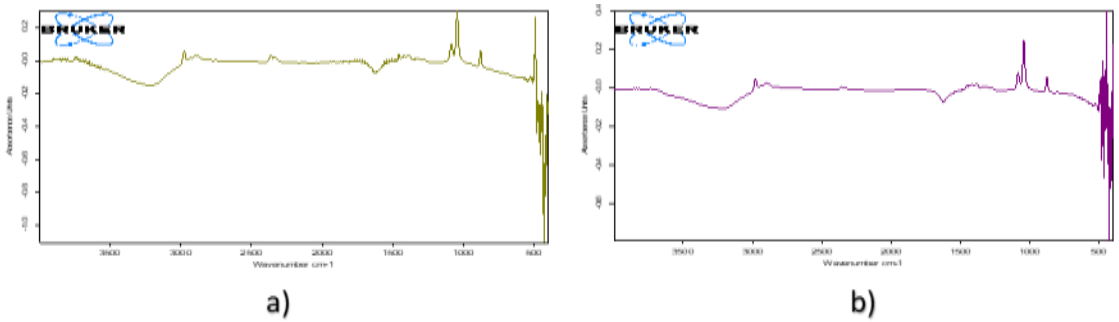


Fig. 3 FT-IR spectra of ferulic acid a) green (standard), and b) purple (PP)

Optimal Conditions validations

The extraction conditions were improved by numerical optimization by determining a point that maximizes the desirability function and assigning a value of 3 to all parameters. TPC, DPPH, and ferulic acid of the pineapple peel have an overall desirability of 0.984, with zero being out of range while one is on target. A maximum level of TPC, antioxidant activity, and ferulic acid were determined in the ferulic acid extract, which was made at the optimum temperature and time of 46 °C and 200 min, respectively.

The optimal parameters proposed by the software were 46°C, 200 min, 3.34 mg GAE/g for TPC and 46.34% DPPH (predicted value). In order to confirm these conditions, four duplicates were performed. The greatest TPC and DPPH production obtained under these circumstances was 3.12 mg GAE/g and 45.94%. On the basis of HPLC estimation, the extraction yield of ferulic acid was 0.471 mg/g. Furthermore, using food-grade NaOH for this study's application in food is a safer choice.

Conclusion

Optimization studies that developed a protocol to achieve the highest yield of total phenol and antioxidant by optimizing the optimum conditions for the extraction of total phenol and

antioxidants from pineapple peel led to a statistically improved alkaline hydrolysis of ferulic acid from pineapple peel waste. The results showed that the temperature and duration of the extraction had a significant effect on the extraction process. The response surface technique was used to determine that 46°C for 200 min was the optimal temperature for pineapple peel to provide the highest production of total phenol content and DPPH. The pineapple peel extract was then subjected to column chromatography under optimal conditions to isolate it. HPLC provided confirmation of the compound's structure. After being purified, pineapple peel had an extraction yield of 0.471 mg/g ferulic acid.

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