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Research Article

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Comparative analysis of Ferulic Acid from Corn Agro-Waste by alkaline hydrolysis

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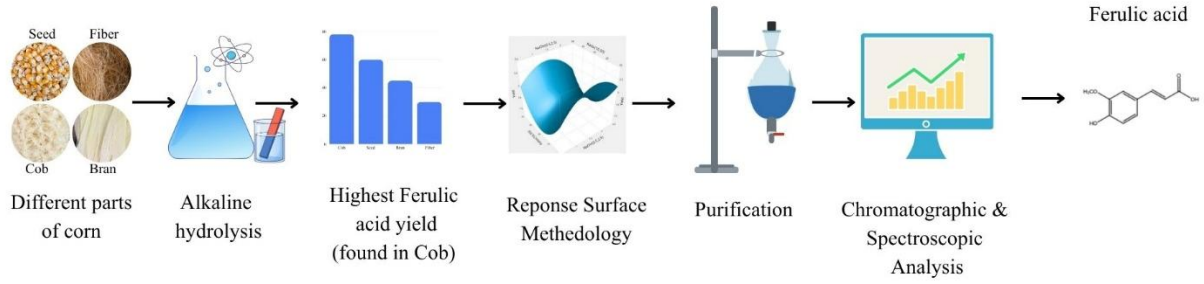
Abstract

Corn agro-waste is an affordable source of ferulic acid (FA), a naturally occurring antioxidant. In this study we compared the release of FA from corn residue using alkaline hydrolysis and optimized its recovery from the most effective method using Response Surface Methodology. Alkaline hydrolysis was used to liberate free FA from corn cob, bran, fiber, and seeds, which were then measured using HPLC. Central Composite Design was used to optimize parameters of corn cob hydrolysis such as temperature, time, sodium hydroxide (NaOH) concentration, and solid-to-solvent ratio. Purified FA was characterized by HPLC, FTIR, NMR, and mass spectrometry. Of all the parts analyzed, the corn cob gave the highest FA release. The quadratic model was significant ($p = 0.0001$, $R^2 = 0.937$), with hydrolysis time the key dominant factor. At the optimum conditions (Time: 33.08 h, Temperature: 30 °C, NaOH Concentration: 1.62 M, Solid-to-solvent ratio: 1:50), the practical yield was about 2%w/w. Purified FA had 94% HPLC purity. The study demonstrates the potential of corn cob as a sustainable source of ferulic acid. RSM-assisted optimization combined with a simple purification approach offers a promising strategy for recovering value-added phenolic compounds from agricultural waste.

Graphical abstract



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1. Introduction

Ferulic acid (FA; Fig. 1) belongs to the hydroxycinnamic acids, a class of phenolic compounds widely found in plant cell walls. It has strong antioxidant, anti-inflammatory, antimicrobial, and UV-shielding activity, which makes it useful across the food, pharmaceutical, cosmetic, and biomaterial industries[1]. FA is also used in food preservation, where it acts as a cross-linking agent, and in sunscreens and skin lotions, where it serves as a photoprotective ingredient [2][3]. Ferulic acid has been identified in many studies to block the PI3K/AKT pathway, ROS generation, and aldose reductase activity. The levels of PPAR γ, CAM, NF-κ B, and p38 MAPK signalling pathways are mostly linked to ferulic acid's anti-inflammatory action. Ferulic acid has anti-fibrosis properties through the TGF-β/Smad and MMPs/TIMPs systems, in addition to protecting vascular endothelium through the ERK1/2 and NO/ET-1 signal[4]. Consumer and industrial preference have recently shifted toward FA of natural origin, particularly that recovered from agro-industrial by-products. FA occurs in many dietary staples, including cereal bran, whole grain products, citrus fruits, bananas, coffee, orange juice, eggplant, bamboo shoots, beetroot, cabbage, spinach, and broccoli [5].

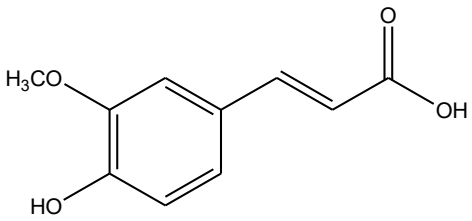

Fig. 1 Structure of Ferulic acid

Corn (Zea mays L.) is among the most commonly grown crops worldwide, and its favourable nutritional profile and functional properties make it a major raw material for both human food and animal feed. Originally domesticated in the Americas, it is now grown worldwide, with the United States, China, and Brazil leading production at annual volumes ranging from approximately 100 to 300 million tonnes [5]. However, only about 5% of the plant biomass is edible kernels, while the remaining 95% is left as residues such as cobs, leaves, husks, and stalks [6].

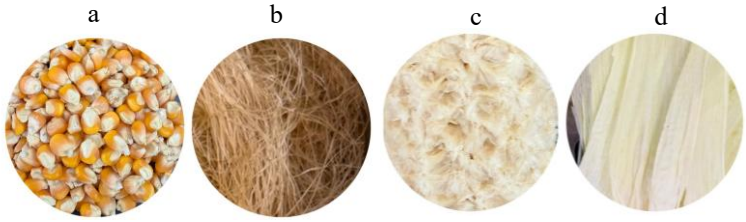

Fig. 2 Different parts of corn: (a) seed, (b) Fiber, (c) Cob, (d) Bran

At present, green extraction techniques, including ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction, are increasingly applied to recover high-value compounds from agro-industrial wastes [7]. The purification of FA from agro-industrial extracts is commonly performed by membrane separation, liquid-liquid extraction, adsorption on activated carbon or polymeric adsorbents, and ion exchange using weakly basic anion-exchange resins bearing amine functional groups [8]. Despite their advantages, these techniques often require specialized equipment and high energy input, which limits their scalability.

Three forms of ferulic acid can be found in plant materials: soluble-free, soluble-conjugated, and insoluble-bound FA as a component of lignocellulosic complexes and arabinoxylan. The majority of this phenolic acid is found in the insoluble-bound form, while the distribution and FA concentration might range across the many plant species[9], [10] . Alkaline hydrolysis, by contrast, remains the most widely used route for liberating FA, because most of it is esterified to arabinoxylans and lignin in the cell wall and must be cleaved before it can be recovered [11]. Under harsher alkaline

conditions, the ester and ether bonds linking phenolic acids to cell wall polysaccharides and lignin are broken, releasing FA from the lignocellulosic matrix[12] .

FA has been reported to be recovered from corn residues by several routes. Enzymatic hydrolysis of sweet corn cob, which holds most of its FA in bound form, has been optimized by response surface methodology[13] . Ultrasound-assisted alkali-alcohol extraction of corn husk has been combined with macroporous resin purification[14].FA enzymatic recovery with extracellular enzymes[15]. Corn cob has also been treated by autohydrolysis followed by a dual-enzyme system. Alkaline hydrolysis of corn cob has been studied for the effects of NaOH concentration, solid-to-liquid ratio, and time [13], [14], but, to our knowledge, the combined effects of hydrolysis time, temperature, NaOH concentration, and solid-to-solvent ratio on FA yield have not been optimized together, and comparisons across different corn residues under identical conditions remain scarce.

Purification of FA after hydrolysis has mostly relied on resins or other adsorbents [18], [19], so a simple route that avoids them is still needed. In the present study, FA was obtained by alkaline hydrolysis from several corn-based agricultural wastes, namely corn cob, bran, fiber and seed (Fig. 2). Corn cob showed the highest conversion efficiency in preliminary screening and was therefore selected for further work. The alkaline hydrolysis was then optimized by response surface methodology (RSM) to maximize FA yield. The crude FA obtained under the optimized conditions was purified by acidification and ethyl acetate extraction.

A central composite design (CCD)is an experimental design used in RSM to investigate the relationship between independent variables and response[20], [21] . CCD was used to optimize alkaline hydrolysis of corn cob with the aim of maximizing the hydrolysis yield. Four independent variables were investigated: hydrolysis time (hr), temperature (°C), NaOH concentration (M), and solid-to-solvent ratio. Each variable was studied at three levels (low, medium, and high) to evaluate its individual and interactive effects on the response.

2. Materials and method

2.1 Materials and chemicals

Corn cob, fiber, bran and seeds were purchased from the nearby market. FA standard was bought from YUCCA Enterprises, Mumbai. Ethyl acetate, Sodium hydroxide, hydrochloric acid, Acetonitrile (AR grade), Acetic acid (AR grade), and methanol were purchased from Sigma-Aldrich.

2.2 Alkaline hydrolysis of different parts of corn

Corn cob, bran, fiber, and seed samples (1 g each) were treated with aqueous sodium hydroxide (NaOH) solution at varying concentrations and kept at room temperature for 48 hrs for alkaline hydrolysis. Following the reaction's completion, the pH of the mixture was neutralized using concentrated hydrochloric acid (HCl), which also facilitated the removal of impurities. An aliquot of the obtained sample was then mixed with methanol in a 1:10 ratio and filtered prior to HPLC analysis. The prepared solution was subsequently used for chromatographic evaluation[22], [23].

2.3 Quantitative analysis of FA by HPLC

For the quantification of FA in different parts of corn, a one-factorial design was used with a JASCO high-performance liquid chromatography system fitted with a reverse-phase C18 column (4.6mmID × 250mmL). FA was eluted isocratically using a mobile phase composition of acetonitrile: water (0.5% acetic acid) at a flow rate of 1 ml/min[24]. The wavelength was tuned to 320 nm, and the injection volume was 20 uL.

2.4 Optimization of FA by statistical method

NaOH concentration (M), hydrolysis time, hydrolysis temperature and solid-to-solvent ratio were the design factors selected for the statistical optimization. As a reaction, the impact of these factors on hydrolysis was evaluated for FA yield. The optimum conditions were identified from the fitted model using JMP software (Edition 19).

2.5 Purification of FA

After alkaline hydrolysis under the optimized conditions, the reaction mixture was passed through a 60-mesh sieve to remove residual solid material. The alkaline hydrolysate was acidified with concentrated hydrochloric acid to pH 1-3 to precipitate FA. Ethyl acetate was then added, and the mixture was stirred gently for 30-45 min to allow FA to partition into the organic phase. The ethyl acetate-rich supernatant was decanted into a separate container, and the aqueous phase was re-extracted with fresh ethyl acetate. The combined ethyl acetate extracts were concentrated under reduced pressure to get crude FA. For further purification, the crude FA was dissolved in methanol and treated with a pinch of activated charcoal at 50° for 10 mins with stirring. The charcoal was removed by filtration, and the filtrate was concentrated and dried to obtain purified FA.

2.6 Spectroscopic analysis

FTIR spectra were recorded on JASCO spectrometer over the range 4000-550 cm^{-1}. Band positions are reported in $cm^{-1.}$ 1H NMR spectra were recorded on a Bruker Avance III spectrometer. About 5mg of sample was dissolved in 0.8 mL of DMSO-d_6. Chemical shift (δ) is given in ppm. Spectra were processed using MestReNova software. The sample was dissolved in methanol for Mass spectrometry to determine molecular mass.

3. Result and Discussion

3.1 Optimization by Statistical Method

RSM was used for optimization of FA extraction efficiency using hydrolysis. Because mild treatment typically results in partial solubilization and severe conditions may produce damaged products, alkali concentration and hydrolysis duration are significant determinants of phenolic compound release in alkaline hydrolysis. Four factors were chosen for this study: NaOH (M), hydrolysis time, hydrolysis temperature and solid-to-liquid ratio. The ranges and the levels of the variables investigated in this study are given in Table 1

Independent variable	Symbol	Coded level -1	Coded level 0	Coded level +1
Time (h)	A	0	24	48
Temperature (°C)	B	30	45	60
NaOH (M)	C	0.5	1.5	2.5
Solid-to-liquid ratio (gm/L)	D	10	30	50

Table 1. Independent variables and their coded and actual levels used in the response RSM for optimization of FA

Table 2 shows the experimental conditions for the RSM. To prevent systematic inaccuracy, the experiments were conducted in a random order. A second-order polynomial equation was fitted to the RSM results by using multiple regression analysis on the experimental data. Therefore, the following is a mathematical regression model for total FA fitted in the coded factors:

Where X_1, X_2, X_3, and X_4 are the coded terms for Time, Temperature, NaOH concentration, and Ratio (solid-to-solvent), respectively, calculated as:

$$X_1 = \frac{\text{Time} - 24}{24}, \quad X_2 = \frac{\text{Temperature} - 45}{15}, \quad X_3 = \frac{\text{NaOH} - 1.5}{1.0}, \quad X_4 = \frac{\text{Ratio} - 30}{20}$$

Time	Temperature	NaOH	Ratio	Yield	Predicted Yield
24	45	1.5	50	2.1	2.23
48	60	0.5	10	1.1	1.26
48	30	0.5	10	1.5	1.48
24	60	1.5	30	1.7	1.55
48	60	2.5	50	0.4	0.88
24	45	0.5	30	1.4	1.52
0	30	0.5	50	0.3	0.25
48	60	2.5	10	1.5	1.25
0	60	0.5	10	0.3	0.34
48	60	0.5	50	1.4	1.09
24	45	1.5	30	2	1.83
24	45	1.5	30	2	1.83
0	30	2.5	10	0.3	0.31
24	45	2.5	30	1.6	1.58
24	30	1.5	30	1.6	1.85
0	60	0.5	50	0.1	0.23
0	60	2.5	10	0.2	0.34
0	30	2.5	50	0.3	0.39
0	60	2.5	50	0.3	0.02
24	45	1.5	10	2.3	2.27
48	30	2.5	10	1.7	1.83
0	45	1.5	30	0.1	0.21
48	30	0.5	50	1.6	1.71
48	45	1.5	30	1.4	1.40
48	30	2.5	50	2.2	1.86
0	30	0.5	10	0.2	-0.02

Table 2. Experimental conditions for the RSM

The adequacy of the quadratic model was evaluated by analysis of variance (Table 3). The model was highly significant ($F = 11.74$, $p = 0.0001$), indicating that the second-order polynomial adequately described the relationship between the variables and FA yield. The lack of fit was not significant ($F = 18.01$, $p = 0.18$), although this test was based on a single degree of freedom for pure error. The coefficient of determination ($R^2 = 0.937$) and adjusted R^2 (0.857) indicated that most of the variation in yield was explained by the model. Among the model terms, time ($p < 0.0001$), the quadratic term of time ($p = 0.0002$) and the quadratic term of solid-to-solvent ratio ($p = 0.03$) were significant, whereas temperature was borderline ($p = 0.05$) and NaOH concentration had no significant linear effect ($p = 0.64$). The negative quadratic term for time shows that yield increased up to an intermediate hydrolysis time and then declined, consistent with degradation of released FA under prolonged alkaline conditions.

Source	Sum of squares	DF	Mean Square	F	P
Model	13.533	14	0.966	11.74	0.0001
Error	0.906	11	0.082		
Lack of fit	0.900	10	0.0900	18.01	0.181
Pure error	0.005	1	0.005		
Total	14.439	25			

Table 3. Analysis of variance (ANOVA) for the quadratic model of FA yield from corn cob

3.2 Effect of process variables on FA yield

The interactive effects of the process variables on FA yield are shown in the three-dimensional response surface plots (Fig. 2). In each plot, two variables were varied within the experimental range while the other two were held at their centre values (Time: 24 h, Temperature: 45 °C, NaOH concentration: 1.5 M, 1:30 solid-to-solvent ratio).

Time and temperature (Fig. 2a): The surface showed pronounced curvature along the time axis. FA yield increased with hydrolysis time up to an intermediate level (about 24–30 h) and then declined at longer times, in line with the highly significant linear and quadratic time terms ($p < 0.0001$ and $p = 0.0002$). Temperature had a smaller, slightly negative effect ($p = 0.051$). The negative time × temperature interaction ($p = 0.064$) indicates that this decline was more marked at higher temperature, which suggests that FA released during prolonged hydrolysis is degraded under combined thermal and alkaline stress.

Time and NaOH concentration (Fig. 2b): The surface was dome-shaped and again dominated by time. The NaOH concentration had no significant linear effect ($p = 0.64$) and no time × NaOH interaction ($p = 1.00$). Yield was highest at intermediate NaOH concentration and reduced slightly toward both extremes, but the quadratic NaOH concentration term was not significant ($p = 0.16$). This shows that, within the range studied, the amount of alkali was less critical than the hydrolysis time.

Time and solid-to-solvent ratio (Fig. 2c): The surface was saddle-shaped. Yield rose and then fell beyond the 40 min time point at every ratio, while the ratio of solid-to-solvent had a smaller influence due to instability of FA. The quadratic ratio term was significant ($p = 0.039$), but the linear ratio term was not ($p = 0.75$), and there was no meaningful time × ratio interaction ($p = 0.87$). The effect of the solid-to-solvent ratio was therefore small compared with that of time.

Temperature, NaOH and ratio (Fig. 2d–f): At the centre time of 24 h, the surfaces were comparatively flat, and the interactions between temperature, NaOH concentration and ratio were not significant ($p = 0.20–0.51$). Higher yields were predicted at lower temperature (30–45 °C) and at intermediate NaOH concentration (Fig. 2d). The highest predicted yields, about 2.4%, occurred at low temperature combined with a low solid-to-liquid ratio (Fig. 2e). The saddle shapes in Fig. 2e and 2f reflect the positive quadratic ratio term, and the small range of predicted yield across the ratio axis indicates that this factor had only a minor effect.

3.3 Optimization and validation

The following optimal conditions were found through numerical optimization of the fitted model: hydrolysis duration of 33.08 hours, temperature of 30 °C, NaOH concentration of 1.62 M, and solid-to-solvent ratio of 1:50. The yield was 2% w/w, was obtained from a confirmation experiment conducted under identical conditions. As a result, the model identified the hydrolysis-friendly area.

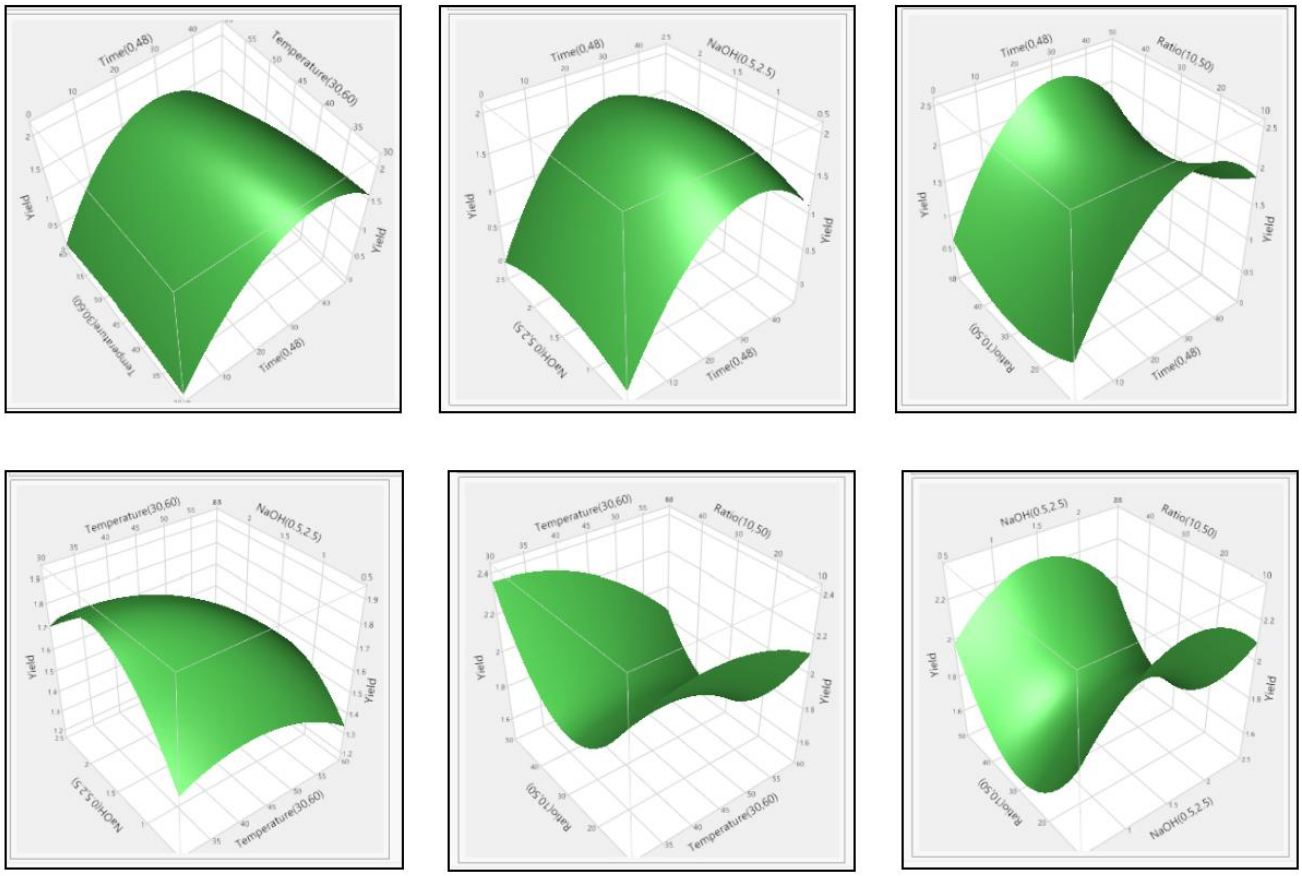

Fig. 3 Three-dimensional response surface plots illustrating the interactive effects of process variables on FA yield: (a) time and temperature, (b) time and NaOH concentration, (c) time and solid-to-solvent ratio, (d) temperature and NaOH concentration, (e) temperature and solid-to-solvent ratio, and (f) NaOH concentration and solid-to-solvent ratio

3.4 Purification of FA

After optimization of the method for extraction of FA, the studies were carried out to increase the purity of FA from corn cob. FA was purified by acid-base treatment. Following completion of the alkaline hydrolysis, the hydrolysate was passed through a 60-mesh sieve to remove the residual solid material. The resulting alkaline hydrolysate was then acidified with concentrated hydrochloric acid, and the pH was adjusted to 1–3 to precipitate FA. After acidification, ethyl acetate was carefully added to the hydrolysate. The mixture was gently stirred for 30–45 min, allowing the FA to partition into the ethyl acetate phase. The ethyl acetate-rich supernatant was carefully decanted and collected in a separate container. The extraction was repeated by adding further ethyl acetate to the aqueous phase. The recovered ethyl acetate extract was collected and concentrated to enrich the FA and facilitate recovery of the solvent. A yellow-coloured powder was subsequently obtained, which was collected as the purified FA fraction.

3.5 Chromatographic and Spectroscopic Analysis of Purified FA

3.5.1 HPLC

The isolated compound was characterized by HPLC. The chromatogram of the 10 µg/mL standard showed a prominent peak at a retention time of approximately 5.8–5.9 min. The sharp and well-resolved peak indicated good chromatographic separation of the analyte. The purity of isolated FA was found to be 94%.

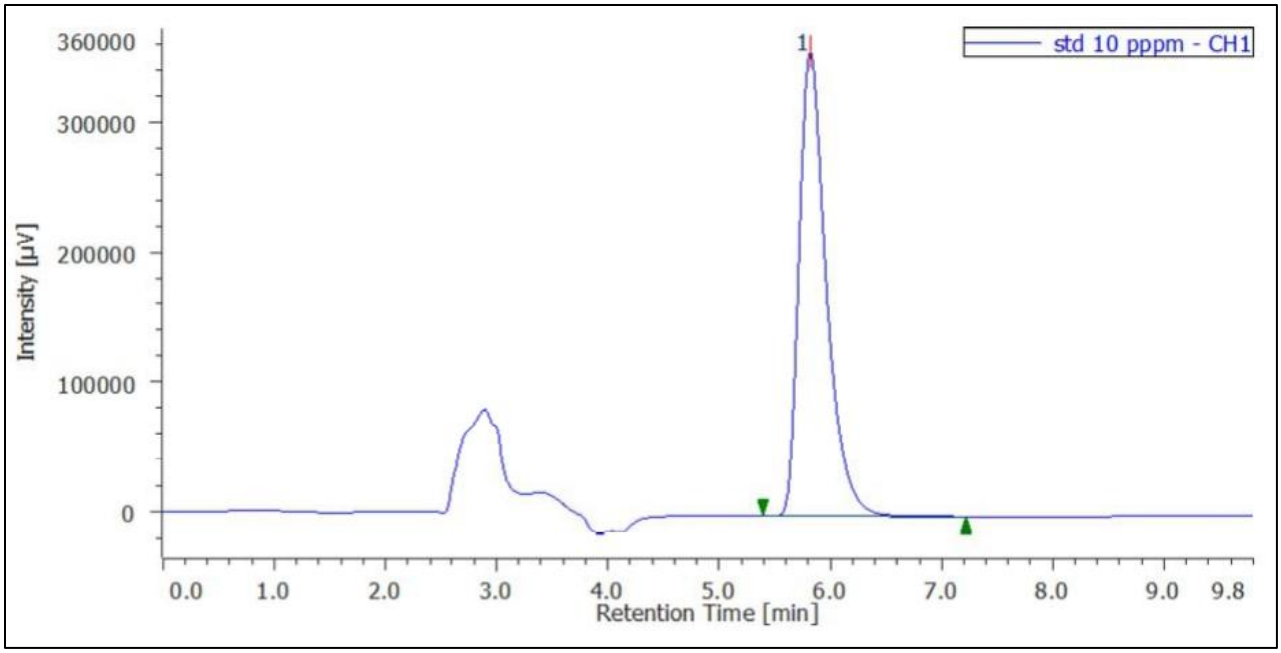

Fig. 4 HPLC chromatogram of isolated FA

3.5.2 IR spectroscopy

The IR spectrum with the peaks at 3430.92 cm^{-1} (O-H Stretching), 1688 cm^{-1} conjugated carboxylic acid C=O stretching, 1616-1511 cm^{-1} aromatic C=C and conjugated alkene, 2894 (C-H Stretching, methoxy group) confirms the basic functional group of FA. The IR spectrum of isolated FA presented in fig 5. As a result, the isolated compound's IR spectrum corresponds with the FA structure.

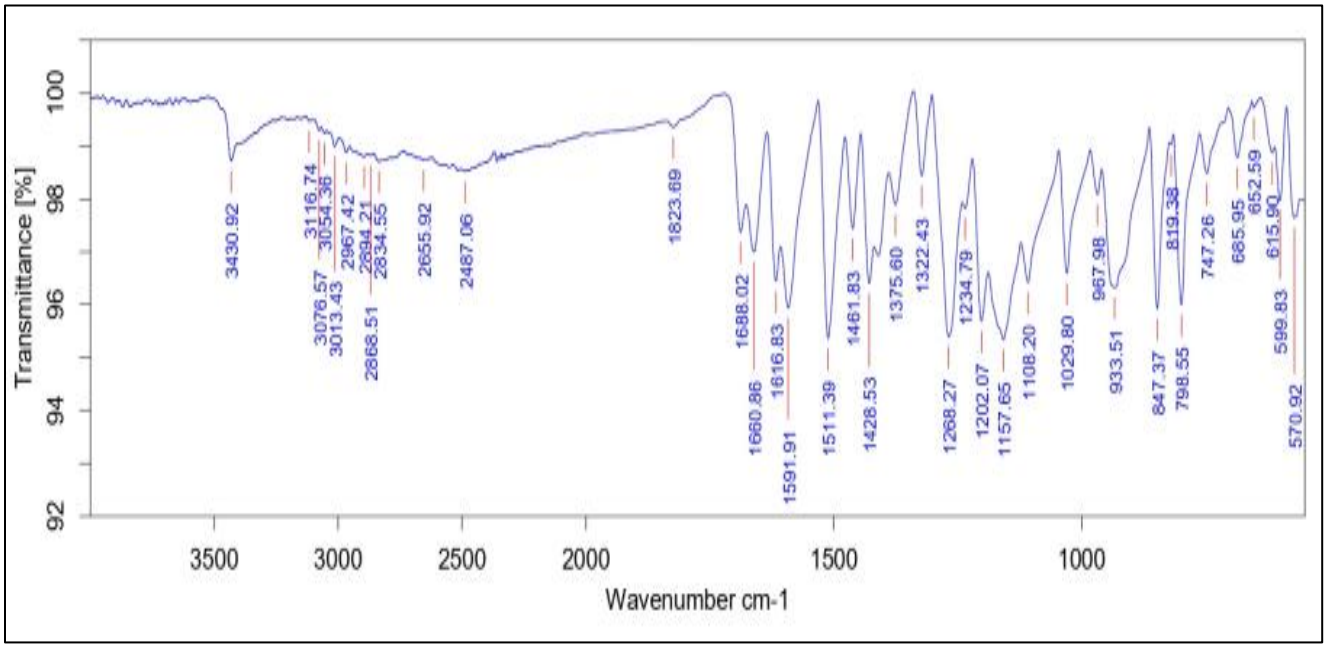

Fig. 5 IR spectrum of isolated FA

3.5.3. NMR spectroscopy

The 1H NMR spectrum (fig 3) confirmed the identity of isolated compound as FA. A three-proton singlet at δ 3.81 corresponds to the methoxy group, while the exchangeable singlets at δ 9.59 and 12.15 were assigned to the phenolic hydroxyl and carboxylic acid protons, respectively[25].

^{1}H NMR (500 MHz, dmso) δ 12.15 (s, 1H), 9.59 (s, 1H), 7.49 (d, *J* = 15.9 Hz, 1H), 7.28 (d, *J* = 2.1 Hz,

1H), 7.08 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.37 (d, *J* = 15.8 Hz, 1H), 3.81 (s, 3H)

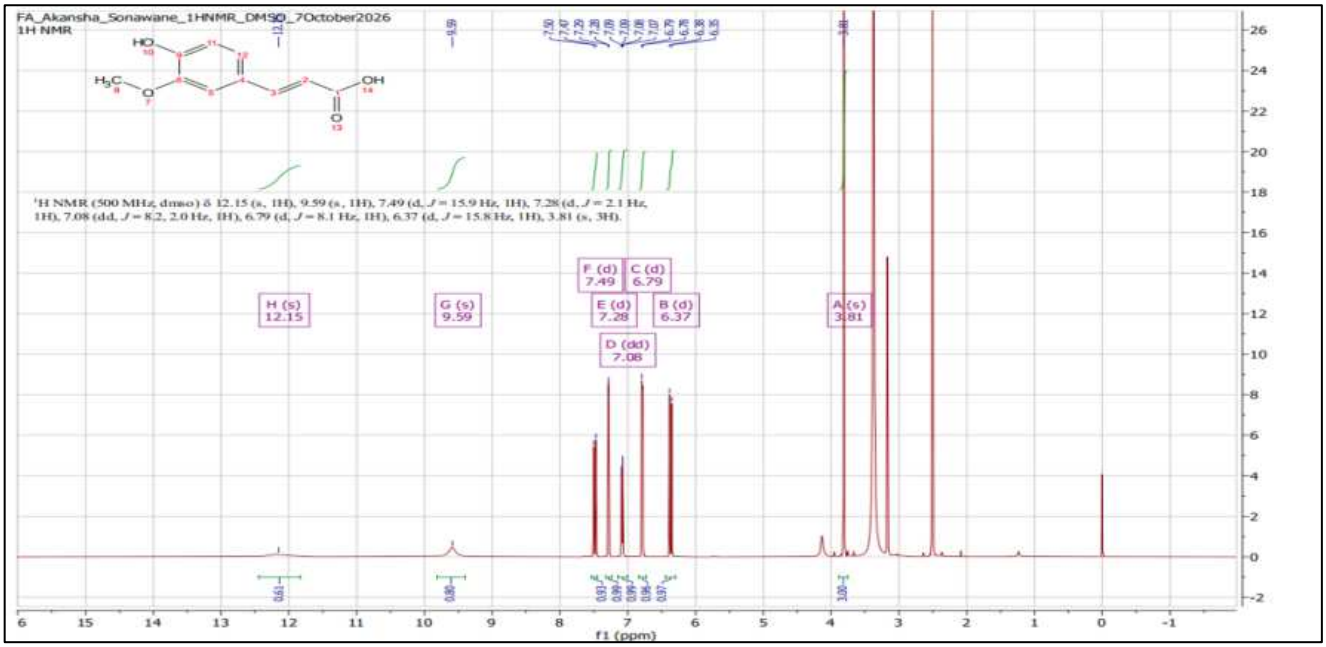

Fig. 6 ^{1}H NMR spectrum of FA

3.5.4. Mass spectroscopy

The purified compound was further characterized by mass spectrometry. The mass spectrum exhibited a prominent base peak at m/z 193, which corresponds to the deprotonated molecular ion $[M-H]^-$ of FA. The molecular weight of FA is m/z 194 (M+, base peak) with the molecular formula $C_{10}H_{10}O_4$.

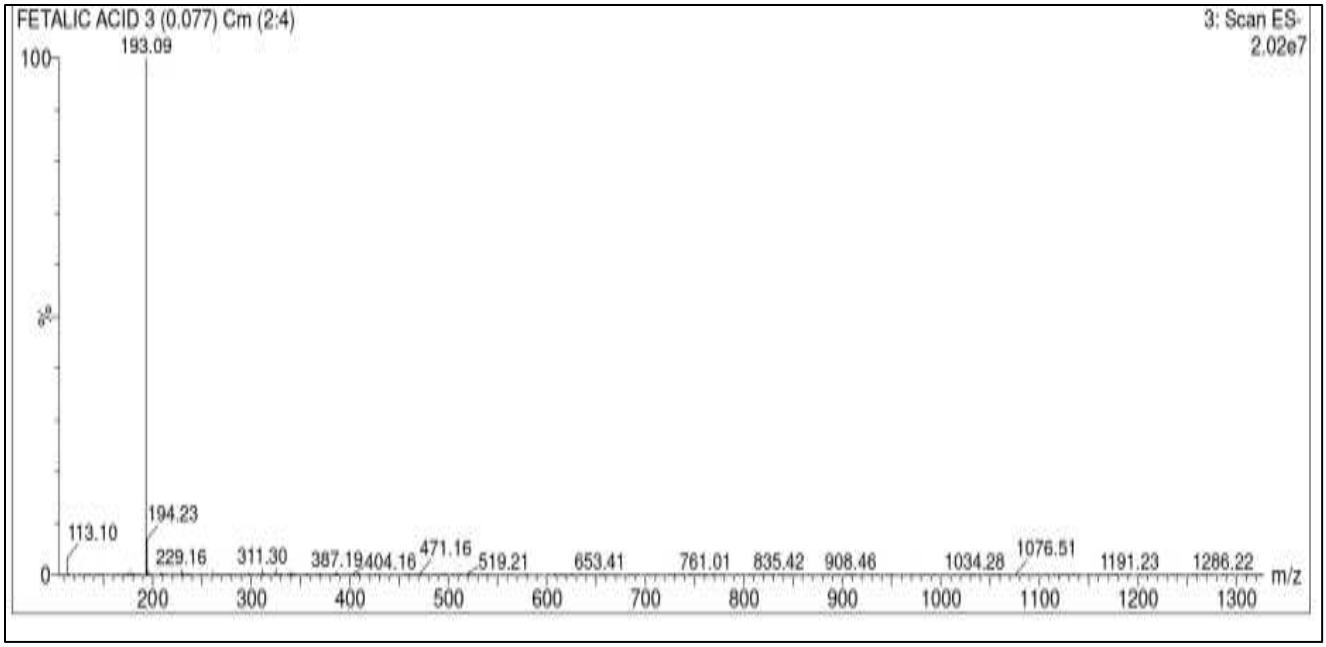

Fig. 7 Mass spectrum of isolated FA

1. Conclusion

FA was recovered from corn agro-waste by alkaline hydrolysis. Corn cob was the most productive of the residues tested (cob, bran, fibre and seed) and was selected for optimization of FA yield. A central composite design with response surface methodology gave a significant quadratic model ($F = 11.74$, $p = 0.0001$; $R^2 = 0.937$, adjusted $R^2 = 0.857$). Hydrolysis time was the dominant variable. Yield increased up to an intermediate time and then declined, consistent with degradation of released FA under prolonged alkaline conditions. Temperature and solid-to-liquid ratio had smaller effects, and NaOH concentration had no significant linear effect over 0.5–2.5 M. The optimum conditions were 33.08 hrs, 30 °C, 1.62 M NaOH and a solid-to-solvent ratio of 1:50. The model predicted a yield of 2.49% under these conditions, and the practical yield was about 2%. FA was purified by acidification, ethyl acetate extraction and activated charcoal treatment, giving a product of 94% purity by HPLC. Its identity was confirmed by HPLC retention time, FTIR (O–H at 3431 cm^{-1}, conjugated C=O at 1688 cm^{-1}, C=C at 1616–1511 cm^{-1}), ^1H NMR and mass spectrometry ($[M-H]^-$ at **m/z** 193). This developed approach has potential as an economically useful method for converting corn residue into value-added phenolic compounds and contributing to sustainable agro-waste valorisation. The findings highlight the potential of corn agro-waste as a renewable feedstock for FA recovery and support the development of resource-efficient strategies for agricultural waste utilisation.

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Author contribution

All the authors have contributed to the study. Akanksha Sonawane - investigation, sample collection, formal analysis, methodology, data interpretation, and original manuscript writing. Megha Palkar, Varad Sankpal- formal analysis and data interpretation. Galvina Pereira: study conceptualization, manuscript review and editing.

Declarations

Competing interest the authors have no competing interests to declare.

Data availability

All data have been included in the article, figures, tables, and supplementary materials.

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