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Microwave-assisted deep eutectic solvent pretreatment of macadamia nut shells and pinewood shavings for effective saccharification and bioethanol production

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ABSTRACT

Due to their unique heating properties, deep eutectic solvents in combination with microwave irradiation in pretreatment are now widely researched for achieving effective biomass fractionation and high sugar yields. In this study, optimisation of the pretreatment of macadamia nut shells (MNSs) and pinewood shavings (PWSs) using choline chloride-lactic acid DES in a microwave was attempted via the response surface methodology approach, to enhance fermentable sugar yield for bioethanol production. A central composite design was employed to optimise microwave power, biomass loading, and pretreatment time. The analysis of variance revealed that microwave (MW) power had the most significant influence on the examined responses (solid yield, cellulose recovery, and delignification). High cellulose recovery (>70 %) was achieved in both biomass although with higher delignification (73.68 ± 1.39 %) in softwood PWSs at the optimised conditions (81.24 % MW power, 8.94 % biomass loading, and 181.43 s) than in hardwood MNSs with lower delignification (39.01 ± 1.15 %) (at 82.55 % MW power, 6.88 % biomass loading and 251.35 s pretreatment time). Structural characterisation revealed a reduction in peaks linked to hemicellulose and lignin, severe pore formation in pretreated PWSs, and a higher crystallinity index (78.41 %) compared to pretreated MNSs (57.99 %). Pretreated PWSs also showed higher cellulose digestibility (60.33 %) and glucose yield (67.03 %) than MNSs (51.01 and 56.67 %, respectively) on hydrolysis with cellulase at 24 mg/g biomass. PWSs hydrolysate supplemented with glucose yielded 4.21 g/L ethanol (44.01 % theoretical yield) using *Saccharomyces cerevisiae* during fermentation. Overall, the MW-assisted CL pretreatment strategy significantly enhanced sugar yields, which could be beneficial for bioethanol production.

1. Introduction

Abundantly available agricultural wastes (AWs), with a huge annual production rate of approximately 5 billion metric tons (Sharma et al., 2023a), stand out as promising second-generation (2 G) feedstocks for renewable energy generation (Dey et al., 2022). As a result, 2 G bioethanol is now the focus of the biorefinery industry in mitigating the over-dependence on non-renewable fossil fuel reserves (Sathish Kumar et al., 2024). However, conventional pretreatment strategies involving the use of solvents and chemicals employed to overcome the recalcitrance of AWs due to complex cell wall linkages have posed challenges due to their high cost and toxicity to human health and the environment (Roslan et al., 2024). In recent years, the green pretreatment processing of feedstock using deep eutectic solvents (DESs) has gained popularity in biorefinery processes owing to their ease of preparation, tunability, biodegradability, and recyclability (Okonkwo et al., 2023). In the production of natural deep eutectic solvents (NADESs), choline chloride (ChCl) is a widely studied hydrogen bond acceptor (HBA) alongside organic acids (lactic, formic, acetic, citric, malonic, etc.), bases (ethylene glycol, imidazole, urea) and polyols (glycerol) as hydrogen bond donors (HBDs) (Hilali et al., 2024). Furthermore, ChCl-based DESs have great potential for delignification of lignocellulosic biomass (LCBs) (Huang et al., 2020), with acidic ChCl-DESs being more effective for lignin and xylan removal, thereby improving the efficiency of enzymatic hydrolysis

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(Ceaser et al., 2023).

A major limiting factor in DES pretreatment, though, is the long pretreatment time, which is uneconomical for industrial applications (Wang et al., 2024b). For instance, the pretreatment of *Eucalyptus* with ChCl:lactic acid (Shen et al., 2019) and poplar wood with ChCl:oxalic acid dihydrate (Liu et al., 2017) at 110°C showed significant biomass fractionation at 6 h and 9 h, respectively. This can be overcome by employing DES pretreatment in combination with MW heating, which reportedly reduces the pretreatment time from hours to a few minutes or even seconds (Wang and Lee, 2021). For instance, pretreatment of poplar wood using a combination of MW irradiation and DES pretreatment was achieved within 3 min, compared to 9 h with DES alone, despite attaining a similar delignification of 80 % (Liu et al., 2017). More importantly, DES's capacity to absorb microwave radiation makes MW-assisted DES pretreatment more efficient (Wang et al., 2024b) than NaOH or H_2SO_4 solution (Zhu et al., 2016). The mechanism of MW irradiation involves rapid heat transfer, which reduces pretreatment time and energy consumption compared with conventional autoclave heating (Aguilar-Reynosa et al., 2017). In addition, the use of MW technique ensures uniform and rapid distribution of heat within the system which ensures the effectiveness of the process, unlike in conventional heating, where slow heating and non-selective heating of the interior of the material via conduction and convection could lead to uneven distribution of heat in a time-consuming and energy-intensive process (Wang et al., 2025a). Evidently, the use of MW irradiation as a heating technique is cost-effective in terms of reduction in time and energy input compared with conventional heating, in addition to its effectiveness in biomass fractionation (Wang et al., 2024b). More so, microwave-assisted biomass pretreatment minimises the production of sugar degradative products such as 5-hydromethymethyl furfural and furfural from xylose (Anoopkumar et al., 2023).

Since the pretreatment process accounts for approximately 40 % of the cost of bioethanol production, with energy and chemicals as major contributors (Ceaser et al., 2024a), any technique that reduces costs and ensures effective pretreatment is crucial. Consequently, studies are now focused on optimising pretreatment procedures (Bibi et al., 2023) using statistical tools to enhance production processes (Manmai et al., 2020). In this regard, Response surface methodology (RSM) is advantageous over the time-consuming and predictable one-factor-at-a-time (OFAT) approach. Furthermore, RSM enables replication with fewer experimental runs, short runtime, and allows for a more systematic assessment of variables (Sharma and Sharma, 2024). Multiple studies have demonstrated the crucial role of response surface methodological optimisation of pretreatment processes in enhancing bioethanol production, as it improves the availability of fermentable sugars (Jose et al., 2024).

The influence of MW-assisted pretreatment of hardwood, softwood, and non-woody biomass has been studied to understand the effects of biomass type using conventional solvents (Mikulski and Kłosowski, 2023). While many studies have shown that sugar yields could be improved in DES and combined DES-MW pretreatment processes, no report exists on comparative MW-DES pretreatment of hardwood (macadamia nut shells) and softwood (pinewood shavings) for enhanced sugar yield. Therefore, the present study is focused on optimising MW-DES pretreatment by investigating the effects of microwave power (MW power), biomass loading (BL), and pretreatment time (PT) on enhanced sugar release and subsequent bioethanol production using *Saccharomyces cerevisiae* in a separate hydrolysis and fermentation (SHF) setup. The pretreated biomass was further evaluated for structural changes using scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) spectroscopy, and X-ray diffraction (XRD). The findings of this study will provide information on possible pretreatment conditions based on biomass type, as well as demonstrate the potential of newer agricultural wastes such as macadamia nut shells in bioethanol production while minimising environmental pollution arising from their improper disposal.

2. Materials and methods

2.1. Materials

Macadamia nut shells (MNSs) were supplied by Wedgewood Farm Makery, Howick, South Africa, while pinewood shavings (PWSs) were collected from a furniture manufacturer in Kwadukuza, KwaZulu-Natal province, South Africa. The MNSs were washed with tap water to remove dirt and nut residue and dried in an oven at 80°C for 48 h (Jung et al., 2020), while PWSs were cleaned and dried in an oven at 60°C for 24 h (Wróbel et al., 2020). Dried biomass was milled with a hammer mill (Retsch GmbH 5657 HAAN, West Germany), sieved to a particle size of < 1 mm using a laboratory test sieve (KINGTEST, South Africa) and kept in air-tight containers for further analyses. Formic acid (98 %), acetic acid, choline chloride (ChCl) (≥95 %), lactic acid (≥85 %), levulinic acid (LA, 98 %), and sulphuric acid (≥98 %) were purchased from Sigma Aldrich (USA). Commercial cellulase (500,000 U/g) was provided by Professor Wang, Tianjin University of Science and Technology, China.

2.2. DES preparation

Choline chloride-lactic acid (CL) with a molar ratio of 1:3 was formulated using a heating-and-stirring method according to the previously developed protocol in our laboratory (Abel et al., 2024).

2.3. Experimental design and optimisation of biomass pretreatment

Firstly, MNSs (hardwood) and PWSs (softwood) were subjected to pretreatment with DES CL 1:3, selected based on preliminary experiments showing its effectiveness in lignin removal (S1a–d, Supplementary material) and cellulose recovery (S2a–d, Supplementary material). Thereafter, the central composite design (CCD) was employed to study the effects of the independent variables on the response and their mutual interactions, to optimise the pretreatment of both biomasses. Independent variables, such as microwave (MW) power (A), biomass loading (B), and pretreatment time (C), were studied at five levels: -α, −1, 0, + 1, and + α (Table 1). A total of 20 experimental runs in both MNSs and PWSs were designed using a central composite design of response surface methodology (RSM) with the Design Expert 11.0 software (Stat-Ease, Inc., USA, trial version). Solid yield, cellulose recovery and delignification were selected as the responses (Table 2). The significance of the model was determined based on the F-test and the p-value ($<$ 0.05), while the fitness of the model was evaluated based on the coefficient of determination (R^2), the adjusted coefficient of determination (adjusted R^2), and the lack of fit test (Ceaser et al., 2024b).

2.4. Compositional analysis

The concentrations of cellulose, hemicellulose, and total lignin were determined according to the NREL standard procedure (Sluiter, 2008). Solid yield of MNSs and PWSs was estimated gravimetrically in relation to the initial dry weight prior to pretreatment, while changes in biopolymer compositions for cellulose, hemicellulose, lignin, cellulose recovery, and delignification were calculated according to Ji et al. (2020) (S3 – S5, Supplementary material).

2.5. Structural and morphological characterisation of biomass

2.5.1. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to analyse the surface morphology of biomass before and after pretreatment. The samples were mounted on aluminium stubs with double-sided carbon adhesive tape and coated with gold sputtering (Quorum Q150R ES Sputter coater, UK). Samples were imaged on the Zeiss Ultra Plus FEG SEM (Germany). The images were acquired at an acceleration voltage of 5 kV with magnifications of x500 and x1000 (Isci et al., 2020).

Table 1
Experimental levels of variables in response surface methodology.

Variable	Unit	Coded symbol	-α	Low (-1)	Zero (0)	High (+1)	+ α
Microwave power	%	A	50.00	60.13	75.00	89.87	100.00
Biomass loading	%	B	5.00	6.01	7.50	8.99	10.00
Pretreatment time	s	C	60.00	108.65	180.00	251.35	300.00

Table 2
Central composite experimental design in MW-assisted DES pretreatment of macadamia nut shells and pinewood shavings.

				MNSs			PWSs		
	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 1	Response 2	Response 3
Run	A: Microwave power (%)	B: Biomass loading (%)	C: Pretreatment time (s)	Solid yield (%)	Cellulose recovery (%)	Delignification (%)	Solid yield (%)	Cellulose recovery (%)	Delignification (%)
18	60.14	6.01	108.65	70.79	83.86	18.84	57.07	81.78	52.44
20	89.86	6.01	108.65	65.22	80.63	26.43	45.94	59.44	57.47
7	60.14	8.99	108.65	76.50	85.32	19.99	56.21	79.45	52.90
8	89.86	8.99	108.65	64.51	74.31	26.75	42.99	60.63	60.61
17	60.14	6.01	251.35	68.08	66.86	23.80	48.46	71.65	58.90
10	89.86	6.01	251.35	76.27	94.96	6.62	36.79	49.23	77.08
1	60.14	8.99	251.35	72.67	66.77	14.80	46.7	69.58	53.25
16	89.86	8.99	251.35	49.20	75.48	39.01	40.07	60.19	68.16
2	50.00	7.50	180.00	73.95	69.13	18.86	64.13	82.27	36.48
4	100.00	7.50	180.00	68.48	69.07	10.48	38.99	48.14	67.42
5	75.00	5.00	180.00	58.99	61.61	34.440	42.25	65.60	70.95
14	75.00	10.00	180.00	60.98	64.25	29.69	46.04	70.42	62.92
15	75.00	7.50	60.00	78.16	81.37	15.98	53.11	77.97	52.80
6	75.00	7.50	300.00	64.10	75.09	23.75	35.07	51.18	75.43
3	75.00	7.50	180.00	67.64	67.73	21.79	43.44	65.87	65.53
13	75.00	7.50	180.00	69.27	77.85	21.25	45.84	69.65	68.26
9	75.00	7.50	180.00	63.52	63.73	33.20	43.15	70.32	73.01
11	75.00	7.50	180.00	61.23	66.11	29.98	39.67	66.53	73.07
12	75.00	7.50	180.00	56.78	60.06	34.54	39.66	57.95	74.24
19	75.00	7.50	180.00	68.29	73.60	20.21	37.47	57.46	75.91

2.5.2. Fourier transform infrared (FTIR) spectroscopy analysis

The differences in functional groups between pretreated and untreated biomass samples were analysed using a Cary 630 FTIR spectrometer (Agilent, USA). The spectra were collected at a scan rate of 40 within the range of 4000–650 cm^{-1}, with 32 scans per spectrum (Amobonye et al., 2021).

2.5.3. X-ray diffraction

X-ray diffraction (XRD) was performed using a Rigaku MiniFlex600 (Rigaku Corporation, Japan) equipped with a sealed ceramic X-ray tube featuring a copper anode to determine the crystalline structure of both the untreated and pretreated biomass samples. The diffraction intensity was measured over a 3°– 80° 2θ range and calculated according to Segal et al. (1959) (S6, Supplementary material).

2.6. Enzymatic hydrolysis of biomass residues

Enzymatic hydrolysis of both the untreated and pretreated biomass residues was performed at 1 % (w/v) solid loading in sodium acetate buffer (50 mM, pH 5.0) at 50°C, 150 rpm for 96 h in a shaking incubator (Multitron, Infors, Switzerland). Cellulase was used at a concentration of 55 FPU/g biomass, and the reaction mixture was sampled every 24 h (Zininga et al., 2024). The hydrolysate was incubated for 5 min in a boiling water bath to deactivate the enzyme and centrifuged at 10, 000 rpm for 5 min. The resulting supernatant was collected and analysed for total reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Glucose concentration was determined using a high-performance liquid chromatography system (Prominence-i LC-2030C 3D Plus, Shimadzu Japan) following the protocol of Okuofu et al. (2023) with slight modifications. The HPLC was equipped with a RID-20A detector and operated with an Aminex HPX-87H column (300 × 7.8 mm, Biorad) using 5 mM H_2SO_4 as the eluent and a flow rate of 0.6 mL/min. The enzymatic hydrolysis efficiency (EHE) in terms of cellulose digestibility and glucose yield was determined according to He et al. (2023) (S7 and S8, Supplementary material).

2.7. Bioethanol fermentation

The PWS hydrolysate, which initially contained 5.5 g/L sugar, was supplemented with glucose to achieve a final sugar concentration of 20.10 ± 0.24 g/L. The final composition was adjusted to pH 5.5 with 1.0 M HCl and autoclaved. After cooling, batch fermentation was carried out in 250 mL flasks at a working volume of 100 mL using a 10 % (v/v) *S. cerevisiae* inoculum, grown on yeast extract, peptone, and glucose (YPG) broth for 16 h. Samples were drawn every 6 h, centrifuged at 10,000 rpm for 10 min and the supernatant was passed through a 0.22 μm cellulose filter before analysing the bioethanol yield and sugar consumption (Gunam et al., 2021). The ethanol produced was analysed by gas chromatography (GC) using a Shimadzu Nexis GC-2030 gas chromatograph (Japan) equipped with a flame ionisation detector (FID) and SH-Rtx-Wax column (30 m × 0.25 mm × 0.25 μm). Nitrogen was used as the carrier gas with an oven temperature of 50°C, while the injector and detector temperatures were maintained at 250°C. The ethanol yield, productivity and fermentation efficiency were calculated according to Zanivan et al. (2022) (S9 – S11, Supplementary material)

2.8. Statistical analysis

Data are represented as mean ± standard deviation of a minimum of three observations, while differences between means were analysed by the ANOVA test and a significant difference was considered at the level of $p < 0.05$. In addition, comparisons between two groups were conducted using a two-tailed unpaired *t*-test with Welch's correction.

3. Results and discussion

3.1. Optimisation of MW-assisted DES pretreatment using central composite design (CCD)

The effects of MW power, biomass loading, and pretreatment time on solid yield, cellulose recovery, and delignification in macadamia nut shells and pinewood shavings were evaluated (Table 2). The solid recovered after the pretreatment (solid yield) of MNSs ranged from 49.20 % to 78.16 %. High levels of process conditions (MW power and time) resulted in a significant decrease in solid yield; however, this was accompanied by enhanced delignification, whereas increasing delignification was observed from lower to higher biomass loading (Runs 10 and 16, respectively). The decrease in solid yield can be attributed to the degradation of biomass components and the removal of lignin (Anita et al., 2020). Interestingly, the highest delignification, 39.01 % (Run 16), corresponded to the least solid yield (49.20 %). On the other hand, lowest delignification (6.6 %) was observed with moderate biomass loading (6.01 %) at higher MW power (89.86 %) and longer pretreatment time (251.35 s) (Run 10) which is contrary to report by Tan et al. (2020) who reported that maximum lignin removal can be obtained using DES pretreatment with the solid loading between 5 –10 %. Due to the high lignin content in MNSs, severe pretreatment may have led to lignin redeposition, thereby reducing lignin removal efficiency. In a recent study, Wang et al. (2024b) reported a similar observation, where severe lignin repolymerisation occurred at high temperatures (160℃) under microwave-assisted pretreatment, resulting in poor lignin dissolution. Moreover, the low solid-to-liquid ratio in the pretreatment mixture (6.01 %) may have been too harsh, leading to ineffective pretreatment, as evidenced by the higher solid yield (76.27 %). Alternatively, a high cellulose recovery (>60 %) was achieved in all runs, with the highest value observed at lower biomass loading, despite high MW power and prolonged pretreatment time. This also coincides with the point of least delignification (Run 10). This observation may have been caused by lignin condensation and redeposition on the pretreated biomass surface, as indicated by the low delignification. This finding aligns with the observations of Zhang et al. (2024), who noted that although prolonged pretreatment at high temperatures can enhance lignin removal and cellulose degradation, recondensation lignin can occur on the cellulose surface.

Similar to MNSs, high levels of MW power and pretreatment time had a positive effect on solid yield and delignification in PWSs, with values ranging from 35.07 % to 64.13 % and 36.48–77.08 %, respectively. It was observed that low solid yield (36.79 %) inversely correlated with the highest delignification (77.08 %) (Run 10). This finding supports the assertion that biomass dissolution and lignin removal can decrease solid recovery, although it resulted in low cellulose recovery (49.23 %). Severe pretreatment conditions can result in a loss of cellulose (Wang et al., 2024a). Under severe pretreatment conditions, high biomass loading could be used to minimise the formation of degradative products and to compensate for the decrease in delignification, as it favoured greater recovery of cellulose (Run 16). Although sufficient time is required to achieve an effective pretreatment, as observed in the high delignification at longer pretreatment times in this study, caution must be taken, as excessive time could cause further degradation of components.

Cellulose recovery was relatively high (about 50 %) in all runs. According to Wang et al. (2025b), such observation may be due to minimal loss in the cellulose relative to hemicellulose and lignin. A low cellulose recovery could also be due to poor separation of the biopolymer components (cellulose, hemicellulose and lignin) as observed at milder pretreatment conditions of low MW power, short time (Runs 18, 7), high MW power, short time (Runs 20, 8) and the central points (Runs 6, 3, 13, 9, 11, 12 and 19). According to Tan et al. (2020), the depolymerisation of cellulose during DES pretreatment is low, which is corroborated by Yadav et al. (2024), who observed that most of the cellulose content in cocoa pod husk remained intact in the solid fraction after ChCl-citric acid and MW-ChCl-citric acid pretreatment. Interestingly, delignification increased by over 75 % when the MW power was doubled (Runs 2 and 4); however, this adversely affected cellulose recovery and solid yield. Thus, it is essential to strike a balance between minimal cellulose loss and high solid yield, as well as high delignification. Moreover, due to increased energy consumption, these processes also become more costly (Li et al., 2019a).

Other studies with higher results than those obtained in this study are either due to differences in HBD, longer pretreatment time or sequential DES-physical pretreatment. For instance, Ceaser et al. (2023) observed optimum conditions of ChCl:formic acid (1:4), 140°C, 800 W, and 14 min at a 15 % solid loading in mixed softwood, with 89.2 %, 90.15 %, and 95.25 % cellulose recovery, delignification, and hemicellulose, respectively. Similarly, high delignification (>80 %) was achieved in oil palm fruit bunches treated with ChCl:formic acid (1:2) at a 10 % solid loading at 120°C in an oil bath, with a longer pretreatment time (Tan et al., 2019). In another study, high delignification efficiency (65.20–79.60 %) was reported for agricultural wastes using microwave-assisted ChCl:lactic acid pretreatment at 800 W and 152°C for 45 s (Chen and Wan, 2018), despite a shorter duration than in the current study. Findings in this study contradict previous studies, which reported that softwood biomass has higher lignin content, mainly guaiacyl units (Pang et al., 2021), and requires more severe pretreatment conditions (Ji et al., 2025) than hardwoods, such as macadamia nut shells or even grass. Even though the lignin content of PWSs in this study is similar to that of most softwoods, its low content may have favoured higher delignification (73.01 %) despite mild pretreatment conditions compared with MNSs, a hardwood. A high lignin content in biomass increases its hardness, resulting in a highly compact structure. In contrast, a low lignin content may imply more loosely bound fibre since lignin acts as a biological binder between individual cells and the fibrils that make up the cell wall (Gani and Naruse, 2007; Teh et al., 2015), thus, necessitating severe pretreatment conditions for effective biomass fractionation as observed in macadamia nut shells than in pinewood shavings.

3.2. Analysis of variance (ANOVA) of response models

Based on the analysis of variance (ANOVA) and correlation coefficient as determined by the F and p values, all models were significant and demonstrated the impact of the investigated factors on the responses in MNSs (Table 3). The ANOVA for solid yield, cellulose recovery and delignification showed that the models were statistically significant ($p < 0.05$) with a non-significant lack of fit. The R^2 values for all three responses were$\geq$ 0.90, and the differences between the adjusted and predicted R^2S were $<$ 0.2, indicating close agreement. Generally, a correlation coefficient (R^2) between 0.70 and 1.0 indicates a good model (Raina et al., 2020), and the closer the value is to 1.0, the stronger the model and the better it predicts the response (Sharma and Dimple, 2019). The non-significant lack of fit for all responses represents the model's ability to explain the experimental results. Since the model predicted for solid yield, cellulose recovery and delignification are in close agreement with the actual results, it thus demonstrates that the quadratic models were fitted with high accuracy and have satisfactory predictive ability.

The second-order polynomial models presented below were derived through multivariate regression analysis of the experimental data set (Equations 1, 2 and 3) as;

$$\text{Solid yield} = 65.96 - 6.19\,A + 0.7384B - 4.28\,C - 1.47AB - 2.82AC - 0.2568BC - 0.1074A^2 - 2.36B^2 + 1.66\,C^2 \quad \text{Eq. 1}$$

$$\text{Cellulose recovery} = 65.41 - 0.1868A - 0.6502B - 4.26\,C - 0.5076AB + 4.05AC + 0.467BC + 2.23A^2 + 0.6053B^2 + 6.18\,C^2. \quad \text{Eq. 2}$$

$$\text{Delignification} = 31.62 + 6.40\,A - 1.29B + 4.02\,C + 0.6912AB + 3.05AC - 1.39BC - 1.59A^2 - 0.1052B^2 - 2.94\,C^2. \quad \text{Eq. 3}$$

where A, B, and C represent the microwave power, biomass loading,

Table 3
Summarised analysis of variance (ANOVA) for cellulose recovery, solid yield and delignification after MW-assisted DES pretreatment in MNSs based on the central composite design model.

	Solid yield		Cellulose recovery		Delignification	
Factor	F-value	p-value	F-value	p-value	F-value	p-value
Model	13.12	0.0002 significant	9.70	0.0007 significant	21.07	< 0.0001 significant
A-Microwave power	62.12	< 0.0001	0.04	0.84	101.31	< 0.0001
B-Biomass loading	0.88	0.37	0.51	0.49	4.12	0.069
C-Pretreatment time	29.66	0.0003	22.13	0.0008	39.97	< 0.0001
AB	2.06	0.18	0.18	0.68	0.69	0.42
AC	7.53	0.02	11.72	0.0065	13.48	0.0043
BC	0.06	0.81	0.16	0.70	2.81	0.12
A^2	0.019	0.89	6.40	0.03	6.59	0.028
B^2	9.56	0.01	0.47	0.51	0.03	0.86
C^2	4.74	0.05	49.14	< 0.0001	22.52	0.0008
Residual						
Lack of fit	0.43	0.81 not significant	0.49	0.78 not significant		0.23 not significant
Pure error						
R-squared		0.92		0.90		0.95
Adjusted R-squared		0.85		0.81		0.91

Significant at 95 % confidence level with p-value less than 0.05.

and pretreatment time, respectively. The significant terms for solid yield were A, C, AC, B^2, while C, AC, A^2, and C^2 were for cellulose recovery. A, C, AC, A^2, and C^2 were significant for delignification, demonstrating their role in influencing the respective responses.

In all models, MW power (A) had the most significant effect on responses, followed by pretreatment time (C) and biomass loading (B). The study by Ceaser et al. (2023) identified temperature as the most significant factor in the pretreatment of mixed softwood using ChCl:formic acid. Since high temperatures are an effect of MW irradiation, both studies are in agreement. This also aligns with the findings of Chia et al. (2024), who reported high delignification at high temperatures, moderate pretreatment times, and high biomass loadings. Similar to MNSs, the ANOVA for solid yield, cellulose recovery, and delignification of PWSs demonstrated statistically significant models ($p < 0.05$) with a non-significant lack of fit (Table 4). The R^2 values for all three responses were$\geq$ 0.88, and the differences between the adjusted and predicted R^2 were < 0.2, indicating close agreement.

A second-order polynomial equation was derived using 20 experimental sets of responses from the CCD as equations 4, 5 and 6 for;

Solid yield = +41.54 – 6.22 A + 0.2990B – 4.43 C + 0.3687AB + 0.7562AC + 0.6662BC + $3.52A^2$ + $0.8949B^2$ + 0.8755 C^2 Eq. 4

Cellulose recovery = +64.41 – 9.55 A + 1.16B – 5.54 C + 2.07AB + 1.17 AC + 1.25BC + $0.3056A^2$ + $1.30B^2$ + 0.0829 C^2 Eq. 5

Delignification = +71.68 + 7.17A – 1.79B + 5.27 C - 0.0737AB + 2.54AC – 2.27BC – $7.03A^2$ – $1.73B^2$ – 2.72 C^2 Eq. 6

where A, B, and C represent the microwave power, biomass loading and pretreatment time, respectively.

The significant model terms for solid yield are A, C, A^2, where A and C represent cellulose recovery, and A, C, A^2 and C^2 represent delignification. This demonstrates their role in influencing the respective responses. Based on the F-value, MW power (A) had the most significant effect on responses, followed by pretreatment time (C) and biomass loading (B) in all models. This is consistent with reports by Ceaser et al. (2023) and Chia et al. (2024), who identified temperature as the most significant factor in pretreatment of biomass for high delignification.

3.3. Interactions of the process factors on the responses in MNSs

The effects of process variables and their interactions on the pretreatment process are represented by the 3-D surface plots (Fig. 1), which aim to maximise cellulose recovery and high delignification while maintaining a relatively low biomass solid yield. An increase in biomass loading at low microwave power resulted in a high solid yield, while the solid yield decreased at higher microwave power (Fig. 1a_1). The

Table 4
Summarised analysis of variance (ANOVA) for cellulose recovery, solid yield and delignification after MW-assisted DES pretreatment in PWSs based on the central composite design model.

	Solid yield		Cellulose recovery		Delignification	
Factor	F-value	p-value	F-value	p-value	F-value	p-value
Model	12.99	0.0002 significant	8.47	0.0013 significant	15.22	0.0001 significant
A-Microwave power	62.21	< 0.0001	53.73	< 0.0001	48.03	< 0.0001
B-Biomass loading	0.14	0.71	0.79	0.39	3.00	0.11
C-Pretreatment time	31.60	0.0002	18.12	0.0017	26.02	0.0005
AB	0.13	0.73	1.48	0.25	0.003	0.96
AC	0.54	0.48	0.47	0.51	3.55	0.089
BC	0.42	0.53	0.54	0.48	2.83	0.12
A^2	20.99	0.0010	0.06	0.81	48.71	< 0.0001
B^2	1.36	0.27	1.05	0.33	2.94	0.12
C^2	1.30	0.28	0.004	0.95	7.32	0.02
Residual						
Lack of fit	0.76	0.62 not significant	0.46	0.79 not significant	0.88	0.55 not significant
Pure error						
R-squared		0.92		0.88		0.93
Adjusted R-squared		0.85		0.78		0.87

Significant at 95 % confidence level with p-value less than 0.05.

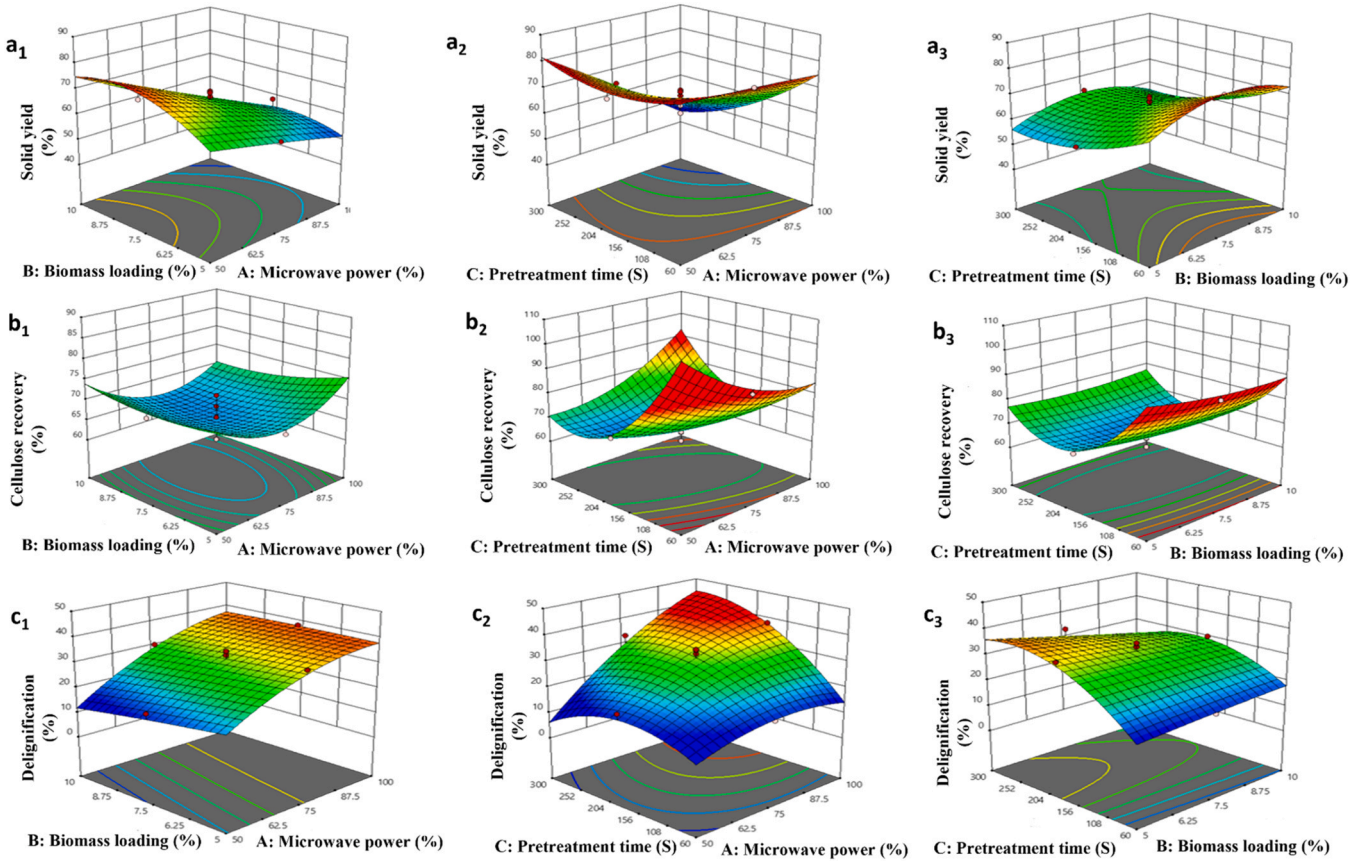

Fig. 1. Response surface plots showing the interactive effect of independent factors in 3-D graphs of MNSs solid residues; a_1: the interaction between biomass loading and microwave power, a_2: pretreatment time and microwave power and a_3: pretreatment time and biomass loading on solid yield (%); b_1: interaction between biomass loading and microwave power, b_2: pretreatment time and microwave power and b_3: pretreatment time and biomass loading on cellulose recovery; c_1: interaction between biomass loading and microwave power, c_2: pretreatment time and microwave power, and c_3: pretreatment time and biomass loading on delignification.

interaction between pretreatment time and microwave power was significant ($p < 0.05$), where the solid yield decreased with increasing pretreatment time and microwave power (Fig. 1a_2). These findings align with the results of Kohli et al. (2020) and Zhong et al. (2022) that an increase in microwave temperature enhanced the removal of the xylan and lignin with a corresponding decrease in solid recovered. On the other hand, the interaction between pretreatment time and biomass loading had a positive effect on solid yield at longer times and lower biomass loading, whereas it had the opposite effect at shorter times and higher biomass loading (Fig. 1a_3). Due to the high lignin content of MNSs, a high biomass loading may have led to a high solid yield, as lignin is the primary cause of biomass recalcitrance. The use of extractive-free MNSs, which have a loose structure, could enhance biomass disruption and improve DES penetration following effective pretreatment. The studies of Ceaser et al. (2023) and Tajmirriahi et al. (2021) confirm that removing extractives from biomass prior to pretreatment enhances the effectiveness of DESs.

Lowering biomass loading favoured an increase in delignification up to 40 % with increasing microwave power (to about 75 %) and remained stable at higher microwave powers (up to 89 %). Similar to the effect on solid yield, both pretreatment time and microwave power favoured delignification (Figure 1 c_1-c_3). Although under severe conditions of high microwave power and longer pretreatment time, the rate of delignification decreased. High biomass loading adversely affected delignification at shorter pretreatment times. Due to the high lignin content of MNSs, a relatively long reaction time may be required to achieve effective fractionation. According to Varilla-Mazaba et al. (2022), a longer time is required to allow DES to penetrate the biomass, thereby facilitating better interaction and more effective breakdown and removal of lignin. The response surface plots further confirmed that microwave power and pretreatment time had significant effects on the responses, as predicted by the models.

3.4. Interactions of the process factors on the responses in PWSs

The interaction between biomass loading and microwave power had a minimal effect on solid yield at higher biomass loading and lower microwave power ($p > 0.05$) (Fig. 2a_1). This is expected, as low microwave power may be insufficient to overcome intermolecular forces between biomass components and facilitate the dissolution of lignin and hemicellulose, potentially reducing solid recovery (Wang et al., 2025a). Even though a high solid yield is beneficial in industrial applications for reduced operating costs and increased product yields (Ceaser et al., 2023), a reduction in solid yield, associated with lignin removal, is observed to improve cellulose retention, as reported in the study. Similar to MNSs, across all process factors and their interactions, cellulose recovery was high; that is, all factors were insignificant ($p > 0.05$), and there was minimal loss of cellulose in the recovered solid (Fig. 2b_1-b_3).

Among the investigated independent variables, the interaction between microwave power and pretreatment time had the greatest influence on the responses, particularly on solid yield and delignification ($p < 0.05$).

An increase in both pretreatment time and microwave power resulted in a lower solid yield, which correlated with higher delignification (approximately 78 %) ($p < 0.05$) (Figure 2c_2). However, a longer pretreatment time had a beneficial effect on delignification at higher

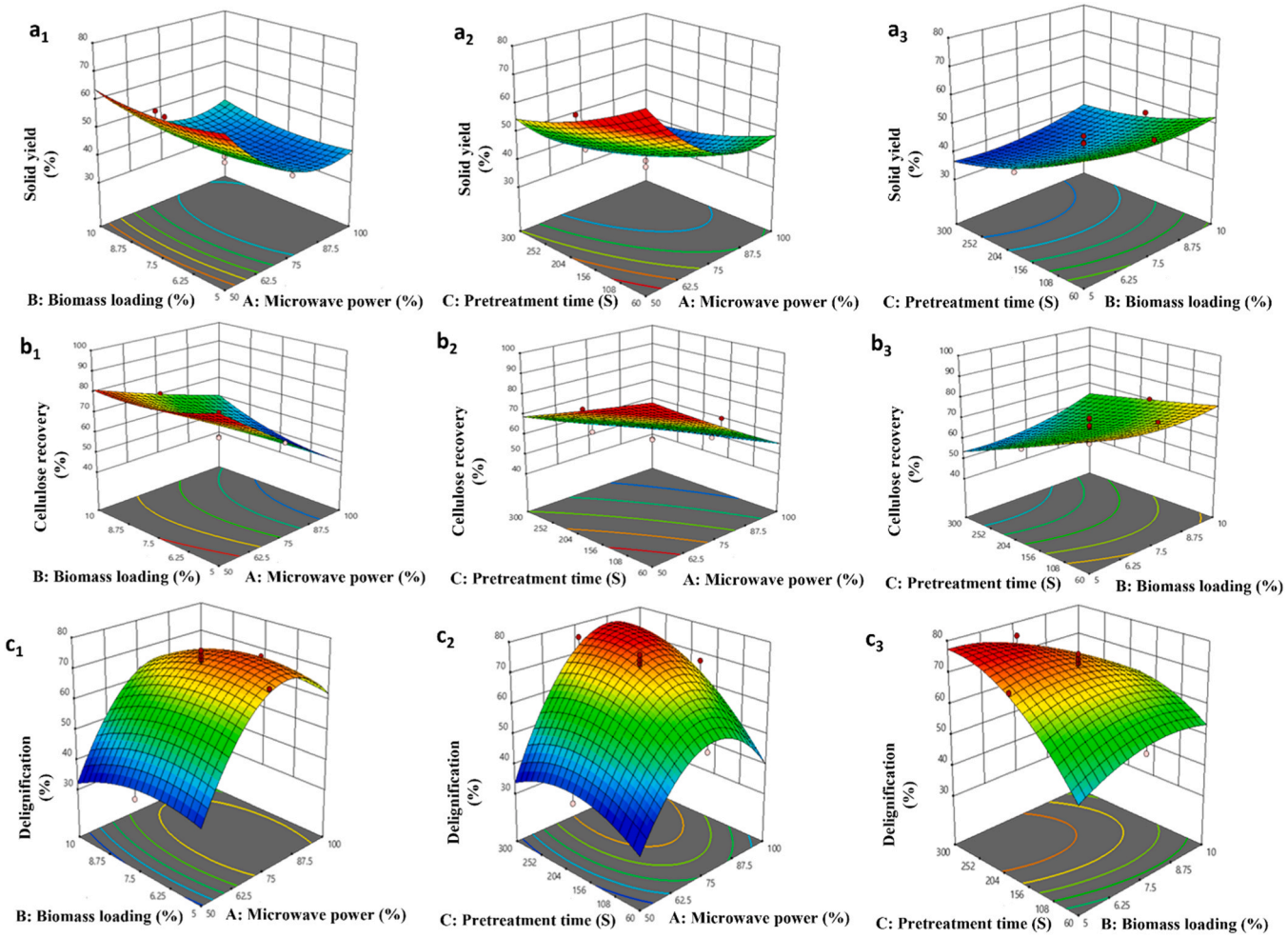

Fig. 2. Response surface plots showing the interactive effect of independent factors in 3-D graphs of PWSs solid residues; a_1: the interaction between biomass loading and microwave power, a_2: pretreatment time and microwave power and a_3: pretreatment time and biomass loading on solid yield (%); b_1: interaction between biomass loading and microwave power, b_2: pretreatment time and microwave power and b_3: pretreatment time and biomass loading on cellulose recovery; c_1: interaction between biomass loading and microwave power, c_2: pretreatment time and microwave power, and c_3: pretreatment time and biomass loading on delignification.

biomass loading than at lower biomass loading (Fig. 2c_3). Even though longer pretreatment time allows DES to interact in a better way, resulting in a greater fractionation of lignocellulosic biomass for better lignin removal (Guo et al., 2018), a low solid loading may result in excessive biomass degradation due to excessive solvent and result in lignin condensation and redeposition on the biomass surface, leading to poor delignification (Zhang et al., 2024). Moreover, severe pretreatment conditions can reduce lignin, due to the onset of thermal degradation of lower-molecular-weight lignin (Chen et al., 2019).

In a previous report, microwave power, irradiation time, and temperature were identified as critical factors influencing biomass digestibility during the pretreatment process (Sharma et al., 2023b). Furthermore, Wang et al. (2024b) identified the interaction between solvents and microwaves as a crucial factor in the deconstruction of biomass.

3.5. Validation of the model

Model validation was achieved by performing the experiment using the combination of factors generated by the point prediction tool of the RSM software and comparing the results with the predicted values to assess any correlation. The optimum conditions predicted for MNSs pretreatment were 82.55 % MW power, 6.88 % biomass loading, and 251.35 s of pretreatment time, resulting in a solid yield of 49.20 ± 0.97 %, cellulose recovery of 75.48 ± 0.65 %, and delignification of 39.01 ± 1.15 %. These values were within the acceptable range of the predicted values of 58.44 %, 70.16 %, and 38.06 %, respectively.

For PWSs, solid yield, cellulose recovery and delignification of 43.15 ± 0.28 %, 70.32 ± 0.81 %, and 73.01 ± 0.53 %, respectively, were obtained at predicted optimum conditions of 81.24 % MW power, 8.94 % biomass loading, and 181.43 s pretreatment time, which are in proximity of predicted values of 40.77 %, 63.78 %, and 70.13 %. Most responses under the optimised conditions were within the 95 % confidence interval, while the MNS solid yield fell within the 95 % tolerance interval, suggesting that the process is not only well-predicted by the model but also consistent and reliable in practice. Thus, the values obtained are considered acceptable, as previously reported by Partida-Sedas et al. (2017). Furthermore, the close agreement between the experimental and predicted responses in both biomasses further demonstrates the model's accuracy in optimising the MW-assisted CL 1:3 pretreatment. In a previous study by Anita et al. (2020), a delignification rate of 50.57 % was achieved in pretreated oil palm empty fruit bunch at optimised conditions of 190°C for 3 min with 1.1 % oxalic acid using RSM. However, the level of delignification in both MNSs (39.01 ± 1.15 %) and PWSs (73.01 ± 0.53 %) at 251 s and 180 s is close to the fractionation range of 41.5 – 92.4 % and time 0.75 — 30 min reported for most MW-assisted DES pretreatment through optimisation (Wang

et al., 2025a).

3.6. Changes in chemical composition of MW-assisted DES CL (1:3) pretreated MNSs and PWSs

Due to differences in the structures of softwood and hardwood biomasses, the suitability and effectiveness of any pretreatment method employed would differ (Kłosowski et al., 2022). As a result, the effectiveness of combined MW-DES pretreatment based on solid yield, changes in biopolymer composition and inhibitory compounds was evaluated (Table 5). The solid recovered was significantly lower in PWSs (43.18 ± 0.28 %) at a shorter time (181.43 s) than MNSs (49.20 ± 0.97 %), even at a longer time (251.35 s). The solubilisation of solids into the liquid fraction occurs during pretreatment of biomass due to the deconstruction of the lignocellulosic structure (Isci et al., 2020). However, the lower lignin content in PWSs implies that less energy input would be required to break down the lignocellulosic biomass structure, which may have contributed to lower solid recovery compared with MNSs with higher lignin content. The increase in cellulose content in both biomass is beneficial, as sugar polysaccharides make the biomass a valuable feedstock in a lignocellulosic biorefinery for producing biofuels and other biobased chemicals (He et al., 2023). The close to 50 % increase in cellulose content of MNSs despite high lignin contents further supports the report by Poy et al. (2023) and Zhao et al. (2017), that all lignin doesn't need to be removed for enzymatic hydrolysis, hence some amount of sugar can still be released during hydrolysis. The decrease in hemicellulose content in both biomasses also exposes more cellulose surface for utilisation. This aligns with the report by Roslan et al. (2024), who observed that high hemicellulose removal during ChCl:oxalic acid pretreatment resulted in increased cellulose content in pineapple peel.

The 37.5 % reduction in lignin content in pretreated PWSs compared with untreated biomass demonstrates the effectiveness of MW-DES pretreatment and this is relatively higher in comparison with the reported values of 23.8 % for corn stover pretreated with choline chloride: formic acid (1:2) at 130°C for 2 h (Xu et al., 2016) and 23.7 % for wheat straw pretreated with eutectic mixture of lactic acid and alanine at 60°C for 24 h (Jablonsky et al., 2019). Higher lignin content in MNSs may have resulted from the redeposition of lignin on the pretreated solid residues, which is in agreement with the report by Semwal et al. (2024).

Cellulose recovery was high (approximately 70 %) in both MNSs and PWSs, but higher in PWSs due to greater delignification. The delignification efficiency of the combined MW-DES pretreatment in this study aligns with the selective solubility of lignin in DESs, thereby promoting enzymatic saccharification (Lobato-Rodríguez et al., 2023). During chemical pretreatment, the degradation of monomeric sugars and the hydrolysis of hemicellulose generate various toxic compounds that act as inhibitors in subsequent steps (Jose et al., 2024). However, the levels of acetic acid in MNSs, as well as those of formic and levulinic acids in PWSs, are below concentrations that can negatively affect downstream processes of saccharification and fermentation.

3.7. Characterisation of pretreated biomass using SEM, FTIR and XRD

Different degrees of structural disintegration in biomass can be attributed to the effectiveness of a pretreatment process (Ramos et al., 2022). The SEM analysis provided a visual confirmation of the extent of morphological changes during pretreatment (Figs. 3 and 4).

The micrographs of untreated MNSs ($MNSs_0$, $MNSs_{00}$) showed a smooth biomass surface, whereas the pretreated ($MNSs_1$, $MNSs_{11}$) showed moderate distortions of the biomass fibril structure. In addition, the interactions of the studied input variables employed in the pretreatment conditions may not be sufficient to disrupt the biomass structure even at optimised conditions (82.55 % MW power, 6.88 % biomass loading, and 251.35 s pretreatment time), however, more severe conditions (in the form of higher MW power and longer pretreatment time) may enhance biomass disintegration. The untreated PWSs ($PWSs_0$, $PWSs_{00}$) showed a smooth, compact and undisturbed fibril structure, while numerous holes and formation of biomass debris/fibres were visible in the pretreated biomass ($PWSs_1$, $PWSs_{11}$). The results showed that optimised MW-assisted CL (1:3) pretreatment at optimum conditions of 81.24 % MW power, 8.94 % biomass loading, and 181.43 s pretreatment time was effective for biomass fractionation. Thus, pretreated PWSs with high hemicellulose and lignin removal showed a higher degree of biomass fractionation, as evidenced by the appearance of more cracks with a loose surface, thereby exposing the cellulose microfibrils. Such structural alterations in biomass can create more active sites, facilitating the effective hydrolysis of cellulosic polysaccharides and enhancing their conversion to fermentable sugars (Chen et al., 2023). Proton-catalysed cleavage of the glycosidic bonds within the carbohydrate polymers facilitates the elimination of hemicellulose. This results in the expansion of the organic structure of lignocellulose and further increase of its internal surface area, ultimately promoting

Table 5
Outcome of biomass residues at optimised MW-assisted DES CL 1:3 pretreatment conditions.

Components	Biomass composition (%)			
	Untreated	MNSs	Untreated	PWSs
Solid yield	-	49.20 ± 0.97	-	43.15 ± 0.28
Biopolymer composition				
Cellulose	26.69 ± 0.15[b]	40.37 ± 1.31[a]	39.93 ± 0.50[b]	65.99 ± 0.48[a]
Hemicellulose	12.16 ± 0.56[a]	10.63 ± 0.61[b]	15.97 ± 0.17[a]	9.74 ± 0.39[b]
Total lignin	41.70 ± 10.46[b]	51.71 ± 0.08[a]	29.26 ± 0.68[a]	18.26 ± 0.51[b]
Cellulose recovery	-	75.48 ± 0.65	-	70.32 ± 0.81
Delignification	-	39.01 ± 1.15	-	73.01 ± 0.53
Inhibitor compounds (g/L)				
Acetic acid	-	0.08 ± 0.00	-	ND
Formic acid	-	ND	-	0.03 ± 0.00
Levulinic acid	-	ND	-	0.04 ± 0.00

Data represent mean ± standard deviation (n = 3). Values with different letters as superscripts are significantly different at $p < 0.05$.
ND – Not detected.

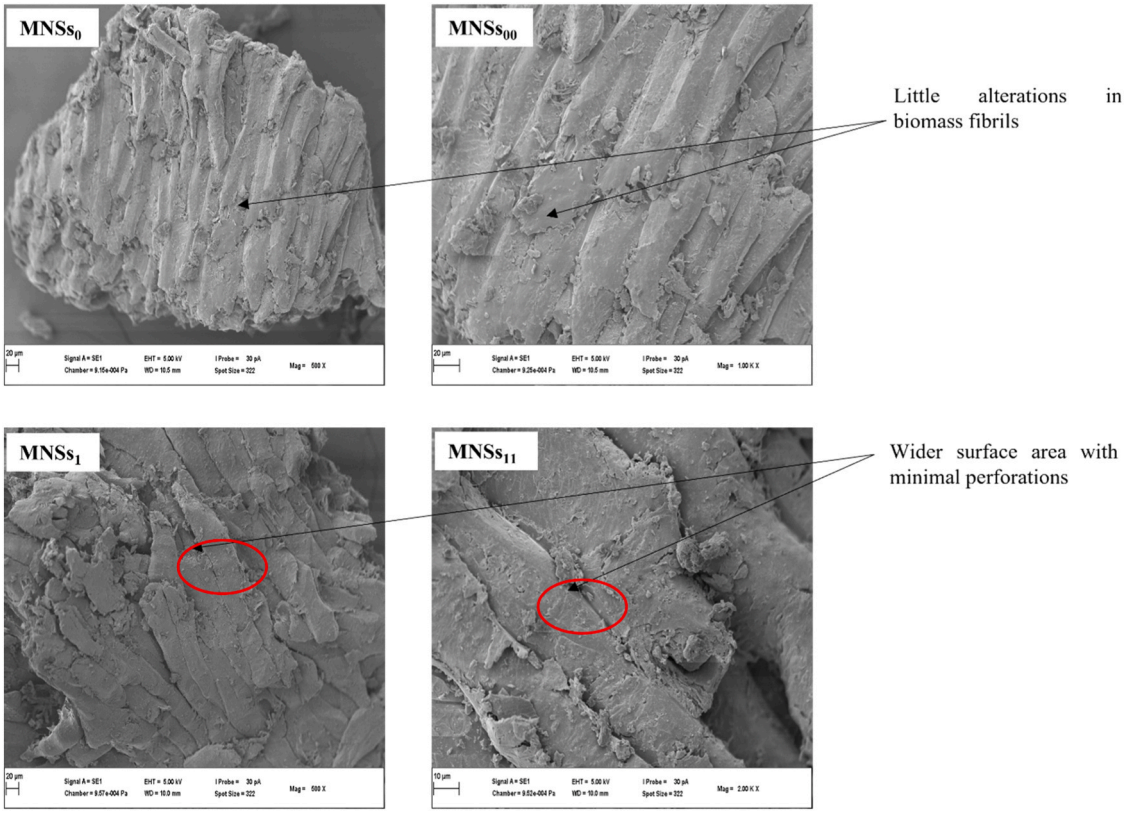

Fig. 3. Micrographs showing untreated ($MNSs_0$and $MNSs_{00}$) and pretreated ($MNSs_1$ and $MNSs_{11}$) MNSs, at x500 and x1000 respectively.

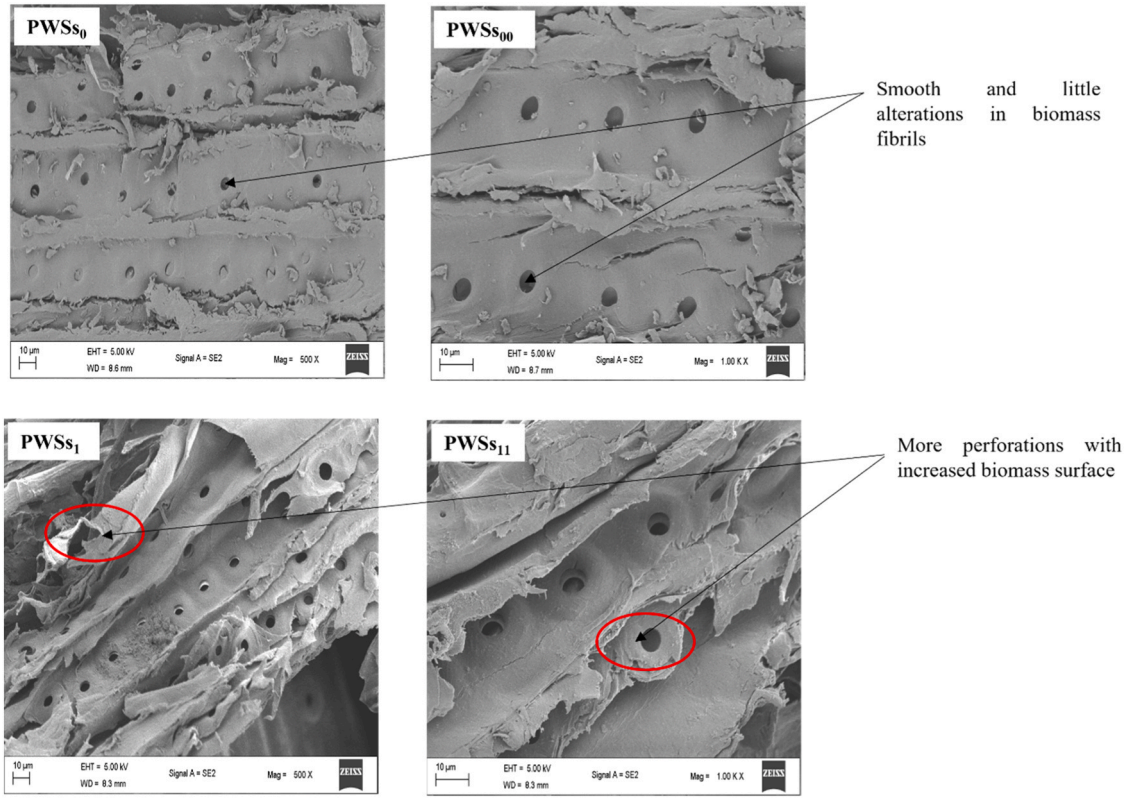

Fig. 4. Micrographs showing untreated ($PWSs_0$ and $PWSs_{00}$) and pretreated ($PWSs_1$ and PWS_{11}) PWSs, at x500 and x1000 respectively.

enzyme action on cellulose and increasing the yield of glucose (Mankar et al., 2021). Similar observations were reported for effective fractionation in MW-assisted acidic DES pretreatment, which led to the formation of a porous surface on sugarcane bagasse (Gan et al., 2024) and roughness in the morphology of oil palm empty fruit bunch (Yaakob et al., 2024). The FTIR spectra revealed various changes and band shifting within the lignin-hemicellulose-cellulose complex at optimised pretreatment conditions in pretreated MNSs ($MNSs_1$) and PWSs ($PWSs_1$) compared with their respective untreated biomass ($MNSs_0$ and $PWSs_0$) (Fig. 5). The broad peaks at 3332.2 cm^{-1} were assigned to O-H stretch, which was attributed to water molecules in cellulose (Zhang et al., 2019), while the bands around 2892.2 cm^{-1} were assigned to asymmetric and symmetric methylene stretching in a saturated aliphatic (alkane/alkyl) group from cellulose (Mjalli and Al-Azzawi, 2021). The intensity of these peaks was higher in pretreated PWSs than in MNSs compared with untreated biomass, suggesting that cellulose exposure was facilitated and accessibility was enhanced by the removal of lignin and hemicellulose during pretreatment. Furthermore, the diminishing peaks at 1722.2 and 1267.3 cm^{-1} in pretreated PWSs indicate structural changes induced by the pretreatment. A similar observation was reported in sugarcane bagasse (Morán-Aguilar et al., 2022) and rice husk (Tang et al., 2023), where the pretreatment resulted in a decrease in the peaks due to lignin removal in the respective biomass. Alterations in the cellulose structure due to pretreatment can cause changes in biomass crystallinity (Isci et al., 2020), thus, for a better understanding of the effect of pretreatment conditions on cellulose, the crystallinity index was determined in both untreated and pretreated biomass (Fig. 6). The XRD spectra revealed that the CrI of pretreated MNSs (57.99 %) was lower than that of untreated biomass (64.12 %) and the observed decrease could be due to a lower rate of removal of the amorphous regions, resulting in less exposure of the cellulose portion. On the other hand, the CrI of pretreated PWSs (78.41 %) was significantly higher than that of untreated biomass (59.88 %), which is linked to the removal of the amorphous hemicellulose and lignin regions, thereby exposing the crystalline cellulose portion. The removal of more amorphous compounds, such as lignin and hemicellulose, leads to increased CrI (Xu et al., 2024). Furthermore, the high lignin content of MNSs (41.70 ± 10.46 %) in this study, compared with PWSs (29.26 ± 0.68 %), may have contributed to their recalcitrance, lower delignification rate at the optimised conditions, and consequently, a decrease in their CrI. Further evaluation of the CrI/cellulose ratio, which represents the ratio of the relative crystallinity of the pretreated residues to the cellulose content, revealed lower ratios in pretreated PWSs (1.19) and pretreated MNSs (1.44) compared with untreated (1.49 and 2.40, respectively). A similar finding was reported by Xu et al. (2023), who found that pretreated

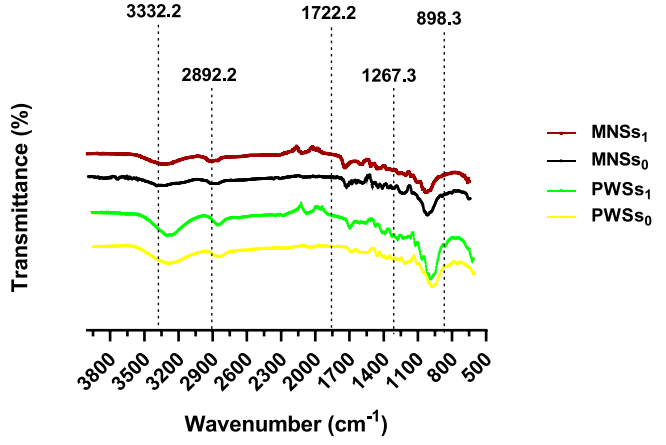

Fig. 5. FTIR spectra of untreated and pretreated MW-assisted CL (1:3) MNSs ($MNSs_0$ and $MNSs_1$) and PWSs ($PWSs_0$, and $PWSs_1$, respectively) at optimised pretreatment conditions.

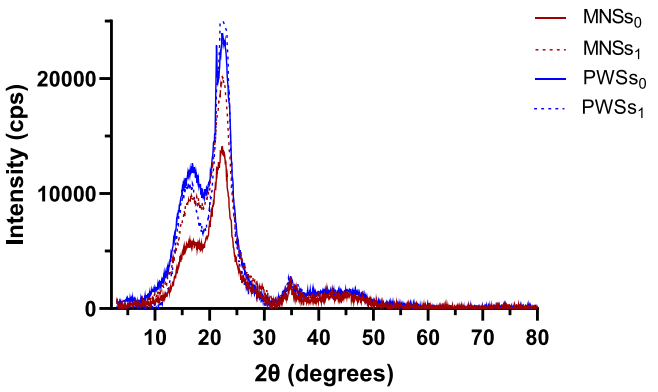

Fig. 6. XRD analysis of untreated and pretreated MNSs and PWSs at optimised conditions.

bamboo shoot shells had a lower CrI-to-cellulose ratio (0.69 – 0.76) than untreated biomass (0.85), suggesting that cellulose content increased more rapidly than CrI, which is beneficial for subsequent enzymatic hydrolysis. Another study by Zhang et al. (2023), also revealed a lower CrI/cellulose ratio in bamboo when pretreated with choline chloride: lactic acid-5 % $CaCl.6H_2O$ at 120°C for 3 h.

3.8. Enzymatic saccharification and fermentation of the resulting hydrolysate

The type of biomass and pretreatment method play significant roles in 2 G biorefinery, with significance in determining the effectiveness of hydrolysis and efficient bioethanol production (Parveen et al., 2025). An effective pretreatment process influences the rate of enzymatic hydrolysis by increasing enzyme accessibility to biomass and thereby increasing the release of fermentable sugars, which serve as substrates for bioethanol production (Semwal et al., 2024). As a result, the fermentable sugar yield in terms of total reducing sugar (Fig. 7), cellulose digestibility, and glucose yield during hydrolysis were evaluated (Table 6). The highest total reducing sugar concentration of 161.16 ± 0.96 mg/g biomass was obtained in PWSs, corresponding to an enhanced cellulose digestibility of 60.33 % and a glucose yield of 67.03 % at 72 h of hydrolysis. MNSs hydrolysate showed lower yields of 65.79 ± 0.74 mg/g biomass total reducing sugar, 51.01 ± 0.03 % cellulose digestibility and 56.67 ± 0.04 % glucose yield than in PWSs. The higher glucose yield in PWSs corresponds to the greater degree of delignification achieved during pretreatment. The glucose yields obtained in the current study are higher than those reported in a previous study, where a glucose yield of 44.94 % was achieved for Chinese fir residue, a type of softwood pretreated with ChCl-formic acid (1:4) at

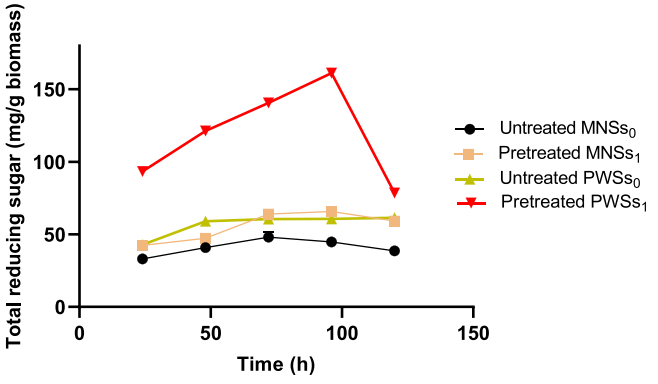

Fig. 7. Outcome of enzymatic hydrolysis in terms of a.) Total reducing sugar yields in both untreated ($MNSs_0$ and $PWSs_0$) and pretreated biomass ($MNSs_1$ and $PWSs_1$).

Table 6
Enzymatic efficiency of both untreated and pretreated MNSs and PWSs.

Enzymatic hydrolysis efficiency (%)								
Time (h)	MNSs				PWSs			
	Cellulose digestibility		Glucose yield		Cellulose digestibility		Glucose yield	
	Untreated	Pretreated	Untreated	Pretreated	Untreated	Pretreated	Untreated	Pretreated
24	15.35 ± 0.01	27.68 ± 0.89	17.26 ± 1.49	30.91 ± 0.76	22.12 ± 0.69	22.36 ± 1.21	24.16 ± 0.89	24.84 ± 0.08
48	16.44 ± 0.11	41.01 ± 0.69	17.88 ± 0.68	46.22 ± 1.26	29.60 ± 0.94	31.99 ± 2.14	33.47 ± 1.25	40.34 ± 2.60
72	29.06 ± 0.18	51.01 ± 0.03	32.29 ± 0.20	56.67 ± 0.04	35.75 ± 1.23	60.38 ± 0.02	39.28 ± 1.29	68.81 ± 0.48
96	21.58 ± 0.32	26.05 ± 2.22	22.63 ± 2.42	24.64 ± 0.77	18.14 ± 0.28	29.86 ± 2.36	20.16 ± 0.31	34.19 ± 0.92
120	10.49 ± 1.01	21.47 ± 0.69	11.66 ± 1.12	20.64 ± 0.65	17.23 ± 0.21	27.12 ± 1.21	17.23 ± 0.11	23.57 ± 0.28

Data represent mean ± standard deviation (n = 3).

150°C for 90 min (Ji et al., 2025). A recent study by Wang et al. (2025a) further supports the efficiency of the optimised MW-CL (1:3) pretreatment in this study, as the resulting enzymatic digestibility (cellulose digestibility and glucose yield) aligns with the widely reported range of 52.8 — 96.3 %. Cellulose digestibility, which is the degree of conversion of cellulose to glucose, was also higher in PWSs. However, higher molar ratios of DES can influence cellulose digestibility, as observed by Li et al. (2019b), who reported 81.90 % cellulose digestibility in softwood (*Pinus bungeana*) after pretreatment with CL 1:10.

The acidic nature of carboxylic acid-based hydrogen bond donors (HBDs) in DES, such as lactic acid, makes them effective for biomass dissolution and for removing hemicellulose and lignin to enhance cellulose digestibility (Srinivasan and Venkatachalam, 2025). In addition, the microwave-absorbing potential of DESs has been identified as a major contributor to intensification of the DES pretreatment under MW conditions (Wang et al., 2024b); as such, even at mild conditions as employed in PWSs, cellulose accessibility was greatly enhanced and favoured the release of higher sugar yields. This was further confirmed by SEM micrographs and XRD analyses, which showed the presence of severe cracks and a loose surface, indicating a higher degree of biomass fractionation and higher crystallinity, respectively. Even though both biomasses are woody, the observed susceptibility of softwood PWSs to the pretreatment process, in contrast to hardwood MNSs, may have been favoured by their lower lignin content, which requires milder process conditions in terms of lower MW power and shorter pretreatment time. However, the use of extractive-free MNSs in pretreatment can loosen the biomass and enhance the penetration of DES during pretreatment, as reported by Tajmirriahi et al. (2021), who stated that the removal of extractives from biomass could be used as a form of pretreatment to enhance subsequent processes. The study by Ceaser et al. (2023) further confirmed this, where the effectiveness of ChCl-formic acid pretreatment was enhanced in extractive-free mixed softwood.

PWSs showed higher cellulose digestibility and sugar yields during enzyme hydrolysis. According to Chen et al. (2024), the performance of DES pretreatment in terms of higher sugar yields can be improved with higher enzyme loadings. Thus, the pretreated PWSs were further hydrolysed using a higher enzyme dosage. At 64 mg/g biomass cellulase, the PWSs hydrolysate yielded 291.99 ± 0.11 mg/g biomass TRSs, equivalent to 5.51 g/L glucose.

Similar results were reported in a recent study by Yadav et al. (2024), which highlights the effectiveness of pretreatment with acidic DES in combination with MW irradiation and obtained high sugar yields of 252.85 mg/g cocoa pod husks. However, the difference in yields compared with the present study is dependent on factors such as biomass type (hardwood, softwood or grass), DES molar ratios, pretreatment conditions (time, temperature, microwave power and biomass loading).

Thereafter, PWSs hydrolysate, supplemented with glucose to yield 20.10 ± 0.24 g/L of sugar, was fermented using *S. cerevisiae* for ethanol production (Table 7). During fermentation, ethanol yield increased until 16 h, at which point glucose was completely utilised. Despite the increase in ethanol yield, fermentation efficiency decreased after 12 h; hence, the optimum time was determined as 12 h, with an ethanol yield of 4.21 ± 0.24 g/L), corresponding to 0.22 ± 0.01 g/g substrate, 0.35 ± 0.02 g/L/h productivity, and 44.01 % theoretical yield. These values were higher than 0.16 g/g ethanol yield, 0.17 g/L/h ethanol productivity, and 30.33 % fermentation efficiency obtained at 48 h by fermenting glucose obtained from a sequential chemical pretreatment and enzymatic hydrolysis of sorghum stalk (Manmai et al., 2020).

The fact that the optimum time in this study is lower implies that the yeast had a high glucose utilisation rate. A lower time to reach optimal ethanol yield is typically considered an advantage, as it reduces process costs, time, and overall fermentation efficiency (da Silva et al., 2024). Further, findings of Okuofu et al. (2022) and Ceaser et al. (2024b) with higher sugar concentration than that in this study revealed higher ethanol yields of 11.57 g/L, 0.38 g/g, 0.19 g/L/h and 0.22 g/g, 0.09 g/L/h, 42.9 %, respectively. This revealed that sugar concentration directly influences fermentation yield. Similarly, artificial hydrolysate

Table 7
Comparison of sugar release and ethanol yield obtained from the combined MW-DES pretreatment in the present study with those reported in the literature.

No	Biomass type	Optimum pretreatment conditions	Sugar yield	Fermentation conditions	Ethanol output	Reference
1	Pinewood shavings	MW-assisted CL 1:3 pretreatment, (81.24 %) 900 MW power, 181.43 s	291.99 ± 0.11 TRS mg/g biomass at 96 h, 20.10 ± 0.24 g/L glucose of supplemented PW hydrolysate	*Saccharomyces cerevisiae* at 12 h	4.21 ± 0.24 g/L ethanol yield, 0.22 ± 0.01 g/g substrate, 0.35 ± 0.02 g/L/h productivity and a theoretical yield of 44.01 %	This study
2	Cocoa pod husk waste	MW-assisted choline chloride/citric acid 1:1, 600 W MW power	TRS: 252.86 mg/g of CPH biomass	-	-	Yadav et al. (2024)
3	Bambara groundnut haulm	CL 1:2 pretreatment at 100°C for 1 h	30.43 g/L	*Saccharomyces cerevisiae* BY4743 at 32 h	11.57 g/L, 0.38 g/g sugar, productivity of 0.19 g/L/h	Okuofu et al. (2022)
4	Mixed softwood	Impregnation with ChCl-formic acid (1:4) and MW-assisted hydrolysis, 1:10 biomass loading, 140°C, 14 min	12 g/L	*Saccharomyces cerevisiae* at 24 h	0.22 g/g ethanol yield, 0.09 g/L/h productivity and 42.9 %, efficiency	Ceaser et al. (2024b)
5	Artificial hydrolysate	-	16.64 ± 0.20 g/L	*Saccharomyces cerevisiae* at 168 h	6.92 ± 0.38 g/L	Sjulander and Kikas (2022)

(16.64 ± 0.20 g/L) was reported to produce a higher ethanol yield (6.92 ± 0.38 g/L) at a longer time (168 h) (Sjulander and Kikas, 2022).

4. Conclusion

The combinatorial microwave irradiation-CL 1:3 DES pretreatment resulted in enhanced bioconversion of softwood PWSs under mild conditions (181.43 s, 81.24 % MW power) compared with MNSs (251.35 s, 82.55 % MW power); with microwave power having the most significant effect on the studied responses. At optimised conditions, higher cellulose content and lower lignin content in pretreated PWSs correlated with high cellulose recovery and delignification when compared with MNSs. Consequently, cellulose digestibility and glucose yield were also higher in softwood PWSs than in hardwood MNSs. Further characterisation of pretreated PWS residues at optimised conditions, using SEM, FTIR, and XRD, revealed structural changes confirming effective biomass fractionation. Supplementation of PWSs hydrolysates enhanced glucose level, which gave a high ethanol yield (4.21 ± 0.24 g/L) achieving a theoretical yield of 44.01 % in a separate hydrolysis and fermentation (SHF). This study offers valuable insights into combinatorial MW-DES pretreatment as an efficient and cost-effective technique for bioethanol production. It also demonstrates the potential of 1 G and 2 G biorefinery integration, as glucose supplementation of PWs hydrolysates enhanced fermentable sugar content and bioethanol yield. Achieving high sugar concentration through supplementation consumes less energy and incurs lower processing costs than evaporation. Thus, agricultural wastes such as PWSs can be promoted as a renewable, bioresource material due to their carbohydrate-rich polymers, thereby promoting waste valorisation.

CRediT authorship contribution statement

Santhosh Pillai: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Neeraj Kumar Singh:** Writing – review & editing, Formal analysis. **Kugen Permaul:** Writing – review & editing, Supervision, Resources. **Prashant Bhagwat:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Ayodeji Amobonye:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Grace Abel:** Writing – original draft, Investigation, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.122263.

Data Availability

Data will be made available on request.

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