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COMPARATIVE ANALYSIS BETWEEN ONE-STEP AND TWO-STEP CHEMICAL PRETREATMENT OF DEFATTED SPENT COFFEE GROUND TO INCREASE SUGAR RECOVERY

ANÁLISE COMPARATIVA ENTRE O PRÉ-TRATAMENTO QUÍMICO EM UMA E DUAS ETAPAS DO BAGAÇO DE CAFÉ DESENGORDURADO PARA AUMENTAR A RECUPERAÇÃO DE AÇÚCARES

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Winda Clarissa Gilbert*
*University Malaysia Sarawak, Sarawak, Malaysia
windagilbert8@gmail.com

Nurashikin Suhaili*
*University Malaysia Sarawak, Sarawak, Malaysia
snurashikin@unimas.my

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Abstract
Coffee is among the most consumed beverages worldwide and widely used as flavoring or ingredient in food products. However, its large-scale consumption generates substantial amounts of waste, particularly spent coffee grounds (SCGs), which pose environmental challenges due to their organic content and resistance to degradation. SCG is rich in lignocellulosic components and polysaccharides, yet the efficient extraction of sugars is hindered by its recalcitrant structure. This study aimed to evaluate and compare various pre-treatment strategies, including one-step acid or alkaline pre-treatments as well as two-step sequential acid-alkaline and alkaline-acid pre-treatments, in order to improve the enzymatic hydrolysis of defatted SCG. SCG was first defatted and then subjected to various pre-treatment protocols. The effectiveness of each method was assessed through reducing sugar yield and surface morphology via SEM, with statistical significance evaluated using one-way ANOVA and Tukey's HSD. The results indicated that one-step acid pre-treatment produced a 1.5-fold increase in sugar yield, while alkaline treatments using KOH or NaOH enhanced sugar production by 2.97 to 3.28 times relative to untreated SCG. The highest sugar yield was obtained through the two-step acid-alkaline pre-treatment, which resulted in a 4.25 to 4.70-fold increase compared to the control. SEM analysis confirmed significant structural disruption and increased porosity after alkaline pre-treatment, thus enhancing enzyme accessibility. The findings contribute to optimising SCG valorisation for

Resumo
O café está entre as bebidas mais consumidas no mundo e é amplamente utilizado como aromatizante ou ingrediente em produtos alimentícios. No entanto, seu consumo em larga escala gera quantidades substanciais de resíduos, principalmente borra de café (BC), que representam desafios ambientais devido ao seu conteúdo orgânico e resistência à degradação. A BC é rica em componentes lignocelulósicos e polissacarídeos, porém a extração eficiente de açúcares é dificultada por sua estrutura recalcitrante. Este estudo teve como objetivo avaliar e comparar diversas estratégias de pré-tratamento, incluindo pré-tratamentos ácidos ou alcalinos em uma única etapa, bem como pré-tratamentos sequenciais ácido-alcalinos e alcalinos-ácidos em duas etapas, a fim de melhorar a hidrólise enzimática da BC desengordurada. A BC foi primeiramente desengordurada e, em seguida, submetida a diferentes protocolos de pré-tratamento. A eficácia de cada método foi avaliada por meio da redução do rendimento de açúcares e da morfologia da superfície via microscopia eletrônica de varredura (MEV), com significância estatística avaliada por meio de ANOVA de uma via e teste HSD de Tukey. Os resultados indicaram que o pré-tratamento ácido em uma única etapa produziu um aumento de 1,5 vezes no rendimento de açúcares, enquanto os tratamentos alcalinos com KOH ou NaOH aumentaram a produção de açúcares de 2,97 a 3,28 vezes em relação ao bagaço de cana-de-açúcar não tratado. O maior rendimento de açúcares foi obtido por meio do pré-tratamento

potential bioconversion into value-added bio-based products while promoting sustainable waste management in the coffee industry.

Keywords: Spent Coffee Grounds. Comparative Analysis. Chemical Pre-treatment. ANOVA Analysis. Sugar Recovery.

ácido-alcalino em duas etapas, que resultou em um aumento de 4,25 a 4,70 vezes em comparação com o controle. A análise por microscopia eletrônica de varredura (MEV) confirmou a significativa desestruturação e o aumento da porosidade após o pré-tratamento alcalino, melhorando assim a acessibilidade das enzimas. As descobertas contribuem para a otimização da valorização do bagaço de cana-de-açúcar para potencial bioconversão em produtos de base biológica de alto valor agregado, promovendo, ao mesmo tempo, a gestão sustentável de resíduos na indústria cafeeira.

Palavras-chave: *Borra de Café Usada. Análise Comparativa. Pré-tratamento Químico. Análise ANOVA. Recuperação de Açúcar.*

1 INTRODUCTION

According to the International Coffee Organization (ICO, 2025), global coffee consumption is rebounding following the COVID-19 pandemic. During the 2022/23 coffee year, consumption declined by 2.0% to 173.1 million bags due to high living costs, reduced disposable income, and stock drawdowns, despite coffee's relatively inelastic demand. However, forecasts for 2023/24 are positive, with a projected 2.2% increase in consumption to 177.0 million bags, driven by anticipated global economic growth exceeding 3.0% and restocking efforts. Although coffee is valued for its flavor and aroma, its production poses environmental issues, particularly from the waste generated in form of spent coffee grounds (SCGs). SCGs are the primary solid waste resulting from coffee brewing with about 650 kg produced per ton of raw coffee beans and 2 kg generated for every kilogram of instant coffee during industrial processing (Saratale *et al*, 2020; Zhao *et al*., 2024). Meanwhile, around 60 million kilograms of SCGs are globally produced daily, contributing to the over 2,000 million tons of waste and by-products generated annually by the coffee industry (Cavanagh *et al*., 2023; Cobo-Ceacero *et al*., 2023). A large portion of SCGs is disposed of in landfills or incinerated, leading to increased greenhouse gas emissions (Roychand *et al*., 2023). SCGs in landfills pose environmental risks as dried SCGs are flammable, and their decomposition can release methane and carbon dioxide, both potent greenhouse gases that contribute to global warming (Forcina *et al*., 2023; Xia *et al*., 2023).

SCGs are lignocellulosic biomass primarily composed of cellulose, hemicellulose,

and significant amounts of polysaccharides including mannans (46.8%), galactose (30.4%), glucose (19.0%), and arabinose (3.8%), which form the complex carbohydrate structure of the material (Mussatto *et al*., 2011). Recently, there has been growing global interest in converting SCG components into a valuable resource for diverse commercial uses. Within the framework of a circular economy, SCGs hold potential across multiple industries. Their bioactive compounds can be applied in the pharmaceutical and cosmetic products while their sugar components can be utilized in the energy sectors. Additionally, the leftover material after extracting these compounds can serve as fertilizer, fuel additive, or raw material for biodegradable products (Sidło and Latosińska, 2024). However, the extraction of sugars from SCG is challenging primarily due to the complex lignocellulosic structure that is rigid, dense, and recalcitrant (Trinh *et al*., 2022). This structure forms a compact and resistant matrix that presents a significant barrier to sugar extraction and limits the release of fermentable sugars (Gosławski, 2024). Hence, an effective pre-treatment strategy is required to improve the accessibility of cellulose and hemicellulose for hydrolysis process.

Pre-treatment techniques are utilized to enhance the hydrolysis of lignocellulose by increasing enzyme access to polysaccharides (Lee *et al*., 2021; Wongsiridetchai *et al*., 2018). The presence of high lignin content hinders enzymatic hydrolysis, while hemicellulose must be broken down to release sugars (Sheng *et al*., 2021; Yoo *et al*., 2020). Therefore, removing lignin and breaking down hemicellulose are crucial steps for successful pre-treatment and efficient hydrolysis. Single-process pre-treatment methods often fail to produce desirable outcomes due to the complex and recalcitrant nature of lignocellulosic biomass. Components such as lignin, hemicellulose, and acetyl groups form a rigid and compact structure that hinders enzyme accessibility to cellulose (Zhang *et al*., 2021). These structural barriers are not effectively disrupted by a single type of chemical, physical, or biological treatment alone, resulting in low sugar yields and inefficient downstream processing (Ravindran *et al*., 2017; Trinh *et al*, 2022). To address this challenge, a sequential or combined pre-treatment strategy has emerged as a promising approach. This method integrates two or more pre-treatment techniques to synergistically remove hemicellulose and lignin, reduce biomass crystallinity, and enhance porosity. Unlike one-step pre-treatments, combined methods are capable of breaking down multiple barriers simultaneously or in succession, thereby improving enzyme accessibility and fermentable sugar recovery from complex biomass.

In a comparative study, it was observed that alkaline pre-treatment of raw SCG with sodium hydroxide yielded a higher sugar concentration, which produced 1.438 mg/mL compared to acid pre-treatment with sulfuric acid that produced 0.453 mg/mL (Jin *et al*., 2020). This showed that alkaline pre-treatment with sodium hydroxide was more effective in enhancing sugar recovery from SCGs compared to acid pre-treatment with sulfuric acid. But, further investigation into this comparison has not been pursued. Several studies have explored combine two-step pre-treatment approaches (Ravindran *et al*., 2017; Trinh *et al*, 2022). These studies reported that sequential pre-treatment methods yielded significantly higher reducing sugar compared to single-step pre-treatment, indicating that a combined approach is more effective in overcoming the structural resistance of SCGs and enhancing enzymatic hydrolysis. However, no studies have reported on a two-step pre-treatment strategy involving both acid and alkali treatments for defatted SCG pre-treatment. Defatting spent coffee grounds is the process of removing residual oils and lipids from the biomass, typically using solvents such as hexane or ethanol, to enhance the efficiency of subsequent extraction or conversion processes by improving accessibility to the structural carbohydrates. Therefore, this study aimed to conduct a comparative analysis between one-step pre-treatment, either using acid or alkali and two-step pre-treatment by combining both acid and alkali of defatted SCG following enzymatic hydrolysis using One-Way ANOVA and Post Hoc Comparison Test: Tukey's HSD as well as to analyze the surface morphology of the raw, defatted and pre-treated samples using scanning electron microscope (SEM).

2 MATERIALS AND METHODS

2.1 Collection, pre-treatment and storage of SCGs

SCGs used in this study were collected from Richiamo Café, located at Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan, Sarawak. After collection, the SCGs were subjected to oven drying at 85 °C for a period of seven days. Once fully dried, the SCGs were finely ground using a mechanical grinder before subsequently passed through a sieve to obtain consistent-sized particles. The sieved SCG were then stored in an airtight container at room temperature to preserve their condition prior to further experimental procedures.

2.2 Defatting of SCGs

The defatting process of SCGs were carried out using a sonication-assisted extraction technique. In this procedure, hexane was used as the solvent for lipid extraction, applied to the SCGs in a solvent-to-solid ratio of 5:1 (v/w). Following the sonication process, the defatted SCG were thoroughly dried in a laboratory oven at 85 °C. Once completely dried, the treated biomass was stored in an airtight container until further analysis.

2.3 Pre-treatment of defatted SCGs

The defatted SCGs were subjected to various chemical pre-treatment methods as outlined in Table 1 below. These included one-step treatments using either 1% (v/v) sulfuric acid (H_2SO_4) at 60 °C for 1 hour, 3% (w/v) potassium hydroxide (KOH) at 90 °C for 2 hours, or 3% (w/v) sodium hydroxide (NaOH) at 85 °C for 1 hour. Additionally, sequential two-step pre-treatments were employed, combining acid and alkali in different orders: KOH–H_2SO_4, H_2SO_4–KOH, NaOH–H_2SO_4, and H_2SO_4–NaOH, each following the same individual treatment conditions:

Table 1

Different conditions of SCG pre-treatments.

Pre-treatments	Condition
Control (Untreated defatted SCG)	No treatment, only enzymatic hydrolysis
H_2SO_4	1% (v/v) H_2SO_4 ; 60 ˚C ; 1 hour
KOH	3% (w/v) KOH ; 90 ˚C; 2 hour
NaOH	3% (w/v) NaOH ; 85 ˚C; 1 hour
KOH-H_2SO_4	3% (w/v) KOH ; 90 ˚C; 2 hours 1% (v/v) H_2SO_4 ; 60 ˚C ; 1 hour
H_2SO_4-KOH	1% (v/v) H_2SO_4 ; 60 ˚C ; 1 hour 3% (w/v) KOH ; 90 ˚C; 2 hours
NaOH-H_2SO_4	3% (w/v) NaOH ; 85 ˚C; 1 hour 1% (v/v) H_2SO_4 ; 60 ˚C ; 1 hour
H_2SO_4-NaOH	1% (v/v) H_2SO_4 ; 60 ˚C ; 1 hour 3% (w/v) NaOH ; 85 ˚C; 1 hour

2.4 Pre-treatment of defatted SCGs

The pre-treated SCG samples were filtered using a tea filter and thoroughly rinsed with distilled water to eliminate residual alkali. They were then dried in an oven at 50 °C for 6 hours. Enzymatic hydrolysis was carried out using a 3% (w/v) substrate loading of the pre-treated defatted SCG in 50 mL of 50 mM citric acid buffer (pH 4.8), incubated in a shaking water bath at 50 °C and 100 rpm for 72 hours. The hydrolysis process included the addition of cellulase (95 FPU/mL) derived from Trichoderma reesei (Sigma-Aldrich), applied at 5% (w/v) relative to the SCG, following the method described by Trinh *et al* (2022). Cellulase activity was assessed according to IUPAC standards (Miller, 1959; Ghose, 1987). To prevent microbial contamination, 0.5 mL of 0.05% sodium azide was added, along with 0.5 mL of 1.0% (v/v) Tween 80 to enhance the efficiency of enzyme (Igbojionu, 2020).

2.5 Total Reducing Sugar (TRS) analysis after pre-treatments

A calibration curve was generated using glucose as the standard. The total reducing sugar (TRS) assay was conducted using dinitrosalicylic acid (DNS). Specifically, 100 µL of the sample was transferred into a test tube and diluted with 2, 900 µL of distilled water to achieve a 1:30 dilution. Subsequently, 1 mL of DNS reagent containing Rochelle salt was added to the mixture. The solution was then boiled for 10 minutes. After cooling, the absorbance was measured at 540 nm using a spectrophotometer. The TRS yield and TRS recovery were determined using the following equation:

$$\text{Total reducing sugar yield, TRS (\%)} = \frac{C_{TRS}V_H}{M_{initial}} \times 100 \qquad (1)$$

$C_{TRS}V_H$ – Total reducing sugar concentration obtained from the calibration curve

$M_{initial}$ – The weight of the initial dry lipid-free biomass before hydrolysis.

2.6 Structural characterization of SCG using SEM analysis

SCGs were examined using a JSM-IT800 Schottky Field Emission Scanning Electron Microscope (SEM) from JEOL. The samples were lightly sprinkled onto black adhesive tape and coated with a thin layer of platinum using an autocoater. SEM imaging was performed at an accelerating voltage of 10 kV, with magnifications of 500×, 2,000×, and 5,000×.

3 RESULTS AND DISCUSSION

3.1 Defatting of SCG and chemical pre-treatment method selection

Reducing sugar yielded from SCG can be obtained without defatting process. However, a study showed that defatting can enhance sugar recovery, as the removal of residual lipids improves the accessibility of chemical agents and enzymes to the lignocellulosic structure (Trinh *et al*., 2022). The presence of oils in untreated SCG can form hydrophobic layers that hinder efficient penetration of hydrolytic agents, leading to lower sugar yields. By implementing a defatting step prior to chemical or enzymatic hydrolysis, the amount of fermentable sugars released in this study was increased, demonstrating that lipid removal is a beneficial pre-treatment step in maximizing the bioconversion efficiency of SCG. Besides, acid pre-treatment using H_2SO_4 was employed primarily to solubilize hemicellulosic sugars from SCG. This approach disrupts the lignocellulosic matrix and specifically targeting hemicellulose by releasing monomeric sugars into the liquid fraction for efficient recovery of reducing sugar (Go *et al*., 2016). H_2SO_4 is effective at hydrolyzing complex carbohydrates in biomass and breaking them down into simpler reducing sugars and thereby improving overall reducing sugar yield. This process occurs under relatively mild conditions, minimizing cellulose degradation while preserving it for further enzymatic hydrolysis (López-Linares *et al*., 2021).

Meanwhile, alkaline reagents such as KOH and NaOH, were selected for their comparatively lower corrosiveness than acidic reagents like H_2SO_4 (Kim *et al*., 2016). Their use is supported by evidence showing their ability to effectively break down the lignin structure in biomass, reducing its recalcitrance and enhancing enzyme accessibility during enzymatic hydrolysis. This, in turn, promotes the release of fermentable sugars.

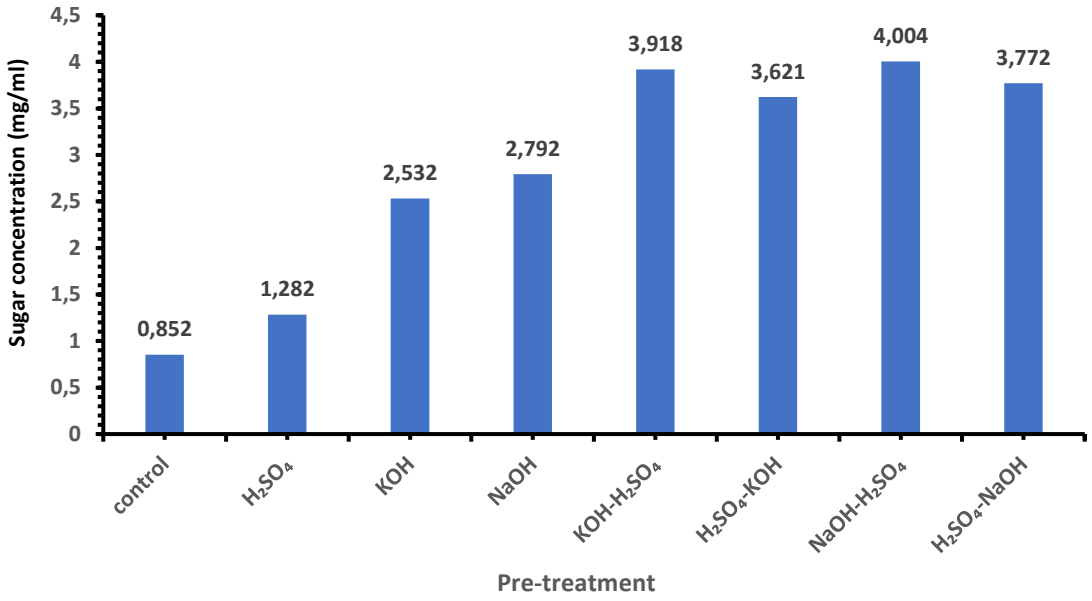
KOH was preferred over NaOH due to its greater effectiveness in removing recalcitrant plant components, especially lignin (Paixão *et al*., 2016). NaOH was particularly effective in breaking the bonds between lignin and hemicellulose within lignin-carbohydrate complexes (LCC) (Kim *et al*., 2016). During this process, NaOH dissociates into sodium (Na^+) and hydroxide (OH^-) ions, with the rising concentration of hydroxide ions accelerating the hydrolysis rate (Modenbach *et al*., 2013). Meanwhile, KOH pre-treatment showed strong potential for practical application. KOH effectively removed lignin and extractives, significantly improving enzyme accessibility to the biomass and thereby enhancing the efficiency of subsequent enzymatic hydrolysis (Chi *et al*., 2019).

3.2 Statistical Analysis between One-step and Two-Step Chemical Pre-treatment

The efficacy of one-step acidic pre-treatment using H_2SO_4, alkaline pre-treatment using KOH and NaOH as well as two-step pre-treatments by combining acid-alkali and alkali-acid sequences were investigated. The average concentration of reducing sugar yield after pre-treatment is a key factor influencing enzymatic hydrolysis efficiency. Effective bioconversion of a lignocellulosic substrate requires adequate exposure of cellulose fibres and the partial removal of hemicellulose and lignin (Ravindran *et al*., 2017). Spent coffee waste was subjected to sugar analysis at each stage of pre-treatment following enzymatic hydrolysis. The summarised data in Figure 1 shows the total concentration of reducing sugar produced after enzymatic hydrolysis of SCG following various pre-treatments, compared to the control.

Figure 1

Total reducing sugar released after enzymatic hydrolysis of pre-treated SCG waste under various pre-treatment conditions.



3.2.1 One-Way ANOVA and Post Hoc Comparison Test: Tukey's HSD

Based on reducing sugar yield, the best pre-treatment strategy for SCG involved a two-step process: 3% (w/v) NaOH at 85 °C for 1 hour, followed by 1% (v/v) H_2SO_4 at 60°C for 1 hour. This approach yielded the highest reducing sugar concentration of 4.00 mg/mL where this represent a 4.7-fold increase compared to the control. Subjecting SCG to sequential chemical pre-treatment resulted in the removal of considerable fractions of hemicellulose and enhance the conversion of cellulose into reducing sugar by increasing its accessibility for enzymatic hydrolysis. The ANOVA analysis were conducted to find if there is any significant difference between the untreated and chemically treated samples. The results for ANOVA analysis is summarized in Table 2. The p-value associated with the F-statistic in the one-way ANOVA is below 0.01, indicating strong evidence that at least one pair of treatments differs significantly than the other.

Table 2

ANOVA analysis of SCG pre-treatment.

Groups	Count	Sum	Average	Variance
(A) Control	3	2.556	0.852	0.001137
(B) H_2SO_4	3	3.846	1.282	0.000907
(C) KOH	3	7.596	2.532	0.000931
(D) NaOH	3	8.376	2.792	0.000496
(E) KOH-H_2SO_4	3	11.754	3.918	0.000849
(F) H_2SO_4-KOH	3	10.863	3.621	0.021619
(G) NaOH-H_2SO_4	3	12.012	4.004	0.000201
(H) H_2SO_4-NaOH	3	11.316	3.772	0.000496

Source of Variation	SS	df	MS	F	P-value	F- critical
Between Groups	31.41567	7	4.487953	1347.936	5.97E-21	2.657197
Within Groups	0.053272	16	0.00333			
Total	31.46894	23				

Further analysis was conducted by performing a post hoc test. The result of the post hoc test is depicted in Table 3. Post hoc tests, also known as multiple comparison tests, assess the statistical significance of differences between group means after conducting an ANOVA that indicates an overall difference (Nanda *et al*., 2021). These methods focus on comparing specific pairs of means, providing researchers with the most relevant insights into the data. One way to address the multiple comparison problem is to analyze each comparison separately using an appropriate statistical method. In this study, Tukey's Honestly Significant Difference (HSD) test was applied to compare each pair of means. This method was used in this study to minimize Type 1 errors during statistical analysis. The Type I error rate (false positive rate) occurs because each additional test increases the chance of incorrectly rejecting a true null hypothesis (Nanda *et al*., 2021; Ostertagová and Ostertag, 2013). Tukey's HSD test is widely accepted as an effective technique for controlling Type 1 errors. Tukey's HSD test was conducted on 28 pairs among the k = 8 treatments to determine which pairs show a statistically significant difference. The critical value of Q-statistic (6.0796) was compared to the calculated Tukey HSD Q-statistic. If the value of the calculated Q-statistic is larger than the critical value, then there is a significant different between the compared pre-treatment at 99% confidence level.

Based on the results, there is a significant difference between raw and defatted SCG samples compared to all treated SCG samples. Additionally, a significant difference

is also observed between acid-treated and alkaline-treated samples, as well as between NaOH- and KOH-treated samples. Besides, there is significant difference between NaOH-H_2SO_4 treated samples with H_2SO_4-KOH and H_2SO_4-NaOH treated samples respectively. Since NaOH-H_2SO_4 treated samples produce a greater sugar concentration than the other two sequences, then it might be a better treatment. Furthermore, there is a significant difference between KOH-H_2SO_4 and H_2SO_4-KOH treated samples but no significant difference between KOH-H_2SO_4 and H_2SO_4-NaOH treated samples whereas H_2SO_4-KOH and H_2SO_4-NaOH treated samples showed no significant difference, suggesting they may have a similar effect on the results. But, KOH-H_2SO_4 treated samples may be the better treatment, as it produces relatively higher sugar concentrations than the other two. Interestingly, there is no significant difference between KOH-H_2SO_4 and NaOH-H_2SO_4 treated samples, suggesting that either can be used or may produce similar results. However, since NaOH-H_2SO_4 treated samples consistently produce more reducing sugar than KOH-H_2SO_4 treated samples, the result suggest that NaOH-H_2SO_4 treated samples is the overall better treatment.

Table 3

Result for Tukey's HSD test.

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD conclusion
A vs B	12.9074	0.0010053	** p<0.01
A vs C	50.4290	0.0010053	** p<0.01
A vs D	58.2335	0.0010053	** p<0.01
A vs E	92.0329	0.0010053	** p<0.01
A vs F	83.1178	0.0010053	** p<0.01
A vs G	94.6144	0.0010053	** p<0.01
A vs H	87.6504	0.0010053	** p<0.01
B vs C	37.5216	0.0010053	** p<0.01
B vs D	45.3261	0.0010053	** p<0.01
B vs E	79.1255	0.0010053	** p<0.01
B vs F	70.2104	0.0010053	** p<0.01
B vs G	81.7070	0.0010053	** p<0.01
B vs H	74.7430	0.0010053	** p<0.01
C vs D	7.8045	0.0010053	** p<0.01
C vs E	41.6039	0.0010053	** p<0.01
C vs F	32.6888	0.0010053	** p<0.01
C vs G	44.1854	0.0010053	** p<0.01
C vs H	37.2214	0.0010053	** p<0.01
D vs E	33.7994	0.0010053	** p<0.01
D vs F	24.8843	0.0010053	** p<0.01
D vs G	36.3809	0.0010053	** p<0.01
D vs H	29.4169	0.0010053	** p<0.01
E vs F	8.9151	0.0010053	** p<0.01
E vs G	2.5815	0.6019860	insignificant

E vs H	4.3825	0.0971818	insignificant
F vs G	11.4966	0.0010053	** p<0.01
F vs H	4.5326	0.0805022	insignificant
G vs H	6.9640	0.0029636	** p<0.01

k = 8; *v* = 16; Q-critical = 6.0796; α = 0.01

3.2.2 Comparison between one-step and two-step pre-treatment

The statistical analysis of Tukey's HSD test prove that there is a significant different between one-step and two-step pre-treatment of defatted SCG. One-step pre-treatment using H_2SO_4 produced 1.50 folds of reducing sugar while one-step alkaline pre-treatment using KOH and NaOH produced 2.97 to 3.28 folds of reducing sugar in comparison to control which according to the multiple comparison test were significantly less compared to two-step pre-treatment that combines acid and alkaline that produces 4.25 to 4.70 folds of reducing sugar. Furthermore, the enzymatic hydrolysis procedure remained consistent across all experiments, with cellulase used to break down cellulose into sugars. The alkaline pre-treatments applied were shown to enhance the conversion of complex polysaccharides, such as cellulose, into reducing sugars like glucose, as indicated by the glucose standard curve in the DNS analysis. This improvement is attributed to the structural and compositional changes induced by both one-step and two-step pre-treatments, which increased the efficiency of cellulase in releasing free sugars during hydrolysis (Zhang *et al*., 2021).

Several studies have shown that enzymatic hydrolysis is a complex process involving enzyme diffusion (Huang *et al*., 2024; Zhang *et al*., 2016). In this process, cellulase molecules diffuse from the solution into SCG, penetrating the pore structures and adsorbing onto specific binding sites where hydrolysis occurs. The enzyme then interacts with the active sites of cellulose on the surface. According to Zhang *et al*. (2021), the rate and extent of hydrolysis depend on how quickly the enzyme moves from the liquid phase to the biomass, which in turn is affected by pore structure, fluid dynamics, and the enzyme concentration gradient. In this study, cellulase was specifically used to convert cellulose into reducing sugars measured as glucose equivalents. However, since SCG contains a higher proportion of hemicellulose, further research is needed to evaluate the use of enzymes capable of directly hydrolyzing hemicellulose into fermentable sugars.

The application of one-step alkaline pre-treatments using KOH and NaOH offers a simplified, straightforward and time-efficient approach which can effectively disrupt

the lignocellulosic structure of SCGs, including breaking down lignin and hemicellulose, leading to increased surface area and improved enzymatic digestibility by enhancing the accessibility of cellulose for subsequent enzymatic hydrolysis to facilitate the release of fermentable sugars (Zhang *et al*., 2021). This method is generally cost-effective due to lower chemical requirements and shorter processing times. Studies have shown that one-step alkaline pre-treatment can achieve high delignification rates, making it suitable for bioethanol and biogas production (Huang *et al*., 2024; Sayoud *et al*., 2025).

However, one-step alkaline pre-treatments have certain limitations. The efficiency of sugar extraction may be lower compared to more intensive methods, and the high pH required for this process can cause corrosion of equipment as the process generate significant amounts of alkaline waste, posing environmental disposal and economic challenges. Moreover, the use of strong bases can lead to the formation of inhibitory compounds, such as phenolic derivatives, potentially hindering downstream bioconversion processes (Bonfim *et al*., 2023; Pereira *et al*., 2022). Additionally, the high alkalinity of the resulting slurry may require extensive neutralization, adding to processing costs. Furthermore, the structural complexity of SCGs may require more robust treatment to achieve optimal yields of desired products.

On the other hand, two-step alkaline-acid pre-treatment process was developed in this study to address these challenges since this method offers a more nuanced approach. Based on the result, the more favourable approach was to involve an initial alkaline hydrolysis using NaOH to solubilized lignin, followed by an acid using H_2SO_4 treatment to breakdown hemicellulose while neutralizing the base at the same time. This the accessibility of cellulose for enzymatic hydrolysis. This sequential approach can lead to higher overall sugar yields and reduced inhibitor formation compared to one-step alkaline pre-treatment, making it more suitable for downstream biological processes (Lee *et al*., 2021; Niglio *et al*., 2019). This result is coherent with multiple studies demonstrated that two-step pre-treatments can significantly increase the yield of fermentable sugars (Ravindran *et al*., 2017; Trinh *et al*., 2022).

Despite the improved efficiency, two-step pre-treatments come with their own set of drawbacks. The process is more complex, requiring more chemicals, increased energy consumption, time-consuming, and involve multiple stages. This translates to higher operational costs and potentially greater environmental impact. Moreover, the use of both acidic and alkaline chemicals necessitates careful handling and neutralization steps to

mitigate environmental impacts. Therefore, while two-step pre-treatments can enhance product yields, the decision to implement such a process should consider the balance between the increased output and the associated economic and environmental costs.

Whether the added complexity of two-step pre-treatment is beneficial, it depends on largely on the specific application and economic considerations. One-step alkaline pre-treatment is advantageous for its simplicity and lower operational costs, making it appealing for large-scale applications where time and resource efficiency are critical. However, its environmental impact and potential for inhibitor formation may limit its suitability for certain applications. On the other hand, the two-step acid-alkaline method, while more resource-intensive, offers superior sugar yields and reduced inhibitor formation, which can justify the additional investment in contexts where high efficiency and product quality are prioritized. Recent studies suggest that the choice of pre-treatment method should align with the intended end-use of the SCG, such as biofuel production, material synthesis, or chemical extraction (Cho *et al*., 2022; Gu *et al*., 2023).

If the primary goal is maximizing sugar yields for biofuel production or other bioconversion processes (Gu *et al*., 2023; Hamedani *et al*., 2022), the higher yields achieved with two-step pre-treatment may justify the increased costs. While two-step pre-treatment can offer superior sugar yields and reduced inhibitor formations, its higher cost and complexity must be weighed against the simpler and more cost-effective one-step alkaline treatment. Further research focusing on minimizing inhibitor formation and maximizing sugar recovery in both methods is crucial for the economic viability of SCG valorization. Meanwhile, for applications where lower yields are acceptable, such as composting or direct combustion (Bonfim *et al*., 2023; Santos *et al*., 2017), the simplicity and lower cost of one-step alkaline pre-treatment may be preferable.

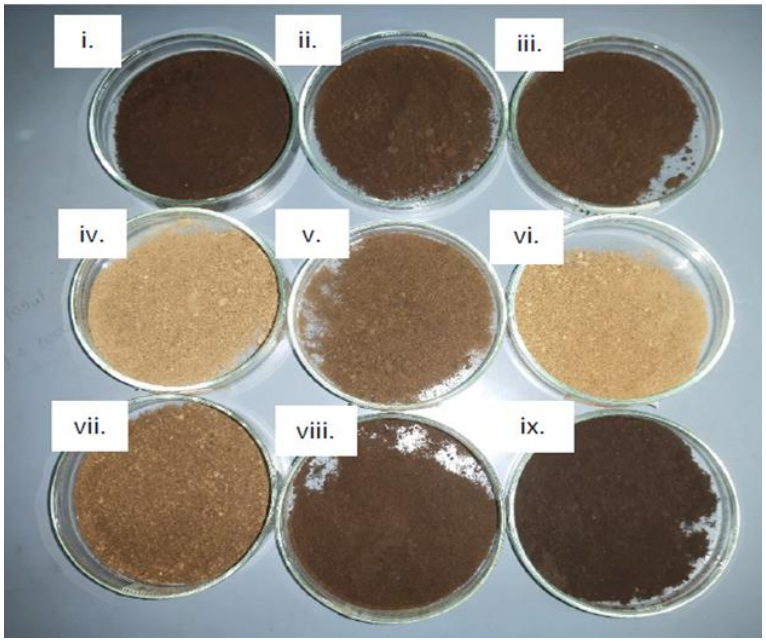
A thorough techno-economic assessment, considering factors such as raw material costs, chemical inputs, energy consumption, and product yields, is essential for determining the most suitable pre-treatment strategy. Ultimately, both one-step and two-step chemical pre-treatment methods have their merits and drawbacks. The decision to adopt either approach depends on a careful evaluation of the specific requirements and goals of the valorization process, such as cost-effectiveness, environmental impact, and desired product quality. Hence, the choice of pre-treatment should be guided by a thorough evaluation of technical, economic, and environmental factors to ensure sustainable and efficient utilization of SCGs.

3.3 Surface and morphological analysis of SCG samples

The samples for raw, defatted, one-step acid or alkaline pre-treated as well as two-step acid-alkali pre-treated samples of SCGs following enzymatic hydrolysis are depicted below in Figure 2. The raw and defatted SCG samples (Figure 2. i-ii) exhibit a denser morphology compared to the particles of chemically pre-treated SCG samples (Figure 2 iii-ix) which consist of thin material sheets powder that resemble sawdust. The raw SCGs has a dark brown, soil-like and dense morphology primarily because of the high concentration of organic compounds according to Hu *et al*. (2022) and Waisarikit *et al*. (2025). The raw SCG loses its deep brown colour after the defatting process due to delignification and decolourization following pre-treatment and enzymatic hydrolysis process, which lead to the extraction of organic compounds (Yang *et al*., 2023). Besides, the alkaline-treated SCG samples had an even lighter brown colour while the particulate of the samples are smaller compared to both raw and defatted SCGs.

Figure 2

Samples of (i) raw SCG, (ii) defatted SCG, (iii) H_2SO_4 pre-treated SCG,
(iv) KOH pre-treated SCG, (v) KOH- H_2SO_4 pre-treated SCG
(vi) H_2SO_4-KOH pre-treated SCG, (vii) NaOH pre-treated SCG,
(viii) NaOH-H_2SO_4 pre-treated SCG, (ix) H_2SO_4-NaOH pre-treated SCG.



Besides, Figure 3 (a-i) depicted the micrographs of raw SCG, defatted SCG, acid-treated, alkaline-treated and acid-alkali treated SCG samples following enzymatic hydrolysis that were observed and analyzed using SEM in order to understand the changes in the surface structure of SCG. SEM micrographs allows for the examination and analysis of the microstructural changes in SCG after one-step and two-step pre-treatment under different conditions. Prior to the analyses, the samples were coated with a thin layer of platinum to improve image quality. These images were obtained by applying an acceleration the voltage of 10 kV, at 500-fold, 2,000-fold and 5,000-fold magnifications. These SEM images highlighted the unique and distinctive surface morphologies of SCG when subjected under different conditions. As shown in Figure 3 (a), the raw SCG display a dense and irregular surface morphology, similar to the report in previous study by Afolabi *et al*. (2020).

Meanwhile, the defatted SCG also exhibit a noticeably irregular yet flatter and smoother surface compared to the raw SCG while featured improve pores and loosen structures with fewer gaps. When viewed closer in Figure 3 (b), the surface of defatted SCG appear to have many uneven, bowl-shaped structures, but the structure still relatively flat and compact. The acid-treated SCG sample using H_2SO_4 develops a clumpy texture with small chunks as tiny pores develop on its uneven surface (Figure 3 c). After one-step alkaline pre-treatment using KOH and NaOH, the SCG particles showed signs of broken components into smaller pieces and had many wrinkles or creases as shown in Figure 3 (d) and Figure 3 (g). Regardless of the pre-treatment conditions, the surface overview of alkaline-treated SCG was smooth with formation of many smaller pores in between the irregular structures. This indicated that the pre-treatment of SCG using KOH and NaOH had transformed the surface structures of the SCG. From these figures, an increment in both the number and size of folds as well as porous sites can be observed. These folds and pores increased the surface area of the coffee grounds which make it easier for enzymes to reach the cellulose and hemicellulose.

Upon further magnification, the pores and tunneling structures for SCG sample after NaOH pre-treatment was also hollower than the SCG samples treated with KOH. Despite the pre-treatment of SCG using alkaline shows a significant pores enlargement as the surface of the SCG became more uneven, the tunneling structure of the SCG remain intact which is why alkaline pre-treatment mainly used for lignin removal since its results in less sugar degradation than acid pre-treatment (Alvira *et al*., 2010; Hudeckova *et al*.,

2018; Titiri *et al*., 2023). Furthermore, two-step acid-alkaline pre-treated SCGs after enzymatic hydrolysis, depicted in Figure 3 (e-f) and Figure 3 (h-i) displayed an even more fractured and fragmented particles. This effect is caused by the dual effect of acid and alkaline pre-treatment as well as an ongoing degradation of cellulose by the enzyme. Enzymatically hydrolyzed two-step alkaline-treated SCGs exhibited different morphological patterns that characterized by tunneling features, pore formation and enlargement, as well as microstructural fragmentation that were significantly smaller and less chunky in comparison to SCGs after one-step acid or alkaline pre-treatment as well as raw and defatted SCGs.

Figure 3

SEM micrographs of (a) raw SCG sample, (b) defatted SCG sample, and (c) H_2SO_4 pre-treated sample.

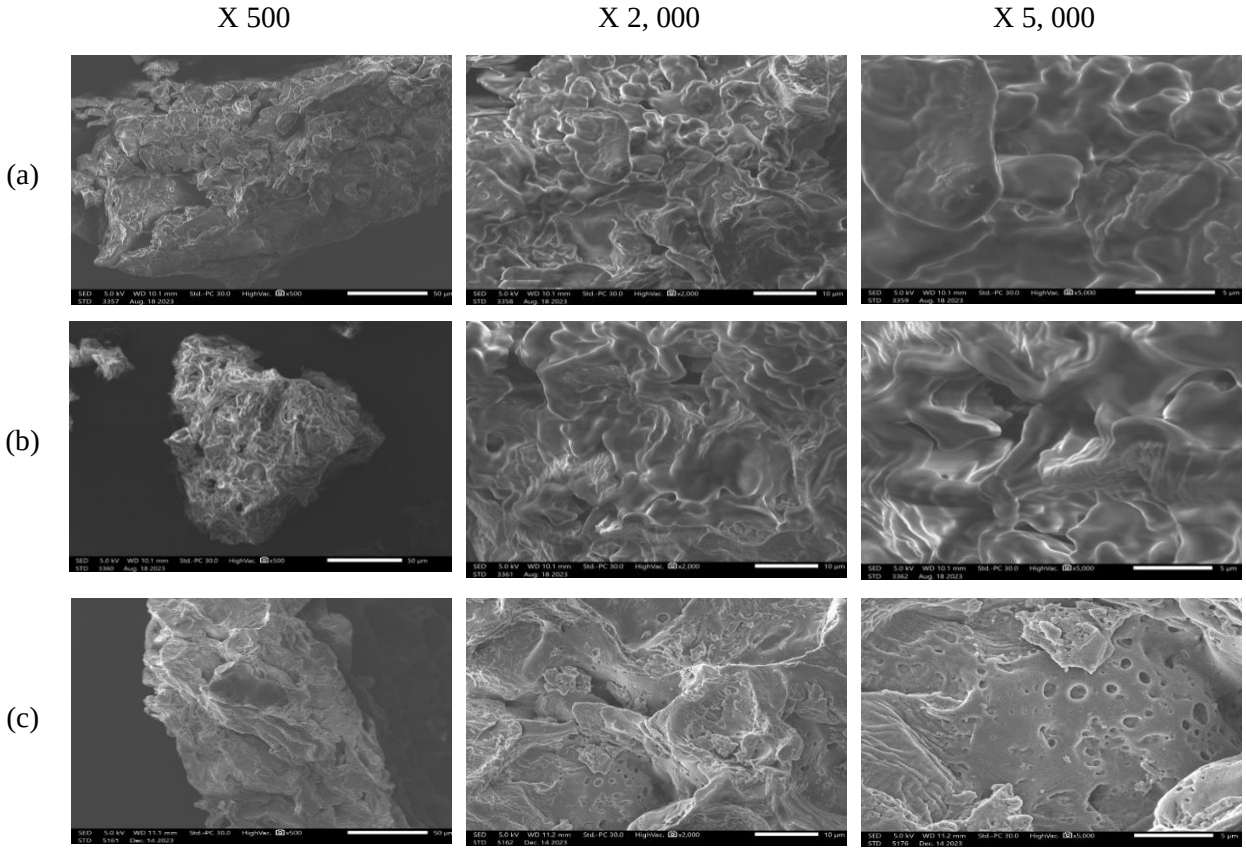
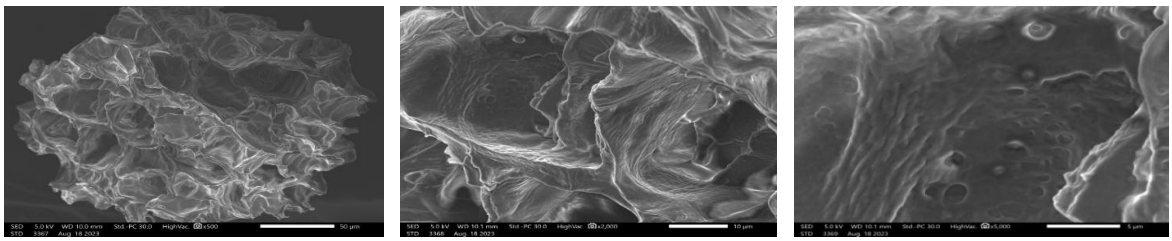


Figure 3

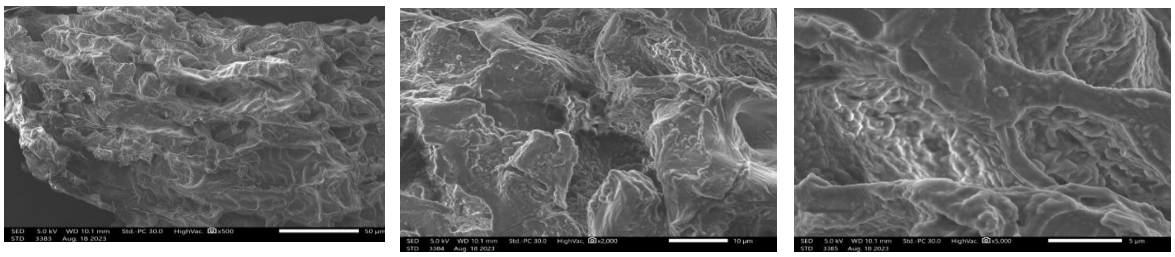
SEM micrographs of (d) KOH pre-treated sample, (e) KOH- H_2SO_4 pre-treated sample, and (f) H_2SO_4-KOH pre-treated sample.

X 500 X 2, 000 X 5, 000

(d)



(e)



(f)

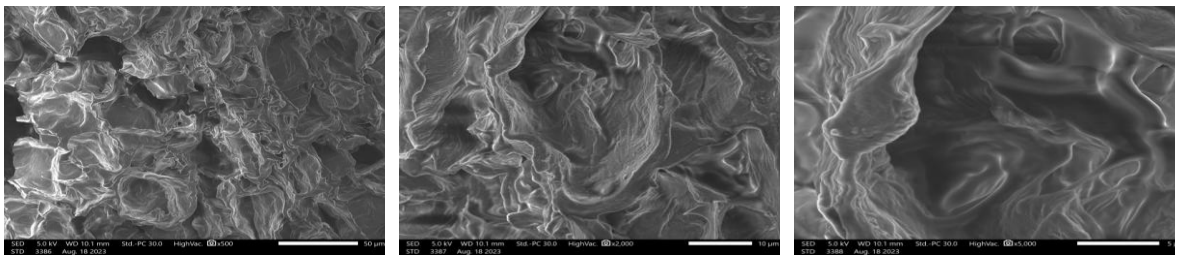
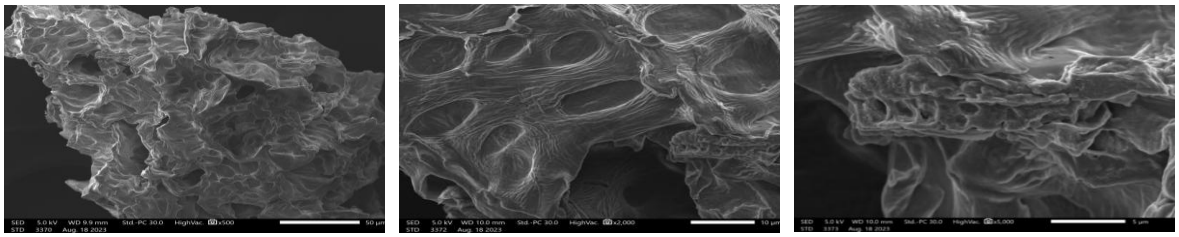


Figure 3

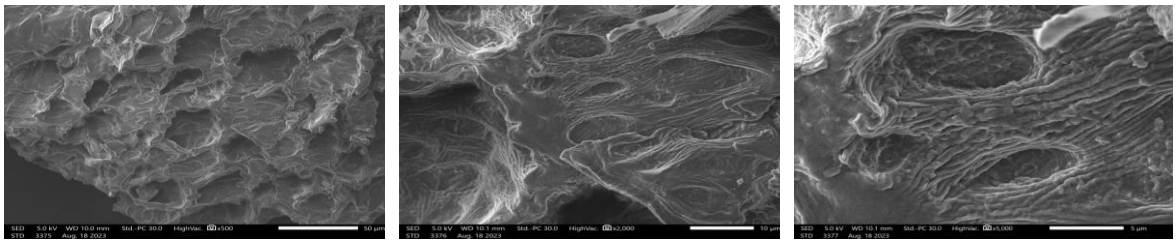
SEM micrographs of (g) NaOH pre-treated sample, (h) NaOH-H_2SO_4 pre-treated sample, and (i) H_2SO_4-NaOH pre-treated sample..

X 500 X 2, 000 X 5, 000

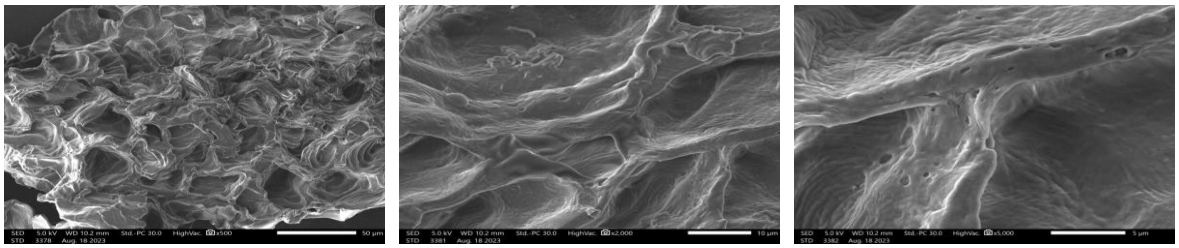
(g)



(h)



(i)



4 CONCLUSION

In conclusion, this study discovered that there is a significance difference between one-step and two-step pre-treatment of the defatted SCG. One-step acidic pre-treatment boosted around 1.50 times reducing sugar production, while a one-step alkaline pre-treatment of defatted SCG using KOH and NaOH yielded an increase of 2.97 to 3.28 times compared to the control. However, these single-step methods yielded significantly less reducing sugars than two-step acid-alkaline pre-treatment, which produced 4.25 to 4.70 times reducing sugar. The two step chemical process resulted in a slightly less than one unit increase in reducing sugar compared to the one-step chemical methods. One-step alkaline pre-treatments using KOH or NaOH offers a simpler, faster, and more direct approach compared to the two-step acid-alkaline pre-treatment. Although two-step treatments were more effective, one-step alkaline pre-treatments offer a more economical alternative with lower environmental impact. The latter, while producing more reducing sugar, requires more chemicals, energy, and time leading to increased operational costs and a larger environmental footprint. The economic viability of SCG valorisation hinges on whether the increased complexity of two-step pre-treatment justifies the added cost. This depends on the specific application and economic factors.

The structural analysis of SCG before and after pre-treatment provided further insights into the effectiveness of alkaline pre-treatment in enhancing sugar recovery. SEM analysis revealed that raw SCG exhibited a dense and irregular surface, while defatted SCG showed a smoother texture with small gaps. Upon alkaline treatment, significant morphological changes were observed, with the development of tunnel-like structures, enlarged hollow pores, and increased surface fragmentation. These modifications indicate successful disruption of the lignocellulosic matrix, making the substrate more accessible to enzymatic hydrolysis. The enhanced porosity and reduced recalcitrance of the pre-treated SCG contributed to the improved efficiency of cellulase action, ultimately leading

to a higher sugar yield. These findings reaffirm the effectiveness of alkaline pre-treatment in breaking down the structural complexity of SCG, facilitating its potential for further bioconversion into value-added bio-based products. It is recommended that future studies explore the use of additional enzymes, such as hemicellulose to enhance sugar yield by specifically targeting the hemicellulosic structure of SCG. Incorporating hemicellulase alongside cellulase could significantly improve the overall concentration of reducing sugars. Furthermore, it is crucial to investigate the formation of inhibitory compounds during pre-treatment and enzymatic hydrolysis, as these by-products can interfere with enzyme activity. Understanding their impact and developing strategies to minimize or neutralize these inhibitors will be vital for optimising the conversion process and ensuring higher sugar recovery from SCG.

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Authors' Contribution

Both authors contributed equally to the development of this article.

Data availability

All datasets relevant to this study's findings are fully available within the article.

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