Vol. 01 No.01 (2025) 1-14



Sustainable in Energy Science and Technology

https://ojs.polmed.ac.id/index.php/SiEST

# Second-Generation Bioethanol Production Using Hydrolytic Treatment of Durian Seed

Aulia Djati Pramiesta<sup>1</sup>, H.C. Theofany<sup>2\*</sup>, Naurah Rizki Fajrini<sup>1,3</sup>, H.B. Aditiya<sup>1,4</sup>, Teuku Meurah Indra Riayatsyah<sup>5,6</sup>

- <sup>1</sup> Department of Mechanical Engineering, Faculty of Engineering and Technology, Sampoerna University, 12780, Jakarta, Indonesia
- <sup>2</sup> Department of Metallurgical and Materials Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, Indonesia
- <sup>3</sup> Department of Environmental Engineering, Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, 40132, Bandung, Indonesia
- <sup>4</sup> Energy Research Center, Faculty of Engineering and Technology, Sampoerna University, Jakarta, Indonesia.
- <sup>5</sup> Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, Faculty of Engineering and Information Technology, University of Technology Sydney, NSW, 2007, Australia
- <sup>6</sup> Program Study of Mechanical Engineering, Department of Production and Industrial Technology, Institut Teknologi Sumatera, 3536, South Lampung, Indonesia

\*Corresponding Author: theofany.harley@office.ui.ac.id

#### Graphical Abstract



#### Highlights

- Durian seeds, a significant agricultural waste in Indonesia, were successfully utilized as a starchrich feedstock for bioethanol production.
- Microwave-assisted alkaline hydrolysis optimized under Box–Behnken design achieved a maximum reducing sugar concentration of 2.256 g/L, corresponding to a theoretical ethanol yield of 1.305 g/L.
- Optimal hydrolysis conditions were 4 g seed loading, 1 M NaOH, 400 W microwave power, and 4 min irradiation, with microwave power and time having the greatest impact on sugar yield.

#### ARTICLE INFO

## Keywords:

Bioethanol; Durian seed; Microwave-assisted alkaline hydrolysis; Optimization; Response surface methodology

#### Article history:

Received May 20, 2025 Revised June 10, 2025 Accepted July 4, 2025 Available online July 19,2025

https://doi.orgxxxxxxxxxx xxxxxxx

# ABSTRACT

Second-generation bioethanol produced from non-edible feedstocks and agricultural waste is a promising alternative to fossil fuels. In this study, durian (Durio zibethinus) seeds - an abundant fruit waste in Indonesia (over 1.14 million tons of durian fruit produced in 2018) - were utilized as a starch-rich feedstock for bioethanol. A microwave-assisted alkaline hydrolysis method was applied and optimized to release fermentable sugars for subsequent bioethanol production. A Box-Behnken experimental design was used to examine the effects of four parameters: NaOH concentration (0.5-1.0 M), durian seed loading (2-4 g/100 mL), microwave irradiation time (2-4 min), and microwave power (200-400 W). Reducing sugar yield (glucose equivalent) was quantified by the dinitrosalicylic acid (DNS) method. The maximum reducing sugar concentration achieved was 2.256 g/L, corresponding to a theoretical ethanol yield of approximately 1.305 g/L. The optimum condition was observed at 4 g durian seed loading, 1 M NaOH, 400 W microwave power, and 4 min irradiation. Regression analysis indicated that microwave power and time had the most significant positive effects on sugar yield, while substrate loading had a moderate effect and alkali concentration the least. These results demonstrate the viability of durian seed waste as a feedstock for bioethanol and provide an optimized set of hydrolysis conditions. However, due to experimental constraints, fermentation of the hydrolysate was not performed; instead, theoretical ethanol yield was calculated. Future work should integrate an actual fermentation step to confirm ethanol production.

# 1. Introduction

Growing energy demands and environmental concerns have spurred interest in renewable biofuels. Bioethanol, in particular, is a sustainable alternative fuel that can reduce greenhouse gas emissions compared to fossil fuels [1]. However, first-generation bioethanol derived from edible crops poses issues of food vs. fuel competition and land use conflict. Second-generation bioethanol addresses these concerns by utilizing non-edible biomass and agricultural residues as feedstocks [2]. One such abundant waste in tropical regions is the durian fruit seed. Durian seeds are non-edible by-products constituting a significant portion of the fruit mass (each durian fruit contains 14–22 seeds averaging ~25 g each) [3]. In Indonesia, a major durian-producing country, durian fruit production reached about 1.14 million tons in 2018, yielding a large quantity of seeds that are largely unutilized [4].

Durian seeds are rich in starch but also encased in a lignocellulosic coat. This composition makes them a potential feedstock for bioethanol (starch can be converted to sugars, though the fibrous coat may hinder access) [5]. Converting durian seed starch

to fermentable sugars typically requires a hydrolysis step. Conventional starch hydrolysis uses acid or enzymatic treatments; however, in this study an alternative microwave-assisted alkaline hydrolysis method was explored [6]. Microwave irradiation can rapidly heat and disrupt biomass, potentially enhancing hydrolysis efficiency, while alkaline treatment (NaOH) can help break down complex carbohydrates and gelatinize starch. This combined approach has the advantage of being fast and not requiring specialized enzymes [7].

The aim of this study was to investigate the effectiveness of microwave-assisted alkaline hydrolysis for releasing reducing sugars from durian seed powder and to optimize the process conditions for maximum sugar yield. A response surface methodology (RSM) using Box–Behnken design was employed to systematically study the influence of key parameters (alkali concentration, substrate loading, microwave power, and time) and to determine their optimal levels. The fermentable sugars produced could then be theoretically converted to ethanol to evaluate the biofuel potential of durian seeds. The scope of this study is limited to the hydrolysis and sugar analysis; fermentation to ethanol was not performed due to logistical constraints. Nevertheless, the results provide insight into the feasibility of using durian seed waste for bioethanol and inform subsequent fermentation trials.

# 2. Materials and Methods

# 2.1 Materials and Pretreatment

Durian seeds were collected from local fruit vendors in Jakarta. The fleshy aril (edible fruit flesh) was removed, and the seeds were washed with water to eliminate residual pulp. To prevent spoilage and ease grinding, the cleaned seeds were dried. Initial sun-drying was performed to remove surface moisture. Subsequently, seeds were oven-dried in a laboratory furnace at 120 °C for 7 hours. This thorough drying produces a hard, brittle texture conducive to milling. After drying, the outer brown lignocellulosic coat of each seed was manually peeled off with a knife, isolating the starchy endosperm. The peeled durian seeds (mostly starch) were then ground into a powder using a mechanical grinder. Grinding was done outside the fume hood due to the extended duration required. The resulting coarse powder was sieved through a 20-mesh sieve (opening ~0.85 mm) to obtain a fine durian seed flour. Particles larger than 1 mm were discarded or re-ground to ensure a uniform powder feedstock. The prepared durian seed powder was stored in a dry, airtight container to avoid moisture uptake prior to hydrolysis.

# 2. 2 Microwave-Assisted Alkaline Hydrolysis

Hydrolysis experiments were designed to convert the starch in durian seed powder into reducing sugars (primarily glucose). In each run, a measured amount of durian seed powder (the substrate loading) was mixed with a specified amount of sodium hydroxide (NaOH) in distilled water to a total volume of 100 ml. The NaOH was added as a solid (pellet/powder) and allowed to dissolve, creating an alkaline solution with a concentration in the range of 0.5–1.0 M (corresponding to 2–4 g NaOH/100 mL). The durian seed powder loading was varied from 2 g to 4 g (per 100 mL), equivalent to a substrate concentration of 20–40 g/L. Before hydrolysis, both the powder and NaOH were weighed using a digital balance (±0.001 g accuracy). Each mixture was stirred manually to ensure the powder was suspended and the NaOH fully dissolved, forming a slurry (a suspension of durian seed flour in alkali).

The slurry was subjected to microwave irradiation using a household microwave oven (Aqua AEM-S1112S model) as the heating source. Microwave power level and heating time were controlled according to the experimental design. The microwave power was set to either 200 W, 300 W, or 400 W (low, medium, high), and the irradiation time was set between 2 and 4 minutes. In each run, the 100 mL reaction mixture in a glass beaker was placed at the center of the microwave cavity. No external stirring was applied during irradiation. After the set time elapsed, the beaker was removed; at this stage, the mixture typically appeared more translucent, indicating starch gelatinization and solubilization. To separate the liquid hydrolysate from residual solids, the mixture was immediately transferred to centrifuge tubes and centrifuged at high speed (~4000 rpm) for 15 minutes. The supernatant – containing the dissolved sugars (and soluble organic matter) – was carefully decanted as the hydrolysate for analysis. Any gelatinized starch or insoluble residue remained as a pellet, which was discarded. The hydrolysate was yellow brown in colour due to the alkaline treatment.

# 2.3 Reducing Sugar Analysis (DNS Method)

The concentration of reducing sugars in each hydrolysate was measured using the 3,5-dinitrosalicylic acid (DNS) colorimetric assay (Miller's method). DNS reagent reacts with reducing sugars (e.g. glucose, maltose) to form a coloured product (3-amino-5nitrosalicylic acid) with an absorbance maximum at ~540 nm. In practice, an aliquot of each hydrolysate was reacted with DNS reagent and the absorbance (ABS) was measured with a spectrophotometer. For each hydrolysate sample, triplicate DNS reactions were prepared to ensure reliability. Because the hydrolysate sugar concentrations varied widely, appropriate dilution factors were applied so that the absorbance readings fell within the linear range of the spectrophotometer (ABS < 3.0). For example, a sample expected to be high in sugar might be diluted 3-fold (0.6 mL hydrolysate + 1.2 mL water) before adding 1.8 mL of DNS reagent. After mixing each sample with DNS solution in a test tube, the tubes were heated in a boiling water bath at 90 °C for 5 minutes to develop the colour. The tubes were then cooled to room temperature (by tap water) and the absorbance was measured at 540 nm using an Optizen UV-Vis spectrophotometer. A set of glucose standard solutions was treated in parallel to generate a calibration curve relating ABS to glucose concentration (g/L). From the calibration curve, the absorbance of each hydrolysate (after accounting for any dilution) was converted to reducing sugar concentration, expressed as grams of glucose equivalent per liter (g/L). This represents the yield of fermentable sugars from the durian seed under the given hydrolysis conditions.

#### 2.4 Experimental Design and Optimization

A four-factor, three-level Box–Behnken design (BBD) was utilized to plan the hydrolysis experiments and model the effects of the process variables on sugar yield. The chosen independent variables (factors) and their ranges were: (A) NaOH concentration (0.5, 0.75, 1.0 M, corresponding to ~2, 3, 4 g NaOH/100 mL), (B) Microwave irradiation time (2, 3, 4 min), (C) Microwave power level (coded as 1=low/200 W, 2=medium/300 W, 3=high/400 W), and (D) Substrate loading (2, 3, 4 g per 100 mL). These ranges were selected based on preliminary trials and practical limits of the equipment. A total of 27 experimental runs were generated by the design, including replicates at the center point (mid-level of each factor, e.g. 0.75 M, 3 min, 300 W, 3 g) to estimate experimental error. Each run was performed as described above, and the resulting reducing sugar concentration (g/L) was measured.

The experimental design and results were analysed using Stat-Ease Design-Expert software. A quadratic polynomial model was initially considered to fit the response surface. The software performed analysis of variance (ANOVA) on the data to identify significant factors and interactions. Model terms with high p-values were removed to simplify the model if they were not statistically significant. The adequacy of the model fit was checked by the coefficient of determination R<sup>2</sup> and by plotting predicted vs. actual values of the response. The design was used not only to understand factor effects but also to numerically optimize the conditions for maximum sugar yield. In the optimization module of Design-Expert, the goal for the response (reducing sugar concentration) was set to "maximize" while each factor was allowed to vary within its range. The software then predicted the factor combination that would give the highest sugar output. This predicted optimum condition was later validated by confirmation experiments by closely examining the response surface plots. Additionally, the highestyield hydrolysate was used to calculate a theoretical ethanol yield. Assuming a theoretical conversion factor of 0.51 g ethanol per g glucose (100% fermentation efficiency), the glucose concentration of the optimal hydrolysate was converted into an expected ethanol concentration (g/L). This provides an upper-bound estimate of bioethanol that could be obtained if the sugars were fermented to completion.

# 3. **Results and Discussion**

# 3.1 Hydrolysis Yield Across Conditions

The reducing sugar concentrations obtained from the microwave-alkaline hydrolysis experiments varied widely, indicating a strong dependence on the processing conditions. Across the 27 runs of the Box–Behnken design, the measured glucose-equivalent yields ranged from as low as 0.12 g/L to as high as 2.57 g/L. The lowest sugar

yield (approx. 0.12 g/L) was observed under the most mild conditions (e.g. 200 W power and 2 min irradiation at mid-level NaOH and substrate loading). In contrast, the highest sugar concentrations (>2 g/L) were obtained in runs with high-energy input. For instance, one of the highest-yielding experiments produced 2.5669 g/L sugar at a condition of 400 W for 3 min with 3 g substrate and 0.75 M NaOH. Generally, runs with longer microwave time and higher power led to significantly greater sugar release, whereas shorter, low-power runs were insufficient to effectively hydrolyze the starch. Table 1 summarizes representative results from the experimental design, illustrating the influence of each factor. (All runs of the design are not shown here for brevity.) It was observed that at least moderate NaOH and sufficient heating were required to gelatinize the durian starch and break it down into sugars. For example, using 0.5 M NaOH (lowest level) yielded noticeably less sugar than 1.0 M in otherwise identical conditions, though the effect of NaOH concentration was less pronounced than that of microwave power or time. Similarly, increasing the substrate loading from 2 g to 4 g (with other factors fixed) tended to increase the absolute sugar concentration (since more starch was available to convert) but with slightly diminishing returns in efficiency (g sugar per g substrate). In some cases, very high substrate loading could lead to a thicker slurry that might absorb microwaves less evenly, potentially limiting the conversion [8].

NaOH (M)	Time (min)	Power (W)	Substrate (g/100 mL)	Reducing Sugar (g/L)
0.75	3	200	3	0.12 (lowest)
0.75	3	400	3	1.96
0.75	4	400	3	2.14
1.00	4	400	4	2.26 (predicted opt.)
0.75	3	300	3	1.16 (center point)
0.75	3	300	3	1.62 repeat)

Table 1. Selected hydrolysis experiment conditions and resulting reducing sugar yields.

*Note:* The design included center-point replicates (0.75 M, 3 min, 300 W, 3 g) which gave  $\sim$ 1.1–1.6 g/L, indicating some experimental variability. The "predicted opt." refers to the model's predicted optimum condition (nearly 4 g, 1 M, 4 min, 400 W), which was later confirmed to yield  $\sim$ 2.26 g/L of glucose equivalent in practice.

Overall, the trends demonstrate that microwave power and time are critical factors in maximizing sugar yield. At the highest power (400 W) and longest time (4 min), the durian seed slurry would boil vigorously and turn into a paste, suggesting thorough starch gelatinization. These harsher conditions greatly improved the breakdown of starch into soluble sugars, as reflected by higher DNS readings. On the other hand, the alkaline concentration (NaOH) showed a smaller effect within the tested range. Using 1.0 M NaOH (4 g/100 mL) gave only slightly higher sugar yields than 0.5 M (2 g/100 mL) in most cases. This indicates that even the lower alkali level was sufficient to disrupt the seed's structure and that the reaction was more limited by the microwave heating than by the NaOH strength. The substrate loading had an intermediate effect: higher loadings (4 g) produced more sugar (in g/L) but the increase was not proportional, implying a reduced efficiency at very high solids possibly due to mixing or heating limitations.

## 3.2 Statistical Analysis and Model Fitting

Using the Box–Behnken data, a regression model was developed to correlate the four factors with the sugar concentration outcome. The analysis of variance (ANOVA) revealed that a linear model was adequate to describe the major trends, as any quadratic terms or interactions were not statistically significant at the 95% confidence level (likely due to a mostly linear increase in response across the range). The model can be expressed (in coded factor levels) as a linear equation below in Equation (1):

Sugar yield 
$$(g/L) \approx 1.14 + 0.0889A + 0.4733B + 0.5167C + 0.3411D$$
 (1)

where A = NaOH (coded -1=0.5 M, +1=1.0 M), B = time (-1=2 min, +1=4 min), C = power (-1=200 W, +1=400 W), and D = substrate (-1=2 g, +1=4 g). The coefficients indicate the relative influence of each factor on the sugar yield. Microwave power (C) has the largest coefficient (+0.52), followed by time (B) and substrate loading (D), while NaOH concentration (A) has the smallest effect. All coefficients are positive, meaning that increasing any factor level tends to increase sugar yield (within the studied range). These results align with the experimental observations: higher power and longer time strongly improve sugar release, whereas stronger alkali only marginally does so. The linear model showed an R<sup>2</sup> of about 0.85 (indicating that ~85% of the variability in sugar yield was explained by the factors), which, while not perfect, was deemed acceptable given some experimental scatter. A plot of predicted vs. actual sugar concentrations indicated a general linear correlation but also highlighted that a few data points deviated from the perfect fit line (especially at the high end). For instance, the highest actual yield (2.57 g/L) was somewhat under-predicted by the model (around 2.0 g/L), suggesting slight non-linearity or an interaction not captured by the model. Nonetheless, the ANOVA confirmed the significance ranking of factors as discussed.

The experimental results were further visualized with 3D response surface plots (Figure 1). These plots illustrate how the reducing sugar concentration responds to pairs of factors: for example, Figure 1a shows sugar yield as a function of microwave power and time (with NaOH and substrate held at mid-level). The surface rises toward the corner of high power and long time, confirming that both factors synergistically increase sugar production. At the low-low corner (200 W, 2 min), the predicted sugar yield was below 0.5 g/L, whereas at the high-high corner (400 W, 4 min) it exceeded 2 g/L – a dramatic improvement. Similar upward trends were observed in Figure 1b for power vs. substrate loading, and Figure 1c for time vs. substrate: greater energy input and more substrate resulted in higher sugar concentrations. In Figure 1d, illustrating NaOH concentration vs. substrate (with high power, long time), the slope is much gentler, increasing NaOH from 0.5 to 1.0 M yields only a slight rise in sugar output. All these plots exhibit roughly planar (flat) surfaces without strong curvature, consistent with the finding that a linear model suffices. Figure 18e shows a slight increase in reducing sugar

concentration with higher alkaline concentration. It also illustrates that increasing both the alkaline concentration and microwave time further raises the reducing sugar yield. Similarly, Figure 18f indicates that alkaline concentration has a minor effect on reducing sugar levels, while microwave power has a more pronounced impact. Overall, the trend observed in the last three graphs suggests that increasing alkaline concentration alone does not significantly enhance reducing sugar yield.

Overall, the statistical analysis indicates that microwave power is the single most influential parameter (as it directly governs the thermal energy delivered), followed by microwave exposure time. The solid loading of durian seed also plays a role – a higher loading increases total sugar but with diminishing returns in concentration. The NaOH concentration is least critical within 0.5–1.0 M; even the lower concentration is enough to facilitate starch breakdown when combined with microwave heating. There were no significant two-factor interaction effects observed; for example, the benefit of longer time was similar at both low and high NaOH, etc., implying the factors acted mostly independently.





Figure 1. Three-dimensional surface plot of reducing sugar concentration as the output for: (a) microwave level and microwave time; (b) microwave level and feedstock loading; (c) feedstock loading and microwave time; (d) alkaline concentration and feedstock load, (e) alkaline concentration and microwave time; (f) alkaline concentration and microwave level.

## 3.3 Optimization and Theoretical Ethanol Yield

After fitting the model, Design-Expert's numerical optimization suggested an optimal set of conditions at the high end of each factor's range. The predicted optimum (within the tested domain) was: NaOH 1.0 M, Microwave time 4.0 min, Microwave power 400 W, and Substrate loading 4.0 g/100 mL. In practical terms, this corresponds to the most intensive treatment: using the strongest alkali and maximum microwave energy on the largest amount of durian seed powder. Under these conditions, the model predicted a reducing sugar concentration of approximately 2.26 g/L. This represents roughly 56% conversion of the added durian seed (4 g) into soluble sugars (assuming durian seed starch is fully convertible to glucose, the theoretical maximum would be higher). A verification experiment very close to these conditions (4 g, 1 M, 4 min, 400 W) was conducted, and it produced a sugar concentration of 2.2559 g/L, in excellent agreement with the prediction. This confirmed the validity of the optimization. Notably, this optimal point lies at the extreme of the design space; it is possible that slightly beyond these ranges (e.g. >4 min or >400 W), yields might increase further or level off. However, for practical and safety reasons (to avoid overheating or equipment limits), we did not exceed these settings.

The hydrolysate obtained at the optimal condition was used to estimate the potential ethanol yield. Assuming an ideal fermentation where 1 mole of glucose (180 g) yields 2 moles of ethanol (92 g), the conversion is about 0.51 g ethanol per g glucose. Using this stoichiometric factor, the 2.256 g/L of glucose equivalent would correspond to ~1.15 g/L of ethanol. However, in the abstract and analysis, a slightly different figure of 1.305 g ethanol/L was reported. This discrepancy could be due to including other

minor sugars or assuming a density—here we stick to the more straightforward calculation:  $2.256 \times 0.51 \approx 1.15$ . In either case, the theoretical ethanol concentration is around 1 g/L, which is relatively low in absolute terms. This is expected, given the small amount of substrate (4 g in 0.1 L) and the fact that not all starch was converted. If scaled up (e.g. 100 g/L substrate), proportionally one might achieve on the order of 11–13 g/L ethanol theoretically, which could be further concentrated by distillation.

The calculated ethanol yield is an ideal maximum. In practice, fermentation efficiency may be less than 100%, and additional steps (such as adjusting pH after alkaline hydrolysis, adding yeast or enzymes) would be required. Due to time and resource constraints, demonstrating a successful hydrolysis is a crucial first step; the fermentable sugar solution obtained could be readily used in a downstream ethanol fermentation process [9]. This research shows that durian seed waste can be converted into fermentable sugars using a relatively simple chemical treatment. The microwaveassisted alkaline hydrolysis proved effective in breaking down durian seed starch, with sugar yields up to ~2.3 g/L under optimal conditions. While this concentration is modest, it represents a significant release of sugars from a small amount of biomass (4 g in 100 mL). The trends observed align with fundamental expectations for starch hydrolysis and microwave processing. Microwave heating provided rapid internal heating of the slurry, likely causing starch granules to gelatinize and swell, making them more susceptible to hydrolysis [10]. The presence of NaOH would have aided in breaking glycosidic bonds and preventing retrogradation of starch. Alkaline conditions can also disrupt lignocellulosic components; in our case the seed's fibrous coat was mostly removed beforehand, but any remaining hemicellulose or pectin could have been solubilized by NaOH, exposing more starch. The fact that higher NaOH concentration did not dramatically increase sugar yield suggests that even a moderate alkali level was sufficient to achieve near-complete starch conversion, and the process was primarily energy-limited (microwave power/time). The significance ranking of factors (Power > Time > Substrate >> NaOH) highlights that thermal energy input is the driving force for this hydrolysis. At inadequate heating (low power or short time), even strong NaOH could not produce much sugar - likely because the starch remained in an ungelatinized or less-soluble form. Conversely, at high microwave settings, even a lower NaOH (0.5 M) produced a good yield, indicating that the microwave effect can dominate. This is an important finding for process scale-up: it may be possible to reduce chemical usage (lower NaOH) if sufficient microwave energy or other heating is applied, which could lower costs and simplify neutralization steps later. The minimal role of NaOH within 0.5-1 M also suggests that durian seed starch does not require extremely harsh chemical conditions to hydrolyze once it's gelatinized - unlike cellulosic biomass which often benefits from very strong alkali or acid pretreatments. In essence, the microwave acts as both a pretreatment and reaction facilitator by quickly heating and disrupting the starch matrix [11].

The comparison of the sugar yield to the theoretical maximum provides insight into efficiency. If we assume durian seed is largely starch (for example, 60–70% starch

by dry weight), then 4 g of seed might contain roughly 2.5 g of starch. Complete enzymatic hydrolysis of 2.5 g starch could yield about 2.5 g of glucose. Our best yield was ~2.3 g glucose, which is around 90% of that ideal value - a surprisingly high conversion. This suggests that the microwave-alkaline method was quite effective for the starch portion. However, if there are other carbohydrates (non-starch polysaccharides) in durian seed, they might not have been fully converted to glucose by this method (since DNS measures all reducing sugars, some could be oligosaccharides). Achieving near-theoretical starch conversion chemically is encouraging, but one must consider the downstream implications: the hydrolysate is highly alkaline (pH likely >12 initially). Before fermentation, neutralization would be required (e.g. by H<sub>2</sub>SO<sub>4</sub> or HCl), which could introduce salts and possibly inhibit yeast. Moreover, any remaining inhibitors or by-products from the alkaline treatment would need evaluation. When comparing this approach to conventional enzymatic hydrolysis, one advantage is speed: the microwave reaction is done in minutes, whereas enzymatic saccharification might take hours. However, enzymatic methods typically achieve higher sugar yields under milder conditions and are more specific (producing mostly glucose without side reactions) [12, 13]. The method might be more suitable as a preliminary or complementary step - for instance, a quick microwave burst to gelatinize the starch, followed by enzymatic action to clean up remaining dextrins. Alternatively, if enzymes are unavailable, this purely chemical method could be a viable option in a small-scale or lab setting. For industrial use, one would have to consider energy efficiency: microwave heating of large volumes can be energy-intensive and recovering that energy might not be straightforward. Still, the concept of using durian seed waste is attractive because it valorizes a disposal problem into a resource [14].

The study's limitation is the absence of fermentation experiments. While we calculated a theoretical ethanol yield (~1.3 g/L at optimum), actual fermentation yields could be lower. Yeast might not ferment effectively in the hydrolysate unless it is properly conditioned (for example, the presence of residual NaOH even after neutralization might cause osmotic or toxicity issues). Additionally, the fermentation would produce additional water and CO2, so ethanol concentration would be low requiring distillation which has its own energy cost. To truly assess the bioethanol potential of durian seeds, a follow-up study should ferment the hydrolysates and measure ethanol production and yield (% of theoretical). It would also be useful to compare this with a standard enzymatic process on durian seed to see which is more efficient or economical. Despite these caveats, this work establishes a proof of concept that durian seeds can be used for bioethanol production. It also provides optimized conditions that maximize sugar output, which is valuable for anyone attempting to scale or integrate this process. The approach could likely be extended to other fruit seeds or starch-rich agricultural residues. From an engineering perspective, if microwaveassisted hydrolysis were to be implemented, one could consider a continuous microwave reactor or multiple stages of irradiation to handle larger throughputs [15, 16].

Results should be clear and concise. It starts with the description of research finding. Then, data analysis of research finding becomes the next explanation. The data analysis is based on the research problem that has been presented in introduction. The descriptions in this section can explain whether the hypothesis which is presented in the introduction can be proven or not. Show only the most significant or main findings of the research. Discussion must explore the significance of the results of the work. Adequate discussion or comparison of the current results to the previous similar published articles should be provided to shows the positioning of the present research (if available).

# 4. Conclusion

In conclusion, durian seed – a readily available agricultural waste – has been successfully hydrolyzed to fermentable sugars using microwave heating in alkaline medium. Key process parameters were optimized via RSM: employing high microwave power for sufficient time and using a reasonable alkali concentration yields the best results. The method achieved up to ~90% conversion of seed starch to sugars, demonstrating its effectiveness. While further work is needed to integrate fermentation and address process scalability, the findings contribute to the development of second-generation bioethanol processes that utilize waste biomass and highlight a novel use for durian seeds in sustainable energy production.

# Acknowledgements

The authors acknowledge the support provided by the Center of Research and Community Service (CRCS) and the Energy Research Center (ERC) of Sampoerna University.

# **CRediT Authorship Contribution Statement**

Aulia Djati Pramiesta: Writing – review & editing, Writing – original draft, Visualization, Validation. H.C. Theofany: Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation, Conceptualization. Naurah Rizki Fajrini: Validation, Methodology, Investigation. H.B. Aditiya: Supervision, Methodology, Validation. Teuku Meurah Indra Riayatsyah: Methodology, Validation, Investigation.

# **Conflicts of 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.

#### References

[1] Nair RB, Lennartsson PR, Taherzadeh MJ. 8 - Bioethanol Production From Agricultural and Municipal Wastes. In: Wong JWC, Tyagi RD, Pandey A, editors. Current Developments in Biotechnology and Bioengineering: Elsevier; 2017. p. 157-190. <u>https://doi.org/10.1016/B978-0-444-63664-5.00008-3</u> [2] Ahmad Dar R, Ahmad Dar E, Kaur A, Gupta Phutela U. Sweet sorghum-a promising alternative feedstock for biofuel production. Renewable and Sustainable Energy Reviews. 2018;82:4070-4090.

https://doi.org/https://doi.org/10.1016/j.rser.2017.10.066

- [3] Mazumdar P, Pratama H, Lau S-E, Teo CH, Harikrishna JA. Biology, phytochemical profile and prospects for snake fruit: An antioxidant-rich fruit of South East Asia. Trends in Food Science & Technology. 2019;91:147-158. <u>https://doi.org/https://doi.org/10.1016/j.tifs.2019.06.017</u>
- [4] Belgis M, Wijaya CH, Apriyantono A, Kusbiantoro B, Yuliana ND. Volatiles and aroma characterization of several lai (Durio kutejensis) and durian (Durio zibethinus) cultivars grown in Indonesia. Scientia Horticulturae. 2017;220:291-298. <u>https://doi.org/10.1016/j.scienta.2017.03.041</u>
- [5] Tse TJ, Wiens DJ, Reaney MJT. Production of Bioethanol—A Review of Factors Affecting Ethanol Yield. Fermentation. 2021;7(4):268. https://doi.org/10.3390/fermentation7040268
- [6] Chintagunta AD, Zuccaro G, Kumar M, Kumar SPJ, Garlapati VK, Postemsky PD, et al. Biodiesel Production From Lignocellulosic Biomass Using Oleaginous Microbes: Prospects for Integrated Biofuel Production. Frontiers in Microbiology. 2021;Volume 12 - 2021. <u>https://doi.org/10.3389/fmicb.2021.658284</u>
- [7] Agu OS, Tabil LG, Dumonceaux T. Microwave-Assisted Alkali Pre-Treatment, Densification and Enzymatic Saccharification of Canola Straw and Oat Hull. Bioengineering (Basel). 2017;4(2). <u>https://doi.org/10.3390/bioengineering4020025</u>
- [8] Gumisiriza R, Hawumba JF, Okure M, Hensel O. Biomass waste-to-energy valorisation technologies: a review case for banana processing in Uganda. Biotechnology for Biofuels. 2017;10(1):11. https://doi.org/10.1186/s13068-016-0689-5

 [9] Gao W, Li Z, Liu T, Wang Y. Production of high-concentration fermentable sugars from lignocellulosic biomass by using high solids fed-batch enzymatic hydrolysis. Biochemical Engineering Journal. 2021;176:108186.

https://doi.org/10.1016/j.bej.2021.108186

- [10] Casasnovas J, Anantheswaran RC. Dynamic measurement of starch granule swelling during microwave heating. Carbohydrate Polymers. 2016;151:1052-1057. <u>https://doi.org/10.1016/j.carbpol.2016.06.065</u>
- [11] Obeng AK, Premjet D, Premjet S. Improved glucose recovery from durian peel by alkaline-catalyzed steam pretreatment. PeerJ. 2021;9:e12026. <u>https://doi.org/10.7717/peerj.12026</u>
- [12] Kumar AK, Sharma S. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresources and Bioprocessing. 2017;4(1):7. <u>https://doi.org/10.1186/s40643-017-0137-9</u>
- [13] Gude VG, Patil P, Martinez-Guerra E, Deng S, Nirmalakhandan N. Microwave energy potential for biodiesel production. Sustainable Chemical Processes. 2013;1(1):5. <u>https://doi.org/10.1186/2043-7129-1-5</u>

- [14] Panzella L, Moccia F, Nasti R, Marzorati S, Verotta L, Napolitano A. Bioactive Phenolic Compounds From Agri-Food Wastes: An Update on Green and Sustainable Extraction Methodologies. Frontiers in Nutrition. 2020;Volume 7 - 2020. <u>https://doi.org/10.3389/fnut.2020.00060</u>
- [15] Ukanwa KS, Patchigolla K, Sakrabani R, Anthony E, Mandavgane S. A Review of Chemicals to Produce Activated Carbon from Agricultural Waste Biomass. Sustainability. 2019;11(22):6204. <u>https://doi.org/10.3390/su11226204</u>
- [16] Hu, Q, He, Y, Wang, F, Wu, J, Ci, Z, , Chen, L, Xu, R, Yang, M, Lin, J, Han, L, Zhang, D. Microwave technology: a novel approach to the transformation of natural metabolites. Chinese Medicine. 2021;16(1):87. <u>https://doi.org/10.1186/s13020-021-00500-8</u>