Generating Low Lipid and Protein Food Waste Hydrolysate by a Dilute Sulfuric Acid Thermohydrolysis for Biorefinery Applications

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ABSTRACT

Food waste holds great potential as a sustainable resource for biorefinery applications. Still, its efficient use is hindered by the simultaneous solubilization of lipids and proteins during polysaccharide hydrolysis, which disrupts downstream fermentation processes. This study investigates the use of dilute sulfuric acid thermohydrolysis to produce food waste hydrolysate with high reducing sugar but low lipid and protein content. The initial food waste was ground and dried before undergoing hydrolysis. Thirty grams of dried food waste were mixed with 1500 mg of oil solidifier, then 180 mL of 5% H_2SO_4 solution added. The mixture was then agitated at 200 rpm and heated to 100°C for 180 minutes. This process resulted in a hydrolysate containing 66.52 mg/mL reducing sugar, 13.66 mg/mL lipid, and 2.02 mg/mL protein. These results demonstrate a viable approach to producing a more fermentation-compatible food waste hydrolysate, contributing to the advancement of sustainable biorefinery technologies and the valorization of food waste as a resource.

Keyword: Reducing sugar content/ Lipid content/ Protein content/ Biorefinery/ Food waste valorization

1. INTRODUCTION

Managing food waste (FW) has become increasingly urgent due to its significant environmental and economic impacts. Globally, more than 2 billion tons of FW are generated annually, contributing to greenhouse gas emissions and resource depletion [1]. In Thailand, whose population exceeds 70 million, FW generation averages 79 kg per capita annually [2]. This trend poses a looming environmental threat for the future. Moreover, conventional disposal methods, such as landfilling and incineration, are unsustainable and waste valuable resources that could be repurposed [3].

FW contains a total sugar content ranging from 25.5 to 143 g/L and a carbon-to-nitrogen (C/N) ratio of 9.3 to 36.4 [4, 5]. Depending on the type, FW's lipid content ranges from 5%-33% [6, 7]. FW also exhibits a total volatile organic content (VOC) ranging from 82% to 96%, providing a significant energy source for microorganisms in biorefinery applications [8]. Utilizing FW in biorefineries presents a promising approach to meeting energy and biochemical needs, addressing environmental challenges, and sidestepping the food-versus-fuel debate [3, 9].

A promising approach to FW valorization is its conversion into hydrolysates, which serve as substrates for various biorefinery applications, including biohydrogen, biomethane, and bioethanol [10-12]. Hydrolysis is crucial for breaking down polysaccharides within organic waste into simpler saccharides, as their large molecular size cannot penetrate bacterial or fungal cell membranes [13]. Thus, FW is more convenient and cost-effective as a substrate than monomer or dimer-based alternatives, particularly after hydrolysis. The composition of FW hydrolysate (FWH) is critical; high levels of lipids and proteins can hinder fermentation and complicate downstream processes. Lipid concentrations above 6 g/L can cause inhibition, rapid pH drops, and floating sludge in the bioreactor [14]. Similarly, protein contents exceeding 8 g/L can result in a low C/N ratio, hindering biorefinery production [15].

Various methods can be employed for FW hydrolysis, including enzymatic hydrolysis, supercritical CO_2 fluid extraction, and dilute acid thermohydrolysis [16-18]. Dilute acid thermohydrolysis is energy efficient, uses readily available chemicals, and is cost-effective compared to other methods [18]. Moreover, H_2SO_4 (sulfuric acid) is commonly used in dilute acid

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thermohydrolysis for various organic wastes because of its lower corrosiveness [18-20]. Dilute sulfuric acid thermohydrolysis has been shown to solubilize low lipids into hydrolysate from various feedstock since lipids have limited solubility in water [18, 21, 22]. When applied for a short duration (60–180 minutes) at lower temperatures (80–160 ^{o}C) and acid concentrations lower than 6%, it prevents protein solubilization into the hydrolysate [23].

This study investigates the use of dilute sulfuric acid thermohydrolysis to generate FWH. By controlling the factors affecting reducing sugars, proteins, and lipids solubilization, we aim to produce an FWH rich in reducing sugars with minimal contamination from lipids and proteins.

2. METHODOLOGY

2.1 Chemicals and initial food waste source

Sulfuric acid, 3,5-dinitrosalicylic acid, and Coomassie Brilliant Blue R 250 were procured from Loba Chemie (Australia), while the oil solidifier (powder) was purchased from SC Johnson (Japan), with the remaining chemicals purchased from Univar Solutions (USA) and RCI Labscan (Bangkok).

The FW used in this study was collected from the canteen around Sirindhorn International Institute of Technology-Thammasat University, Rangsit Campus, Thailand. The FW was collected weekly for a month to account for its variability. Only rice, noodles, flour, vegetables, side dishes, and meat wastes were used; no bones or other hard parts were used. The FW was then ground for 30 seconds, put in a tightly tied plastic bag, and stored in a freezer (-4^{o}C) for further study.

2.2 Characterization of dried food waste

The drying process of the initial FW was carried out at 60°C for 48 hours. Dried FW was then reground and sifted with mesh size 8 to obtain particle size lower than 0.5 mm. The obtained dried FW was characterized by the total solids (TS), total volatile solids (TVS), and ash content, which includes gravimetric moisture measurement at 105°C for three hours and ignition of the dried FW at 550°C for one hour, respectively [24]. At room temperature, the degassed 1:10 mixture of dried FW and deionized water pH was measured using a pH meter [25]. The dried FW protein was extracted using the salt/alkaline method, and the protein content was quantified using the Bradford method [26]. The dried FW crude lipid content was determined using the modified Folch method [27]. The dried FW carbohydrate content was determined using the calculation: carbohydrate (%) = 100 % - (% moisture + % protein + % lipid + % ash) [28].

2.3 Dilute sulfuric acid thermohydrolysis of dried food waste

In this dilute acid thermohydrolysis procedure, about 18-30 g of dried food waste was mixed with 500-1,500 mg of oil solidifier within a 500-mL round-bottom flask, followed by the addition of 180 mL of 1-5 % H_2SO_4 solution. The mixture was then agitated at 0-200 rpm and heated to 80-100°C for 60-180 minutes. The factors level applied are detailed in Table 1. Upon completing hydrolysis, the resulting mixtures were allowed to cool at room temperature and transferred to a 200-mL glass beaker. Twenty mL of deionized water was added to a 500-mL round-bottom flask to recover any remaining mixtures and then combined with the beaker's contents. The mixtures underwent centrifugation at 8,000 rpm and 15°C for 15 minutes to separate suspended solid particles. The supernatant was further filtered through Whatman filter paper no. 1. After neutralizing the supernatant to pH 7 by adding 50% NaOH, the precipitate was separated by centrifugation at 8000 rpm and 15°C for 15 minutes. The clarified FWH was then stored in a refrigerator at 4°C for subsequent analysis.

The analysis encompasses three parameters: reducing sugars, lipid, and protein content within the FWH samples. Reducing sugar content was determined by spectrometry based on a colorimetric reaction with 3,5-dinitrosalicylic acid [18]. Crude lipid content is determined using the modified Folch method, as defined by Min and Ellefson [27]. The protein content was quantified using the Bradford method [26].

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Table 1. The factors level applied in dilute sulfuric acid thermohydrolysis

Factor level	Dried food waste (g)	Oil solidifier (mg)	H_2SO_4 concentration (%)	Temperature (°C)	Agitation (rpm)	Duration (minutes)
Lower	18	500	1	80	0	60
Middle	22.5	1000	3	90	100	120
Upper	30	1500	5	100	200	180

2.4 Data analysis

The FWH reducing sugar, lipid, and protein content (mg/mL) was measured at each factor level and compared to identify which level simultaneously produced the highest reducing sugar content and the lowest lipid and protein content.

Where; CF is carbon footprint (CO_2e per unit product), AD is activity data (mass/volume/kWh/km), and EF is emission factor GHG (CO_2e per unit) is the default emission factor of a given GHG by type of resource use. In addition, the emission factor used is shown in Table 2.

3. RESULTS AND DISCUSSION

3.1 Dried food waste characteristics

FW typically exhibits an acidic pH ranging from 3.9 to 6.7, differing from other organic wastes like manure, green waste, and sewage sludge [29]. Wu et al. [25] reported the prevalence of lactic acid bacteria in FW, which aid in natural fermentation by decreasing pH. Drying FW in this study aimed to stabilize nutrient content and pH. Maintaining moisture below 10% is advantageous as it reduces microbial activity, diminishes biological degradation, and simplifies storage [18]. The dried FW characteristics are shown in Table 2.

Table 2. The dried food waste sample characteristics

pH	Moisture (%)	TS (%)	Ash (%)	TVS (%)	Total carbohydrate (%)	Lipid (%)	Protein (%)
4.37	6.26	93.74	6.96	86.78	59.90	14.85	12.03

*%: w/w

According to Carpenter and Savage [30], FW typically contains 57-85% carbohydrates, 15-38% proteins, and 0-5% lipids on a dry basis, varying by season and location. The carbohydrate content of dried FW corresponds with the existing literature, whereas the protein content is diminished, and the lipid content is elevated. The protein degradation in FW occurs when the food is subjected to cooking processes such as frying, boiling, or steaming [6]. The standard approach involves separating and trans-esterifying grease in FW to produce biodiesel, while the solid portion can be composted into biofertilizer [7]. However, anaerobic digestion or integrated bioconversion approach seems to have more potential to repurpose FW's high carbohydrate content.

Furthermore, the observed TVS value indicates a notably high VOC content. A high VOC content ranging from 82-96% implies a substantial energy source for microorganisms involved in biorefinery processes [29]. Defending the type of FW, studies observed ash contents ranging from 2% to 10% [6]. FW ash content of the sample indicates a high concentration of minerals and metals essential for bacterial growth and activity in fermentation systems [6, 31, 32]. The detailed characterization of FW underscores its potential as a highly promising substrate for biorefinery applications.

This study further processed dried FW using dilute sulfuric acid thermohydrolysis. Two substrates, FWH and post-hydrolysis FW, are recovered through filtration. However, this paper will focus solely on FWH, with post-hydrolysis FW to be investigated in future studies.

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3.2 Food waste hydrolysate characteristics

Reducing sugar, lipid, and protein content in the FWH is important in assessing its potential use as a substrate for biorefinery applications. Figure 1 shows the FWH characteristics based on the level of the factors applied.

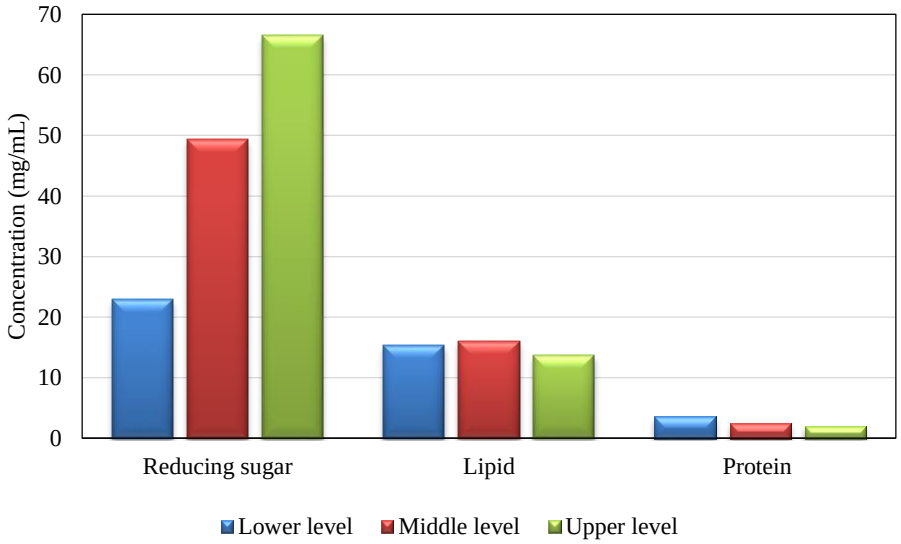

Figure 1. The reducing sugar, lipid, and protein content of food waste hydrolysate based on the level of the factors applied

The FWH-reducing sugar content increases proportionally with the level of factors applied. Hydrolyzing 30 g of dried FW in this study yields more FWH-reducing sugar and lowers water demand than smaller substrate amounts. Vallejos et al. [33] also found that a high substrate loading or low solution-to-solid ratio, between 1-11 mL/g, can significantly improve operational sustainability. Agitation is crucial in hydrolysis, especially with a low solution-to-solid ratio, as it helps maintain uniform concentration, pH, and temperature while reducing particle size [34]. In this study, 200 rpm agitation combined with a particle size of less than 0.5 mm led to a higher reducing sugar content.

The FWH lipid content slightly increases proportionally from the lower to the middle level of the applied factors. However, it decreases when the upper level is used. Higher temperatures and more oil solidifiers enhance oil absorption efficiency [35]. As a result, at 100°C and 1,500 mg oil solidifier, a lower FWH lipid content is observed despite the dried FW weight increases. Nevertheless, this study shows that lipid concentrations in FWH remain significantly high across all experiments, reaching up to three times the maximal level (6 mg/mL) for biohydrogen production. Therefore, the lipid content in hydrolysate is also contingent upon the substrate used.

In contrast to reducing sugar and lipids, raising all factor levels decreases FWH protein content. Laursen et al. [36] support this finding, noting that increased temperature and acidity—beginning at 60°C with 2% citric acid—lead to milk protein aggregation. Similarly, FWH protein aggregation may also occur and can be separated from hydrolysate by filtration and centrifugation. Protein content is important when using hydrolysates for subsequent fermentation applications. Protein content in the hydrolysate above 8 mg/mL leads to an imbalanced C/N ratio, encouraging bacterial overgrowth and protein degradation into ammonium (NH_4) through the consumption of H_2 molecules, ultimately lowering hydrogen production [5, 15]. According to a study by Mustatea et al. [23], extended hydrolysis periods ranging from 18 to 72 hours with 6.26% HCl used under vacuum conditions at 100-160 ^oC are necessary for dilute acid thermohydrolysis for thorough hydrolysis peptide bonds. As a result, the hydrolysis processes in this study can boost the yields of reducing sugars but not proteins. However, the low protein content of FWH is advantageous for subsequent fermentation processes, as an ideal C/N ratio of 10-30 promotes optimal microbial growth [29].

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Despite the upper level of factors applied resulting in a higher reducing sugar and lower lipids and protein in hydrolysate than other levels, further study should investigate different components of FWH, including 5-hydroxymethyl furfural, furfural, and phenolic compounds. These compounds, produced by dilute sulfuric acid thermohydrolysis, are known to reduce the yield of biorefinery applications [37, 38]. Besides, the hydrolysis processes' safety, energy efficiency, and environmental impact should also be considered.

Furthermore, the FWH dilution is a viable alternative approach, where the optimal reducing sugar concentration ranges from 10 to 20 mg/mL, as shown by Wang and Yin [4]. A two- or three-fold dilution can achieve the desired reduction of sugar and lipid concentrations. Selecting compatible or less lipids-sensitive biorefinery applications can also be applied. Lee et al. [39] used leather fleshing waste containing 49% lipids for biomethane production, achieving a yield of 200 mL CH_4/g VS. Additionally, optimizing the configuration of dilute sulfuric acid thermohydrolysis within the range of applied factor levels is essential for achieving the optimal balance of reducing sugars, lipids, and proteins, enhancing biorefinery applications.

4. CONCLUSIONS

Based on laboratory experiments and data analysis in this study. Some conclusions were drawn as follows:

1. The initial FW sample is rich in carbohydrates, indicating a highly promising substrate for biorefinery applications.
2. Dilute sulfuric acid thermohydrolysis effectively extracts significantly reducing sugars from dried FW while minimizing lipid and protein solubilization.
3. Optimum configurations produced an FWH containing 66.52 mg/mL reducing sugar, 13.66 mg/mL lipid, and 2.02 mg/mL protein.
4. Adjusting the dilution or optimizing the process configurations can further refine the content of reducing sugars, lipids, and protein to the desired levels.

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