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Recovery of Glucose and Acetic Acid from *Piper betle* Linn Leaves by Subcritical Water Hydrolysis

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Abstract. *Piper betle* Linn (PBL) leaves contain high carbohydrates, which can be hydrolyzed into glucose and acetic acid by hydrolysis. Subcritical water hydrolysis (SWH) is an environmentally friendly method that uses water as a solvent and is suitable for hydrolysis. Thus, this study aims to evaluate the glucose and acetic acid recovered from PBL leaves using SWH. Using a factorial design, SWH was performed under different process conditions (temperature range 100 to 275°C and time range 5 to 30 min). Glucose and acetic acid were determined using high-performance liquid chromatography (HPLC) with a refractive index (RI) detector. The ANOVA results show that temperature, time, and the interaction of temperature and time significantly impact glucose yield and acetic acid concentration. The particle size of PBL leaves of 220.70± 2.33 µm was effective, and it can be used to recover glucose and acetic acid by SWH. The yield of glucose and acetic acid shows a different trend, with glucose degrading to acetic acid. The highest glucose concentration was achieved at 200°C for 5 min (6.143 mg/g extract), while the highest acetic acid concentration was obtained at 225°C for 20 min (2.856 mg/g extract).

Keywords: Glucose and Acetic Acid, Hydrolysis, PBL leaves, Subcritical Water Hydrolysis, Sugar Degradation,

INTRODUCTION

Piper betle Linn, often called betel leaves, is an abundant plant in Asia. Rahmah *et al.* (2023a) characterized the dried *Piper betle* L. leaves containing 49.6 % carbohydrate. These carbohydrates can be used as substrates to be converted (or hydrolyzed) into desired products such as glucose and acetic acid. In general, sugars such as glucose can be obtained by methods of hydrolysis, such as acid hydrolysis (Cuevas-Aranda *et al.*, 2024; Diedericks *et al.*, 2013; Liu *et al.*, 2012) and enzymatic hydrolysis (Thani *et al.*, 2021; Yang *et al.*, 2023). However, these methods are not satisfactory for sugar recovery as they are

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high cost (especially the cost of enzymes) and could produce many toxic substances (acid hydrolysis) that can harm the environment. To solve this limitation, subcritical water hydrolysis (SWH) becomes an alternative for a better solution to recover glucose and acetic acid from PBL leaves. Hydrolysis is the cleavage of chemical bonds by adding water or a base that provides the hydroxyl ion (OH^-) or hydrogen ion (H^+) in which a chemical bond is cleaved. Two new bonds are created, each containing either the hydrogen atom or the hydroxyl group of the water molecule (Speight, 2018).

Subcritical water is pressurized liquid water with a temperature range of 100 to 374°C and a 1–22.1 MPa pressure. Water exhibits unique properties under these conditions, such as the ability of water (H_2O) to dissociate into hydrogen ions (H^+) and hydroxide ions (OH^-) due to the weakening of the H bonding. This leads to a higher ionization constant, Kw, which gives hydrolysis characteristics to water as a solvent (Cocero *et al*., 2018; Zhang *et al*., 2020). Water is a polar solvent at ambient temperature and pressure due to its high dielectric constant of 80. Water's dielectric constant decreases at high temperatures due to water dissociation, making water a less polar solvent. For example, at 250°C and 25 MPa, water has a dielectric constant of 25, similar to methanol or ethanol for dissolving various organic compounds (Sarker *et al*., 2021). Nowadays, the industry aims to promote sustainability and eco-friendliness process. They are engaged in researching advanced technologies to improve hydrolysis or extraction. The eco-friendly process aims to reduce or eliminate the use of hazardous chemicals, processes, and methods while preventing the generation of waste (Basak and Annapure, 2022).

Ishak *et al.* (2019) used subcritical water treatment under different process conditions (temperature and time) to recover sugars (including glucose) from oil palm trunks (OPT). The highest glucose yield was obtained at 210°C for 5 min (0.08 kg/kg-OPT). Subcritical water was used to hydrolyze the hemicellulose of Holm oak (*Quercus ilex*) and extract glucose and acetic acid (Yedro *et al*., 2017). Glucose and acetic acid are also hydrolyzed by subcritical water and recovered from rice husks (Abaide *et al*., 2019), while Gallon *et al*. (2023) utilized supercritical fluid extraction to recover glucose and acetic acid from *Butia odorata* seed. The presence of organic acid is usually caused by the deacetylation of hemicellulose and the conversion of glucose (Yedro *et al*., 2017). Ishak *et al.* (2019) found that the hydrolysis of OPT using subcritical water at a high temperature (270°C) caused glucose concentration to decrease while acetic acid increased. The degradation of glucose with the formation of acetic acid from syringes in a sequential degradation has also happened at a high temperature of 280°C (Cocero *et al*., 2018; Pan *et al*., 2010). Acetic acid was also recovered with 1.27 ± 0.01 (g/100 g *Butia odorata* seeds residue) by SWH at 260°C for 26 mins (Gallon *et al*., 2023).

At subcritical conditions, the autoionization of water produced H^+ and OH^- ions, which acted as acid or base catalysts, and it was utilized for subcritical water hydrolysis (SWH). This hydrolysis process depends on the temperature and time (Zakaria *et al*., 2017). Studies by Ishak *et al.* (2019) have shown that increased temperature in shorter hydrolysis time in subcritical conditions increases OPT's glucose and acetic acid yield. Glucose yield increased sharply from 200°C then decreased to 230°C, while acetic acid still increased above 300°C.

This study shows that different operation conditions are required for higher glucose and acetic acid yield. Glucose can be degraded at higher temperatures, while acetic acid is produced due to oxidized dehydrated products (Ishak *et al*., 2019). The yield of glucose and acetic acid also depends on the plant's raw material, including the particle size (Ishak *et al*., 2019; Yang *et al*., 2023). However, limited information about the particle size distribution after the sieving of the dried plant material. Therefore, this study aims to evaluate the different process conditions (temperature and time) for the recovery of glucose and acetic acid from PBL leaves using subcritical water hydrolysis in uniform particle size distribution. A laser scattering particle size distribution analyzer was used to analyze the particle size of PBL leaves powder based on the cumulative distribution particle size. The glucose and acetic acid concentrations were analyzed using an ion exclusion high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Raw materials and chemicals

Piper *betle* Linn (PBL) leaves were obtained from the traditional market in Selangor, Malaysia. The young PBL leaves (two weeks old) were harvested from the plants that were more than a year old. The leaves were rinsed with water and dried in an oven at 70°C for 4 hours. The temperature and time of the PBL leaves drying method followed by Pin *et al*. (2009). The drying process of medicinal plants produces dry herbs with higher bioactive compounds than fresh leaves that contain high moisture content (Ebadi *et al*., 2015). Although biomass drying (wheat straw) as raw material can be conducted at 105°C for 4 h (Yang *et al*., 2023) and produced a high yield of total sugar, this study limited the drying of PBL leaves to 70°C to avoid final product degradation. Subsequently, the dried samples were ground and filtered through a 250 µm sieve to increase the surface area of the SWH process. Smaller particle sizes experienced greater pre-treatment severity that tended to render biomass physical and chemical features for better enzymatic accessibility (Yang *et al*., 2023). This powder was placed in a glass bottle and kept in a desiccator to maintain the moisture content at 10.8%. This state is maintained to prevent the formation of clumps, which could disturb the SWH process. This glass was covered with an aluminium foil sheet to avoid light exposure.

Sodium nitrite and potassium nitrate (analytical reagents) in a ratio of 1:1 was used in the SWH as a heating medium for the salt bath. They were purchased from R & M Chemicals. Silicone oil was used as the heating medium for the oil bath and purchased from SYSTERM®.

Particle size analysis

The particle size of the PBL leaves powder was determined in the dry method using a laser scattering particle size distribution analyzer (HORIBA, LA-960, USA). The dispersion method was injector-driven forced dispersion in air using compressed air (air pressure: 0.25 MPa). One gram of PBL leaf powder was placed in the vibrating feeder and then adjusted using vibration power. The powder was ejected through a vacuum-driven forced ejector. The system automatically adjusts the vibration rate to keep the concentration constant during the measurement with the silicone photodiode detector. The diameter was calculated based on a cumulative percentage of 10, 50, and 90%. The analysis was conducted in triplicate.

Subcritical water hydrolysis procedure

Solid loading of PBL leaves powder of 15% (w/w) (ratio of sample: solvent was 3:17) was applied in this study. Approximately 0.75 g of PBL leaves powder (moisture content 10.8%) was added into a stainless-steel reactor cell (Figure 1) with a length of 150 mm (L), an inner diameter of 7.5 mm (ID), a thickness of 1 mm (T) (Swagelok Company, USA), and followed by filling with a 4.25 ml distilled water to achieve 15% (w/w) of solid loading. The reactor was purged with argon gas for 60 seconds and closed tightly.

Hydrolysis was performed using a factorial design at different subcritical process conditions temperature (100-275°C), and time (5-30 min). Heating chambers with an oil bath (for temperature 100 –175°C) and salt bath (for temperature 200 –275°C) were used for subcritical water hydrolysis, as shown in Figure 2. (A and B) (Thomas Kogaku Co Ltd., Japan). The experimental equipment set-up of SWH and the reactor scheme were followed by Rahmah *et al.* (2023b). Once the hydrolysis process was completed using subcritical water in the reactor cell, the extract was centrifuged at 4,000 rpm for 10 min (KUBOTA Corporation, Japan) and filtered through a nylon filter of 0.22µm. The extract (containing glucose and acetic acid) was stored at -15°C for HPLC analysis.

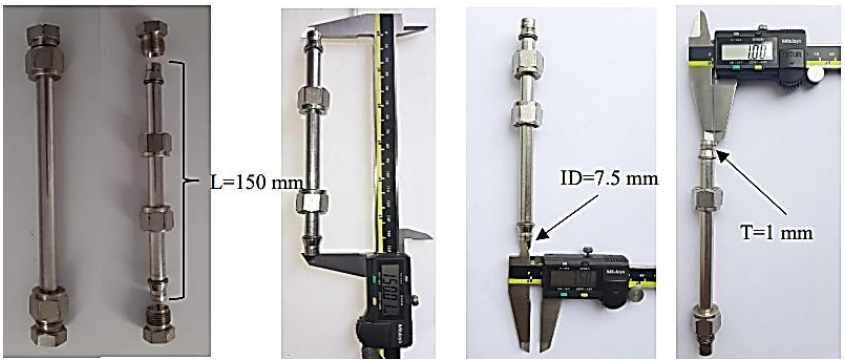

Fig. 1. Electronic photograph of subcritical water reactor

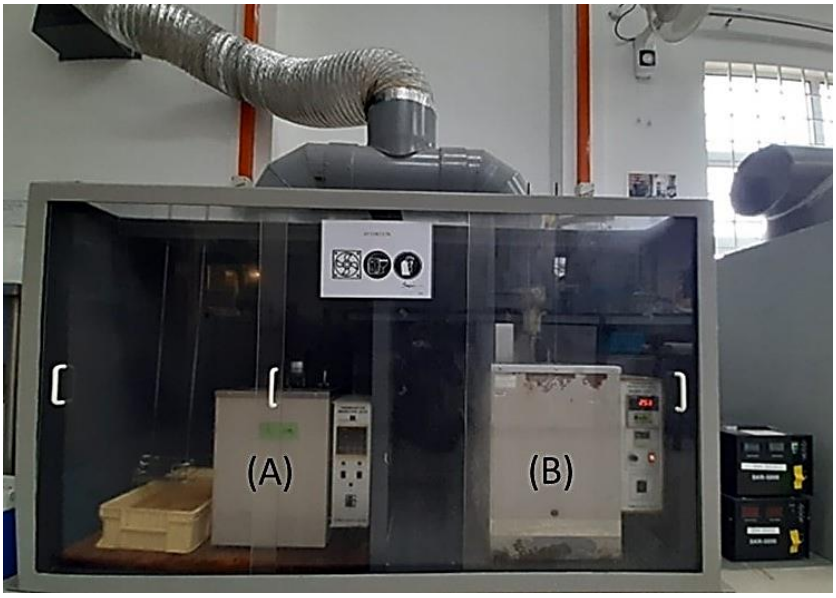

Fig. 2. Electronic photograph of subcritical water instrument: oil bath (A) and salt bath (B)

Glucose and acetic acid analysis

Glucose and acetic acid from the extract of PBL leaves were analyzed using an HPLC method based on Abaide *et al.* (2019) with some modifications. Ion Exclusion HPLC columns packed with an 8% cross-linked hydrogen ion exclusion medium were used for this analysis. HPLC (Agilent, US) equipped with Rezex ROA-Organic Acid H^+ (8%**)** column (length = 300 mm, diameter = 7.8 mm), a refractive index (RI) detector, liquid chromatography (Agilent, US) and column oven (Agilent, US). The flow rate of the chromatographic separations was 0.6 mL/min, the oven temperature was 65°C, and the extract volume was 20 µL. The mobile phase consisted of an isocratic concentration of 0.005 N H_2SO_4. The samples were filtered at 0.22 µm before being injected into the HPLC. The samples' glucose and acetic acid concentrations were identified by their retention time and refractive index detection using the series of their standard solutions. The concentration of glucose (mg/mL) and acetic acid (mg/mL) was obtained from the standard curve of glucose and acetic acid. After obtaining the glucose and acetic acid concentrations, these concentrations were converted to mg/L, and each content of glucose and acetic acid was calculated according to Eq. 1 and 2.

$$\text{Glucose content}\left(\frac{\text{mg}}{\text{g}}\text{extract}\right) = \frac{\text{Glucose conc.}\left(\frac{\text{mg}}{\text{L}}\right)\text{x Vol extract (L)}}{\text{Mass extract (g)}} \quad (1)$$

$$\text{Acetic acid content}\left(\frac{\text{mg}}{\text{g}}\text{extract}\right) = \frac{\text{Acetic acid conc.}\left(\frac{\text{mg}}{\text{L}}\right)\text{x Vol extract (L)}}{\text{Mass extract (g)}} \quad (2)$$

Statistical analysis

The data obtained in this research were analyzed through the ANOVA and Duncan's Multiple Range Test (DMRT) using IBM SPSS Statistics 20. The two-way univariate analysis of variance (two-way ANOVA) was performed to determine the significant differences in factors. A Sig. indicated a significant result or p-value less than 0.05 ($p<0.05$). DMRT, as a post hoc test, was used to know the differences between pairs of means at each factor, as shown by different letters

RESULTS AND DISCUSSION

Piper betle L. (PBL) leaves powder particle size distribution

The dried PBL leaves were ground, and then the powder was analyzed for particle size distribution. The particle size is one of the raw material's physical properties that is responsible for the surface area. The smaller the particle size, the larger the surface area that comes into contact with the solvent so that a highly bioactive compound can be obtained (Makanjuola, 2017). In this study, small particle size is applied as the size of the SWH reactor is small, and there is no agitation between the sample and solvent. At the same time, it helps the subcritical water to achieve the inner part of the PBL leaves matrix to perform hydrolysis. In addition, the short time of SWH (5-30 minutes) needs a small particle diameter to complete the SWH. It was different from conventional hydrolysis of alkaline pre-treated pineapple leaf (dried for 24 hours at 105°C) using enzymatic hydrolysis, which needs a longer time of 24 to 72 hours (Nashiruddin *et al*., 2022). In addition, the small diameter of PBL leaves (below 250 µm) might enhance the hydrolysis of the acetyl group of hemicellulose to produce acetic acid. Hemicellulose molecules had a length of about 20–400 nm and a diameter of 1 nm (Zdunek *et al*., 2014). A similar result was reported by Rizkita *et al.*

(2023) that the extraction of an herbal plant (*Orthosiphon aristatus*) using subcritical water at a particle diameter of 250 µm yielded 284.73 mg of gallic acid equivalent/g of dried sample. Some glucose was linked with gallic acid (called gallotannin), an analog to glucose hydrolysis. Gallotannin is a polymer formed when gallic acid esterifies and binds with glucose. The hydrolysis of gallotannin yields sugar (glucose) and gallic acids (Kim *et al*., 2011; Amarowicz & Janiak, 2019).

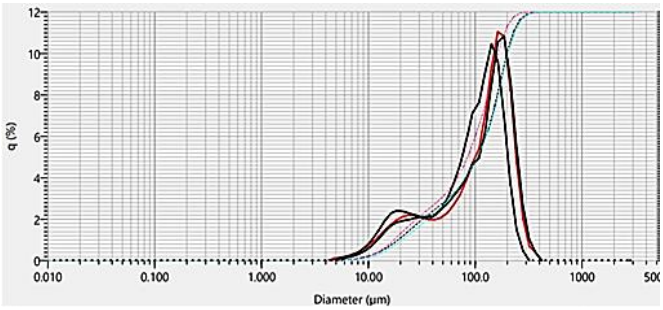

Fig. 3. Particle size distribution

Based on Figure 3, the particle size of the PBL leaves powder in this study is divided into three percentages of cumulative diameter as follows: 21.22 µm (10%), 126.16 µm (50%), and 220.70 µm (90%). Based on the highest percentage (90%) of cumulative diameter, the particle size of PBL leaves powder is 220.70 ± 2.33 µm. Yang *et al*. (2023) studied the effect of different particle sizes (4,000 µm to below 250 µm) of dried wheat straw on the glucose yield via enzymatic hydrolysis. This study found that oven-dried plant material resulted in no significant difference in total sugar yield (g glucose/ g biomass) in the particle size range. However, the particle size below 250 µm showed the highest hydrolysis efficiency (approximately 80%). This study also found that the smaller particle size (250 and below 250 µm) caused more rapid hydrolysis of cellulose to produce glucose than the larger particle size because it experienced greater pre-treatment severity that tended to render biomass physical and chemical features for better enzymatic accessibility. In smaller particle sizes, hemicellulose can also be separated easily to hydrolize an acetyl group to produce acetic acid. Hence, this study proved that the particle size of PBL leaves of 220.70± 2.33 µm was effective, and it can be used to recover glucose and acetic acid by SWH.

Glucose recovery from PBL leaves using subcritical water hydrolysis

A previous study by Rahmah *et al.* (2023a) found that the carbohydrate content is the highest component of the dried PBL leaves (49.6 %). The breakdown of polysaccharides (carbohydrates) into monosaccharides occurs through a hydrolysis process. As Gibson and Newsham (2018), hydrolysis refers to the cleavage or unbinding of chemical bonds by adding water. Subcritical water treatment is a method of autohydrolysis that utilizes subcritical (at high temperature and pressure) for the hydrolysis process to recover sugars. Subcritical water could simultaneously extract several other active ingredients, such as sugar (glucose) and organic acid, from natural products or matrix plants (Cheng *et al*., 2021; Gallon *et al*., 2023; Ishak *et al*., 2019; Valentão *et al*., 2010). In this study, HPLC analyzed glucose recovery from PBL leaf extract using subcritical water hydrolysis. The data is presented in Table 1, and the glucose ANOVA is presented in Table 2. Based on the ANOVA result, process temperature and time significantly influence glucose concentration ($p < 0.05$). Distinct letters analyzed from DMRT indicate significantly different effects across 48 treatments.

Table 1 shows that the glucose concentration at 5 and 10 minutes increased with temperature from 100 to 200°C,

thereafter declining to 275°C. A high yield of glucose can be obtained in a short time at a higher temperature. The high temperature contributed to the high energy required to dissolve the glucose despite the short extraction time. However, increasing the extraction temperature to 275°C led to glucose. P. Cardenas-Toro *et al*. (2014) reported that monosaccharides degrade into organic acids at high temperatures.

At 15 min, there was a fluctuation of glucose concentration from 100 to 200°C. The glucose concentration remains constant at 100 to 150°C, then decreases at 175°C. It increases at 200°C and then decreases until 275°C. This result shows that at 15 min, glucose is stable from 100 to 150°C. At 175°C, glucose concentration decreases because, at this condition, many phenolic compounds are also extracted, which might decrease the solubility of the glucose. Alonso-Riaño *et al*. (2021) revealed that the maximum total phenolic compounds from Brewer's spent grain (a lignocellulosic solid by-product) were obtained at 185°C using subcritical water extraction. A similar result was also obtained with the hydrolysis of glucose from rice husk, where the glucose fluctuated at 260°C (Abaide *et al*., 2019). It was supported by Ishak *et al*. (2019) that glucose concentration was reduced above 210°C. Then, it can be converted to organic acid (P. Cardenas-Toro *et al*., 2014).

At 20 min, glucose increased at 100 to 125°C, then decreased and remained constant until 225°C, then decreased again at 250 to 275°C. Increasing the extraction temperature to 275°C caused the glucose to degrade. At 25 min of extraction time, glucose increased from 100 to 125°C, decreased at 150°C, increased to 175°C, and then decreased until 275°C. At 30 min, glucose increased from 100 to 125°C, remained constant at 150°C, increased at 175°C, then decreased until 275°C. These results indicated that at 20-30 min of hydrolysis/extraction, high glucose concentration was yielded at 125°C, which means that prolonged time in low temperature can dissolve glucose in high amounts due to the long duration of mass transfer without destroying the glucose

Table 1: Glucose concentration in different process conditions of SWH

Time (min)	Glucose concentration mean ± SD* (mg/g extract)							
	Temperature (°C)							
	100	125	150	175	200	225	250	275
5	2.512 ± 0.074^{bcdefg}	4.659 ± 0.032^{klm}	2.868 ± 0.040^{cdefghi}	3.960 ± 0.242^{hijkl}	6.143 ± 0.284^{n}	1.633 ± 1.589^{b}	0.180 ± 0.006^{a}	0.123 ± 0.001^{a}
10	2.973 ± 0.329^{efghi}	4.055 ± 0.281^{ijkl}	3.036 ± 0.182^{fghi}	2.014 ± 0.050^{bcdef}	4.281 ± 0.605^{jkl}	2.094 ± 0.008^{bcdef}	0.139 ± 0.015^{a}	0.126 ± 0.039^{a}
15	3.195 ± 0.086^{fghij}	4.082 ± 0.112^{ijkl}	4.272 ± 0.141^{jkl}	1.719 ± 0.041^{bcd}	4.091 ± 0.140^{ijkl}	1.803 ± 0.108^{bcde}	0.141 ± 0.025^{a}	0.225 ± 0.095^{a}
20	3.047 ± 0.109^{fghi}	4.341 ± 0.108^{jkl}	3.025 ± 0.041^{fghi}	3.632 ± 2.692^{ghijk}	3.025 ± 0.152^{fghi}	2.257 ± 0.023^{bcdef}	0.148 ± 0.001^{a}	0.101 ± 0.022^{a}
25	2.762 ± 0.039^{bcdefgh}	4.962 ± 0.049^{lm}	3.736 ± 0.225^{hijk}	4.983 ± 1.216^{lm}	2.913 ± 0.073^{defghi}	1.706 ± 0.010^{bcd}	0.153 ± 0.031^{a}	0.122 ± 0.022^{a}
30	3.038 ± 0.074^{fghi}	5.544 ± 0.129^{mn}	4.678 ± 0.208^{klm}	6.237 ± 0.438^{n}	2.478 ± 0.057^{bcdefg}	1.657 ± 0.080^{bc}	0.147 ± 0.012^{a}	0.103 ± 0.016^{a}

*) DMRT test symbol with $\alpha = 0.05$. Different letters show significant treatment ($p < 0.05$) in 48 treatments. A similar letter has a similar value statistically.

Table 2. ANOVA result of glucose

Tests of Between-Subjects Effects					
Dependent Variable: Glucose					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	305.365[a]	48	6.362	24.511	.000
Intercept	652.276	1	652.276	2513.084	.000
Temperature	247.199	7	35.314	136.058	.000
Time	4.715	5	.943	3.633	.007
Temperature * Time	53.201	35	1.520	5.856	.000
Block	.250	1	.250	.965	.331
Error	12.199	47	.260		
Total	969.840	96			
Corrected Total	317.564	95			

a. R Squared = .962 (Adjusted R Squared = .922)

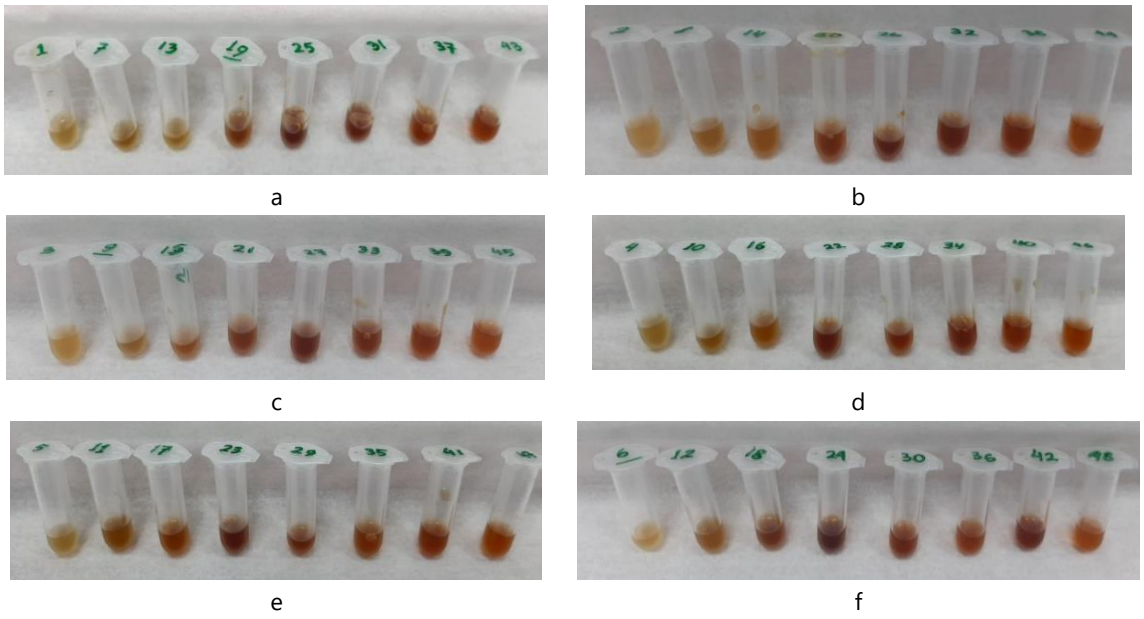

Fig. 4: PBL leaves extract using SWH at different temperature (left to right: 100 to 275°C) and extraction time (a) 5 min; (b) 10 min; (c) 15 min; (d) 20 min; (e) 25 min; (f) 30 min

structure. In contrast, glucose concentration decreased from 200°C (after 5 min) to 275° (5-30 min). High glucose concentrations were achieved at 125°C at 30 min (5.544 ± 0.129 mg/g extract), 175°C at 30 min (6.237 ± 0.438 mg/g extract), and 200°C at 5 min (6.143 ± 0.284 mg/g extract).

In addition, glucose was also detected from the brown color of the PBL leaf extract, as shown in Figure 4. The brown color might be due to the Maillard reaction between glucose and amino acid. According to Alonso-Riaño *et al.* (2021), the maximum amino acid was hydrolyzed at 160°C. This is related to the extracted color that has a darker color (brown), started at 175°C, increased to 200, and then decreased to 275°C. A similar result was also reported by Alonso-Riaño *et al.* (2021) that the increase in browning is directly related to the advanced phases of the Maillard reaction. The darker color of the extracts from the brewer's spent grain was observed with increasing temperature. The color change would

indicate the increase in hydrolysis and decomposition product yield in the aqueous phase.

Quantification of acetic acid

Table 3 shows the concentration of acetic acid at different process temperatures (100 - 275°C) and time (5 – 30 min). The ANOVA shown in Table 4 proved that temperature and time significantly influence acetic acid concentration ($p<0.05$). The acetic acid concentration increased over the times and temperatures, from 5 to 30 min and from 100 to 275°C. Acetic acid recovered highly at high temperatures and extraction time. For instance, at a minimum extraction time of 5 min, extending extraction temperature from 100 to 275°C, the acetic acid concentration increased from 0.694 ± 0.019 to 2.057 ± 0.335 mg/g extract. At 30 min, acetic acid recovery increased from 100 to 275°C (0.815 ± 0.032 to 2.536 ± 0.203 mg/g extract). A similar result was obtained by Pan *et al.* (2010), which was that the optimal temperature for acetic acid production from syringol was 280 °C, which resulted in the lower formation of other products. This organic acid is stable at high temperatures. The presence of acetic acid might be related to the lower pH (4-5) measured in the extract obtained at high temperatures (200 to 250°C) compared to lower temperatures (100 - 175°C) with pH 5-6. In the higher temperature range, the highest content of acetic acid was achieved at 225°C for 20 minutes. This indicates the deacetylation of hemicellulose to produce acetic acid might need a higher temperature and a longer time.

Generally, acetic acid recovery has an opposite trend with glucose. Acetic acid increases when glucose decreases with temperature and vice versa. For example, at the extreme temperature (275°C) and time (30 mins) of extraction, the glucose concentration is very low (0.103 ± 0.016 mg/g extract). In contrast, the acetic acid concentration is very high (2.536 ± 0.203 mg/g extract). This was due to glucose degradation, which formed acetic acid sequentially at a high temperature of 280°C (Cocero *et al*., 2018; Pan *et al*., 2010). Acetic acid can also be obtained from sugar degra-

Table 3. Acetic acid concentration in different process conditions of SWH

Time (min)	Acetic acid concentration mean ± SD* (mg/g extract) (Temperature (°C)							
	100	**125**	**150**	**175**	**200**	**225**	**250**	**275**
5	0.694 ± 0.019^{a}	1.535 ± 0.313^{fghij}	0.806 ± 0.049^{abc}	1.334 ± 0.102^{defg}	1.836 ± 0.105^{hijklm}	1.692 ± 0.069^{ghijkl}	2.228 ± 0.259^{mnopqr}	2.057 ± 0.335^{klmnop}
10	0.995 ± 0.150^{abcde}	1.429 ± 0.181^{efgh}	1.013 ± 0.088^{abcde}	1.280 ± 0.061^{bcdefg}	2.025 ± 0.152^{jklmno}	1.942 ± 0.064^{ijklmn}	2.625 ± 0.185^{rs}	2.272 ± 0.167^{mnopqr}
15	0.847 ± 0.011^{abcd}	1.323 ± 0.152^{defg}	1.372 ± 0.226^{efgh}	0.785 ± 0.726^{ab}	2.805 ± 0.788^{s}	2.001 ± 0.151^{ijklmn}	2.583 ± 0.017^{qrs}	2.625 ± 0.231^{rs}
20	0.828 ± 0.042^{abc}	1.298 ± 0.011^{cdefg}	1.127 ± 0.000^{abcdef}	1.995 ± 0.016^{ijklmn}	1.997 ± 0.031^{ijklmn}	2.856 ± 0.061^{s}	2.421 ± 0.115^{nopqrs}	2.289 ± 0.323^{mnopqr}
25	0.752 ± 0.016^{a}	1.400 ± 0.010^{efgh}	1.327 ± 0.111^{defg}	1.608 ± 0.106^{fghijk}	2.231 ± 0.245^{mnopqr}	2.109 ± 0.037^{klmnopq}	2.514 ± 0.034^{opqrs}	2.598 ± 0.028^{qrs}
30	0.815 ± 0.032^{abc}	1.520 ± 0.160^{fghi}	1.660 ± 0.213^{ghijkl}	2.160 ± 0.154^{lmnopqr}	2.029 ± 0.170^{jklmnop}	2.216 ± 0.066^{mnopqr}	2.435 ± 0.075^{nopqrs}	2.536 ± 0.203^{pqrs}

*) DMRT test symbol with $\alpha = 0.05$. Different letters show significant treatment ($p < 0.05$) in 48 treatments. A similar letter has a similar statistical value.

Table 4. ANOVA result of acetic acid

Tests of Between-Subjects Effects					
Dependent Variable: Acetic_acid					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	37.775[a]	48	.787	18.084	.000
Intercept	299.806	1	299.806	6889.050	.000
Temperature	30.579	7	4.368	100.379	.000
Time	1.578	5	.316	7.253	.000
Temperature * Time	5.521	35	.158	3.625	.000
Block	.097	1	.097	2.232	.142
Error	2.045	47	.044		
Total	339.627	96			
Corrected Total	39.821	95			

a. R Squared = .949 (Adjusted R Squared = .896)

dation in the liquid phase *(Yedro et al*., 2017). It has a similar trend with Abaide *et al*. (2019) that acetic acid by subcritical water hydrolysis of rice husks at 180 and 260°C for 3 min increased from 0.58 ± 0.11 to 21.9 ± 1.7 g/L. Acetic acid also increased from 0.96 ± 0.12 to 1.27 ± 0.01 (g/100 g *Butia odorata* seeds residue) obtained by SWH at 260°C for 26 mins (Gallon *et al*., 2023).

CONCLUSIONS

Due to their high carbohydrate content, PBL leaves can be a potential raw material as the source of glucose and acetic acid. The particle size of dried PBL leaves of 220.70± 2.33 µm was effective for glucose and acetic acid recovery by subcritical water hydrolysis. This study has shown that SWH with suitable process temperature and time can recover a high yield of glucose and acetic acid, which results from the interaction between temperature and time remarkably influencing concentration. In this study, the SWH of PBL leaves yielded glucose in the highest concentration of 6.143 mg/g extract obtained at 200°C and 5 min, while acetic acid with 2.856 mg/g extract obtained at 225°C and 20 min. The current findings show that glucose and acetic acid had an opposite trend: the degradation of glucose forming acetic acid.

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