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Enhanced Recovery of Bioactive Compound from Pineapple Peel Using Ultrasonic-Assisted Extraction with Enzyme Treatment at Varying Extraction Time

Amalia Nur Kumalaningrum[1*], Zulmilafatma Nurlita Arini[2], Jefri Pandu Hidayat[2]

[1]Department of Food Technology, Institut Teknologi Kalimantan, Jl. Soekarno Hatta No.KM 15, Karang Joang, Balikpapan. 76127, Indonesia

[2]Department of Chemical Engineering, Institut Teknologi Kalimantan, Jl. Soekarno Hatta No.KM 15, Karang Joang, Balikpapan. 76127, Indonesia

[*]*Corresponding Author: amalia.nur@lecturer.itk.ac.id*

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Abstract

Pineapple peel is considered waste, but it has numerous beneficial uses due to its rich content of nutrients and bioactive compounds. The selection of extraction methods is essential to produce optimal bioactive compound extracts. Ultrasonic-assisted extraction (UAE) is a modern extraction technique that uses ultrasonic waves to improve the extraction by enhancing the release of compounds into the solvent. The UAE method was performed by varying extraction times at 30, 60, 90, and 120 min. The addition of cellulase enzyme was also applied as an optimization method for the extraction results. The addition of Cellulase elevated the yield, phenolic, and flavonoid content. The highest yield was obtained at an extraction time after 90 minutes. The highest total flavonoid content was 497.8±4.5 µgQE/mL, while the highest total phenolic value was 1007.6±7.6 µgGAE/mL at an extraction of 60 min. However, the extracted samples without enzymes performed the highest results at an extraction of 90 min. The effect of the enzyme resulted in the most potent antioxidant activity (<10 ppm) in all-time extraction. To conclude, the addition of enzymes in the extraction process successfully optimized the extraction of phytochemical compounds from pineapple peel, as evidenced by testing phenolic and flavonoid content and antioxidant activity.

Keywords: Antioxidant activity, Cellulase, flavonoid and phenolic content, Pineapple peel waste, Ultrasonic-assisted extraction

INTRODUCTION

The utilization of pineapple fruit, which is still focused on the fruit's flesh, causes most pineapple peel to contribute to organic waste with a percentage of 30% (Meena et al., 2022). Pineapple peel, which is often counted as waste, has good phytochemical content for the body, such as vitamin C, carotenoids, and flavonoids, making pineapple peel a potential source of natural antioxidants (Waznah et al., 2021). Antioxidants are essential in preventing body cells from exposure to free radicals, which come from air pollution, cigarette smoke, and chemicals contained in food (Martemucci et al., 2022). The availability of pineapple peel, which is relatively abundant but still minimally utilized, makes pineapple peel a promising source of natural antioxidants. Natural antioxidants are preferred over synthetic ones because synthetic antioxidants must be used within the recommended dosage to avoid potential side effects that could be harmful to the body (Mahardika et al., 2023).

These antioxidant compounds can be obtained through the extraction process. Extraction is a process that involves the separation of the desired solute using a particular solvent (Salve and Ray, 2020) Conventional extraction methods, such as solvent extraction, produce low yields and require high energy for a relatively long time, so the efficiency of this process is relatively low (Zhang et al., 2018). Hatam et al. (2013) extracted pineapple peel using maceration, Soxhlet, and reflux methods with 80% ethanol for 2 hours. All conventional methods yielded less than 5%, with phenolic content below 30 µg/mL and flavonoid content below 6 µg/mL, respectively.

The ultrasonic-assisted extraction (UAE) method requires a shorter time, low temperature, and lower solvent usage (Kumar et al., 2021). Ultrasonic waves can damage cell wall permeability and accelerate the release of component targets (Shen et al., 2023). Low operating temperatures can minimize damage to the extracted content, which is sensitive to high heat. Yahya et al. (2019) assessed the effect of sonication in

extracting bioactive compounds from pineapple peel waste. The sonication treatment considerably resulted in higher yield and total phenolic content (29.04 %; 1078.68 ± 1.32 mg GAE/g) than that obtained using Soxhlet reflux extraction (24.95% and 7.98 mg GAE/g).

Besides applying ultrasonic waves, cellulase enzymes are added to optimize the extraction results. The cellulase enzymes added in the extraction process also bring advantages such as lower energy requirements, high extraction rates, and more manageable and environmentally friendly recycling systems (Liu et al., 2021). Pistachio extraction with enzyme assistance contained more phenolic compounds than solvent extraction alone. Adding cellulase enzyme with a concentration of 2.5 U/mL increased the extraction yield by 22% (Ghandahari Yazdi et al., 2019). Furthermore, combining cellulase enzymes added to the UAE process is expected to produce maximum results with more efficient time and energy.

This research examined the impact of combining cellulase enzymes with the UAE method under varying extraction times. The extracted samples were analyzed quantitatively. Quantitative analyses are conducted to measure the total flavonoid and phenol content using a UV-Vis spectrophotometer. The total phenolic content was carried out using the Folin-Ciocalteu method, and the total flavonoid content was carried out using the Aluminum trichloride colorimetric method, where both were analyzed by the spectrophotometric method (Zihad et al., 2019). Antioxidant activity tests were analyzed using the DPPH method. This method's determination of antioxidant activity is expressed in % inhibition or IC_{50}; the amount of antioxidant substances needed to inhibit free radicals is about 50% (Hidalgo & Almajano, 2017).

METHODOLOGY

Materials and instrumentals

The sample used in this research was pineapple peel waste from the North Balikpapan area, Balikpapan City, East Kalimantan. Some of the reagents used for chemical analysis were methanol (Merck), HCl 37% (Merck), NaOH (Merck), Quercetine (Sigma Aldrich), $NaNO_2$ (Merck), $AlCl_3$ (Merck), Folin-Ciocalteu (Merck), Na_2CO_3 anhydrous (Merck), gallic acid (Merck), DPPH (Sigma Aldrich) and cellulase powder (food grade; activity 100,000 IU/g). Some of the main tools used in this study were an ultrasonic bath (GT Sonic), spectrophotometer UV-VIS single beam (Thermo Scientific), food dehydrator, grinder electric (Sonifer), hotplate (Thermo Fisher Scientific), and glassware (pyrex).

Methods

Sample preparation

The harvested pineapple peel samples were thoroughly washed to remove impurities and then sliced into small pieces. The pineapple peel was dried at 60°C until its water content was reduced by 90%. The dried pineapple peel was then mashed, sieved with a size of 100 mesh, and stored in an airtight container at room temperature.

Extraction process

Pineapple peel extraction was carried out using the ultrasonic-assisted Extraction (UAE) method using an ultrasonic bath. The pineapple peel powder was added to distilled water with a ratio of 1:10. The enzyme was added by conditioning the sample solution at pH 5 with an enzyme concentration of 2% (w/w). The sample was homogenized, and extraction was performed at a temperature of 50°C with a power setting of 50 W and time variations of 30, 60, 90, and 120 min. The UAE procedure without enzyme was applied as a comparison. The sample was filtered and evaporated at 60°C until the extract became thick and concentrated. The yield of the thick extract was then calculated using this formula:

$$\%\text{Yield} = \frac{\text{Thick extract (g)}}{\text{Pineapple peel powder (g)}} \times 100\% \quad (1)$$

The concentrated extract was dissolved in 10 mL of distilled water. The sample was stored in the refrigerator until the following analysis process (Kumar et al., 2021; Xu et al., 2018).

Total flavonoid content

A total flavonoid test was conducted by making a quercetin solution that had been diluted with methanol in several concentrations, namely 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL. About 1 mL of each solution was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. The mixed solution was incubated for 5 min. The solution was added with 0.3 mL of 10% $AlCl_3$ and 2 mL of 1 M NaOH, then homogenized for further absorbance measurements to create the standard curve at 415 nm. The absorbance of the samples was measured using the same method as for the standard solutions (Stanković, 2011).

Total phenolic content

Total phenolic testing first begins with forming a standard curve of gallic acid. Gallic acid was diluted with ethanol to 0, 50, 100, 150, 200, 250, and 300

µg/mL. Then, at each concentration of 1 mL, 10% Folin-Ciocalteau reagent was added to as much as 5 mL and 7% Na_2CO_3 as much as 4 mL, and then, it was homogenous. Then, the solutions were allowed to incubate in a dark room for 30 minutes, then continued with the measurement at 760 nm. The samples were analyzed with the same treatment (Manongko et al., 2020).

Antioxidant activity

Antioxidant activity was assessed using the DPPH free radical scavenging method. A DPPH solution of 4 ppm was prepared by dissolving 4 mg of DPPH solid in 100 mL of methanol. Each sample with varying variables was diluted to 100 ppm by dissolving it in methanol. The samples were varied again into 4 concentrations, namely 5, 7.5, 10 and 12.5 ppm. About 2 mL of each sample at different concentrations was taken and mixed with 2 mL of the four ppm DPPH solution, then homogenized, stored in a dark room, and incubated at 30 min. The absorbance was subsequently measured at a wavelength of 519 nm. The blank solution or DPPH control was made by mixing 2 mL of DPPH 4 ppm with 2 mL of methanol (Gulcin & Alwasel, 2023). From the results of absorbance measurements between the sample and the blank solution, the free radical quenching power of the sample would be calculated, known as % inhibition or IC_{50}, which was calculated using the equation (Purwaningsih et al., 2023):

$$\%\text{Inhibition} = \frac{\text{Abs.blank} - \text{Abs.sample}}{\text{Abs.blank}} \times 100\% \quad (2)$$

The results were input into the regression equation, with the extract concentration (ppm) as the X-axis and the % inhibition value as the Y-axis. Using the equation Y = bX + a, the IC_{50} value was calculated using the formula. (Hasanela et al., 2023; Muadifah et al., 2024):

$$IC_{50} = \frac{50 - a}{b} \quad (3)$$

Data Analysis

Data analysis was obtained from the extract yield, total flavonoid, and phenolic content at each various time. The study was repeated three times. Data were analyzed using SPSS 29.0.0.0 (241), the one-way ANOVA method (Tukey test) at a 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Yields of pineapple peel extract

The pineapple peel extraction was performed using the UAE method, which in this study utilized an ultrasonic bath as an extraction medium that delivered ultrasonic waves during the extraction process. The extraction process was conducted using an ultrasonic bath at 50°C and a power of 50W. The pineapple peel extract was prepared by dissolving 5 grams of peel powder into 50 mL of distilled water, creating a solution with a 1:10 ratio. The extraction process was carried out on pineapple peel was given a variation of extraction time: 30, 60, 90, and 120 min. The extraction process was carried out using the cellulase enzyme, which was 2% w/w of the weight of pineapple peel powder so that the effect of adding enzymes and extraction time on pineapple peel in the UAE method could be known.

The results of pineapple peel extraction were evaporated using a hot plate at a temperature of 60°C to reduce the potential damage to the bioactive compound of pineapple peel extract (Istriningsih et al., 2023). The difference in extract yield between samples extracted with and without the addition of cellulase enzyme at different extraction times can be seen in Figure 1.

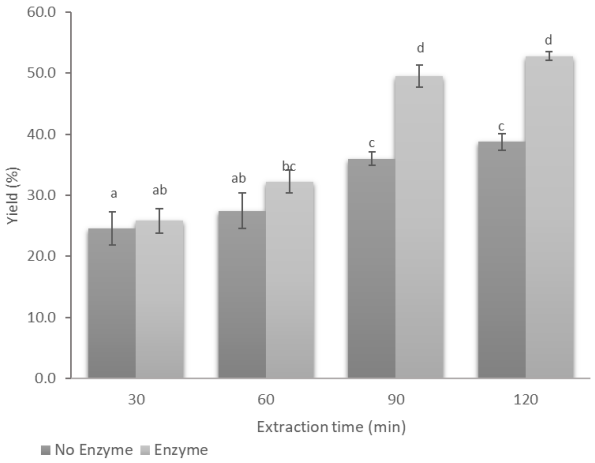

Figure 1. Yield of pineapple peel extractions using the UAE method, with and without enzymes, at various extraction times ($p < 0.05$)

Fig. 1 shows that the amount of pineapple skin extract yield increased along with the increase in extraction time. This phenomenon is because a longer extraction time increases the contact duration between the sample and the solvent, making the extraction process more efficient. Haido et al. (2024) stated that to optimize the extraction process, increasing the extraction time also leads to a higher yield.

The addition of cellulase enzymes produces a higher yield than variables without adding enzymes.

The increase in yield was also observed to differ significantly at 90 min with (49.5±1.8%) and without enzyme (36.0±1.1%), which also showed the highest extraction yield. Adding cellulase enzymes would optimize the extraction process, especially in plant samples. Pineapple peel contains about 24% cellulose (Pereira et al., 2022). Cellulose is a chemical component that makes up the cell wall. The effect of enzymes can damage the cell wall and cause the bioactive components in it to be extracted by the solvent (Fotsing Yannick Stéphane et al., 2022).

The addition of enzymes as a catalyst for the extraction process destroys the cell structure so that the material's permeability will increase and the extraction process will run better (Łubek-Nguyen et al., 2022). The extraction process is followed by adjusting the temperature according to the conditions where the enzyme can work optimally (Mardiah et al., 2019). Enzymes function optimally at lower temperatures, typically between 35–50°C, with a pH adjusted to suit their activity. They perform most effectively in an acidic environment (Panja, 2018).

Total flavonoids and phenolic of pineapple peel extract

The total flavonoid content in pineapple peel extract was measured. The absorbance value of the extracted sample was carried out using UV-Vis Spectrophotometry. Flavonoid compounds possess antioxidant properties because they contain a hydroxyl group linked to the carbon of the aromatic ring, enabling them to combat free radicals. (Hasanela & Souhoka, 2022). Quercetin is a standard for calculating the total flavonoid concentration of the extracted sample. Quercetin itself is chosen as a standard because the content of the flavonol compound group is dominated by up to 75% quercetin and its glycosides(Anggorowati et al., 2016).

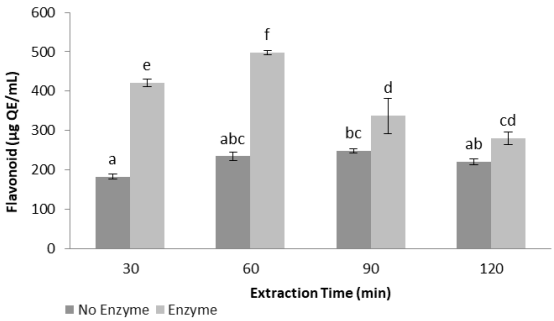

Figure 2. Total flavonoid of pineapple peel extraction using the UAE method, with and without enzymes, at various extraction times ($p < 0.05$)

Extracts with the addition of cellulase enzymes produced higher total flavonoid levels compared to pineapple peel extracts without the addition of enzymes (Fig. 2). Pineapple peel extraction with the addition of enzymes had the highest flavonoid value at the variable of 60 minutes of extraction time, while in extracts without the addition of enzymes the highest value was at the variable of 90 min (253.3±4.9 µgQE/mL). This condition is by the initial purpose of using cellulase enzymes in the extraction process, to be a catalyst in terms of degrading cell walls in pineapple peel so that bioactive compounds can be diffused optimally in an adequate extraction time (Hu et al., 2021). Therefore, the highest flavonoid levels were obtained in the extract by adding cellulase enzymes with an extraction time of 60 min (497.8±4.5 µgQE/mL). In Comparison, there was no significant difference in the extraction time of 60 min and 120 min. There was a decrease in total flavonoids after 60 min of the extraction process. A reduction of flavonoid values was also experienced in previous research conducted by Widyapuri et al. (2022), which stated that the sonication period in extraction using the UAE method would affect the decomposition of the extracted content obtained. This allowed extraction over a longer time to cause deterioration of the flavonoid compound by causing modification or breaking of the flavonoid compound bonds so that the total flavonoid content would decrease.

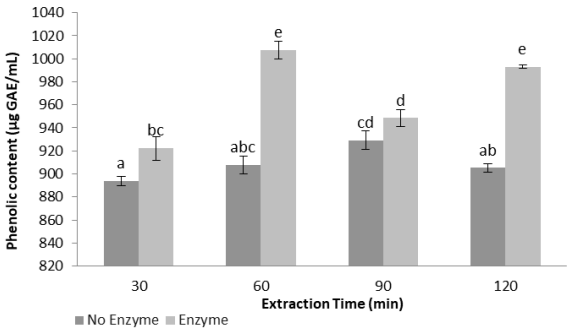

Figure 3. Total phenolic content of pineapple peel extraction using the UAE method, with and without enzymes, at various extraction times

Total phenolic content was determined using the Folin-Ciocalteu method, where absorbance was measured with a UV-Vis spectrophotometer. A standard curve of gallic acid was used, with measurements taken at a maximum wavelength of 760 nm. Without the addition of enzyme, the total phenolic contained reached a higher amount at the time of extraction at 90 min (929.3±8.0 µgGAE/mL). The

highest phenolic content value was obtained in pineapple peel extract with the addition of cellulase enzyme, where the extraction time affected the total phenolic content. The optimum value of total phenolic content with the addition of cellulase enzyme was at 60 min with a total phenolic value of 1007.6±7.6 µgGAE/mL. However, the total phenol decreased after 60 minutes of the extraction process. This condition was similar to the total flavonoid results in Fig. 2. This indicates damage to the phenolic compound content in pineapple skin extract during the heating process and continuous collision due to sonication waves, considering the characteristics of phenolic compounds that are volatile and susceptible to high temperatures (Baturante et al., 2024).

However, the extraction yield (Fig. 1) represented the total mass of extract obtained, it did not necessarily correlate directly with the concentration of bioactive compounds like flavonoids and phenolics. At an extraction time of 90 min, the conditions might be optimal for releasing these compounds from the plant matrix without degrading them. Extended extraction periods can cause the breakdown or oxidation of delicate bioactive compounds. For example, phenolic and flavonoid compounds are vulnerable to environmental influences like temperature, light, and oxygen, potentially diminishing their potency and concentration as time progresses (Gil-Martín et al., 2022). These could explain why test results peaked at 60 min and declined afterward, even if the overall yield continued to increase.

The application of particular conditions, such as introducing cellulase enzyme, facilitated the disintegration of the plant cell wall, leading to an improved release of desired compounds. However, excessive extraction time might dilute these compounds in the total extract or result in the co-extraction of impurities that affect the test outcomes (Shi et al., 2022). The 60-minute extraction time might strike a balance between releasing bioactive compounds and minimizing their degradation or dilution, resulting in higher test values for flavonoid, phenolic, and antioxidant content at that specific time.

Antioxidant activity

The antioxidant activity of compounds in an extract can be evaluated by assessing the extract's ability to inhibit free radicals using the DPPH assay. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method is popular, quick, straightforward, and affordable for measuring antioxidant properties. It is commonly applied to evaluate the ability of substances to act as free radical scavengers (Baliyan et al., 2022). Pineapple peel extract samples varied into several concentrations and were added to the DPPH solution. Then, the absorption was recorded using UV-Vis spectrophotometry at 519 nm. In addition to measuring sample absorption, the control variable wavelength was also measured using a mixture of methanol and DPPH solution. The absorption results obtained were used to calculate the % inhibition. These inhibition values were formed into a linear regression equation so that the IC_{50} value could be obtained. IC_{50} itself is an essential parameter in testing antioxidant activity. The IC_{50} value represents the effective concentration of the extract required to inhibit 50% of the radical activity induced by DPPH (Reviana et al., 2021).

Table 1. IC_{50} (ppm) Pineapple peel extract without and with added enzymes

Extraction time (min)	IC_{50} (no enzyme)	IC_{50} (enzyme)
30	19,348	1,431
60	8,104	0.129
90	7,874	3,407
120	9,756	1,353

A compound is considered a powerful antioxidant if its IC_{50} value is below 10 ppm, strong if the IC_{50} falls between 10 and 50 ppm, weak if the IC_{50} is between 50 and 100 ppm, and inactive if the IC_{50} exceeds 250 ppm (Phongpaichit et al., 2007). Without adding cellulase enzyme, the antioxidant activity test results showed potent antioxidant activity in the 30-minute variable. The 60, 90, and 120 min variables were classified in the powerful antioxidant category, with an IC_{50} value of less than 10 ppm. Meanwhile, pineapple peel extract with cellulase enzyme added an IC_{50} value of less than 10 ppm in all extraction time variables.

A smaller IC_{50} value indicates a stronger ability to scavenge free radicals. Pineapple peel extract with the addition of cellulase enzyme at an extraction time of 60 min (0.129 ppm) had the most potent antioxidant activity. Quantitative testing of flavonoid and phenolic content also produced the highest amount with the addition of cellulase enzyme at an extraction time of 60 min. The addition of cellulase enzyme in the extraction process successfully optimized the absorption of bioactive compounds in pineapple peel as the extracted substrate.

Apart from the addition of enzymes, extraction time also affected the extraction results, which increased yield value at long extraction time, increased. However, regarding phytochemical content, flavonoid, and phenolic compounds generally showed a decrease at extraction times, especially 120 min. This reason was due to the prolonged heating process, and

the continuous application of ultrasonic waves during the extraction process might allow degradation or decomposition of the desired phytochemical compound content during the extraction process. The highest value in quantitative testing carried out on pineapple peel extract samples without the addition of cellulase enzyme was at an extraction time of 90 min. In contrast, the highest test value was observed at an extraction time of 60 minutes in samples treated with the addition of cellulase enzyme. This showed that adding cellulase enzyme to the extraction process using the UAE, which collaborated with the cellulase method, allowed for a shorter extraction time than the UAE without enzymes.

CONCLUSION

This study aimed to investigate the impact of cellulase enzymes on the extraction of bioactive compounds from pineapple peel using the UAE method. The extraction time of pineapple peel was directly proportional to the increase in the amount of % extract yield obtained, where the highest amount of extract obtained was at 120 min. Extraction with the addition of enzymes produced a higher yield than extraction without enzymes. The addition of cellulase enzymes could increase the amount of extracted phytochemical compound, with the highest total flavonoids at about 497.8±4.5 µgQE/mL and the highest total phenolic content was 1007.6±7.6 µg GAE/mL for 60 min of extraction. The addition of cellulase enzyme produced the lowest IC_{50} value at all extraction times in the UAE method, and the lowest IC_{50} was at 60 min of extraction. In conclusion, cellulase enzyme can shorten the extraction time, where the extraction showed optimal results at 60 min of extraction.

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