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Effect of Extraction Temperature on Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Pineapple Peel Extracts

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ABSTRACT

Ananas comosus L. Merr, or pineapple, is recognized for its nutritional and therapeutic benefits, rich in vitamin C and bioactive compounds. However, pineapple waste, including peel, core, and pomace, presents environmental challenges due to inadequate handling practices. This study investigates the influence of drying temperature on the antioxidant properties and phenolic content of pineapple peel. Ultrasonic-assisted extraction with absolute ethanol was employed to extract bioactive compounds. Results indicated that drying pineapple peel at 100°C yielded the highest crude extract (8.74 ± 0.1681%). Additionally, pineapple peel dried at 100°C exhibited the highest total phenolic content (32.23 ± 0.0023 mg GAE/g extract) and total flavonoid content (8.43 ± 0.0004 mg QE/g extract), while the lowest levels were observed at 50 °C (15.90 ± 0.0017 mg GAE/g extract and 5.71 ± 0.0003 mg QE/g extract, respectively). Antioxidant activity evaluated using the DPPH assay showed the lowest IC_{50} value at 100°C (441.10 µg/mL). In conclusion, drying pineapple peel at 100 °C enhances the extraction of phenolic compounds and antioxidant activity, highlighting its potential as a valuable source of bioactive compounds for various applications.

Keywords: Pineapple, antioxidant; free radical scavenging effect; oxidative stress; phenolic.

ABSTRAK

Ananas comosus L. Merr, atau nanas, dikenali kerana manfaat pemakanan dan terapeutiknya, kaya dengan vitamin C dan sebatian bioaktif. Namun, sisa nanas seperti kulit, empulur, dan hampas memberikan cabaran alam sekitar akibat pengurusan yang tidak mencukupi. Kajian ini menyiasat pengaruh suhu pengeringan terhadap sifat antioksidan dan kandungan fenolik kulit nanas. Pengekstrakan berbantu ultrasonik dengan etanol mutlak digunakan untuk mengekstrak sebatian bioaktif. Hasil kajian menunjukkan bahawa pengeringan kulit nanas pada suhu 100°C menghasilkan ekstrak kasar tertinggi (8.74 ± 0.1681%). Selain itu, kulit nanas yang dikeringkan pada suhu 100°C mencatatkan jumlah kandungan fenolik tertinggi (32.23 ± 0.0023 mg GAE/g ekstrak) dan jumlah kandungan flavonoid tertinggi (8.43 ± 0.0004 mg QE/g ekstrak), manakala paras terendah diperhatikan pada suhu 50°C (15.90 ± 0.0017 mg GAE/g ekstrak dan 5.71 ± 0.0003 mg QE/g ekstrak). Aktiviti antioksidan yang dinilai menggunakan ujian DPPH menunjukkan nilai IC_{50} terendah pada suhu 100°C (441.10 µg/mL). Kesimpulannya, pengeringan kulit nanas pada suhu 100°C meningkatkan pengekstrakan sebatian fenolik dan aktiviti antioksidan, menonjolkan potensinya sebagai sumber sebatian bioaktif yang bernilai untuk pelbagai aplikasi.

Kata Kunci: Nanas, antioksidan, kesan perencatan radikal bebas, tekanan oksidatif, fenolik.

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Introduction

Pineapple (*Ananas comosus* L. Merr.), a tropical fruit belonging to the Bromeliaceae family, is widely cultivated in Southeast Asia. Malaysia, a leading agricultural commodity producers in the region, generates about 335,488 tonnes of pineapple annually, along with 67,098 tonnes of leaf waste and 137,550 tonnes of peel waste (Aili Hamzah et al., 2021). Approximately 20,000–25,000 tonnes of pineapple waste are produced per acre after harvesting, highlighting a need for effective waste management strategies (Fouda-Mbanga and Tywabi-Ngeva, 2022). Improper handling and insufficient storage contribute to these large quantities of waste, which can have a negative environmental impact if not properly managed. Pineapple peel rich in bioactive compounds such as flavonoids, saponins, tannins, enzymes, and volatile compounds have been identified from its peel, emphasizing its potential for various applications including bioprocessing and dietary supplementation (Ramli et al., 2020; Hikal et al., 2021). However, the composition of these compounds is influenced by various factors, including climatic and geographical origin, cultivar, ripening stage, and postharvest storage conditions, which can affect their bioactivity and quality (Lasekan and Hussein, 2018). The therapeutic properties of pineapple peel extracts which include protein anti-aggregation, wound healing, anti-proliferative, pro-apoptotic, anti-rheumatic, anti-inflammatory, antioxidant, antimicrobial, anti-diabetic, anti-coagulant, anthelminthic, anti-hyperglycemic, antipyretic, and cardioprotective properties highlight their potential for diverse applications (Awang Rahman et al., 2020).

According to Roshanak et al. (2016), the primary goals of drying are to extend the shelf life, reduce packaging requirements, and lighten the product's mass. Drying prolong the shelf life by inhibiting or slowing down the growth of microorganisms and preventing metabolic reactions that can alter the sensory qualities of the product. Various drying techniques are extensively used including conventional air drying, natural drying (drying in the sun or shade), oven drying, vacuum drying, microwave drying, and freeze drying. However, most of these techniques are costly and energy-intensive (Mohammed et al., 2020). Consequently, selecting an appropriate drying method and conditions is essential to achieving the desired final product quality. Dewi and Simamora (2023) dried pineapple peels at three different temperatures for 12 hours to produce herbal tea. Phytochemical screening indicated the absence of terpenoids, while total bioactive compounds, including phenolics, flavonoids, and alkaloids, were found in higher concentrations at 60°C and 70°C than at 50°C. The tea dried at 60°C demonstrated the highest antioxidant activity, with an IC_{50} value of 448.31 ppm. Additionally, Norhayati et al. (2021) conducted a study to create powders from pineapple peel and core waste from three pineapple species (Josapine, Moris and N36), examining the impact of drying temperatures. Their results showed that drying at 90°C for 7 hours produced a higher phenolic content compared to drying at 50°C and 70°C.

Antioxidants play an important role in mitigating oxidative stress, which results from the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS). These free radicals can damage cellular structure, leading to physiological dysfunction and disease (Yang et. al., 2024). Effective antioxidants, including those found in pineapple peel, help regulate oxidative stress by neutralizing free radicals. Given the high production of pineapple waste and its potential antioxidant activities, this study aims to investigate the effect of temperature variations on the total phenolic content

(TPC), total flavonoid content (TFC) of pineapple peel extracts and their antioxidant activity.

Materials and method

Preparation of Plant Sample

The MD2 pineapple peel (PP) was cleaned, rinsed under running tap water and then cut into small pieces. The samples were divided into four portions to investigate the effects of different drying temperatures. Three of these fractions underwent oven drying at specific temperatures: 50 °C, 75 °C and 100 °C under forced air ventilation in a laboratory oven until fully dried. While drying temperatures of 70-80°C are commonly used, 100°C was chosen based on previous reports indicating that drying at higher temperatures can significantly increase the total phenolic content (Norhayati et al., 2021). Meanwhile, the fourth portion was reserved as fresh samples. Comparing fresh and oven-dried samples allows us to evaluate the impact of drying temperatures on compound stability. Each dried sample was ground to the same particle size using a laboratory blender, except for the fresh sample. To facilitate subsequent analysis, the dried extracts were stored at 4°C.

Sample Extraction

Fifteen grams of fresh / ground samples were weighed and 100% (v/v) absolute ethanol were added at a solid-to-solvent ratio of 1:4. Ethanol is an environmentally friendly solvent that enhances the yield of medium-polar to polar compounds in the extracts (Sharma et al., 2022). Mixture was then sonicated for one hour at room temperature. After filtering the extracts through Whatman No. 1 filter paper (Whatman Ltd., England), the filtrates were concentrated under vacuum. The extraction process was repeated three times. The obtained crude extracts were weighed and stored at 4°C for further analysis.

Determination of Total Phenolic Content

The determination of total phenolic content was carried out as described by Lasunon et al. (2022) with minor modifications. One milligram of extract was dissolved in 1 mL of methanol. An aliquot of 1 mL aliquot of the extract solution was then mixed with 1 mL of the Folin-Ciocalteu reagent. The solution was mixed and incubated at room temperature for 3 minutes. Following this, 3 mL of Na_2CO_3 (10 g/100 mL) and 5 mL of distilled water were added. The absorbance was measured at 764 nm after 30 minutes of incubation in the dark at room temperature. Gallic acid was used as the standard for the calibration curve with concentrations of 0.025, 0.050, 0.075 and 0.1 mg/mL. 100% (v/v) ethanol was used as the blank. Triplicate measurements were carried out and the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram extract.

Determination of Total Flavonoid Content

The total flavonoid content of the extract was determined using the aluminum chloride colorimetric method (Larit et al., 2019). 0.5 mL extracts (1 mg/mL in methanol) were added to 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminium chloride and 4.3 mL of distilled water. The mixture was incubated at room temperature for 40 minutes, after which the absorbance was measured at 415 nm. A calibration curve was prepared using quercetin standards with concentrations of 0.025, 0.050, 0.075, and 0.1 mg/mL. The total flavonoid content was expressed as quercetin equivalents per gram of extract.

Determination of radical scavenging activity (DPPH assay)

The free radical scavenging activity was measured using the DPPH radical scavenging

assay following the protocol of Azizan et al. (2020) with slight modifications. The extracts were diluted in a series of concentrations of 1000, 500, 250, 125, 62.5, 31.25 and 15.625 µg/mL. In the assay, 50 µL of each dilution was mixed with 100 µL of 0.15 mM DPPH solution in a 96-well plate and incubated for 30 minutes. The absorbance was measured at a wavelength of 540 nm using a microplate reader. Methanol was used as the negative control, while ascorbic acid served as the positive control. The DPPH scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Control A0} - \text{Sample A1}}{\text{Control A0}} \times 100$$

where the,
Control A0: absorbance of the blank
Sample A1: absorbances of the sample

Statistical Analysis

The statistical analysis was performed on the data related to extraction yield, total phenolic content (TPC) and total flavonoid content (TFC) using Anova: Single factor, using Microsoft Excel. All data were expressed as the mean ± SEM. The significance level (α) was set at $p < 0.05$. Post-hoc analysis was conducted using RStudio to identify significant differences in extraction yield, TPC and TFC concentrations among all treatments. Means were compared using Tukey's test at $p < 0.05$.

Results and discussion

In this study, we evaluated the percentage yield of pineapple peel (PP) extracts at different drying temperatures using ultrasound-assisted extraction (UAE) and absolute ethanol as the solvent. Ethanol was chosen for its low toxicity and effectiveness in dissolving phenolic compounds (Polania et al., 2023). The percentage yield of pineapple peel (PP) extracts varied with drying temperature. The highest percentage yield of the crude extract was obtained from PP dried at 100°C, compared to drying at lower temperatures. (Figure 1). Specifically, the yields were 4.94 ± 0.86% for fresh samples, 8.32 ± 0.09% for PP dried at 50°C, 8.01 ± 0.17% for PP dried at 75°C, and 8.74 ± 0.16% for PP dried at 100°C. Fresh samples exhibited the lowest yield. Statistical analysis revealed that the yield for fresh PP was significantly different from the dried PP ($p < 0.05$), though no significant difference was observed among the dried samples.

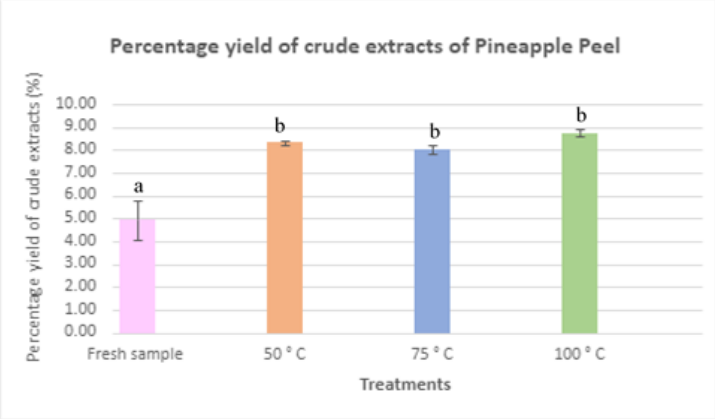

Figure 1: Percentage yield of crude extracts of pineapple peel (PP) extracts

These findings align with Kozłowska et al. (2021), who reported lower extraction yields in fresh herbs compared to dried extracts. Stephenus et al. (2023) reported that fruits dried at higher temperatures with reduced water activity often produce higher extraction yields. An increase in temperature generally enhances the diffusion rate and reduce viscosity, supporting Fakhrulddin et al. (2022), who found increased yields in oven-dried *Caulerpa lentillifera*, at temperatures between 40°C - 80°C.

For total phenolic content, the highest TPC was found in PP dried at 100°C, with a value of 32.23 mg GAE/g. This was followed by PP dried at 75°C (18.94 mg GAE/g), fresh PP (16.36 mg GAE/g), and PP dried at 50°C (15.90 mg GAE/g) (Figure 2). Statistical analysis showed significant differences between the 100 °C dried PP and other treatments ($p < 0.05$). Higher drying temperatures generally increase phenolic content by reducing enzymatic activity and oxidation, as reported by Kalpoutzakis et al. (2023) and Esparza-Martínez et al. (2016). However, excessive heat can degrade phenolic compounds and reduce antioxidant activity (Abhay et al., 2016; Wanderley et al., 2023). Kasunmala et al. (2021) reported that higher temperatures can accelerate the breakdown of polysaccharides and other cell wall components, allowing phenolic compounds that are typically trapped within the cell wall matrix to be more readily extracted, thus enhancing phenolic content. On the other hand, prolonged drying at higher temperatures can lead to the oxidation and degradation of phytochemicals, as reported by ElGamal et al. (2023). Madrau et al. (2009) found that polyphenol-oxidases (PPO) activities occurs at low drying temperatures (55 to 60 °C), slowing polyphenol content accumulation and extending drying times. Meanwhile high temperatures prevent PPO process, avoiding thermal degradation. Shortening drying times can enhance antioxidant activity by reducing prolonged oxygen exposure that causes phenolic breakdown and increased redox activity (Pham et al. 2015).

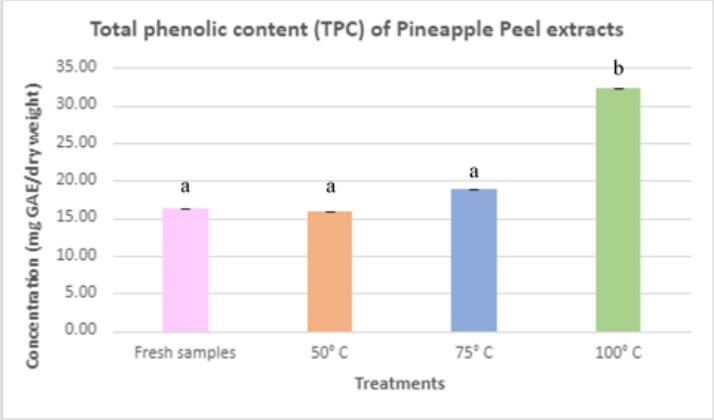

Figure 2: Total phenolic content (TPC) of pineapple peel (PP) extracts.

The total flavonoid content (TFC) exhibited similar trends to the total phenolic content (TPC). The highest flavonoids content was found in PP dried at 100 °C, with 8.41 ± 0.0004 mg QE/g extract, followed by PP dried at 75°C (6.85 ± 0.0004 mg QE/g extract), fresh PP (6.52 ± 0.0003 mg QE/g extract), and PP dried at 50°C (5.71 ± 0.0003 mg QE/g extract) (Figure 3). Higher drying temperatures can enhance flavonoid extraction by disrupting the cell matrix, which may preserve or increase flavonoid content. However, excessive heat and prolonged drying can lead to flavonoid degradation and reduced antioxidant activity due to the oxidation of anthocyanins and polyphenols (Nunes et al., 2016; Vu et al., 2016; Saifullah et. al., 2019; Anthony and Farid, 2022 Dewi and Simamora, 2023).

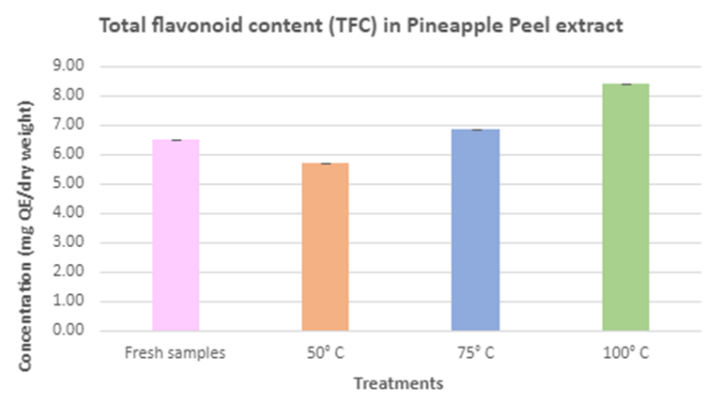

Figure 3: Total flavonoid content (TFC) of pineapple peel (PP) extracts.

Antioxidant activity was assessed using the DPPH radical scavenging activity assay, with ascorbic acid as the positive control. Fresh PP exhibited the highest inhibition percentage at 55.51 ± 0.0067 %, followed by PP dried at 100°C, 75°C, and 50 °C with 52.44 ± 0.0103 %, 52.40 ± 0.0060 % and 51.19 ± 0.0121 % respectively. Ascorbic acid showed an inhibition percentage of 98.22%. and an IC_{50} value for ascorbic acid was 20.33 µg/mL. The IC_{50} values for PP extracts increased with drying temperatures as shown in Table 1.

Table 1. The percentage inhibition and half maximal inhibitory concentration (IC_{50}) values of PP extracts.

Treatment	**Percentage inhibition (%)**	**IC_{50} value (µg/mL)**
Ascorbic acid	98.22	20.33
Fresh sample	55.51	649.28
50 °C	51.19	878.26
75 °C	52.40	791.67
100 °C	52.44	441.10

Higher drying temperatures generally improved antioxidant activity, correlating with increased total phenolic content and the release of bound phenolics (Vidinamo et al., 2021; Polania et al., 2022). This improvement is attributed to the breakdown of cell components that release antioxidants. Additionally, reduced enzyme activity at higher temperatures helps preserve phenolics compounds. However, excessively high temperatures can cause thermal degradation of polyphenols, potentially diminishing antioxidant activity (Antony and Farid, 2022; Lukinac and Jukić, 2022).

Conclusion

The results demonstrate that drying temperature plays a crucial role in the extraction efficiency and the phenolic and flavonoids compounds from pineapple peels. The percentage yield of PP extracts increased with drying temperature, with the highest yield observed at 100°C. Similarly, pineapple peels dried at 100°C exhibited the highest total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. It can be concluded that drying pineapple peels at 100°C provides the most favorable outcomes in terms of extraction yield and bioactive compound concentration. However, it is crucial to balance drying temperatures to avoid excessive degradation of phytochemicals. This study highlights the importance of optimizing drying conditions to maximize the beneficial properties of plant materials while mitigating potential losses in bioactivity.

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Declaration of interest

The author declares no conflict of interest.

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