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Effect of Extraction Methods on Total Phenolic Content and Antioxidant Activity of *Ananas comosus* Peel: A Mini Review

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ABSTRACT

Pineapple (*Ananas comosus*), a tropical fruit belonging to the Bromeliaceae family, is rich in vitamins A and C, flavonoids, tannins, and various phenolic compounds. Pineapple peel is one of the antioxidant sources that could be beneficial for preventing oxidative stress and associated diseases such as anti-inflammatory, anticancer, monitoring nervous system function, and healing bowel movement. However, improper disposal of pineapple peels can have a detrimental impact on the environment, necessitating innovative methods to convert them into valuable products. Despite their numerous potential health benefits, an investigation of various extraction methods for maximizing the antioxidant potential of pineapple peel has been lacking. Therefore, this review aims to evaluate both conventional methods such as maceration and reflux extraction, as well as non-conventional techniques like ultrasound-assisted and microwave-assisted extraction for the preparation of antioxidant-rich pineapple extracts. The data on the total phenolic content and antioxidant activity of pineapple have mainly been obtained from online articles and journals through several online academic databases. The results of this review highlight the varying efficiency of different extraction methods in obtaining bioactive compounds from pineapple peels. Each method presents its distinct strengths and limitations, emphasizing the need for a method-selection approach aligned with specific goals and compounds of interest. These findings underscore the significant influence of extraction methods on the antioxidant potential of pineapple peel, which can contribute to the development of health-promoting products and sustainable waste management strategies.

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

ABSTRAK

Nanas (*Ananas comosus*), sejenis buah tropika daripada keluarga Bromeliaceae, kaya dengan vitamin A dan C, flavonoid, tanin, dan pelbagai sebatian fenolik. Kulit nanas merupakan salah satu sumber antioksidan yang boleh memberi manfaat untuk mencegah tekanan oksidatif dan penyakit yang berkaitan seperti anti-radang, antikanser, memantau fungsi sistem saraf, dan meyembuhkan pergerakan usus. Walau bagaimanapun, pelupusan kulit nanas yang tidak betul boleh memberikan kesan buruk kepada alam sekitar, memerlukan kaedah inovatif untuk mengubahnya menjadi produk yang bernilai. Walaupun terdapat banyak potensi kesihatan, penyelidikan pelbagai kaedah pengekstrakan untuk memaksimumkan potensi antioksidan kulit nanas masih kurang. Oleh itu, kajian semula ini bertujuan untuk menilai kedua-dua kaedah konvensional dan bukan konvensional untuk penyediaan ekstrak nanas yang kaya dengan antioksidan. Data tentang jumlah kandungan fenolik dan aktiviti antioksidan nanas kebanyakannya diperoleh daripada artikel dan jurnal dalam talian melalui beberapa pangkalan data akademik dalam talian. Hasil kajian ini menekankan kecekapan yang berbeza dari pelbagai kaedah pengekstrakan dalam mendapatkan sebatian bioaktif daripada kulit nanas. Setiap kaedah mempunyai kelebihan dan kelemahan yang berbeza, menekankan keperluan untuk pendekatan pemilihan kaedah yang sejajar dengan matlamat dan sebatian yang ingin dicapai. Penemuan ini menggariskan pengaruh kaedah pengekstrakan yang signifikan,terhadap potensi antioksidan kulit nanas yang boleh menyumbang kepada pembangunan produk penjagaan kesihatan dan strategi pengurusan sisa yang mampan.

Katakunci: Nanas, antioksidan, kesan penghapusan radikal bebas, tekanan oksidatif

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1. INTRODUCTION

Antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases (Losada-Barreiro et al., 2022). Antioxidants act as a substance that significantly decreases the adverse effects of reactive oxygen species (ROS), reactive nitrogen species (RNS), or both on normal physiological function in humans (Sharifi-Rad et al., 2020). ROS are produced within the human body by numerous physiological and biochemical processes in the form of superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO). Compounds like thiols and vitamin C counteract this process by stopping chain reactions. Within living organisms, antioxidants like glutathione and enzymes such as catalase and superoxide dismutase, along with dietary sources like vitamins C and E, form intricate defense networks against oxidative stress (Sharifi-Rad et al., 2020).

Pineapple (*Ananas comosus*), is a tropical fruit cherished for its flavor and is a source of both nutrition and potential therapy (Seenak et al., 2021). A ripe and healthy pineapple fruit can fulfill a significant portion of daily vitamin C needs (16.2 %) (Baidhe et al., 2020). Beyond its consumption, pineapple processing yields substantial waste, including the peel, core, pomace, and crown. According to Tran et al. (2023), the production of *A. comosus* fruit for the food and beverage industries results in the release of almost 30 million tons of fruit each year, coupled with a sizable amount of trash. Additionally, pineapple peel contains bioactive compounds like vitamin C, carotenes, phenolic and flavonoid compounds such as catechin, epicatechin, gallic acid, and ferulic acid, showcasing not only antioxidant properties but also diverse biological functions (Meena et al., 2022). These compounds hold the potential to alleviate oxidative stress-related conditions and could be utilised for beneficial purposes (Azizan et al., 2020). Moreover, phenolic compounds have potent abilities to counteract free radical production, contributing to overall well-being (Vargas-Serna et al., 2022).

Bioactive compounds that are responsible for the therapeutic and medicinal values of pineapple peel, particularly antioxidant compounds can be obtained and exploited by means of extraction methods. Extraction is a method of separating the compounds of interest from plant material by adopting standard procedures while considering the possible factors affecting the efficiency of the extraction process such as type of extraction method, drying method, solvent selection, extraction temperature and extraction duration. Extraction methods can be classified into conventional and nonconventional (modern) methods. The conventional method includes maceration, percolation, soxhlet extraction and hydrodistillation, while the modern method includes supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). Each of these methods possesses its own benefits and limitations (Chávez-González et al., 2020). Therefore, this review aims to assess the efficiency of various extraction methods in terms of their total phenolic, total flavonoids and antioxidant potential from pineapple peel. It is hoped that through method comparison, the study seeks to guide researchers and industries in maximizing the value of pineapple peels as a source of valuable bioactive compounds hence promoting sustainable waste utilization.

2. METHODOLOGY

A literature search was conducted to identify relevant research studies on the antioxidant activity of pineapple peel. The search for the research papers involved three databases: PubMed (http://www. ncbi.nlm.nih.gov/pubmed/), Science Direct (http://www. sciencedirect.com) and Scopus (https://www.scopus.com/) from the year 2017 to 2023. However, two research papers published in 2012 and 2013 were also included. The search strategy comprised combinations of the following two keywords: '*Ananas comosus* or pineapple peel' and 'antioxidant' for the three databases. References to all retrieved articles were reviewed based on the total phenolic contents and extraction methods comprising maceration, soxhlet, reflux, ultrasonicated assisted, and microwave assisted extraction, to determine their relevance to the topic or subject of interest.

3. PHYTOCHEMICAL CONSTITUENTS

Phytochemicals are compounds with biological action that are primarily produced by plants. According to Hikal et al. (2021), flavonoids, saponins, tannins, and enzymes are present in pineapple peel extract. The most abundant compound in the extract is bromelain, followed by flavonoids. Additionally, pineapple peel oil contains a total of 57 volatile compounds, comprising 25 esters, nine alcohols, eight acids, seven phenolic compounds, six aldehydes and ketones, one terpene, and one lactone. The majority of the composition consists of alcohols. The primary constituents include phenethyl alcohol (22.18%), 3-methyl-1-butano (6.91%), 2-methyl-1-butano (4.75%), 2,3-butanediol (8.35%), 2-methoxy-4-vinylphenol (5.66%), and octanoic acid (4.29%). Ramli et al. (2020) reported the presence of benzene, 1,3-bis(phenoxyphenoxy), 2-furancarboxaldehyde, 5-methyl, 2-furancarboxaldehyde, 5-(hydroxymethyl), and 4-H-pyran-4-one, 2,3-dihydro-3, and 5-dihydroxy-6-methyl in pineapple peel. Qualitative phytochemical analysis revealed that the peel extracts of pineapple contained protein, alkaloids, flavonoids, tannins, steroids, saponin, inulin, glycosides, phenolic compounds, polyphenols, anthraquinones, quinine, coumarins, terpenoids, triterpenes, oxalate, phytate, and carbohydrates (Awang Rahman et al., 2020).

4. EFFECTS OF EXTRACTION METHODS ON TOTAL PHENOLIC AND ANTIOXIDANT ACTIVITY OF PINEAPPLE PEEL

Several promising methods either conventional or non-conventional are employed for the extraction of bioactive compounds from pineapple peel. Conventional extraction methods often entail extended processing times, high solvent consumption, and involve multiple extraction steps. Moreover, the application of heat during conventional methods can lead to the decomposition or degradation of thermolabile phytochemicals (Lizárraga-Velázquez et al., 2020). In contrast, non-conventional techniques offer advantages such as reduced use of hazardous solvents, higher extraction yields, and shorter processing times to achieve satisfactory results. However, these non-conventional approaches tend to be more intricate and cost-intensive to implement (Lizárraga-Velázquez et al., 2020).

4.1 Maceration

Maceration is the simplest extraction method that involves soaking plant material in a chosen solvent at room temperature for at least three days, with occasional stirring. However, a downside of this method is its lengthy extraction time and low extraction efficiency (Bitwell et al., 2023). Putri et al. (2018) investigated the inhibitory activity of pineapple peel extracts obtained through 24-hour maceration at room temperature. The aqueous extract demonstrated the highest

DPPH activity with an IC_{50} value of 266.02 μg/mL, while the methanol extract exhibited the highest ABTS activity with an IC_{50} value of 46.49 μg/mL. Similar findings were obtained by Saraswaty et al. (2017), who also discovered that the highest DPPH activity was in water extract, both for fresh and dried pineapple peel. In addition, the highest TPC of fresh pineapple peel was also found in water extract, while dried peel favored 75% v/v ethanol extract. In agreement with these results, Lourenco et al. (2021) demonstrated that 80% ethanol extract using a solvent-pineapple peel ratio of 1:1 (w/w) for 25 min at ambient temperature, yielded the highest antioxidant capacity. The resulting extract revealed a TPC value of 11.10 mg gallic acid equivalent (GAE)/g dry extract, 2,2-diphenylpicrylhydrazyl (DPPH) activity of 91.79 mol Trolox/g dry extract and ferric reducing antioxidant power (FRAP) activity of 174.50 mol Trolox/g dry extract. Furthermore, research by Kalaiselvi et al. (2012) revealed that ethanol extracts displayed substantial scavenging activity, reaching 87.10% at a concentration of 2.5 mg/ml when tested using DPPH. Meanwhile, Rashad et al. (2018) compared fermented pineapple peel (FPW) with unfermented (UFPW) from Egypt using methanol extraction at 55°C. FPW exhibited a higher TPC of 120 mg GAE /100 g dry weight compared to UFPW (112 mg GAE /g dry weight) and displayed remarkable scavenging activities of 97% and 86% at an 8 mg/ml concentration. The same concentration also exhibited the highest antioxidant activity in terms of reducing power, beta-carotene-linoleic acid assays and ferrous ion chelating ability.

4.2 Reflux

Compared to maceration, reflux extraction is notably more efficient, requiring less time and solvent. However, it's not suitable for extracting thermolabile natural products (Zhang et al., 2018). Fidrianny et al. (2018) explored various solvent polarities in the reflux extraction of the sample. Their findings highlighted the remarkable efficacy of ethanol extract from Bogor pineapple peel, as evidenced by an IC_{50} value of 0.13 μg/mL in the DPPH assay, categorizing it as a potent antioxidant in comparison to ethyl acetate and n-hexane extracts. The ethanol extract also exhibited an EC_{50} FRAP value of 259.08 μg/mL. Conversely, ethyl acetate peel extract showed the highest TPC at 7.84 g GAE/100 g dry weight, while the ethanol extract had a higher total flavonoid content (TFC) at 0.17 g quercetin (QE)/100 g dry weight. In contrast, a study focusing on the primary polyphenolic extracts from Bali pineapple peel showed that the reflux-extracted methanol peel extract demonstrated an IC_{50} value of 1.13 mg/mL in the DPPH assay, while the total antioxidant capacity was 0.037 g ascorbic acid equivalents/g (Li et al., 2012).

4.3 Soxhlet

The soxhlet extraction method merges reflux extraction and percolation, allowing continuous extraction with fresh solvents. It's an automated technique with high efficiency, needing less time and solvent compared to maceration. However, the elevated temperature and longer extraction can risk thermal degradation (Lizárraga-Velázquez et al., 2020). In the study conducted by Hatam et al. (2013), various extraction techniques were compared, revealing that soxhlet extraction using ethanol as the solvent yielded the highest TPC of 28.78 μg/mL, which was higher than maceration (16.53 μg/mL), while reflux extraction showed the lowest TPC at 16.02 μg/mL. Additionally, for TFC, the soxhlet method yielded the highest concentration at 5.11 μg/mL, followed by reflux at 4.41 μg/mL, with the lowest concentration observed in the maceration extract at 3.51 μg/mL. Madhumeena et al. (2021) concurred with these findings, highlighting that soxhlet extraction yielded a higher TPC of 2.365 mg/g gallic acid equivalent (GAE) compared to maceration and supercritical fluid extraction (SFE) techniques. Moreover, this method exhibited antioxidant activity with a value of 45 mmol/100 g, exceeding those of maceration and SFE. Another study by Samarakoon et al. (2022) involved the extraction of dried and powdered pineapple peels into methanol through soxhlet extraction and sequential partitioning into hexane, dichloromethane (DCM), and aqueous methanol (50%) revealed that 50% methanol fraction exhibited an IC_{50} value of 85.70 µg/mL in terms of antioxidant activity, along with a TPC of 208.899 mg GAE/kg dry weight of plant material. Particularly, the DCM fraction exhibited a high flavonoid content with a TFC of 79.913 catechin (CAT) equivalent/kg dry weight of pineapple peel (Samarakoon et al., 2022).

4.4 Ultra-sonicated assisted extraction (UAE)

Ultrasonic-assisted extraction (UAE), also known as ultrasonic extraction or sonication, harnesses the energy of ultrasonic waves to improve the extraction process. Through ultrasonic waves, cavitation occurs within the solvent, amplifying the dissolution, diffusion of solutes, and heat transfer, thus significantly enhancing extraction efficiency. UAE offers several advantages, including reduced solvent and energy consumption, as well as shorter extraction times, while also maintaining lower extraction temperatures. This method proves particularly advantageous for the extraction of delicate and unstable compounds (Chemat et al., 2017). Liu et al. (2018) conducted a comprehensive exploration of various factors including extraction time, solution-to-peel ratio, and solvent concentration, in relation to total phenolic content (TPC) and total flavonoid content (TFC). Optimal conditions were identified as 65 minutes of extraction using a 75% ethanol solution and a 10:1 solution-to-peel ratio. The DPPH radical scavenging percentage (reached 47%, nearly half of the standard ascorbic acid (91%). However, the pineapple peel extract exhibited remarkable ABTS scavenging ability, at 97%, surpassing the standard (92.8%). In a separate study, Zaki et al. (2018) observed that dried Josephine peels extracted using an ultrasonic bath at 50 °C for 30 minutes retained the highest TPC at 180 W, with 19.8 mg GAE/g dry sample, in comparison to power levels of 450 W, and 850 W employed for microwave drying. Additionally, fresh pineapple peels exhibited a DPPH inhibition of 58% radical species, lower than the inhibition levels of pineapple peels at 180 W (74%) and pineapple core at 180 W (53%) power levels.

Furthermore, Yahya et al. (2019) demonstrated that UAE outperformed soxhlet reflux extraction, resulting in a maximum TPC and TFC reaching 1078.68 mg GAE/g DW and 1276.64 mg QE/g DW, respectively. These values were higher than those obtained using soxhlet reflux extraction (TPC of 7.98 mg GAE/g DW). Another study explored the antioxidant potential of the MD2 pineapple variety through ultrasound-assisted extraction, employing varying ethanol ratios (100%, 50%, and 0%). A substantial TPC of 10.73 mg GAE/g crude extract was observed when employing 50% ethanol. Furthermore, the highest DPPH inhibition rate of 72.67% was achieved with 50% ethanol, exhibiting an IC_{50} value of 339.23 µg/mL (Azizan et al., 2020). In a separate study by Zampar et al. (2022), the optimal conditions for generating extracts with the highest TPC (405.06 mg GAE/100 g) and antioxidant activity as assessed by DPPH and FRAP methods were obtained at 60 °C, 50 W of ultrasonic power and 30 minutes, with ethanol: hydrochloric acid solution ratio of 1:1. Similarly, a study by

Polanía et al. (2022) also revealed the optimal conditions to produce extracts with high TPC and antioxidant activity were at 62 °C, for a duration of 30 minutes and with the use of 58% ethanol. These conditions yielded a TPC of 866.26 mg GAE/g dried mass, along with percentage inhibition values of 80.06 % for ABTS and 63.53 % for DPPH, respectively. These findings collectively underscore the potential of ultrasonic-assisted extraction in obtaining high-quality extracts rich in bioactive compounds (Polanía et al., 2022).

4.5 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) utilises microwave technology to generate heat through interaction with polar compounds, such as water and organic components in plant material. This process involves mechanisms like ionic conduction and dipole rotation. In MAE, heat and mass transfer create a combined effect that accelerates extraction and enhances yield. MAE offers several advantages, including enhanced extract yield, reduced thermal degradation, and selective heating of plant matter. Particularly, MAE is considered an environmentally friendly approach as it reduces organic solvent usage. There are two MAE methods: solvent-free extraction (typically for volatile compounds) and solvent extraction (generally for non-volatile compounds) (Vinatoru et al., 2017). Alias and Abbas (2017) explored the optimum condition for MAE at 250 W microwave power, with temperature variations set at 30 °C, 60 °C, 90 °C and 120 °C. The most favorable condition was identified at 30 °C using deionised water as the solvent. Under this optimise condition, the highest TPC was achieved at 206.46 mg GAE/g dry weight, while the EC_{50} value for DPPH was 13.65 mg/mL. Another study was done by Alias and Abbas (2017) to determine the optimum MAE condition at 750 W microwave power, an operating temperature of 60 °C and a solvent ratio of ethanol: water (v/v 50:50) revealed that the TPC observed was 207.72 mg GAE/g DW, and the EC_{50} value for DPPH was obtained at 13.2 mg/mL. MAE demonstrated it comparability to soxhlet extraction, with a TPC of 28.78 mg GAE/g DW and EC_{50} value of 2.78 mg/L, respectively (Alias and Abbas, 2017). Additionally, by employing a drying temperature of 67 °C, an extraction time of 87 seconds, and a solvent-to-solid ratio of 60.5 mL/g allows maximizing the TPC and antioxidant capacity of the extract (Vargas-Serna et al., 2022). In a study conducted by Harith et al. (2023), MAE at 300W utilizing ethanol revealed a TPC and TFC at 122.74 mg GAE/g PPW and 264.50 mg QE/g PPW. However, the use of choline chloride-glycerol as an extraction solvent resulted in a higher concentration of TPC (7.98 mg GAE/g of DW) compared to traditional solvents. Another experiment utilizing 450 W of microwave power for 2 minutes with ethanol as the solvent yielded the highest TPC of 5803.21 mg GAE/g dry extract and a TFC of 9067.09 mg Quercetin/g dry extract. The DPPH radical scavenging activity reached 93.12%, while the ABTS activity measured 3.19 mg Trolox/g dry extract. The β-carotene bleaching assay showed a 5.74% inhibition rate (Lasunon et al., 2022).

5. CONCLUSION

The abundance of bioactive compounds in pineapple, particularly its phenolic and flavonoids, has ignited considerable interest in its potential as a valuable source of natural antioxidants. This review has explored the relationship between extraction methods and the antioxidant properties of pineapple peel extraction techniques. Extraction methods play an important role in determining the TPC and antioxidant efficacy of these extracts. It's worth noting that different methods with numerous parameters yield extracts with varying concentrations of phenolic and flavonoid compounds, thereby impacting their potential antioxidant activity. MAE and UAE have emerged as efficient options for extracting bioactive compounds from pineapple peel, especially those prone to thermal degradation. These methods also contribute to sustainable waste utilization by reducing solvent usage. Overall, this review highlights the importance of selecting the most suitable extraction method, considering the target compounds and application goals. By comparing these methods, we provide guidance to researchers aiming to utilise the full potential of pineapple peel for bioactive compound extraction and sustainable waste management.

DECLARATION OF INTEREST

The author declares no conflict of interest.

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