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Effect of Alcohol Concentration in Soxhlet Extraction of Bioactive Compounds from Pineapple Peel as an Antioxidant for Cooking Oil

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ABSTRACT

The pineapple peel, as organic waste, is still rarely used and processed widely, even though the benefits of pineapple peel have been studied quite extensively. Pineapple peel contains bioactive compounds that have antioxidant activity, such as flavonoids and phenolic compounds as a source of natural antioxidants. The extraction method using Soxhlet requires less solvent and a shorter time due to increasing temperature. This research aims to determine the optimum method for extracting antioxidant compounds using Soxhlet extraction. Soxhlet extraction was obtained using ethanol solvent at various concentrations of 0%, 55%, and 96%. Quantitative tests were carried out to determine the flavonoid and phenol content. The antioxidant activity test was analyzed using the DPPH free radical scavenger method. The pineapple peel extract resulted in optimum condition in 96% ethanol due to obtaining the highest yield, 11.48%, the highest flavonoid content, 11.99 mg QE/g, and strong antioxidant activity. The phenolic content was achieved in the highest amount after being extracted in 55% ethanol. The pineapple peel showed the potential effect of inhibiting lipid oxidation in cooking oil. The addition of the extracted sample could lessen the formation of free fatty acid in cooking oil by about 38.4%. This research revealed that pineapple peel extract could be a promising natural antioxidant in future applications.

Keywords*: Antioxidants; bioactive compound; free fatty acid; pineapple peel; soxhlet method*

INTRODUCTION

Pineapple is a significant commodity within the agricultural sector's food crop subsector and is still extensively cultivated by farmers. Its high production aligns with the increasing demand for pineapple consumption. During pineapple processing in the industry, only 75% of the fruit is converted into the main product, while the pineapple peel remains a by-product that has not been utilized as a commercial product. Pineapple peel contains phenolic compounds and vitamins with high antioxidant activity. The bioactive compounds in pineapple peel have potential in food preservation as natural alternatives to synthetic antioxidants (Lourenço et al., 2021). Antioxidants are compounds useful for preventing damage due to oxidation. Natural antioxidants generally have lower toxicity than synthetic antioxidants, prompting increased interest in finding natural sources. This interest is driven by concerns over the carcinogenic side effects of synthetic antioxidants (Bellucci et al., 2022).

The Soxhlet extraction process is an efficient, automated continuous extraction method requiring less time and solvent consumption than maceration. The heat applied in this method enhances the extraction of target compounds and shortens processing time compared to maceration. This is due to increasing temperature in Soxhlet extraction, which improves the solubility of insoluble compounds at room temperature. The Soxhlet method also yields a higher concentration of bioactive compounds (Zhang et al., 2018; Lee et al., 2016). One primary advantage of Soxhlet extraction is that the sample can repeatedly contact fresh solvent

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(Osorio-Tobón, 2020). In addition to extraction time and temperature, the type of solvent is essential for achieving optimal yield. Solvents can be single or mixed. Hidalgo & Almajano (2017) stated that the choice of solvent in the extraction process is crucial. Ethanol has been shown to be the best solvent for extraction, compared to water, acetone, hexane, and methanol. Multicomponent solvents provide a more optimal yield, with a water-alcohol mixture producing the best results at a composition of 40%-70%. The best solvent for extraction depends on the type of compounds being extracted. Aliphatic alcohols (e.g., methanol, ethanol) and polar organic solvents (e.g., acetone, ethyl acetate) are the most popular choices for extracting phenolic compounds from plants (Gil-Martín et al., 2022).

In a study by Alaydrus et al. (2019), pineapple peel was extracted using the maceration method with 70% ethanol as a solvent to evaluate its antioxidant activity by preparing extract concentration through specific preparation techniques. The results showed that pineapple peel extract possesses strong antioxidant activity, especially when using particular preparation techniques involving a DPPH solution to reach the required extract test concentration. Given the proven antioxidant benefits of pineapple peel, this study aims to explore the effects of pineapple peel extract on the stability of cooking oil, as no research has specifically investigated the application of pineapple peel extract as an antioxidant in cooking oil.

Cooking oil is a staple in food processing, vulnerable to oxidation, especially when heated during storage, handling, and cooking (Fadda et al., 2022). Oil oxidation produces harmful compounds like hydroperoxides, and fatty acid breakdown products (such as aldehydes, ketones, and epoxides), which degrade oil quality and pose health risks to consumers (Wang et al., 2023). Therefore, antioxidants in cooking oil are essential to slow oxidation and extend the oil's shelf life. Currently, the food industry relies heavily on synthetic antioxidants, but their use often raises concerns due to potential side effects and high costs. Thus, there is a need for safe, effective, and economical natural antioxidant sources(Fadhil et al., 2023). Cooking oil without antioxidants generally lasts 6 months to 1 year, while those with antioxidants can last up to 13 months or even 3 years (Prescha et al., 2014;).

In this study, the Soxhlet method was used to extract pineapple peel. This method could extract compounds with high total phenol and flavonoid content from natural sources. Although this process was relatively time-consuming, it was chosen for its flexibility and capacity to extract relevant compounds applicable to cooking oil. Additionally, antioxidant activity is performed using the DPPH method, and then determination of total flavonoid and phenolic content, and IC_{50} values were carried out using a UV-Vis spectrophotometer. This study was expected to provide new contributions to food technology and health, demonstrating that pineapple peel extract as a natural antioxidant in cooking oil was potentially a safer, more effective, and economical alternative.

METHODS

Material

The materials used for the research were as follows: Pineapple peel from Balikpapan Utara District, Balikpapan City, Ethanol (Sigma-Aldrich, 96%), DPPH 1,1-diphenyl-2-picrylhydrazyl (Merck, 95%), Quercetin (Sigma-Aldrich, 95%), NaOH (Merck, 95%), Methanol (Merck, 99%), Sodium Nitrite (Merck, 99%), Ammonium Chloride (Merck, 99%), Sodium Carbonate (Merck, 99%), and Cooking Oil.

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Equipment

The equipment utilized in this study comprised a Soxhlet extraction apparatus, a heating mantle, a hot plate, a UV-Vis spectrophotometer, and an analytical balance. This research adopts a laboratory experimental approach, involving the preparation of pineapple peel samples obtained from Balikpapan City

Sample Preparation

The collected pineapple peel samples were first cleaned to remove impurities and then cut down to match the texture of fresh pineapple peel. Next, the peels were washed and dried at 60°C until their moisture content was reduced by 90%. After drying, the pineapple peels were ground using a grinder and sifted through a 100-mesh screen. Finally, the ground peels were stored in an airtight container at room temperature.

Extraction Process of Pineapple Peel

The extraction process of pineapple peel was conducted using the Soxhlet method with solvent extraction. Ethanol was prepared at concentrations of 0%, 55%, and 96%. The extraction lasted for 4 hours, maintaining a sample-to-solvent ratio of 1:15. Approximately 10 grams of ground pineapple peel were weighed and placed into a pre-prepared filter paper pouch, which was securely tied to prevent any sample from escaping during the extraction. After extraction, the sample was filtered and evaporated at 60ºC to produce a thick extract. The yield was then determined, and the extract was dissolved in 15 mL of distilled water. The samples were stored in the refrigerator before analysis.

Total Flavonoid Test

A total flavonoid test was conducted by preparing a standard curve using 10 mg of quercetin mixed with 10 mL of methanol. From this mixture, 1 mL was further diluted in 10 mL of methanol. Quercetin was then prepared at a concentration of 100 µg/mL and diluted with methanol to create several concentrations: 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL. About 1 mL of each prepared concentration was added with 4 mL of distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added, and the mixture was incubated for 5 minutes. After incubation, 0.3 mL of 10% aluminum chloride and 2 mL of 1 M NaOH were added, followed by homogenization of the mixture. The absorbance for the standard curve was measured at a wavelength of 415 nm (Stanković, 2011).

Total flavonoid measurements were repeated three times. The total flavonoid equation was calculated as µg quercetin per ml calculated by the equation y = bx ± a, where y is the absorbance of the sample and x is the number of quercetin equivalents (µg/ml). The total flavonoid content of the extract was expressed in mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). The total flavonoid content in all samples was calculated using the formula ((Phuyal et al., 2020):

$$Total\ flavonoid = c\frac{V}{m} \quad (1)$$

c = total flavonoid concentration from the standard curve

V = extract volume (mL)

M = extract mass (g)

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Total Phenolic Test

The total phenolic test began with preparing a standard curve using gallic acid. Gallic acid was diluted with ethanol to create various concentrations of 31.25, 62.5, 125, 250, and 500 µg/mL. For each concentration, 0.2 mL of the gallic acid solution was combined with 15.8 mL distilled water, 1 mL of 50% Folin-Ciocalteu reagent (v/v), homogenized, and then incubated for 8 min. Next, about 3 mL of 5% Na_2CO_3 (w/v) was added to the mixture, which was then mixed again. The final solution was incubated in the dark for 2 hours and subsequently measured at a wavelength of 725 nm (Mu'nisa et al., 2012). Each extracted sample in various ethanol concentrations was prepared and measured under the same conditions as the standard gallic acid solutions. Total flavonoid measurements were performed three times. The calculation results were expressed in mg equivalents of gallic acid per g dry weight using the same method and calculation as in the total flavonoid test.

Antioxidant Activity Test

The spectrophotometric method determined total antioxidants with DPPH (2,2-diphenyl-1- picrylhydrazyl). A total of 1 mL of extract diluted in ethanol was added to 1 mL of DPPH (0.15 mM in ethanol). At the same time, a control consisting of 1 mL DPPH with 1 mL ethanol was prepared. The reaction mixture was mixed well by hand and then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 515 nm, ethanol was used as a blank (Farhan et al, 2012). The DPPH capability of the extract was calculated using the following equation:

$$\%Inhibition = \frac{Absorbance\ blank\ - Absorbance\ sample}{Absorbance\ blank} \times 100\%$$

Absorbance blank = DPPH + ethanol

Absorbance sample = DPPH + sample

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

$$\mathrm{IC50} = \frac{50\ - a}{b}$$

Free Fatty Acid Content

The cooking oil was incubated for 24 hours. Then, 10 grams of the oil was weighed, and 0.761 grams of the extracted sample were added. Next, about 50 mL of 96% alcohol was added, and the mixture was heated for 10 minutes. After that, two drops of phenolphthalein indicator were added to the sample. The sample was then titrated with 0.01 N NaOH, which had been titrated until a light pink color appeared and remained for 30 seconds. This procedure allowed for determining the total acid number, which was an important parameter in evaluating the quality of the oil being tested (Sopianti et al., 2017). The %free fatty acid (FFA) was calculated by using the formula (Soenarno et al., 2024):

$$\%\mathrm{FFA} = \frac{\mathrm{mL\ NaOH}\ \times \mathrm{M\ NaOH}\ \times \mathrm{molecular\ weight\ of\ fatty\ acid}}{\mathrm{weight\ of\ sample}\ \times 1000} \times 100\%$$

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where % FFA = free fatty acid content; mL NaOH = volume titrant NaOH; M NaOH = molarity of the solution NaOH mol/L; moleculer weight of fatty acid (oleic acid) = 282.47 g/mol.

Test Parameters

In this study, the variables were structured as the concepts of the research conducted as follows in Table 1.

Table1. Test parameters

Control Variables	Independent Variables	Dependent Variables
The amount of pineapple peel used, extraction temperature, extraction time, and solvent volume	Solvent Concentration (0%,55%, 96%)	Yield (%), Total Flavonoid, Total Phenolic, IC_{50}, and Total Acid Number

Data Analysis

The data analysis in this study was obtained from the total phenolic and flavonoid content at each solvent concentration used. Data were analyzed using the one-way ANOVA (Tukey test) at a 95% confidence level (p<0.05).

RESULT AND DISCUSSIONS

Yield of Pineapple Peel Extract

A total of 10 grams of sample was extracted with 150 mL of solvent (water (0%), 55% ethanol, and pure ethanol (96%). The extract was filtered with filter paper and evaporated at 60°C until a thick yellow-brown extract remained. From the results of this extraction, the following were obtained:

Table 2. Yield of extracted pineapple peel

Ethanol concentration	Yield (%)
0%	11.19
55%	3.99
96%	11.48

The data in Table 2 indicated that the higher the concentration of ethanol solvent, the greater the solubility of secondary metabolites in pineapple peel. The optimal result was obtained with 96% ethanol solvent. The type of solvent proves to have a significant impact on maximizing the yield achieved. The solvent used can be either a single solvent or a solvent mixture. Several studies have reported that extraction using a water-ethanol solvent mixture with an ethanol proportion in water is more effective (Hidalgo & Almajano, 2017). However, in this research, the single solvent (0% and 96%) showed a higher yield than the mixed solvent (55%). The extraction of pineapple peel in this study denoted that extraction with 55% ethanol did not produce an ethanol extract with an optimal yield.

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Total Flavonoid Content

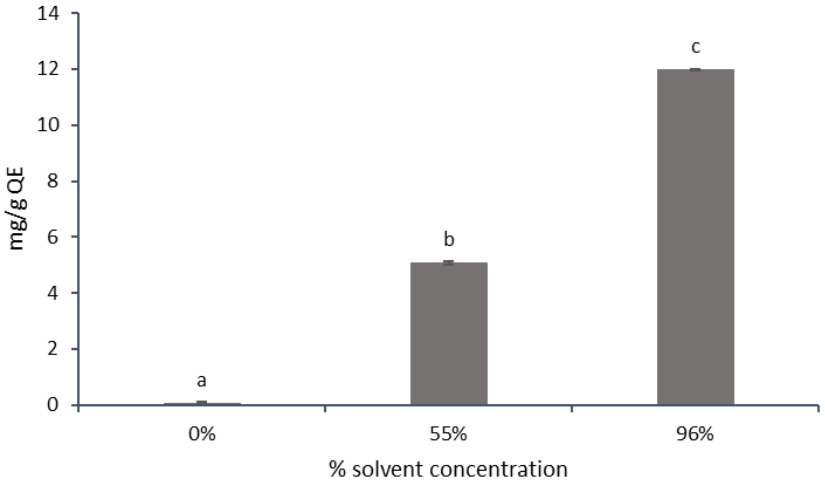

Figure 1. Total flavonoids equivalent to quercetin ($p < 0.05$)

From the results of the total flavonoid test in Fig. 1, it was concluded that the largest flavonoid content was in the extraction with 96% ethanol solvent (11.99 mg QE/g). Although the extraction using 0% ethanol had a higher yield than the sample with 55% ethanol, the pineapple peel extract using ethanol solvent shows a higher flavonoid content. Flavonoid compounds in pineapple peel were easier to extract using ethanol solvent. The greater the concentration of ethanol solvent used, the greater the total flavonoids obtained. This indicated that the flavonoid compounds present in pineapple peel can be effectively extracted using ethanol as a solvent. Ethanol demonstrated a superior ability to selectively extract flavonoids compared to other solvents, resulting in a potentially higher concentration of flavonoids in the extract. Ethanol is a non-toxic and biodegradable alternative that is being increasingly explored in extraction methods to lessen the environmental impact of traditional organic solvents, while still delivering comparable or even enhanced performance (Chaves et al., 2020; Jurinjak Tušek et al., 2022).

Total Phenolic Content

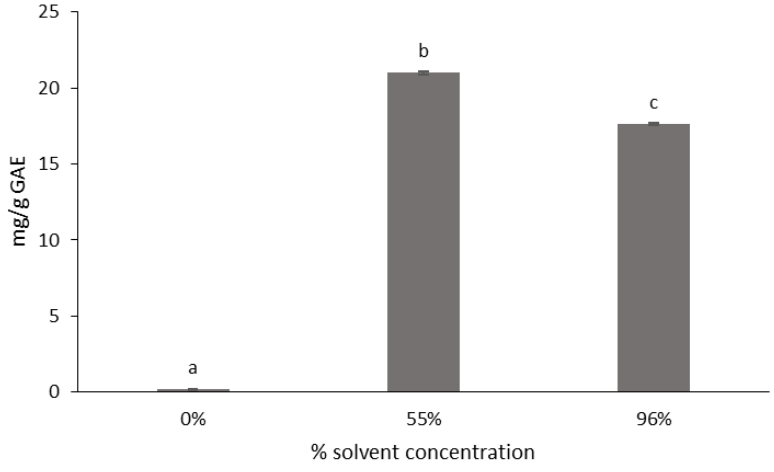

Figure 2. Total phenolic compounds equivalent to gallic acid ($p < 0.05$)

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The data presented in Fig. 2 showed that the total phenolic content significantly increased from 0% to 96% concentration, with a notable difference between the 0% concentration (which used only distilled water) and the concentrations of 55% and 96% ethanol. The results for total phenolic content differed slightly from those for total flavonoid content. Specifically, the extraction using 55% ethanol yielded a higher phenolic content (21.01 mg GAE/g) compared to 96% ethanol, which had a phenolic content of 17.62 mg GAE/g. This indicates that a mixed ethanol-water solvent was particularly effective in extracting phenolic compounds. Ethanol and ethanol-water mixtures were the preferred solvents because they typically extract a significant amount of phenolic acids and flavonoids (Yusof et al., 2020).

Antioxidant Activity

The effectiveness of a sample in counteracting free radicals using the DPPH method is measured by the IC_{50} value. IC_{50} refers to the concentration required to inhibit 50% of DPPH free radicals. A smaller IC_{50} value indicates greater antioxidant activity. A compound is classified as a very strong antioxidant if its IC_{50} value is less than 50, strong if it falls between 50 and 100, moderate between 100 and 150, and weak if it ranges from 151 to 200. (Azizah et al., 2023).

Table 3. IC_{50} value (ppm) of pineapple peel extract

Solvent concentration	**IC_{50} (ppm)**
0%	234.41
55%	206.20
96%	91.34

The results in Table 3 indicated that 96% ethanol showed the highest antioxidant activity in pineapple peel extract, as shown by the lowest IC_{50} value. This concentration was the most effective for extracting antioxidant compounds, particularly flavonoids and phenolics, which are known for their strong antioxidant properties. The extracted pineapple peel in 96% ethanol was classified as a strong antioxidant, whereas the extracted sample using 0% and 55% ethanol solvent was categorized as very low antioxidant activity or might not have any activity as an antioxidant. The 96% ethanol concentration not only effectively extracted antioxidant compounds but also maintained higher stability, preserving their biological activity throughout the extraction process. The DPPH radical scavenging ability of pineapple peel extracts using a shaking incubator showed a declining trend in the sequence of 100%, 80%, 60%, and 40% ethanol concentrations, with the aqueous extract exhibiting the lowest activity. This behavior is likely due to the significant role of phenolic compounds in contributing to the antioxidant properties of plants (Sharma et al., 2022).

Free Fatty Acid Content

Acid number is an important parameter in determining oil quality. This number indicates the amount of free fatty acids in the oil due to the neutralization reaction in which the acidic compounds in the oil react with the basic compounds (KOH/NaOH). In edible oils, free fatty acids (FFA) are considered undesirable because high FFA levels lead to greater losses during refining, reduce the oil's flavor quality and stability, and contribute to rancidity in the final product (Di Pietro et al., 2020). The calculation for this free fatty acid was taken from the titration results, where the vegetable oil titrated with sodium hydroxide (NaOH) will change

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color from yellow to pink. The calculation of free fatty acid levels was determined as the amount of NaOH required during the titration process. The results of the acid number analysis of the oil produced can be seen in Figure 3.

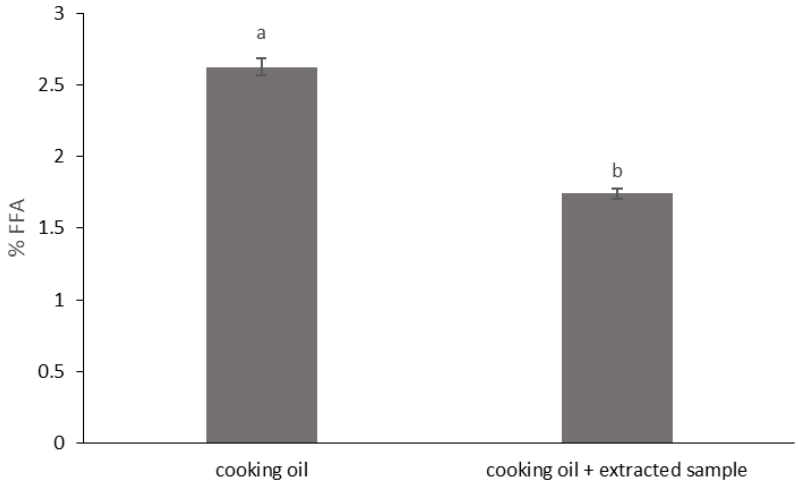

Figure 3. %FFA of cooking oil with and without added extracted sample ($p < 0.05$)

In this research, cooking oil from palm oil was used to observe the ability of the extracted pineapple peel to be an antioxidant. Palm oil contains about 50% saturated fatty acid, mostly palmitic acid (44%), and 40% monounsaturated fatty acid, mostly oleic acid (39.2%)(Mancini et al., 2015). Unsaturated fatty acids are easily oxidized in various ways. When vegetable oil is heated to high temperatures, the unsaturated fatty acids within it undergo oxidation. This process increases the total free fatty acids in the oil, while the content of unsaturated fatty acids, such as oleic acid, gradually decreases. As a result, these acids deteriorate, causing a loss of nutritional value. The primary oxidation products of oils are volatile-free fatty acids(Liu et al., 2022).

The levels of free fatty acids were equivalent to the determination of oleic acid during a 24-hour oxidation process. In Figure 3, the average free fatty acid content in oil treated with pineapple peel extract was approximately 1.62%, while in the oil without the extract, it was about 2.63%. The analysis of free fatty acid levels in both samples indicated that the addition of pineapple peel extract positively influenced the production of free fatty acids during oxidation. The results demonstrated that pineapple peel extract effectively acted as an antioxidant in cooking oil, as shown by a decrease in the acid number of palm oil after the extract was added. When less than 10% (w/w) of the extract was included, there was a reduction of approximately 38.4% in free fatty acids of cooking oil.

CONCLUSION

Based on the research results, it can be concluded that variations in the concentration of solvent extraction significantly affect the yield, total flavonoid, total phenolic content, and antioxidant activity in the Soxhlet method. The use of a single solvent resulted in a higher yield compared to the mixed solvent, and 96% ethanol obtained the highest yield, about 11.48%. The pineapple peel extraction resulted in the highest total flavonoid, 11.99 mg QE/g, after using 96% ethanol. While the highest phenolic content was obtained in 55% ethanol. The extracted

pineapple peel in 96% ethanol showed the lowest IC_{50} and had the ability a strong antioxidant. The extracted pineapple peel proved to decrease the free fatty acid content in cooking oil after incubating for 24 h. It concluded that the pineapple peel extract provided potential natural antioxidants, while the optimum extraction condition was in 96% ethanol.

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