

Antioxidant activity test of packaged juice variants of guava (*psidium guajava* L.) using FRAP (ferric reducing antioxidant power) method

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ABSTRACT

Packaged juice are usually made using only one type of fruit or can be made using two types of fruits or other additional ingredients. Antioxidants are chemical compounds that can be used to protect biological components such as lipids, proteins, vitamins, and DNA. This study aims to determine the level of antioxidant activity in packaged juice drink variants of guava fruit using the FRAP (Ferric Reducing Antioxidant Power) method. The method used in this study is the FRAP method to test antioxidant activity. The results of testing the antioxidant activity with the FRAP (Ferric Reducing Antioxidant Power) method on the packaged juice drink samples, the level of antioxidant activity in juice drink sample A was 88,359 mgAAE/g sample, sample B was 88,202 mgAAE/g sample, sample C was 83,098 mgAAE/g sample, and sample D was 77,317 mgAAE/g sample. This shows that sample A demonstrated the highest antioxidant activity, followed closely by sample B, while samples C and D exhibited lower levels of antioxidant activity.

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INTRODUCTION

Fruit juice or fruit extract is a processed product made from nutrient-rich fruits that provide a refreshing taste. Generally, fruit juice or extract is produced using one type of fruit, although it can also include two types of fruits or other ingredients preferred by consumers. The consumption of fruit or vegetable juice is beneficial for health due to the presence of various chemical compounds that can decrease the risk of degenerative diseases and is rich in antioxidants (Lesjak et al., 2018). Antioxidants are chemical compounds that protect biological components such as lipids, proteins, vitamins, and DNA from damage, rancidity, or color changes caused by oxidation (Suryanto, 2012). Free radicals are reactive compounds known to have unpaired electrons in their outer layer (Winarsi, 2007). The relationship between antioxidants and free radicals is closely linked, as free radicals can be neutralized by consuming or providing antioxidants (Halliwell, 2007).

Guava, scientifically known as *Psidium guajava* L., is a fruit that is abundant in antioxidants and is known for its functional food benefits. Its high content of phenols and other antioxidants has led some researchers to refer to it as a superfruit. While fresh guava trade is restricted globally, there is an increasing availability of processed guava products, such as drinks and preserves, in numerous countries (Angulo-López et al., 2021). Previous studies have highlighted the multiple health benefits of guava, such as its anti-diarrheal, anti-inflammatory, antimutagenic, antioxidant, antidiabetic, antihypertensive, platelet-enhancing, and analgesic properties (El Sohafy et al., 2010; Kumar et al., 2021; Takeda et al., 2022). Additionally, several studies have been conducted to analyze phenolic compounds and antioxidant capacity in various foods and beverages, including legumes, common foods in the United States, and guava fruit juice (Rachmayanti et al., 2021; Wu et al., 2004; Xu & Chang, 2007).

The evaluation of antioxidant activity is crucial for assessing the quality and potential health benefits of fruit juice products. Various standardized methods have been developed for the determination of antioxidant capacity and phenolic compounds in foods and dietary supplements (Prior et al., 2005). Additionally, the analysis of phenolic compounds in different fruits, such as blackberries, has been conducted using high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry (Mertz et al., 2007). Phenolic compounds, including those derived from brewer's spent grain, have also been investigated for their potential antioxidant effects (Verni et al., 2020). Furthermore, different antioxidant methods have been employed to evaluate the antioxidant capacity of blackberries (*Rubus fruticosus* L.) (Albert et al., 2022).

This research aims to test the antioxidant activity in commercially packaged guava juice using the FRAP method to reduce Fe^{3+} to Fe^{2+} . The FRAP method was chosen due to its speed, affordability, use of simple reagents, and no requirement of special equipment to determine total antioxidants (Rachmayanti et al., 2021). It is expected that the results of this research can provide information on the antioxidant activity in guava juice products and provide a basis for the development of healthy and high-quality fruit juice products.

RESEARCH METHOD

This research uses a descriptive laboratory experimental method. The population of this study consists of several samples of packaged guava fruit juice variants (*Psidium guajava* L.) obtained from supermarkets or grocery stores in the city of Batam. Four brands of packaged guava fruit juice variants with 100% vitamin C content were selected as samples for this study. The samples were taken from various supermarkets in Batam that provide different brands of packaged guava fruit juice variants.

Material

The sample of packaged beverage variant made of guava fruit (*Psidium guajava* L.), methanol, NaOH (Sodium hydroxide), KH_2PO_4 (Monopotassium phosphate), oxalic acid, FeCl_3 , TCA (trichloroacetic acid), ascorbic acid, phosphate buffer, $\text{K}_3\text{Fe}(\text{CN})_6$ (potassium ferricyanide) (Merck) and aquabidest (Ikapharmindo).

Sample Preparation

A total of 4 packaged fruit juice samples were selected from various supermarkets in Batam City, with different brands and variants of guava fruit juice. All beverage samples were prepared using standard protocols to prevent antioxidant photodecomposition, and efforts were made to avoid oxygen contact with the samples.

Ferric-Ion Reducing Antioxidant Power

The testing of antioxidant activity using the FRAP method followed the procedures of Benzie et al., Gil et al., Wootton-Beard et al., and Hartati et al., with modification (Benzie & Strain, 1996; Gil et al., 2000; Hartati et al., 2020; Wootton-Beard et al., 2011).

Preparation of Standard Solutions

In this study, a standard curve solution was prepared by first creating a stock solution of ascorbic acid with a concentration of 1.000 ppm. This stock solution was then diluted with 1% oxalic acid to produce several standard solutions with concentrations of 60, 70, 80, 90, and 100 ppm, respectively. The standard solutions were incubated with phosphate buffer and potassium ferricyanide at 50°C for 20 minutes. The determination of the maximum wavelength of the standard ascorbic acid was performed in the range of 650-750 nm.

Measurement of Blank Solution

The measurement of the blank solution was carried out by mixing 1% oxalic acid, 2 M phosphate buffer solution (pH 6), and 1% $K_3Fe(CN)_6$ solution, which were incubated at 50°C for 20 minutes. Then, 10% TCA solution was added, followed by centrifugation for 10 minutes at 3000 rpm, and the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer.

Antioxidant Activity of the Samples

The antioxidant activity was measured using the FRAP method by mixing 25 ml of fruit juice drink with 25 ml of methanol, followed by the addition of 0,2 M phosphate buffer (pH 6) and 1% $K_3Fe(CN)_6$ solution. The mixture was then incubated for 20 minutes at 50°C, followed by the addition of TCA, centrifugation, and measurement of the absorbance at the maximum wavelength using a UV-Vis spectrophotometer. The calibration curve was made using ascorbic acid solutions with various concentrations, and the FRAP value was expressed in mg of ascorbic acid equivalents per gram (mgAAE/g) of fruit juice drink. The test was performed in triplicate.

Data analysis

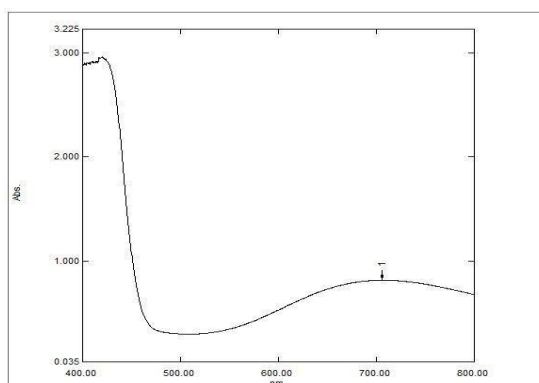
The reduction ability test data was derived by utilizing the linear regression equation of the vitamin C standard curve, and quantified in mgAAE/g of the sample. Furthermore, the ANOVA test was used to compare the data between the samples.

RESULTS AND DISCUSSIONS

The primary objective of this research was to evaluate the antioxidant potential of packaged guava (*Psidium guajava* L.) juice variants using the FRAP assay. Initially, samples of different guava juice variants were procured from various supermarkets and grocery stores in Batam city. The FRAP assay, based on electron transfer instead of hydrogen atom transfer, was employed to determine antioxidant activity. The assay utilizes the pH-dependent reduction of Fe^{3+} to Fe^{2+} , which occurs at an acidic pH of 3.6 to ensure solubility of iron. Under low pH conditions, ionization potential is decreased, and redox potential is increased, which is the dominant reaction mechanism. The reduction of Fe^{3+} to Fe^{2+} produces a colored complex with Fe^{2+} , and the strength of the reduction is directly related to the degree of hydroxylation and the level of conjugation in PH (Cerretani & Bendini, 2010).

Before evaluating the antioxidant activity, the maximum wavelength of the standard and sample solutions was determined. Ascorbic acid, belonging to the group of secondary antioxidants that can scavenge extracellular free radicals, was used as the standard solution. Ascorbic acid contains free hydroxyl groups that act as free radical scavengers, and the presence of polyhydroxy groups enhances antioxidant activity (Raharjo & Haryoto, 2019; Szeto et al., 2002). In this study, the FRAP method was quantitatively used to determine antioxidant activity through UV-Vis spectrophotometry. The maximum wavelength measured ranged from 650-730 nm, and the samples were incubated at 50°C for 20 minutes to optimize the reaction between FRAP and

antioxidant compounds. The absorbance values were observed to ensure that no decrease in



absorbance occurred. The maximum wavelength obtained was 706 nm, as shown in Figure 1.

Figure 1. Maximum Wavelength Of Ascorbic Acid

The addition of TCA aims to precipitate the potassium ferrocyanide complex, FeCl_3 is added to form a green to blue colored complex, and phosphate buffer is added due to its stable effective pH of 6,4-7,4 for this complex. Low pH is used to facilitate the Fe^{3+} reduction process (Birasuren et al., 2013). Absorbance was measured from each concentration of standard solution (60, 70, 80, 90, and 100 ppm). The regression result showed the equation $y = 0,0128x - 0,386$ with an R^2 value of 0,9840, as presented in Figure 2. The sample absorbance values were then inputted into the linear regression equation for vitamin C, and the FRAP value was expressed in mg equivalent of ascorbic acid (AAE) per gram of sample.

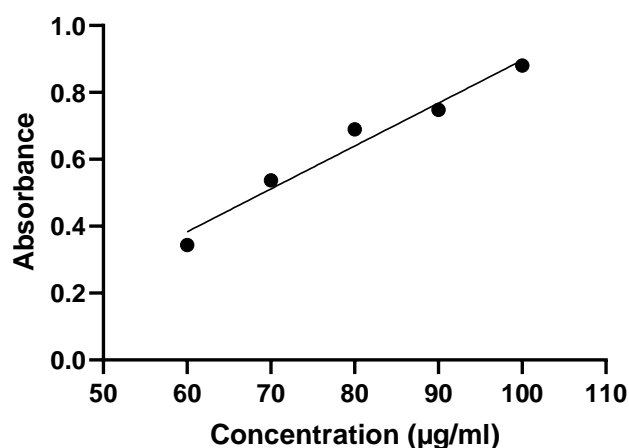


Figure 2 . Graph Of Standard Curve Of Ascorbic Acid

The results of the antioxidant activity testing of packaged guava juice variants using the FRAP method, based on the tested antioxidant levels, are presented in Figure 3. The antioxidant testing revealed that sample A had an antioxidant value of $88,359 \pm 0,078$ mgAAE/g sample, sample B had $88,202 \pm 0,207$ mgAAE/g sample, sample C had $88,098 \pm 0,665$ mgAAE/g sample, and sample D had $77,317 \pm 0,857$ mgAAE/g sample. This indicates that each sample of packaged guava juice exhibited high antioxidant activity.

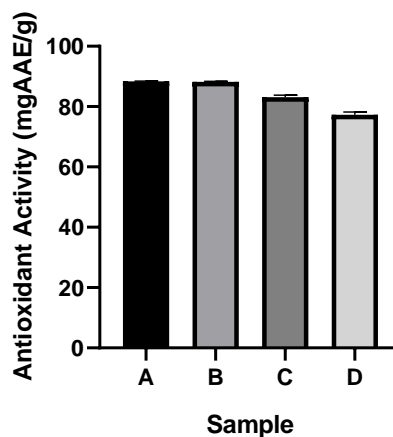


Figure 3. Reducing power of examined samples

The antioxidant activity of four samples was compared using the One-Way ANOVA method. The results indicated a significant difference in antioxidant activity values, with a significance level of $p < 0,05$, specifically 0,0001. The results showed significant differences in the mean values among the samples. The variation in antioxidant activity among the samples can be attributed to the differences in the ingredients contained in each brand of packaged juice. Although the relative differences in ingredient content were not substantial, they were sufficient to result in variations in the antioxidant activity levels (Fernandes et al., 2015; Tambunan et al., 2018). Among the four samples, samples A, B, and C had a thick consistency, retained fruit fibers, exhibited a deeper shade of red, and had a pronounced guava flavor. Only sample D differed, as it had a more liquid consistency, a brighter shade of pink, and a less pronounced guava flavor. Additionally, there were differences in the percentage of guava fruit extract in the four samples, with sample A containing the highest percentage at 38%, sample B containing 35,5% guava fruit extract, sample C containing 35% guava fruit extract, and sample D containing the lowest percentage at 27%.

CONCLUSION

The study reveals varying levels of antioxidant activity among different brands of packaged guava juice with seeds, with sample A demonstrating the highest activity followed by sample B, while samples C and D exhibited lower activity. These findings highlight the influence of brand choice on the antioxidant potential of the juice. However, it is important to acknowledge the limitations of the research and propose suggestions for future studies. Additionally, discussing the implications and research contributions in the conclusion section would provide practical significance and contribute to the existing knowledge in this field. This study offers valuable insights for consumers to make informed decisions when purchasing guava juice.

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