Proximate and Sensory Analysis of Functional Drink from Jackfruit Seed Extract with Citrulline Fortification as a Potential Antidiabetic

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Abstract
The World Health Organization (WHO) predicts that diabetes mellitus in Indonesia by 2030 will increase to 21.3 million people. There was a growing need for natural foods that can be used as a substitute for antihyperglycemic drugs and may lower the blood sugar levels of diabetes Mellitus patients without burdening the kidneys and causing other side effects. Citrulline, one of the amino acids that are useful for the immune system and improving blood flow circulation, is found in the white peel of watermelon in greater levels compared to the citrulline content of other fruits. Previous research has shown that citrulline supplementation significantly lowered fasting blood sugar and HbA1c as well as inflammatory biomarkers, including in obese subjects. Methods conducted in this study include watermelon peel extract making, jackfruit seed drink making, proximate analysis, total phenol testing, organoleptic testing, and data analysis. The results of proximate testing on jackfruit seed drink with additional citrulline (F1) were F1 samples consisting of 92.31% water, 0.47% protein, 0.99% fat, 0.00% ash, and 6.23% total carbohydrates. Sensory analysis revealed that the flavor of the drink was disliked, while the aroma of the drink was somewhat liked. The addition of watermelon peel extract in jackfruit seed drinks may increase nutrition value, antioxidant content, and has the potential to be a functional drink with antioxidant content and blood sugar reduction effects as antidiabetics.
1. Introduction

Diabetes mellitus is a disease that is suffered by many people, especially in Indonesia. The World Health Organization (WHO) predicts that diabetes mellitus in Indonesia by 2030 will increase to 21.3 million people. Indonesia is also ranked seventh with the most people with diabetes mellitus in the world, which is as many as 8.5 million people. People with diabetes mellitus should be treated with consistent antihyperglycemic drugs so that blood sugar levels are controlled (Alfian, 2015). The use of antihyperglycemic drugs will cause side effects to some people such as nausea, dizziness, and increased heart rate. Antihyperglycemic drugs also cannot be consumed excessively in people with diabetes mellitus who experience complications of chronic kidney disease. Therefore, there was a growing need for natural foods that can be used as a substitute for antihyperglycemic drugs and may lower the blood sugar levels of diabetes Mellitus patients without burdening the kidneys and causing other side effects.

Natural ingredients such as some types of fruit can be used to lower blood sugar levels. One of the fruits which may lower blood sugar levels is watermelon. The content in watermelon includes water, vitamin A (carotenoids), vitamin C, vitamins, K, citrulline, and potassium that can lower blood pressure (Rusdiawan dan Habibi, 2020). Citrulline content is more abundant in the white peel of watermelon compared to the content of another fruit citrulline. Citrulline is an amino acid useful for the immune system and can help improve blood circulation (Akhyar dan Pardede, 2019). Citrulline supplementation significantly lowered fasting blood sugar and HbA1c (Abbassazadeh et al., 2021) as well as inflammatory biomarkers (Azizi et al., 2020), including in obese subjects (Azizi et al., 2021). Citrulline found in watermelon serves as a precursor to the formation of NO which will cause insulin secretion, then stimulate glycojenesis and lower blood glucose levels (Amir dan Borang, 2015).

The white part of watermelon peel can lower blood sugar while also reducing food waste. In addition, jackfruit seeds are often thrown away even though jackfruit seeds contain some useful nutrients. Based on data from the Direktorat Gizi, Departemen Kesehatan RI (2009) that at 100 grams of jackfruit seeds contain iron 1.0 mg, vitamin B1 0.20 mg, calories 165 kcal, protein 4.2 grams, fat 0.1 mg, carbohydrates 36.7 mg, calcium 33 mg, phosphorus 200 mg, vitamin C 10 mg, and water 56.7 grams. Therefore, processing jackfruit seeds into a drink may result in a functional drink with high nutrition value.

Jackfruit seed drink contains higher phosphorus and calcium than soy drink while its fat content is lower (Nusa et al., 2015). Consuming jackfruit seed drink can have a good impact on health because it has high nutrition and can be used as a solution for people with lactose intolerant. Jackfruit seeds have various benefits such as preventing anemia, maintaining the health of the heart, improving blood flow, nourishing the eyes, strengthening hair roots, inhibiting facial aging, preventing constipation, and inhibiting colon cancer (Muljawan & Pradana, 2017). The use of jackfruit seeds and watermelon peel citrulline to lower blood sugar levels can be done to replace antihyperglycemic drugs. Previous research conducted by Sugiyanta (Sugiyanta dan Asa, 2013) showed watermelon peel extract was safe to be utilized as a diabetes drug and has been shown to lower serum glucose levels and triglyceride in mice. Therefore, research on jackfruit seed drink added with citrulline from watermelon peel needs to be done because the combination of citrulline synergistic effects and jackfruit seed drink has not been made and tested its effectiveness in lowering blood sugar. Measurements of the effects of citrulline will be made with 2 different formulations namely jackfruit seed drink without citrulline (F0) and jackfruit seed drink with the addition of citrulline (F1). The benefit of this study is knowing the effects of the addition of citrulline to jackfruit seed drinks on the proximate value and sensory score of the drink.
2. Material and Method

2.1 Material

The ingredients used in this study were watermelon white peels, jackfruit seeds, mineral water, vanilla paste, 70% ethanol, filter paper, concentrated H\textsubscript{2}SO\textsubscript{4} and HCl 0.1N, NaOH 45%, selenium, n-hexane, boric acid, aquadest, and methyl red and methyl blue indicators. The tools used in this study are cabinet dryer, grinder, blender, refractometer, spectrophotometer, oven, desiccator, analytical scales, soxhlet extractor, and furnace.

2.2 Extraction of Watermelon Peel

The extraction of watermelon white peels began with the separation of white peel from the flesh of the fruit and the outer peel of a watermelon. Then, the white peel was dried with a cabinet dryer with a temperature of 50 °C for 6 hours or until dry. Next, the dried watermelon peel was ground until smooth (40 mesh). Watermelon peel powder was packed in a sealed container and given 70% ethanol with a ratio of 1:10. Watermelon peel maceration was done for 3x24 hours where every 1x24 hours, the solvent was replaced so that it would not be saturated. The obtained filtrate was then evaporated with a rotary evaporator and concentrated with a water bath until viscous extract was obtained (Setiawan et al., 2018). After the extract was obtained, it would be added into sample F1.

2.3 Sample Production

Jackfruit seed was cleaved and then the seeds are separated. The cleaved jackfruit seeds were then washed and soaked for 12 hours and then boiled for 15 minutes. Next, those seeds were peeled and blended with water with a ratio of 1:4. Jackfruit seed drink was then filtered and boiled at a temperature of 85 °C for 2 minutes and transferred into a bottle when it’s already cold (Masyhura et al., 2021). Watermelon peel extract (4.65 g) was added to 60 mL of jackfruit seed drink that had been made. The amount was obtained based on previous research using citrulline extract then was calculated through dose calculations for humans with an average of 60kg BW (Marlina & Irwanda, 2016).

2.4 Proximate Analysis

Both jackfruit seed drinks (F0 and F1) were tested for their nutritional content with proximate analysis. The proximate analysis consisted of water content analysis by using thermogravimetric method (Legowo et al., 2005), fat content analysis by soxhlet extraction method (Legowo et al., 2005), protein content analysis by Kjeldahl method (Legowo et al., 2005), ash level analysis of Tanur method (Legowo et al., 2005), and carbohydrate content analysis using by difference method (Apriyantono et al., 1989). The proximate analysis was conducted on both samples with two repetitions (duplo).

2.5 Total Phenol Analysis

The total phenols contained in watermelon peel extract were measured by spectrophotometry methods. An extract of 3 mL is taken and added with 2.5 mL of water-soluble 10% Folin Ciocalteu reagent. The solution was then added with 2.5 ml of NaHCO\textsubscript{3} 7.5%, then incubated for 45 minutes with a temperature of 45 °C. Absorbance is measured with a wavelength of 765 nm, and the standard curve of gallic acid is made by the same procedure. Total phenol and absorbance measurements can be read from the standard curve, while the total phenols in extracts can be shown in gallic acid equivalent (GAE) (mg/g). Measurement of total GAE phenols is done with the following formula (Syafitri et al., 2014):

\[ GAE = c \times (V/m) \]

Information:
- c: Total concentration of phenols from the standard curve of gallic acid (mg/L)
- V: Volume of extract (mL)
- m: Extract weight (g)
2.6 Sensory Analysis

Organoleptic was done with a hedonic (fondness) test on both samples. The hedonic test was conducted on 36 panelists with an age range of 18 – 25 years in Tembalang District, Semarang. The hedonic test was done on flavor and aroma parameters. Each panelist was given 2 different samples and a scoring sheet with a score range of 1 – 5: 1 (very dislike), 2 (dislike), 3 (somewhat like), 4 (like), and 5 (very like) (Sumarni et al., 2017). Organoleptic results data were analyzed using an independent T-test (GraphPad Prism 9 Premium) to see whether the difference of flavor and aroma between F0 and F1 is significant.

2.7 Research Subject Sampling Methods

The study was conducted in F0 and F1 products. Organoleptic tests were conducted by panelists from the student population aged 18 – 25 years in Tembalang Subdistrict, Semarang by random sampling method. The total number of subjects in the study was 36 subjects.

3. Result and Discussion

3.1 Total Phenol

Table 1. Total Phenol of Watermelon Peel Extract

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Total Phenol Value (mg GAE/g)</th>
<th>Mean (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.23</td>
<td>4.27</td>
</tr>
<tr>
<td>2</td>
<td>4.30</td>
<td></td>
</tr>
</tbody>
</table>

The total phenol value of watermelon peel extract was obtained at 4.27 mg GAE/g. This value was categorized as a low value which was in line with Ismail’s research (Ismail et al., 2012) on the peel extract of Pinang Yaki, where the lowest total phenol yield on the fruit peel was 3.16 mg/g and the highest total phenol in the fruit seeds was 85.92 mg/g. The low total value of the phenol can be affected by the amount of solvent used. This study used a ratio extract to solvent of 1: 10. According to Maligan (Maligan et al., 2015), the higher the ratio of solvents, the lower the total phenol. The low total value of phenols was also possibly affected by the time of extraction. Aulia & Widjanarko (Aulia & Widjanarko, 2018) found that a longer extraction time will result in a low total phenol. Total phenol analysis was done on watermelon peel extract to make sure it consisted of phenol, indicating that the addition of watermelon peel extract will result in increased antioxidant properties.

3.2 Water Content

Table 2. Water Content of Jackfruit Seeds Drink

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repetition</th>
<th>Water Content (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 A</td>
<td>1</td>
<td>98.40</td>
<td>98.42</td>
</tr>
<tr>
<td>F0 B</td>
<td>2</td>
<td>98.44</td>
<td></td>
</tr>
<tr>
<td>F1 A</td>
<td>1</td>
<td>92.34</td>
<td>92.31</td>
</tr>
<tr>
<td>F1 B</td>
<td>2</td>
<td>92.28</td>
<td></td>
</tr>
</tbody>
</table>

Jackfruit seed drink samples of F0 and F1 had a different water content of 98.42% and 92.31%. The low water content of the F1 sample was caused by a high total dissolved solid value of 5.3 °Brix, while the total dissolved solids in the F0 sample are 1.2 °Brix. According to Ismawati (Ismawati, 2016), the total value of dissolved solids will be higher if the concentration of added extracts is high. Citrulline extract can increase the total value of dissolved solids because citrulline belongs to the protein group. Total dissolved solids come from the breakdown of carbohydrates, fats, organic acids, pigments, as well as the breakdown of simple water-soluble proteins (Permadi et al., 2021).

3.3 Protein Content

Table 3. Protein Content of Jackfruit Seed Drink

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repetition</th>
<th>Protein Content (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Jackfruit seed drink samples of F0 and F1 had different protein levels of 0.24% and 0.47%, respectively. Protein levels of the F0 sample were lower than the F1 sample since citrulline in the F1 sample is one type of amino acid in the protein. The protein content in jackfruit seed drinks was lower than the protein content in jackfruit seeds because the protein content of jackfruit seeds was 4.2%. Jackfruit seed content includes carbohydrates 36.7%, fiber 2.74%, protein 4.2%, and fat 0.1% (Sulistiyaningsih et al., 2019). Decreased protein levels can be caused by the process of processing jackfruit seeds into jackfruit seed drinks. Heating as a processing process in jackfruit seeds causes protein denaturation that can decrease protein levels (Selvi Yulianti et al., 2017).

### 3.4 Fat Content

Table 4. Fat Content of Jackfruit Seed Drink

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repetition</th>
<th>Protein Content (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 A</td>
<td>1</td>
<td>1.31</td>
<td>1.32</td>
</tr>
<tr>
<td>F0 B</td>
<td>2</td>
<td>0.65</td>
<td>0.99</td>
</tr>
<tr>
<td>F1 A</td>
<td>1</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>F1 B</td>
<td>2</td>
<td>0.31</td>
<td></td>
</tr>
</tbody>
</table>

Based on the results of testing, the fat content of jackfruit seed drink in F0 and F1 were 1.32% and 0.99%, respectively. The low-fat content in both samples might be caused by the use of jackfruit seeds as raw materials of the drink. Jackfruit seeds are known to contain low fat, even lower than soybeans. According to Mardina (Mardina et al., 2012), the fat content in jackfruit seed was 0.1% while the fat content in whole soybean seeds was 16%-20.8%. The addition of watermelon peel extract on jackfruit seed drink made its fat content reduced since citrulline in the extract will bind to fat so that fat becomes dissolved (Amertaningtyas et al., 2014).

### 3.5 Ash Content

Table 5. Ash Content of Jackfruit Seed Drink

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repetition</th>
<th>Ash Content (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 A</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>F0 B</td>
<td>2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>F1 A</td>
<td>1</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>F1 B</td>
<td>2</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

The ash content in jackfruit seed drink in F0 and F1 samples was 0.00%. The low ash levels in both samples were caused by the low mineral content in them (Syafar et al., 2019). In general, jackfruit seeds contain several minerals such as calcium, phosphorus, and iron (Indrianti et al., 2019). The reduced mineral content of jackfruit seeds might be due to the processing process in the form of boiling that makes the mineral content shrank in the material (Ariany & Putalan, 2021). However, the low percentage of ash levels resulted in a better quality of both samples of jackfruit seed drink. According to Tubagus (Tubagus et al., 2018), the lower the ash content of a material, the better the quality.

### 3.6 Total Carbohydrate Content

Table 6. Total Carbohydrate Content of Jackfruit Seed Drink

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Content (%)</td>
<td>F0 98.42</td>
</tr>
<tr>
<td>Protein Content (%)</td>
<td>F0 0.24</td>
</tr>
<tr>
<td>Fat Content (%)</td>
<td>F0 1.32</td>
</tr>
<tr>
<td>Ash Content (%)</td>
<td>F0 0</td>
</tr>
<tr>
<td>Total Carbohydrate (%)</td>
<td>F0 0.02</td>
</tr>
</tbody>
</table>
The total carbohydrate content of jackfruit seed drink in F1 and F0 samples were 6.23% and 0.02%. Differences in carbohydrate content in both samples might be influenced by the addition of watermelon peel extract which made the number of solids in the drink increase. The total carbohydrate content in both samples was also affected by other nutrient content where the higher the fat, protein, water, and ash content would lower the total carbohydrate content (Al-Farid et al., 2019). Processing jackfruit seeds into a functional drink requires several stages such as boiling and filtering, which may also lower total carbohydrate levels. The boiling process can degrade carbohydrate molecules into simple sugars (Yulianti et al., 2015) while the filtering process will make jackfruit seed drink separate from its dregs that contain a lot of carbohydrates.

3.7 Sensory Acceptability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F0</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor</td>
<td>2.69 ± 0.786</td>
<td>2.36 ± 0.990</td>
</tr>
<tr>
<td>Aroma</td>
<td>3.47 ± 0.878</td>
<td>3.11 ± 0.919</td>
</tr>
</tbody>
</table>

Table 7. Sensory Score of Jackfruit Seed Drink

![Figure 1. Independent T-Test Result of Flavor and Aroma Sensory Parameter](image)

Description of Organoleptic Score:
Very Dislike (1); Dislike (2); Somewhat Like (3); Like (4); Very Like (5)

The F0 sample had a flavor score of 2.69, higher compared to F1 which only had an average of 2.36. However, this difference wasn't significant (p>0.05), indicating that the addition of citrulline didn't change the flavor parameter of the jackfruit seed drink. The low average value of flavor in jackfruit seed drink with the addition of watermelon peel extract is due to the presence of active compounds. According to Maghfiroh (Maghfiroh et al., 2021), watermelon peel extract contains active compounds, one of which is tannins. Tannin compounds when consumed will form cross-bonds with proteins in the oral cavity so that there will be a sense of flavor (Ilona & Ismawati, 2015).

The results of the organoleptic test on the aroma of jackfruit seed drink showed that F0 had an average score of 3.47 while F1 of 3.11. Although F1 might seem to have a lower aroma score, the difference between the aroma of F0 and F1 wasn't significant (p>0.05), which means that citrulline didn't affect the aroma characteristic of the jackfruit seed drink. This insignificant difference could be affected by the results of organoleptic test flavors that are also didn't
differ significantly since aroma and flavor are directly proportional. According to Nuraini (Nuraini & Merkuria, 2019), flavor and aroma are two organoleptic parameters that are interconnected and also affect each other.

3.8 Potential Antidiabetes Effect

Jackfruit seeds contain fiber, vitamin B complex, as well as phytochemical compounds (Waghmare et al., 2019). Research conducted by Dwitiyanti identified phytochemical compounds in jackfruit seeds extracted with ethanol 70% and obtained phytochemical content including alkaloids, flavonoids, tannins, steroids, saponins, and terpenoids (Dwitiyanti et al., 2019). Flavonoids in jackfruit seeds were known to play a role in controlling blood sugar levels and improving insulin sensitivity. Studies conducted by Russo explained that flavonols increase the expression of insulin receptor mRNA and GLUT 4 (glucose transporter type 4) in muscle tissue that carries glucose into cells (Russo et al., 2019). In addition, flavonoids can also protect β cells of the pancreas from cytokines and inflammation. The results of studies on the mechanism of flavonoids in controlling blood sugar support the results of this study. The decrease in blood sugar levels is caused by the mechanism of action of flavonoids in improving insulin sensitivity and protecting β cells of the pancreas from inflammation. The role of flavonoids from jackfruit seeds in controlling blood sugar can be more effective with the addition of other compounds, such as citrulline. Citrulline is a precursor to the formation of nitric oxide (NO) that secretes insulin and also plays a role in improving the expression and translocation of GLUT 4 in muscle tissue (Abbaszadeh et al., 2021). Research conducted by Marlina (Marlina & Irwanda, 2016) showed a significant reduction in blood sugar in animals who were given watermelon white peel ethanol extract as much as 14 mg/20gr BW and 28 mg/20gr BW. Research by Rezq conducted by feeding animals with a mixture of 10%, 20%, and 30% of watermelon peel extract in feed showed a decrease in fasting blood sugar levels as well as an increase in insulin levels (Rezq, 2017). Thus, watermelon peel extract added to jackfruit seed drink has the potential to have a more effective reduction in blood sugar levels.

4. Conclusion

Based on our research, it can be concluded that the addition of watermelon peel extract in jackfruit seed drinks may increase nutrition value, antioxidant content, and has the potential to be a functional drink with antioxidant content and blood sugar reduction effects as antidiabetics. However, the aroma and flavor in jackfruit seed drink with the addition of watermelon peel extract tends to be less preferred. Therefore, researchers suggest the existence of further studies that can create jackfruit seed drink formula with the fortification of watermelon peel extract that has a better acceptability score with the same potential to reduce blood sugar levels. Further total phenol analysis on the completed product of jackfruit seed drinks may also be analyzed to see the real phenol content on the processed product.

Conflict of Interest

The author declares that there is no conflict of interest in this study.

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