Effect of Storage Time to Ferulic Acid Content in Extracted Banana Stem Waste Juice

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Abstract

Objectives: The present of Ferulic Acid (FA) in plants attract the attention of many researchers, especially in food, pharmaceutical and cosmetic industries. Banana Stem Waste (BSW) was generated from banana plantation.

Methods/Statistical Analysis: In this study, FA was extracted from BSW by using sugarcane press machine. Extracted BSW juice (EBJ) was characterized based on the compositions of total phenolic and glucose. The effect of storage time of EBJ was studied. Design Expert software was used for the experimental design. One factor design was applied for the factorial analysis. Findings: The FA was analyzed by using High Performance Liquid Chromatography (HPLC) and FA yield ranging from 0.1101 mg/g to 0.2274 mg/g. The analysis of the results showed that storing EBJ at room temperature increased the ferulic acid yield however the ferulic acid decreased when the storage time exceed 24 hr.

Application/Improvements: Effect of storage time to extracted ferulic acid concentration will be understood which in turn may be beneficial for the industrial purpose.

Keywords: Banana Stem Waste, Factorial Analysis, Ferulic Acid (FA), Mechanical Extraction, Pre-treatment Process

1. Introduction

The nutrients and phenolic compounds contained in the extracted juice degenerate as they were sensitive to air, light, heat and time. The stability during the storage was essential as its influence the physical appearance, retention of nutritional value and their microbial shelf-life. In order to maintain the stability of the extracted juice, condition such as temperature and storage time need to be controlled.

The storage temperature played an important role in order to maintain the content of phenolic compound during the storage times. From the studies conducted by [1], the amount of phenolic compounds in strawberry was continuously increased when stored at 5 and 10 °C while it was maintained when stored at 0 °C. Besides, the strawberry that was stored at 10 °C also had higher antioxidant content and antioxidant enzyme activities compared to those that stored at 0 and 5 °C. This was due to the levels of the enzyme activities which increased with the increasing of storage temperature.

The level of phenolic compounds contained in the extracted juice can be prolonged if stored at low temperature during the storage time. The used of low temperature lower the degradation rate of phenolic compounds. However, the phenolic compounds in extracted juice were decreased if stored at longer storage time. According to [2], the phenolic compounds in homogenized guava samples were gradually decreased when stored for more than 30 months. Therefore, there is a limit time to store a phenolic compounds and it must be used within reasonable time after extraction to avoid the degradation which reduce their benefit. The objective of this research was to study the effect of storage time in FA extraction from BSW. One Factor At Time (OFAT) analysis and response surface method (RSM) were used to evaluate the significance of different storage time values.
2. Materials and Methods

2.1 Experimental Setups for Banana Stem Waste Mechanical Extraction
The BSW juice was extracted by using sugarcane press machine. The extraction was conducted using the outer part of BSW with one cycle extraction.

2.2 Experimental Setup for One Factor At Time (OFAT) Analysis
In order to study the effect of storage time to FA concentration, the samples of extracted BSW juice (EBJ) were collected and stored in universal bottle at room temperature (25-27 °C) for 0 to 72 h. Then, the samples were analyzed for FA every 24 h.

2.3 Experimental Setup for Response Surface Method (RSM) Analysis
Design Expert software (Version 7.1.3, Stat-Ease, Inc., Minneapolis, MN) program was used in RSM analysis. The experimental table was constructed in one factor design of response surface methodology (RSM) with five levels of numeric factors shown in Table 1. Results from OFAT were utilized to determine the range for RSM analysis. After the extraction, the EBJ was collected and stored at room temperature between 20 to 28 h. Then, the samples were analyzed for FA every 2 h.

Table 1. Values of coded and real values of storage time

<table>
<thead>
<tr>
<th>Coded values</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>+1</th>
<th>+2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real values</td>
<td>20</td>
<td>22</td>
<td>24</td>
<td>26</td>
<td>28</td>
</tr>
</tbody>
</table>

2.4 Analysis of Ferulic Acid (FA) by using HPLC
The concentrations of FA in samples were analyzed by using high performance liquid chromatography (HPLC). The analytical HPLC system employed consisted of an Agilent 1100 HPLC equipped with a diode array detector (DAD). The HPLC pumps, column oven, auto sampler, and DAD system were monitored and controlled using the HP Chem Station computer program. The separation and analysis of FA were carried out using a 5 µm Zorbax SB-C18 (250 mm x 4.6 mm, Agilent Technologies, Palo Alto, CA).

2.5 Characterization of Extracted BSW Juice (EBJ)
The analysis of EBJ was done to determine the compositions of total phenolic and glucose. The sample was obtained from the RSM analysis. The composition of total phenolic was analyzed by using Folin-Ciocalteu reagent. This method relied on the transferred of electrons from phenolic compounds to the Folin–Ciocalteu reagent in alkaline medium. The composition of total phenolic was determined by comparing the absorbance with the standard of Gallic acid. The composition of total phenolic per extract was expressed as Gallic acid equivalent (GAE). The composition of glucose in the EBJ was determined by using dinitrosalicylic (DNS) test. The composition of glucose in the sample was determined by comparing the absorbance with the standard of glucose.

3. Results and Discussion

3.1 Characterization of the Extracted BSW Juice (EBJ)

3.1.1 Total Phenolic in Extracted BSW Juice (EBJ)
Phenolic compounds widely distributed in tissue, cellular and subcellular of the plants. The structure of phenolic compounds consisted of an aromatic ring which contains one or more hydroxyl substituent. The phenolic compounds that contained in the plants can be determined by total phenolic. The composition of total phenolic in EBJ was determined using Folin-Ciocalteu colorimetric method. In this study, the part of BSW used was the outer stem. The total phenolic in EBJ was 22.61 mg GAE/g of dry weight. The value was different than other researchers. Previous study by reported higher total phenolic, about 79.92 and 65.32 mg GAE/g of dry weight, respectively. However, the study conducted by obtained lower than other researchers which only 12.00 mg GAE/g of dry weight. The composition of total phenolic in EBJ was different from other researchers due to the different type of extraction used. In this study, BSW was pressed using mechanical extraction by using sugarcane press machine to extract the juice while other researchers used chemical
Different total phenolic also depend on the part of banana plants. From the study conducted by\textsuperscript{5}, the composition of total phenolic in the outer stem was higher than the pith. The difference in composition of total phenolic depends on the type of banana plants. Total phenolic in plants can be classified into different classes from the simple molecules such as simple phenol to highly polymeric compounds such as condensed tannins. Phenolic acids which were hydroxycinnamic and hydroxybenzoic acids also included in the composition of total phenolic\textsuperscript{11}.

Besides\textsuperscript{9} reported the total phenolic contained in the banana flower was higher than the outer stem. This was due to the accumulation of secondary metabolites in the flower tissues\textsuperscript{6}. Another study by\textsuperscript{10} stated the pulp and the peel also contained phenolic compounds. The total phenolic in the pulp and the peel were 20.47 and 8.08 mg GAE/g of dry weight, respectively. From this, it showed that the total phenolic contained in outer stem was higher than the other part of the plants.

### 3.1.2 Glucose in Extracted BSW Juice (EBJ)

Glucose was a simple sugar that abundant in most of the plants. According to\textsuperscript{12}, glucose composition predominated at a range between 9 to 24% of total sugar in BSW. According to\textsuperscript{13,14}, the composition of glucose were 0.60 and 1.34 mg/mL respectively. The composition of glucose in this study was lower than other researcher due to the different type of banana species used. The composition of glucose in plants also depends on the compositions of cellulose and hemicellulose. In plant, cellulose and hemicellulose was hydrolyzed to simple sugar and increased the composition of glucose\textsuperscript{15}. The bond between FA and hemicellulose was broken and increased their availability\textsuperscript{16}. The BSW sample was cut and extracted by using sugarcane press machine.

### 3.2 Effect of Storage Time for Extracted BSW Juice

#### 3.2.1 OFAT Analysis

The result of FA yields in the extracted BSW juice at different storage times is shown in Figure 1. FA yield was increased from 0.0101 to 0.0149 mg/g with longer storage time from 0 to 24 h. It was increased due to the released of free acids from their bonds during the storage time\textsuperscript{17}. The FA was sensitive to the presence of oxygen at ambient temperature\textsuperscript{18}. Based on the research conducted by\textsuperscript{17}, the FA yield in orange juice was increased when stored at room temperature. However, the FA yield did decrease when the storage time was prolonged up to 72 h. This was due to the oxidation reaction of the FA during processing and storage\textsuperscript{19}.

![Figure 1. FA yield at different storage time of EBJ.](image)

#### 3.2.1 RSM Analysis

One Factor Design (OFD) of RSM analysis was used to optimize the process and product by correlating the interaction between input and output variable (equation 1). It also used to provide a clear understanding of the interactions between the storage time of EBJ and FA yield after the extraction. This method is widely used to analyze and optimize the extraction processes from natural resources\textsuperscript{20}. Compared to OFAT analysis, RSM analysis narrowed the storage time range from 0-24 h to 20-24 h. In OFAT analysis, FA yield was increased when the storage time increased and the highest FA yield was at 24 h.

\[
FA \text{ yield} = 0.22 + 2.41 \times 10^{-3}A - 0.026A^2 \quad (eq. 1)
\]

where, \(A\) is storage time

Figure 2 shows the effect of EBJ storage time on FA yield. The plot shows approximately symmetrical in shape. It shows clear peak, implying that the optimum condition for maximum value of FA yield were attributed by the storage time. Besides, this plot also indicates the maximum point for FA yield is within the experimental region.

The FA yield is affected by the storage condition of EBJ, as shown in Figure 2. The figure shows an increasing FA yield within storage time of EBJ which peaking at 24 h (24 h was encoded as 0.00) with 0.2162 mg/g. Similar to result from OFAT analysis as shown as Figure 1; the
one factor plot proved that longer storage time of EBJ increased the FA yield. However, FA yield decreased when the storage time exceed 24 h. According to \(t\), both temperature and storage time influenced the amount of phenolic compounds in the extracted juice.

The storage condition strongly affects the phenolic content in the extracted BSW juice since it can undergo modifications during storage; due to the hydrolysis, oxidation and complexations. According to the software shown in Table 2, the suggested optimum value for storage time was encoded at 0.05 which in real value is 24.1 hr with the predicted FA yield at 0.2161 mg/g. However, from the experiment, the FA yield was higher than that the predicted (0.2274 mg/g) with the same storage time. Therefore, the selected optimum condition for storage time is 24 h.

![One Factor Plot](image)

Figure 2. Response surface plot of the interaction between pre-treatment temperature (A) and storage time of EBJ (B) and their interaction on FA yield.

<table>
<thead>
<tr>
<th>Name</th>
<th>Goal</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Suggested optimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage time</td>
<td>Is in range</td>
<td>-2</td>
<td>2</td>
<td>0.05</td>
</tr>
<tr>
<td>Ferulic acid yield</td>
<td>maximize</td>
<td>0.1101</td>
<td>0.2274</td>
<td>0.2162</td>
</tr>
</tbody>
</table>

**Table 2. Summary for optimization using RSM (Design Expert software)**

4. **Conclusion**

Longer storage time does increased the FA yield up until maximum point before decreasing, once stored more than 24 h. The maximum FA yield predicted by RSM was 0.2162 mg/g with storage time of 24.1 h. However, the real value from experimental shows higher yields of 0.2274 mg/g with the same storage time. Results from OFAT and RSM do agreed with one another. The International Conference on Fluids and Chemical Engineering (FluidsChE 2017) is the second in series with complete information on the official website and organised by The Center of Excellence for Advanced Research in Fluid Flow (CARIFF). The publications on products from natural resources, polymer technology, and pharmaceutical technology have been published as a special note in volume 224. The conference host being University Malaysia Pahang is the parent governing body.

5. **Acknowledgement**

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6. **References**

7. Shashank K, Abhay KP. Chemistry and biological activities