Biochemical changes of kemiri sunan \textit{[Reutealis trisperma (Blanco) Airy Shaw]} kernels at eight levels of fruit storage duration

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Abstract. Kemiri Sunan \textit{[Reutealis trisperma (Blanco) Airy Shaw]} fruit harvest is carried out according to physiological maturity, indicated by the fruit's natural fall from the tree. The fruit collection period can take up to several days according to the processing mill's minimum capacity. Constraints like this result in fruit collected at an earlier harvest cycle that will undergo a storage process for up to several days. The study was conducted at Pakuwon Experimental Station and Integrated Laboratory, Balittri, Sukabumi, West Java. This study investigates the biochemical changes of Kemiri sunan kernels at eight levels of fruit storage duration. The design used was completely randomized with eight treatments and three replications. The eight treatments were the fruit storage duration: 0 days (without storage) and storage for 2, 4, 6, 8, 10, 12, and 14 days. The variables observed were the kernel’s moisture, ash, oil, protein, peroxide, and free fatty acids (FFA) content. The results showed that fruit storage decreased moisture and oil content but increased FFA content. Storage of fruit up to a maximum of 8 days does not change the kernels' biochemistry, indicating that the fruit is still suitable for further processing.

Keywords: biofuel plants, biochemical properties, harvest cycle

1. Introduction

Today, many nations across the globe are focused on alternative energy sources to address the main problems of rapidly declining oil supplies and negative greenhouse gas emission impacts. However, oil remained its primary source with 96%, accounting for roughly 27.8% of the total global energy consumption for 2016. In the transport industry, the world's ultimate consumption of liquid fuels is expected to account for around 55% by 2040. The top consumer share from 2015 thus remains unchanged [1]. Final energy demand in Indonesia also dominated the industrial and transportation sector in 2018. Energy demand in the transportation sector is influenced by the growth of motorized vehicles [2]. Therefore, discovering an appropriate natural resource that is used as an alternative fuel is important. Appropriate natural resources considered as potential feedstock primarily consist of carbohydrate, lipid, or lignin [3]. Because of their high oil content, lipid-based plant oils are the primary source of biofuel production.

Many investigations have taken user of the feasibility of lipid-based plant oil for biofuels, such as coconut (\textit{Cocos nucifera}), corn (\textit{Zea mays}), oil palm (\textit{Elaeis guineensis}), rapeseed (\textit{Brassica napus}), soybean (\textit{Glycine max}), and sunflower (\textit{Helianthus annuus}) [4–8]. However, given the enormous need for edible oil as food, Kemiri Sunan is believed to be the most excellent source of biodiesel because of
its many benefits. Kemiri sunan [Reutealis trisperma (Blanco) Airy Shaw] is one of Indonesia’s potential lipid-based plants for biofuel. The advantage of the Kemiri sunan plant are as follows: (a) high oil content with more than 50% yield oil [9], (b) this plant categorize as a conservation plant in marginal or degraded land [9], and (c) due to poisonous content in the oil, this categorize as inedible oil [10].

Kemiri sunan oil must be preserved to maintain its quality. Therefore, the storage conditions are crucial in influencing the characteristics of the kemiri sunan oil as oils deteriorate over time. The amount of free fatty acid (FFA) in oil is one of the main aspects of feed selection for biodiesel production. When high FFA oil for the production of biodiesel is utilized, it produces low yields and soap. For this reason, low FFA oil is frequently favored for direct use during biodiesel synthesis in the transesterification process. The storage duration of the fruits is among other variables leading to the increase in FFA.

Fresh kemiri sunan oil has a low content of free fatty acid favorable for biodiesel production; however, high content of oleic unsaturated and linoleic acids in Kemiri sunan oil tends to be oxidized under certain conditions circumstances of storage [11]. Most oil-producing plants such as kemiri sunan, palm, jatropha, and Pongamia oil contain lipase enzymes that increase FFA by catalyzing the hydrolysis process during storage.

Storing kemiri sunan fruit can occur because the number of ripe fruit that falls naturally is limited, while the processing mill has a minimum capacity to carry out the processing. Therefore, the kemiri sunan fruits collected at an earlier harvest cycle will be stored for several days. Moreover, if the location of the production garden is very far from the processing mill, it requires sufficient time for distribution. Therefore, this study aimed to investigate the biochemical change of kemiri sunan kernels at eight levels of fruit storage duration.

2. Methods
2.1. Collection of kemiri sunan fruit
Kemiri sunan fruit collected from Pakuwon Experimental Staton, Balittri, Sukabumi. The physiologically ripe fruit has been taken by the fall of the candlenut fruit from the tree. Storing the Kemiri sunan fruit conducted at room temperature for 14 days, and samples were taken every two days according to the treatment to analyze the moisture, oil, and FFA content.

2.2. Measuring the proximate properties of kemiri sunan kernel
Measuring of the kemiri sunan moisture content following SNI 01-3555-1994. The oil extraction was carried out using the Soxhlet extraction system (BUCHI) with hexane solvent based on AOAC [12]. Determining of the protein content using the Kjeldahl Method [13]. Ash content analysis by furnace based on AOAC [12].

2.3. Measuring the peroxide and chemical properties of kemiri sunan kernel
Kemiri sunan oil used for peroxide and acid value were isolated from the kernel using the Soxhlet extraction system (BUCHI) with hexane solvent. The peroxide value was measured according to SNI 01-3555-1994. A potassium hydroxide solution of 0.1 mol L⁻¹ was made by mixing 5.61 g of KOH with 1 L of ethanol. Free fatty acid values were determined according to the method prescribed by SNI 01-3555-1998. The sample was weighed as much as 5 grams in an Erlenmeyer flask and added 50 ml of 95% neutral alcohol and two drops of phenolphthalein. The solution is titrated using 0.1 N NaOH while being shaken constantly until a faint pink color change occurs, which lasts for 15 seconds. FFA concentration in kernel oil was determined as the oleic acid percentage according to the following formula:

\[
\text{Acid value} = \frac{A \times N \times 56.1}{G}
\]

Where:
A (ml) = the sample volume of Potassium hydroxide standard titrant solution consumed.
N (mol L⁻¹) = represents the Potassium hydroxide standard titrant solution concentration.
G (g) = the sample size.
2.4. Experimental design and data analysis

The experiment was designed in a completely randomized design (CRD) with eight treatments of fruit storage duration and three replications. The eight treatments were the fruit storage duration: 0 days (without storage as control) and storage for 2, 4, 6, 8, 10, 12, and 14 days. The variables observed included the moisture, ash, oil, protein, peroxide, and FFA content of the kernel. The data that has been collected is then analyzed by analysis of variance (ANOVA), and if the results of the F test are significant, then it is continued with the Tukey test. In addition, a regression analysis was also conducted between fruit storage duration and variables that were considered significant based on the ANOVA results.

3. Results and discussion

3.1. Analysis of variance

The analysis of variance showed that the storage duration of the fruit had a significant effect on the moisture content, as well as the oil and FFA content of the kernel. Meanwhile, the fruit storage duration did not affect the ash, protein, and peroxide content of the kernel (Table 1).

Table 1. A probability value of variance analysis result for seven variables of kemiri sunan fruit and kernel.

| Treatments       | Kernel biochemistry |
|------------------|---------------------|
|                  | Moisture content    | Ash content     | Oil content | Protein content | Peroxide level | Free fatty acid (FFA) content |
| Storage duration | 0.011*              | 0.872 ns        | 0.002**     | 0.444 ns        | 0.069 ns        | 0.000**                     |

Note: ns = not significant; * and ** = significant at 5% and 1% level respectively

3.2. Effect of fruit storage duration on the moisture content of Kemiri Sunan kernel

Initially, Kemiri sunan kernel had a moisture content of 48.98%. The effect of fruit storage duration on moisture content is presented in Figure 1. According to Figure 1, the moisture content in the Kemiri sunan kernel was decreased during the storage. The moisture content has been decreased with extended storage time. However, after 12 days, the moisture content in Kemiri sunan was not significantly different from the initial storage period. After 14 days of storage, moisture contents were significantly different from those at the beginning of storage up to 12 days of storage. Based on the regression analysis, moisture content was linearly decreased (0.72% day⁻¹) with increasing storage duration (Figure 2).

![Figure 1](image-url)  
Notes: numbers followed by the same letter are not significantly different according to Tukey's test at 5% level

Figure 1. The effect of fruit storage duration on the moisture content of Kemiri sunan kernel.
3.3. Effects of fruit storage duration on the chemical properties of Kemiri sunan kernel

The quality of kemiri sunan oils has been assessed for their acidity and oxidation product concentration. From the result of fourteen days of storage, it can be concluded that oil content in kemiri sunan has a substantial influence by the storage period. For the oil and FFA content, fruit storage duration up to a maximum of 8 days did not show any significant difference with fruit without storage (control) (Table 2).

Table 2. The effect of fruit storage duration on ash, oil, protein, peroxide, and free fatty acids (FFA) content of kemiri sunan kernel.

| Storage duration (days) | Ash content (%) | Oil content (%) | Protein content (%) | Peroxide level (%) | Free fatty acid (FFA) content (%) |
|------------------------|----------------|----------------|--------------------|--------------------|----------------------------------|
| 0 (control)            | 7.58 a          | 53.43 ab       | 13.52 a            | 6.33 a             | 3.11 b                           |
| 2                      | 11.10 a         | 54.94 a        | 14.69 a            | 7.10 a             | 5.74 b                           |
| 4                      | 8.74 a          | 50.24 abc      | 15.00 a            | 8.95 a             | 8.76 b                           |
| 6                      | 12.24 a         | 43.71 abc      | 16.38 a            | 8.04 a             | 12.45 b                          |
| 8                      | 7.59 a          | 47.71 abc      | 13.14 a            | 8.44 a             | 9.75 b                           |
| 10                     | 8.62 a          | 40.27 bc       | 13.16 a            | 6.92 a             | 34.21 a                          |
| 12                     | 10.15 a         | 36.17 c        | 14.95 a            | 4.79 a             | 27.64 a                          |
| 14                     | 10.13 a         | 40.08 bc       | 13.92 a            | 6.89 a             | 34.84 a                          |

Note: Numbers followed by the same letter in the same column are not significantly different according to Tukey’s test at 5% level.

Results from the fourteen days of the storage period, oil content of kemiri sunan show a significant effect of storage time on kemiri sunan kernel. The oil content of kemiri sunan kernel decreased from 53.3% in fresh condition and became 40.0% oil after 14 days of storage (1.31% day⁻¹) (Figure 3). The reduction in fat content identical to oil also occurred in the storage process of sunflower [13], kambakam (Hopea ponga) [14], oats (Avena sativa) [15], pine [16], soybeans [17], peanuts [18], and cacao [19] seeds.

Free fatty acids (FFA) are a parameter that must be considered in determining the quality of vegetable oil. The presence of FFA in oil is due to the hydrolysis of the triacylglycerols. Temperature promotes this critical response and mostly happens in damaged seeds by lipases when water is present. In order to create fatty acid methyl ester, the free fatty acid has a significant impact on the transesterification of oils with an alcohol (methanol or ethanol) to produce fatty acid methyl (ethyl) esters using an alkaline catalyst. FFA reacts with the catalyst to generate soaps and impedes the separation of the product from the glycerol because viscosity or gels are formed, and the output and transesterification rates are reduced [20]. The increase in FFA due to the fat hydrolysis as a result of the storage process also occurred in studies of the storage of oats [15], pine [16], and soybeans [17] seeds.
Figure 3. Regression analysis of fruit storage duration on the oil content of kemiri sunan kernel (Y = 54.20 – 1.31X; R² = 0.61; p<0.01).

Figure 4. Regression analysis of fruit storage duration on the free fatty acid (FFA) content of Kemiri sunan kernel (Y = 0.18 + 2.41X; R² = 0.77; p<0.01).

Based on Figure 4, it can be seen that there is a trend of increasing FFA levels of kemiri sunan oil along with storage duration with an increased rate of 2.41% day⁻¹. In other words, the duration can decrease the quality of kemiri sunan oil, indicating an increasing level of FFA.

4. Conclusion
The storage of kemiri sunan fruit can reduce the moisture content (0.72% day⁻¹) and oil content (1.31% day⁻¹), but the free fatty acid (FFA) content increases (2.41% day⁻¹). Therefore, the oil should be extracted immediately to prevent further deterioration in quality. However, storage of fruit up to a maximum of 8 days does not affect the biochemical changes in the kernels, so the kernels can be further processed to produce biodiesel based on Kemiri sunan.

Authors Contribution
S Virgian (main contributor); A Aunillah (main contributor); D Pranowo (main contributor); D Listyati (member contributor), M Herman (member contributor), and E Wardiana (member contributor).

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