DEMETER: Journal of Farming and Agriculture
Vol 1, Issue 1, July 2023, Page 23-29
ISSN: 2987-5889 (Media Online)
DOI: https://doi.org/10.58905/demeter.v1i1.106

# Nutrition, Antioxidant And Organoleptic Activities Of Legen Drink (Borassus flabellifer L.) at Different Temperature And Storage Time

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**Abstract.** Siwalan sap (*Borassus flabellifer* L.) is a liquid that comes out of the filter vessels as a result of cob tapping (bunches), both male and female flowers that have a sweet taste. Nira containing high sugar content, and nira ca experience the process of fermentation assisted by microbial activity. This study aims to determine the levels of reducing sugars, pH, total acids and antioxidant activity contained in legen drinks. In addition, to find out the best treatment related to the best temperature and storage time. This study uses a laboratory experimental quantitative analysis method. The research variables included temperature consisting of freezer temperature, refrigerator temperature, room temperature and storage period consisting of 12 hour, 24 hour and 34 hour. The data obtained were analysed based on parametric statistics using analysis of variance (ANOVA) using statistical product and service solution (SPPSS) version 24 and determining the best treatment using effectiveness tests. Temperature and long storage different influential very real to sugar reduction, pH, total acid and antioxidant activity. Interaction between temperature and different storage times had a significant effect on reducing sugars and had a very significant effect on pH, total acid and antioxidant activity. Based on the determination of the effectiveness test, it shows that the temperature $0^0$C and long storage 24 hour and the best treatment with a yield value (NH) of 0,69 with the parameter is sugar reduction 3,98%, pH levels 2,57, total acid 3,83%, antioxidant activity ($IC_{50}$) 12,24 g/ml, color 4,67 (neutral). Aroma 5,4 (like) and taste 5,16 (like).

**Keywords:** Antioxidant, Fermentation, Legen Drink, Nutrition

## 1 Introduction

Indonesia as a country that has the third largest tropical rainforest in the world after Brazil and Zaire, is very rich in biodiversity which is very prospective for development [1], one of which is the lontar or siwalan plant (*Borassus flabellifer* L.). Siwalan is a palm plant that has multi-purpose properties, because from the leaves, stems, fruit, to the cobs, the flowers can be processed into products [2].

Siwalan sap (*Borassus flabellifer* L.) is a liquid that comes out of tapped sieve tubes, both male and female flowers which have a sweet taste. This liquid is usually used to make legen drinks (from the Javanese word legi which means sweet). In general, people consume siwalan sap in a fresh state, which has a distinctive and sweet smell. Fresh sap has a sugar content of >12%, alcohol content <5% and a pH of around 5-6 which means sour. The high sugar content of the sap makes the sap easy to undergo a fermentation process assisted by microbial activity, resulting in alcohol which turns into acid over time [3]. If the fermentation continues, the sap can turn into wine with a slightly bitter taste and can be intoxicating [4].

If fresh sap is stored at room temperature 26$^o$C for 28 hours, 4.3586% alcohol will be formed [5], if preservatives are not added that can inhibit microbial growth, the alcohol content can reach 7% in just 15-20 hours . The process of processing sap into legen is through a fermentation process which is a process of breaking down organic substrates through a metabolic process [2]. According to [6], sugar fermentation in sap is caused by microbial growth originating from dirty environmental conditions such as air, roofs where tapping or other contaminants can contaminate sap during the tapping process. Based on the description above, this study aims to determine the temperature and storage time of legen with the best nutrition, antioxidant and organoleptic activity.

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# 2 Research Method

## 2.1 Material

The main ingredient used in this study was a legen drink which was obtained from Tuban Regency through street vendors in Gedangan District, Sidoarjo Regency. The chemicals used for analysis included Luff Schoorl solution, 0.1 N $Na_2S_2O_3$ (Na-thiosulphate) solution, 20% $H_2SO_4$ solution, 1% starch indicator, 20% KI solution, 4 N HCl solution, 50% NaOH solution, PP indicators, distilled water, buffer, 0.1 N NaOH, 1,1-diphenyl-2-picrylhydrazil/DPPH, 96% ethanol, ethanol pro analysis, and methanol pro analysis.

## 2.2 Tools

The equipment used consisted of an Erlenmeyer flask, burette, filter paper, pipette, pH meter, 100 ml measuring flask, Erlenmeyer flask, analytical balance, 300 ml beaker, spatula, filter paper, digital balance, 30 ml vial, and visible spectrophotometer.

## 2.3 Experimental Design

This study used an experimental design in the form of a completely randomized design (CRD) arranged in factorial/2 factors and each factor consisted of three and two levels. The first factorial is storage temperature (C) consisting of freezer temperature, refrigerator temperature, room temperature and the second factorial is storage time (Y), namely storage time of 12 hours, 24 hours and 36 hours. Each treatment combination was repeated three times so that there were 18 treatments.

## 2.4 Chemical Analysis and Organoleptic Test

The chemical parameters measured in this study included chemical and organoleptic tests. Chemical parameters include reducing sugars [7], pH [8], total acid test [8] and antioxidant tests [9]. Organoleptic tests included color, aroma and taste using 25 panelists with a level of preference scale, namely 1 = really dislike, 2 = dislike, 3 = somewhat dislike, 4 = neutral, 5 = rather like, 6 = like, 7 = like very much .

## 2.5 Data Analysis

The parametric data (total phenol levels, antioxidant activity and vitamin C) obtained were analyzed using analysis of variance (ANOVA) using the SPSS application. If the results of the analysis between treatments show significant or very significant differences, then a follow-up test is carried out with the Least Significant Difference (LSD) test if the Coefficient of Diversity/KK value is below 5% or can be carried out with the Honest Significant Difference test/BNJ if the CC value is 5-10%. or by Duncan's test if the KK value is above 10% [10].

Analysis of non-parametric data obtained from organoleptic tests including color, aroma, and taste were analyzed based on the hedonic test. Then to find out there were differences between treatments, the Kruskal Wallis test was carried out (Ayustaningwarno, 2014), from these two parameters an Effectiveness test was carried out to determine the best treatment [11].

ANOVA results show that the interaction between temperature and storage time has a significant effect on the reducing sugar content of legen drink. It can be seen that the C1Y1 treatment (0$^o$C temperature and 12 hours of storage time) had the highest reducing sugar content of 4.07%, while the lowest reducing sugar content was found in the C3Y3 treatment with an average of 0.38%.

The results showed that the tendency for higher temperatures and longer storage times resulted in lower reducing sugar levels. This is in accordance with research conducted by [12], temperature affects the speed of sugar reduction. The higher the temperature given will also affect the process of increasing sugar reduction. Reducing sugar is formed due to an inversion process, one of which is influenced by temperature and storage time. Table 1 shows that at 0$^o$C a storage time of 12 hours produced a higher reducing sugar content than at 5$^o$C and 28$^o$C with a storage time of 24 and 36 hours.

# 3 Results and Discussion

## 3.1 Reducing Sugar Levels

The results of the research on the average reducing sugar levels in legen drinks can be seen in Table 1 below.

**Table 1.** Average levels of reducing sugar in legen drinks

| Treatment Code | Treatment | The average of reducing sugar levels (%) |
|---|---|---|
| C3Y3 | Temperature 28$^o$C : Storage Time 36 hour | 0,38a |
| C3Y2 | Temperature 28$^o$C : Storage Time 24 hour | 0,71ab |

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| Treatment Code | Treatment | The average of reducing sugar levels (%) |
|---|---|---|
| C3Y1 | Temperature 28ºC : Storage Time 12 hour | 1,27b |
| C2Y3 | Temperature 5ºC : Storage Time 36 hour | 1,90c |
| C2Y2 | Temperature 5ºC : Storage Time 24 hour | 2,67d |
| C2Y1 | Temperature 5ºC : Storage Time 12 hour | 3,15de |
| C1Y3 | Temperature 0ºC : Storage Time 36 hour | 3,69ef |
| C1Y2 | Temperature 0 ºC : Storage Time 24 hour | 3,98f |
| C1Y1 | Temperature 0 ºC : Storage Time 12 hour | 4,07f |
| KK = 8,26 % (BNJ) | | |

Note: The letter behind the number with the same notation on the mean indicates no difference in the 5% BNJ test.

## 3.2 pH

The results of the research on the average pH of legen drinks can be seen in Table 2 below.

**Table 2.** Average pH of legen drinks

| Treatment Code | Treatment | pH Average |
|---|---|---|
| C3Y3 | Temperature 28ºC : Storage Time 36 hour | 1,47a |
| C3Y2 | Temperature 28ºC : Storage Time 24 hour | 1,56ab |
| C3Y1 | Temperature 28ºC : Storage Time 12 hour | 1,63bc |
| C2Y3 | Temperature 5ºC : Storage Time 36 hour | 1,71c |
| C2Y2 | Temperature 5ºC : Storage Time 24 hour | 1,83d |
| C2Y1 | Temperature 5ºC : Storage Time 12 hour | 2,17e |
| C1Y3 | Temperature 0ºC : Storage Time 36 hour | 2,41f |
| C1Y2 | Temperature 0ºC : Storage Time 24 hour | 2,57g |
| C1Y1 | Temperature 0ºC : Storage Time 12 hour | 2,76h |
| KK = 37 % (DUNCAN) | | |

Note: The letter after the number with the same notation on the mean indicates no difference in the 5% DUNCAN test.

Based on the results of ANOVA, it was shown that temperature, storage time and the interaction between different temperature and storage time showed a very significant effect on the pH level of the legen drink. It can be seen that the C1Y1 treatment (0oC temperature and 12 hours storage time) had the highest pH of 2.76, while the lowest pH was in the C3Y3 treatment with an average of 1.47. The higher the temperature and the longer the storage of legen drinks, the lower the pH level.

Changes in the pH value are one result of the fermentation process that occurs due to the accumulation of acids, this is in accordance with research conducted by [13]. Storage with a fairly high temperature (28°C) and long storage (36 hours) causes a very rapid breakdown of acids which causes the pH to decrease. The results of the pH value of the storage time were obtained the longer the storage of sap caused the pH to decrease, resulting in a fermentation process. According to [14], microbes in siwalan sap can grow well at pH 3-6, during the fermentation process it can occur at an optimum pH of 4.3 – 4.7. If the pH is below 3 then the fermentation process will run slowly whereas if the pH is too high it will cause lower microbial adaptation but the fermentation activity increases.

## 3.3 Total Acid Content

The results of the study on the average total acid content of legen drinks can be seen in Table 3 below.

**Table 3.** The average total acid content of legen drinks

| Treatment Code | Treatment | Average total acid levels (%) |
|---|---|---|
| C1Y1 | Temperature 0ºC : Storage Time 12 hour | 3,81a |
| C1Y2 | Temperature 0ºC : Storage Time 24 hour | 3,83a |
| C1Y3 | Temperature 0ºC : Storage Time 36 hour | 3,95b |
| C2Y1 | Temperature 5ºC : Storage Time 12 hour | 4,08c |
| C2Y2 | Temperature 5ºC : Storage Time 24 hour | 4,26d |
| C2Y3 | Temperature 5ºC : Storage Time 36 hour | 4,39e |
| C3Y1 | Temperature 28ºC : Storage Time 12 hour | 4,73f |
| C3Y2 | Temperature 28 ºC : Storage Time 24 hour | 5,13g |

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| Treatment Code | Treatment | Average total acid levels (%) |
|---|---|---|
| C3Y3 | Temperature 28ºC : Storage Time 36 hour | 5,81h |
| KK = 11,50 % (DUNCAN) | | |

Note: The letter after the number with the same notation on the mean indicates no difference in the 5% DUNCAN test.

Based on the results of ANOVA, it was shown that temperature, storage time and the interaction between different temperature and storage time showed a very significant effect on the total legen acid content. It can be seen that the C3Y3 treatment (temperature 28oC and storage time 36 hours) had the highest total acid content, namely 5.67%, while the lowest total acid content was found in the C1Y1 treatment with an average of 3.81%. The higher the temperature and the longer the legen drink is stored, the lower the pH level, which means it is more acidic.

The results showed that the lower the temperature and the shorter the storage time, the lower the total acid would be. This is in accordance with research conducted by [8] that the high average value of total acid is due to the sugar content in the raw material (siwalan sap) if stored for too long it will become lactic acid. Table 3 shows that a temperature of 0oC with a storage time of 12 hours resulted in lower total acid than at 5oC and 28oC with a storage time of 24 and 36 hours.

## 3.4 Antioxidant Activity

The results of the study on the average antioxidant activity of legen drinks can be seen in Table 4 below.

**Table 4.** Average antioxidant activity /IC50 of legen drinks

| Treatment Code | Treatment | IC50 average (µg/mL) |
|---|---|---|
| C1Y1 | Temperature 28ºC : Storage Time 36 hour | 11,37a |
| C1Y2 | Temperature 28ºC : Storage Time 24 hour | 12,24a |
| C1Y3 | Temperature 28ºC : Storage Time 12 hour | 13,96b |
| C2Y1 | Temperature 5ºC : Storage Time 36 hour | 14,51b |
| C2Y2 | Temperature 5ºC : Storage Time 24 hour | 15,08b |
| C2Y3 | Temperature 5ºC : Storage Time 12 hour | 15,56b |
| C3Y1 | Temperature 0ºC : Storage Time 36 hour | 18,85c |
| C3Y2 | Temperature 0ºC : Storage Time 24 hour | 21,16d |
| C3Y3 | Temperature 0ºC : Storage Time 12 hour | 22,92e |
| KK = 12,138 % (DUNCAN) | | |

Note: The letter after the number with the same notation on the mean indicates no difference in the 5% DUNCAN test.

Based on the ANOVA results, it was shown that the different types of temperature and storage time interactions had a very significant effect on the antioxidant levels of legen drink. The lowest IC50 value of 11.37 µg/mL was found in the C1Y1 treatment, in this treatment the temperature and storage time were the lowest, namely 00C with 12 hours of storage. While the highest IC50 level was in C3Y3 (28oC temperature with 36 hours of storage time) with an IC50 value of 22.92 µg/mL. At temperatures of 00C, 50C and 280C with longer storage times (12, 24 and 36 hours), the IC50 values were lower.

The antioxidant activity in legen drinks the higher the IC50 level produced means that the antioxidant activity decreases. The IC50 category resulting from this study is included in the very strong category because the IC50 value is <50 µg/mL. This is in accordance with the opinion of [15], the longer the storage time, the lower the antioxidant activity, this is because the longer it is stored the more contact time with oxygen.

## 3.5 Organoleptic Test

### 1 Color

The results of the color preference test for the legen drink showed that the temperature of 0ºC and the storage time of 36 hours gave the highest color preference value, which was 5.34, which means that the panelists liked the color of the legen drink. The histogram of the average color of the legendary drink can be seen in Figure 1 below

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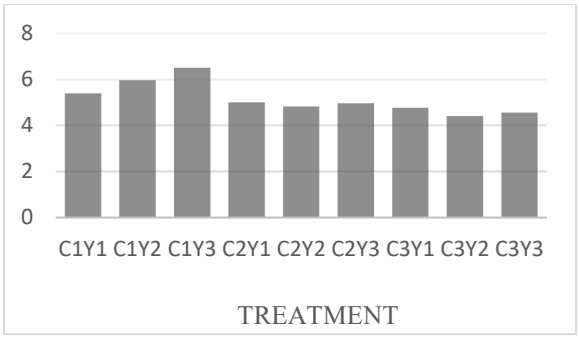


**Figure 1.** The color histogram of the legendary drink

The histogram above shows that the temperature treatment at 0oC and the storage time of 36 hours had the highest color value, namely 5.34, which means that the panelists considered the legendary drink to be quite favorable. Based on the results of the Kruskal Wallis color test, it was found that $p = 0.001 \leq \alpha = 0.05$ indicating a significant difference between each treatment, meaning that temperature and storage time greatly influenced the level of panelist acceptance of legen drink color parameters. Nira damaged marked by a sour taste, frothy and slimy. The formation of froth and mucus causes the clarity of the sap to decrease [4]. Chemically, color changes can be caused by changes in pH or oxidation processes during storage, this affects the color which causes a decrease in sensory values.

## 2 Aroma

The results of the preference test for the aroma of the legen drink showed that the temperature of 0ºC and the storage time of 34 hours gave the highest preference value for the aroma, which was 6.51, which means that the aroma of the legen drink was rated as liked by the panelists. The average aroma of legend drinks can be seen in Figure 2 below.

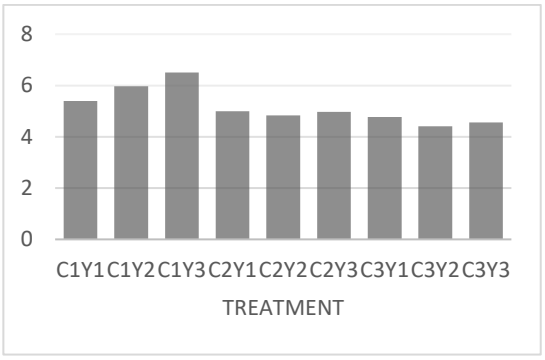


**Figure 2**. Histogram of the aroma of legendary drinks

Figure 2 above shows that the temperature treatment of 0 ºC with a storage time of 12 hours has the highest value of 6.51, which means that the aroma of the legendary drink is rated as liked by the panelists. The panelists' preference level for the aroma of the legendary drink ranged from 4.41 (somewhat like) to 6.51 (very like). Based on the results of Kruskal Wallis aroma, it was found that $p = 0.001 \leq \alpha$ 0.05, showing significantly different values for each treatment, meaning that temperature and storage time greatly influenced the level of panelist acceptance of the parameters of legen's aroma. The aroma of legen drinks is influenced by yeast activity so that a sour aroma appears [14].

## 3 Flavor

The results of the preference test for the taste of the legen drink showed that the temperature of 0 ºC and the storage time of 36 hours gave the highest preference value for the taste of 6.2, which means the panelists liked the taste of the legen drink. The average taste of legendary drinks can be seen in Figure 3 below.

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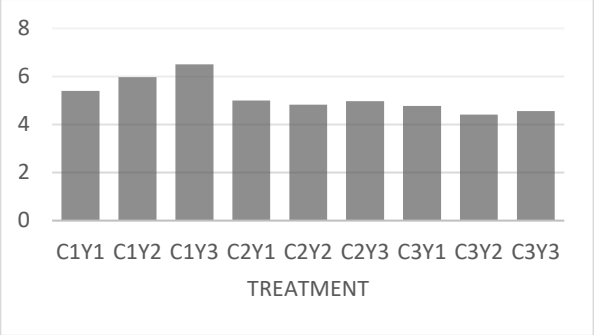


**Figure 3.** Histogram of legendary drink taste

The histogram above shows that the temperature treatment at 0 ºC and the storage time of 36 hours has the highest color value, namely 6.20, which means that the panelists like the legen drink. The lowest value was obtained at room temperature storage which speeds up the fermentation process so that the taste of the legen drink is still sour so that many panelists don't like it [16]. Based on the results of the Kruskal Wallis taste test, it was found that $p = 0.001 \leq \alpha = 0.05$ showed a significant difference between each treatment, meaning that temperature and storage time greatly influenced the level of panelist acceptance of the legen drink taste parameters.

### 3.6 Effectiveness Test

The effectiveness test was carried out to find out the best or most preferred treatment. Based on the effectiveness test results on all research parameters which included chemical tests and organoleptic tests, it was shown that different temperatures and storage times were the best treatment because they had the highest (NH) value. The average NH of all parameters of the effectiveness test research can be seen in Table 5 below.

**Table 5**. The value of the results of the research variable effectiveness test

| Treatment | Result Value (NH) Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | C1Y1 | C1Y2 | C1Y3 | C2Y1 | C2Y2 | C2Y3 | C3Y1 | C3Y2 | C3Y3 |
| Reducing sugar | 0,15 | 0,15 | 0,14 | 0,12 | 0,09 | 0,06 | 0,04 | 0,01 | 0 |
| pH | 0,14 | 0,12 | 0,09 | 0,07 | 0,04 | 0,02 | 0,01 | 0,01 | 0 |
| Total acid | 0 | 0,01 | 0,01 | 0,05 | 0,04 | 0,05 | 0,08 | 0,11 | 0,16 |
| Antioxidant | 0 | 0,18 | 0,03 | 0,05 | 0,06 | 0,01 | 0,13 | 0,15 | 0 |
| Color | 0,14 | 0,07 | 0,11 | 0,19 | 0,04 | 0,03 | 0,01 | 0 | 0 |
| Aroma | 0,06 | 0,08 | 0,12 | 0,03 | 0,03 | 0,02 | 0,02 | 0 | 0 |
| Flavor | 0,06 | 0,08 | 0,12 | 0,03 | 0,04 | 0,02 | 0,01 | 0 | 0 |
| Total | 0,55 | **0,69*** | 0,62 | 0,54 | 0,34 | 0,31 | 0,31 | 0,28 | 0,16 |

Note: * = Best treatment

Based on the effectiveness test on all research parameters showed that the storage temperature was 0 ºC and the storage time was 24 hours and was the best treatment with a Yield Value (NH) of 0.69 with parameter criteria being reducing sugar content 3.98%, pH level 2.57, total acid 3.83%, antioxidant activity level ($IC_{50}$) 12.24 µg/mL, color 4.67 (rather like), aroma 5.4 (like) and taste 5.16 (like).

## 4 Conclusion

The results of the study proved that storage temperature of 0ºC and storage time of 24 hours was the best treatment with a Yield Value (NH) of 0.69 with parameter criteria being reducing sugar content 3.98%, pH level 2.57, total acid content 3.83% , antioxidant activity level ($IC_{50}$) 12.24 µg/mL, color 4.67 (rather like), aroma 5.4 (like) and taste 5.16 (like).

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