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Comparison of Vitamin B1 (*Thiamin hydrochloride*) Level in Brown Rice (*Oryza nivara* S.D.Sharma & Shastry) and Cooked Brown Rice by Alkalimetry Methods

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Abstract: Brown rice (*Oryza nivara* S.D.Sharma & Shastry) is a rice variety that belongs to the Graminae family. Brown rice contains vitamins A, B, C, Zn and B complex. Vitamin B1 relatively unstable compared to other vitamins, which its instability is influenced by pH, temperature and processing. The purpose of this study was to determine the comparison of vitamin B1 levels in brown rice and cooked brown rice. This comparative study of vitamin B1 is important in order to provide information to the public about the correct procedures for processing food containing vitamin B1, such as brown rice. The study began with a qualitative test of vitamin B1 using 10% Pb acetate and 6 N NaOH if a yellow color and brown precipitate formed after heating, the sample was positive for vitamin B1. Determination of vitamin B1 levels in brown rice and cooked brown rice by alkalimetric method using NaOH as a titer that has been standardized in advance with potassium biftalat 0.1 N. Data analysis using the Mann Whitney test is an alternative to the Independent T-test if the t-test requirements are not met. The Mann Whitney test is used to determine whether or not there is a difference between two independent samples. The results of the qualitative test of vitamin B1 in brown rice and cooked brown rice showed that the samples were positive for vitamin B1. The quantitative test results of vitamin B1 levels in brown rice and cooked brown rice obtained an average of 12.40 mg / kg and 4.96 mg / kg. Statistical test results, the significance value (p) = 0.043, where $p < 0.05$ means there is a significant difference in vitamin B1 levels in brown rice and cooked brown rice. The conclusion of this study is that vitamin B1 levels in brown rice are higher than vitamin B1 levels in cooked brown rice.

Keywords: brown rice; cooked brown rice; vitamin B1; titration; alkalimetry.

1. INTRODUCTION

Rice is part of the grain that has been separated from the husk. The husk (*Java merang*) is anatomically referred to as palea (the part that is covered) and lemma (the part that covers). In one of the stages of processing the rice harvest, the grain is milled so that the outer part (husk) is separated from the contents. This white, reddish, purple, or even black content is called rice [1].

Brown rice is a widely grown cereal found in South Asia, Italy, Greece, and the United States. Brown rice (*Oryza nivara*) is one of the rice varieties included in the Graminae family. Brown rice has a rough surface, firm and not fluffy [2]. Brown rice is useful as a functional food because it contains

anthocyanins that have the ability as antioxidants, anticancer [3]. Brown rice is commonly consumed by the community in the form of cooked brown rice. Cooked Brown rice is rice produced from brown rice grains that have properties that are not soft and tender, this is influenced by the amylose and amylopectin content. According to some information, brown rice contains carbohydrates, fat, fiber, folic acid, magnesium, niacin, phosphorus, protein, vitamins A, B, C, Zn and B complex.

The pure form of vitamin B1 is thiamine hydrochloride [4]. Thiamin hydrochloride is one of the vitamins needed to maintain the digestive system, prevent beriberi disease, help metabolize carbohydrates in the body and play a role in the nervous system. The need for vitamin B1 in infants 0.4-0.5 mg / day, children 0.7-1.0 mg / day, adult men 1.2-1.3 mg / day, adult women 1.0-1.3 mg / day, pregnant women 1.5 mg / day and nursing mothers 1.6 mg / day [5].

Vitamin B1 deficiency will cause polyneuritis, which is caused by energy deficiency of nerve tissue [6]. Early symptoms of vitamin B1 or thiamine hydrochloride deficiency are irritability and indigestion as a result of reduced appetite and slowed growth. Symptoms will disappear if the need for vitamin B1 is fulfilled [7].

Vitamin B1 is one type of unstable vitamin. Its stability is affected by pH, temperature and processing. Vitamin B1 is an acidic compound so it can be titrated with a standard solution that is alkaline, which can use NaOH. Washing is an important factor affecting thiamine loss in foodstuffs. In general, before brown rice is cooked, the washing and soaking process is carried out to produce clean brown rice. The washing and soaking process causes a reduction in brown rice thiamine levels which are easily soluble in water. Another factor that can affect the decrease in vitamin B1 levels in brown rice is cooking (regular cooking, high pressure cooking, and microwave cooking). Heat in a warming device is used to raise the temperature of food and functions in stimulating a chemical reaction, but the use of heat for a long time can cause a decrease in the quality of food. Storage for a long time will result in a decrease or destruction of nutritional content such as vitamin B1 in rice [8]. Based on the above, researchers are interested in knowing the difference in vitamin B1 levels in brown rice and cooked brown rice.

In this study, a qualitative test was carried out to determine the presence or absence of vitamin B1 content in brown rice and cooked brown rice using 10% pb acetate and 6 N NaOH if it turns yellow and forms a brown precipitate when heated, then brown rice and cooked brown rice are positive for vitamin B1 and can be continued with a quantitative test. To show the renewal between this research and the research that has been done before, the researcher uses the alkalimetric method to conduct quantitive tests in brown rice and cooked brown rice. This method is most often used because it is cheap, simple, and does not require sophisticated laboratory equipment. Alkalimetry is a method that can be used to determine the levels of acidic compounds, so it is used to determine the levels of vitamin B1 in brown rice and cooked brown rice. The research conducted used an indicator of bromine thymol blue solution [9].

2. MATERIALS AND METHODS

The method used is observational research method, which is research where the researcher only makes observations, without intervening in the variables to be studied [10]. The variable used is a single variable, namely vitamin B1 content in brown rice (*Oryza nivara* S.D.Sharma & Shastry) and brown rice. The population in this study was brown rice (*Oryza nivara* S.D.Sharma & Shastry) taken from Gapoktan Sidomulyo Rice Mill, Godean District, Sleman Regency, Yogyakarta Special Region.

The sample used in this study was 2 kg of brown rice (*Oryza nivara* S.D.Sharma & Shastry) taken from Gapoktan Sidomulyo Rice Mill, Godean District, Sleman Regency, Yogyakarta Special Region.

2.1. Material Collection

2.1.1. Plant Determination

Plant determination aims to establish the truth of the sample, which was used in this study. Determination of brown rice is done by matching its morphological characteristics proven in the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University, Yogyakarta.

2.1.2. Material Preparation

Brown rice (*Oryza nivara* S.D.Sharma & Shastry) and collected cooked brown rice were pulverized using a blender.

2.2. Qualitative Analysis Procedure

2.2.1. Preparation of Solution

Preparation of 10% Pb Acetate Solution : 9.5 g of clear and transparent lead (II) acetate P was weighed and dissolved in fresh water, then boiled into 100 ml of solvent [11]. CO_2 Free Water Generation: Pure water is boiled for 5-10 minutes or more using a stove, allowed to cool and should not absorb CO_2 from the air, then put into a tightly closed flask [11]. Preparation of 6 N NaOH Solution : 127,2 g NaOH was dissolved into 500 ml of CO_2-free distilled water using a beaker glass and stirred until dissolved then put into a tightly closed bottle [12].

2.2.2. Qualitative Test

The sample filtrate of 2 ml was put in a test tube and added 1 ml of 10% Pb acetate solution and 2 ml of 6 N NaOH if a yellow color and brown precipitate formed after heating, the sample was positive for vitamin B1 [13].

2.3. Quantitative Analysis Procedure

2.3.1. Preparation of Solution

Preparation of Potassium Biphthalate Primary Raw Solution : 1.012 g of potassium biphthalate powder was weighed anf dissolved with distilled water. The solution was put into a 50.0 ml volumetric flask and diluted with distilled water to the limit [14]. Preparation of 0.1 N NaOH Secondary Raw Solution : 2 g NaOH P was dissolved in 500 ml carbon dioxide-free water using a glass goblet and then cooled and filtered using filter paper and then put into a 500 ml volumetric flask [11]. Preparation of Phenolphthalein Solution : 0.25 g of phenolphthalein was weighed using an analytical balance then put into a 100 ml volumetric flask and dissolved in 60 ml of 90% ethanol. The solution was added with enough water to 100 ml [11]. Standardization of 0.1 N NaOH with 0.1 N potassium biphthalate ($C_8H_5KO_4$) : $C_8H_5KO_4$ 0.1 N solution was pipetted 5.0 ml into 250 ml erlenmeyer, then three drops of phenolphthalein indicator were added and titrated with NaOH until a change from colorless to pink occured [14]. Preparation of Bromine Thymol Blue Solution : 0.25 g of bromotimol blue P is dissolved in 100 ml of aqueous ethanol P using a glass goblet and filtered with filter paper. The solution was put into a 100 ml volumetric flask [11].

2.3.2. Sample Preparation

10 g of mashed brown rice and cooked brown rice was dissolved in 50 ml of distilled water, then stirred using spoon, continued by filtering sample with filter paper. The separated solution was stored in a glass beaker [15].

2.3.3. Determination of Vitamin B1 Level

Pipette 10 ml of each brown rice and cooked brown rice solution. Then each solution was put into a different beaker glass and 25 ml of CO_2 – free distilled water was added. The solution of brown rice and cooked brown rice is added 3 drops of bromine thymol blue indicator until there is a color change then each solution is titrated with 0.1 N NaOH solution and then calculated the level of vitamin B1 [16].

2.4. Data Analysis

Data on vitamin B1 levels in brown rice and cooked brown rice obtained were then statistically tested using the Independent T-test. Before conducting the Independent T-test statistical test, the data were tested for normality first through the Shapiro Wilk test. The Shapiro Wilk normality test is a test conducted to determine the distribution of random data in a small sample or no more than 50 samples [17]. Data is normally distributed if the significance value is > 0.05. Furthermore, the data was tested for homogeneity through the Lavene Test (homogeneity of variance). Data has a homogeneous variant if the significance value is >0.05. If the data obtained is normally distributed and the variance is homogeneous, then proceed with the Independent T-test statistical test. This Independent T-test test is used to determine the difference in mean values between two groups [18]. If there is data that is normally distributed but the variance is not homogeneous, not normally distributed and not homogeneous, then proceed with the Mann-Whitney test. The Mann-Whitney test is used to determine whether or not there is a difference between two independent samples. The Mann-Whitney test is a non-parametric test which is an alternative to the t-test (parametric test). The α value used is usually 5% (0.05).

3. RESULTS AND DISCUSSION

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.1. Results

3.1.1. Plants Determination

Plant determination was carried out to establish the correctness of the samples used for research, which was proven at the Biology Learning Laboratory, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan Yogyakarta. The result of the analysis confirmed that the plant seeds used in this study were indeed brown rice (*Oryza nivara* S.D.Sharma & Shastry).

3.1.2. Sample

The sample in this study was brown rice (*Oryza nivara* S.D.Sharma & Shastry) taken at Gapoktan Sidomulyo Rice Mill, Godean District, Sleman Regency, Yogyakarta Special Region. The brown rice samples taken were partially used to make cooked brown rice then both samples were reduced in particle size to powder.

3.1.3. Qualitative Test

The qualitative test of vitamin B1 in brown rice and cooked brown rice was carried out by adding 10% Pb acetate and 6 N NaOH. The sample is positive for vitamin B1 if a yellow color and brown precipitate are formed after heating. The results of the vitamin B1 qualitative test on brown rice and cooked brown rice samples showed that the samples were positive for vitamin B1.

Table 1. Identification of Vitamin B1 in Brown Rice and Cooked Brown Rice.

Sample	Results	Description
Brown rice	yellow color brown precipitate	+
Cooked brown rice	yellow color brown precipitate	+

Description :

- : no yellow color formed after the addition of 10% Pb acetate and brown precipitate after heating
+ : yellow color formed after the addition of 10% Pb acetate and brown precipitate after heating

3.1.4. Standardization of 0.1 N NaOH

The standardization of 0.1 N NaOH solution was titrated 3 (three) times and through the calculation of normality (N) the NaOH solution was 0.110 N.

Table 2. Standardization Results of NaOH 0.1 N.

Titration	NaOH Titrant Volume (ml)	Normality of NaOH (N)
Replication 1	4.50	0.111
Replication 2	4.60	0.109
Replication 3	4.50	0.111
Average ($\bar{x}$)	4.53	0.110

3.1.5. Determination of Vitamin B1 Level in Samples

Analysis of vitamin B1 levels in brown rice was carried out by alkalimetric titration with replication 3 times the weight of the sample in one replication was 10 grams, obtaining an average level of 12.40 mg / kg. And then analysis of vitamin B1 levels cooked brown rice obtaining an average level of 4.96 mg / kg. Precision is parameter a measure of the closeness of measurements taken repeatdly with criteria not more than 5% based on ICH. The SD value obtained on the average vitamin B1 content of brown rice and cooked brown rice is 2.147. These results fall into the criteria. Then the CV value on the average levels of vitamin B1 in brown rice and cooked brown rice is 17.31 and 43.28. These results are not included in the criteria.

Table 3. Titration Results on Brown Rice and Cooked Brown Rice Samples per 10 Grams.

Sample	Titration	NaOH titran Volume (ml)	Levels (mg/kg)	SD	CV
Brown Rice	Replication 1	0.40	14.88		
	Replication 2	0.30	11.16		
	Replication 3	0.30	11.16		
	($\bar{x}$)	0.33	12.40	2.147	17.31
Cooked Brown Rice	Replication 1	0.10	3.72		
	Replication 2	0.10	3.72		
	Replication 3	0.20	7.44		
	($\bar{x}$)	0.13	4.96	2.147	43.28

3.1.6. Comparative Analysis of Vitamin B1 Level of Brown Rice and Cooked Brown Rice Rice

Comparative analysis of vitamin B1 levels in brown rice and cooked brown rice begins with a normality test using the Shapiro Wilk test and obtained a significance value (p) = 0.000, which p < 0.05 this means that the data is not normally distributed.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Brown Rice	.385	3	.	.750	3	.000
Cooked Brown Rice	.385	3	.	.750	3	.000

a. Lilliefors Significance Correction

Figure 1. Normality Test Results

Then proceed with the homogeneity test using the Lavene Test (homogeneity of Variance) and obtained a significance value (p) = 1.000, which p > 0.05 means that the data has a homogeneous variant.

Test of Homogeneity of Variances

Vitamin B1 Levels

Levene Statistic	df1	df2	Sig.
.000	1	4	1.000

Figure 2. Homogeneity Test Results

Because the results of the normality test are not normally distributed but the variance is homogeneous, it cannot be continued with the *Independent T-test*. Comparison of vitamin B1 levels of brown rice and cooked brown rice was analyzed using the *Mann Whitney* test as an alternative to the *Independent T-test*.

From the average calculation results, it is known that the average levels of vitamin B1 in brown rice and cooked brown rice are 12.40 mg / kg and 4.96 mg / kg so that it can be interpreted that the levels of vitamin B1 in brown rice are higher than cooked brown rice. From the statistical test data, it is known that the significance value (p) = 0.043, where p < 0.05, this means that there is a significant difference in vitamin B1 levels between brown rice and cooked brown rice.

Table 4. Mann Whitney Test Results of Vitamin B1 Levels in Brown Rice and Cooked Brown Rice.

Brown Rice (Levels)	Cooked Brown Rice (Levels)	t	Sig. (2-tailed)
14.88	3.72	-2.023	0.043
11.16	3.72		
11.16	7.44		
Σ 37.20	Σ 14.88		
$\bar{x}$ 12.40	$\bar{x}$ 4.96		

3.2. Discussion

The sample in this study is brown rice (*Oryza nivara* S.D.Sharma & Shastry) which has been determined at the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad

Dahlan University, Yogyakarta. Samples of brown rice (*Oryza nivara* S.D.Sharma & Shastry) were taken at Gapoktan Sidomulyo Rice Mill, Godean District, Sleman Regency, Yogyakarta Special Region Samples of brown rice taken were partially used to make brown rice then both samples were mashed using a blender.

The study began with a qualitative test conducted on brown rice and cooked brown rice. The purpose of the qualitative test was to determine the presence or absence of vitamin B1 content in brown rice and cooked brown rice. The results showed that brown rice and cooked brown rice samples were positive for vitamin B1 as indicated by the formation of yellow color and brown precipitate after heating. In the cooked brown rice sample, very little brown precipitate was formed due to the low content of vitamin B1. The formation of brown precipitate can occur because thiamine is decomposed by oxidizing substances. Pb acetate is added to oxidize thiamine and Pb^{2+} ions will be reduced to Pb^{+} which will precipitate into a blackish brown precipitate, PbO_2. The purpose of the mixture is heated is to accelerate the reaction [19].

The standardization of 0.1 N NaOH was carried out by titration for 3 replications. The average volume result of the titration obtained was 4.53 ml and the normality was 0.110 N. The reaction that occurs between Potassium Biphalate and NaOH: $KHC_8H_4 + NaOH\ KNaC_8H_4O_4 + H_2O$

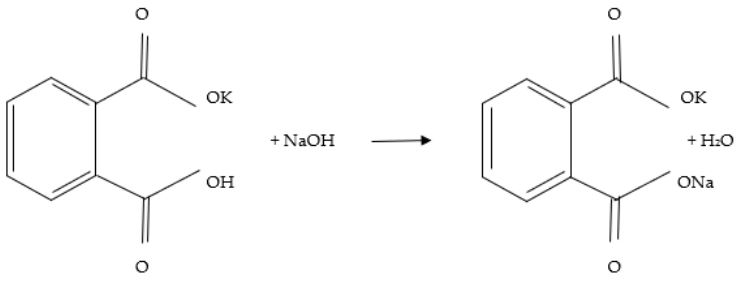

Figure 3. The reaction that occurs between Potassium Biphalate and NaOH.

Determination of Vitamin B1 levels was carried out using the alkalimetric titration method. Alkalimetric titration is the determination of acidic compound levels using basic standards [20]. The indicator used to detect the presence of vitamin B1 in alkalimetric titration is bromine thymol blue indicator. The reaction that occurs between Thiamine HCl (Vitamin B1) and NaOH:

$$C_{12}H_{17}ClN_4O_5.HCl + NaOH\ C_{12}H_{17}ClN_4O_5.NaCl + H_2O$$

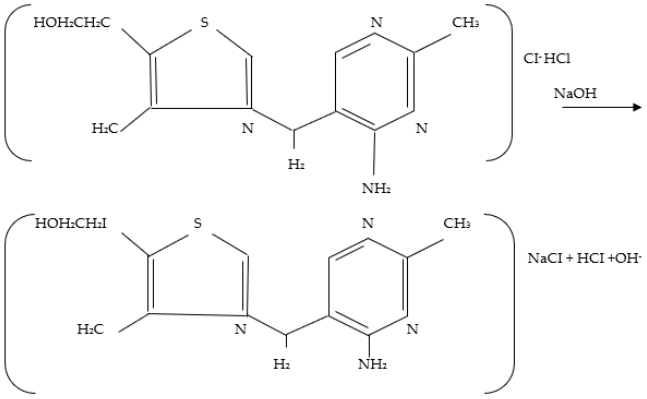

Figure 4. The reaction that occurs between Thiamine HCl (Vitamin B1) and NaOH.

The results of quantitative tests of vitamin B1 in brown rice and cooked brown rice obtained average levels of 12.40 mg / kg and 4.96 mg / kg so that vitamin B1 levels in brown rice are higher than cooked brown rice. From the results of the normality test with the *Shapiro Wilk* test. The *Shapiro Wilk* normality test is a test conducted to determine the distribution of random data in a small sample or no more than 50 samples [17]. The samples used in this study were 2 samples where at the time of determining the level of each sample replicated 3 times. The results of the normality test showed a significance value (p) = 0.000, which p > 0.05 means the data is not normally distributed and the results of the homogeneity test with the *Lavene test* (*homogeneity of variance*) showed a significance value (p) = 1.000, which p > 0.05 means the data has a homogeneous variance. Because the results of the normality test are not normally distributed but the variance is homogeneous, it cannot be continued with the *Independent T-test*. Comparative analysis of vitamin B1 levels of brown rice and cooked brown rice was analyzed using the *Mann Whitney test* as an alternative to the Independent T-test. The Mann-Whitney test is used to determine whether or not there is a difference between two independent samples. From the statistical test, it is known that the significance value (p) = 0.043, which p < 0.05, means that there is a significant difference in vitamin B1 levels between brown rice and cooked brown rice. So it can be said that there is an effect of brown rice processing on vitamin B1 levels in cooked brown rice.

The difference in average levels of vitamin B1 in brown rice with cooked brown rice which is quite significant can be caused by processing brown rice into cooked brown rice such as washing can result in a decrease in vitamin B1 levels in the brown rice. This can occur because vitamin B1 is a vitamin that is easily soluble in water.

In addition, the cooking process of cooked brown rice can also cause the loss of vitamin B1 because the cooking process requires considerable heating [21]. Heat in a warming device is used to raise the temperature of food and functions in stimulating a chemical reaction, but the use of heat for a long time can cause a decrease in the quality of food. Warming for a long time can also result in a decrease or destruction of nutritional content such as vitamin B1 in rice [8]. Vitamin B1 is unstable at high temperatures.

According to the results of research from [15], the highest levels of vitamin B1 in rice before and after washing are black rice (0.379%, 0.302%), brown rice (0.371%, 0.273%), white rice (0.354%, 0.201%), white glutinous rice (0.306%, 0.236%). Based on these results, there is a significant difference in vitamin B1 levels in brown rice before and after washing. Vitamin B1 levels in brown rice before washing are higher than after washing. These results are in line that the washing process can reduce vitamin B1 levels in brown rice. In general, before cooking brown rice, the washing process is carried out to produce clean brown rice. The washing process can reduce the level of vitamin B1 in brown rice which is easily soluble in water.

According to the results of research from [22], the results obtained are the levels of vitamin B1 in brown rice after washing in minute 1 is 0.0262%, in minute 2 is 0.0154%, and in minute 3 is 0.0103%. Based on these results, vitamin B1 levels in brown rice with variations in washing time also experience significant differences. The highest vitamin B1 content of brown rice is found in the 1st minute washing of 0.0262%. Based on this, the brown rice processing that most affects the decrease in vitamin B1 levels is washing. The longer the washing time on brown rice, the more reduced the vitamin B1 content in brown rice.

According to the results of research from [23] the results obtained are the levels of thiamine hydrochloride in cooked brown rice stored on magic-com for 0 hours, 6 hours and 12 hours respectively 5.3 mg/kg; 4.5 mg/kg; 2.8 mg/kg; and in white rice respectively 2.6 mg/kg; 2.0 mg/kg; 1.4 mg/kg. Based on these results, there are significant differences in vitamin B1 levels in cooked brown rice in storage times of 0 hours, 6 hours and 12 hours. The highest level of vitamin B1 in cooked brown rice is found in 0 hour storage time. Storage of cooked brown rice for a long time can reduce vitamin B1 levels because storage on a magic-com requires long heating. The length of heating can cause an unpleasant odor in the rice because vitamin B1 is decomposed into thiophene [24]. In the study conducted by the researcher, cooked brown rice that has finished cooking with magic-com is directly used for research.

Limitations in this study are the lack of previous studies that determine vitamin B1 levels in brown rice and cooked brown rice using the alkalimetric method makes it difficult for researchers to compile research work procedures and compare the results obtained by researchers. Another method that can be used to compare vitamin B1 levels in brown rice and cooked brown rice is uv-vis spectrophotometry or chromatography method.

4. CONCLUSION

Based on the research that has been done, it can be concluded that brown rice and cooked brown rice samples positively contain vitamin B1. Vitamin B1 levels in brown rice and cooked brown rice are 12.40 mg/kg and 4.96 mg/kg. Vitamin B1 levels in brown rice are higher than vitamin B1 levels in cooked brown rice, known significance value (p) = 0.043, where $p < 0.05$ this means there is a significant difference in vitamin B1 levels between brown rice and cooked brown rice. This can be influenced by the cooking time, the amount of water used, the washing time of brown rice.

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