DETERMINATION OF THIAMINE HYDROCHLORIDE AND PYRIDOXINE HYDROCHLORIDE ON RICE WITH WASHING, MILLING AND POUNDING BY SPECTROMETRIC METHODS

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ABSTRACT

Rice is a principal food source for most of the world's population. Rice has many nutrients including thiamin hydrocloride (THC) and pyrodoxine hydrocloride (PHC). Before consumption, rice is usually processed such as milling, washing, and cooking. Determination of THC on IR-64 and Rojolele rice with washing three times has been conducted. Also determined of PHC on the milled IR-64 rice and the pounded IR-64 rice. The THC was determined by spectrophotometric method at 246 nm. Determination of PHC based on the coupling reaction with diazo reagent in alkaline solution to form azo dye then the absorption was measured at 440 nm. The result of determination of THC on IR-64 and Rojolele rice after washing three times can decrease THC level 12,93% and 12,88% respectively. Determination of PHC on the milled IR-64 rice obtained 1.54 ± 0.027 µg/g and on the pounded IR-64 rice 1.80 ± 0.048 µg/g. The result of statistical test with independent T test showed there were significant difference ($\alpha = 0,05$) PHC level on both treatments.

Keywords: Thiamine hydrochloride, pyridoxine hydrochloride, rice, washing, milling, pounding

INTRODUCTION

Rice is a principal food source for most of the world's population, especially in Asia [1]. Rice consumption in Indonesia reached 95% of the population as a functional food ingredient [2]. As a principal food of rice can be processed into rice, various types of cakes or traditional foods in the form of rice flour [3]. Rice is a grain of rice that has been removed from the outer shell with milled or pounded [4].

Based on the color and texture there are three types of rice namely glutinous rice, brown rice and white rice [5]. In Indonesia, the most consumed rice is white rice [6] . There are several variants of white rice on the market, including Pandan Wangi rice, Setra Ramos

rice (IR-64), Rojolele rice and IR-42 rice. Type of IR-64 and Rojolele rice most often consumed by the community because the price is cheap and if cooked not quickly stale.

Rice is a source of energy, rich in protein, vitamin B complex and minerals for the body. The largest nutrient content in rice is dominated by starch about 80-85% [7]. Vitamin B complex contained in rice such as vitamin B_1 (thiamine hydrocloride, THC) and vitamin B_6 (pyrodoxine hydrocloride, PHC). Sources of THC and PHC are commonly found in nuts, beans, meat, fish, egg yolk, milk, and seralia such as rice and wheat [8].

THC has a role as a coenzyme and various energy metabolism reactions. THC is needed to raise appetite, normal growth and play a role in the development of the brain and nervous system [9]. This vitamin is also reported to reduce the damage caused by free radicals and prevent the occurrence of degenerative diseases [10]. PHC has three main forms, namely pyridoxine, pyridoxal, and pyridoxamine. In the liver, this vitamin is converted to pyridoxal 5-phosphate (PLP) which is a cofactor in various reactions of amino acid metabolism. PLP also plays a role in enzymatic reactions to release glucose from glycogen and neurotransmitter synthesis [11].

The daily requirement of vitamins depends on the age, gender and type of work [12]. Deficiency of THC can lead to the occurrence of beri-beri disease, especially countries that use rice staple food. In addition, deficiency of THC can lead to various neurological symptoms and cardiovascular symptoms. Early symptoms such as fatigue, weakness and emotional distress. Prolonged deficiency may cause polneuritis and heart failure or peripheral edema [13]. Deficiencies of PHC are manifested as dermatologic, circulatory, and neurologic changes [14].

Rice to become ready-to-eat food requires several stages of processing such as milling, washing, and cooking. In the process of washing can lead to reduced levels of THC in rice before cooking [15]. THC and PHC are water-soluble and unstable in storage or processing [16].

Determination of THC has been widely performed like using the method of High Performance Liquid Chromatography [17], electrochemical injection flow [9], alkalimetry [18] and spectrophotometry [19]. PHC uses methods such as High Performance Liquid Chromatography [20], Charge transfer complexation [21] and spectrophotometry [22]. The advantages of spectrophotometry are simple, fast, specific and sensitive [23]. Purpose of this

research is to know THC level on IR-64 and Rojolele rice after washing. Also to know the level of PHC on the milled IR-64 rice and the pounded IR-64 rice.

MATERIAL AND METHOD

Material

All reagents and chemicals used were of analytical grade and all solutions were prepared from double distilled water. THC and PHC was obtained from Sigma-Aldrich (China), sulfanilic acic (merck), sodium nitrite (merck), sodium hydroxide (merck), hydrochloric acid, whatman No.1 filter paper, IR-64 and Rojolele rice taken directly from the rice seller in Gresik city.

Instruments

Spectrophotometer UV-1600 PC (singgle beam), with 1 cm matched quartz cells was used for absorption measurement, analytical balance, hot plate, magnetic stirer, and a set of glassware.

PROCEDURE

Reagents and solutions

Hydrochloric acid solution (6.0 N). This solution was prepared by diluting 50 mL of concentrated hydrochloric acid to a 100 mL double distilled water in a volumetric flask. Hydrochloric acid solution (0.1 N) was prepared from HCl 6.0 N solution.

Diazo reagent. This solution was prepared by dissolving sulfanilic acid with HCl 6 N and adding the double distilled water to 100 mL in a volumetric flask.

Sodium nitrite solution (1 %). This solution was prepared by dissolving 1.0000 g of sodium nitrite in 100 mL double distilled water in volumetric flask.

Sodium hydroxide solution (3.0 M). This solution was prepared by dissolving 12 g of sodium hydroxide in double distilled water and adding the double distilled water to 100 mL in a volumetric flask.

Working THC solution (500 μg/mL). This solution was prepared by dissolving 0.2500 g of THC in double distilled water and adding the double distilled water to 500 mL in a volumetric flask. THC solution (250 μg/mL) was prepared from 500 μg/mL stock solution.

Working PHC Solution (100 μg/ml). This solution was prepared by dissolve an accurately 0.0100 g of PHC in double distilled water and adding to 100 mL in a volumetric flask.

Calibration Curve of THC.

Standard solution of THC was prepared with a range concentration of 5-25 µg/mL. THC standard solution were transferred into a series 25 mL volumetric flask. This solution was diluted with 0.1 N HCl to the boundary mark and then the absorbance was measured at a 246 nm.

Callibration Curve of PHC

The diazo reagent (2.0 ml) was transferred into a 25 ml volumetric flask and added 1.0 ml of 1 % $NaNO_2$ solution, then mixed homogenously and allowed to stand for 1 minute while occasionally shaking. Subsequently, the mixture was added 5 ml of PHC standard solution (4.0-20 µg/mL) and 1.0 ml of 3 N NaOH solution then allowed to stand for 10 minutes at room temperature. The volume of the solution is diluted with double distilled water and mixed well. The absorbance of the solution was measured at 400 nm using a reagent blank.

Rice washing water

A certain amount of IR-64 and Rojolele rice samples were weighed and transferred into a beaker glass with the addition of double distilled water. The mixture was stirred using a glass stir bar for 30 seconds then filtered using Whatman No.1 filter paper. The wash water of rice was collected and transferred in a 100 mL volumetrik flask and diluted with double distilled water to the boundary mark.

Rice sample

The preparation of rice sample was conducted according the Sudarmadji's method [24], with slight modification. Rice is milled with a grinding machine and partially pounded with pestle and mortar and then pollinated into flour. A certain amount of rice flour weighed and transferred into a beaker and 90 ml of 0.1 N HCl was added. The mixture was heated for 30 minutes at 100 °C in a water bath while stirring. After cool, the solution was filtered with Whatman No. 1 filter paper. The filtrate was transferred to a 100 ml volumetric flask and diluted with 0.1 N HCl to the marker boundary.

Determination of THC

The THC level was determined using Rohman's proposed method [18], with slight modification. The rice washing water (5.0 mL) was transferred in a 25 ml volumetric flask and diluted with 0.1 N HCl until the boundary marker. The solution was measured by UV-1600 spectrophotometer at 246 nm wavelength using reagent blank.

Determination of PHC

The levels of PHC was determined using diazotization method with modification [25]. The diazo reagent (2.0 ml) was transferred into a 25 ml volumetric flask and added 1.0 ml of 1 % NaNO$_2$ solution, then mixed and allowed to stand for 1 minute while occasionally shaking. Subsequently, the mixture was added 5 ml of rice sample and 1.0 ml of 3 N NaOH solution then shaken and allowed to stand for 10 minutes at room temperature. The solution was diluted with double distilled water and mixed well. The absorbance of the solution was measured at 400 nm using a reagent blank.

RESULT AND DISCUSSION

Determination of Maximum Wavelength

Before to the analysis of THC and PHC on rice, the maximum wavelength was determined. It aims to obtain the wavelength that has the maximum sensitivity and absorbance. Measurements were made by reading the absorbance of THC standard solution using UV-1600 PC sectrophotometer at wavelength 200-400 nm. The measurement results obtained maximum absorbance at 246 nm. In the standard solution PHC measured at 400-800 nm and obtained a maximum wavelength of 440 nm. Each of these wavelengths is used for the determination of THC and PHC on the rice sample.

Calibration Curve

The calibration curve of THC standard solution was obtained good a linear curve according to Lambert-Beer law in the concentration range 5-25 μg /mL. Based on the curve (Fig. 1), obtained regression equation $y = 0.2014x + 0.003$ and the correlation coefficient (r) = 0.9999. Meanwhile, the calibration curve of standard solution PHC was obtained linear in the concentration range 4-20 μg/mL. From the curve (Fig. 2) obtained regression equation $y = 0.0898x - 0.0262$ and correlation coefficient 0,9904. In both calibration curves obtained correlation coefficient value close to one. According Harmita [26], it shows there is a correlation between the concentration of standard solution with absorbance.

Determination of THC

Determination of THC levels was performed on the wash water of IR-64 and Rojolele rice. Each was washed three times, then the washing water was collected for analysis. Every time the washing is done by stirring using a stir bar for half a minute. The duration of stirring is taken from people's habit in general when washing rice before cooking. The result of

determination of THC level on the wah water of IR-64 and Rojolele rice is shown in Table 1 and Table 2.

Based on Table 1 and Table 2 are obtained the average THC levels on the wash water of IR-64 rice with washing once was 0.1821 ± 0.0012 μg/g, twice 0.2825 ± 0.0020 μg/g and three times 0.3258 ± 0.0015 μg/g. While on the wash water Rojolele rice was obtained THC levels by washing once 0.1925 ± 0.0019 μg/g, twice 0.2142 ± 0.0025 μg/g, and three times 0.3538 ± 0.0020 μg/g. From these results it can be seen that the more the number of washing more and more amount of THC dissolved in washing water. Vitamin B_1 (THC) is water soluble and THC content is widely present in aleurone in the surface layer of rice [15]. When rice washed THC on rice, especially on aleurone, it was carried along with water solvent. In the process of washing can reduce THC levels in rice before consumption [27]. Increase of THC levels in IR-64 rice washing water and Rojolele rice is shown in Figure 3. While the decrease of THC levels in IR-64 rice and Rojolele rice is shown in Figure 4.

In addition, the THC levels in rice without washing were also investigated (Table 3). THC levels in IR-64 and Rojolele rice were 2.520 ± 0.006 μg/g and 2.746 ± 0.008 μg/g respectively. From these results it can be seen that THC levels in Rojolele rice yields are slightly larger than IR-64 rice. However, THC levels in both types of rice is still slightly below THC levels in 30 varieties of rice from Philiphina as Villareal and Juliano reported [28]. The range of THC content obtained between 2.85-5.2 μg/g or 0.285-0.52 mg/100 g. While, results of research Kennedy and Burlingame [29] reported, that the thiamine content range of 79 rice varieties was 0.117-1.74 mg/100 g, averaging 0.457 mg/100 g and the largest difference among varieties 1.6 mg/100 g. In this study rice after washing three times on both types of rice respectively decreased of THC levels. The graph of decreasing THC levels with both types of rice is shown in Figure 4.

From Table 3 it can be seen that the more washing of IR-64 and Rojolele rice, the more THC decreases in rice. The percentage of THC decrease after washing three times resulted in IR-64 rice of 12.93%, and Rojolele rice was 12.88%.

THC is one type of vitamins are very unstable. The THC stability in the material is influenced by several factors such as pH, temperature and treatment method. Some rice processing before consumption can be through milling, pulverization, washing, and duration of washing. These factors are very influential to reduce THC levels in food. Washing is an important factor that can accelerate the occurrence of THC solubility in laundry water,

thereby reducing THC levels in rice. According Andarwulan [16], before cooked rice undergoes treatment through milling, pounding dan washing process can reduce THC levels.

PHC with Milling and Pounding

Determination of PHC level was done on the milled IR-64 rice and the pounded IR-64 rice. The milled rice was produced from rice processing by grinding machine. Rice is produced through the process of pounding rice using pestle and mortar. The results of PHC levels on the IR-64 rice milled and pounded are listed in Table 4.

The average results of PHC level on the milled and the pounded IR-64 eice were 1.54 ± 0.027 µg/g and 1.80 ± 0.048 µg/g, respectively (Table 4). From these results, the levels of PHC on the pounded IR-64 rice is higher than the milled IR-64 rice. The result of statistical analysis through the Independent-Samples T test with SPSS 16 for Window program, there are significant differences ($\alpha = 0,05$) PHC level between the milled IR-64 rice and pounded IR-64 rice.

Rice grains before becoming rice undergo processing such as the milling or pounding. The nutrient content of the IR-64 rice including PHC is mostly found in aleuron [18]. During the rice milling process, the aleurone present in the IR-64 rice will be lost by the exfoliating process through the machine until it becomes white rice. The loss of aleurone will be accompanied by a decrease in the amount of PHC present in rice. The pounded IR-64 rice produces a slightly brownish color and does not remove aleurone entirely, thereby lowering only a small amount of PHC in the rice. Rice pounding process is generally done in many rural areas. Rice through the milling process is most in demand by the community because it is considered clean white rice and has a better taste for consumption.

CONCLUSION

Based on this study, the THC levels on IR-64 and Rojolele rice after washing three times decreased THC levels 12.93% and 12.88%, respectively. Whereas the PHC levels on the milled IR-64 rice and the pounded IR-64 rice are 1.54 ± 0.027 µg/g and 1.80 ± 0.048 µg/g respectively. From these results it can be concluded that more washing in IR-64 rice, can reduce THC levels. The levels of PHC on the pounded IR-64 rice is higher yield than milled rice.

ACKNOWLEDGMENT

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Table 1. Levels of THC in the IR-64 rice washing water

Amount of Washing (Time)	C (µg/mL)			Average (µg/mL)	Levels of THC (µg/g)	SD (n= 3)
	1	2	3			
1	0.145	0.147	0.145	0.146	0.1821	0.0012
3	0.228	0.226	0.224	0.226	0.2825	0.0020
3	0.261	0.262	0.259	0.261	0.3258	0.0015

C is consentration of THC obtained from the standard curve

Table 2. Levels of THC in the Rojolele rice washing water

Amount of Washing (Time)	C (µg/mL)			Average (µg/mL)	Levels of THC (µg/g)	SD (n= 3)
	1	2	3			
1	0.154	0.152	0.156	0.154	0.1925	0.0019
2	0.174	0.171	0.169	0.171	0.2142	0.0025
3	0.285	0.283	0.281	0.283	0.3538	0.0020

C is consentration of THC obtained from the standard curve

Table 3. Percentage of THC decrease on IR64 and Rojolele rice after washing

Amount of Washing (Time)	Levels of THC in Rice (% mg/g)		Decrease of THC in Rice (%)	
	IR-64	Rojolele	IR-64	Rojolele
0	2.520 ± 0.006	2.746 ± 0.008	*	*
1	2.338 ± 0.001	2.554 ± 0.002	7.23	7.01
2	2.238 ± 0.002	2.532 ± 0.003	11.21	7.80
3	2.194 ± 0.002	2.393 ± 0.002	12.93	12.88

The data presented are mean value of three repetitions of ± standard deviation (n =3 ± s.d.).
*Not evaluated.

Table 4. Levels of PHC on the IR-64 rice milled and pounded

Type of IR-64 Rice	Levels of PHC (µg/g)	SD (n = 10)
Milled	1.54	0.027
Pounded	1.80	0.048

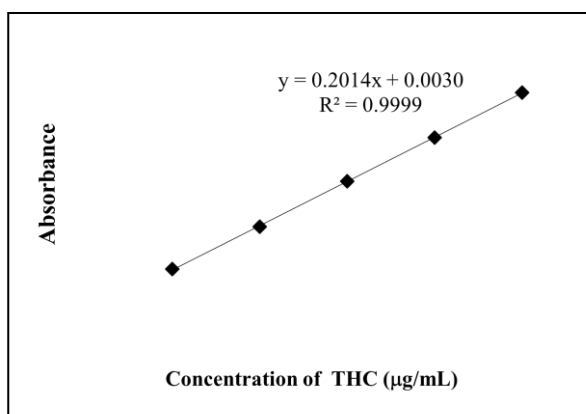The data presented are mean value of ten repetitions

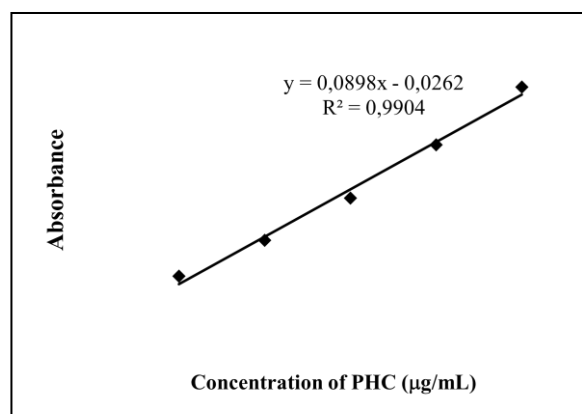**Figure 1.** Calibration curve of THC

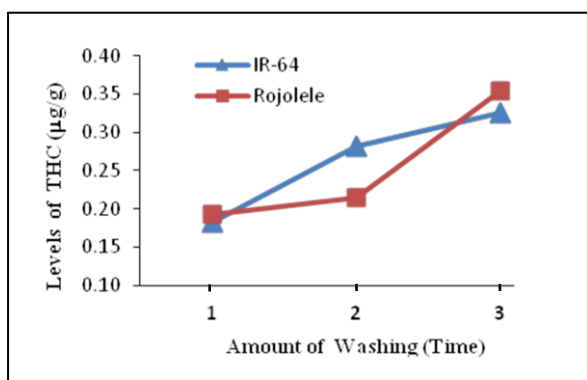**Figure 2.** Calibration curve of PHC

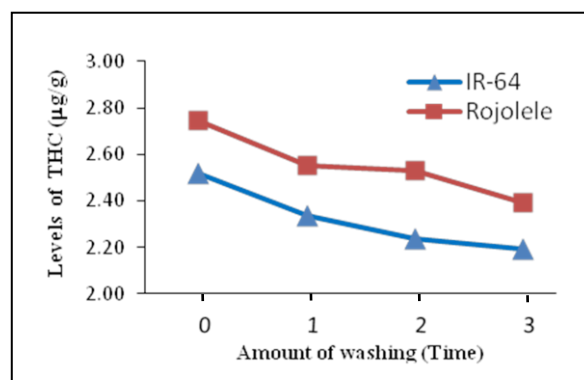**Figure 3.** Increase of THC levels on the IR-64 and Rojolele rice washing water

Figure 4. Decrease of THC levels on the IR-64 and Rojolele rice after washing