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Changes on carbohydrates and protein content in North Sulawesi local rice during storage

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Abstract: *This study aims at determining changes in the level of carbohydrates and protein during the storage process. The results show that during six-month storage, there are decreases in the levels of carbohydrates and protein content in rice grains. Decreased levels of carbohydrate occurred on red rice obtained from Manado Bersehati market (52.23%), followed by red rice from Karombosan market (41.21%). Control rice with insects and fungi protection treatments' experienced the smallest level of carbohydrate decrease (9.20%). The largest protein content decreased occurred in red rice obtained from Bersehati Market (5,45%), followed by red rice from Karombasan market (4.93%). The smallest decrease occurred in the control rice Superwin obtained from Market Bersehati (0.96%). Long period storage affects the quality of rice.*
Keywords: *Carbohydrate, protein, rice storage, food quality*

I. INTRODUCTION

Rice is the important food of the world. Asia ranks number one in terms of rice consumption in the world with an average consumption reaches 30% of the total food staples. Three countries with the largest consumption of rice in Asia are Bangladesh, Laos, and Indonesia. Rice is a commodity that affects social, economic and politics aspects of a country or region [1]. Recently, the global rice crisis has led to serious discussion because rice is one of the world's major food sources [2]. Rice has high nutrient contents because it contains macromolecular compounds such as carbohydrates, proteins, fats, vitamins, and some minerals. Carbohydrates and proteins are the largest components constitute the endosperm of rice [1] [3].

Rice post-harvest storage is one of the crucial problems faced by many countries. Rice as food must possess in proper techniques and conditions to make it suitable for consumption. According to the Indonesian government standards, consumable rice must have these following general requirements: 1) it is free from pests and diseases, 2) it does not smell bad, 3) it is free from a mixture of bran or husks, and 4) it is free from chemical contents that may harm consumers [4]. Rice may experience a loss of quality during storage. The quality may involve milling quality and the aroma of rice [5]. In general, rice will undergo changes after harvest and during storage [6]. Changes on rice grains during storage may be caused by pests and diseases that exist in the barn. It is commonly encompasses insects and fungi. Insects attack on rice can damage the quality of rice. Insects that attack rice will take some nutrients they need from the rice. Way and Bowling (1991) state that the impact of insect infestation in stored rice grains in the warehouse can cause a loss of about 24-35% of the rice [7]. Storage, therefore, plays an important role to provide good rice grains.

Rice is the main food sources for the people in North Sulawesi, Indonesia. Rice production in North Sulawesi has increased from year to year. The vertile lands of North Sulawesi lead to the high productions of rice, crops and agricultural product. There are some active volcanous in North Sulawesi regions which contributes to the lands vertility. According to the data, rice production reached 278,261 tonnes in 2007 and increased to 335,204 tonnes in 2011. According to the available data, the level of rice consumption per capita in 2011 reached 139 kg/capita [8]. The problems faced in the provision of rice is on the rice grains quality due to long storage in the warehouse.

This study aims at determining the carbohydrate and protein content of various types of rice grains which are circulating in the traditional markets in Manado, North Sulawesi, and knowing the changes on the nutrient content as a result of pests and diseases infestation during storage.

II. METHODOLOGY

Materials

Rice grains tested in this study were collected from Bersehati Market, Karombasan Market, and Bahu Market, in Manado North Sulawesi. There were three samples of white rice brands taken from Bersehati Market, namely *Sultan*, *Serang* and *Ladang*, and one variety of red rice. There were two samples of white rice brands taken from Karombasan Market, namely *Memberamo* and *Ladang*, and one variety of red rice. There were three

samples of white rice brands taken from Bahu Market, namely *Memberamo*, *Serang*, and *PL*. As the control group, *Superwin* brand, which is known to be free from rice pests and diseases, was used.

Equipment used in the study included 500 ml Erlenmeyer flask, volumetric flask, pipette, a set of titration tools, Kjeldhal flask, test tubes, micro pipettes, spatulas, and petri dishes. Materials used were HCl 3%, NaOH 30%, CH_3COOH 3%, KI 20%, sulfuric acid 25%, 0.1 N Tio solution, 2 gr of a mixture of 25 ml Selenium and As, PP indicator, and boric acid 2%.

Methods

Rice samples were taken from the three locations and selected markets to obtain samples with the same physical quality and class. Subsequently, the samples were stored for six months, and assessment on protein and carbohydrates was performed periodically every month during the storage period. The procedure to determine the carbohydrates and protein content is as follows:

Determining the Levels of Carbohydrates

Carbohydrates determination was done by weighing 5 gr of rice and put it in a 500 ml erlenmeyer. After that, 200 ml of HCl 3% solution was added and it was boiled for three hours. After three hours, the solution was cooled and neutralized with a solution of NaOH 30% and CH_3COOH 3 % to make the solution slightly acid. The solution was transferred into a 500 ml volumetric flask, and distilled water was added until the volume reaches 500 ml. Furthermore, as many as 10 ml of the solution was taken and put into a 500 ml erlenmeyer, and then a solution of Luff School was added as much as 25 ml and 15 ml distilled water. The mixture was then heated on a stable temperaturefor about 3-10 minutes.The solution was further cooled. Once cooled,as much as 15 mL of KI 20% and of 25 mL H_2SO_4 25% were added. Further, titration was immediately done with $Na_2S_2O_3$ solution of 0.1 N. The same procedure was done for the blank solution.

Determining Protein Contents

Protein contents were determined by weighing 0.51 gr of rice and put it in a 100 ml Kjeldhal flask. Then, 2 gr of a mixture of selenium and 25 ml of concentrated sulfuric acid were added. The solution was heated over an electric heater for 2 hours or until it boiled and the solution became greenish and clear. The solution was then allowed to cool, and then the solution was diluted in the flask until it reached the amount of 100 ml. Subsequently, 5 ml solution was taken and put into the distiller apparatus (distillation apparatus), and 5 ml of NaOH 30% and a few drops of PP indicator (phenolphthalein) were added. Distillation was then performed for about 10 minutes, and a solution of the distillate was collected in a vessel containing of 10 ml of 2% boric acid solution that had been mixed an indicator. The obtained solution was subsequently titrated with HCl 0.01 N. The same treatment was also carried out to the blank solution.

Data Analysis

Glucose level is calculated by the following formula:

$$\text{Glocose level} = \frac{\text{w1 x fp}}{\text{w}} \text{X } 100\%$$

Carbohydrate level = 0,90 x glucose level

In which : w1 = sample weight in mg
W = glucose contained in ml Tio used in mg from the list
fp = dilution factor

Protein content is calculated by the following formula:

$$\text{Protein content} = \frac{(\text{V1} - \text{V2}) \text{ x N x } 0.014 \text{ x fk x fp}}{\text{w}}$$

In which :
W = sample weight in gram
V1 = the volume of HCl 0.01 N used in sample titration
V2 = the volume of HCl 0.01 N used in blank solution titration
N = normality of HCl
fk = conversion factor of protein in general: 6.25 of milk and dairy products
fp = dilution factor

III. RESULTS AND DISCUSSION

Changes in Carbohydrate Levels

Carbohydrates as the main macromolecule constituting rice can undergo changes during storage. From the test results of samples several rice varieties originating from several markets in Manado it was known that there has been a decrease in the levels of carbohydrate during the storage process. Decreased levels of carbohydrate of rice taken from Bersehati Market is shown in Table 1.

Table 1.Carbohydrate levels (in %) of several rice varieties taken from Bersehati Market during storage

No	Rice Varieties	Storage Period (month)						
		0	1	2	3	4	5	6
1.	*Sultan*	75.35	71.85	67.39	61.95	59.45	56.80	54.67
2.	*Serang*	69.24	65.31	59.17	52.43	49.38	46.25	43.20
3.	*Ladang*	70.82	65.22	58.10	54.05	49.90	45.81	41.62
4.	Red Rice	72.68	64.55	56.05	46.95	37.15	28.75	20.45

Each rice variety had different levels of carbohydrates and decreased levels of carbohydrate during storage. The highest percentage of decrease occurred in the carbohydrate content of the red rice variety and the smallest percentage of decrease occurred in *Sultan* rice variety. At the end of the storage period, the red rice variety experienced a decreased level of carbohydrate by 52.23%, while *Sultan* rice variety experienced a decreased level of carbohydrate by 20.68%. Decreased levels of carbohydrates in big percentage on the red rice variety may be caused by the activity of insects and other microorganisms in the rice. Data on previous research shows that there are some insects found in red rice variety at the end of the storage period. Enzymes owned by the insects are used to decompose carbohydrates into simpler compounds when insects using rice as a nutritional ingredient.

As changes in the carbohydrate levels of rice varieties from Bersehati Market, the same change also happened to the varieties of rice obtained from Karombasan Market (Table 2).

Table 2. Carbohydrate levels (in %) of several rice varieties taken from Karombasan Market during storage

No	Rice varieties	Storage period (month)						
		0	1	2	3	4	5	6
1.	*Membramo*	66.83	61.75	55.60	48.24	44.13	40.30	36.45
2.	*Ladang*	71.52	66.40	61.95	55.75	52.61	49.47	46.32
3.	Red Rice	73.46	67.25	60.75	53.65	45.85	38.95	32.25

At the beginning of storage period, red rice variety had the highest carbohydrate content, but at the end of storage period, red rice variety had the lowest carbohydrate content. The largest decrease in carbohydrate levels was experienced by the red rice, that was 41.21%, followed by *Membramo* rice variety as much as 30.38 %, and the smallest carbohydrate decrease was experienced by *Ladang* rice variety as much as 25.20%. High carbohydrate content of red rice variety seems to support the development of insects. Insects survived by using grains of rice as nutrition source. The grains became a source of carbohydrates for insects.

Ladang variety experienced the smallest decrease of carbohydrate levels. This was due to low activities and growth of insects during storage compared to *Membramo* rice and red rice variety. The low activities and growth of insects can be caused by low water levels of *Ladang* variety and the less number of insects at the beginning of storage than the number of insects on *Membramo* rice and red rice. According to Pingale et al. (1957), insect infestation in grains is a common problem during rice storage [9].

Table 3. Carbohydrate levels (in %) of several rice varieties taken from Bahu Market during storage

No	Rice varieties	Storage period (month)						
		0	1	2	3	4	5	6
1.	*Serang*	68.30	65.20	60.58	53.65	50.49	47.44	44.25
2.	*Membramo*	67.75	62.51	57.70	51.93	47.38	42.95	38.39
3.	*PL*	70.29	66.14	60.89	55.09	52.49	49.86	47.76

Table 3 shows different carbohydrate levels on varieties of rice, namely *Serang*, *Membramo*, and *PL*. Based on the calculation, the difference between the carbohydrate levels at the beginning and at the end of storage period, it was found out that the highest carbohydrate decrease was experienced by *Membramo* rice, as much as 29.36%, and the lowest carbohydrate decrease was experienced by *PL* rice, as much as 22.53%.

As a comparison on the effect of insect infestation on grains, the analysis conducted on the control rice, *Supewin* variety, it was found out that the carbohydrate decrease experienced by this variety was better than other varieties (Table 4).

Table 4. Carbohydrate levels(in %) of Superwin rice variety during storage

No	Rice Variety	Storage Period (month)						
		0	1	2	3	4	5	6
1.	*Superwin*	77.15	74.32	72.40	70.65	69.30	68.45	67.95

At the end of the storage period, *Superwin* variety, which is known to be free from insects, experienced a very small decrease in carbohydrate levels, as much as 9.2%. This figure is much smaller compared to the percentage of decrease on carbohydrate levels, as the other varieties are not free from insects since the beginning of storage. This means that insect activities on grains in storage affect much on the decrease of carbohydrate levels. Without the existence of insects of rice grains, changes on carbohydrate levels can be minimized.

Previous research on carbohydrate content in rice grains has been carried out and is interesting to study because the majority of rice grain is composed of carbohydrates. Rice contains about 90% carbohydrate [10]. All types of rice experience decreased carbohydrate levels during storage. Percentage of decreased carbohydrate levels of each type of rice at the end of storage (month 6^{th}) is shown in Table 5.

Table 5. Carbohydrate levels (on %) at the end of storage periods

No	Market	Rice Variety	Initial Carbohydrate Levels (month 0) (%)	Final Carbohydrate Level (month 6) (%)	Decrease in Carbohydrate Levels (%)
1.	Bersehati	*Sultan*	75.35	54.67	**20.68 %**
		Serang	69.24	43.20	26.04 %
		Ladang	70.82	41.62	29.20 %
		Red rice	72.68	20.45	**52.23 %**
2.	Karomba san	*Membramo*	66.83	36.45	30.38 %
		Ladang	71.52	46.32	25.20 %
		Red rice	73.46	32.25	**41.21%**
3.	Bahu	*Serang*	68.30	44.25	24.05%
		Membramo	67.75	38.39	29.36 %
		PL	70.29	47.76	22.53 %
4.	Bersehati	*Superwin* (control)	77.15	67.95	9.20 %

Long time rice storage can affect the physical, chemical and functional quality of rice. Carbohydrates will experience changes leading to damage. The aleurone of rice is more susceptible to damage compared to the endosperm. Degradation of carbohydrates into CO_2 during the storage process usually happens very slowly, except when the humidity reaches 14% [11]. In the period of 3-4 weeks after harvest, rice may change its properties, especially at the temperature above 15°C. Rice aroma may be lost and may be lowered [12]. Fungi are known as one of the causes of damaged rice during storage. Tipples (1995) states the primary fungi which attack rice are *Aspergillus* and *Penicillium* [13]. Humidity, temperature, and retention time are the factors that influence fungal attack against rice grains stored in a warehouse [14]. Keeping rice dry during storage is one of the simplest ways to maintain the quality of rice.

Changes in Protein Levels

Protein found in rice grains and become the important components of in rice grains. Protein in rice grains can reach 10% of total rice weight. Based on the laboratories assessment, there are proteins decreases during rice storage.

Table 6. Protein levels (in %) of several rice varieties taken from Bersehati Market during storage

No	Rice Varieties	Storage Period (month)						
		0	1	2	3	4	5	6
1.	*Sultan*	5.78	5.45	4.94	4.67	4.39	4.10	3.88
2.	*Serang*	5.90	5.42	4.77	4.46	4.18	3.92	3.69
3.	*Ladang*	5.23	4.81	4.25	3.87	3.50	3.15	2.75
4.	Red rice	10.69	9.84	8.67	7.72	6.86	6.02	5.24

Red rice is a rice variety with high protein content. Of the ten varieties of red rice that have been tested previously by Sompong et al. (2011), it has been found out that the protein contents of red rice range from 7.16 to 10.36 [15]. Each type of rice experiences protein content decreased during storage. The largest decrease on protein contents was experienced by the red rice which was 5.45%, while the smallest decline was experienced by Sultan rice as much as 1.90%. Red rice that has greater protein content than other varieties of rice experienced the highest decrease on protein content (Table 6). This is due to the high protein content in red rice,

which becomes good nutrition source for the growth and proliferation of insects. Protein is an indispensable building block needed by insects.

Table 7. Protein levels (in %) of several rice varieties taken from Karombasan Market during storage

No	Rice Varieties	Storage Period (month)						
		0	1	2	3	4	5	6
1.	*Membramo*	6,38	5,98	5,35	4,76	4,38	3,98	3,65
2.	*Ladang*	4,93	4,55	3,80	3,52	3,19	2,85	2,51
3.	Red rice	9,25	8,53	7,43	6,63	5,88	5,10	4,32

Differences on the existing values of protein content in Table 7-8 also shows a decrease in the protein content of rice with increasing storage time. The largest decline on protein contents was experienced by red rice, while *Membramo* variety experienced the smallest decline. Based on the differences between the initial protein content (month 0) and the final protein content (month 6^{th}), red rice experienced 4.93% decrease on protein content, while *Membramo* experienced 2.73% decrease on protein content.

Table 8. Protein levels (in %) of several rice varieties taken from Bahu Market during storage

No	Rice Varieties	Storage Period (month)						
		0	1	2	3	4	5	6
1.	*Serang*	6,04	5,65	5,13	4,83	4,50	4,21	3,90
2.	*Membramo*	6,65	6,20	5,57	5,17	4,79	4,40	4,04
3.	PL	5,07	4,81	4,36	3,98	3,65	3,30	3,02

The protein contents of each type of rice steadily decreased since the initial month storage until the end of storage. The decreased levels of protein content in each type of rice were different. The highest decreased level was experienced by *Membramo* variety, as much as 2.61%, from 6.65% to 4.04%. PL variety experienced decreased protein content as much as 2.05%, which became the smallest. Meanwhile, rice control (*Superwin* rice grains without insect) experienced the smallest deceased protein levels (Table 9). The protein contents of *Superwin* variety, even if it does not contain insects, also decreased with the time of storage.

Table 9. Protein content in Superwin rice.

No	Rice Varieties	Storage Period (month)						
		0	1	2	3	4	5	6
1.	*Superwin*	6,25	6,09	5,80	5,55	5,43	5,35	5,29

Based on the data in Table 6, 7 and 8, the white rice varieties in three markets in Manado have protein contents range from 4.90% - 6.65%. Kennedy and Burlingame (2003) previously report that the ranges of rice protein in Asia are between 4.50% - 15.9 % [16]. The average protein content of rice is Asia is 8.7%. Compared with the data, the rice varieties from the markets in Manado contain lower protein. All varieties of rice from the three locations were stored for 6 months and they experienced decreased protein levels. At the beginning and end of storage, red rice from Bersehati Market has the highest protein content of 10.69% and 5.24%. At the beginning and end of storage periods, *Ladang* variety from Karombasan Market had the lowest protein content, which was 4.93% and 2.51%. Decreased levels of protein content from each of the three varieties of rice taken from the markets are clearly described in Table 10.

Table 10. Percentage of protein decrease of rice grains in North Sulawesi market.

No	Market	Rice Variety	Initial Protein Levels (month 0) (%)	Final Protein Level (month 6) (%)	Decrease in Protein Levels (%)
1.	Bersehati	*Sultan*	5.78	3.88	**1.90 %**
		Serang	5.90	3.69	2.21 %
		Ladang	5.23	2.75	2.48 %
		Red rice	10.69	5.24	**5.45 %**
2.	Karombasan	*Membramo*	6.38	3.65	2.73 %
		Ladang	4.93	2.51	2.42 %
		Red rice	9.25	4.32	**4.93 %**
3.	Bahu	*Serang*	6.04	3.90	2.14 %
		Membramo	6.65	4.04	2.61 %
		PL	5.07	3.02	2.05 %
4.	Bersehati	*Superwin* (control)	6.25	5.29	0.96 %

Sultan variety experienced the smallest changes in protein content characterizing low activities and proliferation of insects. It is also influenced by the condition of the rice having low enough moisture content that is only 12.15 %. Protein in rice is often concentrated in the outer layer than in the inner layer. Martin and Fitzgerald (2002) state the major protein in rice is Oryzenin [10]. In addition, as in other cereals group, a protein found in rice prolamin include, among others, Glubolin and albumin. Marshal and Wadsworth (1994) report during the storage protein contents do not change much. Changes often occur on the physicochemical properties of the protein [11].

Providing good quality of grain rice is important issues in North Sulawesi. In North Sulawesi, rice grain quality is heavily influenced by length of rice storage in warehouse. Length periods of rice grain storage in warehouse are usually motivated by the economic and distribution circulation administration. In such a case, the local and provincial governments should take into consideration several fundamental regulations aspects of rice storage management and market circulation.

IV. CONCLUSION

During storage, the levels of carbohydrates in every variety of rice decreased. The highest decreased level of carbohydrate was experienced by red rice variety from Bersehati Market as much as 52.53%. The protein contents of each type of rice decreased during storage. Red rice from Bersehati Market experienced the highest decreased protein content, which was 5.45 %.

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