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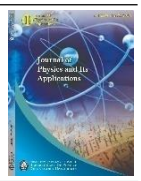

Rice Grain Classification using Fourier Transform Infrared Spectroscopy Technique and Laser Induced Breakdown Spectroscopy

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ABSTRACT

Rice is the main source of carbohydrates for Indonesian people and consumed to fulfill the nutritional needs of the body. The most commonly used method to find out nutrient content in rice grain is a chemical analysis based method that quite difficult and requires a considerable time. Therefore, spectroscopic-based measuring methods are the solution of these problems. A preliminary research is conducted to develop an accurate prediction methods of amylose, phenolic and flavonoids content, also elements contained in rice grain. Chemical analysis method used to determine of amylose, phenolic and flavonoid content then used as a validation. The predictive system is carried out using Partial Least Square (PLS) methods to determine amylose, phenolic and flavonoid content based on Fourier Transform Infrared (FTIR) spectrum. Meanwhile, Laser Induced Breakdown Spectroscopy (LIBS) used to figure out the elements contained in the rice. The Classification of rice grain using Principal Component Analysis (PCA) method based on FTIR and LIBS Spectrum. The results of this research, obtained a prediction system to determine levels of amylose, phenolic and flavonoids with the values coefficient of determination 0.95; 0.86; 0.95 and the RMSE value 1.4; 0.72; 0.44. Based on the spectrum of LIBS obtained from 13 types of rice grain, the elements contained in the rice grain are Mg, Fe, Na, K, Ca, C, H and O. The Classification of rice grain based on FTIR and LIBS spectrum obtained, namely High Quality; Premium and Medium.

1. Introduction

Rice is a staple food for Indonesian people. Based on the data that published by FAO, Indonesia in the 3rd position of the biggest Rice grain producer in the world. There are a lot of Rice grain types in Indonesia, such as ; Pandan Wangi, Setra Ramos, Rojolele, IR 42, C4, Batang Lembang, Mentik Wangi, Red Rice, Black Rice dan Beras Ketan.

Based on SNI 6128:2015, There are 4 types of Rice grain quality classification. The Quality classified by physical condition, such as colors, size, shape and the smell.

As the technology era grows, the method of quality determination has been developed. In 2017, Benjamaporn identified the types of rice grain using image processing methods. Image from the rice grain taken using camera and processed using software. This research identified whether the rice grain in a good condition or contaminated with mold [1-2]. In the same year, Sudhanva done research about variety determination based on area and length. Image processing method also used in tis research. The result was good enough but need improvement on the variety that has same area and length [3].

On the other side, rice consumed to fulfill the nutritional need. Rice contains fiber, mineral, carbohydrate, protein and Vit B. Also, rice good source of magnesium, phosphorus, magnesium, selenium, iron, folic acid, thiamin and niacin. Because of its importance for health, rice grain's nutrient should be considered before we consumed. Such as diabetes patient must be careful when choose the type of rice that to be consumed. Diabetes patient should be concerned with glycemic index, polyphenols and amylose[4].

Technique and method to determine Rice grain's nutrition has been widely developed. The methods that frequently used is chemical based method. In 2019, Nuzul et al done research about glucose determination using Luff-Schoorl method. Luff-Schoorl method followed with chemical sample preparation [5]. There is another method based chemical, named HPLC (*High Performance Liquid Chromatography*). HPLC is chemical analysis that used to separate, identification and quantification of compound. HPLC many used to identify the nutrition of rice grain [6]

Another method that can be used to identify rice grain is an optic and photonic based method named spectrometry. Spectrometry methods investigate and measure spectrum that produced when the wave interacts with material. Measuring amylose content in rice grains is highly valuable, because it

can determine whether it is appropriate for the body's condition, especially in diabetes patients. Rice grains that have higher amylose, have low glycemic index (GI). The Glycemic Index (GI) refers to a numerical ranking system (from 0 to 100) that measures how quickly carbohydrates in a food raise blood glucose levels after being consumed. Glycemic Index (GI) is very suitable and useful for people with diabetes, because it helps manage blood sugar (glucose) levels more effectively. Low-GI foods cause a slower, steadier rise in blood glucose after eating. Amylose determination using spectrometry method has been done in 2018 by Pedro S et al [7]. In 2018 Matsuo et al measure glucose content form rice grain in Japan using spectrometry with infrared range (850 nm-1084 nm)[8]. Not only amylose, Phenolic and flavonoid can be benchmark for rice grain quality. Phenolic and flavonoids have advantage for body, such as; prevent cardiovascular disease, diabetes, cancer, anti-inflammatory and strokes [9]. The high phenolic content of rice is important because it contributes to health benefits through antioxidant, anti-inflammatory, and protection from chronic diseases. Therefore, consumption of dark whole rice (such as black and red rice) is recommended to support a healthy diet. Flavonoid levels in rice are essential for human health due to their antioxidant, anti-inflammatory, and protective effects against various chronic diseases. Consumption of dark-colored whole rice such as red and black rice is highly recommended to increase flavonoid intake in the daily diet. Black rice and red rice have the highest phenolic and flavonoids content. Based on the benefits from the phenolics and flavonoids described before, Phenolic and flavonoid can be benchmark for rice grain quality. Electromagnetic wave in Near-Infrared can be used to analyze Phenolic and flavonoids. In 2018, Aygul Can et al done research about phenolic and flavonoids measurement in olive leaves [10].

Measurement of rice grain's nutrient using chemical methods is not relevant nowadays. The chemical method requires a long time, cost and only can be carried out by the expert. So, it is time to create another method that can be used easily. Furthermore, combining nutrient measurement, element from rice grain and physical appearance can be used to create a rice grain quality determination system.

Based on the background, we measure the nutrient from rice grain namely Amylose, Phenolic and Flavonoid and detect the element in rice grain using spectroscopy method. The type of rice grain that we use are Black Rice, Red Rice, Ciherang, IR-42, IR-62, IR-64, C-4, Membramo, Mentik Wangi, Bengawan, Medium, Karawang and Ketan. Chemical method still used in this study for the validation. The result obtained can be used as parameter of rice grain Quality determination and Classification. Spectroscopy methods that used are Fourier Transform Infrared Spectroscopy (FTIR) and Laser Induced Breakdown Spectroscopy (LIBS).

2. Methods

Amylose, phenolic and flavonoid from rice grain extracted using standard method. Standard curve from amylose, phenolic and flavonoid made to determine the relation between absorbance value and amylose, phenolic, flavonoid and obtain regression equation using absorbance spectroscopy technique. So, amylose, phenolic and flavonoid content from rice grain can be obtained based on the absorbance value.

Grounded rice grain of every type of rice tested using FTIR Spectrometer *Bruker Alpha II*. Wavenumber range that used 4000-600 cm^{-1} or wavelength range 2500 nm – 16666 nm with resolution of 2 cm^{-1}. In this study, wavenumber from FTIR converted to wavelength in order to make it easier to analyze.

Partial Least Square (PLS) is used as a method with a statistical approach to obtain predictive modeling. Determination of amylose, phenolic and flavonoid levels based on absorbance value (chemical methods) which was explained in the previous sub-chapter was used as modeling validation. Modeling was built to determine the correlation between amylose, phenolic and flavonoid levels obtained from the chemical methods with FTIR spectrum of each reference sample. Modeling was then used to predict amylose, phenolic and flavonoid levels in rice based on the FTIR spectrum of rice.

Validation is carried out by comparing the predicted values for amylose, phenolic and flavonoid levels of test samples with the values from chemical method measurements. The R^2 and RMSE value parameters obtained then become benchmarks for the success of the statistical analysis.

Grounded rice is then palletized using a palletizing tool by applying pressure of 15 Torr for 20 minutes. Pallets of each type of rice are then stored and each type is named. LIBS spectrum data was collected at several points in each sample, which were then averaged to obtain one LIBS spectrum. The laser used in LIBS is an Nd: YAG laser with a wavelength of 1064 nm. LIBS spectrum that obtained, used to analyze the element that contained in rice. Some element that may be visible are Ca, Fe, K, C, Cd, Ti, Ma, Si, Li, Ba, Sr, Cr , Na and Al.

FTIR and LIBS spectrum from each type of rice then processed using Principal Component Analysis (PCA) Method. PCA Method can classify data that has similarity of characteristics, so that can be used as rice grain quality analysis based on its classification. Principal Component Analysis (PCA) Method based on FTIR spectrum compared to the result of Principal Component Analysis (PCA) Method based on LIBS spectrum.

The quality of rice is classified based on the content of amylose, phenolics and flavonoids as well as the elements contained in rice. And based on the grouping results of the FTIR and LIBS spectra. Rice grains are classified into 3 group ; High, Premium and Medium. Classification based on the FTIR spectrum, classified based on the content of amylose, phenolics and flavonoids. Classification based on the LIBS spectrum, classified based on the content of elements contained in rice grains.

3. Result and Discussion

Amylose absorbance spectrum obtained with peak wavelength at 670-690 nm. Spectrum that obtained appropriate with previous research [11].

There are 2 spectrums, one is pure amylose and the other amylose extracted from rice grain. Absorbance value from pure amylose used for obtain

standard curve and equation. Absorbance value from extracted rice grain used for amylose determination content based on the equation.

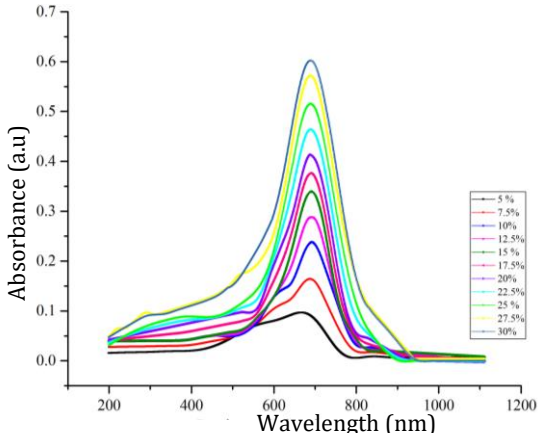

Fig. 1: Pure amylose absorbance spectrum

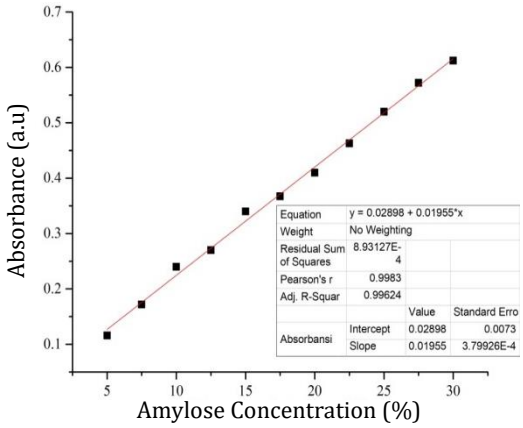

Fig. 2: Amylose standard curve

From the standard curve, equation that obtained is

$$Abs = 0.02898 + 0.01955 \times Concentration \quad (1)$$

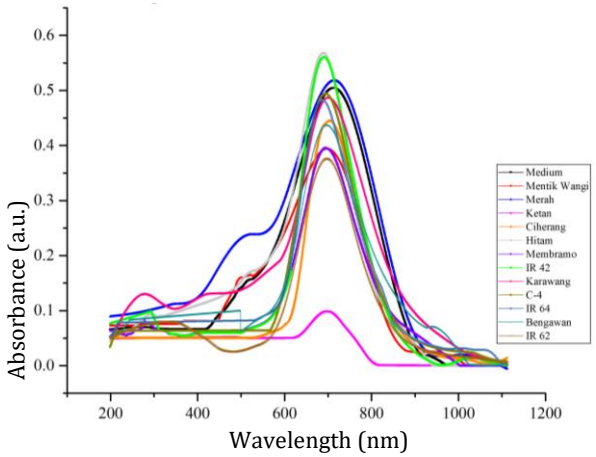

Fig. 3: Amylose absorbance spectrum from rice grain

Table 1 show the result of amylose concentration and compared with known amylose concentration from 6 types rice grain.

Amylose concentration shows texture after cooked. Rice grain with high amylose concentration has little harsh texture, meanwhile rice grain with low concentration has low amylose concentration has fluffier texture.

Moreover, amylose concentration can be consideration for diabetes patient. Diabetes patients recommended to consume rice with high concentration of amylose. Because rice with high concentration of amylose has low Glycemic Index. Rice with high amylose concentration can repair insulin sensitivity and decrease rate glucose absorption.

Table 1: Amylose Concentration

Rice Grain Type	Amylose Concentration (%)	Amylose Concentration Ref (%)
Black Rice	28.76	
Red Rice	27.39	
IR42	27.64	27
IR62	20.45	
Sticky Rice	1.64	
IR 64	23.56	23
C4	20.91	
Medium	26.52	
Karawang	25.70	
Ciherang	22.54	23
Bengawan	21.52	22
Membramo	20.97	19
Mentik Wangi	20.50	20.64

Absorbance spectrum of phenolic has peak wavelength at 390 nm. Same as amylose concentration determination, there are 2 spectrums. One for standard curve, using gallic acid and either extracted from rice grain.

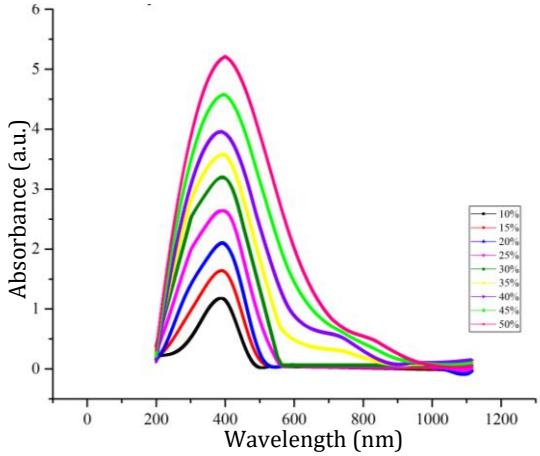

Fig. 4: Pure phenolic standard spectrum

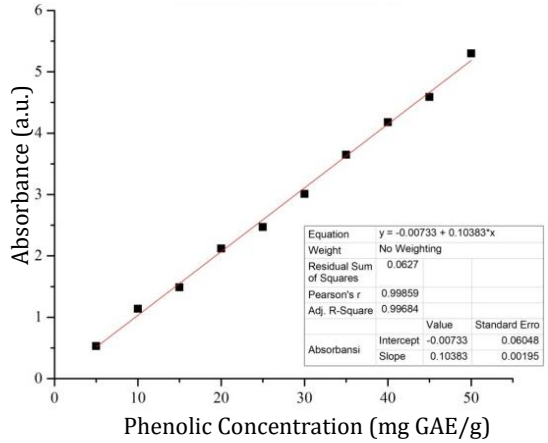

Fig. 5: Phenolic standard curve

Equation that obtained from phenolic standard curve is

$$Abs = -0.00733 + 0.10383 \times Concentration \quad (2)$$

Table 2 show the result of phenolic concentration.

Unit for phenolic concentration in g GAE/ g. GAE is Gallic Acid Equivalent wich means phenolic concentration obtained equivalent to gallic acid per dry weight of sample.

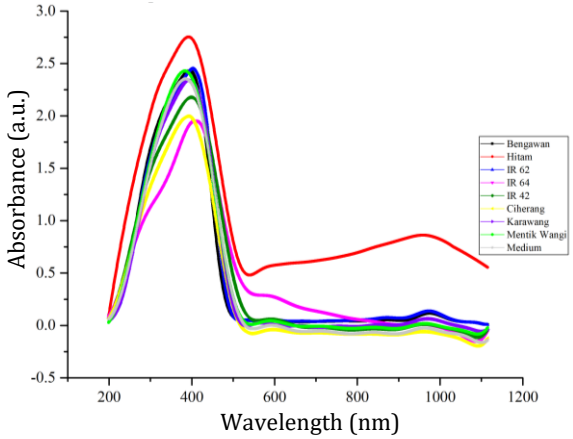

Fig. 6: Phenolic absorbance spectrum from rice grain

Table 2: Phenolic Concentration

Rice Grain Type	Phenolic Concentration (mg GAE/g)
Black Rice	31.50
Red Rice	30.17
IR42	24.56
IR62	27.82
Sticky Rice	23.69
IR 64	27.58
C4	27.37
Medium	24.67
Karawang	24.47
Ciherang	28.17
Bengawan	26.38
Membramo	27.76
Mentik Wangi	27.33

Phenolic concentration contributes as antioxidant. Based on the result, black and red rice have the highest phenolic concentration. Whereas sticky rice has the lowest phenolic concentration.

Absorbance spectrum from pure flavonoid has peak value at 350 nm. Pure flavonoid obtained using Quercetin.

Table 3 show result of Flavonoid concentration based on equation. The unit that used is mg QE/g dry weight. QE is Quercetin Equivalent that means flavonoid content equal with Quercetin content. Quercetin is a flavonoid that commonly found in a fruits and vegetables.

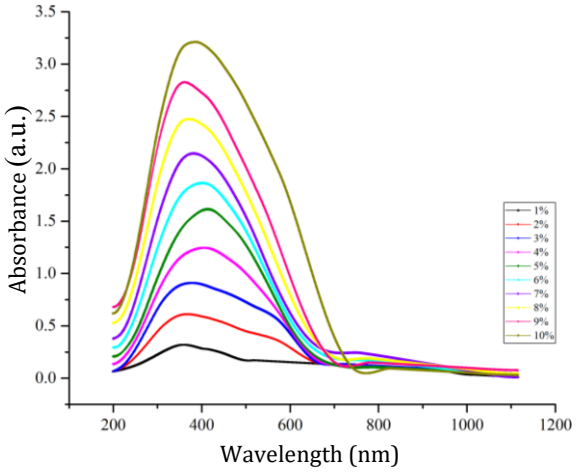

Fig. 7: Pure flavonoid standard spectrum

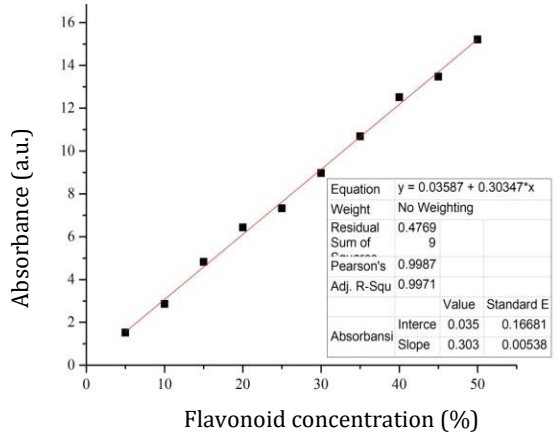

Fig. 8: Flavonoid standard curve

From the standard curve above, obtained equation for flavonoid concentration.

$$Abs = 0.03587 + 0.30347 \times Concentration \quad (3)$$

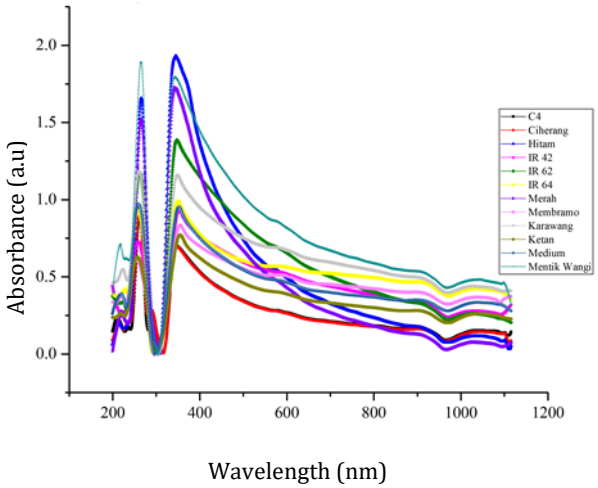

Fig. 9: Flavonoid absorbance spectrum from rice grain

Table 3: Flavonoid Concentration

Rice Grain Type	Flavonoid Concentration (mg QE/g)
Black Rice	6.22
Red Rice	6.45
IR42	3.85
IR62	5.34
Sticky Rice	3.58
IR 64	4.47
C4	4.20
Medium	3.04
Karawang	3.63
Ciherang	4.08
Bengawan	4.87
Membramo	5.47
Mentik Wangi	6.06

The highest flavonoid concentration from these rice grains is black and red rice. Meanwhile, the lowest is medium rice. Phenolic and flavonoid is a compound classified to antioxidant. Antioxidants are really important for health because can prevent cell damage, abnormal cell growth and disease.

Rice grain sample that has been grounded then measured using FTIR spectrometer Bruker ALPHA II. In general, FTIR spectrum from each sample has similarity shape but different transmittance value. The difference transmittance value occur at 9000 nm- 11000 nm, 4000 nm- 4444 nm, 3300 nm- 3600 nm dan 2800 nm – 3125 nm. Some rice grain samples has not same absorbance. For example, at

range 4000 nm – 4444 nm medium rice has not absorbance.

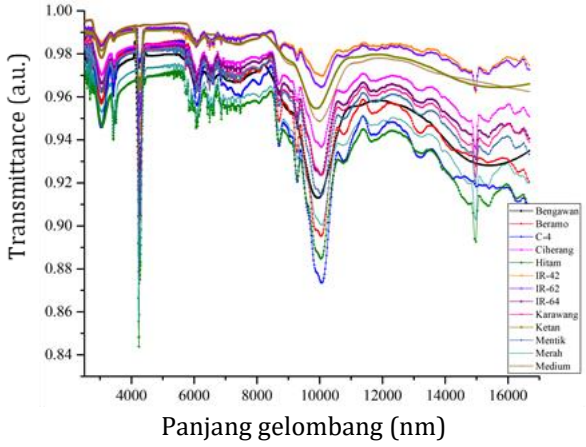

Fig. 10: FTIR Spectrum from Rice grain sample

The presence of compounds can be identified from the FTIR spectrum at certain wavelengths. Absorbance of amylose [12], phenolic [13] and flavonoid [14] known from previous research and compared with obtained data.

Table 4: Wavelength Absorbance for Amylose, Phenolic and Flavonoid

Compound	Wavelength (Reference)	Wavelength (Measurement)
Amylose	9293 nm	9302 nm
	10050 nm	9925 nm
Phenolic	2700 – 3400 nm	2700 – 3400 nm
Flavonoid	6215 nm	6119 nm
	6583 nm	6531 nm

FTIR spectra were obtained with the further aim of replacing chemical methods in measuring amylose, phenolic and flavonoid content. To obtain amylose, phenolic and flavonoid content need to find the correlation between FTIR spectrum from each rice grain sample and amylose, phenolic and flavonoid content based chemical method.

In this research, Partial Least Square used to find the modeling correlation. Modelling obtained by correlating FTIR data in certain wavelength that indicate amylose, phenolic and flavonoid presence with amylose, phenolic and flavonoid content based chemical method. FTIR Spectrum as independent input and amylose, phenolic and flavonoid as dependent input. After the modelling obtained, modelling used as amylose, phenolic and flavonoid content prediction based on FTIR spectrum.

Table 5: Amylose Content Prediction Result

Rice Grain	Actual	Prediction
Black Rice	28.76	29.99
Red Rice	27.39	27.63
IR42	27.64	26.16
IR62	20.45	23.17
Sticky Rice	1.64	2.63
IR 64	23.56	22.67
C4	20.91	19.26
Medium	26.52	25.99
Karawang	25.73	26.03
Ciherang	22.54	23.31
Bengawan	21.52	20.13
Membramo	20.97	21.37
Mentik Wangi	20.50	19.23

Table 5 shows the result of amylose content prediction.

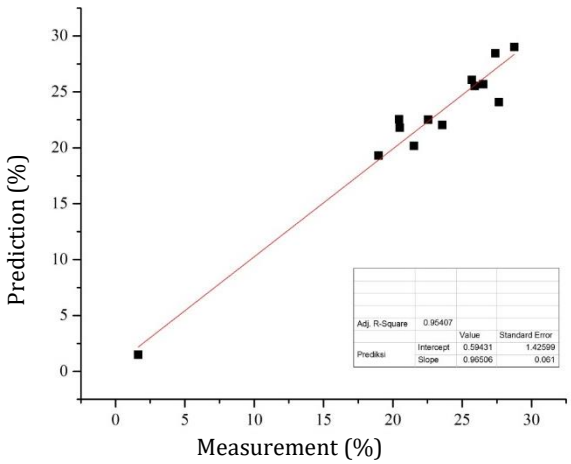

Fig. 11: Graphic of prediction and measurement data

The prediction result of amylose content has an RMSE value 1.25 and Coefficient of Determination (R^2) 0.95. Highest Error occur at rice grain type IR 42.

Table 6 shows the result of phenolic content prediction.

Table 6: Phenolic Content Prediction Result

Rice Grain	Actual	Prediction
Black Rice	31.50	30.60
Red Rice	30.17	29.87
IR42	27.37	26.72
IR62	27.82	27.75
Sticky Rice	23.69	24.10
IR 64	27.58	27.60
C4	27.37	27.67
Medium	24.67	24.39
Karawang	26.46	27.18
Ciherang	28.17	28.35
Bengawan	26.37	26.27
Membramo	27.75	27.36
Mentik Wangi	27.33	28.0488

The results of phenolic content prediction has an RMSE value of 0.72 and Coefficient of Determination (R^2) 0.86. The biggest error occur at rice grain type IR 42.

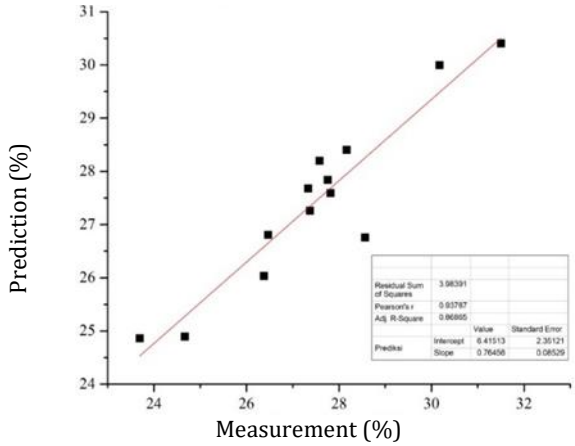

Fig. 12: Graphic of prediction and measurement data

Table 7 shows the results of flavonoid content prediction. The results of phenolic content prediction has an RMSE value of 0.44 and Coefficient of Determination (R^2) 0.95. The biggest error occur at rice grain type IR 42.

Table 7: Flavonoid Content Prediction Result

Rice Grain	Actual	Prediction
Black Rice	6.21	6.77
Red Rice	6.44	6.60
IR42	3.85	4.46
IR62	5.34	4.09
Sticky Rice	3.58	3.62
IR 64	4.47	4.25
C4	4.2	3.91
Medium	2.86	3.58
Karawang	3.63	4.44
Ciherang	4.08	4.24
Bengawan	4.87	4.82
Membramo	5.47	5.19
Mentik Wangi	7.06	6.18

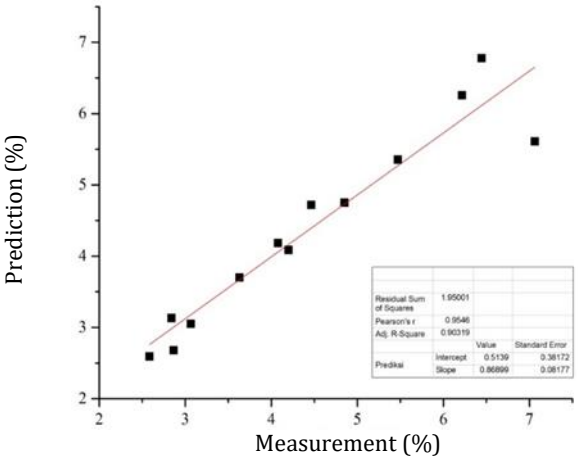

Fig. 13: Graphic of prediction and measurement data

Laser that used in LIBS methods is Nd:YAG laser with wavelength 1064 nm. Grounded rice grain then made into solid pellet using pellet press with 15 Torr pressure and 25 minutes. LIBS data carried out from pellet of each rice in 10 different point then averaged to get one LIBS spectrum data.

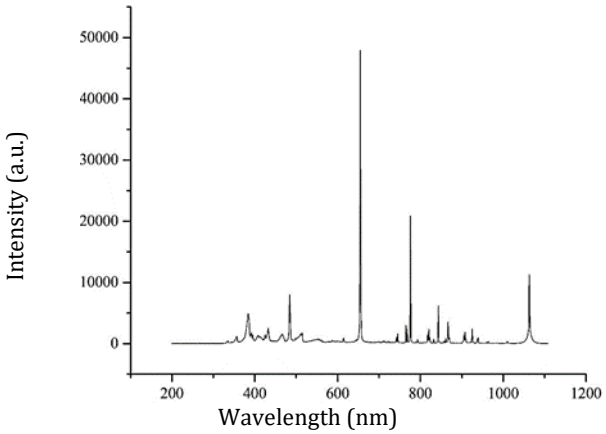

Fig. 14: LIBS spectrum of rice

LIBS spectrum of each rice generally has peak intensity in the same wavelength, the difference is value of intensity. Peak intensity of wavelength indicates the presence of certain element, meanwhile intensity value shows concentration of the element [15].

Element analysis at each peak wavelength was carried out by comparing the LIBS spectrum data obtained with the NIST Spectra Atomic Database. The majority elements contained in rice are Magnesium, Iron, Sodium, Potassium and Calcium.

Classification of 13 types of rice grain carried out by FTIR and LIBS spectrum based on amylose, phenolic and flavonoid content and element contained on rice grains. Classification carried out using Principal Component Analysis (PCA).

13 types of rice grain classified into 3 classes: high, premium and medium.

Table 8: Rice Grain Classification based on Amylose, Phenolic and Flavonoid Content.

Compound	High	Premium	Medium
Amylose	>25%	20%-25%	<20%
Phenolic	> 30 mg GAE/g	25-30 mg GAE/g	<25 mg GAE/g
Flavonoid	>5.5 mg QE/g	4-5.5 mg QE/g	<4 mg QE/g

The picture bellow shows the results of clustering using PCA based on FTIR spectrum.

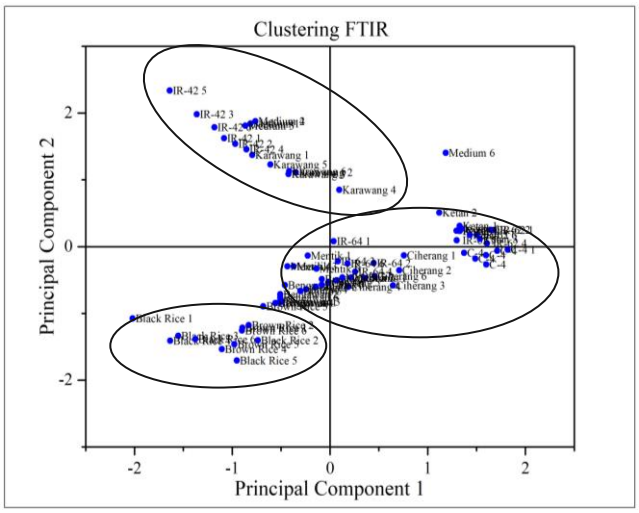

Fig. 15: Clustering based on FTIR spectrum

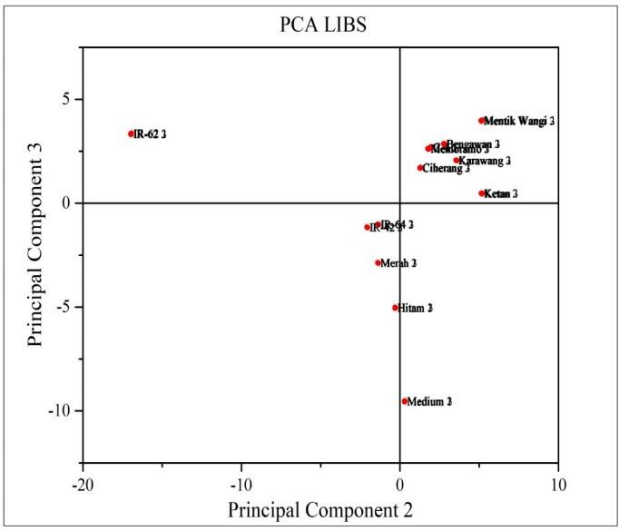

Fig. 16: Clustering based on LIBS spectrum

Same with FTIR spectrum, peak intensity value of LIBS spectrum classified into 3 class, named: High, Premium and Medium. Table 9 shows the classification of rice grain based on LIBS spectrum.

LIBS spectrum also analyzed using PCA method to obtain the classification. From the classification from FTIR and LIBS spectrum based on amylose, phenolic, flavonoid content and element, also combined with result of clustering using PCA

methods 13 rice grains classified into 3 classes showed in Table 10.

Table 9: Peak Values to Classification Conversion

Classification	Mg	Fe	K	Ca	Na	C
High	1300 - 2600	20000 - 40000	2000-4000	2000-2500	1400-2400	1500-2500
Premium	501-1300	10000 - 40000	1000-2000	1600-2000	1000-1400	500-1500
Medium	0-500	0-10000	0-1000	0-1600	0-1000	0-500

Table 10: Rice Grain Classification

Classification	Based on FTIR spectrum	Based on LIBS spectrum
High	Red Rice Black Rice	Red Rice Black Rice IR 64
Premium	Mentik Wangi Membramo Bengawan Ciherang Ketan C-4 IR-64 IR62	Mentik Wangi Membramo Bengawan Ciherang Ketan C-4 Karawang
Medium	IR-42 Medium Karawang	IR-42 Medium IR-62

4. Conclusion

Rice grain classification from 13 types of rice grain has been conducted using FTIR spectroscopy and LIBS. There are bit different with the result of Classification using FTIR and LIBS spectrum. There are 3 types of rice grains classified differently. IR 64 based on FTIR classified into Premium, while based on LIBS classified into High. IR-62 based on FTIR classified into Premium, while based on LIBS classified into Medium. Karawang based on FTIR classified into Medium, while based on LIBS classified into Premium. This could be due to differences in reference. FTIR is based on compounds and LIBS is based on the elements contained. If compared with SNI 6128:2015, where rice grain quality classification based on physical appearance from rice, rice grain classified into premium and medium.

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