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Red-Green-Blue Color Analysis Concerning Rice Husk Incorporation in Rice Bran

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Abstract: We aimed to determine rice husks incorporated in rice bran by using Red-Green-Blue (RGB) color. Rice bran and rice husk samples were analyzed for crude protein and lignin and mixed at a ratio of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50. The phloroglucinol-HCl was added to the samples into Petri dishes and photographed with a smartphone to get RGB pixel values. The data collected were analyzed for mean value, STDV, CV, F-test, regression equation and R^2. The results showed that crude protein and lignin content in rice bran were 12.04±1.27 and 8.69±1.19% with a CV of 10.58 and 13.65% and rice husks were 3.07±0.78 and 15.50±2.83% with a CV of 25.40 and 18.52%, respectively. Rice bran and rice husk mixtures did not have significantly different RGB pixel values, while the addition of phloroglucinol HCl resulted in a highly significant ($p<0.01$) difference. The red value has an R^2 = 0.8195, which is higher than the green (0.8103) and blue (0.3781). Additionally, testing of rice bran samples in the ruminant feed industry indicated that almost all samples of rice bran have been mixed with rice husks. Changes in the color image (red) due to the reaction of lignin with phloroglucinol-HCl that is read by an application on a smartphone can be quantified to predict rice husk in rice bran. This study presents an innovative RGB color analysis technique for detecting rice husk adulteration in rice bran, offering a quick, precise and cost-effective solution to enhance feed quality and protect animal health in agricultural practices.

Keywords: Phloroglucinol-HCl, Rice Bran, Rice Husks, RGB, Smartphone

Introduction

Currently in Indonesia, the demand for rice bran for livestock exceeds availability, especially during the dry season when some farmers are unable to grow rice due to water shortages, resulting in high prices. This condition leads to the counterfeiting of rice bran by adding rice husk (Novita *et al*., 2022). Other ones replace some of the primary constituents of counterfeit feed. Although these other ingredients have almost the same characteristics as the forged feed ingredients, they will still reduce the quality of the feed. This violates consumer protection regulations as stipulated in the law of the Republic of Indonesia No. 8: 1999 (Sonya and Anggraini, 2019).

Rice husk is often used as a mixture of rice bran and contains a lot of lignin and silica, namely 25-30 and 15-20% (Basri *et al*., 2020). These two components inhibit dry matter and protein digestibility, namely <50% (Aquino *et al*., 2020). Additionally, lignin restricts the digestion of structural carbohydrates such as cellulose and hemicellulose by forming stable lignin complexes with carbohydrates and resistance to attack and enzymatic hydrolysis of rumen microbes (Hernaman *et al*., 2022).

Silica is another type of antinutrient found in plant cell walls that can cause low levels of feed intake and physical damage to the animal's tongue due to its hairy structure. It may also decrease forage digestibility by reducing the accessibility of rumen microflora (Rahman *et al*., 2020).

The rice bran adulteration test is more popular in studying color changes with phloroglucinol HCl solution (Rahayu and Susanti, 2023). This method can act as an initial prediction of the rice bran quality based on visual observations and cannot quantify the rice husk added. The lignin content can be estimated from the textured image of rice bran mixed with rice husks using the run-length feature extraction method with the k-nearest neighbor classification yielding an accuracy of 74.55% (Novita *et al*., 2022). However, this technique is not practical because of the high cost. Van Soest (1985) by lignin measurements will be more accurate but time-consuming (1-2 days). The feed producers want a quick transaction regarding the price or the decision to buy rice bran from the supplier with simple procedures and readily available equipment.

Color provides helpful information in predicting product quality (Neupane *et al*., 2019). Color representation, the RGB model, which states the color as a mixture of red, green and blue three-color components, is frequently used to depict the color info of an image (Barbosa *et al*., 2019). Every color in the RGB spectrum is created of dissimilar levels for each of its red, green and blue components (Prabayanti *et al*., 2022). Combining these prime color elements will affect the color outcome (Åsly *et al*., 2019). Rationality pixel color images to determine the nutrient concentration have not been demonstrated.

Many applications can be connected to a smartphone, making it easier to identify an object. The color image of an object can easily be determined with an application installed on the smartphone to be able to quantify. Due to the addition of phloroglucinol-HCl solution, the red color image to materials containing high lignin such as rice husk presumably can be quantified using an application installed on a smartphone.

This study introduces a groundbreaking method for ensuring the quality and safety of animal feed by employing RGB color analysis to detect rice husk adulteration in rice bran. This novel approach not only promises to revolutionize animal production practices by providing a rapid, accurate and cost-effective means of safeguarding feed integrity but also underscores a significant advancement in protecting livestock health.

Materials and Methods

Sample Analysis

Rice bran and rice husk samples (n = 10) were collected from five districts with two huller factory locations, namely Indramayu, Karawang, Subang, Cianjur and Majalengka, respectively. These districts are successively the largest dry grain producers in West Java (BPS, 2022). Each district was obtained from two different huller places/factories. The rice bran used is the result of the first milling of dry grain. Then the rice bran and rice husk samples were analyzed for crude protein and lignin using the proximate analysis method (AOAC, 2010; Soest, 1985) and the samples were also used in testing the color pixel value.

Phloroglucinol-HCl Solution

The phloroglucinol-HCl solution was obtained by solving 10 g of phloroglucinol in 200 mL of ethanol using a magnetic stirrer (DLAB MS7-7) at 1500 rpm. Then the solution was dissolved with 800 mL of 2 N HCl solution until evenly distributed using a magnetic stirrer and stored in a closed dark container. All chemicals used in this study were of analytical grade and were purchased from Merck, PT Merck TBK Indonesia.

RGB Pixel Value Measurement Procedure

The rice husk samples were finely ground to a size of 30 mesh and mixed into the rice bran homogeneously. The ratios of rice bran and rice husk were (1) 100:0, (2) 90:10, (3) 80:20, (4) 70:30, (5) 60:40 and (6) 50:50. Then, 1 g of the mixture was placed in a 5 cm diameter petri dish, 4 mL of phloroglucinol HCl solution was added, stirred until homogeneous, left for 10 min and placed in a closed box with a lighting system on LED lights with a power of 7 watts. The Color Grab 3.9.2 application was downloaded and installed on a smartphone with a camera of 64 megapixels from Samsung Electronics Co. Ltd. Then the camera was placed exactly in the middle of the Petri dish, which is in a closed room box, the distance to the object is about 20 cm. Once the object is locked, it is photographed and the value of the color pixel is determined in the application. Color grab 3.9.2. has the following primary colors, RGB. Score the pixel value for each color on a scale of 0-255. A white color test is carried out by placing plain white paper as an imaging object in the test box. This is used as the standard color when the white color is close to the highest score of 255 and then testing the pixel value of the treatment object is next. The same procedure is carried out for samples from the ruminant feed industry.

Data Analysis

The data collected were analyzed descriptively by calculating the mean, Standard Deviation (STDV), Coefficient of Variations (CV) and F-test using the IBM SPSS statistic (21st version) software. In addition, the regression equation, coefficient of determination (R^2) and figure of regression were used in Microsoft Excel 2016. Based on the best R^2 analysis results, a regression equation was used to create a formula to predict the amount of rice husk mixed into rice bran.

Results and Discussion

The rice bran and rice husk from five districts in West Java contained crude protein and lignin as revealed in Table 1. This result is still in the category of acceptable data uniformity. According to Aronhime *et al*. (2014), the CV is believed to be very good when it is 10%, good when the CV is between 10-20% and the value is accepted when the CV is between 20-30% and weak when it is >30%.

Direct viewing showed that there was no change in color from no. 1-6. From the results of reading the application, the color values of each color are presented in Table 2. The table shows that all the average color values of each color and the ratio of rice bran and rice husks have a low CV value of >10%, meaning that the ratio of rice bran and rice husk produces a stable color image. Then the F-test was carried out for the ratio of rice bran and rice husk, which showed a non-significant difference. This condition shows that the data for each color is homogeneous.

Rice bran and its mixture (Fig. 1) reacted with phloroglucinol-HCl to produce a gradual color change resulting in a more intense red color, as well as higher levels of rice husk in rice bran from 1-6 (Fig. 2).

Table 1: Crude protein and lignin content of rice bran and rice husks from five districts with two huller factory locations

Sample	Rice Bran Crude protein (%)	Rice Bran Lignin (%)	Rice Husk Crude protein (%)	Rice Husk Lignin (%)
1	12.91	08.85	NA	13.26
2	12.09	07.92	01.77	20.06
3	13.57	08.67	01.34	13.26
4	13.02	08.62	02.78	15.73
5	10.52	08.09	02.85	16.84
6	11.66	07.09	02.95	14.81
7	10.68	09.01	03.79	17.26
8	10.04	11.82	02.68	17.29
9	13.06	08.02	03.57	10.27
10	11.81	07.89	02.03	15.71
Mean	12.04±01.27	8.69±01.19	3.07±00.78	15.50±02.83
CV (%)	10.58	13.65	25.40	18.25

NA = Not Available

Table 2: The average color value at different ratios of rice bran and rice husk pre-reacting with phloroglucinol-HCl

Color value	Rice bran: Rice husk 100:0	90:1	80:20	70:30	60:40	50:50	F-test
Red	188.60±10.05	189.20±8.11	188.30±5.38	189.90±6.69	190.30±5.62	190.10±8.10	NS
CV (%)	6.87	4.83	3.64	4.27	3.44	4.82	
Green	175.00±12.03	176.10±8.50	173.90±6.33	174.80±7.47	174.90±6.01	174.70±8.42	NS
CV (%)	11.80	8.06	6.62	7.18	5.40	6.50	
Blue	113.8±13.43	115.2±9.28	113.2±7.50	113.4±8.14	113.9±6.15	114.8±7.47	NS
CV (%)	6.89	4.97	3.63	4.28	3.39	4.74	

Values are expressed as mean (n = 10), NS = Non-Significant (p>0.05)

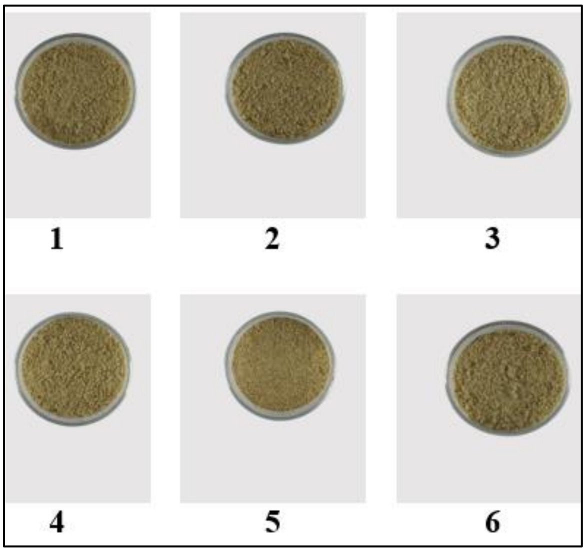


Fig. 1: The mixture of rice bran and rice husks at different ratios pre-reacting with phloroglucinol-HCl

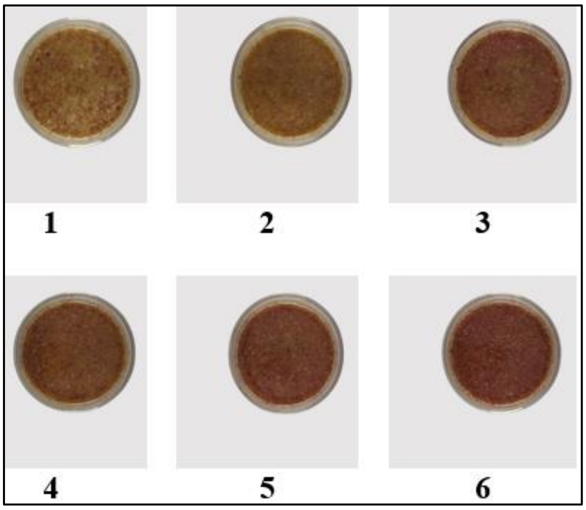


Fig. 2: The mixture of rice bran and rice husks at different ratios post-reacting with phloroglucinol-HCl

Then the color change can be determined by the RGB value shown in Table 3. Then the color change can be determined by the RGB value (Table 3). This RGB value for each rice bran and rice husk ratio shows CV <10%, which means that the data is very good (Table 3). However, after the F-test, there was a very significant difference ($p<0.01$).

The linear regression equation shows that the higher the rice husk in the rice bran, the lower the RGB value (Fig. 3a-c). Additionally, red and green have a strong R^2, while blue is in the moderate category (Hair *et al*., 2009). Out of the three colors, red is the best regression model, because it has the highest R^2. The red regression equation is used to predict the amount of rice husk mixed into rice bran using the following equation:

$$Rice\ Husk\ (\%) = \frac{R-165}{-0.892} \tag{1}$$

where, R = red pixel value.

This formula will find a negative value if the red value reaches more than 165 and assumes pure rice bran.

Furthermore, the formula is applied to test samples in the ruminant feed industry and the results are shown in Table 4. From the table, information is obtained that almost all rice bran has been mixed with rice husks. Only one sample, namely no. 6, was believed to be close to pure rice bran.

Rice bran contains moderate amounts of crude protein and lignin, while rice husk is low in protein and high in lignin, each data provides variation even though it is still acceptable. This is believed to be due to differences in rice species, environment and cultivation techniques at each sampling location. Several factors can directly or indirectly affect the nutritional quality of plants such as soil factors (pH, available nutrients, texture, organic matter content and soil-water relations), weather and climate factors (temperature, rainfall and light intensity), plants and cultivars, postharvest handling and storage, fertilizer application and cultural practices (Hornick, 1992).

Rice bran consists of the pericarp, aleurone, seed coat (rice husks) and nucellus (Sapwarobol *et al*., 2021). Subsequently, rice husks contribute lignin, causing rice bran to contain moderate levels of lignin. The other components in rice brans have a high protein content, fat, dietary fiber, vitamins, minerals and phytochemicals (Sapwarobol *et al*., 2021). The percentage and composition of rice bran depend on milling and the rice variety (Ilias *et al*., 2020; Cao *et al*., 2021).

Table 3: The average color value at different ratios of rice bran and rice husk post-reacting with phloroglucinol-HCL

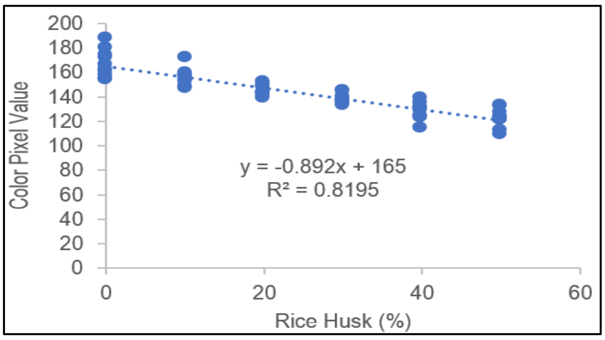
Color value	Rice bran: Rice husk 100:0	90:10	80:20	70:30	60:40	50:50	F-test
Red	166.70±6.99	155.80±4.44	145.20±4.10	138.20±7.11	127.90±7.591	22.40±**	
CV (%)	6.92	4.49	3.06	2.97	5.56	6.20	
Green	120.00±13.07	107.04±8.86	93.04±7.62	85.00±5.73	71.00±8.49	66.20±9.64**	
CV (%)	11.4	28.25	8.16	6.75	11.95	14.56	
Blue	65.08±7.13	62.05±6.29	60.08±5.09	59.8±4.73	54.06±6.69	51.08±6.07**	
CV (%)	10.84	10.07	8.38	7.91	12.25	12.93	

Values are expressed as mean (n = 10) and different letters within rows represented significant differences (p<0.01) or **

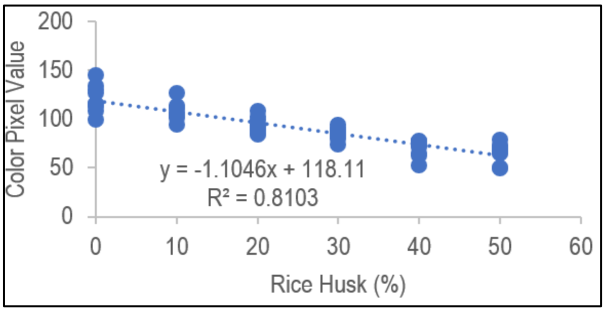
Table 4: Prediction of rice husk in rice bran using the formula in samples from the ruminant

Sample (%)	Red pixel value*	Rice husk (%)	Range
1	135.7±0.58	33	30-40
2	116.7±0.58	54	50-60
3	139.3±3.51	29	20-30
4	132.2±3.51	37	30-40
5	143.3±4.16	24	20-30
6	157.0±5.20	09	00-10
7	119.3±5.03	51	50-60
8	125.3±3.21	45	40-50
9	112.3±3.79	59	50-60
10	109.7±1.15	62	60-70
11	108.7±1.15	63	60-70
12	115.7±3.51	55	50-60
13	121.7±1.53	49	40-50
14	148±13.08	19	10-20
15	91.7±3.51	82	80-90
16	95.7±8.14	78	70-80

*Values are expressed as mean (n = 3) and (Prabayanti *et al*., 2022) stated that digital image processing based on a Green-Blue (RGB) color examination was practiced to measure the intensity of wood color



(a)



(b)

Fig. 3: (a) The regression equation and R^2 of red color value at different ratios of rice bran and rice husk; (b) The regression equation and R^2 of green color value at different ratios of rice bran and rice husk

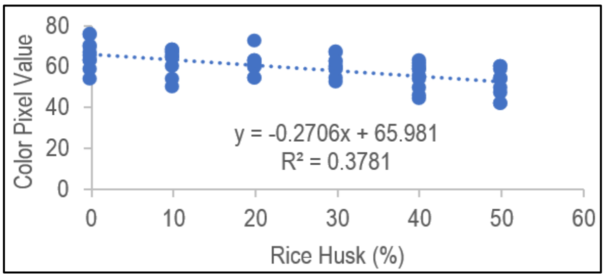


Fig. 3c: The regression equation and R^2 of blue color value at different ratios of rice bran and rice husk

The high lignin content of rice husks provides structural strength and rigidity to the protective coverings of rice grains. Subsequently, lignin is a complex aromatic polymer in the cell wall, which increases as plants mature. This compound consists of phenylpropane units (Rinaldi *et al*., 2016) that fill the gap between cellulose and hemicellulose (Zakzeski *et al*., 2010). In the rumen, lignin limits the enzymatic access of microbes to fiber polysaccharides by acting as a physical barrier and cross-linkages to polysaccharides by ferulate bridges (Sharma *et al*., 2019), which affects fiber digestibility (Rahman *et al*., 2020).

The cinnamaldehyde end group of lignin will react with phloroglucinol-HCl to give a red-purple color. The higher the lignin content in the substrate, the darker the red color from pink to purple (Fig. 2). This color image change is read by applications based on Red, Green and Blue (RGB) colors. Subsequently, RGB color is the basic color of all objects in everyday life and is also a color that is often used in the field of digital image processing (Prabowo and Abdullah, 2018). These colors can be combined in various proportions to get any color in the visible spectrum. Each level is measured by a decimal number ranging from 0-255 (256 levels for each color) (Toomanian, 2022). If the color value is RGB 0,0,0 indicates a dark/black condition, while RGB 255, 255, 255 means white (Rizani, 2021). Color can be represented in a three-dimensional RGB color space and each color will have an RGB value with a range of values from 0-255 (Karma, 2020).

Rice bran mixed with rice husk will not change the color image (Fig. 1) and has no significant difference in the color values (Table 2), making it difficult to distinguish between pure rice bran and its mixture. However, when phloroglucinol-HCl was added, there was a color change resulting in a very significantly different color value ($p<0.01$) for the treatment of rice bran mix with rice husk (Table 3). Rice bran and rice husk are taken from various locations that are suspected of having variations in rice species, cultivation management and environment but have an RGB value with CV<10%. This means that the sample location provides variation but is not significant, which is supported by lignin analysis data (Table 1) reported to be acceptable.

A red value gives the highest response observed at the highest coefficient of determination (R^2) compared to other colors. This strengthens the statement of Rahayu and Susanti (2023) that the reaction of lignin with phloroglucinol-HCl produces a red color image. The addition of high rice husks to rice bran resulted in lower red values, resulting in a decreasing linear regression equation (Fig. 3a). This means that the higher the rice husk, the more it will result in an intense red color image or produce a lower red color value. Thus, the red color can predict the amount of rice husk in the rice bran with the formula from the derivative of the linear regression equation (Fig. 3a) namely (R-165)/-0.892 (Prabayanti *et al*., 2022).

Images are digitally stored in an array form digital numbers which is the result of the quantification of the brightness level of each pixel image maker. Image is a continuous function of light intensity in the two-dimensional plane. Source light illuminates objects, objects reflect return a portion of the beam of light. This reflected light is captured by optical devices, for example, the eye in humans, cameras, scanners and others so the shadow of that object is called the recorded image. An image is a collection of thousands of dots (pixels) that are infinitesimal and each of those dots has a certain color (Prabowo and Abdullah, 2018).

Using the formula from the regression equation for the red pixel value, it is believed that in the ruminant feed industry, there almost all samples have been counterfeiting of rice bran from suppliers to which about 10-90% of rice husk has been added. Several reasons may be due to high prices and high demand for rice bran and it is difficult to physically distinguish between rice bran and rice husk. During the dry season, the need for concentrate is high, they ignore the quality of the rice bran.

This test method is very fast, simple and cheap and all the materials, equipment and applications needed are easily available. The time needed for testing is only 15-20 min, making it very fast. With this technique, ruminant feed producers can quickly decide to reject or buy at a reasonable price. Current image analysis ensures product quality control without any further information (Prabayanti *et al*., 2022).

Conclusion

The protein and lignin content of rice bran and rice husk samples from various places has acceptable variations. The mixture of rice bran and rice husk added with phloroglucinol-HCl solution produces a significant color image change (RGB). The red color image produces the highest coefficient of determination, so it is used to predict the content of rice husk in rice bran. Changes in the color image (red) read by an application on a smartphone can predict the amount of rice husk in rice bran. This study presents an innovative technique for detecting rice husk adulteration in rice bran, offering a quick, precise and cost-effective solution to enhance feed quality and protect animal health in agricultural practices.

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Author's Contributions

Iman Hernaman: A leader in research who prepares research, conducts research, collects data, interprets data and writes manuscripts.

Urip Rosani, Hidayat Tanuwiria and Muhammad Rifqi Ismiraj: Assisted in conducting research, collected data, interpreted data and written manuscripts.

Tidi Dhalika, Atun Budiman and Rahmat Hidayat: The co-authors who assisted in conducting research, data collection and data analysis.

Budi Ayuningsih: Assisted in conducting research and financial administration.

Nahrowi: Acts as a reviewer of journal manuscripts and provides advice in carrying out research.

Ethics

This study was conducted based on orders from ruminant feed industry players, especially mini feed mills which have limited capital and felt there was an economic loss due to the adulteration of rice bran with rice husk. The samples collected were obtained with the approval of the huller mill owner and the management of the ruminant feed industry. In addition, the sampling location is kept confidential.

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