Two Alternative Methods to Predict Amylose Content of Rice Grain by Using Tristimulus CIE Lab Values and Developing a Specific Color Board of Starch-iodine Complex Solution

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Abstract: Amylose content was predicted by measuring tridimensional Commission Internationale de l’Eclairage (CIE L* a* b*) values in starch-iodine solutions and building a regression model. The developed regression model showed a highly significant relationship ($R^2=0.99$) between the L* a* b* values and the amylose content. Apparent amylose content was strongly and negatively correlated with L* a* b* values. This method could be used to predict amylose content in rice. The conversion of L* a* b* values to red, green, blue (RGB) values and to color hexadecimal codes allowed reproducing the colors of starch-iodine solution and making an explicit color board. Using this specific color board, we could sort entries into their respective classes and easily estimate their apparent amylose content.

Key words: Amylose content, Amylose-iodine colorimetry, Color chart, Commission Internationale de l’Eclairage (CIE L* a* b*), Rice.

Amylose content in rice grain is one of the most important parameters for predicting rice grain eating quality. Quality may vary with the consumer’s behavior or preferences. Amylose is often used for tenderness prediction in rice. It may also be helpful for genotype selection in breeding.

Starch-iodine colorimetry has been widely used to determine amylose content in rice grain and other crops. The procedure was firstly proposed by McCready and Hassid (1943) for potato starch, and was modified several times in order to increase the reproducibility (Fitzgerald et al., 2009). Many methods have derived from this method (Knutson, 1985). There are also other methods based on different technologies, such as amperometric titration (BeMiller, 1964); near infrared reflectance (Delwiche et al., 1995) and more recently polymerase chain reaction (Bergam et al., 2001) for amylose prediction in rice starch. However, most of those methods are time consuming or require expensive equipment (Ronoubigouwa et al., 2009). Batey and Curtin (1996) suggested a method using high performance liquid chromatography.

The human eye is useful to distinguish colors. Color estimation with the human eye using a color chart is a practical qualitative and quantitative method in many circumstances, for example, the characterization of sea water color and soil by Munsel color, and chlorophyll estimation through IRRI’s leaf color chart, although training may be required to reduce subjective interpretations.

Colorimetry, the most widely used method for quantification of amylose and amylopectin in starch, is based on the absorbance spectra, using a UV spectrophotometer. A low-cost method substituting the spectrophotometer with a color chart has been reported (Ronoubigouwa et al., 2009). Since starch-iodine binding results in a colorful solution that strongly depends on the amylose/amylopectin proportion in starch, alternative methods based on tristimulus values obtained with a tristimulus color meter, or a color board, L* a* b* and red, green, and blue (RGB) values may be used.

This study explores a new way to quantify amylose content in rice using Commission Internationale de l’Eclairage (CIE L* a* b*) measurements. A specific chart board was created to classify rice amylose content into the five standard classes or more based on true digital colors, after converting CIE L* a* b* values to RGB values which have similarity with human vision.
Materials and Methods

1. Starch-iodine solution

Five genotypes with known amylose content determined by colorimetry based on the ability of amylose to bind with iodine (California Rice Commission, 2009a, 2009b): Calmochi (0%), Calamylow (6%), M-206 (17%), L-206 (22%), L-205 (25%), provided by the California Cooperative Rice Research Foundation, were used to make a standard curve and computing regression equation for amylose content determination. Milled rice grains were powdered with a faience pestle and mortar. Rice grain powder was then collected in a paper envelop and dried for 1 hr in a dry oven at 135ºC. Then 100 ±0.01 mg of dried rice powder was collected from envelop and put in a conical flask, to which 1 mL of 95% ethanol and 9 mL of 1M NaOH were added. The suspension was put in vigorously boiling water bath for 10 min. Flasks were allowed to cool at laboratory ambient temperature for 10 min, and then distilled water was added to make 100 mL solution. A 5 mL aliquot of the solution was transferred to a 100 mL volumetric flask, and to adjust pH, 1 mL of 1N acetic acid was added. Then 2 mL of 0.2% iodine solution (I2: 2 g/KI: 20 g/liter) and distilled water were added to make exactly 100 mL. Three additional amylose levels (3%, 11.5% and 14%) were prepared by mixing the amylose iodine solution as indicated in Table 5.

2. Measurements

Spectrophotometer measurements were made at 620 nm (Juliano, 1971) after the above starch-iodine solution was incubated for 30 min. A color meter (Chroma Meter CR-200, Minolta Co.) was used as an alternative method to check amylose content through L*a*b*. One milliliter of the starch-iodine solution was transferred into small white caps (Average L*, a* and b* of caps was approximately 71.92, −1.92 and −1.87, respectively). The color meter measurement unit was placed on the top of the caps to minimize any external light interference. Three successive measurements were performed on each cap and three for every entry. L*a*b* values were recorded and software was used to make the conversion to the RGB values and to find the relative color of the solution.

3. Data computing and color conversion, identification and reproduction

Excel Microsoft Office 2007 (Microsoft Inc.) was used for data assortment. Computing and statistical analysis (model linear regression equation and correlation) were performed by Statgraphics Centurion 16 (Statpoint Technologies Inc.). RGB conversion was performed by the free software, OpenRGB version 2.01.80406. (Logicol S.A.R.L.). R, G and B converted values were processed in ColorSchemer Studio version 2 for reproduction and color board making.

Results and Discussion

1. Estimation of amylose content by spectrophotometry

A linear regression equation was made from measurements at 620 nm. A statistically significant multiple linear regression (p < 0.05) was found between absorbance at 620 nm and the apparent amylose content of known genotypes, at 95% confidence level. The simple linear regression equation of the fitted model was:

\[ \text{Amylose content (\%)} = 1.48989 + 46.1109 \times \text{Abs620}; \]

where Abs620 is the absorbance at 620 nm in spectrophotometer. 

\[ R^2 = 0.90, \text{p}<0.01 \]

There was a highly significant (p <0.01) relationship between amylose content and absorbance at 620 nm. Since the correlation coefficient was 0.98, there was an indication of a strong relationship between the absorbance values and the amylose content. The analysis of variance of model (1) and (2) showed a highly significant relationship (p<0.01) between absorbance values and the apparent amylose content (Table 1 and Table 2).

2. Estimation of amylose content by colorimeter: L*a*b* and RGB values

A multiple linear regression model was computed using CIE L*a*b* values. The model’s regression equation was:

\[ \text{Amylose content (\%)} = 26.8306 - 0.318911 \times \text{L*} + 1.43819 \times \text{a*} - 0.925163 \times \text{b*} \]

L* is the intensity of lightness (0 stands for yields black);
a* is green (negative values) red (positive values); b* is blue (negative values) and yellow (positive values).

In this model, $R^2=0.99$, indicating that there was a strong relationship between $L^*a^*b^*$ values and the amylose content. The analysis of variance of the model showed a highly significant relationship ($p < 0.01$) between $L^*a^*b^*$ values and the apparent amylose content (Table 3).

Observed versus predicted values (Fig. 1) showed that the model could be used to predict amylose content. A strong and negative correlation was established between the amylose content and $L^*$, $a^*$ and $b^*$ values. This means that the darker the solution (decreasing $L^*$ value) the higher the amylose content, and the greener the solution (from $a$ to $-a$, $L^*$, $a^*$ and $b^*$ color space representation), the higher the amylose content. On the other hand, the lighter the solution, the lower the amylose content. The $b^*$ value showed highly negative significant correlation (Table 4). This means that the more bluish from ($b$ to $-b$) the solution, the higher the amylose content. $L^*a^*b^*$ values showed significant positive correlations with the amylose content. The results showed that $L^*a^*b^*$ values can be used to predict amylose content of rice, obtained by starch Table 2. Analysis of variance of the fitted linear regression model (2) for amylose estimation by spectrophotometry.

| Source   | Sum of Squares | Df  | Mean Square | F-Ratio | P-Value |
|----------|----------------|-----|-------------|---------|---------|
| Model    | 56.708         | 1   | 56.708      | 619.60  | 0.00    |
| Residual | 2.014          | 22  | 0.092       |         |         |
| Total (Corr.) | 58.721   | 23  |             |         |         |

Table 3. Analysis of variance of the fitted multiple linear regression model (3) for amylose estimation by colorimeter ($L^*a^*b^*$).

| Source   | Sum of Squares | Df  | Mean Square | F-Ratio | P-Value |
|----------|----------------|-----|-------------|---------|---------|
| Model    | 1672.840       | 3   | 557.613     | 4341.95 | 0.00    |
| Residual | 2.568          | 20  | 0.128       |         |         |
| Total (Corr.) | 1675.408   | 23  |             |         |         |

Table 4. Correlation between amylose content and $L^*a^*b^*$ values.

| Amylose content | $L^*$  | $a^*$  | $b^*$  |
|-----------------|--------|--------|--------|
| Amylose content | 1      | -0.9414** | -0.9429** | -0.997** |
| $L^*$           | 1      | 0.7794** |        | 0.9564** |
| $a^*$           | 1      |        | 0.9253** |
| $b^*$           | 1      |        |        |

Values with * and ** in the table indicate significant at $P<0.05$ and highly significant $P<0.01$ correlations, respectively.

Fig. 1. Relationship between predicted and observed amylose content. Predicted values were by $L^*$, $a^*$ and $b^*$ values.
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3. Difference in amylose prediction between different models

For amylose content prediction in Calmochi, models (2) and (3) were closer to the data provided with the genotype; model (1) showed a slight overestimation of amylose content 2.2% and 5.3% in Calmochi and Calmochi/Calamylose (A+B) instead of as 0% and 3% (Table 5). For Calamylow, model (3) was closer to the predictable amylose content; models (1) and (2) showed respectively 1.3 and 1.7 more points, the expected value. Same kind of differences occurs for low and intermediate amylose content with an advantage of the L*a*b* method to be very close to the predicted data. Highest difference was observed in low and high amylose content ranges for model (1) and (2), which tend respectively to underestimation and overestimation. Although these differences occurred, the results did not affect the standard classification and all checked genotypes were found to be in their respective classes. The model (2) increased the relationship for the prediction. In current conditions, the model (3) was the best for prediction.

However, iodine-amylose based methods have some limitations, due to the iodine binding capability of long branches of amyllopectin that may lead to an iodine complex coloration.

### Table 5. Comparison of amylose values from different models and methods.

| Entry            | True amylose content (%) | (1)         | (2)         | (3)         | Panel of representative colors |
|------------------|--------------------------|-------------|-------------|-------------|-------------------------------|
| Calmochi (A)     | 0                        | 2.2±0.1     | 0.1±0.1     | 0.0±0.1     | HEX: #9E9294                 |
|                  |                          |             |             |             | RGB: 158, 146, 148            |
|                  |                          |             |             |             | RGB: 142, 132, 136            |
| A+B              | 3                        | 5.3±0.2     | 4.7±0.3     | 3.3±0.1     | HEX: #8E8488                 |
|                  |                          |             |             |             | RGB: 120, 121, 128            |
| Calamylow (B)    | 6                        | 7.3±0.1     | 7.7±0.1     | 5.6±0.1     | HEX: #827980                 |
|                  |                          |             |             |             | RGB: 130, 121, 128            |
|                  |                          |             |             |             | HEX: #73707A                 |
|                  |                          |             |             |             | RGB: 115, 112, 122            |
| B+C              | 11.5                     | 10.9±0.1    | 12.2±0.2    | 11.9±0.1    | HEX: #73747F                 |
|                  |                          |             |             |             | RGB: 115, 116, 127            |
|                  |                          |             |             |             | HEX: #069DB78                |
|                  |                          |             |             |             | RGB: 105, 109, 120            |
|                  |                          |             |             |             | HEX: #616B79                 |
|                  |                          |             |             |             | RGB: 97, 107, 121             |
|                  |                          |             |             |             | HEX: #495162                 |
|                  |                          |             |             |             | RGB: 73, 81, 98               |
|                  |                          |             |             |             | RGB: 115, 116, 127            |

(1) Amylose content (%) = 0.682136 + 47.5078 × Abs620;
(2) Amylose content (%) = (6.2535 + 1.8305 × ln(Abs620))²;
(3) Amylose content = 45.0244 − 0.630471 × L* − 1.61567 × a* − 0.0679672 × b* (by colorimeter).

Abs620 means absorbance at 620 nm; a: relative color references computed obtained by OpenRGB and ColorSchemer Studio.

![Fig. 2. Relative color board derived from apparent amylose content characterization (obtained after conversion of L*a*b* to R, G and B).](image-url)
overestimation of amylose content (Chen and Bergam, 2007). Fitzgerald et al. (2009) mentioned that, although the standard solution was modified and the wave length changed from 540 nm to 640 and 720 nm, the interference of iodine-amylopectin complex, still remains one of the major limitations of iodine-amylose colorimetry based methods. Consequently, the method we describe here may also have limitations. The prediction of amylose using these methods might be more suitable to compare cultivars with similar amylopectin composition. Moreover, the described method may not be applicable to genotypes with amylose content exceeding the higher standard sample.

4. Color identification, reproduction and amylose content color determination for board

There are many systems for digitally representing and reproducing visible colors. The color identification was done using OpenRGB where L*a*b* data obtained from the colorimeter were input. A function provided the correspondent RGB values (0−255). The function match was applied to have panel of 8 colors and their hexadecimal names. This allowed choosing visually the matching colors by closely comparing with the solution. As amylose content is generally classified into five classes, five groups of colors were retained for the color board (Fig. 2). The software has the function which allows recalling color panel simply by typing the names, so this would permit classification within the 5 classes. A color board was made by using 8 solutions with different amylose concentrations and the software.

However, further improvement of the board in the range 3−17% is needed, since this range is very important for amylose classification.

Conclusion

The use of a colorimeter (L*a*b*, RGB and Hexadecimal codes) was helpful to predict amylose content with high precision and accuracy (Table 5, Fig. 1). This is an innovative way for amylose prediction in rice. It also definitively allows the establishment a specific color board and strengthens the use of a cheap color board to estimate apparent amylose content in rice grain (Ronoubigouwa et al., 2009), which can be used for determination of amylose estimation during first screening of breeding process. Both methods may also be applied to other starch based crops.

Acknowledgments

The present research was made possible by the Ministry of Education and Culture of Japan and the Tokyo University of Agriculture and Technology, through a special funding and an exchange program agreement with University of California Davis. Gratitude is addressed to Mr. Kameron Chun, the Manager of the Laboratory of Chemistry, Department of Biological and Agricultural Engineering, University of California Davis, for his kind assistance.

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