

Estimation of Alkali Spreading Value and Gelatinization Temperature of Some Philippine Rice Varieties Using Digital Photometry

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Gelatinization temperature (GT) is an important property of the rice grain which affects its cooking and eating qualities. The most reliable method for determining GT needs expensive equipment, namely differential scanning calorimeter (DSC). An alternative method is digital photometry (DP) which uses an inexpensive digital camera and free-access image processing software to determine the alkali spreading value (ASV) and GT of milled rice. The starch gel area of the images of alkali-gelatinized grains was measured after a given gelatinization time; the images were then taken with a digital camera inside a fabricated light box and analyzed using ImageJ. Literature GT values based on DSC (GT_{DSC}) for eight rice varieties were correlated with experimental values of relative increases in gel area after gelatinization, i.e., RIA_f and RIA_i based either on initial area or final area, respectively. The natural logarithm of RIA_i (ln RIA_i) was found to be highly correlated with GT_{DSC} values and was numerically equal (within experimental uncertainty) to published ASV values. The correlation equation of ln RIA_i versus GT_{DSC} allowed determination of GT values for three additional rice varieties and correctly predicted GT values for 29 rice varieties based on literature ASV data. Thus, DP can be used as a cheap and rapid method of estimating ASV and GT. DP values were similar to the conventional visual scoring method for ASV determination, but is less subjective, more precise and provides a permanent record of data.

Key words: alkali spreading value, differential scanning calorimetry, digital photometry, gelatinization temperature, image analysis, milled rice

Abbreviations: ASV – alkali spreading value; DSC – differential scanning calorimetry; GT – gelatinization temperature; NSIC – National Seed Industry Council; PSB – Philippine Seed Board; Rc – rice; RIA_f – relative increase in gel area (based on final area); RIA_i – relative increase in gel area (based on initial area)

INTRODUCTION

Rice Starch Gelatinization Temperature

Starch undergoes gelatinization upon addition of sufficient water and heat. A general mechanism for starch gelatinization had been proposed wherein the integrated water molecules in the starch granule first strip off resulting in disorganization of the amorphous region. When the amount of water becomes limited, the remaining partially hydrated or crystalline part of the starch granules melts as temperature increases (Donovan 1979). This melting process has been explained as a conformational transition of the polymer chains in the

starch granules from intramolecular double helix to coil conformation (Colonna and Mercier 1985). However, none of proposed theories can fully explain the exact mechanism of sequential structural changes of starch during gelatinization (Ratnayake and Jackson 2009).

The peak temperature at which starch absorbs heat is the gelatinization temperature (GT). Jennings et al. (1979) reported that GT ranges from 55 to 79°C and classified rice grains based on GT as low (<70°C), intermediate (70–74°C), and high (>74°C). The cooking quality of rice is highly influenced by GT, such that a low-GT rice variety requires less amount of water and cooks faster than one with high GT. Moreover, apparent amylose content (AC)-GT combination has been reported to predict the cooked

rice texture of Philippine rices, which include released modern varieties (Roferos et al. 2006), traditional varieties and farmers' specialty rices (Juliano et al. 2009; Tũaño et al. 2015), elite rice breeding lines (Tũaño 2013) and market rice samples (Tũaño et al. 2016), as evidenced by Instron hardness of freshly cooked and staled rice (Juliano 2007; 2010; Tũaño et al. 2011; Tũaño 2013; Tũaño et al. 2014). GT, which is mainly influenced by the amylopectin chain ratio allowing rice classification as having S-type or L-type amylopectin, affects cooked rice texture (fresh and staled) in that intermediate-AC rices with intermediate GT tend to have softer cooked rice than those with low GT while low-AC rices with low GT tend to have softer cooked rice than those with intermediate GT (Perez et al. 1993; Nakamura et al. 2006; Tũaño et al. 2011). With this information about GT of milled rice, farmers and consumers may be able to determine the eating quality of rice which allows selection of a suitable variety for planting and consumption, respectively. Therefore, there is need to develop simpler and less expensive but reliable methods for determining GT of rice.

Methods for Determining Rice Starch Gelatinization Temperature

Conventional determination of GT uses either an amylograph, a photometer measuring birefringence endpoint temperature (BEPT), or a differential scanning calorimeter (DSC). Halick et al. (1960) determined the GT of 20 rice samples using an amylograph and found the results to be accurate. Also, Russell and Juliano (1983) measured the GT of eight rice starches and found a high correlation between DSC GT values and photometric GT values using the BEPT method. Gill et al. (2010) reported that the DSC method (Nakamura et al. 2006) is probably the most versatile among all the methods used to study gelatinization of starch because the heat flow during transition of starch can be studied over a wide range of moisture content and temperature and requires less time. The DSC, BEPT and amylograph methods of determining rice starch GT are reliable, albeit expensive, due mainly to equipment purchasing and maintenance costs.

An alternative method for milled rice GT determination is the alkali spreading test wherein milled rice grains are incubated at room temperature for 23 h in a Petri dish containing dilute aqueous KOH (Little et al. 1958). During alkali spreading, KOH gelatinizes starch, particularly its amorphous region, causing degradation of the long linear and branched chains of amylose and amylopectin; this results in rice grain gelatinization. GT classification of rice varieties is done using the alkali spreading value (ASV) which is visually determined by a

trained laboratory technician after the 23-h alkali spreading test. ASVs of 1–2, 3, 4–5 and 6–7 correspond to high, high-intermediate, intermediate and low GT types, respectively (Little et al. 1958; Juliano 2010). This method is inexpensive, but is not very accurate in determining actual GT of milled rice due to the subjective nature of visual scoring of grain spreading. A promising improvement to subjective estimation of starch gel area after alkaline gelatinization of starch is digital photometry (DP) combined with image processing. In this method, the area of the starch gel after gelatinization for 23 h is quantitatively measured from the starch gel images resulting in greater accuracy for determining ASVs. Mariotti et al. (2010) correlated ASV with DP parameters and showed that the application of image analysis allows a faster and more accurate method, eliminates the subjectivity of the visual determination of GT, as well as provides a "digital history" of the gelatinization phenomena.

The present study deals with the application of DP, combined with image analysis using free-access software (ImageJ), to quantify the degree of starch gel spreading in terms of parameters that measure the relative increase in gel area (RIA). These experimental parameters were correlated with published ASV and GT values for several rice varieties (Tuano et al. 2014). The mathematical basis of the correlations observed in this study and in the previous report of Mariotti et al. (2010) is also discussed.

METHODOLOGY

Light Box Design and Fabrication

The light box, 35-cm long with rectangular ends (18.5 cm x 24 cm) the design of which is similar to that used by Suzuki et al. (2006) and Yanos et al. (2013), was made of plywood and painted white on the inside. The light source consisted of two light emitting diodes (Apollo Brand, Model No. E-27 LED GLB 3W DL, 220V, Day Light) which were positioned at the sides of the box away from a 14-megapixel Olympus v160 digital camera slightly above a 9-cm wide Petri dish.

Alkali Spreading of Milled Rice Grains and Image Collection

Eight rice varieties/breeding lines were used for correlation studies, namely NSIC Rc13, NSIC Rc19, Improved *Malagkit Sungsong 2*, PSB Rc14, NSIC Rc222, IR64, PR34627-7-12-3-1-3 (A), and *Ballatinao*; three rice varieties/breeding line were used as test samples, namely

NSIC Rc170, NSIC Rc108, and IR2071-137-5. Alkali spreading of rice starch gel was measured by first placing and arranging five milled grains of each rice variety in a 9-cm wide Petri dish containing 20 mL of aqueous KOH (1.7% w/v). The 44 Petri dishes (four per rice variety) were then positioned separately, such that when the light box was placed over one Petri dish and transferred to the other, the Petri dishes were not moved. Moreover, the Petri dishes were placed on a wide non-reflective black surface with white circle markings.

The images of the Petri dishes were taken, along with a 2.5-cm stick, using the camera in *Super Macro* mode which was mounted on the light box. One image can be analyzed within 5 min and the technique can be learned easily by an analyst even with no experience in computer techniques. The final images were then taken after 23 h of incubation at room temperature. Prior to determination of the reproducibility of the method, this experiment was repeated twice at two different days using two freshly prepared solutions of aqueous KOH (1.7% w/v).

Image Processing and Analysis of Alkali-gelatinized Milled Rice Grains

Free-access software (ImageJ version 1.48v) was installed on a Lenovo Idea Pad S410p, operated with Windows 8.1 and used for image analysis. The image was first dragged to the software's interface, enhanced by adjusting its contrast and gamma to 10 and 5, respectively, and bright outliers were removed with at most 10 pixels of radius. The resulting image was then inverted and converted to an 8-bit image and then to binary format. These settings were recorded into a plug-in device, allowing all the images to be enhanced at the same time with a single click. A scale was then set such that the unit pixel was expressed in cm. This procedure was specifically done by placing a straight line with the same length as the 2.5-cm stick on the image. The known length of the stick was inputted on the cell dedicated for the *known distance*, which can be found by clicking *Analyze* and then *Set Scale*. Finally, the area of the grains was measured through the *ROI Manager* tool in the software.

Correlation of Gel Area Parameters with Gelatinization Temperature

The calculated gel areas were converted into two related derived quantities which are proportional to the increase in rice grain area after gelatinization, namely RIA_i (relative increase in gel area - based on initial area) and RIA_f (relative increase in gel area - based on final area). These gel area parameters are defined below in Equations 1 and 2:

$$RIA_i = (A_f - A_i) / A_i = (A_f / A_i - 1) \text{ (Eq. 1)}$$

$$RIA_f = (A_f - A_i) / A_f = (1 - A_i / A_f) \text{ (Eq. 2)}$$

where A_i and A_f are the initial and final gel areas, respectively. Equations 1 and 2 have the same numerator but with A_i and A_f as denominators, respectively. The data calculated for the eight rice varieties were then correlated with GT values obtained using DSC data (GT_{DSC}) reported by Tuaño et al. (2014). GT values of the three test samples were then calculated using the equation generated from the correlation curve. These were then compared with the above mentioned DSC data of Tuaño et al. (2014).

Statistical Analysis for Method Assessment

The experimental method used in this study was assessed by analyzing the raw and correlated data using IBM SPSS Statistics Version 22. The method was tested for reproducibility by comparing their variances using the Levene test.

RESULTS AND DISCUSSION

Construction of Light Box

The light box was constructed with a fixed distance (at optimal focus) of the camera from the rice grains and with uniform light source prior to image processing before and after alkali spreading. Because the rice grains must not be disturbed prior to alkali spreading, the box was opened at its lower portion; thus, all rice varieties were simultaneously analyzed by just moving the light box from one sample holder to the other. Furthermore, the light source of the box was modified and positioned in order to avoid excessive lighting on the lens of the camera and at the sides of the sample holder.

Alkali Spreading of Rice Grains and Image Analysis

During image processing, the camera was manually set to *Super Macro* mode; the zoom position was fixed in order to make sure that the images were taken at the same zoom values. Furthermore, the camera did not have an option for changing f-stop and exposure time settings so these were automatically set at $f/3.4$ and $1/30$ sec, respectively. The amount of light that reached the camera was uniform for all images taken; the latter were enhanced and made binary prior to analysis. The camera settings were optimized in order to produce highly defined images of the rice grains that allowed (using

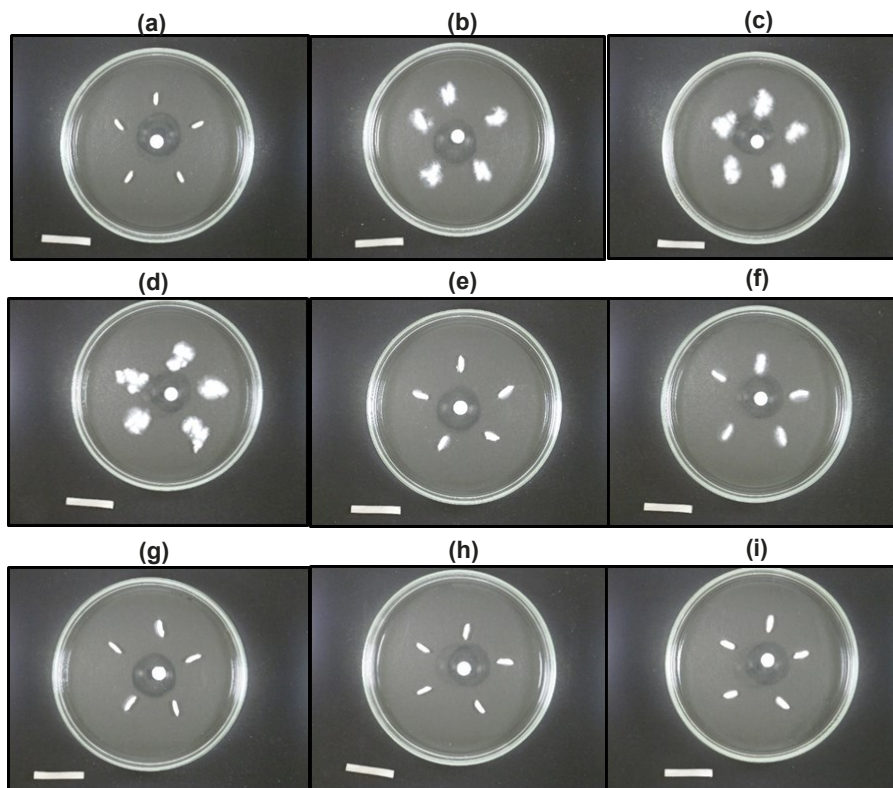


Fig. 1. Sample images before alkali spreading of (a) NSIC Rc13 and after alkali spreading for 23 h of (b) NSIC Rc13, (c) NSIC Rc19, (d) IMS 2, (e) PSB Rc14, (f) NSIC Rc222, (g) IR64, (h) PR34627-7-12-3-1-3 (A) and (i) *Ballatinao*.

ImageJ software) easy detection and measurement of rice grain areas before and after alkali spreading. Images of the rice grains in the Petri dishes after alkali spreading for the eight rice varieties are shown in Figure 1. Only one image/variety (Fig. 1a) for the intact (ungelatinized) rice

correlation against GT.

The correlation of RIA_f values with ASV and GT for rice has been reported by Mariotti et al. (2010) from data obtained using a visco-amylograph; these authors used the abbreviation RDA for RIA_f . The mean RIA_f values of

grains was included in order to save space and because there was no significant difference among the images of the eight ungelatinized milled rice grain samples.

Correlation of Experimental Data with Gelatinization Temperature

The calculated quantities RIA_i and RIA_f were determined from the measured gel areas of the rice grains after alkaline gelatinization. Values of the natural logarithm of RIA_i ($\ln RIA_i$) ranged from 3.6 to 6.5, which is the usual range for most rice varieties (Table 1). The plot of $\ln RIA_i$ against GT_{DSC} for the eight rice varieties (Fig. 2; data from Tuaño et al. 2014) had a higher absolute correlation coefficient (0.935) than the non-logarithmic plot of RIA_i (0.909). The degree of starch gel spreading has been traditionally quantified as ASV and was found to have a high

correlation against ASV. The correlation of RIA_f values with ASV and GT_{DSC} (Fig. 3 and 4) which showed linear correlation coefficients of 0.970 and 0.932, respectively. RIA_f was also highly correlated with $\ln RIA_i$ (Fig. 5). The results indicated that $\ln RIA_i$ is numerically equal to ASV. The correlation coefficient was statistically the same, namely 0.970, for linear plots of $\ln RIA_i$ and RIA_f with ASV. The use of DP and calculation of $\ln RIA_i$ from the digital images after milled rice grain gelatinization, as described

Table 1. Alkali spreading value (ASV), natural logarithm of relative increase in starch gel area - based on initial area ($\ln RIA_i$) and alkali-spreading data on gel areas (A_i and A_f) for eight rice varieties/elite breeding lines.

Rice Variety/Line Designation	A_i , cm ²	A_f , cm ²	$\ln RIA_i$	ASV*
NSIC Rc13	0.128 ± 0.011	0.699 ± 0.121	6.09 ± 0.17	6.0
NSIC Rc19	0.119 ± 0.007	0.783 ± 0.133	6.31 ± 0.22	6.0
Improved <i>Malagkit Sungsong 2</i>	0.143 ± 0.008	1.133 ± 0.159	6.53 ± 0.15	6.0
PSB Rc14	0.146 ± 0.012	0.258 ± 0.042	4.28 ± 0.39	4.6
NSIC Rc222	0.142 ± 0.010	0.410 ± 0.100	5.17 ± 0.36	5.0
IR64	0.132 ± 0.010	0.234 ± 0.054	4.25 ± 0.43	4.5
PR34627-7-12-3-1-3 (A)	0.142 ± 0.011	0.199 ± 0.030	3.63 ± 0.37	3.6
<i>Ballatinao</i>	0.144 ± 0.012	0.228 ± 0.024	4.03 ± 0.27	3.6

*Data from Tuaño et al. (2014).

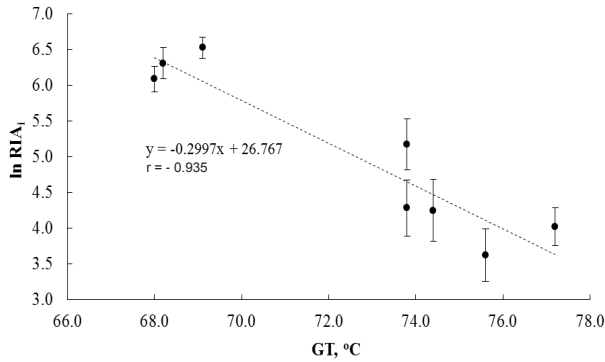


Fig. 2. Correlation of $\ln RIA_i$ with GT_{DSC} .

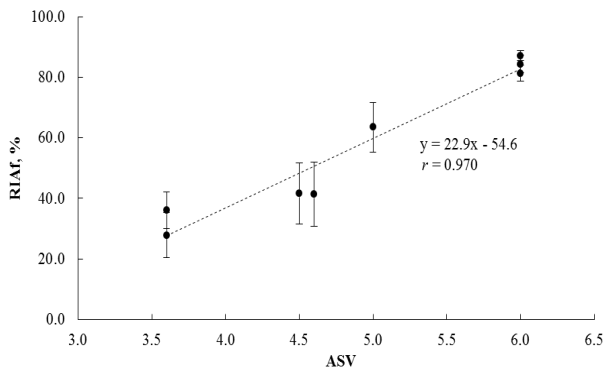


Fig. 3. Correlation of RIA_f with ASV.

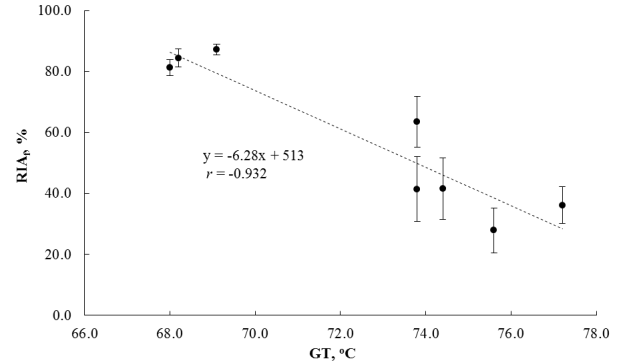


Fig. 4. Correlation of RIA_f with GT_{DSC} .

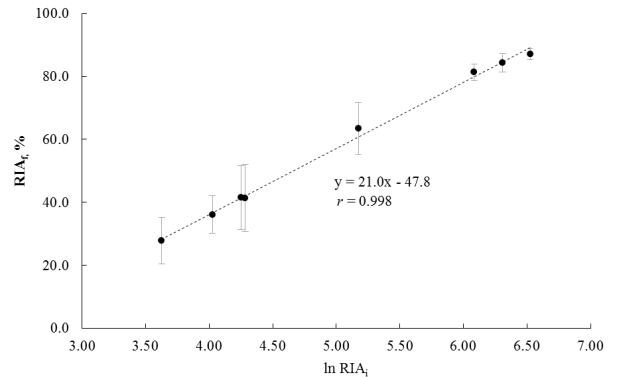
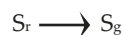


Fig. 5. Correlation of RIA_f with $\ln RIA_i$.

in this paper is quantitative, less subjective and appears to be more reliable, compared to the conventional visual method of determining ASVs. The DP method may be improved and automated to make it faster and more practical than the visual method. Furthermore, a permanent record of the calculated gelatinization parameters (such as RIA values) for many rice samples may be made and stored such that detailed statistical analyses of the DP data are possible.

In order to derive the equations which were used as basis for the calculated starch gel area parameters, consider the following reaction for gelatinization of starch in a raw rice grain:



where S_r is raw starch from a dry milled rice grain (~12–14% moisture) and S_g is gelatinized starch. The pseudo first-order rate equation for gelatinization is:

$$-d[S_r]/dt = d[S_g]/dt = k[S_r] \quad (\text{Eq.3})$$

where the brackets denote molar concentrations and the rate constant k contains the concentration of water which is large and may be assumed to remain constant. Consider the following mass conservation equation in

terms of substrate and product concentrations, $[S_r]$ and $[S_g]$, respectively:

$$[S_r] + [S_g] = C \quad (\text{Eq. 4})$$

where C is a constant. Rearrangement of Equation 4 and substitution into Equation 3 results in:

$$(d[S_g])/dt = k(C - [S_g]) \quad (\text{Eq.5})$$

which can be rearranged further and integrated to give

$$\ln ([S_g]/[S_r]) = -kt \quad (\text{Eq.6a})$$

The ratio of concentrations of gelatinized and raw starches ($[S_g]/[S_r]$) is correlated with the ratio of the corresponding final and initial areas (A_f/A_i) of the rice grain during gelatinization as measured in the ASV method. Based on literature data RIA_i , which is equal to $(A_f/A_i - 1)$ as shown in Equation 1, may be approximated by A_f/A_i with less than 5% error. Among 793 rices (which include modern varieties, traditional cultivars, rice breeding lines, market samples), $A_f/A_i \geq 20$ for 99% of the rices and $A_f/A_i \geq 100$ for 74% of the rices (Juliano 2010; Tuaño 2013; Tuaño et al. 2014, 2015, 2016). Calculation of ASV using the alkali spreading test involves grain starch gelatinization for a fixed time period, which is usually 23 h. This means that time t in Equation 6a is fixed and the

left-hand side of the equation, which is proportional to $\ln RIA_i$, is negatively correlated with the gelatinization rate coefficient k . The latter is dependent on rice variety, especially on the GT; the lower the GT the greater is the value of k .

Equation 6a can be written in terms of A_f/A_i , which is denoted as y , as follows;

$$\ln RIA_i = \ln (y-1) \approx \ln y \approx [(y-1)/y] = -kt \quad (\text{Eq. 6b})$$

The first equality in Equation 6b is the same as Equation 1. The approximation for $\ln (y-1)$, being equal to $\ln y$, is discussed above based on literature values of A_f/A_i and the approximation for $\ln y$ is based on the first term of its power series expansion where y is greater than or equal to $\frac{1}{2}$. It should be noted that $[(y-1)/y]$ is equal to RIA_f , which is defined by Equation 2, and is the same as the RDA parameter of Mariotti et al. (2010) who reported the linear relationship of RDA (or RIA_f) with ASV and GT for several rice varieties. This has been shown also in the present study to be true for eight rice varieties (Fig. 3 and 4). $\ln RIA_i$ is approximately equal to RIA_f as shown in the left-hand side of Equation 6b). However, $\ln RIA_i$ has the advantage (unlike RDA) of being numerically equal to ASV as shown in Table 1. Thus, $\ln RIA_i$ values obtained using the DP method described in the present study can be substituted for and compared with ASVs obtained using the visual scoring method.

Calculation of the Gelatinization Temperatures of the Test Samples

Additional three rice varieties were also subjected to alkali spreading in order to determine values of $\ln RIA_i$ and RIA_f . Corresponding values of GT were predicted

Table 2. Estimated gelatinization temperature (GT; in °C; mean \pm SD) from calculated values of natural logarithm of relative increase in starch gel area - based on initial area ($\ln RIA_i$) and relative increase in starch gel area - based on final area (RIA_f) for three additional rice varieties/breeding line.

Rice Variety/Line Designation	GT _{DSC} *	GT _{$\ln RIA_i$} #	GT _{RIA_f} #
NSIC Rc170	65.6	65.3 \pm 0.8	67.0 \pm 0.3
Difference		-0.3	1.4
NSIC Rc108	75.1	75.1 \pm 1.4	75.1 \pm 1.5
Difference		0.0	0.0
IR2071-137-5	75.8	76.2 \pm 1.2	76.5 \pm 1.0
Difference		0.4	0.7

*Data from Tuaño et al. (2014).

#Average values from two separate digital photometric trials.

and presented in Table 2 using the equations shown in Figures 2 and 4 ($GT_{\ln RIA_i}$ and GT_{RIA_f}). The estimated GT values from digital photometry were close to the GT values obtained using DSC. Calculated GT values from $\ln RIA_i$ were also close to those calculated from RIA_f (or RDA) (Table 2).

Table 1 shows the equivalence of $\ln RIA_i$ and ASV values for eight rice varieties included in the present study. However, this equivalence may be further tested for other rice varieties using the following correlation equation between $\ln RIA_i$ and GT which was generated using the experimental eight varieties (Fig. 2):

$$\ln RIA_i = -0.2997 (GT) + 26.767 \quad (\text{Eq. 7})$$

If $\ln RIA_i$ is really equivalent to ASV, substitution of $\ln RIA_i$ with the literature value of ASV (determined using the visual alkali spreading test) in Equation 7 should give a calculated GT value for the specified rice variety, which is statistically the same as the experimental GT obtained using DSC measurement. This is shown in Table 3 for 29 rice varieties wherein the GT values calculated using Equation 7 based on the experimental ASV values are compared with the experimental GT values obtained using DSC (Tuaño et al. 2014). The mean difference between the GT values calculated using Equation 7 (wherein $\ln RIA_i$ values were substituted by literature ASV values) and experimental GT_{DSC} values was 1.8°C. This value is three times the mean standard deviation of 0.6°C for GT_{DSC} reported by TUAÑO et al. (2014).

An additional advantage of using $\ln RIA_i$ as gel-spreading parameter, rather than RIA_f or RDA (Mariotti et al. 2010), is that Equation (7) obtained in the present study between $\ln RIA_i$ and GT can be used to estimate the value of GT for a given rice variety whose ASV value has been determined experimentally using the visual screening test; this results in a faster and simpler method.

Assessment of the Digital Photometric Method

Calculated values of $\ln RIA_i$ for two separate trials of the DP experiment were tested for reproducibility (Table 4); the null hypothesis showed that the results are equal at $p < 0.01$ as shown by the Levene test, indicating that the DP method used to estimate milled rice GT in this study is reproducible.

SUMMARY AND CONCLUSION

Gelatinization temperature (GT) is an important physicochemical property which affects rice grain quality. However, existing methods for GT determination involve

Table 3. Estimated gelatinization temperature (GT; in °C; mean) values for 29 rice varieties/elite breeding lines calculated using correlation equations from digital photometry (DP) and published alkali spreading value (ASV) data and their comparison with actual GT values via differential scanning calorimetry (DSC).

Rice Variety/Line Designation	ASV*	GT _{DSC} *	GT _{ASV} #	Difference
Improved <i>Malagkit Sungsong 2</i>	6.0	69.1	69.3	0.2
NSIC Rc13	6.0	68.2	69.3	1.1
NSIC Rc19	6.0	68.0	69.3	1.3
<i>Ballatinao</i>	3.6	77.2	77.3	0.1
<i>Malgaya Special 12</i>	3.7	77.3	77.0	-0.3
PR25218-9-3-3-4 (G)	3.9	77.4	76.3	-1.1
PR36900-B-20-2 (G)	3.3	76.7	78.3	1.6
PR36930-B-20-1-1 (G)	3.3	76.9	78.3	1.4
RD4	3.0	77.6	79.3	1.7
<i>Tapol</i>	3.8	76.5	76.6	0.1
IR24	7.0	65.9	66.0	0.1
NSIC Rc160	6.0	64.7	69.3	4.6
NSIC Rc170 ⁺	6.0	65.6	69.3	3.7
NSIC Rc218	6.0	66.5	69.3	2.8
<i>Sinandomeng</i>	6.0	65.2	69.3	4.1
IR2071-137-5 ⁺	3.0	75.8	79.3	3.5
<i>Kasturi</i>	3.6	73.6	77.3	3.7
NSIC Rc108 ⁺	3.0	75.1	79.3	4.2
NSIC Rc130	3.6	5.1	77.3	2.2
PR34627-7-12-3-1-3 (A)	3.6	75.6	77.3	1.7
PR35766-B-24-1	3.5	74.8	77.6	2.8
PSB Rc52	3.2	75.2	78.6	3.4
NSIC Rc222	4.5	74.4	74.3	-0.1
IR64	4.8	73.9	73.3	-0.6
NSIC Rc142	4.7	73.5	73.3	0.1
NSIC Rc172	4.6	73.8	74.0	0.2
PSB Rc14	5.0	70.0	72.6	2.6
PSB Rc18	4.7	74.8	73.6	-1.2
PSB Rc82	5.0	73.8	72.6	-1.2
Mean Difference				1.8

*Data from Tuaño et al. (2014).

⁺Used as test samples in this study.[#]Calculated using Equation 7 wherein the ASV values reported by Tuano et al. (2014) were substituted for the calculated values of $\ln RIA_i$.

either expensive equipment, such as DSC or amylograph, or a highly subjective test for estimating gel area after grain gelatinization, such as visual scoring of alkali spreading value. In the present study, literature GT values for eight rice varieties obtained using DSC were correlated with values of relative increase in gel area (RIA_i and RIA_t) after gelatinization with alkali. The RIA

values were determined from images of the rice grains before and after alkali spreading using an inexpensive digital camera and a fabricated light box followed by image analysis using ImageJ software. Values of $\ln RIA_i$ were found to be numerically equal to ASV values of the studied rice varieties. The correlation equation of $\ln RIA_i$ with GT_{DSC} allowed reasonable estimation of GT values

Table 4. Differences in experimental values of natural logarithm of relative increase in starch gel area – based on initial area (ln RIA_i) for two separate digital photometric trials using 11 rice varieties/elite breeding lines.

Rice Variety/ Line Designation	ln RIA _i	
	Trial 1	Trial 2
NSIC Rc13	6.06 ± 0.24	6.07 ± 0.17
NSIC Rc19	6.25 ± 0.32	6.31 ± 0.22
Improved <i>Malagkit Sungsong 2</i>	6.45 ± 0.14	6.60 ± 0.12
PSB Rc14	4.28 ± 0.46	4.29 ± 0.32
NSIC Rc222	5.05 ± 0.30	5.29 ± 0.38
IR64	4.32 ± 0.48	4.18 ± 0.39
PR34627-7-12-3-1-3 (A)	3.58 ± 0.36	3.66 ± 0.37
<i>Ballatinao</i>	3.88 ± 0.28	4.14 ± 0.18
NSIC Rc170	7.12 ± 0.27	7.27 ± 0.22
NSIC Rc108	4.33 ± 0.39	4.18 ± 0.42
IR2071-137-5	4.11 ± 0.72	3.92 ± 0.21

for three additional rice varieties. This correlation equation also allowed prediction of GT values for 29 rice varieties based on experimental ASV data which closely correspond to GT values directly obtained from DSC data, where both experimental ASV and GT data were obtained from the literature.

The use of DP and calculation of ln RIA_i from the digital images after milled rice grain gelatinization is less subjective and appears to be more reliable for determining ASV compared to the conventional visual scoring method. Furthermore, the DP method requires much cheaper equipment compared to the DSC and amylograph methods for obtaining ASV and GT data needed in rice breeding programs; it also provides a permanent quantitative record of experimental data.

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REFERENCES CITED

- COLONNA P, MERCIER C 1985. Gelatinization and melting and maize and pea starches with normal and high amylose genotypes. *Phytochem* 24: 1667–1674.
- DONOVAN JW. 1979. Phase transitions of the starch-water system. *Biopolymers* 18: 263–275.
- GILL P, MOGHADAM TT, RANJBAR B. 2010. Differential scanning calorimetry techniques: Applications in biology and nanoscience. *J Biomol Tech* 4: 167–193.
- HALICK JV, BEACHELL HM, STANSEL JW, KRAMER HH. 1960. A note on the determination of gelatinization temperatures of rice varieties. *Cereal Chem* 37: 670–672.
- JENNINGS PR, COFFMAN WR, KAUFFMAN HE. 1979. Rice Improvement. Los Baños, Laguna, Philippines. International Rice Research Institute. 186+ p.
- JULIANO BO. 2007. Rice Chemistry and Quality. Nueva Ecija, Philippines: Philippine Rice Research Institute. 402 p.
- JULIANO BO. 2010. Grain Quality of Philippine Rice. Nueva Ecija, Philippines: Philippine Rice Research Institute. 60 p.
- JULIANO BO, PEREZ CM, RESURRECCION AP. 2009. Apparent amylose content and gelatinization temperature types of Philippine rice accessions in the IRRI gene bank. *Philipp Agric Scientist* 92: 106–109.
- LITTLE RR, HILDER GB, DAWSON EH. 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. *Cereal Chem* 35: 111–126.
- MARIOTTI M, FONGARO L, CATENACCI F. 2010. Alkali spreading value and image analysis. *J Cereal Sci* 52: 227–235.
- NAKAMURA Y, SATO A, JULIANO BO. 2006. Short-chain distribution of debranched rice starches differing in gelatinization temperature or cooked rice hardness. *Starch/Stärke* 58: 155–160.
- PEREZ CM, VILLAREAL CP, JULIANO BO, BILIADERIS CG. 1993. Amylopectin-staling of cooked nonwaxy milled rices and starch gels. *Cereal Chem* 70: 567–571.
- RATNAYAKE WS, JACKSON DS. 2009. Starch gelatinization. *Adv Food Nutr Res* 55: 221–68.
- ROFEROS LT, FELIX ADR, JULIANO BO. 2006. The search for the grain quality of raw and cooked IR64 milled rice among Philippine Seed Board Rice varieties. *Philipp Agric Scientist* 89: 58–70.

- RUSSELL PL, JULIANO BO. 1983. Differential scanning calorimetry of rice starches. *Starch/Stärke* 35: 382–386.
- SUZUKI Y, ENDO M, JIN J, IWASE K, IWATSUKI M. 2006. Tristimulus colorimetry using a digital still camera and its application to determination of iron and residual chlorine in water samples. *Anal Sci* 22: 411–414.
- TUAÑO APP. 2013. Physicochemical characterization and starch fractionation of selected Philippine rices with atypical grain quality. [MS Biochemistry Thesis]. Los Baños, Laguna, Philippines. University of the Philippines Los Baños. 97+ p. (Available at the UPLB Main Library and PhilRice Main Library).
- TUAÑO APP, AOKI N, FUJITA N, OITOME NF, MERCA FE, JULIANO BO. 2014. Grain and starch properties of waxy and low-apparent amylose Philippine rices and of NSIC Rc222. *Philipp Agric Scientist* 97: 329–339.
- TUAÑO APP, PEREZ LM, PADOLINA TF, JULIANO BO. 2015. Survey of grain quality of Philippine farmers' specialty rices. *Philipp Agric Scientist* 98: 446–456.
- TUAÑO APP, REGALADO MJC, JULIANO BO. 2016. Grain quality of rice in selected retail stores and supermarkets in the Philippines. *Int J Philipp Sci Technol* 9: 15–22.
- TUAÑO APP, UMEMOTO T, AOKI N, NAKAMURA Y, SAWADA T, JULIANO BO. 2011. Grain quality and properties of starch and amylopectin of intermediate- and low-amylose Indica rices. *Philipp Agric Scientist* 94: 140–148.
- YANOS AA, BAUTISTA MN, ANGELIA MRN, DEL ROSARIO EJ. 2013. Digital photometric determination of protein using Biuret, Bradford and Bicinchoninic acid reagents. *Philipp Sci Lett* 6: 168–175.