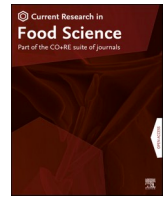


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Analysis of apparent amylose content of market milled rice via digital image photometry using a smartphone camera

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ABSTRACT

Apparent amylose content (AC) of market milled rice was analyzed through digital image photometry (DIP) utilizing a smartphone camera and a free-access software (ImageJ). The DIP-AC method was validated using seven test samples and applied to a set of 17 commercially available milled rices varying in AC. A light box was constructed to accommodate a cuvette and a smartphone while ImageJ was used for digital image analysis towards quantifying AC. Smartphone camera settings were also optimized using the red, green, and blue (RGB) values of the digital images of amylose-iodine blue solutions. ISO 100 combined with shutter speed 1/640 was the optimum and most suitable settings combination when B values were used to generate calibration curves yielding a high degree of linearity ($r = 0.995\text{--}0.998$). Validation showed the DIP-AC method to be accurate based on the conventional ultraviolet–visible (UV-vis) spectrophotometric AC assay. It was also found to be repeatable and precise for non-waxy rice samples only, yielding RSD values below 7% among all replications made within one day and across different days. With the optimized DIP-AC assay, limits of detection and quantitation of AC that is capable of iodine binding at alkaline pH and influencing cooked rice texture, were 0.2% and 0.4% (milled rice basis at 12–14% moisture), respectively. The reported DIP-AC method can be a reliable and accurate assay for determining AC of non-waxy milled rice alternative to UV-vis spectrophotometry. Further refinement of the DIP-AC method is warranted to improve precision in measuring AC of milled waxy rice.

1. Introduction

Several analytical techniques have been developed for the quantification of different analytes in a variety of sample matrices including industrial, environmental, and food samples. Spectroscopy is one of the most common analytical techniques based on the interaction between electromagnetic radiation and matter which can be used in accurate quantitative measurements of various substances (Royal Society of Chemistry, 2017). Different analytes have distinct capabilities of absorbing or transmitting light and the amount of light absorbed by a substance is directly proportional to its concentration based on the Beer-Lambert's Law (Christian et al., 2014). In the analysis of several food components and properties, the ultraviolet (UV) and visible (vis)

regions of the electromagnetic spectrum are two of the most important regions used in spectrophotometric analyses and the most reliable and commonly used instrument in colorimetric determinations is the UV-vis spectrophotometer. However, procurement of said equipment involves high acquisition price and its maintenance cost is usually expensive. A relatively new analytical technique called digital image photometry (DIP) has been used to quantify specific analytes in various samples through examining the color properties of digital images of colored solutions or sample surfaces using free-access image analysis software such as ImageJ (Abramoff, 2004; Simpson, 2010). The software allows one to evaluate a digital image and generates a sample solution's red, green, and blue (RGB) values which are then plotted into graphs, incorporated into derived equations, and interpreted consequently to yield

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quantitative data. DIP has been used to quantify certain analytes in various sample matrices such as manganese and chromium in steel (Vedad et al., 2015), iron and residual chlorine in water samples (Suzuki et al., 2006), chemical oxygen demand of untreated and treated wastewater samples from a hog slaughterhouse (Mallari et al., 2018), xylose in liquid fermentation medium of four different yeast strains (Alojado-Rubianes and del Rosario, 2019), proteins in liquid solutions (Yanos et al., 2013), anthocyanins in solutions (Van Buren et al., 1974), and total phenolics in aqueous extracts of brown and red algae (Sinad and del Rosario, 2013). DIP has also been applied in a number of experiments related to agriculture and food science such as determining the: (a) degree of harvest maturity and ripeness of papaya (Calegario et al., 1997; Bron and Jacomino, 2006; Domingo et al., 2012), (b) color

and quality of red wine (Almela et al., 1995), and (c) gelatinization temperature and alkali spreading value of milled rice grains (Tuño et al., 2018), among others. In addition, through DIP along with certain chromaticity experiments, the color, pigment content, and antioxidant capacity of pigmented corn cultivars (Libron et al., 2021) and some fruits and vegetables (McGhie and Ainge, 2002; Cömert et al., 2020) have been analyzed, as well as the variability of insect and plant colors (Byers, 2006). These studies have shown the potential of digital image photometry as an alternative to spectrophotometric methods in measuring physical and chemical properties of different food crops, including rice, the primary staple cereal in many countries, especially in Asia.

Starch is the major component of rice grains composed of amylose

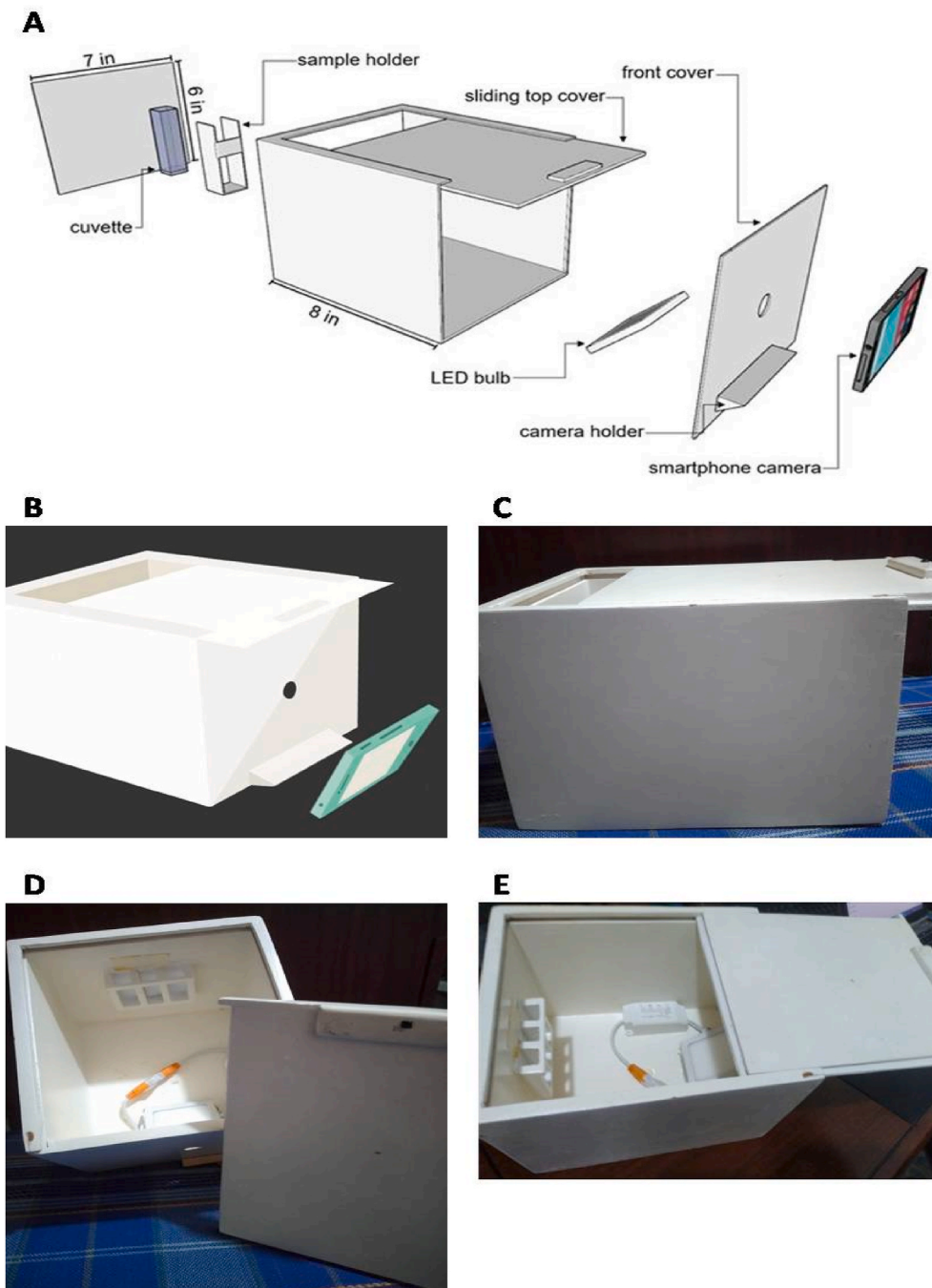


Fig. 1. Schematic representation of the fabricated light box with dimensions and locations of the sample holder, LED bulb, and smartphone camera (A and B). Photos (side and top views) of the actual light box constructed and used in this study (C–E).

and amylopectin. Milled rice amylose level varies depending on the type and variety of rice and consequently affect its eating and cooking quality (Juliano, 2007; Bao, 2019; Juliano and Tuño, 2019). Apparent amylose content (AC) is considered one of the main determinants of rice cooking and eating quality aside from amylopectin properties such as gelatinization temperature, gel consistency, amylopectin staling, and pasting viscosity (Juliano, 2007, 2010; Bergman, 2019). Rice varieties, breeding lines, and market milled rice samples in the Philippines may be classified in terms of AC as follows: waxy or glutinous rice (0–2%), very low (2–10%), low AC (10–17%), intermediate AC (17–22%), and high AC (>22%) (Juliano et al., 2012; Tuño et al., 2015, 2016). Higher AC results in firm, dry, and separated grains when cooked while lower AC gives soft and moist cooked rice (Juliano, 2007; Bergman, 2019). Milled rice AC is commonly measured in several rice breeding programs and grain quality laboratories using iodine colorimetry either at acidic or alkaline pH (Juliano, 1971; Juliano et al., 2012) while enzymatic, chromatographic, calorimetric, and dual wavelength iodine binding assays have also been developed and used in some laboratories (Yun and Matheson, 1990; Mestres et al., 1996; Zhu et al., 2008; Fitzgerald et al., 2009; Bergman, 2019). However, these methods require relatively more expensive and sophisticated equipment, procedures, and/or reagents, hence, amylose-iodine colorimetry remains to be the primary method of choice of most grain quality evaluation analysts in terms of milled rice AC measurement, despite the many modifications employed to improve it (Williams et al., 1958; Perez and Juliano, 1978; Juliano et al. 1981, 2012; Zhu et al., 2008; (Fitzgerald et al., 2009; Tuño et al., 2015)).

The present study deals with the validation and application of DIP in analyzing the AC of commercially available milled rice samples using a smartphone camera and free-access image analysis software. To the best of our knowledge, this is the first report on the use a smartphone camera for rice AC analysis among the other pioneering works on DIP as applied to rice grain quality evaluation such as the estimation of rice gelatinization temperature (Tuño et al., 2018). In this study, a light box for sample holding and digital image capture was constructed (Fig. 1) while ImageJ was used for image processing and analysis. The smartphone camera-based DIP method for milled rice AC analysis yielded reproducible, precise, and accurate results as compared to the conventional UV–vis spectrophotometric method. Digital image photometry proved to be an innovative technique to measure AC of milled rice using a commonly used gadget nowadays such as smartphones and may be utilized in food analysis laboratories as an alternative to spectrophotometric AC assay.

2. Materials and methods

2.1. Rice samples, chemicals, and sample preparation

All of the samples ($n = 17$) used in this study were open-bin milled rices purchased from selected supermarkets in Dasmariñas City, Cavite, Philippines in February 2018. These were ground to pass a 60-mesh sieve, transferred into polyethylene zip-lock plastic bags and stored in a -10°C freezer until further analysis. All reagents used were analytical grade and distilled water was used in all experiments and replications.

2.2. Fabrication of the light box

A light box was fabricated using plywood (0.5 inch thick) following the specifications reported by Yanos et al. (2013), with slight modifications. The plywood was painted white all throughout and the light box had the following dimensions: $8 \times 7 \times 6$ inches. A sliding top cover was also made for easier sample transfer and to prevent the entry of stray light into the box. A hole fitted for the smartphone camera was drilled on the front cover of the light box. The source of white light was a 3-W rectangular Akari LED bulb placed 7-in away from the cuvette holder at the inner central portion and 1.5-cm below the hole for the smartphone camera (Fig. 1).

2.3. Preparation of blue-colored milled rice amylose-iodine solutions for AC analysis

AC of the market milled rice samples was determined according to the method of Juliano et al. (2012), with slight modifications. Milled rice flour (50 mg) was soaked in 1 mL of 95% ethanol and 4.5 mL of 1 M NaOH in a 50-mL conical screw-cap tube and allowed to stand undisturbed overnight for 16 h. Afterwards, distilled water (44.5 mL) was added to bring the solution up to 50 mL, and 0.5 mL aliquot was transferred into a 10-mL test tube containing 5 mL distilled water. Then, 0.1 mL of 0.9 M NH_4Cl was added and the solution was mixed thoroughly using a vortex mixer followed by addition of 0.2 mL of iodine solution (0.15% I_2 in 1.5% KI). The solution was then diluted to 10 mL using 4.2 mL distilled water. For the development of the calibration curves (with AC expressed in % of milled rice; dry basis), standard solutions of amylose at varying concentrations (1, 2, 5, 10, 15, 20, 25, 30, and 35% in 0.09 M NaOH) were used by diluting 100% (w/v) amylose stock solution. This was initially prepared using 50 mg Avebe potato amylose dissolved in 50 mL of 0.09 M NaOH. These were all treated similarly as mentioned earlier to obtain blue-colored amylose-iodine standard solutions. Standards and samples were prepared in triplicate for all replications and analyzed via UV–vis spectrophotometry vis-à-vis digital image photometry as described below.

2.4. AC measurement via UV–vis spectrophotometry

Aliquot from the resulting solution in Section 2.3 was transferred into a glass cuvette and absorbance was read at 620 nm within 20–60 min using Lambda 25 UV–vis spectrophotometer (PerkinElmer Pte., Ltd., Massachusetts, USA). Absorbance for each standard was plotted against the concentration and the resulting calibration curve was used to calculate for the AC of the samples. All spectrophotometric determinations were done in triplicate.

2.5. Optimization of DIP method for milled rice AC

The same set of amylose standard solutions was used in the optimization of the smartphone camera settings which was done in triplicate. Upon color development in Section 2.3, blue-colored amylose-iodine solutions were photographed inside the light box using a 12-megapixel Huawei P10 smartphone camera (Huawei Co., Ltd., Shenzhen, China). The smartphone camera settings ISO and shutter speed were varied for every replicated determination for the whole set of standard solutions. The captured digital images (Fig. 2) were then stored in a laptop computer (HP Pavilion 14 Laptop PC with Windows 10 operating system) and processed using ImageJ. The digital images' RGB values were obtained from ImageJ and converted into percentage values. These values were then plotted against the actual concentrations of the amylose standards to generate the calibration curves. Correlation coefficient (r) for each calibration curve was noted with respect to the corresponding smartphone camera settings combinations used. The camera settings combinations that yielded r values near 1.0 and t_{calc} values lower than the corresponding t_{crit} values per test run ($n = 3$ or 6) were selected and used in the succeeding experiments. All DIP determinations were done alongside UV–vis spectrophotometric measurements at 620 nm using Lambda 25 UV–vis spectrophotometer (PerkinElmer Pte., Ltd., Massachusetts, USA).

2.6. AC measurement via digital image photometry

Aliquot from the resulting solution in Section 2.3 was then transferred into a glass cuvette and placed in the cuvette holder inside the fabricated light box within 20–60 min. Digital image of each solution was captured through a 12-megapixel camera of Huawei P10 smartphone (Huawei Co. Ltd., Shenzhen, China). Smartphone camera parameters were set according to the most suitable combined settings

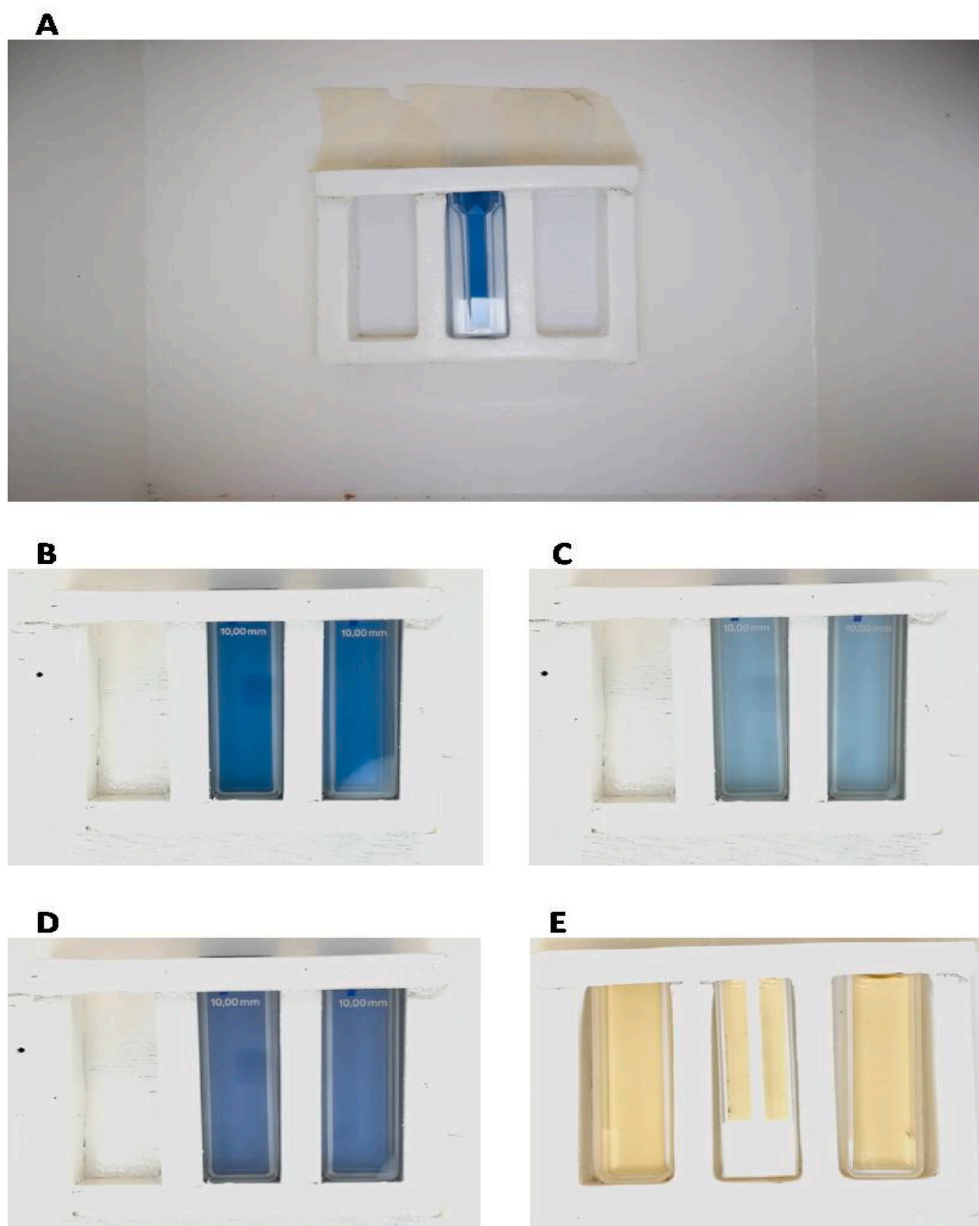


Fig. 2. Digital images of the amylose-iodine solutions of selected milled rice samples and amylose standards captured using smartphone camera settings ISO 100 and shutter speed 1/640: (A) non-waxy sample (single cuvette's full view); (B) 30% amylose standard (2 cuvettes' close up view); (C) 10% amylose standard (2 cuvettes' close up view); (D) non-waxy sample (2 cuvettes' close up view); (E) waxy sample (3 cuvettes' close up view).

selected in Section 2.5. Digital images were stored in a laptop computer and RGB values were determined after image processing via ImageJ as described below.

First, the digital photos (Fig. 2) were opened as a virtual stack by selecting 'File' in the left portion of the ImageJ toolbar, followed by clicking 'Import', then 'Image Sequence'. The folder containing the photos was located and accessed and the 'Use virtual stack' button was ticked. These command sequences would open all the photos in the selected folder as a stack according to their arrangement in the folder. To see the RGB channels of the photos, the following sequence of options was clicked, 'Image > Color > Make composite'. Then, the cursor was hovered towards a specific region for image analysis. The left mouse button was pressed and held to drag the cursor forming a rectangular region of interest within the digital image. Then, 'T' in the keyboard was pressed and the region was entered into a new window called the Region of Interest (ROI) manager. This was repeated for each photo and image parameter (i.e. R, G, and B values) in the whole image sequence

selection. The RGB values were obtained through the ROI manager and were displayed in another dialogue box. This was done by clicking the command sequence: 'Any of the 12-digit image area code > Ctrl + A > Measure'. The whole RGB values file was then saved as a spreadsheet by clicking 'File > Save As ...' and stored in a specific folder. The RGB values of the photos were then retrieved using Microsoft Excel and converted to %R, %G, and %B. The percent RGB values obtained from the digital images of the amylose standard solutions were then plotted against the actual concentrations to generate calibration curves which were subsequently used to interpolate and determine the AC of the milled rice samples via DIP. All digital image photometric determinations were done in triplicate.

2.7. Validation of DIP method for milled rice AC

The procedures for validating the DIP method for AC analysis were based on the requirements set by ICH and USP (Huber, 2007). The

accuracy of the DIP method was assessed by comparing the DIP AC values for seven milled rice test samples with their corresponding spectrophotometric AC values using paired sample two-tailed *t*-test at 95% confidence level and analysis of variance at $\alpha = 0.05$. Similarly, t_{calc} value per sample was calculated and compared with the t_{crit} value at $n = 3$ or $n = 6$, where appropriate. A higher t_{crit} compared to t_{calc} would denote no significant difference between the AC values being compared per sample. Corresponding data obtained from UV–vis spectrophotometry (i.e. absorbance) and digital image photometry (i.e. %R, %G, %B) for the standards were plotted and correlation coefficients (*r*) were evaluated through linear regression analysis. DIP-AC method's repeatability (RSD_r) was determined using the same seven test rice samples analyzed repeatedly (in triplicate) within the same day and by the same analyst, then, the standard deviation (SD) and relative standard deviation (RSD) were determined for all samples. RSD of less than 7% was considered acceptable rendering a method repeatable with respect to each sample in the set. Intermediate precision or intra-laboratory reproducibility (RSD_R) was represented by the SD and RSD values obtained per sample for each test run done in different analysis days and/or by two different analysts within the same laboratory. RSD of not more than 10% would render a method reproducible within said laboratory using the same set of test samples (Huber, 2007).

2.8. Statistical analysis

All data collected from the DIP AC assays employed in the method validation experiments were statistically evaluated using paired sample two-tailed *t*-test or analysis of variance (ANOVA), where appropriate, with the conventional UV–vis spectrophotometric method as basis. Likewise, data obtained from the application of the validated DIP AC assay on the whole sample set of market milled rices were statistically analyzed using *t*-test as compared to spectrophotometric AC results. Statistical mean comparisons were considered to show no significant difference when $p\text{-value} > 0.05$. Mean AC values were expressed as mean \pm SD, where appropriate. All statistical analyses were employed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 for Windows as similarly done in another study of a different group in the research team (Team APPT) published recently (Tuño et al., 2021).

3. Results and discussion

3.1. Optimization of smartphone camera settings

Optimization of the DIP method for AC was done by screening the linearity coefficients (*r*) of the calibration curves for %R, %G, and %B using combinations of ISO and shutter speed of the smartphone camera (Table 1). Results showed that %G calibration plots had *r* values lower than 0.9000 ranging from 0.1524 to 0.8792. These were generally lower than those obtained from the calibration curves generated using %R and %B. It was also observed that the combinations of smartphone camera settings that yielded the top three calibration curves with highly linear

Table 1

Correlation coefficients (*r*) of amylose standard calibration curves generated from digital images' red (R), green (G), and blue (B) values using different combinations of smartphone camera settings ISO and shutter speed and using Image J for image processing and analysis.

ISO	Shutter Speed	Correlation Coefficient (<i>r</i>)		
		%R	%G	%B
50	1/320	0.9964	0.8513	0.9986
100	1/640	0.9953	0.7634	0.9982
125	1/320	0.9940	0.2670	0.9858
160	1/1000	0.9968	0.7776	0.9989
200	1/1250	0.9961	0.8792	0.9977
250	1/640	0.9939	0.1524	0.9925

and positive *r* values were those of ISO 50 with shutter speed 1/320, ISO 100 with shutter speed 1/640, and ISO 160 with shutter speed 1/1000, considering %B (Table 1). Taking these three smartphone camera settings combinations, AC of the seven test rice samples was measured using %R, %G, and %B and compared with the UV–vis spectrophotometric AC (Table 2). Results showed that ISO 100 combined with shutter speed 1/640 had the lowest absolute t_{calc} values for all the samples ranging from 0.1 to 1.5 utilizing the %B image parameter. The other two image parameters (%R and %G) were observed to yield highly scattered AC data and large t_{calc} values (data not shown). AC obtained using %G ranged from 8.9% to 188.2% while %R gave 0.0–23.6% AC, across all the three combinations selected from the optimization experiment (Tables 1 and 2). For ISO 100 with shutter speed 1/640, %G generated significantly higher AC than spectrophotometric AC for all the test rice samples. On the other hand, AC values obtained for all the test samples using %R were relatively lower than the spectrophotometric AC results. This might be due to the blue-to-deep blue solutions obtained from rice amylose-iodine binding at alkaline pH, where the brown color of rice amylopectin-iodine complex is at the minimum (Zhu et al., 2008; Juliano et al., 2012). Additionally, the highly positive correlation between functional amylose levels in rice and the solutions' blue color intensities was reflected as measured by the %B parameter over %R and %G, without possible interference from amylopectin (Table 1) (Juliano et al., 2012), similar to the case of intense blue-colored solutions of Bradford dye-protein complexes in the study of Yanos et al. (2013). These indicated that %B was the most suitable image parameter for use in milled rice AC determination via DIP using the optimized settings of ISO 100 and shutter speed 1/640 for a Huawei P10 smartphone camera and were utilized in succeeding experiments.

3.2. Validation of DIP method for milled rice AC

AC determination for the seven test rice samples was performed on four different analysis days (twice per day) aside from the optimization experiments and these were termed as “test runs” from this point onwards. The two test runs with the highest linearity and accuracy, as compared to spectrophotometric AC, were used to assess the repeatability (RSD_r) and intra-laboratory reproducibility (RSD_R) or intermediate precision of the DIP-AC method. Repeatability was assessed for all image parameters %R, %G, and %B to determine the degree of scatter among multiple measurements for all the test rice samples analyzed under the same set of laboratory and analysis conditions (i.e. optimized smartphone camera settings and experimental set-up) (Tables 3 and 4) while intra-laboratory reproducibility was evaluated under the same operating conditions on different analysis days one month apart and done by different analysts (Table 5). Test run Day 1 results showed that in terms of %B, AC measurements using DIP had lower RSD as compared to UV–vis spectrophotometry as well as those obtained from %R and %G results, indicating %B as the most accurate image parameter for AC analysis via DIP (Table 3). All AC values from UV–vis spectrophotometry on test run Day 1 were repeatable having RSD lower than 7% ranging from 1.4% to 6.9% and mean RSD of 3.6%. Notably, only 5 out of 7 samples showed precise DIP-AC measurements using %B. These were all non-waxy rices showing RSD lower than 7% and ranging from 0.4% to 4.4%, indicating repeatable measurements. %R and %G were generally considered not repeatable yielding RSD greater than 7% and mean RSD of 12.6% and 25.6%, respectively, across all test rice samples. AC of the two waxy test rice samples (Malagkit 17 and MPRS Malagkit HB) measured via DIP were erratic resulting in RSD higher than 7% and as high as 70.6%, though %B yielded closer AC to those obtained from UV–vis spectrophotometry. Among the three image parameters, %B still yielded the lowest RSD for the waxy samples (Table 3). Data from a similar experiment done on test run Day 2 showed higher RSD for all waxy test rice samples in all image parameters, particularly %B, averaging at 29.5% (Table 4). When only non-waxy samples were considered, only %R and %B were noted to have RSD lower than 7% with %B

Table 2

Mean apparent amylose content (AC; %) for each test rice sample analyzed by UV–visible spectrophotometry and digital image photometry using % red (R), green (G), and blue (B) values and smartphone camera settings (ISO and shutter speed) combinations with highest linearity of calibration curves and lowest t_{calc} values.

Test Rice Sample	Mean Apparent Amylose Content (%)												
	UV–visible Spectrophotometry	ISO 50; 1/320			t_{calc} (for % B)	ISO 100; 1/640			t_{calc} (for % B)	ISO 160; 1/1000			t_{calc} (for % B)
		%R	%G	%B		%R	%G	%B		%R	%G	%B	
Passion <i>Maalsa</i>	26.3	22.3	43.6	24.8	1.6	23.6	47.9	26.1	0.8	22.8	167.5	28.9	6.5
Platinum <i>Sinandomeng</i>	25.8	20.7	50.9	24.1	1.2	22.0	56.4	25.4	1.0	20.6	182.4	27.1	1.2
Equal <i>Sinandomeng</i>	24.2	19.3	50.7	22.8	13.4	20.5	56.0	24.1	0.1	18.9	188.2	25.6	1.6
Jasmine	15.5	11.3	31.0	13.5	3.7	13.1	34.9	15.3	0.3	11.2	129.6	15.8	0.3
Malagkit 3	5.9	3.7	15.7	5.0	1.2	4.7	15.5	5.8	0.4	3.2	70.6	5.5	2.0
Malagkit 17	2.3	0.1	10.0	1.3	1.3	1.6	8.7	2.4	0.4	0.7	37.4	1.9	0.4
MPRS Malagkit HB	2.1	0.0	8.9	1.0	2.6	1.8	7.5	2.4	1.5	0.8	32.3	1.9	0.7

Table 3

Apparent amylose content (AC; %) of seven test rice samples analyzed in triplicate during test run Day 1 via UV–visible spectrophotometry and digital image photometry using % red (R), green (G), and blue (B) values and the relative standard deviation per sample.

Test Rice Sample	Replicate Number	Apparent Amylose Content (%)					Relative Standard Deviation (%)				
		UV–visible Spectrophotometry			Digital Image Photometry		UV–visible Spectrophotometry			Digital Image Photometry	
		%R	%G	%B	%R	%G	%R	%G	%B		
Passion <i>Maalsa</i>	1	26.7	23.6	49.1	26.2	1.3	1.8	8.9	0.4		
	2	26.0	23.2	51.5	26.0						
	3	26.1	24.1	43.2	26.0						
Platinum <i>Sinandomeng</i>	1	26.8	24.3	46.9	26.6	3.3	10.6	17.8	4.4		
	2	25.5	19.7	66.9	24.4						
	3	25.2	21.9	55.5	25.2						
Equal <i>Sinandomeng</i>	1	23.7	20.9	54.6	24.2	2.0	2.0	2.7	0.9		
	2	24.6	20.1	57.5	23.8						
	3	24.4	20.6	55.8	24.1						
Jasmine	1	14.7	12.3	40.8	15.1	5.1	9.8	25.2	1.8		
	2	15.6	12.5	39.1	15.2						
	3	16.3	14.6	24.8	15.6						
Malagkit 3	1	6.1	4.1	20.0	5.7	4.0	11.1	26.6	3.2		
	2	5.7	5.0	14.6	6.0						
	3	5.9	4.9	11.9	5.6						
Malagkit 17	1	2.3	2.1	1.6	2.1	2.4	27.5	70.6	11.1		
	2	2.3	1.5	12.1	2.6						
	3	2.2	1.2	12.5	2.4						
MPRS Malagkit	1	2.2	2.0	8.2	2.6	6.9	25.3	27.4	12.1		
	2	2.1	2.1	5.2	2.4						
	3	1.9	1.3	9.1	2.1						

yielding the lowest RSD values, averaging at 4.2%. These imply that the DIP method for AC determination might be limited to non-waxy milled rices only considering precision and repeatability of measurements. However, the observed limits of detection (LOD) and quantitation (LOQ) for the DIP-AC method using %B image parameter for all test runs were 0.2% and 0.4% (milled rice basis), respectively, which cover the whole waxy-to-non-waxy rice AC spectrum. This might explain the still accurate AC measurements for waxy test samples compared to UV–vis spectrophotometric AC as basis, despite low precision (Table 5). These observations might be due to the very faint yellow or faint yellowish-brown color of the waxy rice solutions upon addition of I_2/KI reagent at alkaline pH (Juliano et al., 2012; Tũaño et al., 2014) leading to inconsistent %B measurements through the ImageJ software. This could be also explained by the fact that at alkaline pH, where amylopectin and fat interferences are at the minimum, only functional amylose (i.e. amylose + long-chain amylopectin; capable of effectively binding iodine and highly and significantly influencing cooked rice texture, both freshly cooked and retrograded) can form stable amylose-iodine blue complex, hence the term apparent amylose content (Hizukuri et al., 1989; Horibata et al., 2004; Hanashiro et al., 2005; Juliano et al., 2012). True-to-type and pure waxy rice varieties essentially have amylopectin only and do not contain functional amylose affecting the variable %B values observed for the waxy test rice samples in this study (Inouchi

et al., 2005; Tũaño et al., 2014). Similar observation was previously noted in the development of the field test for AC type determination validated using 61 Philippine rice varieties and applied to 1,600 market rice samples (Tũaño et al., 2009). When milled rice grains were stained with I_2/KI solution, distinct purplish blue-to-deep blue color was observed for non-waxy rice grains allowing their classification into the non-waxy AC types, including those with very low AC. However, milled waxy rice grains, as expected, did not produce any trace of the blue amylose-iodine complex on the surface and remained to be opaque white grains upon staining (Tũaño et al., 2009). In general, AC determination via DIP was most precise and reproducible using %B over %R and %G, among non-waxy rice samples only. It exhibited RSD values lower than 7% indicating small degree of scatter among the AC data generated for each non-waxy test rice sample in two separate test runs rendering the method repeatable (Tables 3 and 4).

DIP-AC data from two different test runs employed a month apart by two analysts (within the same laboratory) were evaluated for intermediate precision or intra-laboratory reproducibility (RSD_R). Mean AC values from both DIP experiments using the %B image parameter were compared. DIP-AC determination using %B showed RSD_r lower than 1.5% with mean RSD_r of 0.6%, corroborating repeatability tests earlier mentioned. However, RSD_R higher than 10% were observed for the three waxy test rice samples averaging at 19.1% while all non-waxy test rice

Table 4

Apparent amylose content (AC; %) of seven test rice samples analyzed in triplicate during test run Day 2 via UV–visible spectrophotometry and digital image photometry using % red (R), green (G), and blue (B) values and the relative standard deviation per sample.

Test Rice Sample	Replicate Number	Apparent Amylose Content (%)				Relative Standard Deviation (RSD; %)			
		UV–visible Spectrophotometry		Digital Image Photometry		UV–visible Spectrophotometry		Digital Image Photometry	
		%R	%G	%B	%R	%G	%B		
Passion <i>Maalsa</i>	1	25.6	22.4	78.4	26.4	2.4	0.5	4.1	0.9
	2	26.8	22.3	72.4	25.9				
	3	25.8	22.5	74.4	26.2				
Platinum <i>Sinandomeng</i>	1	25.8	20.3	66.8	23.8	2.2	6.2	7.1	5.9
	2	26.4	22.9	76.7	26.8				
	3	26.9	21.4	73.7	25.2				
Equal <i>Sinandomeng</i>	1	25.0	18.1	93.4	23.6	0.7	9.5	18.1	6.1
	2	25.0	21.4	95.5	26.6				
	3	24.7	21.6	67.7	25.1				
Jasmine	1	17.7	11.2	73.2	16.1	3.9	4.7	3.7	3.9
	2	17.2	11.1	73.4	16.1				
	3	16.4	10.3	68.7	15.0				
<i>Malagkit</i> 3	1	7.0	−0.7	49.5	4.0	3.5	150.3	6.8	25.0
	2	7.5	2.2	46.9	6.5				
	3	7.4	1.7	53.6	6.4				
<i>Malagkit</i> 17	1	3.3	−2.3	39.2	2.0	7.8	35.6	13.8	40.0
	2	3.9	−1.9	50.9	3.0				
	3	3.7	−3.7	49.7	1.4				
MPRS <i>Malagkit</i>	1	3.4	−2.5	47.4	2.3	13.9	31.7	8.1	20.7
	2	3.3	−3.2	55.1	2.2				
	3	2.6	−1.7	48.8	3.1				

Table 5

Comparison of mean apparent amylose content (AC; %) of seven test rice samples obtained from for two different test runs of digital image photometric AC assay done in triplicate and a month apart within the same laboratory as well as repeatability (RSD_r) and intra-laboratory reproducibility/intermediate precision (RSD_R) of the measurements per sample.

Test Rice Sample	Apparent Amylose Content (%)		RSD _r (%) ^a	RSD _R (%) ^b	Significantly Different? ^c
	Digital Image Photometry				
	Test Run 1	Test Run 2			
Passion <i>Maalsa</i>	26.1 ± 0.1	26.2 ± 0.2	0.2	0.7	No
Platinum <i>Sinandomeng</i>	25.4 ± 1.1	25.3 ± 1.5	1.3	4.6	No
Equal <i>Sinandomeng</i>	24.1 ± 0.2	25.1 ± 1.5	0.9	4.5	No
Jasmine	15.3 ± 0.3	15.7 ± 0.6	0.5	3.1	No
<i>Malagkit</i> 3	5.8 ± 0.2	5.6 ± 0.6	0.4	15.8	No
<i>Malagkit</i> 17	2.4 ± 0.3	2.1 ± 0.9	0.6	25.7	No
MPRS <i>Malagkit</i>	2.4 ± 0.3	2.5 ± 0.5	0.4	15.9	No

^a RSD_r - relative standard deviation; repeatability.

^b RSD_R - relative standard deviation; intra-laboratory reproducibility/intermediate precision.

^c No indicates "no significant difference between the AC values being compared per row" based on $t_{crit} > t_{calc}$ via *t*-test and *P* value > 0.05 via ANOVA.

samples yielded RSD_R lower than 7%, averaging at 3.3% (Table 5). Despite the high RSD_R for waxy test rices, statistical analysis showed that AC measured for all samples in these two test runs had no significant difference with each other. Linearity was also evaluated through the *r* values of the calibration curves for these two test runs vis-à-vis parallel UV–vis spectrophotometric runs. The latter yielded exactly the same *r* value of 0.9996 across two analysis days (Table 6). Notably, both %R and %G showed negative *r* values indicating inverse relationship of

Table 6

Correlation coefficients (*r*) of amylose standard calibration curves generated from digital images' % red (R), green (G), and blue (B) values using smartphone camera settings ISO 100 and shutter speed 1/640 for two different test runs done in triplicate and a month apart within the same laboratory.

Test Run	UV–visible Spectrophotometry	Correlation Coefficient (<i>r</i>)		
		%R	%G	%B
Day 1	0.9996	−0.9933	−0.9244	0.9975
Day 2	0.9996	−0.9896	−0.7990	0.9945

these image color parameters with AC. Also, %R showed a relatively more linear curve than %G (based on *r* values of the calibration plots) which might explain the erratic AC values obtained using %G. On the other hand, %B showed highly positive linearity coefficients for both test runs implying that among the three image parameters, %B had the highest degree of linearity closest to 1.0, making it most suitable for milled rice AC determination via DIP (Huber, 2007). These results confirm the observations on linearity of calibration curves in the earlier optimization experiments.

Accuracy of the DIP-AC method was assessed by statistically comparing the mean AC of all DIP determinations with those obtained from the conventional colorimetric AC assay at alkaline pH via UV–vis spectrophotometry (Juliano et al., 2012) at 95% confidence level (Table 7). Absolute error and percent relative error of the DIP-AC results relative to UV–vis spectrophotometric AC (per sample) were also calculated. Absolute error for the seven test rice samples ranged from 0.1 to 0.4. Percent error relative to UV–vis spectrophotometry ranged from 0.8% to 5% with the highest noted for the three waxy test rice samples. AC via DIP showed no significant difference with UV–vis spectrophotometry yielding *p*-values greater than 0.05 (data not shown). Also, the computed t_{calc} values were small and lower than the t_{crit} values (Table 7), despite higher than 2% relative error for waxy rices. The latter might be attributed to the nature of the samples being waxy wherein even small decimal deviations in AC would result in high increase in % error. Overall, the optimized DIP-AC method using %B and the smartphone camera settings ISO 100 and shutter speed 1/640 was found to be accurate, precise, repeatable, and reproducible relative to the

Table 7

Comparison and accuracy testing of mean apparent amylose content (AC; %) of seven test rice samples based on the conventional UV–visible spectrophotometric AC assay at alkaline pH (Juliano et al., 2012).

Test Rice Sample	Apparent Amylose Content (%)			Relative Error (%)	t _{calc}	Significantly Different? ^a
	UV–visible Spectrophotometry	Digital Image Photometry	Absolute Error			
Passion <i>Maalsa</i>	26.3 ± 0.4	26.1 ± 0.1	0.2	0.8	1.5	No
Platinum <i>Sinandomeng</i>	25.8 ± 0.8	25.4 ± 1.1	0.4	1.5	1.2	No
Equal <i>Sinandomeng</i>	24.2 ± 0.5	24.1 ± 0.2	0.1	0.7	0.5	No
Jasmine	15.5 ± 0.8	15.3 ± 0.3	0.2	1.4	0.7	No
<i>Malagkit</i> 3	5.9 ± 0.2	5.8 ± 0.2	0.1	2.4	0.6	No
<i>Malagkit</i> 17	2.3 ± 0.1	2.4 ± 0.3	0.1	4.1	0.6	No
MPRS <i>Malagkit</i>	2.6 ± 0.1	2.5 ± 0.3	0.1	5.0	0.4	No

^a No indicates "no significant difference between the AC values being compared per row" based on t_{crit} > t_{calc} via t-test and P value > 0.05 via ANOVA.

conventional UV-vis spectrophotometric AC assay. DIP-AC assay using %G gave AC values that were inaccurate and inconsistent aside from failing to generate highly linear calibration curves. On the other hand, calculating AC using %R, though consistent and having relatively linear calibration plots, also yielded inaccurate AC relative to UV-vis spectrophotometry.

3.3. AC of market milled rice via DIP using a smartphone camera

Applying the validated DIP-AC method using a smartphone camera, AC of open-bin milled rices (n = 17) collected from supermarkets near the DLMSHI campus in Dasmariñas City, Cavite, Philippines was analyzed. Results showed no significant difference between the AC measured via DIP and via UV-vis spectrophotometry for all the market milled rice samples (Table 8). Mean absolute error was 0.2 for waxy rices and 0.4 for non-waxy rices, while mean % error relative to UV-vis spectrophotometric AC was 11.6% for waxy rices and 3.4% for non-waxy rices. All DIP measurements for non-waxy milled rices showed

high precision with SD ranging from 0.1 to 1.6. Mean AC obtained from UV-vis spectrophotometric method was found similar to those obtained from the validated DIP-AC method. Using the AC classification ranges for Philippine rice (Juliano et al., 2012; Tuño et al., 2015), 10 samples were classified as high AC, 2 as intermediate AC, 2 as low AC, 1 as very low AC, and 2 as waxy. However, the three samples with the lowest AC actually had opaque white grains, characteristic of waxy rices and identified as *Malagkit* (Filipino term for waxy rice) in the supermarket source (Table 8). All of these *Malagkit* samples had over 10% relative error and one was misclassified as very low AC (*Malagkit* 3), while the other two were classified as waxy (*Malagkit* 17 and MPRS *Malagkit* HB). They had AC higher than the 0–2% range for Philippine waxy rices in both the DIP and UV-vis spectrophotometric AC assays, despite having mainly opaque grains. This may imply that prior to grinding the samples into flour; the bulk milled rice grains for these three might have few non-waxy translucent grains as impurities which might have pulled their AC values a little over the AC range for waxy rices. Nonetheless, the very low-AC rice sample (*Malagkit* 3) must be reclassified as such since its

Table 8

Comparison and accuracy testing of mean apparent amylose content (AC; %) of 17 market milled rice samples based on the conventional UV–visible spectrophotometric AC assay at alkaline pH (Juliano et al., 2012).

Market Milled Rice Sample	Market Source	Apparent Amylose Content (AC; %)			Relative Error (%)	AC Type ^a	Significantly Different? ^b
		UV–visible Spectrophotometry	Relative Error (%)	Absolute Error			
Passion <i>Maalsa</i>	SM Savemore <i>Salitran</i>	26.3 ± 0.3	26.1 ± 0.1	0.2	0.4	High	No
Platinum <i>Sinandomeng</i>	SM Savemore <i>Salitran</i>	25.8 ± 0.9	25.4 ± 1.1	0.4	4.4	High	No
MPRS <i>Maharlika</i>	Waltermart Dasmariñas	25.4 ± 0.8	25.5 ± 0.6	0.1	2.5	High	No
Equal <i>Sinandomeng</i>	Puregold Jr. Dasmariñas	24.2 ± 0.5	24.1 ± 0.2	0.1	0.9	High	No
Equal <i>Dinurado</i>	Puregold Jr. Dasmariñas	24.0 ± 0.6	24.0 ± 0.8	0.0	3.5	High	No
GS Supreme Regular	SM Hypermarket Kadiwa	23.8 ± 0.4	23.7 ± 1.1	0.1	4.6	High	No
Platinum <i>Sinandomeng</i> <i>Mabango</i>	SM Hypermarket Kadiwa	23.7 ± 0.3	24.7 ± 1.0	1.0	4.4	High	No
Equal LG R-60	Puregold Jr. Dasmariñas	23.3 ± 1.1	23.6 ± 1.1	0.3	4.6	High	No
Commodore	SM Savemore <i>Salitran</i>	22.6 ± 0.4	21.1 ± 0.6	1.5	2.7	High	No
California WG	SM Savemore <i>Salitran</i>	22.4 ± 0.9	21.3 ± 1.3	1.1	1.4	High	No
GS Supreme	SM Savemore <i>Salitran</i>	21.4 ± 0.4	21.1 ± 1.6	0.3	4.7	Intermediate	No
MPRS <i>Dinurado</i> WHT	Waltermart Dasmariñas	20.3 ± 0.1	20.3 ± 0.6	0.0	3.0	Intermediate	No
Jasmine	SM Savemore <i>Salitran</i>	15.5 ± 0.8	15.3 ± 0.3	0.2	1.8	Low	No
MPRS Thai Jasmine	Waltermart Dasmariñas	12.8 ± 0.3	13.0 ± 1.4	0.2	0.9	Low	No
<i>Malagkit</i> 3	SM Savemore <i>Salitran</i>	5.9 ± 0.2	5.8 ± 0.2	0.1	10.9	Very Low	No
<i>Malagkit</i> 17	Puregold Jr. Dasmariñas	2.3 ± 0.1	2.4 ± 0.3	0.1	11.1	Waxy	No
MPRS <i>Malagkit</i> HB	Waltermart Dasmariñas	2.1 ± 0.1	2.4 ± 0.3	0.3	12.1	Waxy	No
Average (non-waxy)		21.2 ± 0.5	21.0 ± 0.8	0.4	3.4		
Average (waxy)		2.2 ± 0.1	2.4 ± 0.3	0.2	11.6		

^a Philippine rice AC classification - High >22%; Intermediate 17–22%; Low 10–17%; Very Low 2–10%; Waxy 0–2% (Juliano et al., 2012; Tuño et al., 2015).

^b No indicates "no significant difference between the AC values being compared per row" based on t_{crit} > t_{calc} via t-test and P value > 0.05 via ANOVA.

relatively higher AC than that of pure waxy rice might be expected to affect the texture and eating quality of the bulk milled rice grains when cooked due to the contaminating non-waxy grains. This was similarly observed in previous studies dealing with the survey of grain quality of open-bin milled rices in various retail stores and supermarkets in the Philippines (Tuano et al., 2016; 2020).

4. Concluding remarks

Validation and application of digital image photometry (DIP) for milled rice AC analysis, using seven test rice samples and 17 commercially available milled rice samples, respectively, showed that the DIP-AC method was accurate, precise, repeatable, and reproducible as compared to the conventional UV–vis spectrophotometry, especially for non-waxy milled rices. A fabricated light box was able to accommodate a cuvette and a smartphone camera for use in milled rice AC measurement via DIP. ISO 100 combined with shutter speed 1/640 was the most suitable settings combination for the smartphone camera used in this study (Huawei P10) giving similar AC values with those obtained using the UV–vis spectrophotometry, when the %B image parameter was considered. Optimization of smartphone camera settings, using amylose standard solutions and screening of image parameters with respect to the most linear calibration curve, must be done in case a different brand or model of smartphone camera shall be used for AC assay via DIP. However, similar sample preparation, color development, light box fabrication, image capture, processing, and analysis as described in this study may be employed. Some limitations of the present DIP-AC method were the precision and reproducibility in measuring waxy milled rice AC and further refinement experiments are warranted. DIP-AC method may also be applied in the analysis of a larger and more diverse set of rice samples including released varieties, farmers' selections, traditional rice cultivars, hybrid rice varieties, and elite breeding lines, in order to capture a representative majority of the rice types available in the field. This will increase the reliability and accuracy of the developed DIP-AC assay for use in grain quality evaluation and food analysis laboratories. Inter-laboratory testing and validation of DIP-AC assay is also necessary to show the reproducibility and wide applicability of the method.

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Conflict of interests

The authors declare no conflict of interest.

CRediT authorship contribution statement

Arvin Paul P. Tuano: Conceptualization, Supervision, Interpretation of the results, Writing – original draft, Writing – review & editing. **Gabrielle A. Castrillo:** Conceptualization, Performed the experiments, Interpretation of results. **Gabriel Angelo V. Viola:** Conceptualization, Performed the experiments, Interpretation of results. All authors have read and approved the final manuscript prior to submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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