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Determination of apparent amylose content, pasting properties and gel texture of rice starch by near-infrared spectroscopy

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Abstract: For breeding rice with improved quality, apparent amylose content (AAC), rapid visco analyser (RVA) pasting viscosities and gel texture properties may be routinely measured. As a direct measurement is time-consuming and expensive, rapid predictive method based on near-infrared spectroscopy (NIRS) is useful for measurement of these quality parameters. In this study, calibration models were developed using modified partial least-squares regression with different mathematical treatments based on the grain and flour spectra of non-waxy rice alone or in combination with waxy rice. The results showed that calibration models built with flour spectra are more robust than those with grain spectra, and with total rice including waxy rice are superior to those with only non-waxy rice. Some starch quality parameters, such as AAC, setback viscosity (SB), pasting temperature (PT), hardness (HD) and cohesiveness (COH) could be predicted with sufficient accuracy by NIRS based on flour spectra, whereas only AAC and PT could be predicted with sufficient accuracy based on grain spectra. All the models reported here are usable for rough sample screening (cold paste viscosity and breakdown viscosity), sample screening (SB, PT and COH) and for most applications (AAC and HD) for routine screening of a large number of samples in the early generation selection in breeding programs. However, for accurate assay of the pasting viscosity and gel textural parameters, direct instrumental measurement should be employed in later generations.

Keywords: amylose; near-infrared spectroscopy (NIRS); rice; starch; viscosity

INTRODUCTION

A number of genetic studies have proved that the quality traits of cereal crops, such as starch property of rice grain, have high heritability, indicating that early selection in a breeding programme for improvement of the end-use quality is necessary and efficient to obtain the desired target trait.[1,2] However, the crossbred lines are propagated and selected from generation to generation. The lines that are promoted to the next generation need to be those that have properties as close as possible to the specific target quality. Thus a problem arises as how to efficiently select these lines from thousands of candidates during the time interval between harvest and sowing of the next generation at the lowest cost.[3]

A rapid technique, near infrared spectroscopy (NIRS), has been widely used in screening of the quality traits in cereal breeding programs.[3–12] This technique has several well-known advantages, e.g. high speed of analysis, no sample preparation required, low cost per test and concurrent analysis of multiple constituents, over the conventional lab methods.[3] For the starch traits of rice, the amylose content measured by iodine-blue colorimetry is prone to inter-laboratory variability because of the complexity of the procedure and its reliance on amylose and amylopectin standards for establishing references curves.[4] With pasting viscosity properties performed with a rapid visco analyser (RVA), fewer than five samples per hour can be run and gel textural property measurement also requires complex equipment and procedure.[4] Application of NIRS to the estimation of the starch quality is an alternative choice in favour of fast measurement. It should be mentioned that these traits represent the most important parameters for evaluation of the quality of cooked rice and other starch-based foods. The amylose content of rice is considered to be the most important factor influencing the cooking and textural qualities of cooked rice. Amylography, such as tested by an RVA can help

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improve differentiation of rice varieties with similar apparent amylose content. The textural properties of starch gels formed during amylography reflect their retrogradation properties.[4]

Some reports have been published concerning establishing the NIRS calibration equations for starch quality in rice.[4–13] NIRS calibration for amylose content gave favourable results for unground brown or milled rice[5,6,11] and single brown rice,[12] as well as flour.[4] Delwiche *et al.*[6] found unsatisfactory results for the RVA parameters when the spectra were from milled grain, but Bao *et al.*[4] found favourable results for breakdown and setback viscosity of RVA parameters when the spectra were from flour. Meadows and Barton[8] obtained high regression coefficient values between NIR spectrum and the point chosen from the RVA profile. However, the viscosity values at these points are really low, so whether these points can reflect the whole pasting behaviour is unknown. Windham *et al.*[13] applied NIRS to predict rice texture attributes whose variation arose from differences in degree of milling, although the predication ability is generally low. Meullenet *et al.*[9] found that the sensory parameters, cooked rice adhesion to lips, hardness, cohesiveness of mass and toothpack could be satisfactorily predicted by NIRS.

Sample preparation is important for establishment of reliable NIRS calibration equation. The lower predication in the studies by Windham *et al.*[13] and Delwiche *et al.*[6] might be caused by the milled grain used.[4] The spectrum from brown rice grain gave lower prediction ability than that from milled grain or flour.[4,5,10–12] Our previous study[4] assumed that including waxy rice would give less accurate prediction for RVA profiles because of distinct RVA profiles between waxy and non-waxy rice starches.[8,14] However, direct comparisons of the calibration robustness between unground milled grain and flour samples, and between non-waxy rice alone and including waxy rice are rare.

The objectives of the present study were to develop calibration equations from spectra collected from milled grain and flour using modified partial least-squares regression with different mathematical treatments. The results of this research will contribute to a more efficient application of NIRS in rice breeding programmes.

MATERIALS AND METHODS

Rice samples

A total of 516 rice accessions have been used to study the genotypic diversity of the apparent amylose content, pasting viscosity and gel texture properties.[15] Four hundred and sixty-two accessions which had enough grains and flour samples were used in this study to collect NIRS spectra. We divided these rice accessions into two groups: (1) a non-waxy rice group, in which only the non-waxy samples were used in the analysis; and (2) the total rice group, in which all the rice, including 13 waxy rice samples, were used in the analysis.

Spectroscopic analysis

Both the grain and flour of milled rice of each accession were scanned with a visible/near-infrared scanning spectrometer NIR System model 5000 monochromator (Foss-NIR System, Inc., Silver Spring, MD) to obtain their reflectance spectra under the control of the software WinISI II Project Manager version 1.50. Each sample was scanned in duplicate (rotating the ring cup to a different position) in a small ring cup (internal diameter 35 mm, depth 8 mm). The spectrum was collected continuously over a wavelength range of 1100–2498 nm, and was recorded as $\log(1/R)$ at 2 nm increments. The average spectrum of each sample was used for further analysis.

Calibration and validation

We conducted calibration and validation for grain and flour spectra, respectively, hereafter called grain set and flour set. All sample spectra were divided into two subsets, one subset (calibration subset) was used to develop the calibration equation through different mathematical treatments, and the other (external validation subset) was used to evaluate the calibration equation (Table 1). Each subset (calibration and validation) contained the same rice samples for grain and flour spectra set, thus facilitating comparison of the calibration models developed from grain and flour set. Calibration and validation were conducted on the WinISI II Project Manager version 1.50. Different mathematical treatments with scatter correction of SNV-D (standard normal variate and detrend) were applied for calibration.[16] For example, the mathematical treatment 1, 4, 4, 1, i.e. $D = 1, G = 4, S_1 = 4$ and $S_2 = 1$ denotes that D is the derivative order number (that is 0 indicates no derivative operation, 1 means the first derivative, and so on), G is the gap (the number of data points over which derivation is computed), S_1 is the number of data points in the first smoothing and the S_2 is the number of data points in the second smoothing which is normally set at 1 for no second smoothing. Modified partial least squares (MPLS) were used to develop the regression equations. The major statistics are standard error of calibration (SEC) and the coefficient of determination (R^2) for calibration, coefficient of determination (1-VR) and standard error of cross-validation (SECV) for cross-validation.[17] The prediction ability of each equation was tested based on coefficient of determination (r^2) and standard error of performance (SEP).[17]

Reference analysis

The apparent amylose content (AAC), pasting viscosity characteristics and gel texture properties have been reported elsewhere (see Bao *et al.*[15] for detailed methods).

Table 1. Means, ranges and standard deviations (SD) of reference values for the starch property parameters of rice

	Calibration				External validation			
Parameter	No.	Range	Mean	SD	No.	Range	Mean	SD
Non-waxy rice group								
AAC (%)	300	7.9–32.6	23.3	6.6	152	9.8–31.7	24.5	6.2
PV (RVU)	300	102.5–305.4	245.9	29.9	152	157.2–296.5	247.4	26.0
HPV (RVU)	300	77.8–248.1	175.7	28.2	152	106.8–245.0	181.1	26.1
CPV (RVU)	300	145.0–412.6	312.3	45.3	152	225.8–393.3	322.9	40.5
BD (RVU)	300	24.0–133.4	70.2	22.0	152	24.5–141.3	66.3	20.0
SB (RVU)	300	−43.2–194.8	66.3	40.0	152	−44.8–143.7	75.5	37.3
PT (°C)	300	67.5–81.5	74.8	3.0	152	68.6–80.8	75.3	2.7
HD (*g*)	300	9.4–61.1	28.9	14.4	152	10.4–58.7	30.3	13.4
COH	300	0.46–0.76	0.60	0.08	152	0.47–0.74	0.59	0.07
Total rice group								
AAC	310	1.8–32.6	22.6	7.5	155	2.1–31.7	24.1	6.9
PV	310	81.7–305.4	243.0	34.3	155	115.5–296.5	245.6	29.4
HPV	310	43.0–248.1	172.8	32.5	155	48.8–245.0	179.3	29.1
CPV	310	56.5–412.6	305.6	58.0	155	62.3–393.3	318.8	50.0
BD	310	24.0–133.4	70.2	21.9	155	19.6–141.3	66.3	20.3
SB	310	−70.9–194.8	62.6	44.4	155	−69.3–143.7	73.2	40.8
PT	310	67.5–81.5	74.7	3.1	155	68.6–80.8	75.2	2.8
HD	310	1.4–61.1	28.1	14.8	155	0.8–58.7	29.8	13.8
COH	310	0.46–0.94	0.60	0.09	155	0.47–0.82	0.59	0.08

AAC: apparent amylose content; PV (peak viscosity), HPV (hot paste viscosity), CPV (cold paste viscosity), BD (breakdown viscosity), SB (setback viscosity) and PT (pasting temperature) are pasting properties; HD (gel hardness) and COH (gel cohesiveness) are gel texture properties; RVU: RVA unit.

RESULTS

Reference data and spectra analysis

The means, ranges and standard deviations for each starch quality parameter of the calibration and validation subsets are shown in Table 1. The non-waxy rice accessions show high genetic diversity,[15] thus it is not surprising that wide ranges exist in each subset for all parameters. There were small differences in means, ranges and standard deviations (SD) between the calibration and the validation subsets. After adding 10 and three waxy rice to the calibration set and validation set, total rice group had wider ranges, larger SD, but smaller means than the non-waxy rice set (Table 1).

Both the unground milled grain and flour of each rice accession were scanned to obtain the NIRS spectra. The spectra of grain of waxy and non-waxy rice were quite different (Fig. 1). In the raw spectra, it could be seen that waxy rice displayed a ‘W’ shape of the profile at wavelengths from1932 to 2292 nm, while the non-waxy rice displayed relatively linear lines (Fig. 1A). The difference became more distinct after first (Fig. 1B) and second (data not shown) derivative treatments. However, the spectra from flour samples were apparently similar, all displaying a ‘W’ shape of the profile from 1932 to 2292 nm (Fig. 1C and D). Even after first (Fig. 1D) and second (data not shown) derivative treatments, there were only small differences between waxy and non-waxy rice samples. However, all the spectral region of 1100–2498 nm was applied to develop calibration equations.

Calibration and validation for the grain set

The statistics of calibration, cross-validation and external validation for grain set are summarized in Tables 2 and 3, respectively.

Apparently, different mathematical treatments produced a different coefficient of determination for calibration (R^2) and coefficient of determination for cross-validation (1-VR), indicating choosing a suitable treatment will improve the calibration capacity. The AAC and PT could be best modeled with the highest R^2 ($\sim$0.9) of calibration and 1-VR ($\sim$0.8) of cross-validation in both of non-waxy and total rice groups (Table 2). The calibration equations for SB, HD and COH were modelled with a little lower R^2 ($\sim$0.80) and 1-VR ($>$0.65). The calibration R^2 and 1-VR was rather low for BD (0.58) and CPV (0.65), indicating these two parameters could not be modelled with enough accuracy. In general, both of R^2 and 1-VR in total rice group were 5% higher than those in non-waxy rice group (Table 2), indicating higher calibration capacity for the total rice, which might be due to its larger genetic diversity as shown in the ranges (Table 1).

The external validation generally resulted in a slightly smaller coefficient of determination for external validation (r^2) (Table 3) than R^2 in calibration or 1-VR in the cross-validation (Table 2). Again, AAC and PT could be predicted with the highest accuracy ($r^2 = 0.75$) in both non-waxy and total rice groups. The external validation produced $r^2 > 0.64$ for HD in some math treatments of the both groups. However, the validations were only successful for

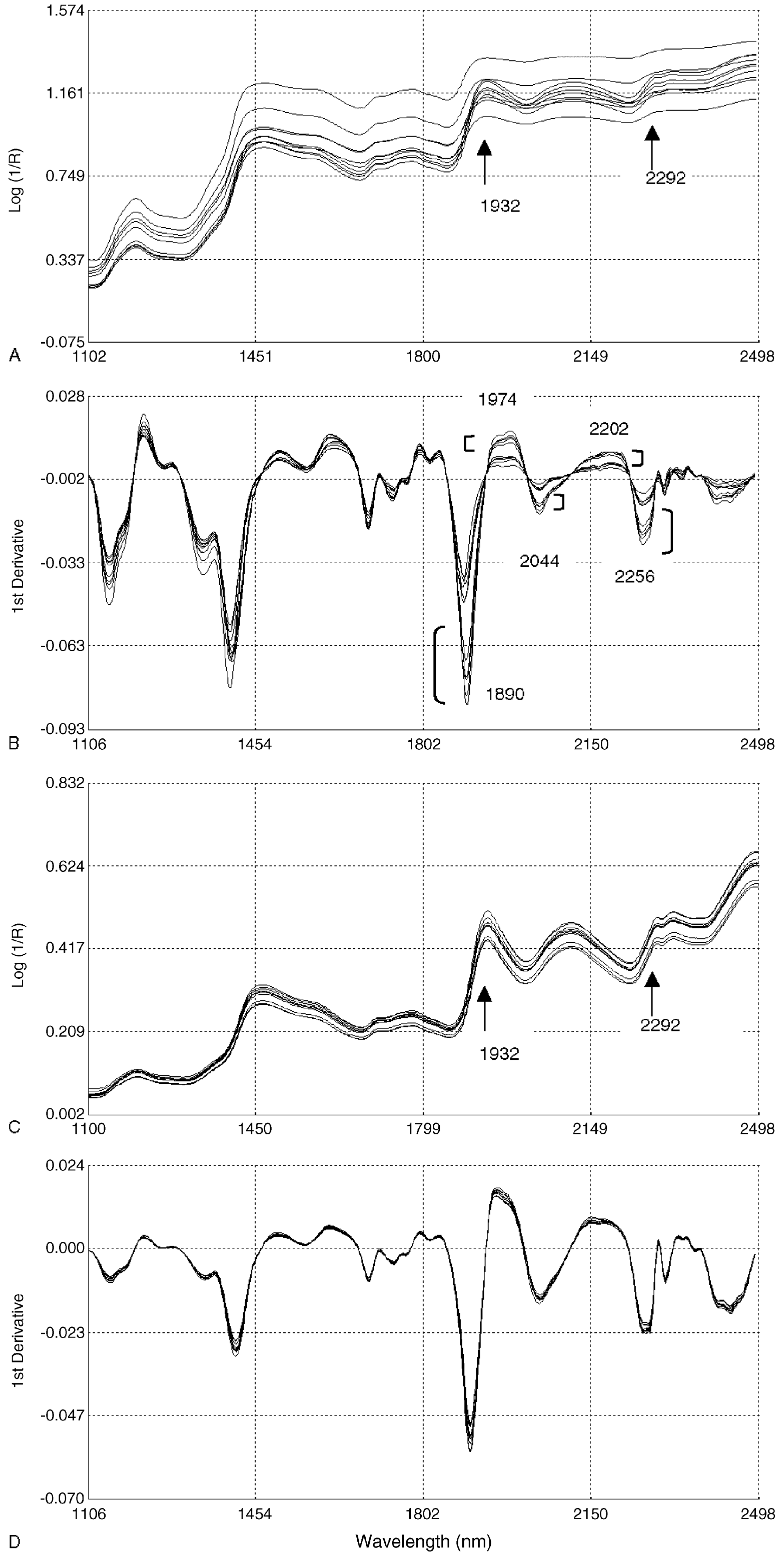

Figure 1. Typical NIRS spectra of grain (A, B) and flour (C, D) of waxy (BP011, BP029, BP216, BP225 and BP307) and non-waxy (BP001, BP002, BP003, BP004 and BP005) rice accessions with raw (A, C) and first (B, D) derivative treatments. Note that the spectra of the waxy grain displayed a distinct 'W' shape at the wavelength from 1932 to 2292 nm, whereas the non-waxy showed a relatively linear line at this region. The brackets in B indicated the spectra of waxy rice.

SB ($r^2 = 0.64$) and COH ($r^2 = 0.65$) with the first derivative mathematical treatment of 1,5,5,1 for total rice group. Among all mathematical treatments, all models seemed to be poor for CPV and BD. Similarly, the total rice group had larger r^2 than the non-waxy rice group except BD and HD (Table 3), confirming the results obtained from calibration (Table 2). According to the guidelines for interpretation of r^2 outlined by Williams and Norris,[18] models for AAC, PT and HD were usable for sample screening because their r^2 ranged from 0.66 to 0.81, responsible for the relative predictive determinant (RPD) of 1.71–2.42.[18,19]

Table 2. Calibration and cross-validation statistics for starch property parameters in the grain set

		Non-waxy group					Total rice group				
		Calibration			Cross-validation		Calibration			Cross-validation	
Parameter	Mathematical treatment	Factors	SEC	R^2	SECV	1-VR	Factors	SEC	R^2	SECV	1-VR
AAC	1,4,4,1	13	2.22	0.88	3.20	0.76	15	1.98	0.93	2.95	0.84
	1,5,5,1	13	2.19	0.89	2.98	0.79	15	2.02	0.92	2.81	0.85
	2,4,4,1	14	2.07	0.90	3.09	0.78	14	2.03	0.93	3.40	0.79
	2,5,5,1	14	2.01	0.91	2.89	0.81	12	2.05	0.92	2.99	0.84
CPV	1,4,4,1	5	34.51	0.33	36.35	0.25	12	24.86	0.80	32.79	0.65
	1,5,5,1	5	35.03	0.31	36.27	0.26	12	26.30	0.78	33.79	0.63
	2,4,4,1	3	33.39	0.38	36.53	0.25	7	25.36	0.80	34.74	0.62
	2,5,5,1	5	31.53	0.43	33.92	0.33	7	29.06	0.72	36.72	0.56
BD	1,4,4,1	5	13.10	0.62	13.93	0.57	5	13.08	0.61	13.82	0.57
	1,5,5,1	5	10.31	0.77	14.11	0.58	5	13.12	0.61	13.69	0.58
	2,4,4,1	5	13.40	0.60	14.68	0.51	4	12.24	0.65	13.47	0.57
	2,5,5,1	4	13.44	0.59	14.42	0.52	4	12.63	0.63	13.58	0.57
SB	1,4,4,1	13	17.10	0.80	23.46	0.62	12	17.69	0.83	23.50	0.70
	1,5,5,1	13	17.05	0.81	22.88	0.65	12	19.06	0.81	24.54	0.68
	2,4,4,1	5	18.22	0.77	25.08	0.57	7	22.03	0.72	26.33	0.60
	2,5,5,1	12	16.79	0.81	24.99	0.57	7	18.64	0.81	24.09	0.68
PT	1,4,4,1	14	0.94	0.90	1.44	0.77	15	1.04	0.89	1.39	0.80
	1,5,5,1	14	0.99	0.89	1.41	0.78	15	0.95	0.90	1.35	0.81
	2,4,4,1	7	1.11	0.86	1.55	0.73	10	0.92	0.91	1.43	0.78
	2,5,5,1	11	1.42	0.78	1.65	0.70	10	1.34	0.81	1.55	0.74
HD	1,4,4,1	12	6.14	0.81	8.07	0.68	7	5.38	0.86	7.48	0.74
	1,5,5,1	11	5.49	0.85	8.00	0.68	12	5.23	0.87	6.88	0.78
	2,4,4,1	5	5.44	0.86	8.42	0.66	12	7.01	0.77	8.57	0.66
	2,5,5,1	7	5.06	0.88	7.90	0.70	10	5.73	0.85	7.80	0.72
COH	1,4,4,1	11	0.05	0.66	0.05	0.59	13	0.04	0.78	0.05	0.67
	1,5,5,1	12	0.04	0.77	0.05	0.65	14	0.04	0.79	0.05	0.69
	2,4,4,1	11	0.05	0.62	0.05	0.56	5	0.05	0.65	0.05	0.60
	2,5,5,1	5	0.05	0.67	0.05	0.61	6	0.05	0.72	0.05	0.65

However, the models for SB, COH, BD and CPV were only usable for rough sample screening, as their r^2 ranging from 0.50 to 0.64, which was responsible for the RPD of 1.41–1.70.

Calibration and validation for the flour set

A summary of the statistics of calibration and cross-validation for parameters were shown in Table 4. In general, the calibration R^2 and cross-validation 1-VR in the flour set were higher than their corresponding values in the grain set in both the non-waxy and total rice groups. Accordingly, the standard error of calibration (SEC) and standard error of cross-validation (SECV) became smaller as compared with the respective values in the grain set. Both of which indicated that calibration equations modelled with flour spectra were more robust than with grain spectra.

In non-waxy group, the calibration and cross-validation produced the highest R^2 and 1-VR values (>0.95) for AAC, followed by HD, COH and PT (>0.83), and all other traits including BD had $R^2 > 0.65$ and 1-VR > 0.6. The similar trend in the calibration capacity were also obtained in the total rice group, but the R^2 and 1-VR in total rice group were generally similar to or higher than those in non-waxy rice group except for the BD, which suggest calibration equations developed from total rice group were more robust than those from non-waxy group. The R^2 and 1-VR values increased to 0.98 and 0.97 for AAC in the total rice group (Table 4). The largest increase came from CPV, which had 0.85 of R^2 and 0.82 of 1-VR, a ∼0.15 larger than those in the non-waxy group (Table 4).

It was found that the external validation r^2 values from the flour set (Table 5) were much higher than the corresponding values in the grain set (Table 3). Accordingly, the standard error of performance (SEP) became smaller than the corresponding values in the grain set. Both of which indicated that calibration equation modelled with flour spectra were more robust than with grain spectra.

Independent external validation proved that AAC could be predicted by NIRS very accurately with $r^2 = 0.90$ in the non-waxy group and 0.93 in the total rice group. Validation for HD and COH also gave r^2 higher than 0.80 in both the non-waxy and total rice groups, while for SB and PT, the r^2 was higher than 0.7 in both sets. The calibration equations for CPV were not validated successfully in the non-waxy rice, but was a little successful in all rice groups ($r^2 = 0.65$), and poor predication for BD was also observed in non-waxy group ($r^2 = 0.55$). According

Table 3. External validation statistics for starch property parameters in the grain set

		Non-waxy rice group		Total rice group	
Parameter	Mathematical treatment	SEP	r^2	SEP	r^2
AAC	1,4,4,1	3.24	0.73	3.54	0.73
	1,5,5,1	3.38	0.71	3.54	0.74
	2,4,4,1	3.78	0.66	3.47	0.75
	2,5,5,1	3.43	0.70	3.53	0.74
CPV	1,4,4,1	32.84	0.34	36.14	0.50
	1,5,5,1	32.83	0.35	34.95	0.53
	2,4,4,1	32.44	0.37	38.16	0.46
	2,5,5,1	32.69	0.36	37.45	0.47
BD	1,4,4,1	13.80	0.52	14.87	0.47
	1,5,5,1	13.36	0.58	15.03	0.46
	2,4,4,1	14.97	0.45	15.49	0.42
	2,5,5,1	15.16	0.44	15.42	0.43
SB	1,4,4,1	26.61	0.55	26.34	0.61
	1,5,5,1	25.07	0.60	24.99	0.64
	2,4,4,1	26.95	0.50	27.54	0.56
	2,5,5,1	25.93	0.56	26.89	0.58
PT	1,4,4,1	1.48	0.71	1.40	0.75
	1,5,5,1	1.40	0.74	1.40	0.75
	2,4,4,1	1.59	0.67	1.39	0.76
	2,5,5,1	1.77	0.59	1.55	0.70
HD	1,4,4,1	8.05	0.65	8.30	0.65
	1,5,5,1	7.66	0.69	8.43	0.65
	2,4,4,1	8.79	0.61	9.17	0.58
	2,5,5,1	7.93	0.67	8.59	0.63
COH	1,4,4,1	0.05	0.50	0.05	0.62
	1,5,5,1	0.05	0.52	0.05	0.65
	2,4,4,1	0.06	0.33	0.06	0.38
	2,5,5,1	0.06	0.41	0.06	0.53

to the guidelines for interpretation of r^2 outlined by Williams and Norris,[18] models for AAC and HD were usable with caution for most applications, for COH, SB, and PT were usable for sample screening, whereas for CPV and BD were usable for rough sample screening.

DISCUSSION

In our previous study,[4] we hypothesised that different samples used in the development of calibration models may lead to different capacity in the calibration for RVA, because RVA profiles between waxy and non-waxy rice are very different.[8,14] The spectrum between waxy and non-waxy grains also displayed different profiles, especially in the region from 1932 to 2292 nm (Fig. 1). After being ground to flour, both of waxy and non-waxy flours displayed similar spectrum profiles (Fig. 1). In the present study, we compared the calibration capacity from the non-waxy rice and the total rice including 10 waxy rice (Table 1), the results indicated that models developed from total rice groups are more robust than those from the non-waxy rice group for most of the traits (Tables 2 and 4), reflecting the higher R^2 and 1-VR parameters in calibration and cross-validation. This is also true when comparing the r^2 in independent validations (Tables 3 and 5). Thus, the hypothesis is denied, indicating that samples with a larger range and SD may lead to more reliable calibration equations.

Different sample preparations cause different NIRS prediction accuracy. Our previous study[4] used flour samples to establish NIRS calibrations for RVA, and the results were better than those of Delwiche *et al.*[6] We hypothesised that it was likely due to the differences in rice sample preparation because we used milled rice flour samples, while Delwiche *et al.*[6] used milled whole-grain samples. In the present study, it is clearly shown that the equations developed from the flour spectra are superior to those from the grain spectra for all the traits (Tables 2–5). For AAC, no matter what samples are used, good results are always obtained.[4–6,10,11] Even using single brown rice, favourable calibration models for AAC were also developed.[12] Similarly, Wu and Shi[20] recently reported that models developed for AAC using brown rice and milled rice grains were slightly poorer than those using their corresponding flour samples. However, grain samples are not always successful for prediction of RVA profiles as results reported here (Table 3) and previously,[6] but flour samples give better prediction results.[4,8] In addition to the differences in the spectra mentioned above, there is another reason behind the measurement of the spectrum, i.e. different sample sizes between grain and flour groups. Obviously, the grain sizes of the milled

Table 4. Calibration and cross-validation statistics for starch property parameters in the flour set

		Non-waxy group					Total rice group				
		Calibration			Cross-validation		Calibration			Cross-validation	
Parameter	Mathematical treatment	Factors	SEC	R^2	SECV	1-VR	Factors	SEC	R^2	SECV	1-VR
AAC	1,4,4,1	14	1.05	0.97	1.45	0.95	12	1.02	0.98	1.36	0.97
	1,5,5,1	14	1.06	0.97	1.39	0.95	13	1.07	0.98	1.40	0.96
	2,4,4,1	11	1.34	0.96	1.88	0.92	11	1.29	0.97	1.79	0.94
	2,5,5,1	11	1.22	0.97	1.56	0.94	10	1.13	0.98	1.55	0.96
CPV	1,4,4,1	7	22.39	0.71	23.67	0.68	7	21.99	0.85	23.76	0.82
	1,5,5,1	7	23.39	0.69	24.29	0.66	7	22.59	0.84	24.16	0.81
	2,4,4,1	6	23.08	0.70	26.30	0.60	5	24.78	0.80	26.89	0.77
	2,5,5,1	6	21.99	0.72	24.31	0.66	5	23.96	0.82	25.87	0.78
BD	1,4,4,1	6	12.52	0.65	13.45	0.60	8	13.13	0.61	13.13	0.55
	1,5,5,1	7	12.60	0.65	13.35	0.61	9	12.83	0.62	14.01	0.55
	2,4,4,1	6	11.97	0.65	13.74	0.54	5	14.25	0.50	15.13	0.43
	2,5,5,1	6	12.53	0.65	13.92	0.56	6	14.08	0.52	14.85	0.46
SB	1,4,4,1	7	18.79	0.75	20.80	0.70	7	18.80	0.79	19.73	0.77
	1,5,5,1	7	18.78	0.75	20.09	0.71	7	19.19	0.78	19.96	0.77
	2,4,4,1	6	16.66	0.80	19.75	0.72	7	19.45	0.77	21.22	0.73
	2,5,5,1	7	15.99	0.82	19.77	0.73	7	16.79	0.83	19.18	0.78
PT	1,4,4,1	11	1.03	0.88	1.30	0.82	11	1.04	0.89	1.36	0.81
	1,5,5,1	11	0.97	0.90	1.25	0.83	11	1.08	0.88	1.36	0.81
	2,4,4,1	7	1.21	0.84	1.64	0.71	9	1.06	0.88	1.55	0.74
	2,5,5,1	8	1.19	0.84	1.51	0.75	9	1.08	0.88	1.49	0.77
HD	1,4,4,1	11	3.99	0.92	4.72	0.89	10	4.10	0.92	5.06	0.88
	1,5,5,1	11	4.00	0.92	4.63	0.89	10	4.13	0.92	4.91	0.89
	2,4,4,1	10	4.47	0.90	5.62	0.84	9	4.41	0.91	5.71	0.85
	2,5,5,1	9	4.42	0.90	5.37	0.86	9	4.27	0.92	5.45	0.86
COH	1,4,4,1	9	0.03	0.87	0.03	0.84	9	0.03	0.87	0.03	0.84
	1,5,5,1	9	0.03	0.86	0.03	0.84	9	0.03	0.86	0.03	0.84
	2,4,4,1	8	0.03	0.87	0.04	0.81	8	0.03	0.88	0.04	0.82
	2,5,5,1	8	0.03	0.87	0.03	0.81	9	0.03	0.89	0.03	0.83

rice still vary with different rice genotypes. Whereas in the flour group, all flours passed through a 100-mesh sieve, thus the homogenous sample sizes may lead to a better calibration performance.

It should be mentioned that only 13 waxy rice were involved in this study, the limited waxy accessions may explain why the improved prediction ability was not so large. It is expected if including of more waxy rice in the analysis will improve prediction capacity for some of the parameters for which a large difference between non-waxy models and full set models, such as CPV. Furthermore, peak viscosity (PV) and hot paste viscosity (HPV) (Table 1) can not be modelled with all kind of samples, whether including more waxy rice will help establishment of NIRS models needs further investigation.

NIRS equations can be used to select germplasm for some specific quality targets either for breeding purpose to improve rice quality or for the purpose of finding suitable starches for manufacturing specified food products. Wu and Shi[20] recently developed models for cooking characteristics with intact rice grain, brown rice grain and flour, milled rice grain and flour, which allows early selection based on a single plant could be easily conducted in rice breeding programs. In addition, the NIRS technique can be used in rice processing to monitor the quality on line at every milling stage, since NIRS applications in quality control in grain milling increasingly call for on-line analyses during production process itself.[21]

In conclusion, this study showed that calibration models built with flour spectra are more robust than those from grain spectra, and calibration models from total rice including waxy rice are superior to those from only non-waxy rice. Some starch quality parameters, such as AAC, SB, PT, HD, and COH could be predicted with sufficient accuracy with NIRS based on flour spectra, whereas only AAC and PT could be predicted with sufficient accuracy based on grain spectra. All the models reported here are usable for rough sample screening (CPV and BD), for sample screening (SB, PT and COH) and for most applications (AAC and HD). However, for accurate assay of the pasting viscosity and gel textural parameters, direct instrumental measurement should be employed in later generation.

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Table 5. External validation statistics for starch property parameters in the flour set

		Non-waxy rice group		Total rice group	
Parameter	Mathematical treatment	SEP	r^2	SEP	r^2
AAC	1,4,4,1	1.95	0.90	1.85	0.93
	1,5,5,1	1.96	0.90	1.83	0.93
	2,4,4,1	2.15	0.88	2.10	0.91
	2,5,5,1	2.11	0.89	2.05	0.91
CPV	1,4,4,1	30.16	0.46	30.24	0.65
	1,5,5,1	30.48	0.46	30.27	0.65
	2,4,4,1	32.30	0.41	32.02	0.60
	2,5,5,1	32.19	0.41	31.43	0.62
BD	1,4,4,1	13.83	0.55	16.22	0.42
	1,5,5,1	13.67	0.55	15.21	0.46
	2,4,4,1	14.16	0.51	17.15	0.32
	2,5,5,1	13.46	0.56	16.96	0.34
SB	1,4,4,1	21.37	0.68	20.74	0.74
	1,5,5,1	20.70	0.71	20.85	0.74
	2,4,4,1	24.12	0.60	23.37	0.68
	2,5,5,1	21.81	0.68	21.83	0.72
PT	1,4,4,1	1.51	0.70	1.56	0.70
	1,5,5,1	1.47	0.72	1.53	0.71
	2,4,4,1	1.68	0.64	1.74	0.63
	2,5,5,1	1.71	0.63	1.70	0.64
HD	1,4,4,1	5.23	0.85	5.32	0.85
	1,5,5,1	5.22	0.85	5.27	0.85
	2,4,4,1	6.54	0.76	6.19	0.80
	2,5,5,1	6.21	0.79	5.98	0.81
COH	1,4,4,1	0.03	0.78	0.03	0.82
	1,5,5,1	0.03	0.80	0.03	0.82
	2,4,4,1	0.04	0.76	0.04	0.78
	2,5,5,1	0.03	0.79	0.04	0.80

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