Performance of Fourier transform near infrared spectrophotometer for determining moisture and protein contents of single kernel thai "KDML105" brown rice

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Introduction

Fourier transform near infrared spectroscopy (FT-NIR) is a method that has the added advantages of improved speed and resolution, or signal-to-noise ratio, over the conventional dispersive NIR method. However, the disadvantage of FT-NIR is the relatively high cost of instrumentation.[1]

Rice (*Oryza sativa* L.) is the most important cereal crop in the developing world and is also the staple food for two-thirds of the world's population.[2] About 95% of the world's rice is produced in developing countries and 92% of it is in Asia.[3] Low amylose rice, Kao Dok Mali 105 (KDML105) is a popular variety in Thailand due to the aroma and the soft texture of the cooked rice. In order to achieve desired quality, in a similar way to that of other crops, rice has been cultivated and selected based on physico-chemical properties. Quality improvement of rice is necessary in a breeding programme. If the chemical composition of kernels can be measured non-destructively, screening before cultivation is possible. An advantage of single kernel NIR analysis is the ability to detect attributes that may only be present in a few kernels. Specific kernels can then be used to propagate specific traits in breeding programmes.

As mentioned in previous works done in various countries, non-destructive screening of single kernels is an important factor in successful plant breeding. The single kernel technique would also facilitate various studies on rice. In this work, we evaluated the ability of a high resolution Fourier transform (FT)-NIR spectrophotometer equipped with a six channel single kernel sample cell in reflectance mode, for measuring moisture and protein contents in Thai brown rice.

Materials and methods

Samples

A total of 100 single grains of Thai brown rice (KDML 105) were used as samples. To expand the moisture range, an oven drying method at 45 °C was applied. The samples were randomly separated into five groups. Three single kernels of each group were randomly chosen from each plant location, and were dried for 0 min, 20 min, 60 min, 180 min, and 360 min. The kernels were kept individually in sealed glass vials to prevent moisture change after treatment. Prior to spectral acquisition, the vials were kept in the instrument laboratory (25 °C) for at least 12 hours, to allow the temperature to equilibrate.

Spectral acquisition

A commercially available NIR spectrophotometer Model NIRFlex N500 (Buchi Labortechnik AG, Flawil, Switzerlan) equipped with a six channel single kernel sample cell was used to measure

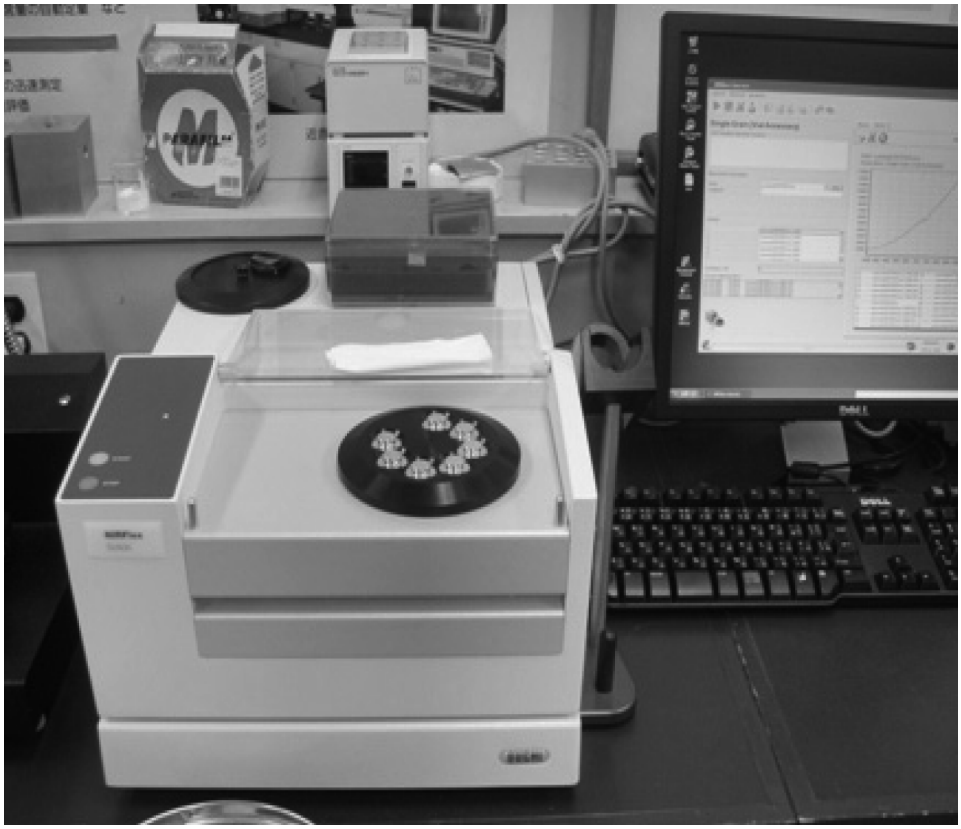

Figure 1. NIR spectrophotometer Model NIRFlex N500 (Buchi Labortechnik AG, Flawil, Switzerland) equipped with a six channel single kernel sample cells.

the NIR spectra in the region from 4000 cm^{-1} to 10000 cm^{-1} in reflectance mode (Figure 1). The resolution used was 16 cm^{-1} with an averaged number of 32 scans.

Chemical analysis

Moisture content was measured by AACC method 44–19 air oven method,[4] drying at 135 °C. The kernels were dried in a hot-air oven at 135 °C for 15 hours. For protein content determination, the kernels were dried at 135 °C for 15 hours before determination. Protein content (N × 5.95) of each kernel was determined by the AACC method 46–30 crude protein combustion method[4] with a "Leco FP-528" (LECO Corporation, Miami, USA) nitrogen analyser. The protein content was reported on a dry weight basis.

Data analysis

The samples were divided into calibration and validation sets according to their constituent values using an odd-even method. Partial least squares (PLS) regression was used to develop a calibration equation. Unscrambler software (Camo, Oslo, Norway) was used to perform the calibration.

Results and discussion

NIR spectra of Thai "KDML105" brown rice single kernels

The original [log (1/*R*)] reflectance spectra of Thai "KDML105" brown rice single kernels in wavenumber region of 4000–10000 cm^{-1} are shown in [Figure 2(a)]. [Figure 2(b)] shows the original spectra of single Thai brown rice kernels of varying moisture content levels and presents important absorption bands of main constituents such as water (5184 cm^{-1}) and protein (5753 cm^{-1}), reported by Osborne *et al*.[5] and Williams.[6]

Baseline shifts occurred in all original spectra of brown rice due mainly to the differences in kernel size, in a trend similar to the research on transmittance measurement of Satsuma orange reported by Kawano *et al*.[7] Baseline shifts can be removed by second derivative pre-treatment as shown in [Figure 2(c)].

Appropriate wave-number region

PLS calibrations for moisture and protein content were developed to determine the appropriate wave-number region by which to analyse single kernels. The wave-number regions were varied in order to get the best calibration. The original spectra plots [Figure 2(a)] and second derivative spectra of single Thai brown rice kernels of varying moisture content levels [Figure 2(c)], using the whole wave-number region of 4000–10000 cm^{-1} revealed that the spectral data beyond 8000 cm^{-1} were rather noisy, and showed no significant bands. PLS calibrations verified that the use of the longer wave-number region from 8000–10000 cm^{-1} was of no benefit.

The PLS results for moisture and protein contents, developed with the wave-number region of 4000–8000 cm^{-1} showed the smallest value for the standard error of prediction (*SEP*) and bias, compared with other wave-number region, and was concluded to be the appropriate wave-number region for analysing of brown rice single kernels.

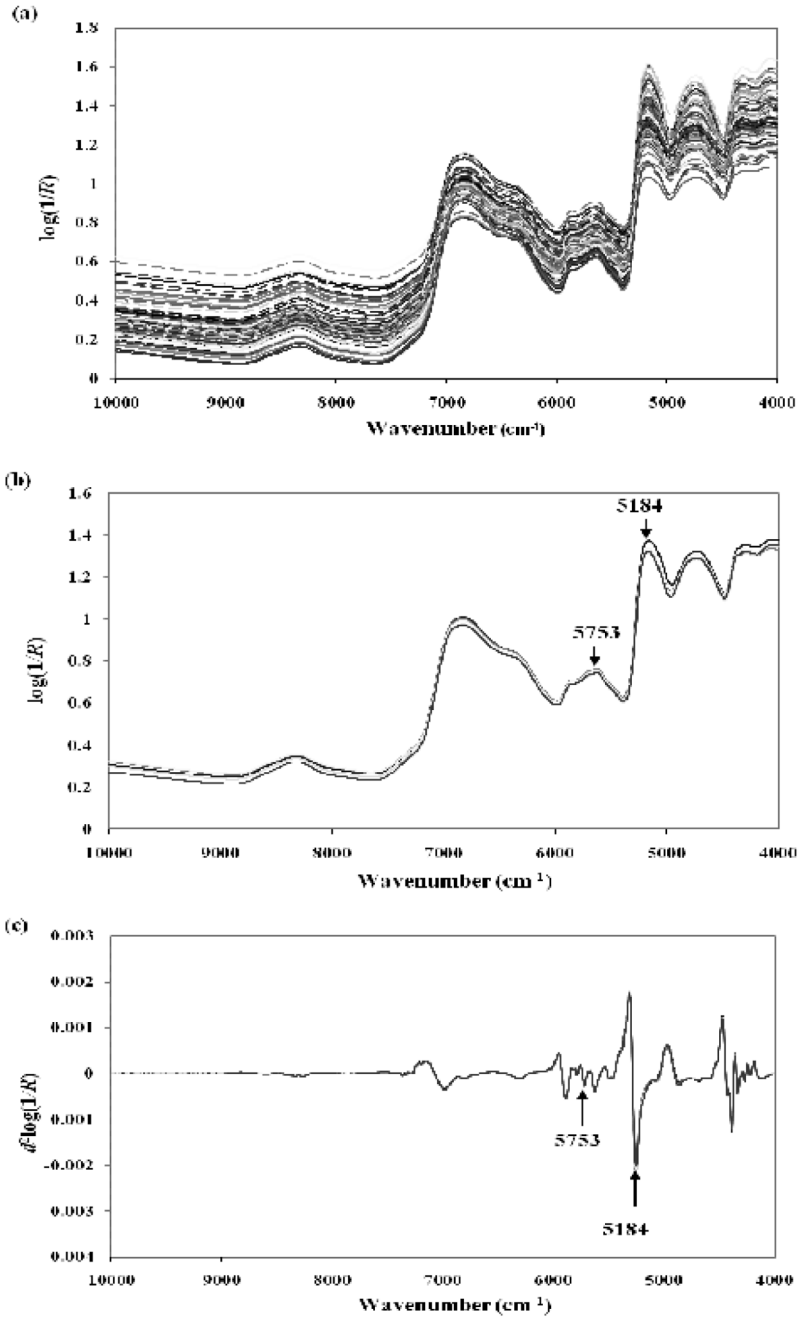

Figure 2. Original spectra of all samples (a). Original spectra of single Thai brown rice kernels of varying moisture content levels (b). Second derivative spectra of single Thai brown rice kernels of varying moisture content levels (c). Arrows indicate absorption due to water (5184 cm^{-1}) and protein (5753 cm^{-1}).

Table1. PLS calibration results for predicting moisture and protein contents in single kernels of Thai brown rice using spectra measured in the wave-number region of 8000–4000 cm^{-1}.

Constituents	Pretreatment methods	*F*	*R*	*SEC* (%)	*SEP* (%)	Bias (%)
Moisture	Smoothing + 2nd derivative	5	0.88	0.5	0.6	−0.0
Protein	Smoothing + 2nd derivative	5	0.86	0.2	0.2	−0.0

F: Number of factors used in the calibration equation; *R*: Multiple correlation coefficients; *SEC*: Standard error of calibration; *SEP*: Bias-corrected standard error of prediction; Bias: The average of difference between actual value and NIR-value

Appropriate pretreatment method

The PLS calibration and validation result of various pretreatments for moisture and protein contents in the wave-number region of 4000–8000 cm^{-1}, showed that the standard error of calibration (*SEC*), the standard error of prediction (*SEP*), and the bias values generated by the water and protein calibrations after pretreatment by smoothing plus second derivative were essentially the same as those obtained with other pretreatment methods, but the number of factors was the lowest. Therefore, the appropriate pretreatment method for analyzing brown rice single kernels was considered to be smoothing plus second derivative method (Table 1).

Scatter plots between actual and predicted moisture and protein content values, were developed from data produced from the spectra after pretreatment by smoothing plus second derivative, over the wave-number region of 4000–8000 cm^{-1} By the paired t-test, there were no significant differences between the actual and NIR-predicted value, indicating that the system developed was

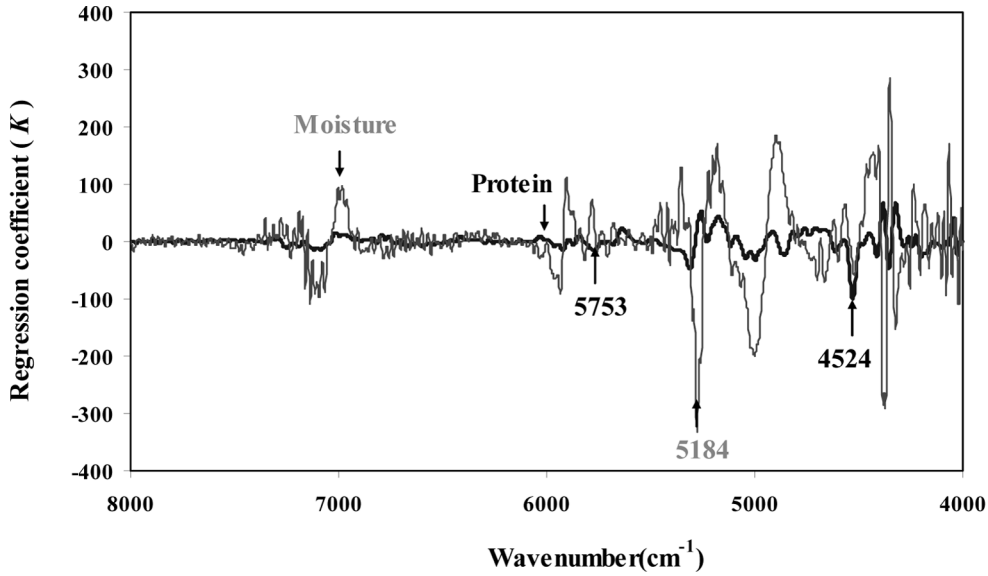

Figure 3. Regression coefficient plots from PLS calibration for predicting moisture content, using MSC plus second derivative spectral pretreatment measured in the wavelength region of 8000–4000 cm^{-1}.

sufficiently accurate. For the calibration structures the PLS regression coefficient plots of moisture and protein content are shown in Figure 3.

Dominant peaks could be observed in the regression coefficient plots. For moisture content, the dominant peak indicated a water band at 5184 cm^{-1} while a protein band peak occurred at the wave-number of 5753 cm^{-1}.

Conclusion

Using a PLS calibration method an effective non-destructive system was developed for single kernel FT-NIR analysis of Thai "KDML105" brown rice, for protein and moisture content over the 4000–8000 cm^{-1} wave-number region. Smoothing plus second derivative pretreatment was found to be an appropriate spectral pretreatment method.

References

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