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Determination of Apparent Amylose Content in Japanese Milled Rice Using Near-Infrared Transmittance Spectroscopy

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The objective of the present study was to develop a method to analyze apparent amylose content (AAC) of Japanese milled rices using near-infrared transmittance spectroscopy (NIT). Samples (n=110, varieties=37), harvested in 1996, were collected at various sites throughout Japan. Whole-grain milled rice was scanned using a near-infrared range (833-1050 nm with 8 nm steps and 27 wavelengths) transmittance filter type spectrometer. The AACs of samples were in the range of 13.1% to 20.7% (SD: 1.53). The wide range AAC (0-35.3%) partial least squares (PLS) model was found to be inadequate for accurate prediction of the narrow AAC range (13.2-20.7%) of the rice samples. The statistical performance of PLS modeling (11 factors) for narrow range AAC analyses gave a standard error of cross-validation (SECv) of 0.78 and square of regression coefficient (R^2) of 0.74. The AAC model was applied to 20 unknown samples of products from different crop year (1997), and gave a standard error of prediction (SEP) of 1.25, R^2 of 0.49 on the validation set. These results suggested that this model based on NIT spectroscopy could be applied for rapid and nondestructive measurement of narrow range AAC of Japanese milled rices.

Keywords: *japonica*, amylose, near infrared transmittance, filter type NIT

Simple and rapid analytical methods have been necessary to measure the amylose content of milled rice, because consumers of northeast Asian countries (Japan, and northern regions of China and Korea) prefer low amylose rice for cooked rice. The rice industry (breeding, distribution, processing, consuming) has begun to use a non-destructive analysis technique in connection with the eating quality of rice. Near infrared technology is used for classifying rice samples into qualitative groups such as poor taste, better taste and best taste (Kawamura *et al.*, 1997). Milled rices in the Japanese rice market are characterized by low apparent amylose content (AAC), and AAC can be used as one of the indices for eating quality of rice. The narrow AAC range of milled rices needs to be accurately determined by non-destructive evaluating systems.

A simplified colorimetric method for AAC of milled rice was developed at the International Rice Research Institute (Juliano, 1971) based on the standard method of Williams *et al.* (1958). The apparent content of amylose is measured based on the affinity of iodine for amylose. Due to recent advances in the determination of molecular structures of starches, the amylose content using the colorimetric method should be more correctly labeled as apparent amylose content (Takeda, 1993). The selection of low AAC rices in Hokkaido, Japan's northernmost island, provided a rice of improved eating quality (Inatsu, 1988).

Methods using near-infrared transmittance (NIT) spectroscopy for prediction of AAC in milled rices have recently been developed and whole-grain brown milled rices have been examined (Villareal *et al.*, 1994; Kobayashi *et al.*, 1994; Delwiche *et al.*, 1996). The partial least squares (PLS) in chemometrics modeling also was used to quantitatively related NIT spectra (Lindberg *et al.*, 1983). These methods have employed calibrations for AAC based on a wide range of rice subfamilies such as *indica, japonica* and *javanica* rices. Practical considerations for using NIR applied to AAC have been discussed by Barton *et al.* (1997). These NIR methods utilized monochromator type spectrometers, which are expensive and sophisticated systems, thus, their main application is for laboratory-scale AAC analyses.

In all previous studies using NIT for AAC analyses, only grating monochromator systems have been used. These were employed to determine a wide range of AACs of rices from glutinous to high-amylose rices. There was no significant correlation between the values of reference and the values of NIR prediction in Japanese milled rice AAC using the NIR technique (Sasaki, 1997).

The objective of the present study was to develop a narrow

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range AAC calibration especially for Japanese milled rice, using a simple filter-type NIT apparatus for whole-grain milled rice of narrow AAC range. The chemical assignment of NIT bands and loadings in the PLS model are discussed, and the model was applied to narrow range AAC of Japanese milled rice produced in different crop years.

Materials and Methods

Sample and preparation The samples of the calibration set consisted of *indica, japonica* and *glutinous* type rices. The short-grain *japonica non-glutinous* type rices (110 samples; 37 varieties), harvested in 1996, were collected in 37 prefectures throughout Japan. The long-grain *indica* type milled rices (7 samples; 7 varieties) and short-grain *glutinous* milled type rices (3 samples; 3 varieties), harvested in 1996, were grown in Thailand. Short-grain *japonica* type rices (20 samples; 15 varieties), harvested in 1997, were used for model validation.

Milling of the *japonica* type brown rice samples was carried out to a milling yield of 90%, w/w using a friction type rice milling machine (VP-31T, Yamamoto Co., Tendou). Broken kernels were removed by a cylinder separator (type TRG, Satake Co., Higashi-hiroshima).

Chemical analyses The milled rice samples were ground with a cyclone grinder (model 3010-018, Udy, Ft. Collins, Co., USA) equipped with a 50-mesh screen. Before AAC determination, the moisture content of the ground samples was determined in duplicate by an oven using 3 g of rice powder at 135°C for 1 h. The AAC (%) was determined in duplicate on 50-mesh milled rice flour by the iodine colorimetric method of Juliano (1971). The repeatability of error of the AAC reference method was less than 0.5%. Amylose (Amylose typeIII, Lot 17H3893, Sigma Chemical Co., St. Louis, MS) and amylopectin (glutinous rice amylopectin, Shimada Co., Niigata) were mixed (amylose to amylopectin weight fraction of 0/100, 10/90, 15/85, 20/80, 30/70 and 40/60) for calibration of AAC. Absorbance of iodine solution after 20 min incubation with iodine (I_2: 2.0 g and KI: 20.0 g diluted to 1.0 *l* with distilled water) at 26.5°C (Ohtsubo, 1995) at 620 nm was measured using a spectrophotometer (model U-2010, Hitachi Co., Katsuta).

Near-infrared transmittance spectroscopy The whole-grain samples of milled rice were scanned using an NIT filter type spectrometer (Grainspec A™, Foss Development, York, UK). The light source was a tungsten filament lamp and the detector was a silicon photodiode array. Spectra were recorded for each sample from 833 to 1050 nm, using 27 wavelength points at every 8 nm. Milled rice grains (300 g) were automatically supplied to the measuring photocell by the transport system. Each batch was scanned ten times, and the ten scans were averaged to form one spectrum (log (1/T)) for each sample.

Modeling procedure A commercial multivariate analysis program (Unscrambler 6.11, Camo AS, Trondheim, Norway) was employed to develop a PLS calibration model for AAC using all 27 wavelengths in PLS.

With full cross-validation, the same samples were used for both model estimation and testing. The method consisted of leaving out one sample from the calibration data set and calibrating the model on the remaining 119 samples, then predicting the value for the left-out sample and computing the prediction residual. The process was repeated with another subset of the calibration set and so on, until each sample had been left out once. Mathematical development of PLS calibration was documented elsewhere (Lindberg *et al.*, 1983; Kim 1994).

The optimum number of PLS components was determined when the value of least standard error of full cross-validation (SECv) reached a minimum in a plot of SECv versus number of PLS components in full cross-validation. Performance of the PLS model was evaluated using the SECv and the ratio of standard deviation of the reference values of the validation set to the SECv of the validation set (RPD) (Williams & Sobering, 1993). Furthermore, we evaluated our PLS model using the standard error of prediction (SEP) as an index of the prediction of different crop year samples.

Results and Discussion

Diversity of AAC of milled rice Figure 1 shows the distribution of AAC in the calibration set. The range of AAC of milled rice was from 0 to 35.3%. The standard deviation (SD) of 4.24 was similar to the SD of 3.97 of Delwiche *et al.* (1996).

Figure 2 shows the distribution of AAC of just the *japonica* type calibration samples. The range of AAC was

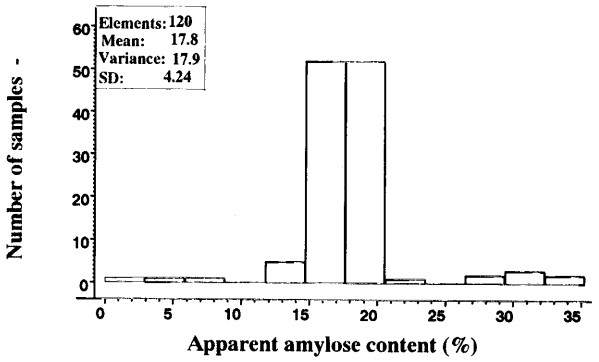

Fig. 1. Distributions of reference values of apparent amylose content in 120 milled rice calibration set samples (*indica, japonica* and *glutinous* type rices).

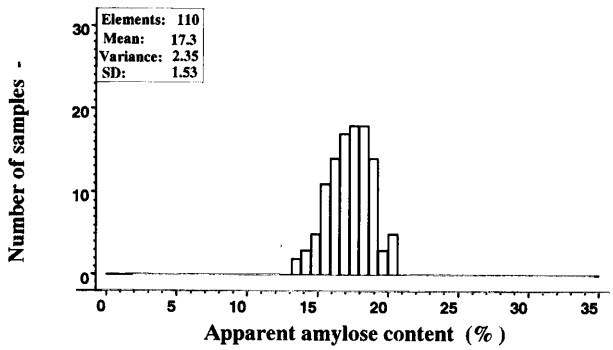

Fig. 2. Distributions of reference values of apparent amylose content in 110 milled rice calibration set samples (*japonica non-glutinous* type rices).

from 13.1 to 20.7%, with SD of 1.53. The AACs of unknown samples were from 13.4 to 19.8%, with SD of 1.68 (Fig. 3). This range fell in the low amylose category of 12.1-20.0%, as defined by Juliano and Villareal (1993).

The SD of all 120 samples was 2.7 times higher than the value of the SD of the *japonica* type samples alone. Wide variation in quality preferences within a country or region of a country is reported for Brazil, China, India, Madagascar, Pakistan, Philippines, Thailand, and USA (Juliano & Villareal, 1993).

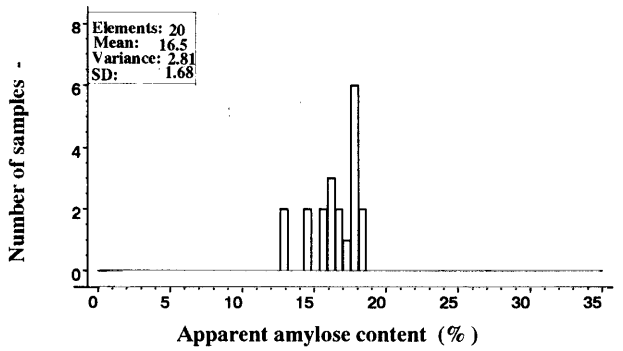

Fig. 3. Distributions of reference values of apparent amylose content in 20 milled rice validation sets (*japonica non-glutinous* type rices).

Comparison of two PLS loading spectra for AAC in samples of whole-grain Figure 4 shows the loading plots for 1st, 2nd, and 3rd PLS components of the wide AAC range (0-35.3%) of milled rice. Sample score from the 2nd PLS component had the highest correlation with AAC (Fig. 4, r= 0.4) and the loading, with large intensity related to C-H in the CH_2 band at 933 nm (Osborne *et al.*, 1993). The 1st PLS component had the second highest correlation with AAC (r=0.27). Loading 1 had significant intensity related to C-H and C-C at 841 nm (Osborne *et al.*, 1993). The 3rd PLS component was not correlated with AAC (r=0).

Figure 5 shows the loading plots for 1st, 2nd and 3rd PLS components of the narrow AAC range (13.2-20.7%) of Japanese milled rice. Sample score from the 3rd component had the highest correlation with AAC (Fig. 5, r=0.50) and the loading, with large intensity related to C-H and C-C at 841 nm (Osborne *et al.*, 1993). The 1st PLS component had

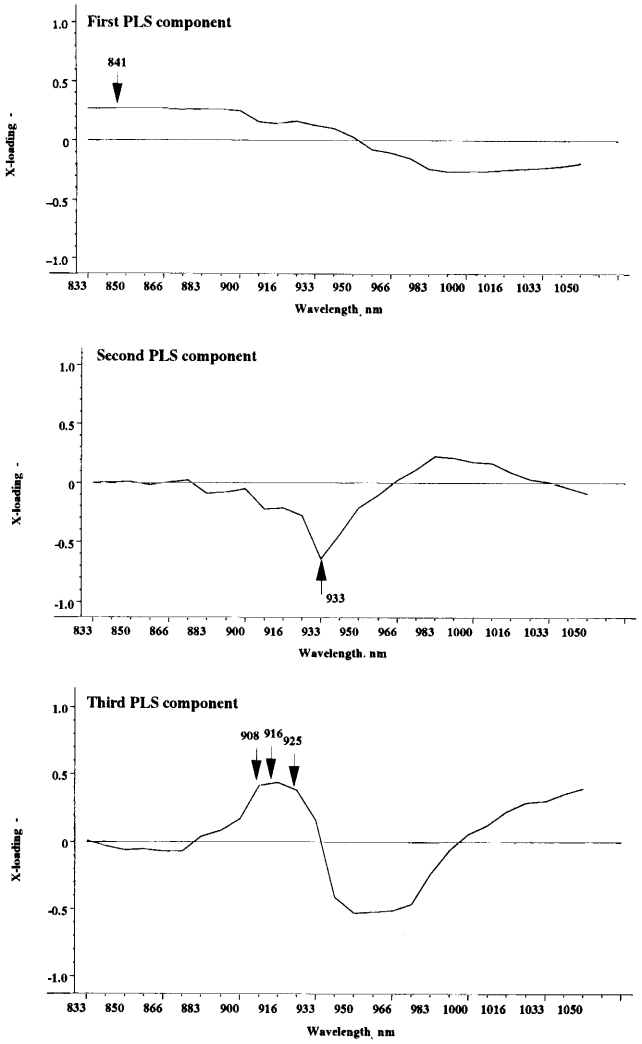

Fig. 4. PLS loading spectra for wide AAC range in milled rices (*indica, japonica* type and *glutinous* type rices). AAC range: 0-35.3 (%).

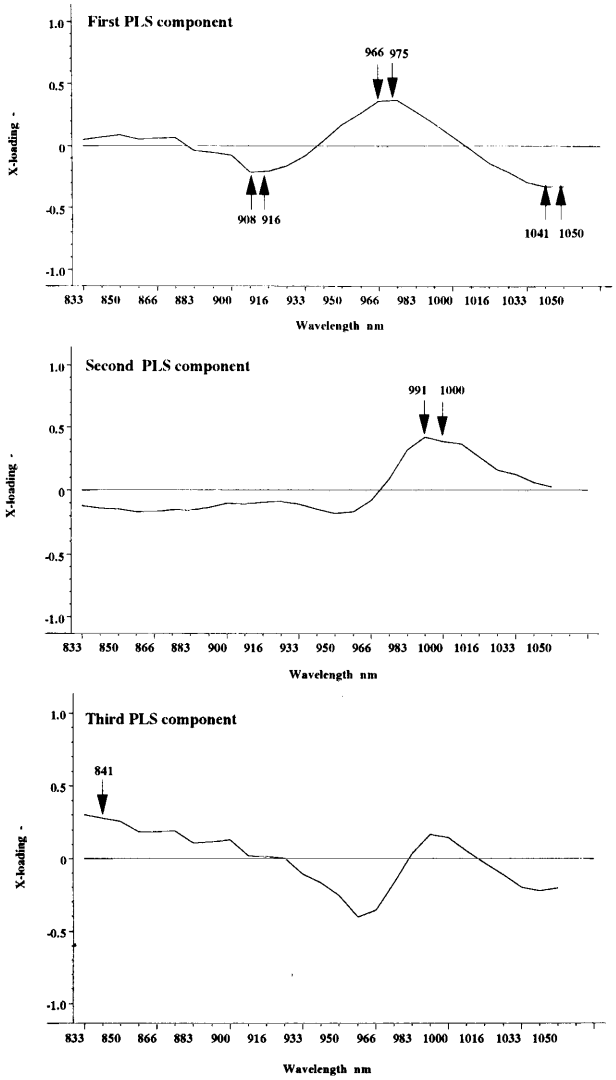

Fig. 5. PLS loading spectra for narrow AAC range in milled rices (*japonica* type rices). AAC range: 13.1-20.7 (%).

the second highest correlation with AAC (r=0.31). Loading 1 had significant intensity related to C-H at 908 and 916 nm, and due to O-H in ROH, H_2O band at 966 and 975 nm (Osborne *et al.*, 1993).

These results show that the wavelengths necessary for determination of AACs using wide AAC range rices such as *indica* and *japonica*, and those necessary for narrow AAC range rices such as Japanese rices, are markedly different. There seems to be significant correlation between O-H in ROH and H_2O bands and AACs for the group of samples covering the Japanese rices. Development of an accurate AAC prediction model for Japanese milled rices requires measure AACs of milled rice over a narrow range.

Comparison of the two calibration model Figure 6 shows the NIT-prediction over the wide range of AAC. Figure 7 shows the relationship between the SECv and the numbers of PLS components for the cross-validation of wide AAC range samples. The SECv became minimum with 13 PLS components.

The performance of calibration based on a wide range AAC model was not as good when it was applied to narrow range AAC of milled rice (Fig. 8). It seems that the PLS model of wide range AAC relies on only the C-H and C-C related wavelengths. We could not obtain accurate predictions for the Japanese milled rices of narrow range AACs by directly adopting this calibration. Thus, it is necessary to develop a model using just the narrow range of Japanese milled rices as samples for the calibration set.

Figure 9 shows the relationship between the SECv and the number of PLS components for the cross-validation of the *japonica* type samples alone. The SECv was reached minimum with 11 PLS components.

The calibration equation proposed is shown in Fig. 10. NIT-prediction versus reference of AAC is shown in Fig. 11. The performance of this PLS calibration model (11 PLS components) was SECv of 0.78, R^2 of 0.74 and RPD of 2.01 on the full cross-validation set. The SECv of 0.78 on the best

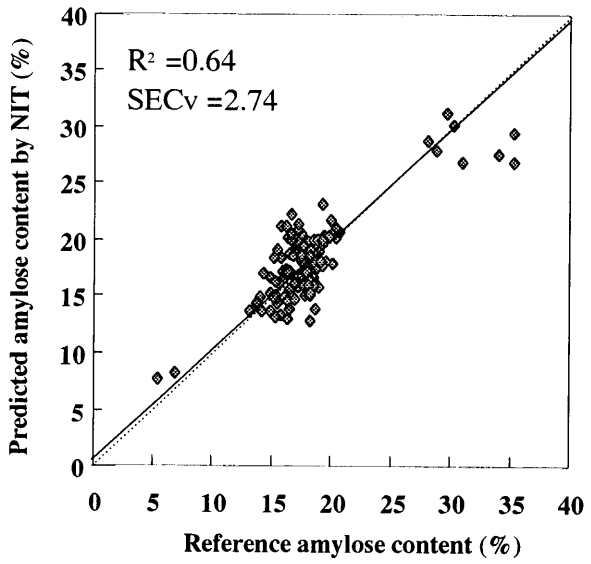

Fig. 6. Relationship between reference amylose content in milled rice and predicted amylose content in milled rice. AAC range: 0-35.3%.

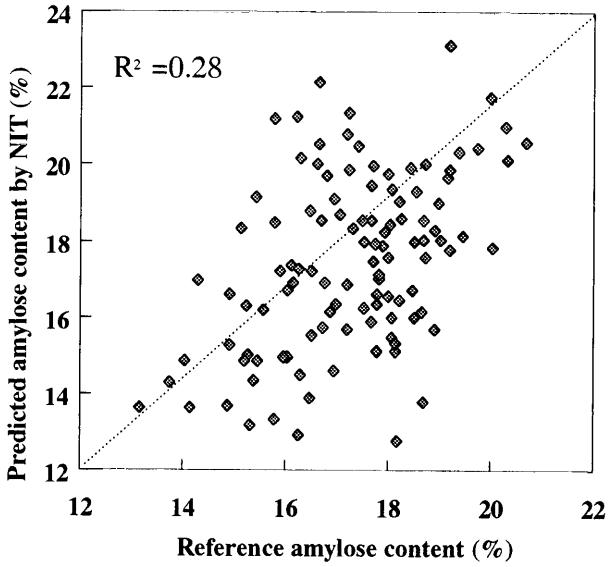

Fig. 8. Relationship between reference amylose content in Japanese milled rice and predicted amylose content in milled rice using the AAC analysis of wide AAC range rices.

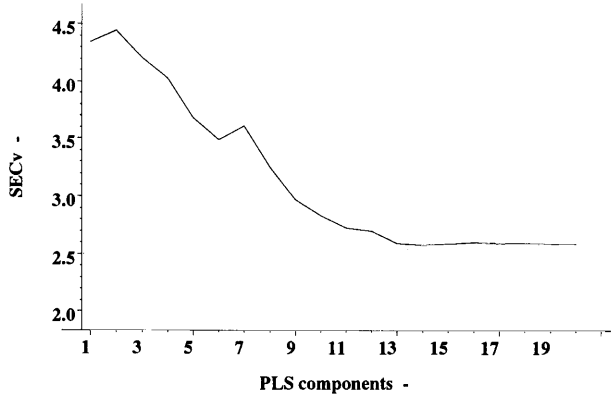

Fig. 7. Plot of SECv versus PLS components. Development of PLS calibration model for the AAC analysis of wide AAC range rices.

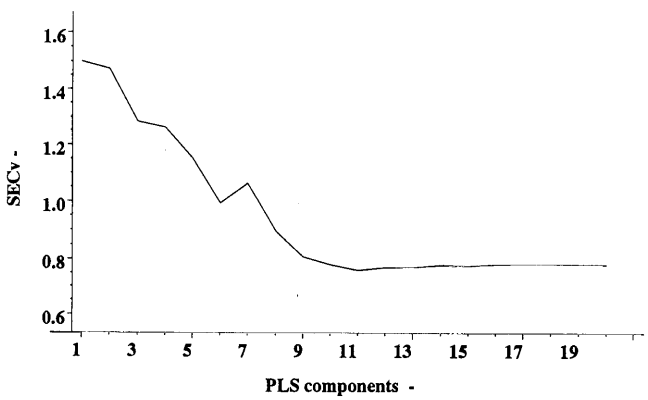

Fig. 9. Plot of SECv versus PLS components. Development of PLS calibration model for the AAC analysis of narrow AAC range rices.

$$AAC = -1160x_1+2710x_2+2120x_3-2920x_4-2170x_5-1200x_6-703x_7 +3070x_8+4420x_9+144x_{10}-4810x_{11}-2690x_{12}+4850x_{13}+259x_{14} -3190x_{15}+938x_{16}-1720x_{17}+3490x_{18}-2240x_{19}+1370x_{20}-3250x_{21} +1480x_{22}+3440x_{23}-1730x_{24}-416x_{25}-2120x_{26}+2000x_{27}+48.6$$

x_1:833nm,x_2:841nm,x_3:850nm,x_4:858nm,x_5:866nm,x_6:875nm,x_7:883nm,x_8:891nm, x_9:900nm,x_{10}:908nm,x_{11}:917nm,x_{12}:925nm,x_{13}:933nm,x_{14}:942nm,x_{15}:950nm, x_{16}:958nm,x_{17}:967nm,x_{18}:975nm,x_{19}:983nm,x_{20}:992nm,x_{21}:1 000nm,x_{22}:1 008nm, x_{23}:1 017nm,x_{24}:1 025nm,x_{25}:1 033nm,x_{26}:1 042nm,x_{27}:1 050nm,B_0:constant

Fig. 10. Calibration model of PLS for AAC analysis (Number of PLS components: 11).

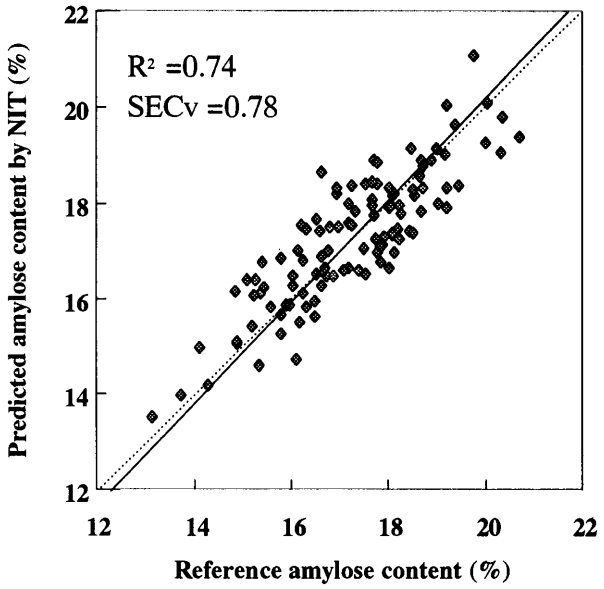

Fig. 11. Relationship between reference amylose content in milled rice and predicted amylose content in milled rice using developed PLS model. (AAC range: 13.1–20.7 (%)).

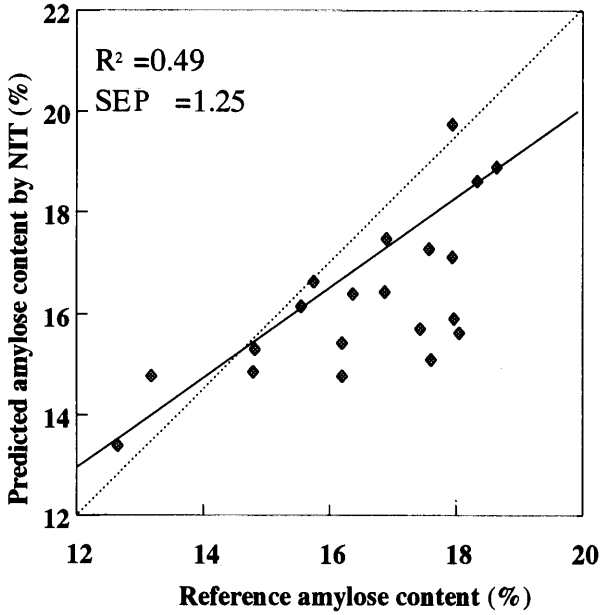

Fig. 12. Relationship between reference amylose content in milled rice and predicted amylose content in milled rice using developed PLS model (AAC range: 13.4–19.8 (%)).

calibration model determined here was the same as an SEP of a previous model (Villareal *et al.*, 1994, SEP: 0.78), while, there was no significant relationship between the reference of narrow range AAC and prediction value of NIR (Sasaki, 1997)

The developed AAC model of a single year crop (1996) of *japonica* type samples was applied to 20 samples of a different crop year (1997) (Fig. 12). The performance of this calibration model was SEP of 1.25 and R^2 of 0.49. This adaptability could be explained because the repeatability error of our reference method was less than 0.5%, the AAC calibration was enhanced due to the sample diversity of Japanese milled rices, and loading 1 of the developed AAC model had significant intensity in relation to O-H (Fig. 5).

These results suggested that the present model based on NIT spectroscopy could be applied for rapid and nondestructive measurement within narrow range AAC of Japanese milled rices. Thus, the present AAC analysis based on NIT spectroscopy could be used as one index of the quality of rice.

Conclusions

A rapid and simple technique for AAC analysis using NIT spectroscopy was developed based on near-infrared transmittance spectra, with AAC determined by the iodine colorimetric method. The wide range AAC (0–35.3%) PLS model was found to be inadequate for accurate prediction of the narrow AAC range (13.2–20.7%) of Japanese milled rice samples, because the first model developed using wide range AAC samples seemed difficult to apply to narrow range AAC rices (R^2 of 0.28). The statistical performance of the PLS model (11 PLS components) for the narrow range of AAC analyses gave SECv of 0.78, and R^2 of 0.74 and RPD of 2.01. The performance of this model was SEP of 1.25, and R^2 of 0.49 on the validation set. The present AAC analysis based on NIT spectroscopy could thus be used as one of the quality indices for quality of rice.

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