Establishment of an Accurate Starch Content Analysis System for Fresh Cassava Roots Using Short-Wavelength Near Infrared Spectroscopy

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ABSTRACT: Short-wavelength near infrared spectra in the interactance mode were collected from intact cassava roots and cassava flesh, using two portable spectrometers for the spectral regions of 720−1050 and 850−1150 nm, respectively. All starch prediction models were developed using the partial least squares regression. Good prediction performance was obtained from the cassava flesh (cross-section cut root) measurement with a correlation of prediction ($r_p$) of 0.917 and standard error of prediction (SEP) of 1.73%, for both spectrometers. For the intact root, the prediction models were satisfactorily accurate with $r_p$ values of 0.687 and 0.772 and SEP of 3.151 and 2.803%, respectively. Moreover, the performance measurement of all optimum models was also evaluated according to ISO 12099:2017(E). The results showed that the predicted values were not significantly different from the actual values obtained from the standard method at 95% confidence intervals. These results showed the feasibility of using portable spectrometers to predict the starch content of fresh cassava roots.

1. INTRODUCTION

Cassava (Manihot esculenta Crantz) is one of the most drought-resistant crops that can grow in low-fertility and degraded soils but still produces reasonable yields. Furthermore, it has flexibility in harvest time and seasons.1 The cassava root is considered as an important source of carbohydrates and the third highest energy-giving diet after rice and wheat.2 Utilization of the cassava root is numerous. The direct uses of the roots are processed into various food forms for human consumption, animal feed, including ethanol production. The starch obtained from the roots is used as the industrial raw material for binding agent or thickening agent in food and non-food industries such as in paper, textile, plywood, glue, and bioplastic industries.3,4 However, the uses of fresh cassava roots are limited by the shelf life of the roots after harvest due to the rapid postharvest physiological deterioration resulting in degraded and undesirable quality attributes in the market.5 The more the importance given to the high quality of the roots after harvest, the better the utilization of these products and therefore more benefits for growers, manufactures, and consumers.

The cassava is an important economy crop of Thailand and its quality traits depend on the starch content. The starch content in the fresh roots determines their end use and price. Thai cassava growers usually determine harvest maturity based on a subjective assessment of the root size (length, surface, diameter, and volume) and estimate harvest time as the time ranges from planting to harvesting at around 8−12 months.6 However, these attributes depend on the cultivar and the prevailing conditions during the season. A non-invasive, field portable system capable of assessment of starch thus has potential use in gauging the time of harvest or delay in harvest.

Near infrared (NIR) spectroscopy has been widely adopted in food and agriculture industries. The most attractive advantage of the technique is the non-invasive and rapid determination of several quality attributes at the same time without or with only minimal sample preparation. Many studies have been reported in recent years on using NIR spectroscopy for the spectral region of 1100−2500 nm for the rapid estimation of starch content of flour prepared from sweet potato, taro, and yam. It was found that NIR could be used to predict starch contents in sweet potato, taro, and yam, with $R^2$ of 0.71, 0.76, and 0.84, respectively.7−9 In addition, the flour
prepared from tropical root and tuber crops (cassava, sweet potato, taro, and yam) was analyzed for their starch content. Models were developed separately from each crop species and by combining data from all species. Results showed acceptable performance for the measurement of starch in both cases with $R^2$ ranging as high as 0.82–0.91.

Although the previous NIR studies have shown satisfactory results for starch measurement in the roots, flour samples must be prepared in order to remove water in the raw material and make uniform, which is time consuming and instruments required. The water must be removed from samples before being measured at the long-wavelength NIR (1100–2500 nm) region because strong absorption bands of water OH groups overlap the absorbance bands of the substance of interest. In contrast, short-wavelength NIR spectroscopy (SWNIR; 700–1100 nm) is used for high-moisture sample assessment because of lower absorptivity by water in these regions. The SWNIR spectroscopy can be applied to raw materials and can reveal information on the internal attributes of intact samples such as potato, apple, apricot, orange, pear, and peach. The measurement modes most often used for the prediction in raw materials are reflectance and interactance. NIR light passes through the peel (rind of a fruit or vegetable) in both reflectance and interactance modes. This acts as a barrier and prevents the penetration of the infrared radiation to the flesh which can affect the prediction results. The optical depth penetrated into the intact fruit is typically 2–4 mm when using the reflectance and 10–30 mm for the interactance within the SWNIR regions. Surprisingly, despite the economic importance of cassava crops, very few studies have been conducted on cassava and no research has been reported on measuring the starch content of intact roots and cassava flesh using SWNIR regions. Spectral measurements for these regions offer considerable advantages because they can be done more quickly and conveniently using a low-cost portable spectrometer.

The objective of this work was to assess the feasibility and efficiency of SWNIR spectroscopy for predicting the starch content in fresh cassava roots. This information is useful for tapioca starch factories, including cassava growers and other relevant factories for starch evaluation in the cassava roots before and after harvesting.

2. RESULTS AND DISCUSSION

Starch content values for the cassava root samples obtained from the polarimetric method were converted from a dry weight basis to a fresh weight basis. It was found that the starch values were approximately the mean value of 32.79% (standard deviation = 4.69%) and range between 16.11 and 40.14% and standard error of the laboratory of 0.84%. The samples showed a large variability of the starch content for cassava roots in this trial. These results confirm that the selected roots cover the range of starch content of 32–35% in fresh weight that is usually found at harvest maturity. Before developing models, aberrant spectra (outliers) in each measurement were detected and removed. The outliers are the samples containing interferences which may be induced by interface errors, sensor malfunctions and fouling, and bad sampling or sample presentation and have a negative influence on the modeling. The outliers show up in the scores plot, which were obtained from the principal component analysis (PCA), as points outside the normal range of variability (Figure 1).

Table 1. Descriptive Statistics of the Calibration and Validation Set for Starch Prediction

| spectrometer  | sample          | sample set | range (º) | mean ± SD | number of samples |
|--------------|-----------------|------------|-----------|-----------|-------------------|
| model A      | cassava flesh   | calibration | 16.11–40.14 | 32.90 ± 4.54 | 127               |
| 720–1050 nm  | intact root     | validation | 20.46–39.51 | 33.05 ± 4.25 | 63                |
| model B      | cassava flesh   | calibration | 16.11–40.14 | 32.92 ± 4.58 | 124               |
| 850–1150 nm  | intact root     | validation | 20.46–39.51 | 33.05 ± 4.29 | 60                |

*Expressed in percentage on a fresh weight basis (%FW).

![Figure 1. PCA scores plot for all samples obtained from intact root measurement in the wavelength range of 750–1050 nm with the detected outliers.](https://dx.doi.org/10.1021/acsomega.0c01598)
content using SWNIR spectra for both spectrometers are listed in Table 2. Second derivative absorbance spectra using the number of different smoothing points had various degrees of effect on the calibration and prediction performance of partial least squares (PLS) models, as measured by correlation coefficient for calibration ($r_c$) and standard error for the calibration (SEC), correlation coefficient for prediction ($r_p$) and standard error for prediction (SEP), and the ratio of sample SD to SEP (RPD). Furthermore, the number of smoothing point depends on digital resolution of an instrument for the second derivative pretreatment. More detailed spectra data require a substantial amount of smoothing because the second derivative processing amplifies the interference that affects a stable and reliable calibration model.

| sample          | pretreatment | PCs | RPD  | calibration | validation |
|-----------------|--------------|-----|------|-------------|------------|
|                 |              |     |      | $r_c$ | SEC (%) | $r_p$ | SEP (%) | bias (%) |
| cassava flesh   | 2nd derivative (41 points) | 5   | 2.46 | 0.918 | 1.798  | 0.916 | 1.730  | −0.362 |
|                 | 2nd derivative (51 points) | 5   | 2.46 | 0.915 | 1.833  | 0.917 | 1.726  | −0.355 |
|                 | 2nd derivative (61 points) | 5   | 2.45 | 0.914 | 1.838  | 0.916 | 1.731  | −0.346 |
|                 | 2nd derivative (71 points) | 5   | 2.42 | 0.914 | 1.837  | 0.914 | 1.756  | −0.340 |
| intact root     | 2nd derivative (41 points) | 6   | 1.28 | 0.728 | 3.132  | 0.645 | 3.317  | 0.168  |
|                 | 2nd derivative (51 points) | 6   | 1.33 | 0.706 | 3.236  | 0.678 | 3.186  | 0.257  |
|                 | 2nd derivative (61 points) | 6   | 1.35 | 0.695 | 3.286  | 0.687 | 3.151  | 0.279  |
|                 | 2nd derivative (71 points) | 6   | 1.34 | 0.689 | 3.314  | 0.679 | 3.167  | 0.253  |

| sample          | pretreatment | PCs | RPD  | $r_c$ | SEC (%) | $r_p$ | SEP (%) | bias (%) |
|-----------------|--------------|-----|------|------|---------|------|---------|---------|
| cassava flesh   | 2nd derivative (101 points) | 3   | 2.03 | 0.928 | 1.707  | 0.874 | 2.111  | −0.664 |
|                 | 2nd derivative (121 points) | 3   | 2.18 | 0.925 | 1.741  | 0.891 | 1.968  | −0.598 |
|                 | 2nd derivative (141 points) | 3   | 2.33 | 0.920 | 1.792  | 0.906 | 1.838  | −0.487 |
|                 | 2nd derivative (161 points) | 3   | 2.48 | 0.917 | 1.821  | 0.917 | 1.730  | −0.415 |
| intact root     | 2nd derivative (101 points) | 4   | 1.45 | 0.760 | 3.032  | 0.738 | 2.972  | 0.247  |
|                 | 2nd derivative (121 points) | 5   | 1.52 | 0.779 | 2.922  | 0.761 | 2.845  | 0.281  |
|                 | 2nd derivative (141 points) | 5   | 1.54 | 0.773 | 2.959  | 0.772 | 2.803  | 0.215  |
|                 | 2nd derivative (161 points) | 6   | 1.51 | 0.778 | 2.930  | 0.760 | 2.860  | 0.160  |

$^a$PCs: number of latent variables in the calibration equation, RPD: the ratio of sample SD to SEP, $r_c$: correlation coefficient of calibration, SEC: standard error of calibration, $r_p$: correlation coefficient of prediction, SEP: standard error of prediction, bias: the average of the residual.

Figure 2. PLS regression coefficients for optimum calibrations base on absorbance data for cassava flesh collected from wavelength ranges at (a) 720–1050 and (b) 850–1150 nm and for intact root collected from wavelength ranges at (c) 720–1050 and (d) 850–1150 nm.
Among all spectra pretreatment methods, the best starch prediction model was developed using 2D plus 51 smoothing and 2D plus 161 smoothing for the wavelength ranges of 720–1050 and 850–1150 nm of cassava flesh, respectively. Table 2 shows that the best correlation for the calibration model was 0.915 and the SEC was 1.833% for 720–1050 nm, compared with the best correlation of 0.917 and the SEC of 1.821% for 850–1150 nm. In predicting the starch content for the validation set of cassava flesh, both starch prediction models had excellent accuracies, with $r_p$ values of 0.917 and 0.917, SEP of 1.726 and 1.730%, RPD of 2.46 and 2.48 for 720–1050 and 850–1150 nm, respectively. For the intact roots, the pretreatment of 2D plus 61 smoothing (720–1050 nm) and 2D plus 141 smoothing (850–1150 nm) achieved the best prediction performance. The results were not as good as those for cassava flesh. The values of $r_c$ and SEC were 0.695 and 3.286% for 720–1050 nm, which were less than that from 850–1150 nm ($r_c = 0.772$ and SEC = 2.968%). When the model was used to predict the starch content for intact roots, both spectrometers provided acceptable predictions with $r_p$.

Figure 3. X-loading weight plots of optimum models for starch content in fresh cassava root measurement of cassava flesh using wavelength ranges of (a) 720–1050 and (b) 850–1150 nm and measurement of intact root using wavelength ranges of (c) 720–1050 and (d) 850–1150 nm.

Figure 4. Scatter plots between predicted and actual values of starch content in fresh roots measured from cassava flesh (top) and intact root (bottom) based on wavelength region at 720–1050 nm (left) and at 850–1150 nm (right).
were also obvious bands of water OH groups at 747 nm, the combination bands associated with the first overtone associated with OH stretching (990 nm) of starch, the third overtone associated with CH 2 stretching (930 nm), the bands associated with CH stretching (900, 910, and 914 nm), the bands associated with CH deformation (1040 nm), and the second overtone associated with CH stretching (1100–1200 nm). Moreover, there were also obvious bands of water OH groups at 747–776 and 970–975 nm. These confirmed the influence of OH bands of water on the starch prediction of fresh cassava root.

Further analysis on X-loading weights would enable to ascertain the influence wavelengths for determining the starch content and thus gain a better understanding of light interaction with the root tissue. Figure 3 shows the X-loading weights for the prediction models of the starch content of a fresh cassava root. The X-loading spectra of various PLS factors showed vibration bands relevant to the starch prediction which began to present at PLS factor 3 of all models, except for the cassava flesh model in ranges of 850–1150 nm (PLS factor 2). The wavelengths were located at 747–776, 902–917, 931–935, 970–975, 990–993, 1036–1054, and 1092–1131 nm. These bands were associated with starch and water. It was again observed that the peaks at 747–776 and 970–975 nm region were influenced by absorption of water in prediction of starch content. These confirmed that CH, OH bands of starch and the OH bands of water influenced the prediction of the starch content of the fresh cassava root.

Results from the reference method (actual values) of the validation set were plotted against NIR predicted values to give a visual impression of the performance of the calibration. Figure 4 shows deviations of single samples visualized in a scatter plot between actual and predicted values. When comparing the predictive ability of the models from cassava flesh versus models for intact roots, it is interesting that all starch prediction equations were good with RPD parameters above 2 for cassava flesh measurement (Table 2). The RPD was used to evaluate model prediction accuracy. The RPD values are between 2 and 2.5 allowing for approximate quantitative predictions to be made. Indeed, if NIR spectra are collected from intact samples, it could result in reduced accuracy due to the heterogeneity of the samples.25 In the same way as the calibration results, cassava flesh spectra performed better than intact root spectra in predicting starch content. One possible explanation was that peel of the root affected the SWNIR measurement of the intact root. The thickness of the peel acts as a barrier which prevents the penetration of the infrared radiation to the starchy flesh. However, RPD between 1.5 and 2.0 for the intact root can be used for rough predictions. It was pointed out that this measurement can be conducted to assess starch content of in-ground cassava roots prior to harvesting.

In order to further investigate the applicability of the model, the performance measurement of the equation is conducted in accordance with ISO 12099:2017(E). Three confidence limits were calculated to determine the limits for accepting or rejecting equation performance. If bias was lower than $T_{b,i}$, it indicated that the bias was not significant. An SEP value lower than $T_{UE}$ indicated that the SEP was low enough for practical acceptance. The $t_{obs}$ was lower than $T_{(1−α/2)}$ which was obtained from the value of the $t$-distribution with a probability of $α = 0.05$. It indicated that the slope was not significantly different from 1. The results of statistics for performance measurement are summarized in Table 3. Therefore, all calibration models could be used for determining starch content of the fresh cassava root with an acceptable accuracy. Predicting starch content values were not significantly different from actual values at 95% confidence intervals. These results demonstrated that the SWNIR technology could be used for determining starch content of fresh cassava roots and screening roots in the field to estimate a proper harvesting time.

### 3. CONCLUSIONS

The results from this study demonstrated that SWNIR spectroscopy could be used to predict the starch content with excellent accuracy from cassava flesh (cross-section cut root) measurement. The models which were established from intact root spectra remained interesting. Although it was not as accurate as the cassava flesh measurement, but if considered in

| Table 3. Statistics of Model Performance Measurement Followed in ISO 12099:2017(E) |
|-----------------|----------------|----------------|-----------------|
| wavelength      | sample         | parameters | calculated value | result     |
| 720–1050 nm     | cassava flesh  | bias       | $T_{b} = \pm 0.435$ | $−0.355$   | pass       |
|                 |                 | SEP        | $T_{UE} = 2.187$ | $1.726$    | pass       |
|                 |                 | $t_{obs}$  | $t_{(1−α/2)} = 1.999$ | $1.447$    | pass       |
|                 | intact root     | bias       | $T_{b} = \pm 0.807$ | $0.279$    | pass       |
|                 |                 | SEP        | $T_{UE} = 3.931$ | $3.151$    | pass       |
| 850–1150 nm     | cassava flesh  | bias       | $T_{b} = \pm 0.447$ | $−0.415$   | pass       |
|                 |                 | SEP        | $T_{UE} = 2.179$ | $1.730$    | pass       |
|                 |                 | $t_{obs}$  | $t_{(1−α/2)} = 2.000$ | $1.560$    | pass       |
|                 | intact root     | bias       | $T_{b} = \pm 0.724$ | $0.215$    | pass       |
|                 |                 | SEP        | $T_{UE} = 3.543$ | $2.803$    | pass       |
|                 |                 | $t_{obs}$  | $t_{(1−α/2)} = 2.001$ | $1.628$    | pass       |

$T_{b,i}$: bias confidence limits, $T_{UE}$: unexplained error confidence limits, $t_{obs}$: observed $t$ value, calculated from eq 5, $t_{(1−α/2)}$: $t$ value obtained from table $t$-distribution for a probability of $α = 0.05$, SEP: standard error of prediction.
terms of nondestructive testing, it is likely that these models would do better for estimating starch content of in-ground cassava roots because it can continue growing. However, there are many strategies that will help to develop a more accurate starch prediction model. Further improvement in the lighting/detector configuration, calibration methods, different spectral preprocessing, and modeling methods is needed to be considered in order to improve the starch content prediction accuracy for intact roots.

4. MATERIALS AND METHODS

4.1. Cassava Root Samples. Cassava root samples, 200 in total, were collected from farmers’ field in Ratchaburi and Kanchanaburi provinces in Thailand during the harvest season. Harvest took place around 8–12 months after planting. The cassava grew in those field sites with various soil types based on appropriate varieties and different field treatments according to local farmers’ protocols. This study used four commercial varieties consisting of Kasetsart 50, Rayong 5, Rayong 11, and Huaybong 80 that are certified from the Department of Agriculture, the Ministry of Agriculture and Cooperatives, Thailand. All root samples were washed to remove adhered soil. Thereafter, the samples were air dried to remove adhered water and equilibrated at ambient temperature for 1 day before spectral measurements. Spectral measurements were first taken from intact roots and then from cross-section cut roots, using two portable spectrometers. After the spectral measurements had been completed, wet lab chemistry analysis was performed to measure the starch content of each root sample.

4.2. SWNIR Spectra Collection. Spectra were collected from all samples using two spectrometers which were model A (STS-NIR, Ocean Optics Inc., USA) for the spectral region of 650–1100 nm and model B (Maya2000 Pro-NIR, Ocean Optics Inc., USA) for the spectral region of 800–1200 nm. However, the first and last wavelength regions (650–720 and 1050–1100 nm for model A; 800–850 and 1150–1200 nm for model B) were omitted because of the existence of considerable noise in these regions. Both spectrometers were operated in the interactance mode (Figure 5a). A bifurcated interactance probe was used to transfer light from the light source to the sample and reflected light from the sample to detector mounted on the device. The tungsten halogen light source with optimum power 7 W was set for the model A spectrometer and 20 W for model B spectrometer. Preliminary tests (unpublished) showed that the cassava root that had its periderm layer removed by a kitchen knife before spectrum measurement showed better precision and prediction results.

Fortuitously, the periderm layer which is about 1 mm thick came off easily while it was being harvested or washed. Therefore, this layer was peeled off at the spectral collecting positions. Spectral measurements were taken at six positions along the cassava root and around equatorial locations on the sample which was divided into three equal parts along the length of the cassava root. The point of spectrum collection was the middle of each part and on the opposite side (Figure 5b). Two scans were acquired from the same position on intact roots for both spectrometers. Then, the average spectrum was calculated from the spectra of each root sample. After spectral measurements for the intact sample had been completed, the root was cut in the transverse direction (cross sections) into three sections using a stainless steel kitchen knife. Spectral measurements were made using each spectrometer from the three section. Two scans were taken at two positions that were approximately equidistant from the center and the outside edge of each piece (Figure 5c), and the spectra were averaged. Integration times were 30 and 15 ms for model A and model B spectrometers, respectively. All measurements were conducted on single roots in order to compare sample preparation between intact root and cross-section cut root, and to eliminate in-batch variation using two portable spectrometers operated in the interactance mode for the SWNIR regions. Reference spectra were acquired from a white ceramic plate measurement in order to calculate the relative absorbance of each root sample.

4.3. Reference Measurement. Each cassava root including the peel were chopped with a chopper (DPA130, SEB, France). The sample was divided for moisture and starch content analysis. Five grams of the chopped sample was used to analyze the moisture content and was dried in an oven at 105 °C for 5 h. Each sample was done in duplicate. To analyze the starch content, the remaining samples were oven-dried at 50 °C until moisture content was lower than 12% or about 24 h. The dried samples were ground into flour just after oven-drying. In order to achieve a homogenized sample, ground samples were passed through four sieves (mesh nos. 10, 12, 16, and 25) until their particle size was less than 0.707 mm. One hundred grams of the flour samples was packed into a vacuum bag and sent to the Cassava and Starch Technology Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand, for starch content analysis using the polarimetric method of the European Economic Community. The sample (5.0 g) was put in a 200 mL volumetric flask and added with 50 mL of 1.128% hydrochloric acid. The flask was
shaken until the sample was saturated and then a further 50 mL of 1.128% hydrochloric acid was added. Next, the sample in the flask was immersed in a boiling water bath and swirled vigorously for 3 min to prevent flocculation. After 15 min, the flask was removed from the boiling water bath, 60 mL of cold water was added, and it was cooled immediately to 20 °C. Then, 20 mL of sodium phosphotungstate solution was added into the flask and shaken vigorously to form the precipitate. After adjusting the volume with 20 °C distilled water, the solution was mixed thoroughly and filtered through a dry filter paper (Whatman no. 1). The first 25 mL of the filtrate was discarded. The following filtrate was transferred to a 100 mm tube of the polarimeter. The specific optical rotation of 184.0° was used for calculation.

### 4.4. Data Analysis and Model Development

PCA was initially performed using all available samples in order to evaluate the variation among the samples and to eliminate outlier samples. The outliers were determined as those points outside the normal range of variability in the PCA scores plot. After removing the outliers, all remaining samples were first sorted for starch content in the ascending order. The samples were then divided into a calibration set and validation set by a ratio of 2:1 (i.e., every third sample was taken out for validation). A calibration model for starch prediction was developed for the calibration samples only.

PLS regression was used for modeling and prediction, and the modeling was performed by the Unscrambler v9.7 (CAMO Software AS, Norway) software. To increase the spectral resolution and remove baseline offset and baseline slope (tangent) arising from scattering effects, second derivative of the spectra was calculated using a Savitzky Golay second derivative (2D) fit over 20 nm. After the calibration model had been developed, statistical parameters including correlation coefficient of the calibration set (r_c) and the validation set (r_v), the SEC and SEP data sets, bias (predicted fault), and the RPD were calculated. These parameters were used to assess the performance of each calibration model for predicting the starch content of the roots.

#### 4.5. Performance Measurement of the Prediction Model

The performance of the best prediction model shall be determined by a set of validation samples according to the International Organization for Standardization (ISO) 12099:2017(E) for animal feeding stuffs, cereals, and milled cereal products-guidelines for the NIR spectrometry. Statistical parameters for performance measurement are bias, SEP, and slope. The significance of the bias is checked by a t-test. If the bias value is less than the bias confidence limits (BCLs), the bias is not significantly different from zero. The calculation of BCLs or T_b is defined in eq 1.

\[ T_b = \pm \frac{t_{(1-\alpha)/2,2} \times \text{SEP}}{\sqrt{n}} \]  

(1)

The SEP expresses the accuracy of routine NIR results corrected for the mean difference (bias) between routine NIR and the reference method. If SEP is less than the unexplained error confidence limits (T_{UE}), the SEP can be accepted, whereas the T_{UE} are calculated from an F-test as

\[ T_{UE} = \text{SEC} \sqrt{F_{(a,\nu,M)}} \]  

(2)

where α is the probability of marking a type I error; t is the appropriate Student t-value for a two-tailed test with degrees of freedom associated with SEP and the selected probability of a type I error; n is the number of independent samples; SEP is the standard error of prediction. SEC is the standard error of calibration; \( \nu = n - 1 \) is the numerator degrees of freedom associated with the SEP of the test set in which n is the number of samples in the validation process; M = n_l − p − 1 is the denominator degrees of freedom associated with the SEC in which n_l is the number of calibration samples and p is the number of terms or PLS factors of the model.

The slope, b, of the simple regression \( y = a + bx \) is often reported in NIR publications. The slope must be calculated with the reference values as the dependent variable and the predicted NIR values as the independent variable. From the least squares fitting, the slope is calculated as

\[ b = \frac{s_{b\bar{y}}}{s_b^2} \]  

(3)

where \( s_{b\bar{y}} \) is the covariance between the reference and predicted values; \( s_b^2 \) is the variance of n predicted values.

The intercept is calculated as

\[ a = \bar{y} - b\bar{x} \]  

(4)

where \( \bar{y} \) is the mean of the reference values; \( \bar{x} \) is the mean of the predicted values; and b is the slope.

As for the bias, a t-test can be calculated to check the hypothesis that \( b = 1 \)

\[ t_{obs} = |b - 1| \sqrt{\frac{s_b^2 (n - 1)}{s_{res}^2}} \]  

(5)

where b is the slope; n is the number of independent samples; \( s_b^2 \) is the variance of the n predicted values; and \( s_{res}^2 \) is the residual SD as defined in

\[ s_{res} = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - (a + b\bar{x}))^2} \]  

(6)

where a is the intercept eq 4; b is the slope eq 3; \( y_i \) is the i-th reference value; and \( \bar{x} \) is the i-th predicted value obtained when applying the multivariate NIR model.

The slope, b, is considered as different from 1 when \( t_{obs} \geq t_{(1-\alpha/2,2)} \), where \( t_{obs} \) is the observed t-value, calculated according to eq 5; \( t_{(1-\alpha/2,2)} \) is the t-value obtained from table t-distribution for a probability of \( \alpha = 0.05 \).

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Notes
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