Introduction

Half of the populations in the world have rice as the main food source. Protein, as one of the important components of rice nutrition quantity, rice protein content influence on cooking time, rice texture and nutritional value (Juliano, 1985). Protein content in milled rice has traditionally been determined by Kjeldahl analysis and more recently by the combustion method. Both methods are replacement of spent chemicals, catalysts and reagents and require the expensive apparatus (Delwiche et al., 1996).

Near infrared spectroscopy (NIRS) is nondestructive technique, rapid, accurate and precise method, that is alternative to wet chemistry procedures for determining the concentration of various constituents in food and agricultural products (Williams and Norris, 2001). NIRS was used for prediction of rice chemical composition such as amylose (Ripon et al., 2006), protein (Delwiche et al., 1996), moisture (Theanjumpol et al., 2006), and fat...
content (Wang et al., 2006). In addition, identify aromatic Thai rice varieties (Theanjumpol, et al., 2005). The main variation in sensory texture attributes is related to amylose and protein content in rice (Juliano, 1985). Therefore, the objective of this study was to determine protein content in Thai milled rice by NIRS.

Materials and Methods

Five rice cultivars; Khaodawkmali105 (KDML105), RD15, Homsuphanburi (HSP), Pathumthani1 (PTN1) and Chainat1 (CNT1) were grown at Faculty of Agriculture, Chiang Mai University on November 2005. Samples were prepared and analysed at Postharvest Technology Institute, Chiang Mai University. A commercially available NIR spectrophotometer, “NIRSystems6500” (Foss NIRSystems, Silver Spring, USA), equipped with spinning module was used to measure the whole milled rice spectra in the long wavelength region from 1100 nm to 2500 nm. The spectra were obtained at 2 nm intervals with the average scan of 32 times. Conducting the chemical analysis to determine the protein content of milled rice by the Dumas combustion method (Sweeny and Rexroad, 1987) with the automated LEKO CN analyzer model CN2000 (LECO, St. Joseph, MI) at Institute of Agriculture Chemistry, University of Goettingen, Germany. The protein conversion factor of 5.95 was used for calculation the protein content (FAO, 2003). Partial least squares regression (PLSR) was used to develop the calibration equation which performed by the Unscrambler® version 7.6 software (Camo, Oslo, Norway).

Results and Discussion

The range of protein content in milled rice was from 6.64 to 9.03 %. In Table 1 showed the characteristic of the sample in calibration and validation set to develop the calibration equation.

Table 1: Characteristic of calibration and validation sample set of milled rice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calibration set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sample</td>
<td>64</td>
<td>55</td>
</tr>
<tr>
<td>Protein content</td>
<td>6.64-9.03</td>
<td>6.65-8.89</td>
</tr>
<tr>
<td>Mean</td>
<td>7.72</td>
<td>7.68</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>Unit</td>
<td>%wet basis</td>
<td></td>
</tr>
</tbody>
</table>

The original reflectance spectra of five milled rice cultivars in long wavelength region (1100-2500 nm) were shown in Figure 1. The clearly peak at 1454 and 1928 nm due to water band and 2088 nm was starch band, which is the major component in rice (Osborne et al., 1993). The spectra treated with multiplicative scatter correction (MSC) to reduce the light scattering effect which caused from grain shape and size. Also, transformation with Savitzky-Golay second derivative (10 nm averaging for left and right side) was use to remove baseline shift, overlapping peak and resolution broad absorbance band on the reflectance spectra (Williams and Norris, 2001). The spectral beyond 2300 nm were quite noisy, to reduce noise by transforming with Savitzky-Golay smoothing (5 nm averaging for left and right side). Then, the treated spectra showed the clearly negative peak at 1430, 1704, 1902 and 2070 nm (Figure 2).
The PLSR calibrations were developed in wavelength region from 1100 nm to 2250 nm. The regression coefficient plot showed the high value at 1726 nm which influenced to the protein content calibration equation (plot not shown in this paper), similar to Rittiron et al. (2004, 2005) were found protein band at 1735 nm and 1726 nm in single brown and milled Japanese rice kernel respectively. The selected protein calibration equation showed their value of the correlation coefficient (R), standard error of calibration (SEC), standard error of prediction (SEP) and Bias were 0.95, 0.15 %, 0.19 % and 0.04 respectively. In addition, the value of the ratio of standard deviation of reference data in validation set to SEP (RPD) was 2.52 (Table 2). Similar to the research of Delwiche et al. (1996) which developed NIR reflectance equation of protein content of whole grain milled rice were grown at United States.

Table 2  PLSR calibration results for protein content using spectra treated with second derivative, smoothing and multiplicative scatter correction (MSC).

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Wavelength region (nm)</th>
<th>F</th>
<th>R</th>
<th>SEC</th>
<th>SEP</th>
<th>Bias</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second derivative</td>
<td>1100-2500</td>
<td>6</td>
<td>0.96</td>
<td>0.14</td>
<td>0.23</td>
<td>0.04</td>
<td>2.03</td>
</tr>
<tr>
<td>Second derivative + Smoothing</td>
<td>1100-2250</td>
<td>6</td>
<td>0.96</td>
<td>0.15</td>
<td>0.21</td>
<td>0.05</td>
<td>2.25</td>
</tr>
<tr>
<td>MSC + Second derivative</td>
<td>1150-2500</td>
<td>6</td>
<td>0.96</td>
<td>0.14</td>
<td>0.22</td>
<td>0.05</td>
<td>2.09</td>
</tr>
<tr>
<td>MSC + Second derivative + Smoothing</td>
<td>1100-2250</td>
<td>6</td>
<td>0.95</td>
<td>0.15</td>
<td>0.19</td>
<td>0.04</td>
<td>2.52</td>
</tr>
</tbody>
</table>

F: number of factors used in the calibration equation.

R: multiple correlation coefficients.

SEC: standard error of calibration.

Bias: average of difference between actual value and NIR value.

*: indicate significant difference at 95% confidence.

Table 2  PLSR calibration results for protein content using spectra treated with second derivative, smoothing and multiplicative scatter correction (MSC).

Figure 3  Scatter plots for predicting protein content in the calibration sample set (a) and validation sample set (b) using PLSR calibration equation.
The scatter plots between the actual values and NIRS predicted values of calibration and validation set were shown in Figure 3. The standard errors in both set had obtained quite 0.2%. This indicated that the model for protein content was the accurate.

Summary

The predicted calibration equation from this experiment has high values of the correlation coefficient and low values of the standard error of calibration and the standard error of prediction. Therefore, milled rice protein content determination by NIRS can be used to replace traditional method in routine use and useful primary analysis in the agriculture and food industries with rapid and high accuracy results.

Acknowledgements

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Literature cited


