

การหาปริมาณโปรตีนในข้าวสารด้วยเนียร์อินฟราเรดสเปกโทรสโกปี Determination of protein content in Thai milled rice by near infrared spectroscopy

ศิริพร ริพล¹ สงวนศักดิ์ ธนาพรพูนพงษ์² ปาริชาติ เทียนจุมพล¹ และสุชาดา เวียรศิลป์²
Siraporn Ripon¹, Sa-nguansak Thanapornpoonpong², Parichat Theanjumpol¹ and Suchada Vearasilp²

Abstract

The protein content determination in five cultivars of Thai milled rice; Khaodawkmal105 (KDML105), RD15, Pathumthani1 (PTN1), Homsuphanburi (HSP) and Chainat1 (CNT1) were assayed by near infrared spectroscopy (NIRS). Each milled rice cultivar was random and measured the spectrum by NIRSystems 6500 in wavelength region from 1100 nm to 2500 nm. The protein content of milled rice was determined by Dumas combustion method. The range of protein content was 6.64 to 9.03 %. Partial least squares regression (PLSR) was used to develop the calibration equation for prediction. The correlation coefficient (R), standard error of calibration (SEC), standard error of prediction (SEP), and ratio of standard deviation of reference data in validation set to SEP (RPD) from the equation were 0.95, 0.15 %, 0.19% and 2.52 respectively. This result was found that the calibration equation from this experiment obtained has high values of R and low values of SEC and SEP. Therefore, it can be concluded that NIRS could be used to determine the protein content of milled rice.

Keywords: milled rice, near infrared spectroscopy (NIRS), protein content

บทคัดย่อ

การตรวจหาปริมาณโปรตีนในข้าวสารไทย 5 พันธุ์ ได้แก่ ข้าวดอกมะลิ105 กข15 ปทุมธานี1 หอมสุพรรณบุรี และชัยนาท1 โดยเนียร์อินฟราเรดสเปกโทรสโกปี ทำการสุ่มตัวอย่างข้าวสารแต่ละพันธุ์มาวัดสเปกตรัมด้วยเครื่อง NIRSystems 6500 ในช่วงความยาวคลื่น 1100-2500 นาโนเมตร และนำมาวิเคราะห์หาปริมาณโปรตีนด้วยวิธี Dumas combustion ได้ปริมาณโปรตีนในช่วง 6.64 ถึง 9.03 % นำค่าที่ได้มาสร้างสมการทำนายด้วยเทคนิค partial least squares regression (PLSR) พบว่ามีค่าสัมประสิทธิ์สหสัมพันธ์ (R) ค่าความคลาดเคลื่อนมาตรฐานในการทำนายปริมาณโปรตีนในกลุ่ม calibration (SEC) ค่าความคลาดเคลื่อนมาตรฐานในการทำนายปริมาณโปรตีนในกลุ่ม validation (SEP) และสัดส่วนของค่าเบี่ยงเบนมาตรฐานของปริมาณโปรตีนในกลุ่ม validation ต่อค่า SEP (RPD) เท่ากับ 0.95, 0.15 %, 0.19 % และ 2.52 ตามลำดับ จากผลการทดลองพบว่าสมการที่ได้ให้ค่าสัมประสิทธิ์สหสัมพันธ์สูง และให้ค่าความคลาดเคลื่อนมาตรฐานในกลุ่ม calibration และกลุ่ม validation ต่ำ ดังนั้นจึงสามารถใช้เนียร์อินฟราเรดสเปกโทรสโกปีหาปริมาณโปรตีนของข้าวสารได้

คำสำคัญ: ข้าวสาร เนียร์อินฟราเรดสเปกโทรสโกปี ปริมาณโปรตีน

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

¹ สถานีวิจัยการหลังการเก็บเกี่ยว มหาวิทยาลัยเชียงใหม่ เชียงใหม่ 50200

¹ Postharvest Technology Institute, Chiang Mai University, Chiang Mai, 50200

² ภาควิชาพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ เชียงใหม่ 50200

² Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200

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; Khaodawkmalii105 (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 LECO 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

Variable	Calibration set	Validation set
Number of sample	64	55
Protein content	6.64-9.03	6.65-8.89
Mean	7.72	7.68
Standard deviation	0.50	0.47
Unit	%wet basis	

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).

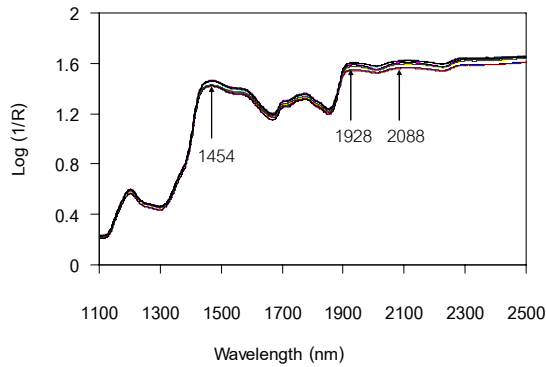


Figure 1 The original spectra of five milled rice cultivars.

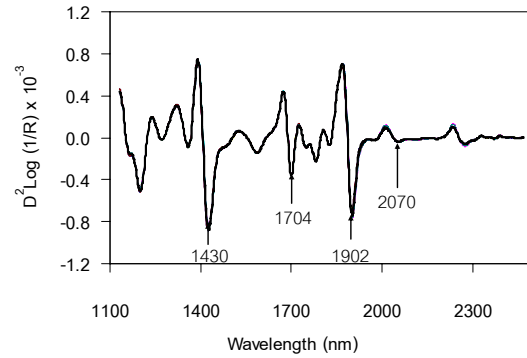


Figure 2 Five milled rice cultivars spectra treated with multiplicative scatter correction (MSC), Savitzky-Golay second derivative and smoothing.

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).

Pre-treatment	Wavelength region (nm)	F	R	SEC	SEP	Bias	RPD
Second derivative	1100-2500	6	0.96	0.14	0.23 ^{ns}	0.04	2.03
Second derivative+ Smoothing	1100-2250	6	0.96	0.15	0.21 ^{ns}	0.05	2.25
MSC + Second derivative	1150-2500	6	0.96	0.14	0.22 ^{ns}	0.05	2.09
MSC + Second derivative +Smoothing	1100-2250	6	0.95	0.15	0.19*	0.04	2.52

F: number of factors used in the calibration equation.

SEC: standard error of calibration.

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

*: indicate significant difference at 95% confidence

R: multiple correlation coefficients.

SEP: standard error of prediction, tested by the method of Fearn (1996).

RPD: ratio of standard deviation of reference data in validation set to SEP.

^{ns}: mean no significant difference at 95% confidence.

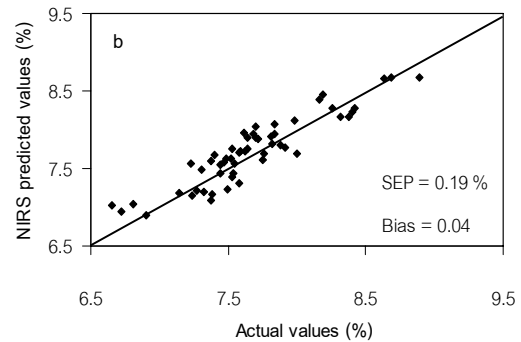
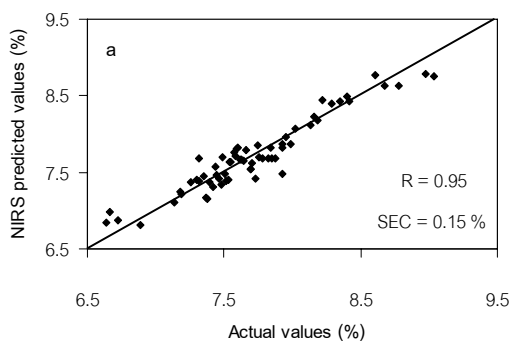


Figure 3 Scatter plots for predicting protein content in the calibration sample set (a) and validation sample set (b) using PLSR calibration equation.

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

We are grateful to Institute of Agriculture Chemistry, University of Goettingen, Germany for determination the protein content of milled rice by the Dumas combustion method.

Literature cited

- Delwiche, S.R., K.S. Mckenzie and B.D. Webb. 1996. Quality characteristics in rice by near-infrared reflectance analysis of whole grain milled samples. *J. Cereal Chem.* 73: 257-263.
- Fearn, T. 1996. Comparing standard deviations. *NIR news.* 7: 5-6.
- Food and Agriculture Organization (FAO). 2003. Food energy-methods of analysis and conversion factors. Publishing Management Service. Rome, Italy. 87 p.
- Juliano, B.O. 1985. Rice: Chemistry and Technology. 2nd ed. The American Association Cereal Chemists, Inc., St. Paul, Minnesota. 774 p.
- Osborne, B.G., T. Fearn and P.H. Hindle. 1993. Practical NIR Spectroscopy: with Applications in Food and Beverage Analysis. 2nd ed. Longman Singapore Publisher (Pte) Ltd, Singapore. 227 p.
- Ripon, S., S. Thanapornpoonpong, S. Veerasilp and P. Theanjumpol. 2006. Prediction of Amylose Content of Milled Rice by Near-Infrared Reflectance Spectroscopy. 32nd Congress on Science and Technology of Thailand (STT.32). Queen Sirikit National Convention Center, Bangkok, Thailand. Available: http://www.scisoc.or.th/stt/32/sec_c/paper/stt32_C1_C0279.pdf.
- Rittiron, R., S. Saranwong and S. Kawano. 2004. Useful tips for constructing a near infrared-based quality sorting system for single brow-rice kernel. *J. Near Infrared Spectrosc.* 12: 133-139.
- Rittiron, R., S. Saranwong, and S. Kawano. 2005. Detection of variety contamination in milled Japanese rice using a single kernel near infrared technique in transmittance mode. *J. Near Infrared Spectrosc.* 13: 19-25.
- Sweeney, R.A. and P.R. Rexroad. 1987. Comparison of the LECO FP 228 "Nitrogen Determination" with AOAC copper Kjeldahl method for crude protein. *J. AOAC International.* 70: 1028-1030.
- Theanjumpol, P., S. Ripon, S. Karaboon, K. Suwapanit, S. Thanapornpoonpong and S. Veerasilp. 2005. Aromatic Thai Rice Identification by Near-Infrared Reflectance Spectroscopy. Tropentag 2005, The Global Food & Product Chain - Dynamics, Innovations, Conflicts, Strategies. University of Hohenheim, Stuttgart, Germany. Available: [http:// www.trogentag.de/2005/abstracts/full/385.pdf](http://www.trogentag.de/2005/abstracts/full/385.pdf).
- Theanjumpol, P., R. Rittiron, S. Thanapornpoonpong and S. Veerasilp. 2006. High Accuracy Moisture Determination in Milled Rice cv. Khao Dawk Mali 105 by Near Infrared Spectroscopy. *J. of Agriculture.* 22(3): 189-277.
- Wang, H.L., X.Y. Wan, J.C. Bi, J.K. Wang, L. Jiang, L.M. Chen, H.Q. Zhai and J.M. Wan. 2006. Quantitative Analysis of Fat Content in Rice by Near-Infrared Spectroscopy Technique. *J. Cereal Chem.* 83: 402-406.
- William, P. and K. Norris. 2001. Near-Infrared Technology in the Agricultural and Food Industries. 2nd ed. America Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA. 296 p.