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Development of a method for quantitative determination of γ-oryzanol using near infrared spectroscopy

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Abstract

γ-Oryzanol is a natural bioactive component found in rice bran oil. Quantitative analysis of the compound is usually done by UV-spectrophotometric or chromatographic methods. This study evaluated the potential of near infrared spectroscopy (NIRS) transmission analysis for determining γ-oryzanol in commercial rice bran oil using standard γ-oryzanol in 3 different solvent systems, i.e. in hexane, in purified medium chain fatty acids (MCF), and in hexane containing 10% MCF. The results showed that NIRS analysis using standard γ-oryzanol in hexane allowed estimation of γ-oryzanol content in commercial rice bran oil with higher accuracy than the others. The predicted performance of the developed model measured by the ratio of performance to deviation (RPD) was 5.93, which indicated that the method effectively provided an alternative for determination of γ-oryzanol in rice bran oil.

Keywords: NIRS, rice bran oil, γ-oryzanol, method development, rapid method

1. Introduction

γ-Oryzanol is a natural component found in rice bran oil and shows several health related functions, such as decreasing of low density of lipoprotein (LDL), inhibiting dietary cholesterol absorption (Berger *et al.*, 2005). γ-Oryzanol refers to a group of several esters of ferulic acid and phytosterols (Xu and Godber, 1999). Various analytical methods for quantifying γ-oryzanol contents are available, such as high performance liquid chromatography (HPLC) and ultraviolet (UV)-spectrophotometry. HPLC is generally applied to measure individual components of γ-oryzanol (Chen and Bergman, 2005; Cho *et al.*, 2012; Huang and Ng, 2011; Lu *et al.*, 2014; Pascual *et al.*, 2013; Pestana *et al.*, 2008; Pestana-Bauer *et al.*, 2012; Shammugasamy *et al.*, 2014), while UV-spectrophotometry is preferred for determining the total content of γ-oryzanol because the method is simpler and more rapid than HPLC (Bhatnagar *et al.*, 2014; Bucci *et al.*, 2003). It is also recommended by CODEX for determination of γ-oryzanol content in rice bran oil (CODEX, 2015).

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Near Infrared (NIR) spectroscopy is one of the most popular non-destructive analytical methods for many practical and industrial applications because it is fast and easy to extend to in-line/on-line analysis (Osborne *et al.*, 1993). It has been applied to wide industrial areas, such as food and agricultural, biotechnology, and pharmaceutical fields (Blanco and Peguero, 2010). Because NIR spectroscopy can be used to measure various major compositions such as protein, ash, crude oil, and moisture content of raw materials (Bangchi, Sharma and Chattopadhyay, 2016), it should be an advantage if the industry can apply it for other analyses. Therefore, the objective of this study was to develop an NIR spectroscopy method for determination of γ-oryzanol which is a major bioactive compound found in rice bran oil.

2. Materials and Methods

2.1 Chemical reagents

γ-Oryzanol (98.5%) was obtained as a gift from Tsuno Rice Fine Chemical (Wakayama, Japan). Medium chain fatty acids (MCF, 99%) was a gift from Siam Fat and Oil (Samut Sakhon, Thailand). Analytical grade hexane (Macron, USA) and n-heptane (Univar, Australia) were used.

2.2 Rice bran oil samples

A total of 27 individual rice bran oil bottles from 3 commercial brands were used for model validation. Rice bran oil samples were collected from local markets in Thailand and kept at 4°C in amber bottles flushed with nitrogen gas. Each sample was used for the reference analysis of γ-oryzanol by UV-spectrophotometry and also for NIR analyses.

2.3 UV-spectrophotometry analysis

γ-Oryzanol content of the rice bran oil sample was determined by spectrophotometric method according to the method described in CODEX (2015) by dissolving 0.2 g of rice bran oil sample in 100 mL of n-heptane and reading the absorbance at 314 nm in 1-cm quartz cuvette using a spectrophotometer (Genesys 10uv, Thermo Scientific, USA). γ-Oryzanol content (%) was calculated by using equation (1):

$$\gamma\text{-Oryzanol content (\%)} = 100 \times (1 / W) \times A \times (1 / 359) \qquad (1)$$

where, A = absorbance of the sample, W = weight of the sample in gram, 359 = specific extinction ($E^{1\%}_{1\ cm}$) for γ-oryzanol.

2.4 NIR spectra acquisition

Three standard γ-oryzanol sample sets at the concentrations of 100 to 1000 mg/L were prepared in order to establish suitable calibration models: (a) set of 100 standard γ-oryzanol solution samples in hexane, (b) set of 100 standard γ-oryzanol solution samples in MCF, and

(c) set of 100 standard γ-oryzanol solution samples in hexane containing 10% MCF. For the rice bran oil samples, 1 g of oil was weighted in 10 mL glass volumetric flask and diluted with 10 mL of hexane. All samples were transferred to a quartz sample container with a path length of 2 mm and measured for the absorbance spectra using an FT-NIR Spectrometer (MPA, Bruker Optics, Germany) in the transmission mode. The spectra acquisition was carried out in the wave number range of 12500–4000 cm^{-1} at every 8 cm^{-1} with 16 scan times.

2.5 NIR spectra pretreatment and calibration model development

The spectra of 100 standard γ-oryzanol samples were used as a calibration set and the spectra of 27 rice bran oil samples were used as a validation set. The spectra of standard γ-oryzanol were fitted to the γ-oryzanol content using the partial least square regression (PLSR) to develop the correspondent NIR prediction equation. Optimized NIR prediction equations were obtained using OPUS Spectroscopy Software (Version 7, Bruker Optics, Germany) with various pretreatment methods including vector normalization (SNV), first derivative, second derivative, multiple scatter correction (MSC), first derivative + SNV, and first derivative + MSC. Best calibration models were selected based on higher squared correlation coefficient (R^2) and ratio of performance to deviation (RPD) and lower root mean square error of prediction (RMSEP) expressed in the same unites as concentration of γ-oryzanol since those parameters are commonly used to evaluate the performance of prediction model (Osborne, Fearm and Hindle, 1993).

3. Results and Discussion

3.1 γ-Oryzanol content in rice bran oil

Mean, standard deviation (SD), minimum, and maximum values of the γ-oryzanol contents in commercial rice bran oil samples determined by UV-spectrophotometry are shown in Table 1. The measured γ-oryzanol contents were from 2760.27 to 6496.18 mg/L rice bran oil, which were close to those reported on the labels. Note that the measurement values was convert from mg/kg to mg/L by using a rice bran oil density of 0.9067 kg/L obtained experimentally.

Table 1 γ-Oryzanol content (mg/L rice bran oil) quantified by UV-spectrophotometry

Brand	Mean (n=9) (mg/L rice bran oil)	SD (n=9) (mg/L rice bran oil)	Minimum (n=9) (mg/L rice bran oil)	Maximum (n=9) (mg/L rice bran oil)	Label (ppm*)
Brand 1	2807.28	34.45	2760.27	2854.45	2500
Brand 2	5336.60	31.48	5305.10	5415.79	5000
Brand 3	6389.29	124.29	6192.26	6496.18	6000

Note: * The unit used on labels

3.2 NIR calibration equation

To obtain the calibration equation for each solvent system, NIR spectral data and chemical data from 100 standard samples were used. The samples of NIR spectra from 3 different solvent systems are shown in Figure 1. It should be noted that spectra of pure solvent systems were almost identical to those added with γ-oryzanol in the tested concentration range and cannot be differentiated by naked eyes (data not shown).

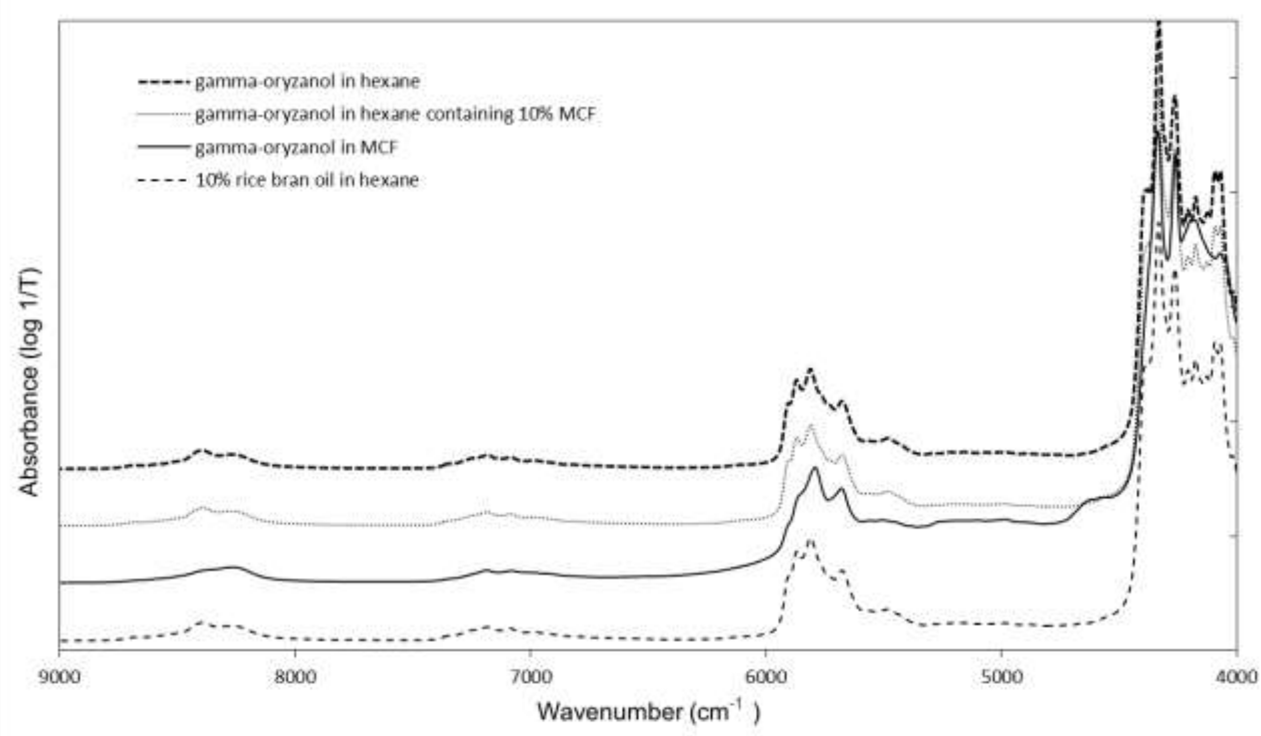

Figure 1 Examples of NIR spectra obtained from standard γ-oryzanol (100 mg/L) in different solvent systems and from 10% rice bran oil in hexane.

Calibration models for γ-oryzanol content from each solvent systems was built by the PLSR method using an automatic optimization procedure in the OPUS software. Calculated statistical parameters of the best calibration model for each solvent system are shown in Table 2. The calibration equation obtained from the γ-oryzanol in hexane gave better results than MCF and hexane containing 10% MCF, as shown in Figure 2–4. The selected wavenumber range for the calibration equation obtained from the γ-oryzanol in hexane was 7502.2 to 6800.2 cm^{-1} which reflected the fact that the region around 6915 and 7057 cm^{-1} represents the –CH stretching vibration and deformation of the aromatic molecules while the region around 7042 cm^{-1} represents the first overtone of –OH groups of aromatics and the range between 7067 to 7352 cm^{-1} arises from the –CH stretching and deformation of the $-CH_2$, $-CH_3$ molecules whereas the region around 7092 cm^{-1} can be attributed to the first overtone of the –OH stretching vibration (Osborne, Fearm and Hindle, 1993). We attempted to improve the accuracy of the model in hexane by adding 10% MCF to the solvent system or changing the solvent system to 100% MCF to stimulate the rice bran oil system. However, the results were not better than those from the model in hexane.

Table 2 Statistic descriptors of calibration and external validation by NIR

Model	Calibration							
	Pretreatment	**LV**	**Region (cm^{-1})**	**N**	**Range (mg/L)**	**R^2**	**RMSEE**	**RPD**
Hexane	2st derivative	6	7502.2-6800.2	100	1009.00-100.90	0.9999	3.55	85.2
MCF	SNV	1	5450.2-4246.7	100	1003.00-100.30	0.1495	289	1.01
Hexane containing 10% MCF	1st derivative+ SNV	7	9403.8-6098.2	100	1012.00-101.20	0.9998	4.48	67.6

Model	Validation				
	N	**R^2**	**RMSEP**	**RPD**	**Bias**
Hexane	27	0.9716	25.4	5.93	0.645
MCF	27	0.9340	143	1.071	-26.2
Hexane containing 10% MCF	26	0.8566	56.1	2.66	6.47

Note: N: number of samples; RMSEE: root mean square error of external validation; RPD: ratio of performance to deviation; RMSEP: root mean square error of prediction; LV: latent variable

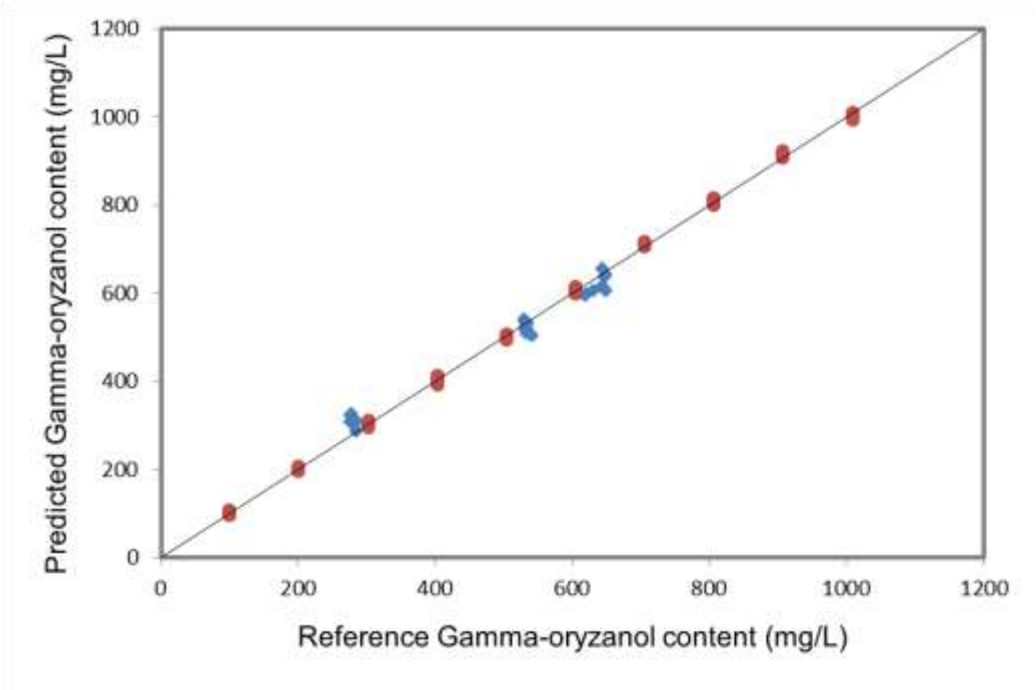

Figure 2 Reference values versus NIR predicted values plots for γ-oryzanol content from the prediction equation obtained using γ-oryzanol in hexane. (●: calibration set; ◆: validation set)

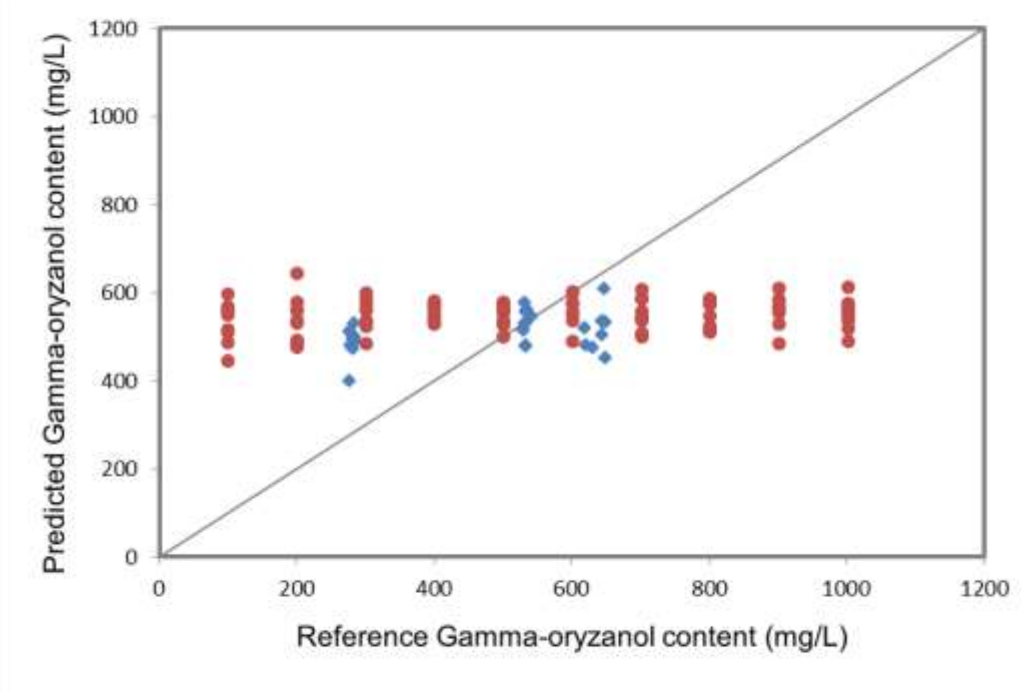

Figure 3 Reference values versus NIR predicted values plots for γ-oryzanol content from the prediction equation obtained using γ-oryzanol in MCF. (●: calibration set; ◆: validation set)

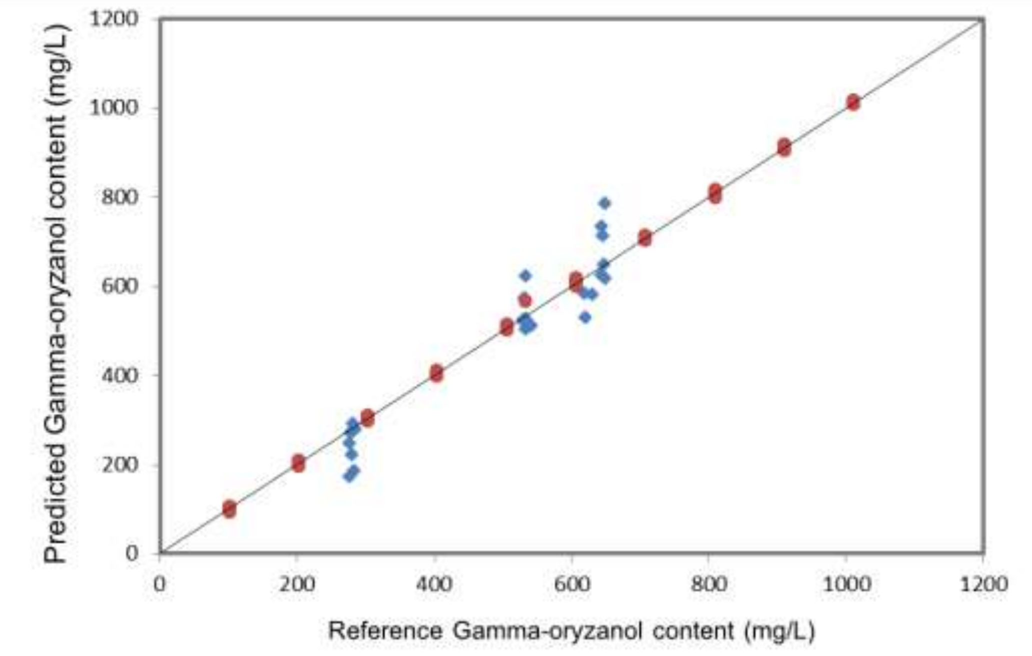

Figure 4 Reference values versus NIR predicted values plots for γ-oryzanol content from the prediction equation obtained using γ-oryzanol in hexane containing 10% MCF (●: calibration set; ◆: validation set)

3.3 External validation

After obtaining the calibration equations, model validation was carried out using an external validation set containing 27 rice bran oil samples from 3 different commercial brands. The statistical results of the validation are also shown in Table 2. Strong correlation between NIR predicted values and reference data for γ-oryzanol was obtained from the calibration model built from γ-oryzanol in hexane (Figure 3). Note that one outlier was removed from the validation set. The model built from γ-oryzanol in hexane clearly showed a better prediction performance than the model built in hexane containing 10% MCF and the model built in MCF with an R^2 value of 0.9716, an RPD of 5.93, and an RMSEP of 25.4 mg/L. Szlyk *et al.* (2005) used NIR spectroscopy for determination of α-tocopherol in edible oils after extraction with ethanol. The calibration model was obtained from standard α-tocopherol in absolute ethanol. The result in this study indicated that it is possible to use the model obtained from γ-oryzanol in hexane to quantify the γ-oryzanol content in rice bran oil.

4. Conclusion

This study demonstrated the potential of NIR spectroscopy combined with chemometrics methods for the rapid determination of γ-oryzanol in rice bran oil with minimal sample preparation. This study will be useful for the effective utilization of NIR spectroscopy as a high throughput measurement method for rice bran oil industry. Although a promising result was obtained, it is necessary to extend the study with more samples in order to obtain a model with the greatest applicability.

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