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Study on evaluation of gamma oryzanol of germinated brown rice by near infrared spectroscopy

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Germinated brown rice (GBR) is rich in gamma oryzanol which increase its consumption popularity, particularly in the health food market. The objective of this research was to apply the near infrared spectroscopy (NIRS) for evaluation of gamma oryzanol of the germinated brown rice. The germinated brown rice samples were prepared from germinated rough rice (soaked for 24 and 48 h, incubated for 0, 6, 12, 18, 24, 30 and 36 h) and purchased from local supermarkets. The germinated brown rice samples were subjected to NIR scanning before the evaluation of gamma oryzanol by using partial extraction methodology. The prediction model was established by partial least square regression (PLSR) and validated by full cross validation method. The NIRS model established from various varieties of germinated brown rice bought from different markets by first derivatives+vector normalization pretreated spectra showed the optimal prediction with the correlation of determination (R^2), root mean squared error of cross validation (RMSECV) and bias of 0.934, 8.84×10^{-5} mg/100 g dry matter and 1.06×10^{-5} mg/100 g dry matter, respectively. This is the first report on the application of NIRS in the evaluation of gamma oryzanol of the germinated brown rice. This information is very useful to the germinated brown rice production factory and consumers.

Keywords: Germinated brown rice; gamma oryzanol; near infrared spectroscopy.

1. Introduction

Germinated brown rice (GBR) contains more chemicals and functional components including gamma-ogyzanol and gamma aminobutyric acid than the ungerminated brown rice.[1] In the rice bran, there is vitamin E (α-tocopherol and tocotrienol) and gamma-ogyzanol.[2]

Gamma oryzanol is a naturally occurring component in rice bran and germ. It is a complex mixture of furalate, esterified with sterols or triterpene

alcohols.[3] Gamma oryzanol in rice bran was about 13 to 20 times (w/w) greater than tocopherol and tocotrienols.[4]

Gamma oryzanol was shown to be able to reduce cholesterol absorption.[5] It was appropriate for the treatment of the inflammatory process[6] and it could inhibit linoleic acid and cholesterol oxidation.[7,8] In addition, it is a potential antioxidant for food, pharmaceutical and cosmetic industries.[9,10]

From Kim *et al.*,[1] the rough rice of Ilpumbyeo variety was soaked in water at 15°C for three days and the results showed that the gamma oryzanol content of different parts (rough rice, hull, brown rice and sprout) was increased by 1.13 and 1.20 fold in germinated rough rice and GBR, respectively.

The near infrared spectroscopy (NIRS) technique can provide rapid results in seconds or continuously online, rather than in hours or days, with an accuracy and reproducibility equivalent to most reference methods and other advantages of NIR include its low cost per test, low labor costs, no required chemicals to purchase or dispose of, great flexibility in sample presentation and the capability of testing many constituents simultaneously.[11]

NIRS has been applied to many types of agricultural products. Researchers have utilized NIRS to analyze chemical components of rice, such as amylose content, protein content, lipid content,[12–16] fatty acid of rough rice,[17] lipid content of milled rice[18] and amino acid in brown rice.[19]

The objective of this research was to apply NIRS for evaluation of gamma oryzanol of the GBR.

2. Materials and Methods

2.1. *Rice samples*

The GBR samples were prepared by a factory of P.J. Brand germinated rough rice in Chonburi Province, Thailand and purchased from local markets in Thailand.

2.1.1. *Preparation of GBR by a factory*

Rough rice of *Oryza sativa* L., cultivar Khao Dawk Mali 105 (KDML 105) was collected from a field of P.J. Brand germinated rough rice factory in Chonburi Province, Thailand. Rough rice was soaked in water at room temperature for 24 or 48 h. The water was changed every 4 h and drained at the end of soaking. The rice was kept in polypropylene sag for incubating at seven different duration (0, 6, 12, 18, 24, 30 and 36 h) to obtain the germinated rough rice (GRR). The GRR was dried using fluidized-bed technique by superheated steam which would reduce the moisture content of the rice to around 19%wb. Then the rice was spread on a screen for 3 h at room temperature and the moisture content would reduce to about 13–14%wb. After that the GRR sample was dehusked before the experiment and it would be called "GBR-Oryzanol adjusted" in this paper. The rough rice sample used was 10 kg for 1 treatment. There was one control condition and 15 treatments with 4 replicates resulted in 68 samples in total. In addition, there were 20 samples from the rough rice sample prepared by the commercial production condition (soaked for 48 h and incubated for 24 h), hence, 88 samples from a factory.

2.1.2. *Germinated brown rice from local markets in Thailand (MGBR)*

All GBR of different brands were purchased from local markets in Thailand and stored in the laboratory at room temperature. These type of samples would be called "market-germinated brown rice (MGBR)" in this paper. Each brand bought was separated into four subsamples. There were 16 brands for KDML 105 variety (68 samples, MGBR–KDML 105) and 16 brands for other varieties such as Red Jasmine rice, Hom Nil, Kam Doi Muser, RD6, Kam Doi Mae jam, Tang Doi, Khao Doi (short grain), Khao Na (short grain), Dok Pradu, Dang Pa Tam, Hom Dang Sukhothai, Hom Sin Lek, Hom Dam Pathumrat, Nheaw Kam and Nheaw RD6 (68 samples, MGBR-various varieties). Hence, 136 samples from local markets.

2.2. *Near infrared spectroscopy experiment*

2.2.1. *Sample scanning*

Each GBR sample was poured from the vacuum bag into the quartz-sampling cup of a rotary diffuse reflectance holder (Bruker Ltd., Germany). NIRS was measured with Fourier transform near infrared (FT-NIR) spectrometer (Bruker Ltd., Germany) in reflection mode on 12 500–4000 cm^{-1} (800–2500 nm). Each rice sample was scanned 64 times at a resolution of 16 cm^{-1}. The scan results were averaged and

recorded in absorption mode (log $1/R$) for each sample. The quartz-sampling cup was cleaned with a vacuum cleaner prior to subsequent use.

2.2.2. *Spectrum pre-treatment and NIRS model establishment*

The NIRS models for predicting the gamma oryzanol content of GBR were established using the partial least squares regression (PLSR). The software for multivariate analysis (OPUS, v. 7.0.129) was used in both spectrum pre-processing and model development. The NIR spectra used to model development were not pre-processing spectra or pre-processing spectra using any of the following methods: constant offset elimination, straight line subtraction, vector normalization (SNV), min-max normalization, multiplicative scatter correction (MSC), first derivatives, second derivatives, first derivatives+straight line subtraction, first derivatives+SNV and first derivatives+MSC.

After model development, the outlier samples were identified by the software and they were removed.

The optimum model was selected by coefficients of determination (R^2), root mean squared error of cross validation (RMSECV), ratio of standard error of validation to the standard deviation (RPD) and bias.

2.3. *Analysis of gamma oryzanol*

The gamma oryzanol content was determined by following the method of Lilitchan *et al.*,[20] by using partial extraction methodology. After scanning, 50 g of GBR was ground by cereal grinder (Oku San No, Malaysia). The ground sample was weighed into two identical vials (0.5 g each) and extracted with n-hexane using different volumes (4 and 8 mL) by vigorous agitation on the vortex mixer for 1 min, at room temperature. The mixture was centrifuged at 2500 rpm for 10 min. The absorbance of two supernatants was measured at 314 nm using a GENESYS 10S UV–Vis spectrophotometer (USA). The gamma oryzanol content in the extracts were quantified against the standard curve. Total gamma oryzanol was calculated by solving two simultaneous equations:

$$k = \left(\frac{x}{v}\right)\left(\frac{w}{y-x}\right), \tag{1}$$

where:

k = A constant,

x = The amount of gamma oryzanol in the extract (g),

y = The amount of gamma oryzanol in bran,

v = The volume used for extraction (mL),

w = The weight of bran used for extraction.

There are two unknowns (k and y), thus the extraction of two identical samples with different volumes of solvent are necessary. In order to simplify the analysis and the calculation, the solvent used for the second extraction is doubled ($V_2 = 2V_1$) and the amount of solute in the two extracts are x_1 and x_2. Although the k value for solid extraction is slightly varied, as the amount of solvent used for extractions is different, it is assumed that the change does not affect the accuracy of this study. Thus, by assuming $k_1 = k_2$, the total amount of gamma oryzanol (y) can be calculated:

$$y = \frac{x_1 x_2}{2x_1 - x_2}. \tag{2}$$

2.4. *Overall precision test*

The overall precision, i.e., reproducibility, of the reference test was determined by selecting samples, 10 as blind duplicates (5 pairs) along with the normal experiment. The reproducibility is the standard deviation (SD) of the differences between the values of blind duplicates. In addition, the repeatability of the reference test was determined by the SD of the differences between the values of another 10 as duplicates (5 pairs) which were not blind samples.

3. Result and Discussion

The six groups of GBR samples included GBR-Oryzanol adjusted, MGBR, MGBR- KDML 105, MGBR-various varieties, GBR-Oryzanol adjusted+ MGBR-KDML 105 and GBR-Oryzanol adjusted+ MGBR that were used for model development. In the outlier identification process, there were 4, 3, 4, 4, 5 and 6 outliers that were removed, respectively. The minimum (Min), maximum (Max), mean, and SD values of gamma oryzanol content of GBR of different sample sets are shown in Table 1. The range of gamma oryzanol content was from 6.06×10^{-4} to 3.27×10^{-3} mg/100 g dry matter.

The statistics of prediction performance of the PLS model for gamma oryzanol content of GBR

Table 1. Minimum (Min), maximum (Max), mean and SD of gamma oryzanol content of the GBR.

Parameter	Number of samples	Min (mg/100 g dry matter)	Max (mg/100 g dry matter)	Mean (mg/100 g dry matter)	SD (%)
GBR-Oryzanol adjusted	84	9.87×10^{-4}	2.11×10^{-3}	1.44×10^{-3}	2.82×10^{-4}
MGBR	133	6.06×10^{-4}	2.23×10^{-3}	1.24×10^{-3}	4.10×10^{-4}
MGBR–KDML 105	64	6.06×10^{-4}	2.23×10^{-3}	1.31×10^{-3}	4.32×10^{-4}
MGBR-various varieties	64	6.87×10^{-4}	1.87×10^{-3}	1.12×10^{-3}	3.46×10^{-4}
GBR-Oryzanol adjusted +MGBR–KDML 105	151	6.06×10^{-4}	2.23×10^{-3}	1.38×10^{-3}	3.59×10^{-4}
GBR-Oryzanol adjusted+MGBR	218	6.06×10^{-4}	3.27×10^{-3}	1.32×10^{-3}	4.15×10^{-4}

Table 2. Statistics of prediction of gamma oryzanol content of GBR by PLS models.

Parameter	Pre-processing	Range	PLS factor	R^2	RMSECV	RPD	Bias
GBR-Oryzanol adjusted	First derivatives+MSC	9403.8–7498.3 4605.4–4242.9	4	0.610	1.78×10^{-4}	1.60	7.12×10^{-7}
MGBR	Second derivatives	9403.8–7498.3 6102–5168.6	8	0.535	2.79×10^{-4}	1.47	-3.40×10^{-6}
MGBR–KDML 105	SNV	8454.9–7498.3 6102–4597.7	9	0.768	2.06×10^{-4}	2.08	-6.09×10^{-6}
MGBR-various varieties	**First derivatives+SNV**	**9403.8–7498.3 6102–4597.7**	**10**	**0.934**	$\mathbf{0.88 \times 10^{-4}}$	**3.91**	$\mathbf{1.06 \times 10^{-5}}$
GBR-Oryzanol adjusted +MGBR–KDML 105	MSC	6102–4597.7	9	0.482	2.63×10^{-4}	1.39	-7.23×10^{-6}
GBR-Oryzanol adjusted +MGBR	First derivatives+straight line subtraction	6102–4597.7	9	0.536	2.53×10^{-4}	1.47	2.23×10^{-6}

R^2 — Coefficient of determination; RMSECV — Root mean squared error of cross validation; RPD — Ratio of standard error of validation to the SD; Bias — Average error of prediction.

developed using six different groups of samples is shown in Table 2. The optimum models for gamma oryzanol content evaluation of MGBR-various varieties derived from 10 PLS factors from the spectra in the range of 9403.8–7498.3 cm^{-1} and 6102–4597.7 cm^{-1} that were pre-processed by the first derivatives+SNV methods showed the coefficients of determination (R^2), root mean square error of cross validation (RMSECV), ratio of standard error of validation to the standard deviation (RPD) and bias of 0.934, 0.88×10^{-4} mg/100 g dry matter, 3.91 and 1.06×10^{-5} mg/100 g dry matter, respectively.

Williams[22] indicated that R^2 of 0.92–0.96 implied that the model was usable in most applications, including quality assurance.

The scatter plots (reference data (X) with prediction data (Y)) of gamma oryzanol content are shown in Fig. 1.

Furthermore, the prediction of gamma oryzanol content of MGBR-KDML 105 provided R^2 value in prediction which were high R^2 values (0.768) and low bias (-6.09×10^{-6} mg/100g dry matter). For the other groups, the prediction of gamma oryzanol content showed low R^2 (0.535–0.610).

Williams[22] indicated that R^2 of 0.66–0.81 implied that the model was okay for screening and some other "approximate" calibration.

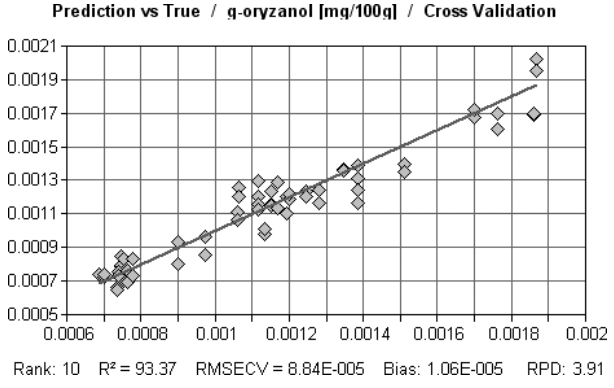

Fig. 1. Comparison of gamma oryzanol content of MGBR-various varieties between NIRS prediction and reference lab.

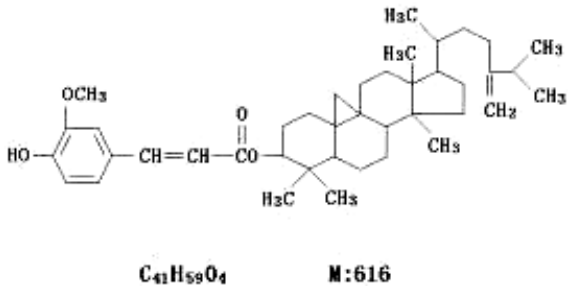

Fig. 2. Chemical structure of gamma oryzanol.[23]

Gamma oryzanol is a group of ferulic acid esters of phytosterols and triterpene alcohols.[21] The chemical structure of gamma oryzanol is shown in Fig. 2.

Figure 3 shows the regression coefficient plot of gamma oryzanol content of MGBR-various varieties group. It could be seen that the high regression coefficient was illustrated at the absorption bands of **8714**, **8378**, 6090, 5936, 5311, **5122**, 5048, 4912, 4815, 4728 and 4633 cm^{-1} (**1148**, **1194**, 1642, 1685, 1883, **1952**, 1981, 2036, 2077, 2115 and 2158 nm). The vibration at 8714 cm^{-1} (1148 nm) and 8378 (1194 nm) might be due to the combination vibration of C–H stretch second overtone of CH_3. According to Osborne and Fearn,[22] the absorbance bands of the bond vibration are at 1152 and 1195 nm. Furthermore, the vibration at 5122 cm^{-1} (1952 nm) might be due to the combination vibration of C = O stretch second overtone of $–CO_2R$. The bond vibration was indicated by Osborne and Fearn[24] at 1950 nm. These bonds are in the gamma oryzanol chemical structure. Therefore, the vibration of oryzanol was effected and included in the model.

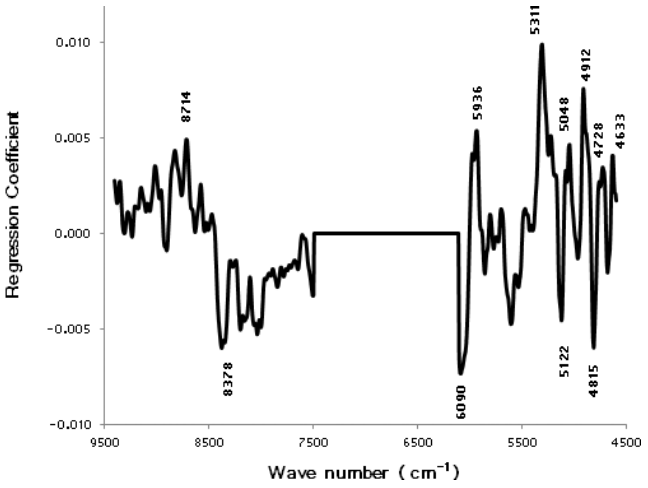

Fig. 3. Regression coefficient plot of gamma oryzanol content of MGBR-various varieties.

Table 3 shows the overall precision of reference testing for gamma oryzanol of GBR samples. The SD of differences of all duplicates, i.e., repeatability was 0.25×10^{-3} mg/100 g dry matter and the SD of differences of blind duplicates, i.e., reproducibility was 0.48×10^{-3} mg/100 g dry matter.

4. Conclusion

From the study, the NIRS of GBR could be an alternative technique to evaluate the gamma oryzanol content of various varieties of market-germinated brown rice (MGBR-various varieties) because the model was usable in most applications, including quality assurance. Future work should be focused on the real application of the models generated in this study to predict the gamma oryzanol of samples drawn from the product of different factories and labeled it on the packages.

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Table 3. Repeatability and reproducibility of reference test for gamma oryzanol of GBR. (mg/100 g dry matter).

Repeatability SD				Reproducibility SD			
Sample number	Duplicate.a	Duplicate.b	Diff. a−b	Sample number	Duplicate.a	Duplicate.b	Diff. a−b
5, 17	1.29×10^{-3}	1.55×10^{-3}	-0.26×10^{-3}	11, 18	1.24×10^{-3}	1.23×10^{-3}	0.01×10^{-3}
24, 33	1.19×10^{-3}	0.79×10^{-3}	0.40×10^{-3}	28, 34	1.55×10^{-3}	0.86×10^{-3}	0.69×10^{-3}
32, 51	1.14×10^{-3}	1.24×10^{-3}	-0.09×10^{-3}	38, 52	1.48×10^{-3}	2.07×10^{-3}	-0.59×10^{-3}
59, 63	1.95×10^{-3}	1.95×10^{-3}	1.62×10^{-6}	53, 64	1.72×10^{-3}	1.38×10^{-3}	0.34×10^{-3}
75, 87	1.51×10^{-3}	1.66×10^{-3}	-0.15×10^{-3}	79, 88	1.06×10^{-3}	1.13×10^{-3}	-0.07×10^{-3}
		SD	0.25×10^{-3}			SD	0.48×10^{-3}
		Mean	-2.06×10^{-5}			Mean	0.07×10^{-3}

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