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Rapid quantitative analysis of daidzein and genistein in soybeans (*Glycine max* (L). Merr.) using FTIR spectroscopy and multivariate calibration

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ABSTRACT

Soybean with the scientific name of *Glycine max* (L.) Merr. is nutritious vegetable food sources in Indonesia, and it may be processed into various kinds of food products. Soybeans are a rich source of isoflavones, rather than nuts and meat products. The research was aimed at quantifying daidzein (DN) and genistein (GN) in various soybean varieties applying Fourier transform Infrared (FTIR) spectroscopy coupled with two multivariate calibrations [principal component regression (PCR) and partial least square (PLS)]. Before being used as variables during quantification processes, some pretreatments of FTIR spectra including the selection of wavenumbers and spectral derivative (high-order derivatives) were carried out to get the best prediction models for the correlation between the actual values of DN and GN. The actual values of DN and GN were determined with High-performance liquid chromatography (HPLC). The results revealed that PLS provides a better modeling than PCR for the relationship between actual values and FTIR spectroscopy-predicted values. The first derivative spectra at wavenumbers of 3,600–2,800 and 1,500–780 using PLS regression were preferred for the quantification of DN and GN in soybean. PLS calibration model yielded R^2 for the relationship between HPLC-based actual values (*x*-axis) and FTIR-predicted values (*y*-axis) of DN and GN, which were 0.9947 and 0.9900, respectively. FTIR spectroscopy method-combined PLS regression provides fast and acceptable results as indicated by high accuracy and precision during quantification of DN and GN in soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is a popular health nutritious vegetable food widely consumed in Indonesian. The economic value of soybean is very important for Indonesian society because various processed soy-based food products become their daily consumption. Isoflavone (aglycone form of glycosides) such as daidzein (DN) and genistein (GN) is reported to be more responsible for biological activities than glycoside form because aglycones had a good biological activity and are also easy to be absorbed (Izumi *et al*., 2000). DN and GN have potential protective effects on mammae cancers (Dhananjaya *et al*., 2012), estrogenic effect (Islam *et al*., 2008), and protective action against osteoporosis (Morabito *et al*., 2002).

High-performance liquid chromatography (HPLC) is an analytical technique widely used for the analysis of DN and GN in soybean samples or soybean-based food products (Hong *et al*., 2011; Magiera and Sobik, 2017; Shao *et al*., 2011; Yatsu *et al*., 2016). However, an HPLC analysis needs complex sample preparation and skillful analysis; therefore, fast and reliable analytical techniques based on Fourier transform Infrared (FTIR) spectroscopy methods combined with chemometrics are developed for the analysis of DN and GN in soybeans (Mulsow *et al*., 2015). FTIR spectroscopy is based on the interaction between infrared radiation and samples to get specific peaks corresponding to the absorption of functional groups present in the analyzed samples (Rohman, 2012).

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FTIR spectroscopy coupled with multivariate data analysis (chemometrics) of multivariate calibrations can be used for the qualitative and quantitative analysis of compounds of natural ingredients (Rohman *et al*., 2014). FTIR spectroscopy-based techniques have been used for the quantification of curcuminoids in *Curcuma* syrups (Prabaningdyah *et al*., 2018) and analysis of curcumin and demethoxycurcumin in some extracts of turmeric (*Curcuma longa* L.) and java turmeric (*Curcuma xanthorrhiza)* (Lestari *et al*., 2017; Rohman *et al*., 2015). Based on this application, the correlation between FTIR spectroscopic and HPLC methods emerged very flexible for the analysis of DN and GN. Using a literature study, there are no reports regarding the employment of FTIR spectroscopy-based techniques for the analysis of DN and GN. For this reason, this study was aimed to optimize and develop FTIR spectroscopy coupled with partial least square (PLS) and principal component regression (PCR) for the analysis of DN and GN. As actual values of DN and GN in soybeans, the validated HPLC method was used.

MATERIALS AND METHODS

The reference standards of DN and GN were purchased from Sigma (Aldrich, St. Louis, MO). HPLC instrument was used for the quantitative analysis of DN and GN. Soybean samples were obtained from Balitkabi (Balai Penelitian Tanaman Aneka Kacang dan Umbi), Malang, East Java, Indonesia.

Preparation of soybean samples

Approximately 2.5 g of soybean samples were extracted with 25 ml of water–methanol (50:50 volume/volume) at room temperature for 3 days with immediate shaking. The mixture was then filtered, and then, 1.0 ml of sample solution was diluted with 9.0 ml of water–methanol (50:50 volume/volume). This solution was subjected to filtration using 0.45-µm polyvinylidene fluoride or polyvinylidene difluoride and finally introduced into HPLC system.

HPLC analytical conditions

Reserved-phase HPLC for the quantification of DN and GN in soybean samples was performed on HPLC waters, using column Sun Fire ™C-18 (150 × 4.6 mm, with internal diameter 5 µm), equipped with guard column Waters Symmetry ™C18 (20 × 4.4 mm, with an internal diameter of 5 µm). The composition of mobile phase consisted of a mixture of methanol and 0.1% acetic acid (53:47 volume/volume) delivered isocratically using a flow rate of 1.0 ml/minute. The volume of injection was 10 µl. For the preparation of standard solutions, 10 mg of reference standards were accurately weighed and then diluted with mobile phase to get stock solution of DN and GN (100 µg/ml). Aliquots of the solution (2, 3, 4, 5, 6, 7, 8, 9, and 10 µg/ml) were prepared and injected into the HPLC equipment. The solutes containing DN and GN were detected by using photodiode array at 254 nm. HPLC running time was 20 minutes with retention times (t_R) of DN and GN which were 6.342 and 10.088, respectively.

FTIR spectroscopy analysis

The analysis of samples using FTIR spectroscopy was performed according to Wulandari *et al*. (2018). The powdered samples were directly placed on an attenuated horizontal total reflectance (Smart iTR™) accessory. The specification of measurement is as follows:

Software:	OMNIC ver. 9.7
Wavenumbers of scanning:	4,000–650 cm^{-1}
Number of scanning:	32 scans
Spectral resolution:	8 cm^{-1}
Spectral background:	air (environment) spectrum

Model prediction of DN and GN

The prediction of DN and GN was facilitated by multivariate calibrations of partial least-square regression (PLSR) and PCR. The levels of DN and GN obtained during HPLC were used as actual values, and then, the levels of DN and GN were predicted using the variables of absorbance values at the region of optimized wavenumbers. The correlation models for actual values and predicted values were regressed using PLSR.

Data analysis

The software of TQ Analyst from Thermo Fisher Scientific Inc. (Madison, WI) was used for data analysis applying multivariate calibrations including PLS and PCR. Statistical parameters used are as follows:

Coefficient of correlation (R) for correlation between HPLC actual values and FTIR predicted values which indicated the accuracy of the model.

The errors of root mean square error of calibration (RMSEC) and error of prediction (RMSEP) indicating the precision of the model (Miller and Miller, 2005).

RESULTS AND DISCUSSION

Using two models of multivariate calibrations, PLS and PCR were employed for the correlation between HPLC actual values of DN and GN as determined and FTIR predicted values using variables of absorbance values at certain FTIR spectra regions. The HPLC chromatogram obtained during the separation of DN and GN is shown in Figure 1. HPLC conditions used can provide a good system because it has an asymmetry value of 1.2. The relative standard deviation (RSD) values for peak area and peak height were obtained to meet the requirements of maximum RSD value, namely, ≤ 2%. In addition, HPLC used was validated previously by Sulistyowati *et al*. (2019).

FTIR spectra at the wavenumbers of 4,000–650 cm^{-1} of soybean pulverized containing DN were overlaid as shown in Figure 2. Each peak at specific wavenumbers (1/λ) in these spectra could be attributed by functional groups present in soybean samples. A wide peak at 1/λ 3,272 cm^{-1} was the vibration of -OH stretching, associated with hydrogen bond of -OH. Two peaks at 1/λ 2,925 and 2,855 cm^{-1} were coming from vibrations of CH_3- and CH_2- functional groups in stretching modes. A peak at 1/λ 1,743 cm^{-1} originated from carbonyl (C=O) groups (Rohman *et al*., 2015). The presence of these functional groups indicated the presence of DN and GN in soybean samples.

For quantitative analysis using FTIR spectroscopy, some optimizations were performed by selecting the FTIR spectral treatment and derivatization treatments. Based on the optimization applying wavenumbers' region and FTIR spectral treatments (normal, first, and second derivatives) and relying

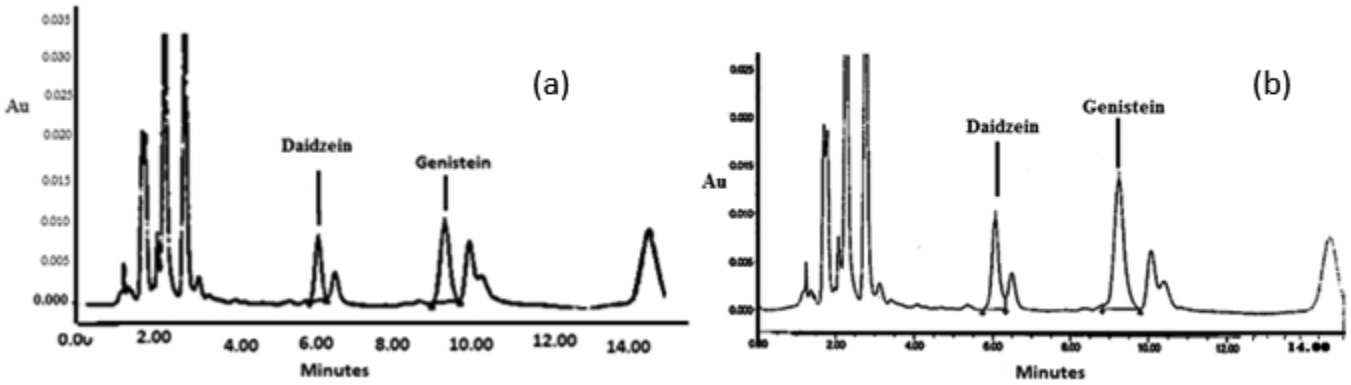

Figure 1. Chromatogram by HPLC with the SunFire™C-18 (150 × 4.6 mm, 5 µm): (a) chromatogram of soybeans extract containing DN and GN and (b) chromatogram spiking performed using 1 ppm of DN and GN standard.

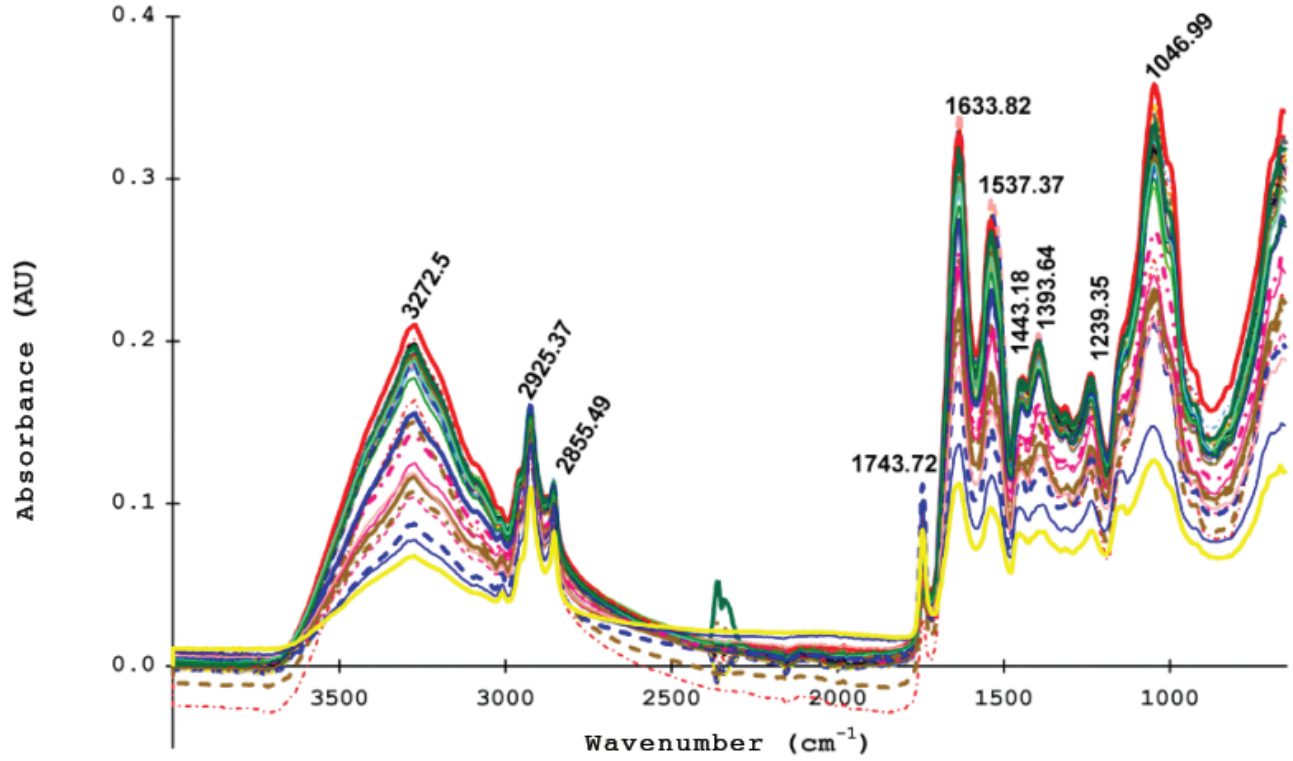

Figure 2. The overlay of FTIR spectra of soybean pulverized containing DN and scanned at midinfrared region (4,000–650 cm^{-1}).

on the highest coefficient of correlation (R-value) and lowest values of RMSEC and RMSEP, DN and GN were subjected to quantification using the first derivative spectra at combined 1/λ of 3,600–2,800 cm^{-1} and 1,500–780 cm^{-1} with 10 factors. The selection of the wavenumbers of samples is important because the measurements with not suitable wavenumber regions or less informative are able to reduce the performance of PLS/PCR modeling (El-Gindy *et al*., 2006). Tables 1 and 2 show the performance of multivariate calibrations of PLS and PCR models applying either normal or derivative spectra (first and second) for the quantitative analysis of DN and GN in soybeans together with some statistical performances, consisting of the number of factors, *R*-values, and error values (RMSEC and RMSEP).

The values of *R* obtained for the determination of DN using PLS were 0.9943 (calibration model) and 0.9961 (validation model), whereas the error values were 0.865% (calibration) and 0.994% (validation), respectively (Fig. 3). The R-values for the determination of GN were 0.9882 (calibration) and 0.9936 (validation) with the error values of 0.874% (calibration) and 1.01% (validation), respectively (Fig. 4). Figure 5 shows the correlation between HPLC actual results (*x*-axis) and FTIR calculated values (*y*-axis) in validation samples which resulted

Table 1. The characteristic performances of PLS for modeling the correlation between HPLC actual values and FTIR predicted values of DN and GN.

Wavenumber (cm^{-1})	Spectral treatment	Number of factors	DN				GN			
			Calibration		Validation		Calibration		Validation	
			RMSEC	R	RMSEP	R	RMSEC	R	RMSEP	R
4,000–650	Normal	10	2.74	0.9416	3.98	0.9349	4.13	0.6911	5.25	0.7318
	Derivative 1	6	3.55	0.8997	3.62	0.9369	2.83	0.8691	4.15	0.8347
	Derivative 2	7	2.67	0.9447	6.17	0.7962	2.07	0.9324	4.91	0.7673
3,600–650	Normal	10	2.67	0.9446	3.54	0.9508	4.1	0.6973	5.31	0.7309
	Derivative 1	6	3.35	0.9115	3.23	0.9524	2.83	0.8684	4.22	0.8283
	Derivative 2	7	2.46	0.9532	6.02	0.8043	0.607	0.9943	4	0.8541
3,600–780	Normal	9	2.83	0.9377	3.77	0.9364	1.84	0.9466	3.22	0.9231
	Derivative 1	10	1.22	0.9887	1.44	0.9905	2.88	0.8636	3.75	0.8739
	Derivative 2	10	0.881	0.9941	4.22	0.9073	0.579	0.9949	3.38	0.8967
1,789–780	Normal	9	2.87	0.9358	3.61	0.9491	2.07	0.9322	2.7	0.9477
	Derivative 1	10	1.31	0.9869	1.68	0.9855	1.6	0.96	1.9	0.9762
	Derivative 2	9	1.66	0.9789	3.54	0.9334	3.14	0.8353	3.76	0.8899
1,500–780	Normal	10	2.52	0.9507	3.68	0.9462	2.32	0.9137	3.13	0.9159
	Derivative 1	9	1.12	0.9905	1.43	0.9902	0.875	0.9882	1.12	0.9923
	Derivative 2	8	1.66	0.9789	3.28	0.9493	1.33	0.9724	2.36	0.9611
3,600–2,800 and 1,789–780	Normal	9	3.2	0.9196	3.18	0.9543	4.09	0.6991	5.23	0.7647
	Derivative 1	8	0.86	0.9734	1.57	0.9873	2.45	0.9031	2.72	0.9522
	Derivative 2	7	2.48	0.9524	3.99	0.9163	2.78	0.8737	4.23	0.832
3,600–2,800 and 1,500–780	Normal	9	3.09	0.9254	3.27	0.9507	2.29	0.9166	3.01	0.9226
	Derivative 1	10	0.865	0.9943	0.994	0.9961	2.27	0.9179	2.53	0.9562
	Derivative 2	5	3.78	0.8855	4.96	0.8662	2.33	0.9135	3.87	0.861
3,000–2,800 and 1,789–780	Normal	6	4.52	0.8314	4.96	0.8863	2.43	0.9052	3.14	0.9207
	Derivative 1	10	1.26	0.988	1.25	0.9919	1.02	0.9839	1.57	0.9794
	Derivative 2	7	2.58	0.9486	3.63	0.9315	3.16	0.833	3.77	0.8939
3,000–2,800 and 1,500–780	Normal	7	3.77	0.8867	4.28	0.9191	2.49	0.9002	3.19	0.9155
	Derivative 1	10	0.996	0.9925	1.26	0.9921	0.874	0.9882	1.01	0.9936
	Derivative 2	4	4.22	0.855	4.84	0.8705	2.76	0.8756	3.7	0.8726

R = coefficient of correlation; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction.

Table 2. The performance of multivariate calibrations of PCR for modeling HPLC actual values and FTIR predicted values of DN and GN.

Wavenumber (cm^{-1})	Spectral treatment	Number of factors	DN				GN			
			Calibration		Validation		Calibration		Validation	
			RMSEC	R	RMSEP	R	RMSEC	R	RMSP	R
4,000–650	Normal	10	6.18	0.6504	7.32	0.7253	4.28	0.6634	5.42	0.7366
	Derivative 1	10	5.63	0.7225	6.2	0.7827	3.78	0.7503	5.19	0.7221
	Derivative 2	10	6.98	0.514	8.47	0.5225	4.62	0.5891	6.31	0.5422
3,600–650	Normal	10	6.2	0.6484	7.38	0.7183	4.29	0.6603	5.52	0.7219
	Derivative 1	10	5.52	0.7351	5.85	0.81	3.73	0.7582	4.86	0.7608
	Derivative 2	10	7.06	0.4991	8.48	0.514	4.68	0.5749	6.64	0.469
3,600–780	Normal	10	6.17	0.653	7.22	0.7402	4.27	0.6651	5.41	0.7427
	Derivative 1	10	5.36	0.7527	5.37	0.8483	3.77	0.7523	4.22	0.8347
	Derivative 2	10	6.7	0.5687	7.59	0.6503	4.4	0.638	5.32	0.7226
1,789–780	Normal	10	5.64	0.7214	6.6	0.7567	3.6	0.7771	4.5	0.8226
	Derivative 1	10	5.34	0.7544	5.84	0.8266	3.63	0.7721	3.94	0.8662
	Derivative 2	10	6.09	0.6642	7.31	0.6792	3.82	0.7437	4.54	0.8113
1,500–780	Normal	10	4.78	0.8092	6.09	0.8111	2.81	0.871	3.67	0.8805
	Derivative 1	10	5.23	0.7662	6.21	0.7858	3.7	0.7615	4.69	0.7839
	Derivative 2	10	5.25	0.7647	5.54	0.8306	3.72	0.7596	4.65	0.7976

Continued

Wavenumber (cm^{-1})	Spectral treatment	Number of factors	DN				GN			
			Calibration		Validation		Calibration		Validation	
			RMSEC	R	RMSEP	R	RMSEC	R	RMSP	R
	Normal	10	6.19	0.6494	7.44	0.7381	4.28	0.6625	5.54	0.7238
3,600–2,800 and 1,789–780	Derivative 1	10	5.33	0.7559	5.82	0.8268	3.57	0.7812	4.1	0.8531
	Derivative 2	10	6.34	0.6274	6.75	0.7501	4.12	0.6937	4.88	0.7786
	Normal	10	5.67	0.7172	6.18	0.8943	4.15	0.687	5.02	0.8455
3,600–2,800 and 1,500–780	Derivative 1	10	5.47	0.7404	6.47	0.7658	3.77	0.7514	4.94	0.7656
	Derivative 2	10	5.58	0.7281	5.29	0.871	3.97	0.7189	5.05	0.7548
	Normal	10	6.07	0.6664	7.39	0.7081	4.29	0.6616	5.84	0.661
3,000–2,800 and 1,789–780	Derivative 1	10	5.32	0.7573	5.75	0.8339	3.61	0.7747	3.92	0.8687
	Derivative 2	10	6.29	0.6357	6.6	0.7616	4.11	0.6952	4.76	0.7918
	Normal	10	4.95	0.7945	6.49	0.7982	3.11	0.8386	4.38	0.8355
3,000–2,800 and 1,500–780	Derivative 1	10	5.06	0.7837	5.38	0.8546	3.56	0.7828	4.24	0.8355
	Derivative 2	10	5.68	0.7166	5.59	0.8425	4.06	0.7047	4.98	0.7621

R = coefficient of correlation; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction.

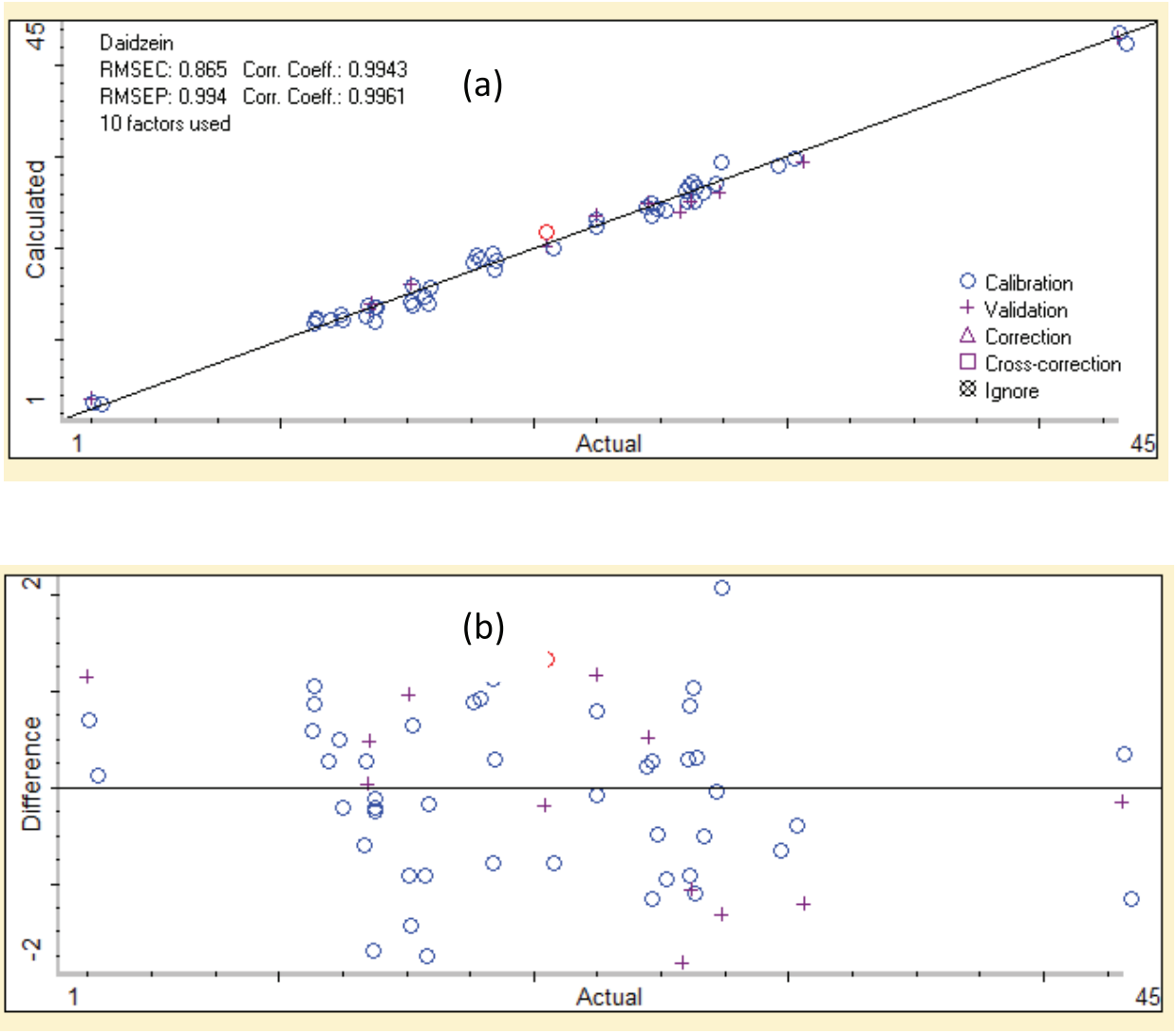

Figure 3. PLS model for the relationship between HPLC actual values (*x*-axis) and FTIR calculated values of DN. (a) Calibration model; (b) residual analysis.

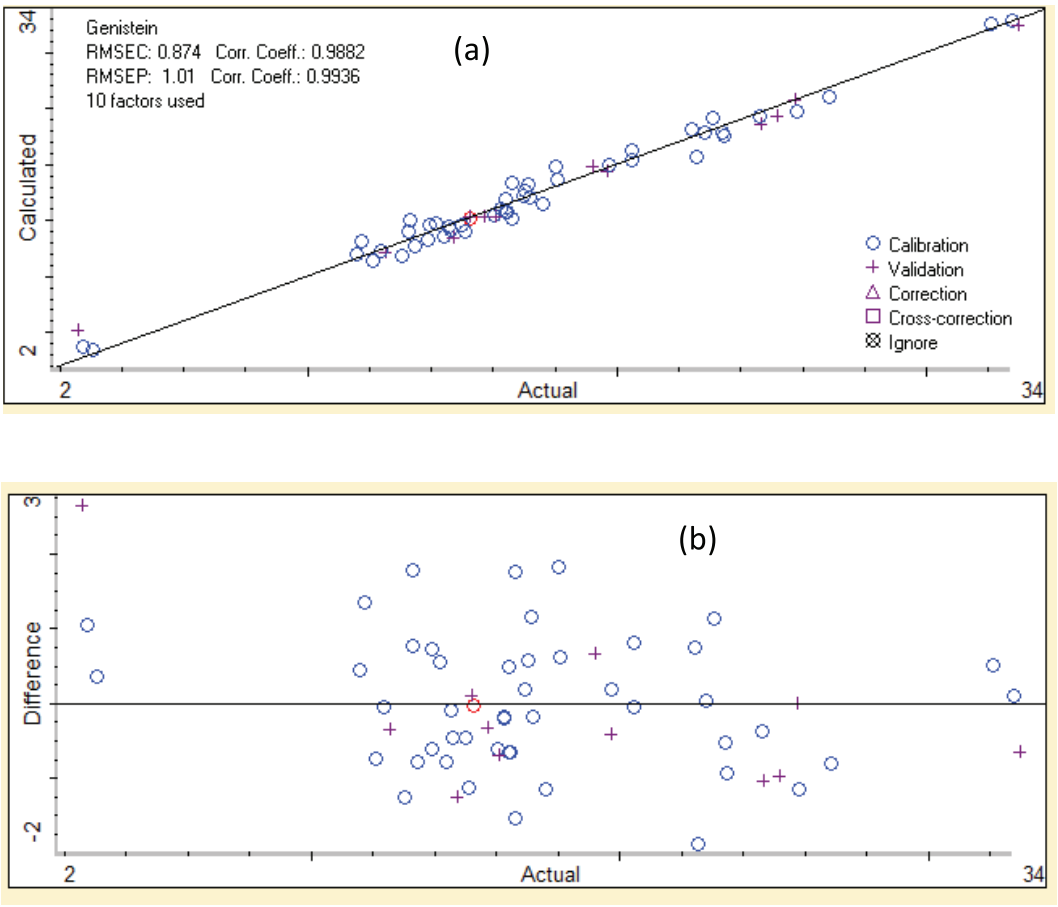

Figure 4. PLS model for the relationship between HPLC actual values (*x*-axis) and FTIR calculated values of GN. (a) Calibration model; (b) residual analysis.

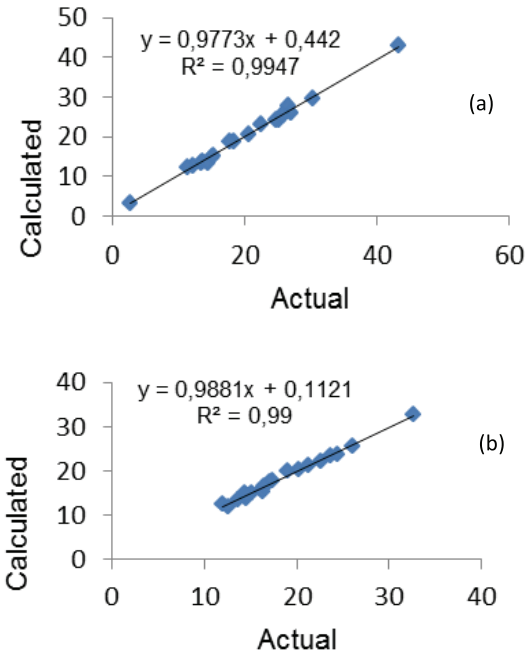

Figure 5. Scatter plot for the relationship between HPLC actual values of DN and GN and FTIR predicted values. (a) Daidzein; (b) GN. R^2 = coefficient of determination.

in $R^2 > 0.99$. This indicated that PLS with appropriate spectral treatments is accurate and precise methods for the quantification of DN and GN in soybeans (Sim *et al*., 2004).

CONCLUSION

HPLC was used successfully for the analysis of DN and GN in soybean samples, and the concentrations obtained were used for actual values during the prediction of DN and GN in soybean samples. FTIR spectroscopy using the first derivative spectra at combined 1/λ of 3,600–2,800 and 1,500–780 cm^{-1} coupled PLS model could be used as a rapid and reliable method for the quantitation of DN and GN in soybeans. FTIR spectroscopy offered the reliable method for the analysis of DN and GN in soybeans with the main advantages of being rapid, minimum sample preparation, and being environmental friendly. The developed method could be prolonged to be used in the analysis of soybean-based food products such as soybean milk and soy sauce.

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CONFLICT OF INTEREST

Authors declared that they do not have any conflicts of interest.

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