วารสารสมาคมวิศวกรรมเกษตรแห่งประเทศไทย
ปีที่ 26 ฉบับที่ 1 (2563) 19-27
Available online at www.tci-thaijo.org/index.php/TSAEJ

บทความวิจัย
ISSN 1685-408X (Print)

Improving Detection Ability of Near Infrared Spectroscopy to Detect the Low Concentration Phorbol Esters in Jatropha Seed

Nadaphast Koomklang[1,2], Ponsiri Liangsakul[1], Ronnarit Rittiron[3]*, Patcharin Tanya[1], Peerasak Srinives[4]

[1]Department of Agronomy, Faculty of Agriculture, Kasetsart University – Kamphaeng Saen, Nakhon Pathom, 73140
[2]Agricultural Research and Development Programme, Faculty of Agricultural, Kasetsart University-Kamphaeng Saen, Nakhon Pathom, 73140
[3]Department of Food Engineering, Faculty of Engineering, Kasetsart University-Kamphaeng Saen, Nakhonpathom, 73140
[4]The Royal Society of Thailand, Sanam Suea Pa, Dusit, Bangkok, 10300
*Corresponding author: Email: fengror@ku.ac.th

Abstract

Phorbol esters (PEs) is the crucial toxic compound of jatropha that can be tumor promotors, even in low concentration. The reference methods for determining the PEs was HPLC. The method requires an experienced analyst, chemical solvent, many steps of sample preparation, high cost and time consumption. However breeding jatropha for animal feed and biodiesel production needs rapid and efficient methods to determine the phorbol esters (PEs) content of a large number of seed samples. In the current study, a calibration set of 130 samples of jatropha interspecific breeding lines (*J. curcas x J. integerrima*) was investigated using NIRS technique to determine phorbol esters (PEs) contents. In the pre-concentration step, the effective performance of calibration models for PEs prediction were compared between with and without using dry-extraction spectroscopy for near infrared technique (DESIR). The model for the PEs obtained using DESIR showed the correlation coefficient for the validation set (R_v), standard error of prediction (SEP) and ratio of standard error of validation to the standard deviation (RPD) of 0.80, 0.24 mg g^{-1} DW and 1.70. While the parameters without DESIR were 0.62, 0.27 mg g^{-1} DW and 1.26, respectively. Statistical testing for performance measurement based on ISO12099:2017 of the model for PEs prediction, the NIR-predicted values using DESIR were not different from the actual values at the 95% confident interval. Thus NIRS using DESIR could potentially be used for predicting PEs contents in jatropha seed.

Keywords: Jatropha interspecific breeding lines, Phorbol esters (PEs), Near-infrared spectroscopy (NIRS), Dry-extraction spectroscopy for near infrared technique (DESIR)

1. Introduction

Jatropha curcas L. (jatropha, physic nut), an oil-bearing shrub belonging to the Euphobiaceae family. This plant has spread beyond its original distribution because of its hardiness, easy propagation, drought endurance, high oil content, rapid growth adaptation to wide agro-climatic condition. Seed of *J. curcas* also contains high amount of protein (50-60% w/w) (Flores et al., 2012). With these special features, *J. curcas* is a potential plant for alternative fuel, biomass and feeds. (King et al., 2009). However, the utilization of *J. curcas* as a multipurpose crop is limited because it contains toxic compounds.

The crucial toxic compound in *J. curcas* is phorbol esters (PEs), because PEs is a persistent chemical that can be destroyed by heat at over 150 °C together with some other treatments (Sadubthummarak et al., 2013). Six isomers of PEs isolated from jatropha and all compounds show tumor promoting property (Gubitz et al., 1999).

Received: June 23, 2019
Revised: August 14, 2019
Accepted: August 14, 2019
Available online: November 7, 2019

In order to make maximum use of *J. curcas* especially oil, seed cake and wood, and increase the commercial value of this plant for the farmers, a specific cultivar is needed. The new varieties should have low PEs content to decrease the toxicity in seeds (Sujatha, 2006). In general, PEs is determined by HPLC. However, this method required time consumption (at least 30 min per sample, not included sample preparation step), chemicals and skill analysis. Therefore, breeding *J. curcas* for animal feed and biodiesel production requires rapid and efficient methods to assess the PEs content in a large number of samples.

Near-infrared spectroscopy (NIRS) is an effective method widely used for analysing the composition contents in a number of crops. It has been proven to be a powerful analytical tool due to speed, accuracy and cost-effectiveness. These environmental friendly techniques should minimize the chemical waste consuming in pretreatment of the samples. Moreover, this methods is able to determine many components simultaneously. For all these advantages, this method has been widely used in various fields, such as routine analysis, quality control and on-field measurement (Burns and Ciurczak, 2008; Thyholt and Isaksson, 1997; Osborne et al., 1993).

The concentration of PEs content in jatropha seeds is very low (at ppm level), while the NIRS technique can normally assess the concentration at only percent level due to the usual limits of detection of NIRS is 0.1 %. (Siesler et al., 2006; Lima et al., 2007). Therefore, in the current study, a dry-extraction spectroscopy for near infrared (DESIR), a pre-concentration technique, has been used to prepare samples before collecting the NIR spectra. With this technique, the analyte was extracted from samples in the form of solution to reduce the effect of the other components in sample. Then the extracted solution was dried onto a solid substrate with low absorptivity. The solvent was removed in order to increase the concentration of the analyte (Acharya et al., 2012; Zhang et al., 2013).

Since there is no published report on determination of PEs in seed of jatropha using NIRS, this study aimed to assess the ability to detect the low concentration PEs in seeds of the jatropha breeding lines which were developed from hybridization between *J. curca* and *J. integerrima* obtained from crossing between the F2 plants as well as multiple crossing between the selected progenies derived from crossing between the selected plants in the jatropha breeding project of Kasetsart University. We also want to compare the effectiveness of the calibration model for PEs predicting between with and without DESIR in sample preparation steps.

2. Materials and methods

2.1 Sample preparation

One hundred and thirty interspecific lines were developed from hybridization between *J. curcas* (Thai local cv "Chai Nat"; female parent) and *J. integerrima* (Thai local dwarf ornamental type; male parent) in the jatropha breeding project of Kasetsart University. Seeds from each line were oven-dried at 45 ^{o}C for 3 days, then ground using a cyclone sample mill grinder (DxFillMachine model DXM-2000, China) until it passed through a stainless steel screen with aperture size of one mm. The ground seeds of each line was separated in two sets, one for NIRS without DESIR analysis and the other for PEs residual extraction.

The PEs residual was used for NIRS with DESIR analysis. PEs extraction procedure was carried out according to the method described in Hass and Mittelbach (2000) and Makkar et al. (2007) with some modifications. Briefly, 4.00 grams of ground seed were weighed on filter paper and transferred into a thimble. The samples were extracted with methanol in a soxhlet extractor for 4 hours 15 minutes then transferred to a round bottom flask. The methanol in the extracted samples were removed by vacuum rotary evaporator. The residue was dissolved in a minimum volume of methanol (HPLC grade), then transferred into a 25 ml amber colored glass vial. The solvent was dried with N_2 gas blowing and kept at 4^{o}C until use.

2.2 Near- infrared spectroscopy measurements

Prior to collecting the spectra, the homogenized ground samples were oven-dried at 60 ^{o}C for 24 h then kept in a desiccator to cool to room temperature. Four g of each sample was put in a 30 ml glass beaker and placed on top of the window of the NIR spectrometer (Buchi NIRFlex FT-NIR model N-500, Switzerland) as shown in Figure 1(a). The absorbance spectra were collected in diffuse reflectance mode at 32 random positions on each sample and calculated for the average spectra. Three NIR measurements were made on each

sample over the wavelength region 1000-2500 nm with 1 nm spectral resolution and an integrating time of 23 s.

2.3 Dry extraction spectroscopy for near infrared (DESIR)

The dried residue of PEs was dissolved with methanol in a 25 ml volumetric flask and adjusted to a final volume of 25 ml. One ml of the aliquot was taken by a micropipette to a 47 mm diameter glass microfiber filter (GF/A) that was placed in a 50 mm diameter glass petri dish. The filter was held for 40 min in hot air oven at 70 ^{o}C and stored in a desiccator to avoid absorbing the moisture until cool down to room temperature. Diffuse reflectance NIR spectra were measured at the bottom of sample petri dish (Figure 1b). The NIR absorbance spectra were collected in three replicates under the same condition of NIR without DESIR measurements.

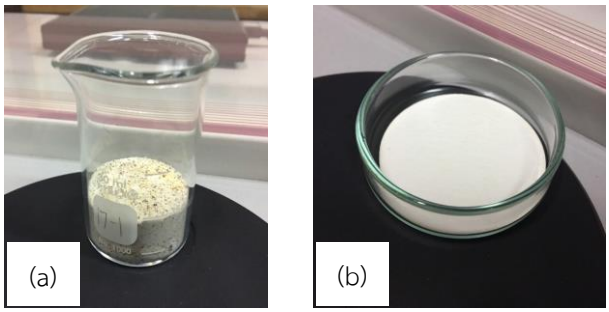


Figure 1 NIR spectral acquisition of (a) ground sample and (b) DESIR sample.

2.4 Reference method for PEs analysis

PEs content was determined in duplicates of each sample following the method described by Wink et al. (1997) with a slight modification. The residual of the PEs was filtered by 0.2 µl nylon filter and loaded into a HPLC (Waters 600E, USA). HPLC analysis was fitted with a reverse-phase C_{18} 150 x 5 mm. octadecyl as functional group, particle size 5 mm. The column was thermally controlled at 25°C with an isocratic solution mixture 80:20 (v/v) acetonitrile: water at a flow rate of 1 ml min^{-1}. The detector wavelength was set at 280 nm. A total of 20 µl of each samples was injected.

The PEs peaks appeared between 6-12 min and the running time for HPLC analysis is 25 min per sample. The area of each peak was measured using HPLC-DAD. A calibration curve was prepared using phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich). The PEs was dissolved in methanol as an external standard at the concentrations of 10, 20, 30, 40 and 50 ppm, respectively. The peak areas of PEs in each sample were summed and changed to PMA equivalent as the total amount of PEs (Devappa et al., 2010). Each analysis was conducted in duplicate and the results were represented as means ± standard deviation (SD).

2.5 Calibration development

Calibration was developed based on the full spectrum from 1000 to 2500 nm. The spectrum of each sample was measured in triplicate, then averaged. Raw spectra data from NIR spectrophotometer were treated using the Standard Normal Variate (SNV) and a second derivative to solve overlapped peak, remove baseline offset and linear background from scattering effects and enhance the information related to chemical components. Calibration models for determining PEs (with and without DESIR) were constructed from the relationship between absorbance spectra and concentration of the component from chemical method using the software NIR cal (ver. 5.4: Build 3000). Multivariate calibration was done by partial least squares regression (PLSR). The optimal number of partial least squares component was defined by a coefficient of variation (CV) and SD. In order to evaluate the performances of the calibration model, 309 ground seed samples from 130 line were used to construct models for determining PEs without DESIR. A total of 215 samples were used as calibration set for model development and the rest (94 samples) was used as a validation set for accuracy verification.

After that, the samples were extracted for PEs analysis. Since some lines had small number of seeds, each ground sample could be prepared into 2-3 samples of PEs residual. The 600 and 91 samples of PEs residual were obtained and then used to construct models for determining PEs using DESIR. The 478 samples were used as a calibration set and 213 samples were used as validation set.

2.6. Statistical testing for performance of the measurement

The constructed calibration models for predicting the contents of PEs (with and without DESIR) were evaluated from a validation set based on the statistic parameters, correlation coefficient (R), standard error of prediction (SEP), slope and bias as detailed in

ISO12099:2017. In this study, comparison of the calibration model performance for PEs prediction between with and without DESIR technique in sample preparation steps were studied. The bias, SEP and slope (observed t-value) value were compared with the bias confidence limit (T_b), unexplained error confidence limit (T_{UE}) as given by Eq. (1)-(2), and $t_{(1-\frac{\alpha}{2})}$, which is the *t*-value obtained from Table *t*-distribution for a probability of α = 0.05, respectively.

$$T_b = \pm \frac{t_{(1-\frac{\alpha}{2})} SEP}{\sqrt{n}} \quad (1)$$

Where; α is the probability of making a type I error; *t* is the appropriate *t*-value for two tailed test with the degree of freedom associated with SEP and the selected probability of a type I error; n is the number of independent samples. If value of the bias is lower than T_b, the bias is not different from zero.

The SEP express the accuracy of NIR results corrected for the mean difference (bias) between NIR results and reference methods. If the SEP is lower than the unexplained error confidence limit (T_{UE}), the SEP can be accepted.

$$T_{UE} = SEC\sqrt{F_{(\alpha, v, M)}} \quad (2)$$

Where SEC is the standard error of calibration; v is $n_v - 1$; where, n_v is the number of validation groups; M is n_c-P-1 where, n_c is the number of calibration groups and P is the number of PLS factors of the model; α is the probability of making a type I error;

$$t_{obs} = |b - 1| \sqrt{\frac{S_{\hat{y}}^2 (n-1)}{S_{res}^2}} \quad (3)$$

where *b* is the slope; n is the number of independent samples. The slope is considered as different from 1 when $t_{obs} \geq t_{(1-\frac{\alpha}{2})}$ where t_{obs} is the observed *t*-value, calculated according to the Eq. (3); $S_{\hat{y}}^2$ is the variance of n prediction values; S_{res} is the residual standard deviation values, as given by Eq. (4);

$$S_{res} = \sqrt{\frac{\sum_{i=1}^{n} [y_i - (a + b\hat{y}_i)]^2}{n-2}} \quad (4)$$

where, n is the number of independent samples; a is the y- intercept calculated as; $a = \bar{y} - b\bar{\hat{y}}$; b is the slope of regression; y_i is the i^{th} reference value; $\hat{y}$ is the i^{th} predicted value obtained when applying the multivariate NIR model. For equation performance checking as in ISO12099:2017. If value of the bias is lower than T_b, SEP is lower than T_{UE} and observed *t* – value for slope are lower than $t_{(1-\frac{\alpha}{2})}$, the performance of the calibration model is acceptable.

3. Results and discussion

3.1 Phorbol esters analysis

The equation obtained from the calibration curve for quantification of PEs was [Y] = 1200[X] + 3340 as shown in Figure 2. Where [X] is the sum of the chromatographic peak areas and [Y] is the PEs concentration (ppm).

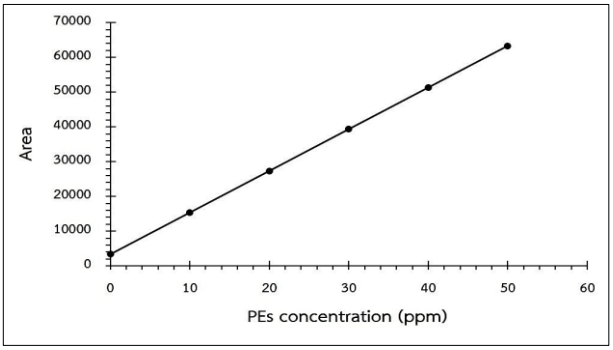


Figure 2 HPLC calibration curve of standard phorbol esters.

The results showed good linearity (R = 0.9996) over the concentration range 10.00 – 50.00 ppm. Figure 3 shows the HPLC/DAD (λ = 280 nm) chromatogram of the extracts from seed of the breeding lines showed five principal peaks with retention times in the range of 6 - 11 minutes, which corresponded to the absorption spectra of PEs reported by Hass and Mittelbach (2000), Makkar et al. (2009), and Devappa et al. (2010).

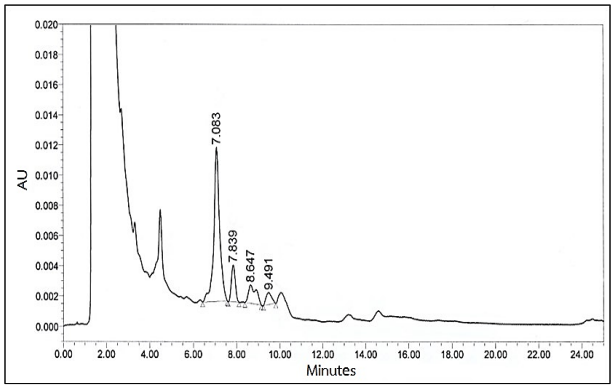


Figure 3 HPLC-UV ($\lambda = 280$ nm) chromatograms of the extracts from ground seeds of jatropha lines.

3.2 NIR analysis

The original spectra of the ground seed and dried residue PEs on glass microfiber filter from NIR spectrometer are shown in Figure 4.

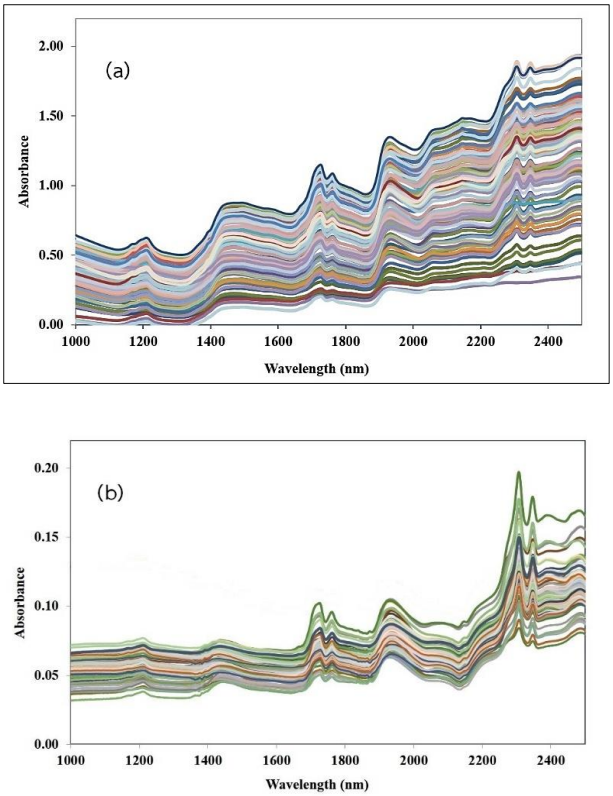


Figure 4 (a) The original spectra of the ground seed of jatropha and (b) dried residue PEs on glass microfiber filter from NIR spectrometer over the wavelength region of 1000-2500 nm.

In the case of ground seed (Figure 4a), baseline shift and scaling (intensity variation) were observed due to light scattering effect. Particle size variation is a main factor affecting the spectra. Scattering is a source of error by changing the intensification of the absorption bands. NIR spectra of the samples prepared by the DESIR technique showed less baseline shift and scaling variation (Figure 4b) than those without the DESIR due to the reduction in sample-to-sample variation. To suppress the effect, the NIR spectra of the ground seed of the lines and dried residue PEs on glass microfiber filter (DESIR) were treated with SNV and the second derivative are shown in Figure 5.

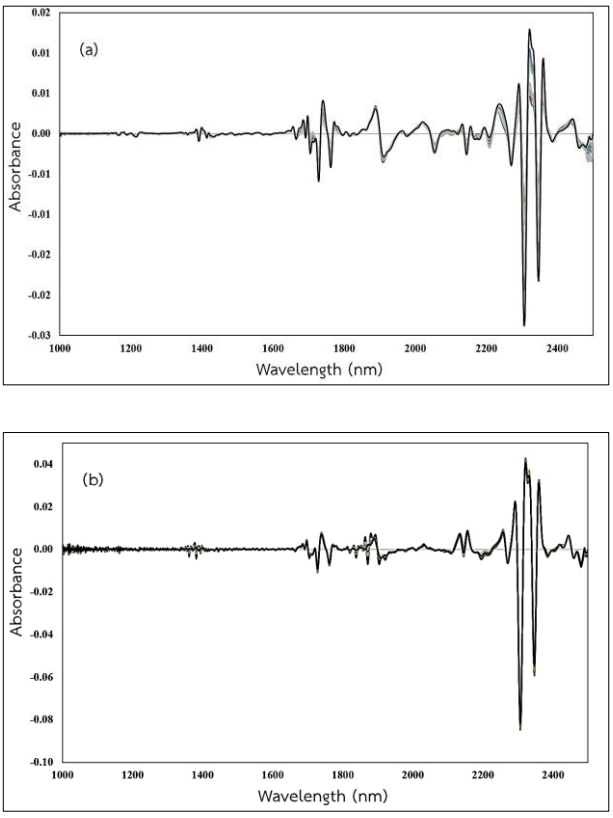


Figure 5 (a) Spectra of the ground seed of jatropha and (b) the dried residue PEs on glass microfiber filter from NIR spectrometer after treated with SNV and second derivative.

Figure 5 shows two absorption peaks between 2310 – 2350 nm, which corresponded to the absorption of oil, the major component in jatropha seed (Osborne et al., 1993). Whereas the other components such as protein, the peak was observed between 2160-2200 nm. The absorption peaks of PEs were unclear even if DESIR was applied, because PEs are not the major components.

3.3 Descriptive statistics and model performance

The descriptive statistics for PEs content were shown in Table 1. PEs concentrations in this study ranged from

0.25 – 2.23 mg g^{-1} DW. The standard error of calibration (SEC), standard error of prediction (SEP), correlation coefficient of calibration (R_c), and correlation coefficient of validation between predicted and reference values (R_v) of the calibration model for PEs content are presented in Table 2.

Table 2 showed the SEC and SEP of calibration model for prediction of the PEs content of the ground seeds with and without DESIR at the values of 0.23, 0.24 and 0.23, 0.27, respectively. The SEP was very low for PEs prediction using DESIR and showed high agreement between the predicted and actual values (R_v = 0.8). While the calibration model for PEs prediction without using DESIR showed lower agreement between the predicted and actual values (R_v = 0.62).

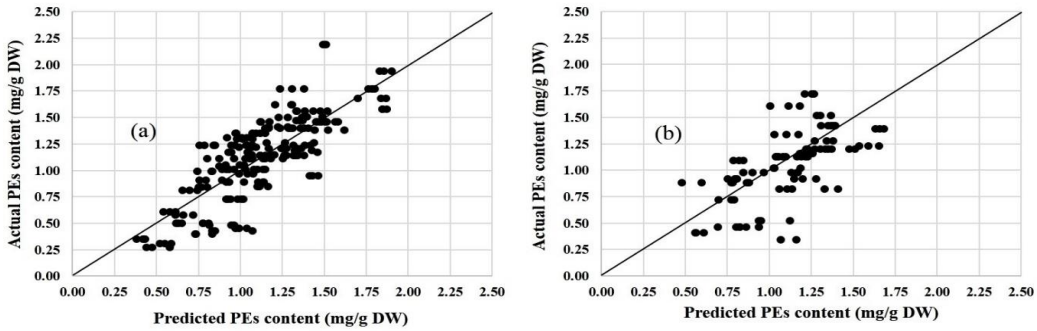
The scatter plot of the NIR predicted PEs (with and without DESIR) were shown in Figure 6. Most of the data are closely dispersed to the target line in both the calibration and validation sets, indicating a well agreement between the NIR predicted and actual values. The statistic values of the calibration models for PEs prediction were also tested for the performance following ISO12099:2017(E), as shown in Table 3. All the statistics obtained from the calibration prediction model for PEs (DESIR) content satisfactorily passed the criteria of ISO12099:2017(E)

Table 1 Characteristics of PEs content of the ground seed and DESIR samples of the interspecific Jatropha lines in the calibration set and validation set.

Compound	Sample set	Number of spectra	Range (mg g^{-1} DW)	Average (mg g^{-1} DW)	SD (mg g^{-1} DW)
PEs (ground seed)	Calibration set	215	0.27-2.19	1.12	0.392
	Validation set	94	0.34-1.72	1.05	0.344
PEs (DESIR)	Calibration set	478	0.25-2.23	1.08	0.404
	Validation set	213	0.28-2.05	1.10	0.410

Table 2 Descriptive statistics in the development of calibration models for PEs (with and without DESIR), oil and protein in the ground seed of the interspecific Jatropha lines.

Compound	PC	Calibration			Validation			
		slope	R_c	SEC (mg g^{-1} DW)	R_v	SEP (mg g^{-1} DW)	Bias	RPD
PEs (Ground seed)	8	0.64	0.80	0.23	0.62	0.27	- 0.061	1.26
PEs (DESIR)	8	0.67	0.82	0.23	0.80	0.24	0.020	1.70



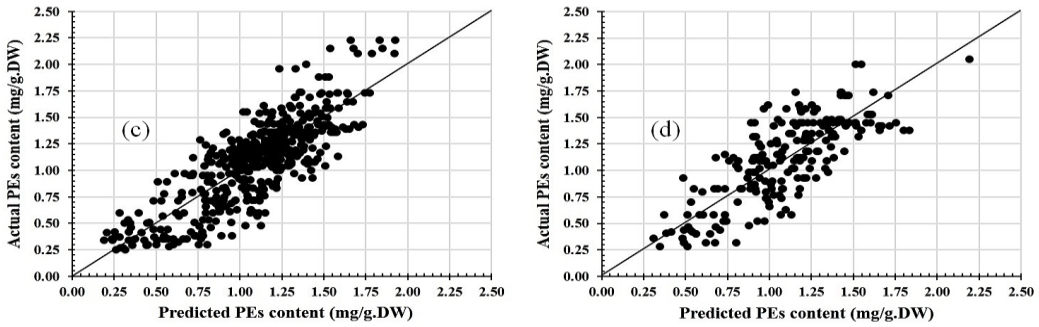


Figure 6 Scatter plot of the NIRs predicted PEs values obtained from PLS calibration model and the actual PEs values in (a) calibration set of ground seed, (b) validation set of ground seed, (c) calibration set of DESIR and (d) validation set of DESIR.

The bias of the calibration models using DESIR for PEs are lower than T_b obtained from calculation, indicating that they are not significantly different from zero. The SEP of calibration models for PEs (DESIR) are lower than T_{UE}, confirming that the SEP are low enough for practical acceptance. The t_{obs} for slope test calculated from the calibration models of PEs (DESIR) were lower than $t_{(1-\frac{\alpha}{2})}$, which was obtained from the value of *t*-distribution with a probability of α = 0.05. This indicated that the NIR-predicted values obtained from NIR spectrometers using DESIR are not significantly different from the actual values at the 95% confidence interval.

Table 3 The statistics performance measurement in ISO 12099:2017(E).

Model	Parameters	Criterion	Calculated value	Result
PEs (Ground seed)	Bias	$T_b = \pm$ 0.0563	- 0.0610	Not pass
	SEP	T_{UE} = 0.2685	0.2750	Not pass
	t_{obs} for slope testing	$t_{(1-\frac{\alpha}{2})}$ = 1.9710	1.9891	Not Pass
PEs (DESIR)	Bias	$T_b = \pm$ 0.0329	0.0197	Pass
	SEP	T_{UE} = 0.2527	0.2435	Pass
	t_{obs} for slope testing	$t_{(1-\frac{\alpha}{2})}$ = 1.9712	1.7869	Pass

T_b = bias confidence limit, T_{UE} = unexplained error confidence limit, t_{obs} = observed t-value, $t_{(1-\alpha/2)}$ = the *t*- value obtained from the t-distribution table at the probability of α = 0.05, SEP = standard error of prediction

To apply the NIRS technique in predicting PEs concentration, Roque et al. (2017) recently reported using NIRs to estimate the concentrations of PEs in seeds of Jatropha. In their study, 100 samples of jatropha intact seeds were directly collected for NIR spectrum without any sample preparation step. The oil extracted from seeds was analyzed for actual PEs content by HPLC, using PMA as an external standard. The NIR model from their study was not able to predict the PEs content due to extremely high prediction error.

The RPD is a ratio of standard deviation of the reference data in validation set to SEP and used for assessing the ability of calibration. The RPD is applied to compare the performance of models which have difference of sample sets or number of samples. A higher value of RPD implies the more reliable model (Rittiron, 2017)

Table 2 and 3, the results showed that using DESIR could reduce SEC, SEP and bias, while increase the agreement between the predicted and the actual values (R_v = 0.62 - 0.80 and RPD values = 1.26 - 1.70). Whereas the calibration model for predicting PEs without DESIR technique could not pass the standard criteria of ISO12099:2017(E). The calibration model for predicting the PEs content without using DESIR is not acceptable for the prediction purpose. Predicting PEs using DESIR for sample preparation give a more accurate prediction than the calibration model of ground seed

as reported earlier by Montes et al. (2013). Their regression coefficient and RPD are lower (0.66 and 1.27) than our study (0.80 and 1.70). The DESIR technique needs to extract PEs from sample therefore PEs concentration increases comparing to the original concentration in the ground sample and absorbance of another components become zero or less important. The DESIR technique has proven to increase the performance of NIR spectroscopy for trace analysis.

4. Conclusions

NIR spectroscopy only was not suitable for predicting the low concentration of PEs content in jatropha seed. However, NIR spectroscopy with a special sample preparation technique called DESIR could predict PEs content accurately and faster than HPLC. This research is useful for the researchers, especially plant breeders to screen for a large number of samples in jatropha improvement program.

Acknowledgement

This work was supported by the Chair Professor Grant of Thailand's Nation Science and Technology Development Agency (Grant no. P-11-00599)

Reference

Acharya, U.K., Subedi, P.P., Walsh, K.B. 2012. Evaluation of dry extract system involving NIR spectroscopy (DESIR) for rapid assessment of pesticide contamination of fruit surface. American Journal of Analytical Chemistry 3, 524-533.

Burns, D.A., Ciurczak, E.W. 2008. Handbook of Near-Infrared analysis. (3rd ed.). New York: CRC Press of Taylor & Francis Group.

Devappa, R.K., Makkar, H.P.S., Becker. K. 2010. Optimization of conditions for the extraction of phorbol esters from Jatropha oil. Biomass Bioenergy 34, 1125-1133.

Flores, P.L., Tintoréa, S.G., Feriaa, J.S., Ortízb, G.D., Guerreroc, L.C., Ayalaa, A.M. 2012. Biochemical evaluation of protein fractions from physic nut (*Jatropha curcas* L.). Grasas y aceites 63(3), 253-259.

Gübitz, G.M., Mittelbach, M., Trabi, M. 1998. Exploitation of the tropical oil seed plant *Jatropha curcas* L. Bioresource Technology 67, 73-82.

Hass. W., Mittelbach, M. 2000. Detoxification experiments with the seed oil from *Jatropha curcas* L. Industrial Crops and Products 12, 111-118.

ISO 12099. 2017. Standard Method for Animal Feeding Stuffs, Cereals and Milled Cereal Products - Guidelines for Application of Near Infrared Spectrometry. The International Organization for Standardization. (1st ed.). Available at: https://www.iso.org/search.html?q=12099. Accessed on 11 June 2018.

King, A.J., He, W., Cuevas, J.A., Freundenberger, M., Ramiaramanana, D., Graham, I.A. 2009. Potential of Jatropha curcas as source of renewable oil and animal feed. Journal of Experimental Botany 60, 2897-2905.

Lima, K.M.G., Raimundo Jr., I.M., Pimentel, M.F. 2007. Improving the detection limits of near infrared spectroscopy in the determination of aromatic hydrocarbons in water employing a silicone sensing phase. Sensors and Actuators B 125, 125-229.

Makkar, H.P.S., Becker, K. 2009. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. European Journal of Lipid Science and Technology 111, 773-787.

Makkar, H.P.S., Francis, G., Becker, K. 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 1(9), 1371-1391.

Montes, J.M., Technow, F., Bohlinger, B., Becker, K. 2013. Grain quality determination by means of near infrared spectroscopy in *Jatropha curcas* L. Industrial Crops and Products 43, 301-305.

Osborne, B.G., Fearn, T., Hindle, P.H. 1993. Practical NIR Spectroscopy with Application in Food and Beverage Analysis. Singapore: Longman Scientific and Technical.

Rittiron, R. 2017. Establishment of near infrared spectroscopy system for international standard. In: Establishment of NIR system for routine analysis (pp.16-17). Nakhon Pathom, Thailand: Department of Food Engineering, Faculty of Engineering at Kamphaeng Saen, Kasetsart University.

Roque, J.V., Dias, L.A.S., Teófilo, R.F. 2017. Multivariate calibration to determine phorbol esters in seeds of *Jatropha curcas* L. using near infrared and ultraviolet spectroscopies. Journal of the Brazilian Chemical Society 28, 1506-1516.

Sadubthummarak, U., Parkpian, P., Ruchirawat, M., Kongchum, M., Delaune, R.D. 2013. Potential treatments to reduce phorbol esters levels in jatropha seed cake for improving the value added product. Journal of Environmental Science and Health 48, 974-982.

Siesler, H.W., Ozaki, Y., Kawata, S., Heise, H.M. 2006. Near-infrared spectroscopy. (3rd ed.). Weinheim, Germany: Wiley-VCH.

Sujatha, M. 2006. Genetic improvement of *Jatropha curcas* L. possibilities and prospects. Indian Journal of Agroforestry 8, 58-65.

Thyholt, K., Isaksson, T. 1997. Near infrared spectroscopy of dry extracts from high moisture food products on solid support-a review. Journal of Near Infrared Spectroscopy 5, 179-193.

Wink, M., Koschmieder, C., Sauerwein, M., Sporer, F. 1997. Phorbol esters of *Jatropha curcas*-biological activities and potential applications. In: Gübitz, G.M., Mittelbach, M., Trabi, M. (Eds.), Biofuels and Industrial Products from *Jatropha curcas* (pp. 160-166). Austria: Dbv-Verlag for the Technical University of Graz.

Zhang, X., Ren, Y., Du, Y., Li, X., Geng, J., Li, W., Iqbal, J. 2013. Assessment of ability to detect low concentration analyte with near-infrared spectroscopy based on pre-concentration technique. Chemometrics and Intelligent Laboratory System 124, 1-8.