Determination of Tocopherols and Tocotrienol in Encapsulated Vitamin E Powder by NIR Spectroscopy

Laichheang Yort¹, Saowaluk Rungchang^{1,2}, Riantong Singanusong^{1,2}, Chayanid Sringarm¹, Pantaree Phudud¹, Sujitra Funsueb³, Sila Kittiwachana³, Phumon Sookwong³, and Sudarat Jiamyangyuen^{1,2,4,*}

1Department of Ago-Industry, Faculty of Agriculture, Natural Resources, and Environments, Naresuan University, Phitsanulok, Thailand ²Centre of Excellence in Fats and Oils, Faculty of Agriculture, Natural Resources, and Environments, Naresuan University,

Phitsanulok, Thailand

³Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

⁴Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand

Email: yortl61@nu.ac.th (L.Y.); saowalukr@nu.ac.th (S.R.); riantongs@nu.ac.th (R.S.); chayanids62@nu.ac.th (C.R.);

pantareep63@nu.ac.th (P.P.); sujitra.funs@gmail.com (S.F.); sila.k@cmu.ac.th (S.K.); phumon.s@cmu.ac.th (P.S.);

sudarat.j@cmu.ac.th (S.J.)

*Corresponding author

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Abstract—Vitamin E is necessary fat-soluble vitamin that is especially susceptible to oxidation, hence requiring the preservation techniques. Encapsulation performs a dual function by stabilizing vitamin E and altering its physical state from liquid to powder, so improving its practicality and enabling its quantification. NIR (Near Infrared Spectroscopy) is employed as an analytical tool for scanning and characterizing the unique spectral signature of the molecule contained within each sample. Additionally, the application of chemometric methods in the processing of NIR data allows for the recognition and quantification of substances present in the provided sample. The objective of this study is to investigate the effectiveness of utilizing NIR as a method for predicting the quantity of tocopherol (Toc) and tocotrienol (T3) in encapsulated vitamin E. The PLS (Partial Lease Square Regression) models developed using NIR exhibited statistical results in terms of prediction performance for Toc (RPD = 2.55, R^2 = 0.98, Q^2 = 0.84, RMSEE = 1.19, RMSEP = 2.43) and T3 (RPD = 3.75, $R^2 = 0.98$, $Q^2 = 0.92$, **RMSEE = 5.74, RMSEP = 9.96**). These are obvious indications of accuracy utilizing NIR from their high RPD, high R², and Q², coupled with low values of RMSEE and RMSEP. The established predictive models were subsequently utilized for determining the concentrations of Toc and T3 in the vitamin E encapsulating powder, consequently presenting its suitability for analytical purposes. The models have the potential to be utilized for broad testing in laboratories, as well as in the context of industrial processing.

Keywords—Tocopherol, Tocotrienol, NIR spectroscopy, chemometric

I. INTRODUCTION

Vitamin E is a naturally occurring lipid-soluble vitamin. Vitamin E is composed of eight derivatives, namely α -, β -, γ -, and δ -tocopherols, along with their four tocotrienols [1]. The primary sources of its origin are predominantly derived from seeds, grains, and vegetable oils. According to the findings of Ye *et al.* [2], vegetable oil deodorizer distillate, which is a byproduct of the refining process, serves as the main supply for commercial manufacture. In contrast to soybean oil and palm oil, Rice Bran Oil Deodorizer Distillate (RBODD) is relatively less recognized within the food industry. The reason for this phenomenon could be attributed to the larger scale of oil manufacturing processes. The high susceptible of Vitamin E to oxidation necessitates significant stabilizing measures. The process of encapsulation enhances the stability of oil-soluble vitamins, as proven by Fan *et al.* [3]. The

selection of a wall material in the design stage of encapsulation is of major significance due to its major effect on the result. The raw materials of the encapsulation comprise of maltodextrin, gum arabic, sodium caseinate, pectin, starch, chitosan, and whey protein. Gum arabic and maltodextrin are commonly employed as encapsulating agents [4].

Vitamin E can be effectively determined in different liquid media, such as oils, serum, human milk, and foods, through the application of various analytical methods, including High-Performance Liquid Chromatography (HPLC), Fourier-Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, Ultraviolet-Visible spectroscopy (UV-VIS), and spectrophotometric techniques. In contemporary times, a number of methodologies have been developed for the purpose of examining the occurrence of vitamin E in cosmetics, food packaging, and food items [5].

The spectroscopy technique known as Near Infrared (NIR) spectroscopy utilizes the identification of overtones and combinations of fundamental vibrations derived from the C-H, N-H, O-H, and S-H bands. The observed vibrations appear within the spectral region ranging from 14,000 to 4,000 cm-1. The incorporation of chemometric devices is crucial for the collection of relevant information in NIR. Previous study has investigated the effectiveness of NIR spectroscopy techniques in determining the presence and concentration of tocopherol, tocotrienol, and their respective derivatives [6-10]. Chemometrics is an analytical field that mainly deals with obtaining significant and valuable information from data. Partial Least Squares regression (PLS) is a chemometric methodology utilized for the purpose of establishing the relationship between observed data and prediction data, as mentioned by Diaz et al. [11].

Previous investigations on the determination of tocopherol and tocotrienol were conducted using materials that contained other compounds. In contrast, the present study utilized crude extracts of tocopherol and tocotrienol obtained from RBODD for the purpose of determination. Therefore, it would be fascinating to develop an expedited, and efficient analytical method capable of evaluating the vitamin E concentration. Additionally, it is imperative for the analytical methodology to possess the ability to detect the differences in derivatives of vitamin E contained within encapsulated vitamin E samples, considering their potentially diverse structural characteristics.

The primary objective of this research was to examine the effectiveness of applying NIR spectroscopy with chemometric as a predictive tool for determining the levels of tocopherol and tocotrienol in encapsulated vitamin E derived from RBODD. The chemicals under investigation are able to be observed through study by conducting a comparative analysis of the spectra obtained from the significant NIR spectral region. The present effort attempts to offer substantial insights that may contribute to accelerate the development and expansion of this scientific discipline.

II. MATERIALS AND METHODS

A. Materials

The provision of Rice Bran Oil Deodorizer Distillate (RBODD) was gratefully provided by Surin Bran Oil Co., Ltd., Surin, Thailand. The control in the study was tapioca starch, which was designated as DE-0. The maltodextrin samples used in this study, namely DE-7, DE-10, and DE-16, were generously provided by WGC Co., Ltd. Nakhon Pathom, Thailand. The Gum Arabic (GA) used in this study originated from Sigma-Aldrich, Germany.

B. Sample Preparation

The extraction of Vitamin E was conducted in accordance with the methodology described in an earlier study by Yort *et al.* [12]. The extraction of the compound from RBODD was performed using ethanol at a weight-to-volume ratio of 1:9.5. The mixture used for extraction underwent reflux at a temperature of 80 ± 5 °C for a total of 30 minutes. This was followed by incubation at a low temperature of -26 °C for a period of 24 hours. The collection of Vitamin E extract (VEE) was performed by evaporating ethanol at a temperature of 40 °C under vacuum conditions utilizing a rotary evaporator.

VEE encapsulation method was modified from Sahlan et al. [13]. VEE from RBODD was utilized as a core material for the encapsulation process. The wall materials utilized for encapsulation consisted of DE-0, which represented as tapioca starch, and DE-7, DE-10, and DE-16, which respectively represented maltodextrin types with varying degrees of dextrose equivalent (DE-7, DE-10, and DE-16). The experimental design was 4×3 factorial in which there are 4 different DE (0, 7, 10, and 16) and 3 durations of ultrasonic emulsification time (UT:0, 30, and 60 min). Totally, 12 treatments were conducted with 3 replications. Encapsulation vitamin E was prepared by forming GA:DE:water:VEE (1:1:4:2). After obtaining the vitamin E emulsion from the previously mentioned formula, an ultrasonic bath of 150 watt at 37 kHz (Elma Elmasonic S 60 H, Elma Schmidbauer GmbH, Germany) was used for the difference in duration time mentioned earlier. The emulsion was frozen at -40 °C for 18 h and lyophilized at -80 °C for 24 h. The encapsulated vitamin E obtained was ground to powder by blender (model HGB2WT, Waring Commercial, Torrington, USA) and sieved through a 20 mesh (850 µm) and collected in a vacuum aluminium bag until analysed. The final Vitamin E Encapsulated Powder (VEP) was then obtained.

C. NIR Spectroscopy Analysis

The experimental setup involved the utilization of a

Matrix-F Fourier transform Near-Infrared (NIR) spectrometer manufactured by Bruker Fibre Optics in Ettlingen, Germany. The instrument was outfitted with a 1.0 m fiberoptic diffuse reflectance probe and an extended thermoelectrically cooled InGaAs (indium gallium arsenide) detector. The light that is dispersed in various directions is gathered and directed by a fiberoptic cable towards the spectrometer. The NIR spectra of VEP samples was collected in rotating reflectance mode at 16 cm⁻¹ spectral resolution with a background with 32 scans and recorded in the wave range of 4,000–12,000 cm⁻¹. A quantity of 150 g of sample was placed into a quartz cup $[100(\emptyset) \times 20(H) \text{ mm}]$. The NIR absorbance was measured in order to facilitate subsequent data analysis.

D. Vitamin E determination

Quantitative analysis of tocols was determined using an Agilent HPLC 1100 and a fluorescence detector constituted the apparatus (Model 1046A, Hewlett Packard, CA). VertiSepTM UPS SILICA column (4.6 × 250 mm, 5 µm, Vertical Chromatography Co., Ltd., Bangkok, Thailand) was used as the separation columns tested. An isocratic elution of mixture of hexane, tetrahydrofuran, and isopropanol (93:6:1) was used as the mobile phase. The column was maintained at a constant temperature of 30°C, and the flow rate was 0.5 mL/min. A fluorescence detection of tocols was achieved (excitation at 294 nm and emission at 326 nm). One gram of sample was diluted with dichloromethane, then adjusted the volume to 1.00 mL. 0.45 µm nylon syringe filter was used to filter the solution. The chromatographic analysis was using 5.0 µL of sample injection.

E. Data analysis

A partial least squares (PLS) model was developed utilizing the recorded NIR spectral data [14,15]. In this study, test set validation was exclusively utilized for modelling the NIR spectral data. There are 72 spectra of NIR from scanning the vitamin E encapsulated powder. The samples were separated into calibration and test sets at a ratio of 2:1 (48:24 samples). The samples were organized in ascending order based on their modelling response, ranging from the minimum to the maximum. The test set included every second sample from a set of three samples.

One of the key components of PLS analysis is to the decomposition of the spectral data matrix x into a linear combination of scores matrix T and loadings matrix P. The process of decomposing x is performed by iteratively regressing the score against the provided concentration vector y, as described by Ritthiruangdej *et al.* [16]. PLS modelling is a statistical technique that results in the estimation of a regression vector, denoted as b, commonly known as the PLS model. As a result, it turns into

$$y = xb + e \tag{1}$$

where X represents the observed spectrum, y represents the concentration of the relevant analyte, and e denotes the residual. The comprehensive information can be located in another source [16]. The regression vector b contains the essential data for determining the concentration of the analyte, while being less influenced by interference from other chemical components that are not directly related to the

quantity of y.

Various statistical measures were calculated in order to evaluate the effectiveness of the prediction models that were constructed. The quality predictions of the training set or auto-prediction were assessed using the coefficient of determination for calibration (R^2) and the Root Mean Square Error of Calibration or Estimation (RMSEE). The predictive accuracy of the models in predicting the test sets or unknown samples was evaluated using the coefficient of determination for prediction (Q^2) and the Root Mean Square Error of Prediction (RMSEP). Additionally, the Standard Deviation (SD) of the response values and the root mean square error (RMSE) were utilized to generate the Ratio of Prediction to Deviation (RPD), which was used to standardize the predictive accuracy. The calibration models were optimized using the leave-one-out cross validation approach. The chemometric computations and statistical analyses were conducted using OPUS version 7.8 software, developed by Bruker Optics.

III. RESULT AND DISCUSSION

A. Spectral Analysis

The average with standard deviations of the NIR data obtained from encapsulated vitamin E sample, as well as the first derivative, are shown in Fig. 1. The obtained NIR raw spectra are very comparable to those that have already been published for tocopherol determination [10, 17, 18]. The spectral region ranging from 4400 to 4200 cm-1 reveals the presence of highly considered bands, which can be attributed to the vibrational activity of CH, CH₂, and CH₃ bonds within the combination band region. Within the spectral range of 5,350 to 5,000 cm⁻¹, vibrational phenomena associated with the OH bonds found in water molecules can be observed, specifically in the first overtone area and combination band region. The bands exhibiting the highest intensity are located within the spectral range of 6,000 to 5,600 cm^{-1} and can be assigned to the vibrational modes of CH, CH₂, and CH₃ chemical bonds in the first overtone area. The band exhibiting the highest level of intensity within the spectral range of 7,622 to 6,507 cm^{-1} can be attributed to the vibrational action of OH bonds, occurring in the second overtone region of water molecules. The spectral bands seen in the wavenumber range of 9,000 to 8,000 cm⁻¹ are potentially associated with the vibrational modes of CH, CH₂, and CH₃ bonds in the second overtone area.

B. Distribution of Calibration and Validation Reference Data for NIR Prediction Models of the Parameters for Encapsulated Vitamin E Properties

Table 1 summarizes the relevant characteristics of encapsulated sample sets utilized for the calibration model of tocopherol (Toc) and tocotrienol (T3) content. Data regarding Toc and T3 demonstrated a normal distribution located around the mean. The calibration set had 48 samples, whereas the validation set comprised 24 samples. For calibration and validation, the value of Toc ranged from 25.91 to 47.33 mg/g and 26.82 to 44.55 mg/g respectively. The T3 values ranged from 119.25 to 240.08 mg/g for calibration and from 134.73 to 225.32 mg/g for validation.

quality parameter of vitamin E encapsulated powder

	Test set validation (NIR)							
Parameters	Calibration set (n = 48)				Validation set (n = 24)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Toc (mg/g)	34.04	6.98	25.91	47.33	33.88	6.28	26.82	44.55
T3 (mg/g)	168.79	37.20	119.25	240.08	165.22	35.17	134.73	225.32



Fig. 1. NIR spectra from VEP samples. (a) average and SD spectra and (b) 1st derivative spectra.

C. Quantitative Analysis of Encapsulated Vitamin E Using PLS Regression

The current study involved the development of Partial Least Squares (PLS) regression models for the purpose of predicting several forms of vitamin E, specifically Toc and T3. The models were constructed utilizing the gathered NIR spectra (X) of encapsulated vitamin E, alongside the matching quantitative outcomes (Y) acquired from reference analyses (as shown in Table 1). Fig. 2 shows the scatter plot representing the calibration and validation of parameters, demonstrating the relationship between the predicted values and the observed values. The preprocessing of NIR spectra included the application of the Standard Normal Variate (SNV) technique. The models exhibiting elevated levels of resilience and variability were generated by combining the contents of all contained samples of vitamin E. The model selection for each parameter was determined by the analysis of statistical parameters, such as the coefficients of determination for calibration (\mathbf{R}^2) and validation (\mathbf{Q}^2) , Relative Percent Difference (RPD) values, and Root Mean Square Error of Estimation (RMSEE) and Root Mean Square Error of Prediction (RMSEP). Following the information presented in the study conducted by Okere et al. [18], it is established that models demonstrating RPD values lower than 1.5 qualify as possess inadequate reliability. On the other hand, models with RPD values within the range of 1.5 to 2.0 demonstrate their potential effectiveness for approximate prediction objectives. Moreover, it is widely accepted in the academic community that RPD values ranging from 2.0 to 2.5 are considered appropriate for making quantitative predictions. Consequently, RPD levels that exceed 3 are seen to be acceptable.





This study offers a comprehensive analysis of the results of the developed models in regard to different quality outcomes. R^2 for the NIR calibration exhibited notable values of 0.98 for both Toc and T3, indicating a satisfactory correlation between the anticipated and observed values. Moreover, it was noted that the RMSEE exhibited a low value of 1.19 for Toc and 5.74 for T3. This further strengthens the evidence supporting the accuracy of the calibration model. The regression statistics were strongly influenced by the models created within the NIR spectral range. In order to evaluate the accuracy and reliability of the calibration model mentioned earlier, it is necessary to utilize a validation model to conduct testing. The prediction model was tested using the calibration model and the validation dataset provided in Table I. The prediction coefficient Q2 for Toc and T3 were 0.84 and 0.92, respectively, accompanied with low RMSEP values of 2.43 and 9.96.

The present investigation showed Toc and T3 RPD values of 2.55 and 3.75, respectively. In contrast to a previous investigation carried out by Cayuela and García [6], which utilized NIR spectroscopy to predict the levels of α -Toc (RPD = 2.37), β -Toc (RPD = 1.04), γ -Toc (RPD = 1.91), and Toc (RPD = 2.01) in olive oil by the implementation of Partial Least Squares (PLS) regression. Furthermore, the latest research conducted by Xu *et al.* [10] investigated the determination of Toc in Brassica napus seeds using NIR. The findings of this study revealed RPD of 1.97 and Q2 of 0.64.



Fig. 3. PLS coefficient of NIR spectra for (A) Toc and (B) T3 using SNV as data preprocessing.

Fig. 3 shows the regression coefficients of PLS in relation to the wavenumber of the NIR with Toc and T3. The previous coefficients apply to the components Toc and T3 of vitamin E that have been encapsulated. Determining the value of a particularly significant region is a complex task as it involves the computation of these values based on several functional categories. The spectral region of interest, namely covering from 4,000 to 6,000 cm⁻¹, was observed to have absorption bands that are linked to the functional groups CH₂ and CH₃. The observed absorption bands are attributed to combination bands inside the 1st overtone area, and they are recognized as important constituents of the functional group found in vitamin E. The findings of this study align with the results reported by Antónia *et al.* [17], wherein they similarly detected numerous peaks (ranging from 4,456 to 4,359 cm⁻¹) matching the absorption bands of CH₂ and CH₃ in tocopherol derived from olive pomace. Similarly, Páscoa *et al.* [18] have successfully discovered the absorption bands of CH₂ and CH₃ inside the combination band region (4,035–4,961 cm⁻¹) and the 1st overtone region (5,389–6,504 cm-1). The results presented in Fig. 1 also provide additional support for the importance of the vitamin E region, which was detected within the wavenumber range of roughly 4,000–6,000 cm⁻¹.

IV. CONCLUSION

The prediction capacities of NIR spectroscopy in predicting tocopherol and tocotrienol have been found to be superior in the context of Partial Least Squares (PLS) analysis. Regarding the calibration, it can be observed that the NIR predictions demonstrate a significant level of precision, as evidenced by their elevated RPD, substantial Q^2 , and low RMSEP. The spectral band covering wavenumbers 4,000–6,000 cm⁻¹, which includes the combination bond and 1st overtone region, retains considerable importance in the analysis of vitamin E through the application of NIR spectroscopy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally in this study. Laichheang Yort conducted the experiment, analyzed the data and wrote paper. Sudarat Jiamyangyuen, Saowaluk Rungchang, Riantong Singanusong, Sila Kittiwachana, and Phumon Sookwong conceptualized the topic and revised the final paper. Chayanid Sringarm, Pantaree Phudud, and Sujitra Funsueb analyzed the data. All authors approved the final version.

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