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Research Article

Prediction of α-Solanine and α-Chaconine in Potato Tubers from Hunter Color Values and VIS/NIR Spectra

Shimeles Tilahun,[1,2,3] **Hee Sung An**,[1] **In Geun Hwang**,[4] **Jong Hang Choi**,[1] **Min Woo Baek**,[1] **Han Ryul Choi**,[1] **Do Su Park**,[5] **and Cheon Soon Jeong**[1]

[1]*Department of Horticulture, Kangwon National University, Chuncheon 24341, Gangwon, Republic of Korea*
[2]*Agriculture and Life Science Research Institute, Kangwon National University, Chuncheon 24341, Gangwon, Republic of Korea*
[3]*Department of Horticulture and Plant Sciences, Jimma University, Jimma 378, Ethiopia*
[4]*Frusen Co. Ltd, Yongin 702, Republic of Korea*
[5]*Horticulture Section, School of Integrative Plant Science, College of Agriculture and Life Sciences, Cornell University, Ithaca 14853, NewYork, USA*

Correspondence should be addressed to Cheon Soon Jeong; jeongcs@kangwon.ac.kr

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The glycoalkaloids contents of potato tubers are usually measured by the destructive analysis that consumes time and requires expensive high-performance equipment. This study was carried out to determine the possibility of nondestructive estimation of α-solanine and α-chaconine content in potato tubers. Visible/near-infrared (VIS/NIR) spectra, color values, and the reference α-solanine and α-chaconine were measured from 180 tubers of 'Atlantic' and 'Trent' potato cultivars with eight replications at two-week intervals during the storage up to ten weeks. The partial least square (PLS) regression method was used to develop models correlating color and spectra data to the measured reference data. Regression coefficient (r) between color variables (Hunter a^*, a^*/b^*, and $(a^*/b^*)^2$) and the actual measured values of a-solanine and a-chaconine content were 0.74, 0.62, and 0.62 and 0.70, 0.58, and 0.57, respectively, for the prediction set. Concurrently, equations were developed from color variables in multiple regression with r-values of 0.76 and 0.71 for α-solanine and α-chaconine, respectively. Additionally, the selected PLS model of VIS/NIR spectra had promising predictive power for α-solanine and α-chaconine with r-values of 0.68 and 0.63, respectively, between measured and predicted samples. Taken together, although it requires further studies to improve the prediction power of the developed models, the results of this study revealed the possibility of using VIS/NIR spectra and color variables for the prediction of α-solanine and α-chaconine contents from intact unpeeled potato tubers with chemical-free, fast, and cheap assessment methods.

1. Introduction

Potato (*Solanum tuberosum* L.) is a starchy tuberous crop that belongs to the Solanaceae family. Potato cultivars are grown worldwide with highly diverse tuber shapes and colors [1, 2]. Potato is the world's fourth-largest important food crop following maize (corn), wheat, and rice [3] with a worldwide production of 376.82 million tons from 19.2 million ha valued at $111.06 billion. According to FAOSTAT [3], potato production in the Republic of Korea was 631,596 tons valued at $121.66 million from 24,041 ha in 2016. Sustainable production of potato ensures long-term food security due to its high productivity and generation of more food per unit area and per unit time than maize, rice, and wheat [4]. Potato tubers are rich sources of energy due to their starch content (60–80% of the dry matter). Besides, tubers are also rich in potassium, calcium, vitamin C, and protein with good amino acid balance [5].

However, potatoes are more susceptible to quality degradation by water loss, diseases, or build-up of defense

molecules due to the hydrated and metabolically active cells in tubers as compared to dry-stored crops [6]. The abundant group of defense molecules that help potatoes in deterring pests and diseases are glycoalkaloids (GAs) [6–9], yet their toxicity and bitterness are detrimental to the quality of the product. Glycoalkaloids are a class of nitrogen-containing steroidal glycosides that usually found in the genus *Solanum*; 95% of the total glycoalkaloids content in *Solanum tuberosum* primarily consists of trisaccharide steroidal glycoalkaloids α-chaconine and α-solanine [10, 11]. Total glycoalkaloid levels are highly variable in different potato cultivars and are influenced by postharvest factors such as light, mechanical injury, and storage [2, 6]. Greening occurs with an associated increase in the amount of glycoalkaloid when potato tubers are exposed to light [12]. Potato tubers that contain over 200 mg kg^{-1} total glycoalkaloids of fresh tuber weight possess a bitter off-flavor and may cause gastroenteric symptoms, coma, and even death [2, 9, 13–15]. The toxicity of glycoalkaloids could be related to their anticholinesterase activity and disruption of cell membranes, producing neurological disorders and gastrointestinal disturbances, respectively [16]. According to Friedman and McDonald [17], the estimated highest safe level of total glycoalkaloids for human consumption is about 1 mg kg^{-1} body weight; a level that may cause acute toxicity and a lethal dose is 1.75 and 3–6 mg kg^{-1} body weight, respectively.

Although glycoalkaloids are perceived as potentially toxic, studies suggest that they may also possess anticarcinogenic effects, depending on the dose and conditions of use [2]. Friedman et al. [18] and Friedman [19] reported the concentration-dependent anticarcinogenic effects of α-chaconine and α-solanine against human cancer cells. Therefore, it is necessary to establish guidelines limiting the glycoalkaloid content of new cultivars before releasing them for commercial use to obtain the optimum benefit without the potential toxicity to human beings [20].

The common methods used to determine the secondary metabolites of potatoes are destructive and time-consuming and require high-performance laboratory equipment. The development of a rapid, low-cost, reliable, and reproducible analytical method that avoids the extensive sample preparation is inevitable. Pasquini [21] reported that qualitative and quantitative information can be derived from NIR range spectra from the interaction between the spectra and organic compounds that form the substance. NIR in the wavelength range between 700 nm to 110 nm could be used to determine the carbohydrate content [22] and sugar content [23] of potatoes. López-Maestresalas et al. [24] also reported nondestructive detection of black spots in potatoes by VIS/NIR (400–100 nm). The sprouting capacity of potatoes was also predicted by using NIR spectroscopy in intact potato tubers [25]. However, the prediction of glycoalkaloids from color variables and VIS/NIR spectra of intact potato tubers has not yet been reported. The objective of this study was, therefore, to develop models suitable to predict the mass fraction of α-solanine and α-chaconine content of intact potato tubers based on VIS/NIR spectra and color variables.

2. Materials and Methods

The graphical abstract summarized the overall experimental processes and contents of this article.

2.1. Plant Materials. 'Atlantic' and 'Trent' potato cultivars were obtained from Haitai-Calbee snack factory, Korea. Each potato tuber was selected carefully for its freedom from defects, and relatively uniform size potato tubers were then subsequently stored at room conditions (22°C, 12-hour shift of light-dark cycles) to simulate the consumers' practice and to allow greening. Subsampling was done at every 2-week interval and continued up to the 10th week of storage. Samples for reference analysis (α-solanine and α-chaconine) were prepared after taking VIS/NIR spectra data and color reading from intact tubers. Samples for reference analysis were frozen by liquid nitrogen and stored in a deep freezer (−80°C) until analysis [26].

2.2. Color Measurement and Analysis. Hunter a^*, b^*, and L^* color variables were determined using a CR-400 chroma meter (Minolta, Tokyo, Japan). Hunter a^* value indicates chromatic redness and it ranges from red (+ values) to green (− values), b^* shows yellowness chromatic parameter that ranges from yellow (+ values) to blue (− values), and L^* is the lightness parameter that indicates the degree of lightness of the sample that ranges from 0 (black) to 100 (white) [27]. Color variables were measured eight times from the surface of each tuber and the average was determined. Measurements were taken during the storage period until the 10th week at a 2-week interval. Thirty tuber samples ('Atlantic' and 'Trent'; 15 each) were used during each sampling day. The tuber samples from the first and second sampling dates (60 tubers) were used for the prediction set and tuber samples from the last four consecutive sampling dates (120 tubers) were used for the prediction set. A total of 180 tuber samples were used for the experiment.

2.3. VIS/NIR Spectra Measurement and Analysis. The transmittance spectra were acquired from the intact tuber in the spectral region of 500–1100 nm with three (12 V/100 W) halogens lamp as a source of VIS/NIR light by using VIS/NIR spectrometer (Life & Tech, Co., Ltd., Yongin, Korea) (Figure 1(a)) as indicated by Tilahun et al. [26]. A tuber holder was used to keeping the tuber right above the detector (Figure 1(b)). The integration time was set to 100 ms and the measurement was done 8 times at different tuber directions per a tuber to reduce noise from being included. A total of 3500 data were saved for each measurement at 0.2 nm spectrum resolution. NIR spectrometer was connected to a computer for data transmission. A total of 1440 spectra readings were obtained from tubers throughout the storage period. Outliers were excluded and a total of 1100 spectra were used for analysis (Figure 2). Half of the samples (550 spectra readings) were used for the calibration set, and the remaining half (550 spectra readings) were used for the prediction set. Transformation of the original spectra was

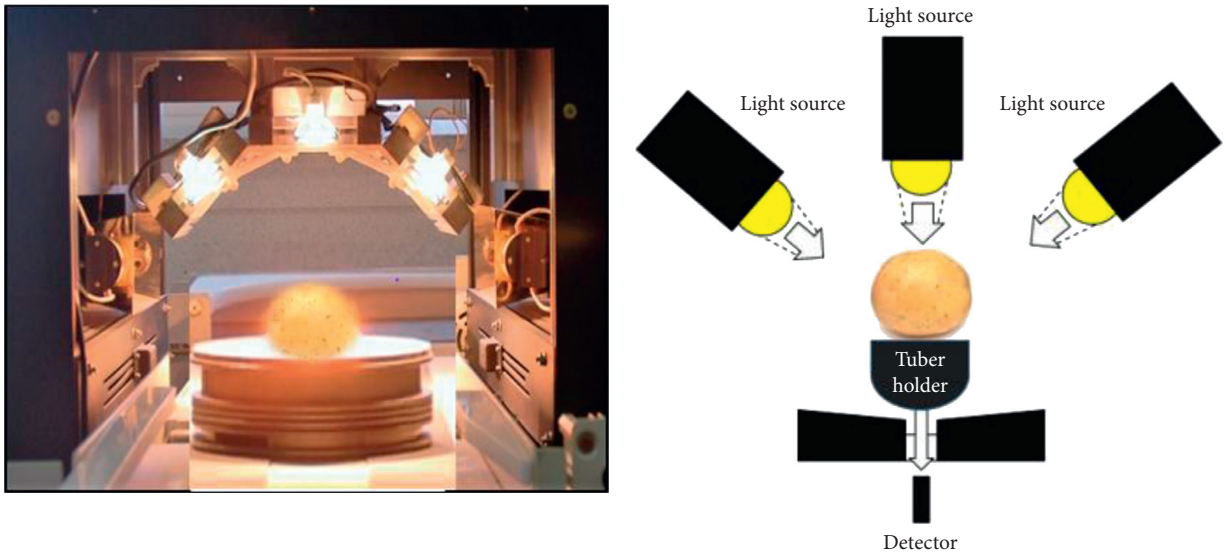

Figure 1: VIS/NIR spectrometer (a) and measurement system (b) during transmittance spectra measurement of intact potato tubers.

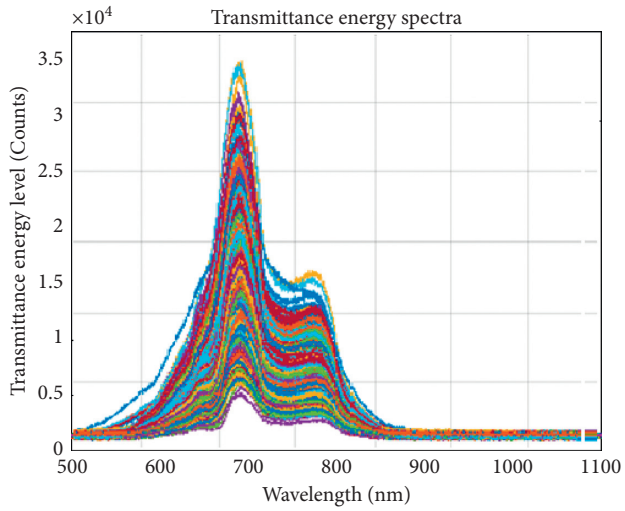

Figure 2: Transmittance energy spectra curves obtained from potato tubers by using VIS/NIR spectrometer.

done by the Hanning window, standard normal variate (SNV), multiplicative scattering correction (MSC), and first derivatives to reduce systematic noise and remove unwanted information. The prediction was performed based on the lowest predicted residual error sum of squares (PRESS) value to select the optimal number of latent variables in the PLS model. Partial least square (PLS) regression analysis was performed with MATLAB R2012b (version 8.0.0.783, The Math Works, Inc., Natick, MA, USA) to establish a linear relationship between spectral data and measured references. RMSECV (root mean square of standard error in cross-validation), RMSEP (root mean square of standard error in prediction), and coefficient of determination for calibration (R^2) and prediction (r) were used to evaluate the performance of the developed PLS models. A predictive model with few bias values and lower RMSECV/P is considered to be a good prediction model.

2.4. Extraction and Quantification of Glycoalkaloids for Reference Analysis. The extraction of glycoalkaloids was done from fifteen tubers for each potato cultivar at two-week interval during the 10-week storage period. Peeling of the sample tubers was not done as glycoalkaloids are mainly found in the potato skin or close to the skin [28, 29]. Extraction of 0.5 g of homogenized sample was made as stated by Tilahun et al. [29] and glycoalkaloids were analyzed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [30]. The spectrometer was adjusted as described by Zywicki et al. [30], Nie et al. [31], and Tilahun et al. [29] for detection of α-solanine and α-chaconine.

3. Results and Discussion

3.1. Color Variables vs. the Measured Reference Analysis. Measurements of Hunter's L^*, a^*, and b^* were taken during the experiment. However, the PLS model for Hunter's L^* and b^* values in the calibration data set had lower R^2 (<0.31) for both α-solanine and α-chaconine. Hence, we did not include Hunter's L^* and b^* values for PLS model development in the prediction data set. Instead, the PLS model for a^*/b^* and $(a^*/b^*)^2$ values in the calibration data set had higher R^2 (>0.69) for both α-solanine and α-chaconine (Tables 1 and 2). Consequently, we included a^*/b^* and $(a^*/b^*)^2$ values for PLS model development for the prediction data set. Although no metabolic connection between chlorophyll and accumulation of glycoalkaloids has been established, the greening of tubers occurs along with the concomitant increase of glycoalkaloids [12]. Therefore, a measurement that encompasses Hunter's a^* value could be a

Table 1: Statistics for calibration and prediction of α-solanine (mg kg^{-1}) using potato tubers color variables.

Set	Parameters	Sample number	Mean	Range	SD	R^2	RMSEC	RPD
	Reference α-solanine		32.40	1.70–129.46	29.28	—	—	—
	Hunter's a*		33.47	0.00–91.00	23.50	0.78	13.97	2.09
Calibration	Hunter's a*/b*	960	33.21	0.00–130.08	24.72	0.74	13.09	2.24
	Hunter's $(a^*/b^*)^2$		32.39	7.29–137.96	26.52	0.82	12.34	2.37
	Multivariate		32.38	0.13–122.80	29.28	0.85	11.24	2.60
Set	Parameters	Sample number	Mean	Range	SD	r	RMSEP	RPD
	Reference α-solanine		28.74	9.58–61.37	10.38	—	—	—
	Hunter's a*		39.18	12.32–63.84	11.15	0.74	11.93	0.87
Prediction	Hunter's a*/b*	480	39.58	10.75–78.97	12.99	0.62	13.54	0.77
	Hunter's $(a^*/b^*)^2$		33.60	10.22–93.77	15.54	0.62	10.98	0.95
	Multivariate		33.92	7.61–80.82	14.40	0.76	9.09	1.14

SD: standard deviation; RMSEC: root mean square error of calibration; RMSEP: root mean square error of prediction; RPD: residual predictive deviation; R^2: coefficient of determination in calibration; and r: coefficient of correlation in prediction data set.

Table 2: Statistics for calibration and prediction of α-chaconine (mg kg^{-1}) using potato tubers color variables.

Set	Parameters	Sample number	Mean	Range	SD	R^2	RMSEC	RPD
	Reference α-chaconine		18.69	0.93–59.36	15.60	—	—	—
	Hunter's a*		18.98	0.00–48.74	12.43	0.69	8.42	1.85
Calibration	Hunter's a*/b*	960	18.87	0.00–68.32	12.88	0.71	8.25	1.89
	Hunter's $(a^*/b^*)^2$		18.68	6.04–71.86	13.36	0.74	7.98	1.95
	Multivariate		18.70	1.68–65.30	13.77	0.78	7.34	2.12
Set	Parameters	Sample number	Mean	Range	SD	r	RMSEP	RPD
	Reference α-chaconine		14.85	8.32–25.69	4.25	—	—	—
	Hunter's a*		22.17	8.40–34.82	5.72	0.70	8.00	0.53
Prediction	Hunter's a*/b*	480	22.34	7.70–42.36	6.60	0.58	8.66	0.49
	Hunter's $(a^*/b^*)^2$		19.29	7.52–49.60	7.83	0.57	6.98	0.61
	Multivariate		19.43	5.39–42.32	4.46	0.71	6.30	0.67

good indicator of glycoalkaloids content as it indicates chromatic values that range from red to green [27]. Tilahun et al. [26] also used the same color variables (a*, a*/b*, $(a^*/b^*)^2$) to predict carotenoids in intact tomato fruit.

Tables 1 and 2 show the means and ranges of reference (measured) α-solanine and α-chaconine obtained by the destructive analysis in the calibration and prediction data sets. Meanwhile, α-solanine and α-chaconine that are estimated by using color variables in the calibration and prediction data sets are also presented in Tables 1 and 2. For α-solanine, R^2, RMSECV, and RPD values of the calibration data set ranged between 0.74–0.85, 11.24–13.97, and 2.09–2.60, respectively. In the prediction data set, the corresponding values were 0.62–0.76, 9.09–13.54, and 0.77–1.14, respectively, for r, RMSEP, and RPD (Table 1). The highest R^2 was found for multivariate PLS model ($R^2 = 0.85$), followed by Hunter's $(a^*/b^*)^2$ ($R^2 = 0.82$), Hunter's a* (0.78), and Hunter's a*/b* ($R^2 = 0.74$) in the calibration data set. For the prediction data set, the PLS models for Hunter's a*/b* and $(a^*/b^*)^2$ had the lowest coefficient of correlation ($r = 0.62$), followed by Hunter's a* ($r = 0.74$) and multivariate PLS model ($r = 0.76$) (Table 1 and Figure 3). The RMSECV values for Hunter's a*, a*/b*, $(a^*/b^*)^2$ and multivariate PLS models were 13.97, 13.09, 12.34, and 11.24, respectively (Table 1 and Figure 3). The highest RPD value of a calibration data set was obtained for a multivariate PLS model (2.60) followed by Hunter's $(a^*/b^*)^2$ (2.37), a*/b* (2.24), and a* (2.09), respectively (Table 1).

The statistics for α-chaconine also showed similar trends with α-solanine and the values for R^2, RMSECV, and RPD in the calibration data set were ranged between 0.69 –0.78, 7.34–8.42, and 1.85–2.12, respectively. In the prediction data set, the values for r, RMSEP, and RPD were ranged between 0.57–0.71, 6.30–8.66, and 0.49–0.67, respectively (Table 2). Interestingly, the highest R^2 (0.78) and r (0.71), lowest RMSEC (7.34) and RMSEP (6.30), and the highest RPD (2.12) and (0.67) values were found in calibration and prediction data sets, respectively, with multivariate PLS model (Table 2 and Figure 4).

Following the predictive analysis in multiple regression, Hunter's a*, a*/b*, and $(a^*/b^*)^2$ values were found to have high predictive p-values in the prediction of both α-solanine and α-chaconine from color variables. The following equations were found to be the best equations:

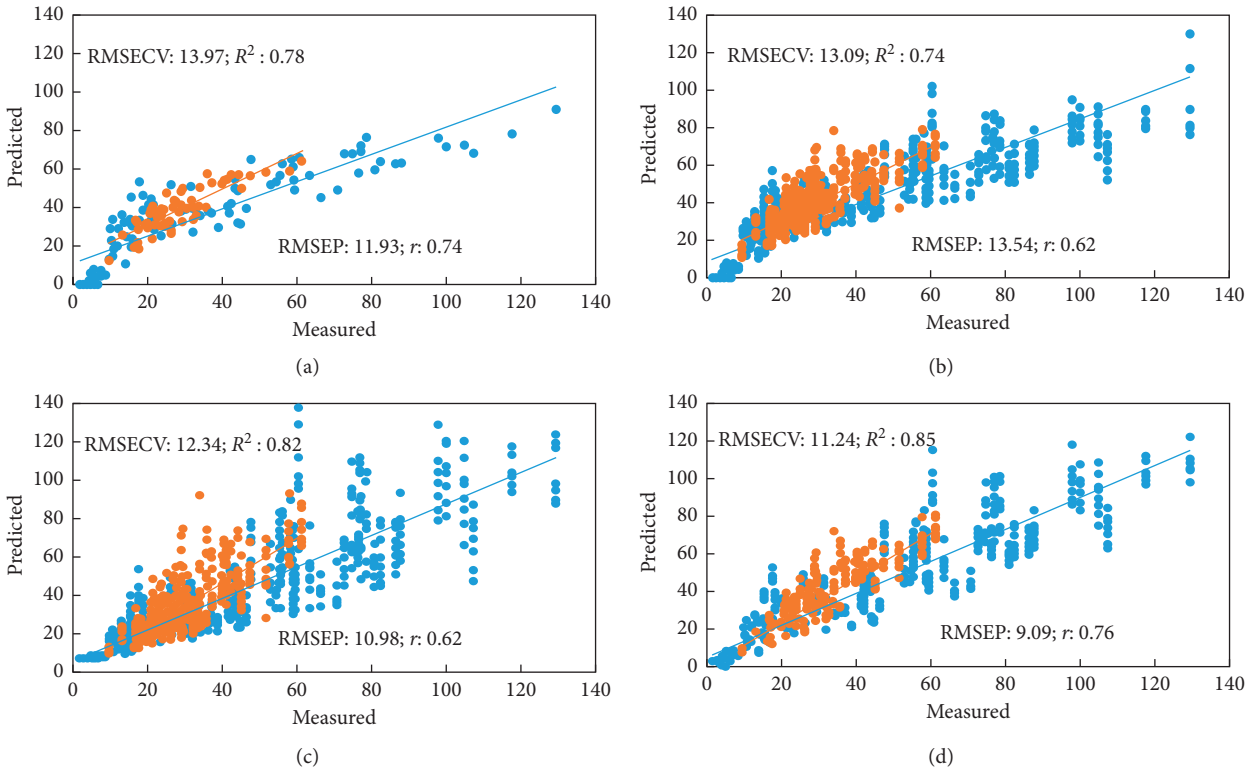

Figure 3: Measured vs. predicted scores of α-solanine (mg kg^{-1}) in the calibration (blue) and prediction (red) sets with PLS models using (a) Hunter's a* values; (b) Hunter's a*/b* values; (c) (a*/b*)^2 values; and (d) multivariate values.

$$\alpha\text{-solanine}\left(\text{mg kg}^{-1}\right) = 2.46 - 12.75\left(a^*\right) + 226.85\left(\frac{a^*}{b^*}\right) + 1068.11\left(\frac{a^*}{b^*}\right)^2,$$
$$\alpha\text{-chaconine}\left(\text{mg kg}^{-1}\right) = 3.17 - 8.01\left(a^*\right) + 146.29\left(\frac{a^*}{b^*}\right) + 550.70\left(\frac{a^*}{b^*}\right)^2. \quad (1)$$

The measured reference vs. predicted scores of both α-solanine and α-chaconine in the calibration and prediction sets with multivariate PLS models had shown a promising result to use the model. For the prediction data set, a multivariate PLS model had the highest coefficient of correlation (0.76) for α-solanine and (0.71) for α-chaconine (Tables 1 and 2 and Figures 1 and 2). However, it could not be claimed that this technique can be adopted with all potato cultivars as the cultivars used in the present study have white-colored tubers. Hence, further studies are needed on various cultivars having different colored tubers to develop more robust models.

3.2. VIS/NIR Spectra vs. the Measured Reference Analysis. The current demand for quality products relies on the adoption of environmentally friendly nondestructive technologies like VIS/NIR spectroscopy [4, 26] and it has gained broad acceptance for food quality evaluation [32]. VIS/NIR spectra have been reported as rapid, low-cost, and reliable method for estimation of lycopene and β-carotene in tomato [26, 33]. Bonierbale et al. [34] also reported the estimation of total and individual carotenoid concentrations in *Solanum phureja* cultivated potatoes by NIR spectroscopy. In this study, the transmittance energy spectra of intact potato tubers were recorded in the wavelength of 500–100 nm as shown in Figure 2. PLS models were also developed to predict α-solanine and α-chaconine based on VIS/NIR spectra of intact potato tubers and promising results were recorded. R^2 and RMSEC for measured vs. VIS/NIR values of α-solanine in the calibration set were 0.69 and 7.87, respectively (Figure 5(a)). Meanwhile, R^2 and RMSEP for reference vs. VIS/NIR values of α-solanine in the prediction set were 0.68 and 7.93, respectively (Figure 5(b)). On the other hand, R^2 and RMSEC for measured vs. VIS/NIR values of α-chaconine in the calibration set were 0.64 and 3.94, respectively (Figure 6(a)), while R^2 and RMSEP for reference vs. VIS/NIR values of α-chaconine in the prediction set were 0.63 and 3.97, respectively (Figure 6(b)). Several efforts have been made to predict different physicochemical properties of potato tubers by using VIS/NIR spectroscopy.

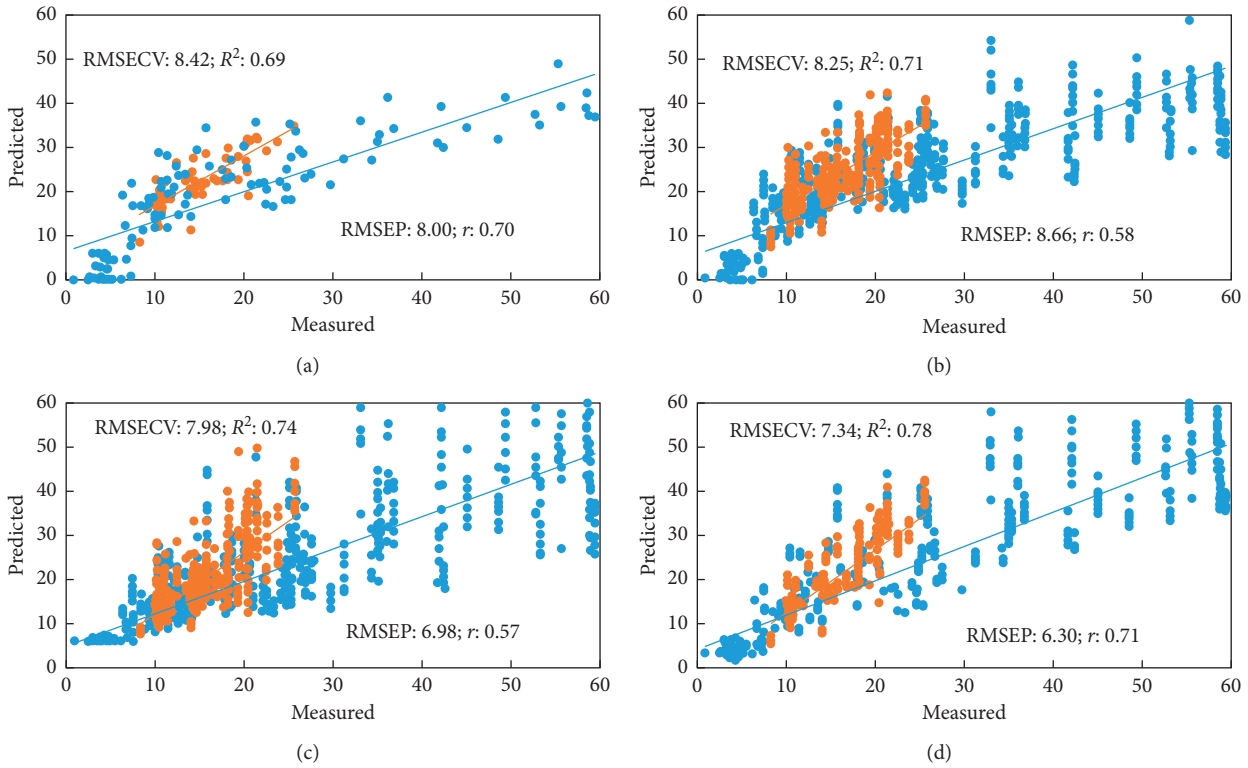

Figure 4: Measured vs. predicted scores of α-chaconine (mg kg^{-1}) in the calibration (blue) and prediction (red) sets with PLS models using (a) Hunter's a* values; (b) Hunter's a*/b* values; (c) $(a^*/b^*)^2$ values; and (d) multivariate values.

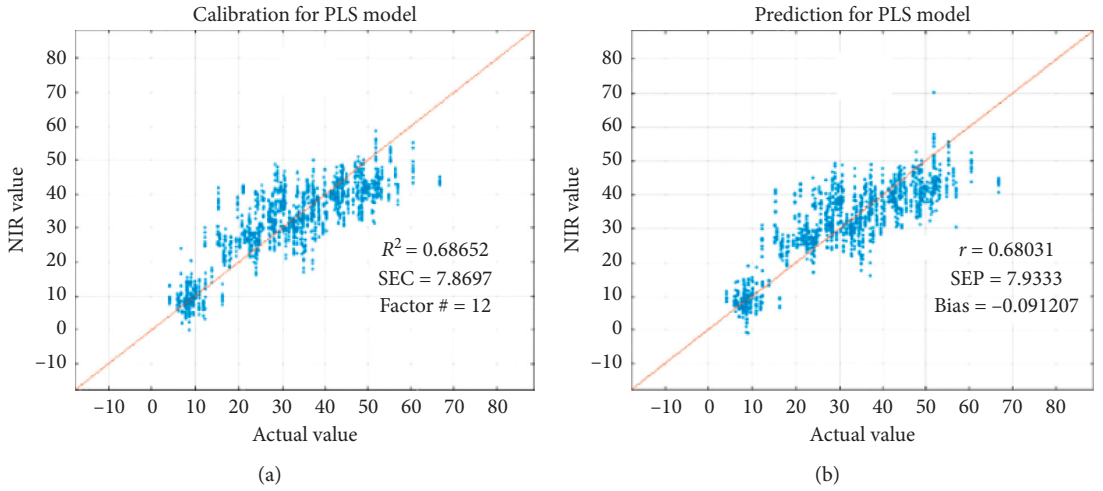

Figure 5: Measured vs. VIS/NIR values of α-solanine (mg kg^{-1}) in the calibration (a) and prediction (b) sets with PLS models. SEC: standard error of calibration; SEP: standard error of prediction.

For instance, NIR spectra were used to predict the sprouting capacity [25] and internal defects [35, 36] of tubers. Also, different infrared-based researches were reported on the prediction of quality-related parameters like dry matter content [37, 38], carbohydrate [22], and sugar content [23, 39]. Similarly, Haase [40] reported the NIR reflectance-based prediction of overall processing related quality parameters from ground raw tubers. The sensory texture of cooked potatoes was also estimated by the use of NIR spectroscopy [41, 42]. Although it requires further studies to improve the prediction power of the developed models, the results of this study revealed the possibility of

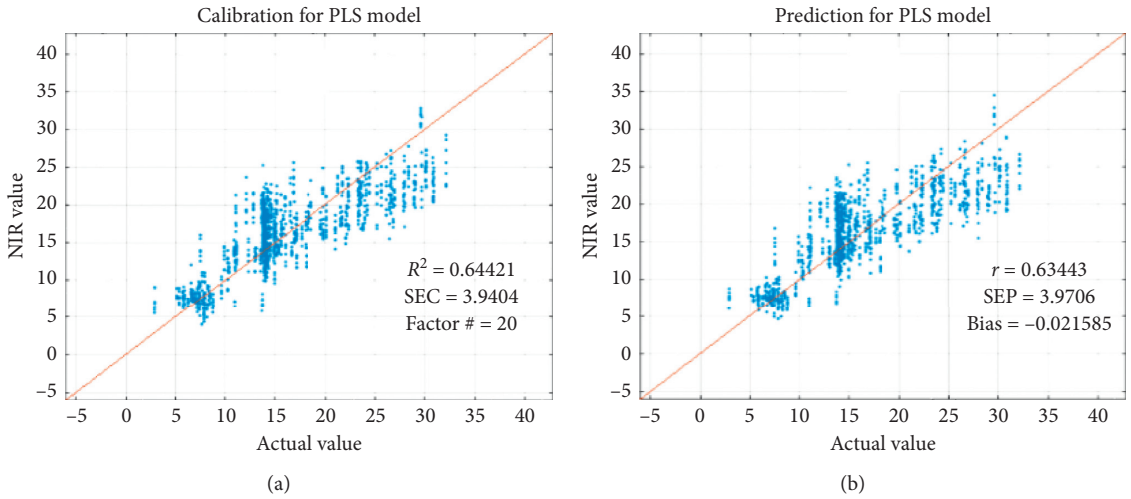

Figure 6: Measured vs. VIS/NIR values of α-chaconine (mg kg^{-1}) in the calibration (a) and prediction (b) sets with PLS models.

using VIS/NIR spectra for the prediction of α-solanine and α-chaconine from intact unpeeled potato tubers.

4. Conclusions

The present study indicates the attempts made to predict α-solanine and α-chaconine in intact unpeeled potato tubers with chemical-free, fast, and cheap assessment methods. Models were developed by using Hunter color values and VIS/NIR spectra. Prediction of α-solanine was relatively better than α-chaconine with both color and VIS/NIR-based techniques. Our models could be a promising alternative to the costly and time-consuming destructive analysis for breeders during cultivars screening before releasing them for production. The developed models could be used easily in the field with the use of portable chroma meter and in the agricultural processing centers to sort tubers on a conveyor belt with the use of a VIS/NIR spectrometer. However, it could not be claimed that the developed models can be adopted with all potato cultivars as the cultivars used in the present study have white-colored tubers. Hence, the developed models need to be tested further on independent data sets from white-colored potato cultivars, and further studies are needed on various cultivars having different colored tubers to develop more robust nondestructive methods for the estimation of the glycoalkaloids content of the intact potato tubers.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

S.T., H.S.A., and C.S.J. contributed to conceptualization. S.T. and H.S.A. performed methodology. S.T., J.H.C., H.R.C., and M.W.B. executed experiment. S.T. and I.G.H. were responsible for software. Formal analysis was performed by S.T. and I.G.H. C.S.J. contributed to resources. Original draft was prepared by S.T. Review and editing were carried out by D.S.P. and C.S.J. C.S.J. contributed to supervision. Project administration was carried out by M.W.B. Funding was acquired by C.S.J. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Graphical abstract is provided in this section. (*Supplementary Materials*)

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