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Determination of the Betacyanin and Betaxanthin Contents of Red Beet (Beta Vulgaris) Powder Using Partial Least Square Regression Based on Visible-Near Infrared Spectra

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Abstract

Red beet (Beta vulgaris) contains betalain, which comprises red-violet betacyanin and yellow betaxanthin with esthetic and health benefits. Betacyanin and betaxanthin are usually detected using common chemical analysis, which requires a long time, skilled analysts, and sample destruction. For fast and accurate measurement, this study utilized a portable low-cost Visible-Near Infrared Spectra (Vis-NIR) spectrometer at 350 - 1000 nm combined with partial least square regression to predict the betacyanin and betaxanthin contents of red beet powder. The best calibration models for betacyanin and betaxanthin had R^2c of 0.89 and 0.919, respectively, and standard error of calibration SEC of 0.108 and 0.037 mg/g, respectively. The models were able to predict the contents of both pigments with R^2p of 0.87, standard error of prediction SEP of 0.108 mg/g, and the ratio of prediction to deviation RPD of 2.52 for betacyanin and R^2p of 0.84, SEP of 0.056 mg/g, and RPD of 2.47 for betaxanthin. When applied to external unknown data, the models predicted the contents of betacyanin and betaxanthin with R^2 of 0.98 and root mean square error of 0.107 and 0.055 mg/g. Moreover, the predicted values were not significantly different at 95 % confidence.

Keywords: Betacyanin, Betaxanthin, Red beet, Partial least square regression, Visible-Near Infrared, Spectroscopy

Introduction

Beetroot belongs to family Chenopodiaceae and has a color range from yellow to red; the root is the edible part [1]. One of the varieties of beetroot is red beet, Beta vulgaris subspecies vulgaris (conditiva), which has lower sugar content than its fellow altissima subspecies known as sugar beet [2]. Red beet is usually consumed raw as salad, baked or boiled as food, or processed as beverages. Although rich in macronutrients (carbohydrate, protein, and fat), micronutrients (minerals and vitamins), and total fiber and calories, the distinctiveness of red beet lies in its phytochemicals [3]. The main phytochemicals in red beet are ascorbic acid, carotenoids, phenolic acids, and flavonoids [4]. Red beet also contains abundant pigments, one of which are betalains [5], natural plant pigments that include red-violet betacyanin and yellow betaxanthin [6].

Betalain has esthetic and health benefits. In terms of esthetics, it is used as a natural colorant [7] that is water soluble and nontoxic, making it safe for food applications [8,9]. In terms of health-promoting effects, betalain in red beet has anticancer, antiviral, anti-inflammatory, and antioxidant activities [10]. Betalain comprises betacyanin and betaxanthin pigments, the ratio of which affects the redness and varieties of the plants [11]. Betalain pigments are extracted using conventional methods, such as maceration and Soxhlet, and nonconventional methods, such as aqueous extraction, ultrasound-assisted extraction, pulse electric field, microwave-assisted extraction, cryogenic freezing, gamma irradiation, or membrane technology [10,12]. The composition of betalain is usually measured using a UV-Vis spectrophotometer [13,14], a high-performance thin-layer chromatograph, and a high-performance layer chromatograph [15]. These methods require sample preparations, skilled analysts, expensive equipment, and produce chemical waste, making them impractical for routine and huge sampling.

Apart from chemical analysis, infrared spectroscopy at near and mid-infrared regions has been used for the quantification of phytochemical content in agriculture products. Infrared spectroscopy can determine anthocyanin content in soybean seed [16], cherries [17], açaí, and palmitero-juçara palms [18]. Another spectroscopy method is hyperspectral imaging (HSI) at infrared region, which has been used to quantify anthocyanin in black rice [19], or total pigments in red meat [20]. These point-mode (FT-NIR or FT-IR) or spatial-mode (HSI) spectroscopies are accurate for predicting phytochemicals, but the initial price of instruments is considerably expensive. Meanwhile, a Vis-NIR spectroscopy working at the wavelength of 350 - 1000 nm is affordable and has been used to predict the contents of lycopene and carotene in tomatoes [21] or chlorophyl in apples [22]. The portable type of Vis-NIR spectrometer is cheap and suitable for the quality [23,24] and quantity detection [25,26] of agricultural products. However, the use of a Vis-NIR spectroscopy for the quantification of phytochemicals in red beet has not been studied.

Spectra, either for classification or prediction, are analyzed by multivariate analysis commonly using partial least square regression, a linear regression model that can handle multicollinearity in spectroscopy data. This research aims to predict the betacyanin and betaxanthin contents of red beet powder using a Vis-NIR with PLSR.

Materials and methods

Red beet samples and chemicals

Red beet samples were purchased from the local farmers of Hampyeong, Haenom, Jeju Seogwipo, and Jeju City in South Korea, transversely sliced in 1 cm-thick portions, and dried in a convection dryer at 70 °C for 24 h. The samples were then ground to create the powder form of red beet and dried again in the dryer at 50 °C for 510 min. The initial moisture content of fresh red beet was around 87 % (wb), and the moisture content of dried powder was around 13 % (wb). Standards for betacyanin and betaxanthin were purchased from Sigma (St. Louis, MO, USA).

Betacyanin and betaxanthin content analysis

Betalain pigments were distinguished as betacyanins, which have a red-violet color, and betaxanthins, which have a yellow-orange color. Betacyanin and betaxanthin contents (in mg) were measured as previously described [27]. Briefly, pigment extraction was done by mixing 25 mL methanol and 1 g beet powder was placed in a 100-mL Erlenmeyer flask and stirred at 180 rpm for 30 min at room temperature. The mixture was then centrifuged at 10,000 rpm and 10 °C, and the resulting supernatant was analyzed for betacyanin and betaxanthin pigment content. Standard solutions of betacyanin and betaxanthin absorptions were determined using a spectrophotometer (Spectronic Genesys-5, Thermo Electron, Waltham, MA) at λ = 480 and λ = 540 nm, respectively for calibration. The calibration curve was used to determine the concentration of betacyanin and betaxanthin in the sample.

Spectrum measurement

The spectra of powdered samples were scanned using a Vis-NIR spectrometer (Flame-T-Vis-NIR Ocean Optics) equipped with a tungsten halogen light (360 - 2400 nm, HL-2000-HP-FHSA Ocean Optics) and a reflection probe (QR400-7-Vis-NIR Ocean Optics). Spectra in reflectance mode were collected using OceanView 1.6.7 software with an integration time of 150 ms, scanning average of 50, and boxcar width of 1. White and black reference spectra were also captured to calibrate the sample spectra.

PLSR

The obtained reflectance spectra were imported to Unscrambler®X software (CAMO, Oslo, Norway) for PLSR analysis to develop calibration models. The reflectance spectra data were used as predictors (X-variables), and the betacyanin and betaxanthin contents were applied as responses (Y-variables). Two PLSR models were developed, namely, PLSR development model and external validation model. The flow of PLSR development model and the external validation steps for predicting betacyanin and betaxanthin contents are shown in **Figure 1**.

In the PLSR development model, datasets categorized as known samples were divided into calibration and prediction sets. The PLSR calibration model used original spectra and various preprocessing spectra, namely, Savitzky Golay 1st derivative (SG1D), standard normal variate (SNV), and multiple scatter correction (MSC). The best calibration model was selected according to the coefficient of determination (R^2) and SEC. The selected PLSR model was then applied to predict betacyanin and betaxanthin contents using a prediction dataset that was not previously employed in developing the calibration model. The performance of the PLSR prediction model was evaluated according to R^2, SEP, and RPD.

The flow of external validation in **Figure 1** was explained as follows. The best PLSR method was used to predict betacyanin and betaxanthin contents (mg) using the regression coefficients obtained from the best selected PLSR model. The model was then saved and used to predict the betacyanin and betaxanthin contents of new unknown samples (their betacyanin and betaxanthin contents were not measured beforehand). The pigment contents in these samples were only measured using their reflectance spectra captured by a Vis-NIR spectroscopy. After the measurement of spectra, the actual betacyanin and betaxanthin contents of the unknown samples were then determined using the conventional procedure [27]. The predicted and actual betacyanin and betaxanthin contents were then compared in terms of R^2 and RMSE.

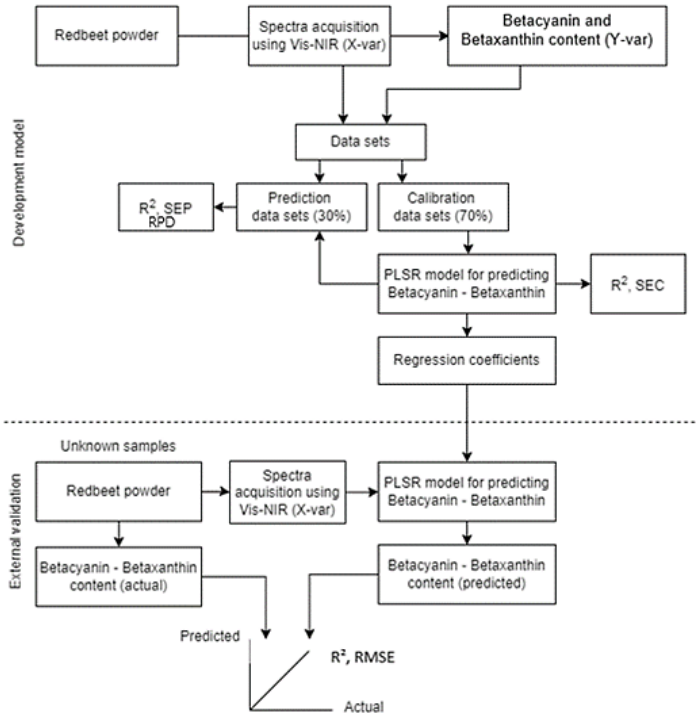


Figure 1 Flow for PLSR development model and external validation path for predicting the betacyanin and betaxanthin contents of red beet powder.

Results and discussion

Betacyanin and betaxanthin contents of red beet

Betacyanin and betaxanthin are the compounds providing red-violet and yellow-orange colors, respectively, in red beet plants. **Table 1** shows that the betacyanin and betaxanthin contents of red beet range at 0.33 - 1.893 and 0.168 - 0.780 mg/g, respectively. The amount of betacyanin is higher than that of betaxanthin, which is similar to previous values of 0.04 - 0.21 and 0.02 - 0.14 %, respectively [28]. **Table 1** also shows that red beet of Hampyeong (HP) variety has relatively lower betacyanin and betaxanthin compared with other varieties.

Table 1 Betacyanin and betaxanthin contents (mg/g) of red beet.

Sample	Betacyanin (mg/g)		Betaxanthin (mg/g)	
	Range	Mean ± SD	Range	Mean ± SD
HN	0.455 - 1.893	0.961 ± 0.340	0.305 - 0.780	0.490 ± 0.122
HP	0.330 - 1.368	0.741 ± 0.419	0.168 - 0.593	0.334 ± 0.137
JS	0.668 - 1.655	0.926 ± 0.263	0.293 - 0.630	0.400 ± 0.114
JC	0.443 - 1.230	0.798 ± 0.254	0.230 - 0.505	0.364 ± 0.101
All	0.330 - 1.893	1.072 ± 0.732	0.168 - 0.780	0.469 ± 0.236

Note: Hampyeong (HP), Hainan (HN), JC, Jeju Seogwipo (JS)

Spectrum exploration

The spectrum profile of red beet powder is shown in **Figure 2**. Spectra below 400 nm and above 950 nm were omitted due to noise. In general, JS and HN have higher reflectance intensity than HP and Jeju City. However, all the spectra exhibit a similar trend. High reflectance is noticeable from 600 nm and above, and high absorbance (low reflectance) can be observed at 450 - 600 nm due to the presence of anthocyanin, carotenoid [29], and betalain [30].

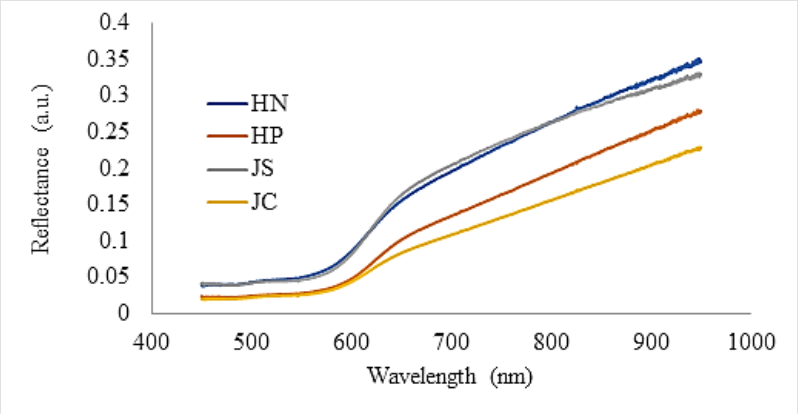


Figure 2 Spectra of red beet powder showing a similar trend using original Vis-NIR spectra. (Legend: Hampyeong, HP; Hainan, HN; Jeju City, JC; and Jeju Seogwipo, JS).

Development model for the determination of betacyanin and betaxanthin using PLSR

Table 2 lists the calibration and prediction results of PLSR for the determination of betacyanin and betaxanthin contents. The calibration models generate R^2c of 0.849 - 0.896 for original spectra and preprocessed spectra such as SG1D, SNV, and MSC. The good PLSR calibration models were obtained using original and SG1D based on the similar results of R^2c (0.89) and low SEC of 0.10 mg/g. However, PLSR using SG1D is considered as a better model because it has a lower latent variable (LV) of 4 compared with that using the original spectra. Therefore, the PLSR calibration model using SG1D spectra was used to predict betacyanin content, and the results are R^2p of 0.877 and low SEP of 0.108 mg/g. The PLSR results for the determination of betaxanthin content (mg/g) are also displayed in **Table 2**. The best PLSR calibration model was obtained using SG1D with R^2c of 0.919, SEC of 0.037 mg/g, and LV of 7. This model was then applied to predict betaxanthin content, and the results are R^2p of 0.844 and SEP of 0.056 mg/g. **Table 2** shows that no significant difference can be observed for SEC and SEP, indicating that the obtained PLSR models are not overfitted [31] and thus are applicable for determining betacyanin and betaxanthin contents. Moreover, the RPD values obtained using SG1D spectra are 2.52 and 2.47 for betacyanin and betaxanthin, respectively. These values indicated that the model is reliable for quantitative prediction.

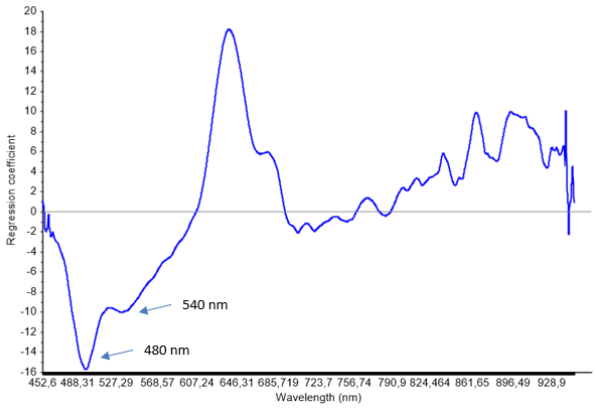
Table 2 Results of PLSR for the determination of betacyanin and betaxanthin contents using calibration and prediction datasets.

Processing method	Calibration			Prediction		
	R^2c	SEC (mg/g)	LV	R^2p	SEP (mg/g)	RPD
Betacyanin						
Original	0.896	0.107	6	0.825	0.129	2.10
SG1D	0.894	0.108	4	0.877	0.108	2.52
SNV	0.874	0.118	7	0.788	0.139	1.86
MSC	0.849	0.129	6	0.731	0.159	1.55
Betaxanthin						
Original	0.782	0.061	6	0.808	0.062	2.08

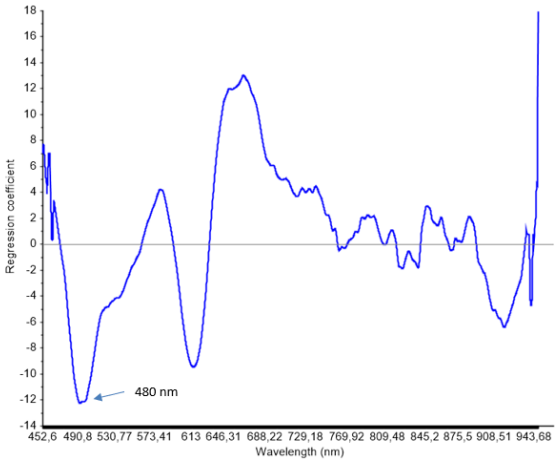
Processing method	Calibration			Prediction		
	R^2c	SEC (mg/g)	LV	R^2p	SEP (mg/g)	RPD
SG1D	0.919	0.037	7	0.844	0.056	2.47
SNV	0.824	0.054	7	0.791	0.065	1.97
MSC	0.848	0.051	7	0.806	0.062	2.09

Note: R^2c = coefficient of determination of calibration; SEC = standard error of calibration; R^2p = coefficient of determination of prediction; SEP = standard error of prediction.

One study reported that the anthocyanin content in soybeans in terms of total anthocyanin, cyaniding-3-glucoside, and delphinidin-3-glucoside was 0.267, 0.606, and 0.97 mg/g, respectively [16]. In this research, the betacyanin and betaxanthin contents were 0.330 - 1.893 and 0.168 - 0.780 mg/g, respectively (**Table 1**). With similar amount of compounds, the PLSR model developed using FT-NIR (R^2 of 0.88 - 0.90 and SEP of 9.4 - 19.5 %) and FT-IR (R^2 of 0.86 - 0.88 and SEP of 9.7 - 21.8 %) [16] are comparable with the PLSR model developed using Vis-NIR spectroscopy in the current research (**Table 2**). For the detection of the chlorophyll contents of eucalyptus leaves, Vis-NIR spectroscopy yielded R^2 of 0.95 and SEC of 1.35 [32]. Given the similar performance, this finding confirmed the utilization of the portable, fiber optic Vis-NIR spectroscopy in the present research. Moreover, the performance of the PLSR obtained in the current study is comparable with that of the models for predicting lycopene and β-carotene in tomatoes using VIS-NIR spectroscopy [21]. All these results proved the applicability of Vis-NIR spectroscopy in determining micro components, such as phytochemicals.



(a) Betacyanin



(b) Betaxanthin

Figure 3 Regression coefficients (B) of PLSR developed using SG1D for the determination of (a) betacyanin and (b) betaxanthin contents.

For betacyanin and betaxanthin quantification, the best PLSR models were obtained using SG1D preprocessing. This method removes the baseline offset and provides less distinct peaks; however, the peaks are still visible [33]. The regression coefficients (B) of the PLSR developed using SG1D spectra are shown in **Figure 3**. The B values were used to determine which bands have a high correlation with betacyanin and betaxanthin contents. As shown in **Figure 3**, the regression coefficient profiles of betacyanin and betaxanthin show similar peaks at 480 and 600 nm. However, another peak is observed for betacyanin at 540 nm due to the conjunction of the substituted aromatic nucleus to 1,7-diazaheptamethinium chromophore [34].

External validation of betacyanin and betaxanthin PLSR models

The best PLSR calibration models for the determination of betacyanin and betaxanthin contents of red beet powder were developed using the original spectra processed with SG1D method. The developed PLSR models were then applied to predict or to validate the model using external data that have not been previously used in the calibration models. The statistics of samples are shown in **Table 3**. The range of betacyanin and betaxanthin contents for the external validation sets are within their ranges in the calibration sets (**Table 1**). The R^2 and RMSE for the determination of betacyanin and betaxanthin contents are shown in **Figures 4(a)** - **4(b)**, respectively.

Table 3 Betacyanin and betaxanthin profiles of red beet powder used for external validation.

Sample	Betacyanin (mg/g)		Betaxanthin (mg/g)	
	Range	Mean ± SD	Range	Mean ± SD
HN	0.455 - 1.668	0.944 ± 0.340	0.305 - 0.780	0.507 ± 0.142
HP	0.330 - 1.367	0.870 ± 0.486	0.168 - 0.593	0.338 ± 0.153
JS	0.668 - 1.293	0.880 ± 0.222	0.293 - 0.630	0.401 ± 0.122
JC	0.442 - 1.230	0.805 ± 0.290	0.230 - 0.505	0.363 ± 0.107

For the external validation models, the same R^2 of 0.98 and RMSE of 0.107 and 0.055 mg/g are obtained for betacyanin and betaxanthin, respectively. The high R^2 and low RMSE values indicated the robustness and reliability of the PLSR models for predicting the betacyanin and betaxanthin contents of red beet powder.

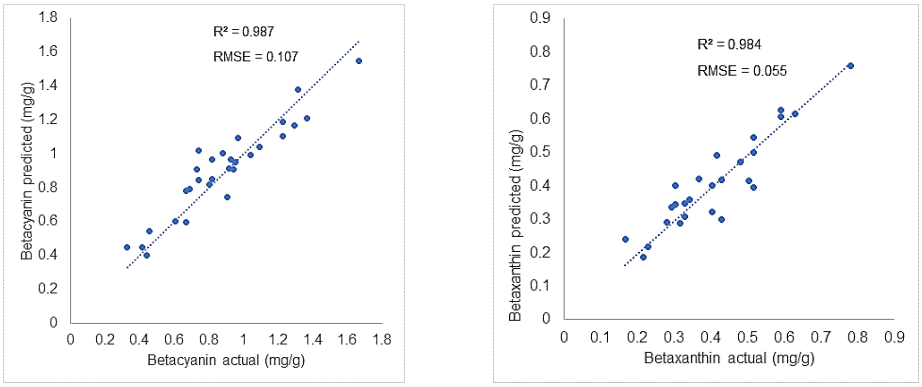


Figure 4 Actual and predicted (a) betacyanin (mg/g) contents and (b) betaxanthin (mg/g) contents using non-targeted red beet powder samples.

Conclusions

With the progress on functional foods, information on the use of phytochemicals as natural colorants is important. Phytochemicals, specifically betacyanin and betaxanthin from red beet, were determined nondestructively using a portable Vis-NIR spectrometer, an affordable device for small food industries.

The Vis-NIR spectroscopy performed well in predicting betacyanin and betaxanthin contents ranging from 0.33 to 1.893 and 0.168 to 0.780 mg/g, respectively. The best calibration and prediction models for betacyanin showed R^2c of 0.89, SEC of 0.108 mg/g, R^2p of 0.87, SEP of 0.108 mg/g, and RPD of 2.52, and those for betaxanthin generated R^2c of 0.919, SEC of 0.037 mg/g, R^2p of 0.84, SEP of 0.056 mg/g, and RPD of 2.47. After the model was applied to unknown data, the betacyanin and betaxanthin contents were predicted with R^2 of 0.98 and RMSE of 0.107 and 0.055 mg/g. T-test showed that the predicted values were not significantly different at 95 % confidence. This study confirmed the effectiveness of a Vis-NIR spectroscopy in quantifying phytochemicals in powder samples.

References

[1] N Chhikara, K Kushwara, P Sharma, Y Gat and A Panghal. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chem.* 2019; **272**, 192-200.

[2] J Wruss, G Waldenberger, S Huemer, P Uygun, P Lanzerstorfer, U Muller, O Höglinger and J Weghuber. Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *J. Food Compos. Anal.* 2015; **42**, 46-55.

[3] D Babarykin, G Smirnova, I Pundinsh, S Vasiljeva, G Krumina and V Agejchenko. Red beet (Beta vulgaris) impact on human health. *J. Biosci. Med.* 2019; **7**, 61-79.

[4] T Clifford, G Howatson, DJ West, EJ Stevenson and N Building. The potential benefits of red beetroot supplementation in health and disease. *Nutrients* 2015; **7**, 2801-22.

[5] HB Koubaier, A Snoussi, I Essaidi, MM Chaabouni, P Thonart and N Bouzouita. Betalain and phenolic compositions, antioxidant ctivity of Tunisian red teet (*Beta vulgaris* L . *conditiva*) roots and stems extracts. *Int. J. Food Properties* 2014; **17**, 1934-45.

[6] A Gengatharan, GA Dykes and WS Choo. Betalains: Natural plant pigments with potential application in functional foods. *LWT Food Sci. Tech.* 2015; **64**, 645-9.

[7] EFSA Panel on Food Additives and Nutrient Sources added to Food. Scientific opinion on the re-evaluation of beetroot red (E 162) as a food additive. *EFSA J.* 2015; **13**, 4318.

[8] N Mohammed, PS Ishwarya and P Nisha. Ice-cream as a model system to evaluate the food colorant functionality of red beet extract emulsion. *J. Culinary Sci. Tech.* 2023; **21**, 960-80.

[9] V Raikos, A McDonagh, V Ranawana and G Duthie. Processed beetroot (*Beta vulgaris* L.) as a natural antioxidant in mayonnaise: Effects on physical stability, texture and sensory attributes. *Food Sci. Hum. Wellness* 2016; **5**, 191-8.

[10] Y Fu, J Shi, SY Xie, TY Zhang, OP Soladoye and RE Aluko. Red beetroot betalains: Perspectives on extraction, processing, and potential health benefits. *J Agr. Food Chem.* 2020; **68**, 11595-611.

[11] AA Szopińska and M Gawęda. Comparison of yield and quality of red beet roots cultivated using conventional, integrated and organic method. *J. Horticultural Res.* 2013; **21**, 107-14.

[12] GA Cardoso-Ugarte, ME Sosa-Morales, T Ballard, A Liceaga and MFS Martín-González. Microwave-assisted extraction of betalains from red beet (*Beta vulgaris*). *LWT Food Sci. Tech*. 2014; **59**, 276-82.

[13] A Faridah, D Syukri and R Holinesti. Simple characterization of betalain compound from red pitaya (*Hylocereus Polyrhizus*) peel solution. *Int. J. Adv. Sci. Eng. Inform. Tech.* 2015; **5**, 207-11.

[14] AP Preczenhak, B Orsi, GPP Lima, JV Tezotto-Uliana, IO Minatel and RA Kluge. Cysteine enhances the content of betalains and polyphenols in fresh-cut red beet. *Food Chem.* 2019; **286**, 600-7.

[15] B Kusznierewicz, M Mróz, I Koss-Mikołajczyk and J Namieśnik. Comparative evaluation of different methods for determining phytochemicals and antioxidant activity in products containing betalains - Verification of beetroot samples. *Food Chem.* 2021; **362**, 130132.

[16] HZ Amanah, R Joshi, RE Masithoh, MG Choung, KH Kim, G Kim and C Byoung-Kwan. Nondestructive measurement of anthocyanin in intact soybean seed using Fourier Transform Near-Infrared (FT-NIR) and Fourier Transform Infrared (FT-IR) spectroscopy. *Infrared Phys. Tech*. 2020; **111**, 103477.

[17] CS Pappas, C Takidelli, E Tsantili, PA Tarantilis and MG Polissiou. Quantitative determination of anthocyanins in three sweet cherry varieties using diffuse reflectance infrared Fourier transform spectroscopy. *J. Food Compos. Anal.* 2011; **24**, 17-21.

[18] MRC Inácio, KMGD Lima, VG Lopes, JDC Pessoa and GHDA Teixeira. Total anthocyanin content determination in intact açaí (Euterpe oleracea Mart.) and palmitero-juçara (Euterpe edulis Mart.) fruit using near infrared spectroscopy (NIR) and multivariate calibration. *Food Chem.* 2013; **136**, 1160-4.
[19] HZ Amanah, C Wakholi, M Perez, MA Faqeerzada, SS Tunny, RE Masithoh, C Myoung-Gun, K Kyung-Hwan, L Wang-Hee and C Byoung-Kwan. Near-infrared hyperspectral imaging (NIR-HSI) for nondestructive prediction of anthocyanins content in black rice seeds. *Appl Sci.* 2021; **11**, 4841.
[20] Z Xiong, DW Sun, A Xie, H Pu, Z Han and M Luo. Quantitative determination of total pigments in red meats using hyperspectral imaging and multivariate analysis. *Food Chem.* 2015; **178**, 339-45.
[21] S Tilahun, DS Park, MH Seo, IG Hwang, SH Kim, HR Choi and CS Jeong. Prediction of lycopene and β -carotene in tomatoes by portable chroma-meter and VIS/NIR spectra. *Postharvest Biol. Tech.* 2018; **136**, 50-6.
[22] M Zude-sasse, I Truppel and B Herold. An approach to non-destructive apple fruit chlorophyll determination. *Postharvest Biol. Tech.* 2002; **25**, 123-33.
[23] MFR Pahlawan, BMA Murti and RE Masithoh. The potency of Vis/NIR spectroscopy for classification of soybean based of colour. *IOP Conf. Ser. Earth Environ. Sci.* 2022; **1018**, 012015.
[24] DAS Saputri, MFR Pahlawan, RE Masithoh and BMA Murti. Vis/NIR spectroscopy for non-destructive method in detecting soybean seeds viability. *IOP Conf. Ser. Earth Environ. Sci.* 2022; **1038**, 012043.
[25] RE Masithoh, HZ Amanah, WS Yoon, R Joshi and BK Cho. Determination of protein and glucose of tuber and root flours using NIR And MIR spectroscopy. *Infrared Phys. Tech.* 2021; **113**, 103577.
[26] RK Wati, MFR Pahlawan and RE Masithoh. Development of calibration model for pH content of intact tomatoes using a low-cost Vis/NIR spectroscopy. *IOP Conf. Ser. Earth Environ. Sci.* 2021; **686**, 012049.
[27] SV Gokhale and SS Lele. Betalain content and antioxidant activity of beta vulgaris: Effect of hot air convective drying and storage. *J. Food Process. Preservation* 2014; **38**, 585-90.
[28] P Ninfali and D Angelino. Nutritional and functional potential of Beta vulgaris cicla and rubra. *Fitoterapia* 2013; **89**, 189-99.
[29] MN Merzlyak, AE Solovchenko and AA Gitelson. Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biol. Tech.* 2003; **27**, 197-211.
[30] EA Macavilca and L Condezo-Hoyos. Assessment of total antioxidant capacity of altiplano colored quinoa (*Chenopodium quinoa* willd) by visible and near-infrared diffuse reflectance spectroscopy and chemometrics. *LWT* 2020; **134**, 110182.
[31] RE Masithoh, MF Pahlawan and RK Wati. Non-destructive determination of SSC and pH of banana using a modular Vis/NIR spectroscopy: Comparison of Partial Least Square (PLS) and Principle Component Regression (PCR). *IOP Conf. Ser. Earth Environ. Sci.* 2021; **752**, 012047.
[32] J Liu, L Zhang and B Datt. Study on chlorophyll content in visible-near infrared spectroscopy. *Int. J. Rem. Sens.* 2021; **632**, 2741-59.
[33] RE Masithoh, HZ Amanah and BK Cho. Application of fourier transform near-infrared (FT-NIR) and fourier transform infrared (FT-IR) spectroscopy coupled with wavelength selection for fast discrimination of similar color of tuber flours. *Indonesian J. Chem.* 2020; **20**, 680-7.
[34] J Chandrasekhar, G Sonika, MC Madhusudhan and KSMS Raghavarao. Differential partitioning of betacyanins and betaxanthins employing aqueous two phase extraction. *J. Food Eng.* 2015; **144**, 156-63.