

Seedlessness detection in ‘White Malaga’ table grapes using near-infrared spectroscopy

^{1,2}Kanchanomai, C., ²Nakano, K., ³Kittiwachana, S., ³Krongchai, C., ²Ohashi, S.,
⁴Maniwara, P., ^{4,5}Theanjumpol, P. and ^{1*}Naphrom, D.

¹*Division of Horticulture, Department of Plant and Soil Sciences, Faculty of Agriculture,
Chiang Mai University, 50200 Chiang Mai, Thailand*

²*Graduate School of Science and Technology, Faculty of Agriculture, Niigata University, 950-2181 Niigata, Japan*

³*Department of Chemistry, Faculty of Science, Chiang Mai University, 50200 Chiang Mai, Thailand*

⁴*Postharvest Technology Research Centre, Faculty of Agriculture, Chiang Mai University, 50200 Chiang Mai, Thailand*

⁵*Postharvest Technology Innovation Centre, Ministry of Higher Education, Science, Research and Innovation,
10400 Bangkok, Thailand*

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Abstract

‘White Malaga’ table grapes are seeded and widely grown in Thailand. They are converted by induction into seedless grapes to increase their value. It is difficult to identify seedlessness in table grapes without destroying the grape berry. The present work thus described a quick and non-destructive method for detecting and predicting seedlessness in ‘White Malaga’ table grapes by using near-infrared (NIR) spectroscopy together with chemometric analysis. The NIR spectra of 280 grape samples were recorded after harvest. Firmness, total soluble solids (TSS), pH, titratable acidity (TA), tartaric acid, number of seeds, and relevant physical properties were analysed. The width and weight of plant growth regulator (PGR) treatments were significantly lower than those in the untreated grapes, while the length, firmness, TA, and tartaric acid were not significantly different. Partial least square (PLS) regression was used to investigate the prediction. Classification models, namely principal component analysis (PCA) and quadratic discriminant analysis (QDA), were used to identify seedlessness. It was found that, QDA, as a representative of linear classification, resulted in the best classification of seeded and seedless performance, where the percentages of predictive ability (%PA), the percentages of model stability (%MS), and the percentages of correctly classified (%CC) were 97.27, 98.57, and 96.23%, respectively, for the training set with no pre-processing. Therefore, the NIR spectroscopy technique can be a non-destructive technique for seedlessness detection in ‘White Malaga’ table grapes.

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Introduction

‘White Malaga’ is a major seeded table grape cultivated in Thailand. It is firm with green skin, and has a sweet taste. However, an undesirable characteristic of this cultivar is the existence of seeds. Gibberellic acid (GA₃) is an endogenous phytohormone that is important for growth and development characteristics of plants such as seed germination, flower induction, stem and leaf elongation, and fruit and seed development. Exogenous GA₃ can induce seedlessness in grapes. It causes seed abortion by reactive oxygen species, a decrease in antioxidant enzymatic activities, and an

alteration in the expression of genes related to seed development (Cheng *et al.*, 2013). ‘Delaware’ and ‘Muscat Bailey A’ wine grapes produced seedless berries following the application of 100 mg/L GA₃ at 12 - 17 days before full maturation (Sugiura and Inaba, 1966; Kimura *et al.*, 1996). Cytokinin, N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU), is a plant growth regulator (PGR) that is commonly applied in many vineyards to develop the size of the grape berries. Treatment with 4 mg/L CPPU on ‘Thomson’ seedless table grape berries of 6 mm diameter had a great effect on increasing their size (Maoz *et al.*, 2014). Brassinosteroids (BRs) are a group of PGR that have various effects on plant growth and

*Corresponding author.

Email: daruni.n@cmu.ac.th

development. Treatment with 1.0 mg/L BRs for two applications over 14 days after pollination and variation periods decreased bunch compactness, increased berry size, changed colour, and altered the concentrations of anthocyanins and polyphenols in 'Flame' seedless grapes (Champa *et al.*, 2015).

Near infrared (NIR) spectroscopy is a technique that uses the spectra of near infrared wavelengths, and could be used to detect quality parameters by non-destructive sampling. It can evaluate the internal starch, total soluble solids (TSS), oil, acidity, firmness, pH, and other physiological properties of fruit and vegetable products such as passion fruit (Maniwaru *et al.*, 2014), citrus (Gomaz *et al.*, 2006), and mango (Saranwong *et al.*, 2004). NIR spectroscopy has three different detection modes namely transmittance, interactance, and reflectance, which allow NIR measurement to provide non-destructive identification of samples in both liquids and solids (Maniwaru *et al.*, 2014; Torres *et al.*, 2017). Shortwave NIR (700 - 1,100 nm) possesses penetration power; so, it is possible to investigate the internal characteristics of samples without destroying them (Williams and Norris, 2001; Magsawa *et al.*, 2012; Theanjumol *et al.*, 2014; Ncama *et al.*, 2018). Chemometric is a form of mathematical analysis that was developed to create a calibration and validation model for spectra from the NIR technique. NIR together with a suitable chemometric method seems to be one of the best analytical tools for the analysis of fruit quality (Kanchanomai *et al.*, 2019). Recently, several classification methods were employed to classify agricultural products such as linear regression by partial least squares-discriminant analysis (PLS-DA) which was used to classify mandarin fruit from different canopy positions (Magsawa *et al.*, 2012). Visible-short-wave near-infrared (Vis-SWNIR) spectroscopy with partial least-squares (PLS) regression can detect and predict the potassium concentrations in fresh lettuce (Xiong *et al.*, 2020). The NIR spectroscopy technique can be a good predictor of both chemical and physical parameters. The calibration and validation of PLS models in transmittance mode at wavelengths of 299 and 1,100 nm on banana were found to be 0.88 and 0.81% Brix for TSS, 0.85 and 0.78 for the acid-Brix ratio (ABR), 0.88 and 0.83 for pH, and 0.90 and 0.87 for dry matter (DM), respectively (Jaiswal *et al.*, 2012). The PLS models from interactance mode on passion fruit provided better prediction results than those from transmittance mode. Both modes also

provided good prediction results for physical parameters such as peel firmness (Maniwaru *et al.*, 2014). Theanjumol *et al.* (2019) described that a supervised self-organising map (SSOM), a representative of non-linear classification, resulted in the best classification performance on physiological disorder for granulation in 'Sai Num Pung' tangerine fruit, where the percentages of predictive ability (%PA), model stability (%MS), and correctly classified (%CC) were 93.7, 95.3, and 94.0%, respectively.

The success of seedless grape induction by GA₃ application depends on the grape cultivar, flowering stage, ambient temperature, GA₃ concentration, and the number and timing of applications. The seedless percentage is not 100%, which makes prediction difficult and affects the price (Fellman *et al.*, 1991). Concerning the cellulose component in grape seed, NIR should be able to detect the spectra from them, and possibly identify and predict seedlessness in grapes without destroying the samples. No other previous studies have focused on this research topic. Therefore, the aim of the present work was to develop a simple and non-destructive method for the detection of seedlessness and other qualities of 'White Malaga' table grapes using NIR and multivariate chemometric analysis.

Materials and methods

PGR application

The experiments were performed at Monsoon Valleys Vineyard, Hua Hin, Prachuap Khiri Khan Province, Thailand. The seedless treatments were applied with PGR to 'White Malaga' table grapes inflorescences 'by soaking. Intact inflorescences were selected on 29 November 2019. PGR was not applied to the seeded treatment (Tr.1), while seedless treatments consisted of a first PGR application, at the flowering stage, of 25 mg/L GA₃, and a second application, 14 days later (13 December 2019), of 25 mg/L GA₃, 5 mg/L CPPU, and 1 mg/L BRs (Tr.2, 3, and 4, respectively). Berry samplings were performed on 3 March 2019. For the seeded treatment (Tr.1), 100 berries were picked, and 60 were picked from each of the three seedless treatments. In total, 280 berry samples were analysed at the Postharvest Technology Research Centre, Faculty of Agriculture, Chiang Mai University, Chiang Mai Province, Thailand.

Analysis methods

Physical and chemical measurements

For physical analysis, firmness was determined by texture analyser (TA XT plus, Massachusetts, USA), and seedlessness was determined by cutting open the berries and counting their seeds. For chemical analysis, reference data of each sample were obtained by TSS with a refractometer (Atago, PAL-1, Tokyo, Japan), and pH and TA by automatic titration (Titroline, Camlab Co. Ltd., Cambridge, United Kingdom).

Spectra collection and NIR analysis

The reflectance spectra of grape samples were collected using a NIR spectrometer (MPA, Bruker, Germany). Each spectrum was acquired in the wavenumber of 3,996 - 12,489 cm^{-1} for a total of 1,102 wavenumbers at the middle of each berry sample. To develop the calibration and validation models, the relationship between NIR spectra and all measurement properties of samples were studied using linear regression analysis with a PLS model by Unscrambler software V.10.5 (CAMO, Oslo, Norway). PCA and QDA model calculation were carried out using in-house MATLAB scripts (MATLAB V7.0, The Math Works Inc., Natick).

Multivariate data analysis

To establish a classification model for identifying the presence of seeds in the studied grapes, the recorded NIR profiles were used as predictive parameters, and the total number of seeds in fruits was used as a class membership. The QDA model was used as a classification model in this investigation. QDA is among the most well-known classifications, and based on the calculation of the Mahalanobis distance to each class centroid. A test sample can be categorised into a class membership, where it is the most similar to or has the smallest distance to the class centroid. Bootstrap methodology was used to evaluate the reliability of the developed NIR-chemometric model (Theanjumol *et al.*, 2019). To identify the bootstrap training samples, two thirds of the grape samples were randomly selected and used for establishing the QDA model. The rest of the samples were used as test samples to evaluate the predictive performance of the model. This algorithm was repeated 100 times, and model statistics were based on the majority vote, including %PA, %MS, and %CC, calculated to evaluate the predictive ability of the classification model (Krongchai *et al.*, 2017).

For comparison, the NIR spectra were pre-processed by standard normal variate (SNV) and first derivative data pre-processing prior to this analysis.

Results and discussion

Grape physical and chemical quality

From Table 1, the mean width and weight of the untreated samples (Tr.1) were 17.77 mm and 5.74 g, which were significantly greater than those in the PGR treatments (Tr.2, 3, and 4), while the length, firmness, TA, and tartaric acid content were not significantly different. TSS and pH of treatments 3 and 4, which was applied with CPPU and BRs, were lower than other treatments which could be related to late development. Maoz *et al.* (2014) reported that CPPU application on a 10 mm diameter berry had a greater influence on delaying berry maturation of 'Thompson' seedless table grapes. Champa *et al.* (2015) reported that BRs (1.0 mg/L) delayed ripening in 'Flame' seedless table grapes. When we picked all treatments at the same time, the berry development of Tr.3 and Tr.4 was still not complete. Therefore, their TSS and pH were lower than those of Tr.1 and Tr.2. The number of seeds in Tr.1 was higher than those of Tr.2 and Tr.3, but not different from that of Tr.4. Exogenous BRs increased grape development, which also increased seed development (Champa *et al.*, 2015). Thus, BR treatment still caused seeded grapes, although they received exogenous GA_3 .

The linear relationship between the number of seedless 'White Malaga' table grapes treated by GA_3 and other chemical qualities, namely TSS, pH, TA, and tartaric acid, ranged between 0.0104 and 0.0783 (Table 2). The linear relationship was not significant, as evidenced by the very low R^2 . This showed that seedlessness was not related to any other chemical qualities. The correlation between seed hardness and the physicochemical composition of the grapes showed that their physical qualities, namely berry weight and bunch weight, had a closer relationship with seedlessness than chemical qualities did (Verma *et al.*, 2014).

NIR spectra analysis

Calibration and validation results of physical and chemical quality

Chemometric analysis with all NIR spectra for all quality parameters was performed using a partial least square (PLS) model with linear regression as shown in Table 3. The interpretation of R^2 from PLS

Table 1. Physical and chemical properties of ‘White Malaga’ table grapes affected by plant growth regulators (PGRs).

	Tr.1		Tr.2		Tr.3		Tr.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Width (mm)	17.77 ^a	0.67	15.03 ^b	0.26	14.67 ^b	0.17	15.23 ^b	0.32
Length (mm)	25.31 ^{ns}	3.11	26.28 ^{ns}	0.87	25.68 ^{ns}	0.51	24.17 ^{ns}	2.03
Weight (g)	5.74 ^a	0.99	4.09 ^b	0.25	3.57 ^b	0.20	3.63 ^b	0.44
Firmness (N)	458.51 ^{ns}	50.09	460.9 ^{ns}	54.65	398.91 ^{ns}	103.36	467.65 ^{ns}	24.3
TSS (%)	19.8 ^a	2.41	21.77 ^a	1.84	17.15 ^{ab}	4.23	14.75 ^b	1.16
pH	3.94 ^a	0.06	3.87 ^{ab}	0.13	3.82 ^{ab}	0.10	3.74 ^b	0.07
TA (%)	3.82 ^{ns}	0.67	3.56 ^{ns}	0.13	3.03 ^{ns}	0.14	3.25 ^{ns}	0.17
Tartaric acid (%)	4.47 ^{ns}	0.79	4.17 ^{ns}	0.15	3.54 ^{ns}	0.16	3.81 ^{ns}	0.19
No. of seeds	0.97 ^a	0.67	0.37 ^b	0.26	0.42 ^b	0.28	0.92 ^a	0.10

Tr.1 = Control, Tr.2 = GA₃ on 3rd application, Tr.3 = GA₃+CPPU on 3rd application, Tr.4 = GA₃+BRs on 3rd application, SD = standard deviation, TSS = total soluble solids, and TA = titratable acidity. Means with different superscript letters are significantly different based on Duncan’s multiple range test ($p < 0.05$).

Table 2. Linear relationship between seedlessness and other chemical properties.

	Regression equation	R ²
Seedlessness and TSS	Y = -1.1314X + 19.573	0.0407
Seedlessness and pH	Y = -0.0264X + 3.8787	0.0104
Seedlessness and TA	Y = 0.0254X + 0.3245	0.0783
Seedlessness and tartaric acid	Y = 0.0298X + 0.3803	0.0783

TSS = total soluble solids, and TA = titratable acidity.

Table 3. Relationships between NIR data and measurement properties of grape samples as provided by PLS analysis.

	Pre-processing	Factor	Slope	Offset	RMSE	R ²
Width	Original	6	0.5793	6.7091	1.1169	0.5793
			0.4790	8.3339	1.3127	0.4228
Length	Original	5	0.2860	17.8844	2.7553	0.2860
			0.2389	20.1584	2.9270	0.1978
Weight	Original	5	0.4133	2.6255	1.0210	0.4133
			0.3722	2.8192	1.0783	0.3517
Firmness	SGD1	1	0.0802	4.0696	1.0618	0.0802
			-0.0748	4.7491	1.2216	NA
TSS	SGD1	6	0.8811	2.2114	1.2999	0.8811
			0.8263	3.1690	1.6682	0.8063
pH	SGD1	4	0.5153	1.8680	0.1249	0.5153
			0.3662	2.4460	0.1582	0.2280
TA	Original	3	0.1130	0.3087	0.0589	0.1130
			0.1014	0.3128	0.0597	0.0970
Tartaric	Original	3	0.1188	0.3596	0.0685	0.1188
			0.1055	0.3651	0.0697	0.0955
TSS/TA	SGD1	4	0.5392	25.3313	10.2500	0.5392
			0.4063	32.5261	12.4587	0.3243

Values in normal text were obtained from calibration models, while those in bold text were obtained from validation models. SGD1 = Savitzky-Golay derivative 1, TSS = total soluble solids, and TA = titratable acidity.

of 0.50 - 0.64 was sufficient for the rough screen, 0.66 - 0.81 was sufficient for screening, and 0.83 - 0.90 was usable with caution for most applications (Williams and Norris, 2001). Using the model for prediction, TSS presented a very satisfactory performance, with a calibration value of R^2 , Root Mean Square Error (RMSE), offset, and slope of 0.811, 1.2999, 2.2114, and 0.8811, respectively, while the validation values were 0.8063, 1.6682, 3.1690, and 0.8263, respectively, by pre-treatment Savitzky-Golay derivative 1 (SGD1). The rough screen model for prediction was pH, with a calibration value R^2 , RMSE, offset, and slope of 0.5153, 0.1249, 1.8680, and 0.5153, respectively, while the validation values were 0.3662, 0.1582, 2.4460, and 0.2280, respectively. Cavaco *et al.* (2009) stated that the PLS prediction model by NIR reflectance mode on pear was not suitable for physical parameters such as firmness. NIR spectroscopy is an efficient non-destructive technique for rapid seedlessness detection in Japanese table grapes (Kanchanomai *et al.*, 2020).

NIR spectral data exploration for seedlessness

The NIR spectra recorded from the studied grape samples are presented in Figure 1(A), and their absorbances were parallel for whole wavenumbers, which showed uniform reflections. The corresponding PCA analysis of this NIR dataset is illustrated in Figure 1(B). The PCA score plot clearly revealed the differentiation between seedless grapes and seeded grapes by PC1, where most of the seedless grapes were clustered on the top-right side of the PCA space. In contrast, the seeded grapes were placed on the opposite side of the PCA space. Average NIR spectra were grouped into four classes in Figure 1(C), and two classes in Figure 1(D). The absorbance of average spectra of seedless grapes was clearly higher than one, two, and three seeds. These results revealed that NIR measurement could be used as a tool for discriminating the difference between seedless and seeded grapes.

Classification of grape seed numbers

The classification results based on the developed QDA models are summarised in Table 4. Due to the choice of training samples for developing the model, the characteristics of the developed models were not the same each time the set of training samples changed. In other words, the selection of

training data heavily affected the characteristics of the developed model; hence, different data sets could result in different optimal solutions for the same problem. Therefore, bootstrap methodology was adopted to generate several prediction models, and model statistics were comprised of %PA, %MS, and %CC. In general, %PA indicated how often the samples were correctly classified. The interpretation of %PA and %MS could imply that there were strong outliers in the dataset (samples having a high %MS but low %PA).

In the case where the classification task was to categorise the grape samples into two classes (seedless and seeded), for the test set, the %CC resulting from the QDA models pre-treated using different data pre-processing ranged from 93.35 to 94.82%, where the QDA model with SNV data pre-processing resulted in the highest classification rate of 95.44%. This result matched the sample distribution in Figure 1(E), where most of the samples were placed correctly within class regions of the first two PC score plots. In addition, the %CC values of the test samples were slightly lower than those from the training samples, thus demonstrating that the developed models were not prone to overfitting. With the relatively high %PA and %MS, the established classification model could be well used to identify if grapes were seedless, whereas data pre-processing had less of an effect on the predictive ability. This could be because the NIR detection probe was directly attached to the surface of the fruit samples. As a result, the variation due to the scatter effect should have a lesser effect on the recorded NIR spectra; therefore, data pre-processing appeared to have less influence on model prediction.

When the classification task was adopted for a more complex task, where the grape samples were categorised into four different classes based on the number of seeds found inside the fruits, the classification results seemed to be slightly higher where the %CC of the test samples were in the range of 94.25 - 95.44%. It was expected that the classification decision for the QDA with four classes should be more complex than that for the two classes; however, in this case, most of the grape samples were categorised into classes 1 (seedless, 81 fruits) and 2 (seeded, 153 fruits). There were only 43 and three fruits having two and three seeds, respectively. With fewer training samples, the test samples could be easily identified if they were not so far away from the

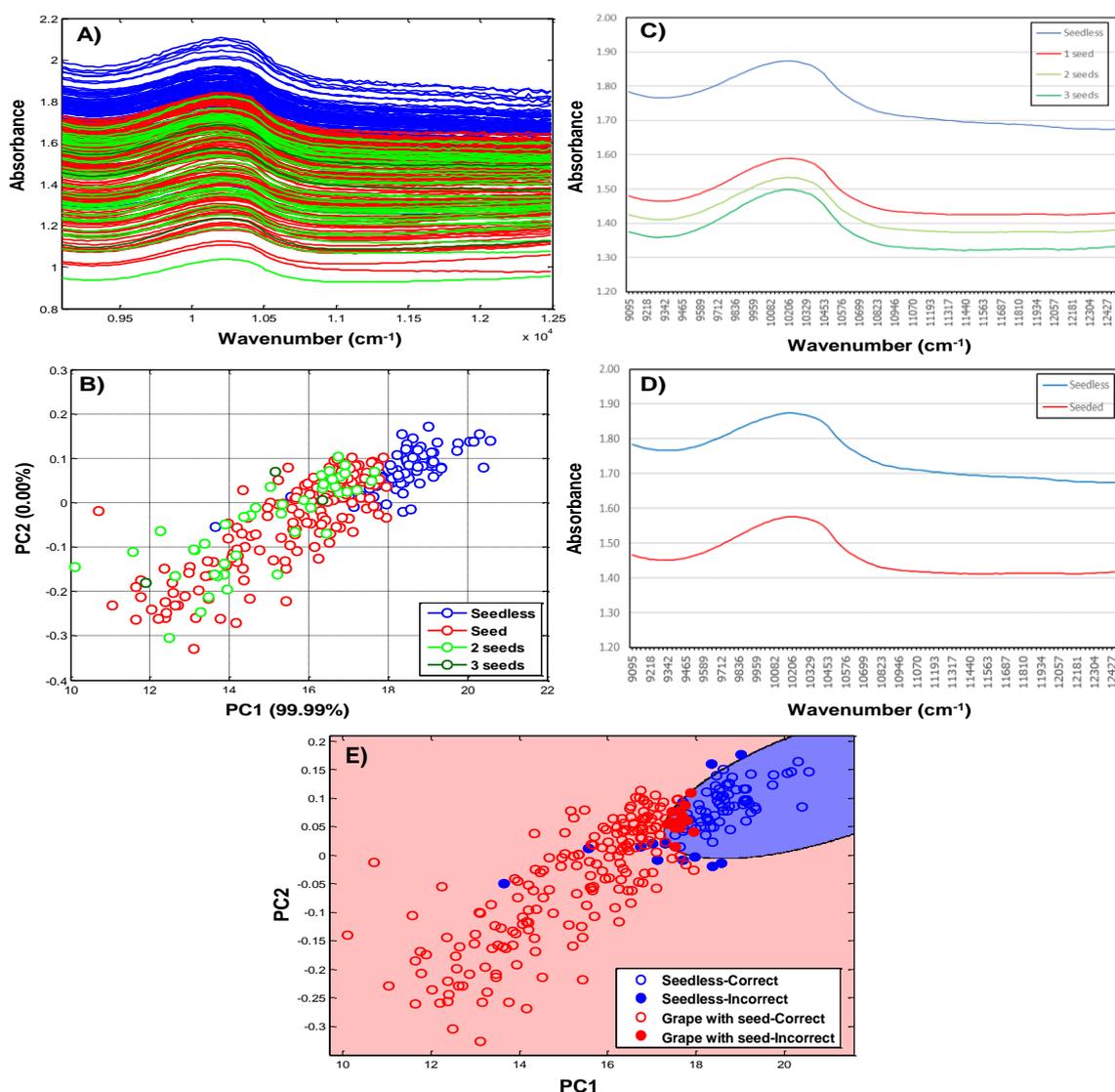


Figure 1. (A) NIR spectra of all grape samples, (B) PCA score plot where the samples were labelled based on the number of seeds in the fruit, (C) Average spectra of four classes, (D) Average spectra of two classes, and (E) Quadratic discriminant analysis (QDA) boundary of the two-class classification (seedless grapes and grapes with seeds).

Table 4. Predictive performance of QDA models on two classes (seedless and seeded) and four classes (0 seed, 1, 2, and 3 seeds) by best pre-processing on train and test set.

Classes	Pre-processing	%PA		%MS		%CC	
		Train	Test	Train	Test	Train	Test
2	None	97.27	94.60	98.57	95.71	96.23	94.64
	SNV	94.08	92.30	95.00	93.21	95.68	94.82
	1 st Derivative	81.86	82.27	81.79	82.14	92.82	93.35
4	None	81.32	78.86	82.50	80.00	97.02	95.30
	SNV	78.91	77.36	79.29	78.21	96.88	95.44
	1 st Derivative	68.32	68.86	68.21	69.29	94.63	94.25

%PA = percentage of predictive ability, %MS = percentage of model stability, %CC = percentage of correctly classified, and SNV = standard normal variate.

centroids. Therefore, they were easily identified as being correctly classified. However, the classification regions for classes 1 and 2 were slightly reduced, thus resulting in a slightly higher prediction error for the remaining samples.

NIR spectroscopy with suitable chemometric analysis can detect C-H, N-H, and O-H bonds (Lui *et al.*, 2016). These bonds are components of moisture, cellulose, oil, and crude protein in grape seeds (Yalcin *et al.*, 2016). To summarise, based on the high value of %PA, %MS, and %CC from QDA results, the developed NIR-chemometric model could be suitable for seedlessness detection in PGR-treated seedless grapes, and is considered a robust model for grape growers and traders.

Conclusion

NIR spectroscopy with suitable chemometric analysis successfully identified seedlessness in 'White Malaga' grapes. The performance of several classification methods was compared. QDA, possessing the predictive ability of linear modelling, provided the best classification results. The development of a NIR-chemometric model could be used to non-destructively detect and quantify the number of seeds in 'White Malaga' table grapes.

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