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Infrared Spectroscopy for the Quality Assessment of Habanero Chilli: A Proof-of-Concept Study †

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Abstract: Habanero chillies (*Capsicum chinense* cv Habanero) are a popular species of hot chilli in Australia, with their production steadily increasing. However, there is limited research on this crop due to its relatively low levels of production at present. Rapid methods of assessing fruit quality could be greatly beneficial both for quality assurance purposes and for use in breeding programs or experimental growing trials. Consequently, this work investigated the use of infrared spectroscopy for predicting dry matter content, total phenolic content and capsaicin/dihydrocapsaicin content in 20 Australian Habanero chilli samples. Near-infrared spectra (908–1676 nm) taken from the fresh fruit showed strong potential for the estimation of dry matter content, with an R^2_{cv} of 0.65 and standard error of cross-validation (SECV) of 0.50%. A moving-window partial least squares regression model was applied to optimise the spectral window used for dry matter content prediction, with the best-performing window being between 1224 and 1422 nm. However, the near-infrared spectra could not be used to estimate the total phenolic content or capsaicin/dihydrocapsaicin content of the samples. Mid-infrared spectra (4000–400 cm^{-1}) collected from the dried, powdered material showed slightly more promise for the prediction of total phenolics and the ratio of capsaicin-to-dihydrocapsaicin, with an R^2_{cv} of 0.45 and SECV of 0.32 for the latter. The results suggest that infrared spectroscopy may be able to determine dry matter content in Habanero chilli with acceptable accuracy, but not the capsaicinoid or total phenolic content.

Keywords: total phenolics; capsaicinoids; *Capsicum chinense* cv Habanero; mid-infrared spectroscopy (MIRS); near-infrared spectroscopy (NIRS)

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1. Introduction

Habanero chillies (*Capsicum chinense* cv Habanero) are some of the hottest commonly consumed chillies in Australia. The pungency of chilli arises from capsaicinoids, which are compounds classified as *N*-vanillylamides of branched fatty acids. The two most abundant capsaicinoids present in chilli are capsaicin and dihydrocapsaicin; however, a number of other capsaicinoids may be present in minor amounts. There is an ongoing interest in developing new Habanero chilli varieties with higher capsaicin contents, as these form a niche high-value market sector.

Capsaicinoid contents are generally measured using high-performance liquid chromatography (HPLC), which provides a high level of specificity and accuracy. However, this technique is time-consuming and expensive, which means that it may not be suitable for the routine assessment of large number of samples. Hence, there is recent interest in using rapid analytical techniques such as infrared spectroscopy for the quality assurance/analysis of chilli. Near-infrared (NIR) spectroscopy has previously been used for the estimation of capsaicinoid content [1] and total phenolic content in chilli [2]. Furthermore, NIR spectroscopy has an extensive history of use for food quality analysis [3,4]. Hence, this study aimed to conduct a proof-of-concept investigation into the potential application of infrared spectroscopy for the quality analysis of Habanero chilli and to compare the relative performance of NIR and mid-infrared (MIR) spectroscopy for this purpose.

2. Methods

Twenty samples of Habanero chilli were sourced from Austchilli (Bundaberg, Queensland), incorporating a wide range of environmental variability. Near-infrared spectra between 908 and 1676 nm were collected from the fresh, intact chillies using a MicroNIR OnSite handheld spectrometer (Viavi, Santa Rosa, CA, USA). Duplicate spectra were collected from opposite sides of each chilli, providing four spectra per sample (n = 80 spectra in total). The chillies were subsequently oven-dried and ground to a fine powder. Mid-infrared spectra were collected from the dried, ground chilli powder in triplicate using a Bruker Alpha Fourier transform infrared (FTIR) spectrophotometer (Ettlingen, Germany) fitted with an attenuated total reflectance (ATR) module (4000–400 cm^{-1}). Polar compounds were extracted from the dried, powdered samples in duplicate using 90% methanol, following previously described protocols [5]. The total phenolic (TP) content of the extracts was measured as previously reported for other matrices [5], with the results being expressed as gallic acid equivalents (GAE) per 100 g (dry weight basis). Capsaicin and dihydrocapsaicin contents were analysed in the methanolic extracts using HPLC, following the method of Waite and Aubin [6]. Chemometric analysis was performed in the Unscrambler X software (version 10; Camo Analytics; Oslo, Norway). Moving-window PLS-R was conducted using a custom script in R Studio running R 4.0.2.

3. Results and Discussion

Figure 1 shows the NIR spectra of the chilli samples. The major peaks were centred at approximately 1447, 1193 and 976 nm, corresponding to the OH second overtone, CH_3 second overtone and OH third overtone, respectively. Following Standard Normal Variate (SNV) normalisation, two of the spectra were identified as outliers and removed.

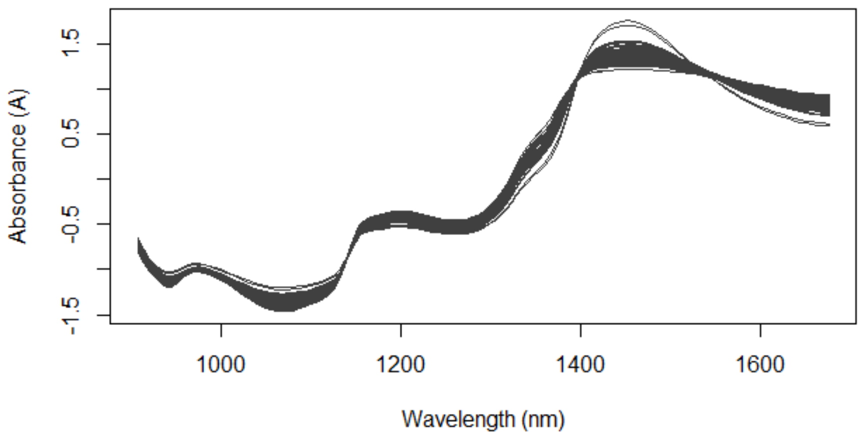

Figure 1. NIR spectra (908–1676 nm) of the Habanero chilli samples following SNV smoothing.

As can be seen in Table 1, only the prediction of dry matter gave acceptable model statistics when using the NIR spectra, with an R^2_{cv} of 0.65 and standard error of cross-validation (SECV) of 0.50%. The comparison of results obtained using NIR spectroscopy and the reference DM method is presented in Figure 2. Although the model statistics could be further improved, these results suggest that NIR spectroscopy may be useful for the rapid, in-field estimation of dry matter content. This could be used to monitor the maturity of the chilli crop and determine the optimum time for harvest. However, PLS-R prediction of other parameters showed poor R^2_{val} values and high SECV values, indicating that the NIR spectral range used was not able to detect the functional groups responsible for these compound classes (i.e., total phenolics, capsaicinoids).

Table 1. Optimum model statistics for the prediction of various parameters in Habanero chilli using the full NIR spectra (908–1676 nm) with different spectral pre-processing methods (n = 78 spectra).

Parameter	Spectral Pre-Processing	Factors	R^2_{cal}	SEC	R^2_{val}	SECV	RPD
DM ^	SNV + 1d5	5	0.70	0.44	0.65	0.50	2.02
TP	SNV + 2d11	3	0.29	152	0.21	162	1.14
Capsaicin	SNV	2	0.19	589	0.15	611	1.08
Dihydrocapsaicin	SNV + 1d11	7	0.57	171	0.36	215	1.05
Capsaicin-to-dihydrocapsaicin ratio	SNV + 1d5	7	0.48	0.31	0.28	0.36	1.19
Sum capsaicinoids	SNV + 1d5	2	0.21	767	0.15	811	1.08
Pungency (SHU)	SNV + 1d11	2	0.20	12,380	0.15	12,887	1.09

^ One outlier sample (n = 4 spectra) was excluded.

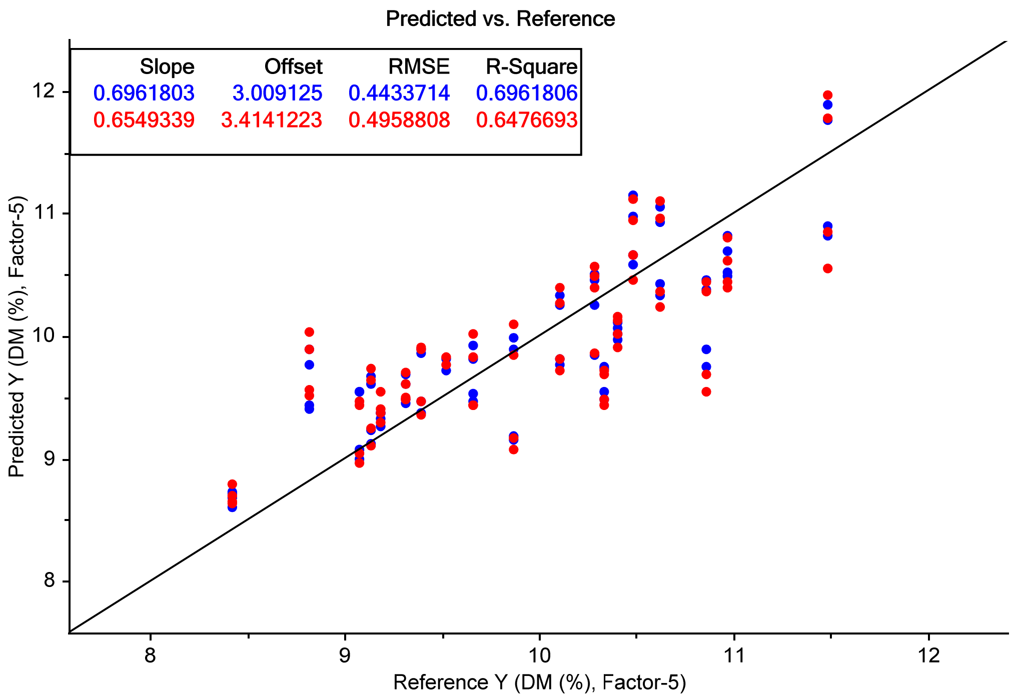

Figure 2. Calibration (blue) and cross-validation (red) results for the prediction of dry matter content in Habanero chilli using NIR spectroscopy.

Moving-window PLS-R was implemented to determine the optimum range of wavelengths to use for the prediction of dry matter content, following the method of Anderson et al. [7]. The procedure used the SNV+1d5 pre-processed spectra, with a step increment of 6 nm throughout the NIR spectrum. The optimum model was found between wavelengths of 1224 and 1422 nm, with an R^2_{val} of 0.67, RMSECV of 0.46% and RPD of 2.20.

As shown in Table 2, the model performance for all parameters aside from dry matter was slightly higher when using MIR compared to NIR spectra; nevertheless, model performance remained quite poor. The best performing MIR model was for the capsaicin-to-dihydrocapsaicin ratio, which showed an R^2_{val} of 0.45, SECV of 0.32 and RPD of 1.34.

Table 2. Optimum model statistics for the prediction of various parameters in Habanero chilli using the full MIR spectra (4000–400 cm^{-1}) with different spectral pre-processing methods (n = 60 spectra).

Parameter	Spectral Pre-Processing	Factors	R^2_{cal}	SEC	R^2_{val}	SECV	RPD
DM	SNV + 1d11	5	0.75	0.50	0.28	0.86	1.17
TP	2d11	7	0.93	48	0.37	145	1.28
Capsaicin	SNV + 2d11	6	0.91	196	0.35	530	1.25
Dihydrocapsaicin	1d21	7	0.77	127	0.29	222	1.02
Capsaicin-to-dihydrocapsaicin ratio	SNV + 1d11	6	0.84	0.17	0.45	0.32	1.34
Sum capsaicinoids	SNV + 2d11	6	0.90	268	0.31	722	1.21
Pungency (SHU)	SNV + 1d11	7	0.88	4715	0.32	11,348	1.24

4. Conclusions

This proof-of-concept study sought to investigate the potential of NIR and MIR spectroscopy towards the rapid quality assessment of Habanero chillies, including the prediction of dry matter content, total phenolic content and capsaicin/dihydrocapsaicin content. Spectra collected using a handheld NIR instrument showed strong potential for the estimation of DM content, but not for TP or capsaicinoid content. The major benefits of using handheld instrumentation include portability, speed of measurement (almost instantaneous) and low cost (virtually no ongoing costs). This means that NIR instrumentation could potentially be applied for use in the in-field assessment of fruit maturity. Furthermore, the method is non-destructive, meaning that samples can be analysed at different points throughout the harvest season to determine the optimum time for harvest. MIR spectroscopy did not perform well for the estimation of capsaicinoid content, although it performed slightly better for the estimation of TP content and the capsaicin-to-dihydrocapsaicin ratio.

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