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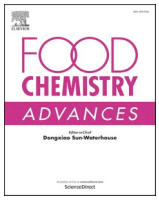

Nondestructive assessment of maturity in cantaloupe using Raman spectroscopy with carotenoids as biomarkers

Ganga K. Sah [a], Nicolas Goff [b], Jashbir Singh [a], Kevin M. Crosby [a], Dmitry Kurouski [b,*], Bhimanagouda S. Patil [a,*]

[a] Vegetable and Fruit Improvement Center, USDA National Center of Excellence, Department of Horticultural Sciences, Texas A&M University, 1500 Research Parkway, Suite A120, College Station TX 77845-2119, USA
[b] Department of Biochemistry and Biophysics, Texas A&M University, 300 Olsen Blvd, College Station, TX 77845, USA

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ABSTRACT

Currently, there is no reliable, non-destructive way to assess the maturity of cantaloupes (*Cucumis melo* L.). Here, we investigated the extent to which Raman spectroscopy can be used to determine cantaloupe maturity. Five cantaloupe cultivars were grown and harvested at 13, 26, and 39 days after anthesis. Raman spectra from cantaloupes were acquired and partial least-square discriminant analysis (PLS-DA) was used to predict maturity based on the collected spectra. The PLS-DA model predicted maturity with up to 100 % accuracy in the cultivars studied. HPLC analysis of lutein and β-carotene in cantaloupe rind showed an increase in the concentration of carotenoids with maturity. The same trend was observed in the vibrational bands originating from carotenoids in the acquired Raman spectra. Based on this, Raman spectroscopy can primarily detect the changes of carotenoids in different cultivars of cantaloupe rind, which can be used for non-invasive and non-destructive assessment of fruit maturity.

1. Introduction

Cantaloupes (*Cucumis melo* L.) are climacteric fruits and belong to the Cucurbitaceae family. Fruits are generally harvested at maturity, based on their visual appearance, such as slip separation, netting turning from green to yellow, flower end softening, and their aroma (Beaulieu & Lea, 2007). However, cantaloupe maturity is difficult to determine based on these criteria; indeed, mature and fully mature cantaloupes can have similar skin color (Quamruzzaman et al., 2022). Erroneous estimates of maturity can cause field losses of fruits and vegetables of around 20 % (Johnson et al., 2019), low quality, and lower consumer acceptance (Maietti et al., 2012).

Cantaloupe is a rich source of phytochemicals such as carotenoids, especially β-carotene (a precursor of vitamin A), polyphenols, and flavonoids, which accumulate in pulp and rind during maturation, along with many other chemical changes (Gómez-García et al., 2021; Singh et al., 2022; Zhou et al., 2020). Carotenoids are responsible for the color and some health benefits of cantaloupes. Because they accumulate during ripening, carotenoids could be useful biomarkers for assessing cantaloupe maturity; however, measuring the carotenoid contents in fruits generally requires destructive techniques such as high-performance liquid chromatography (HPLC). For example, carotenoids from the rind of cantaloupe fruits were extracted and measured using UV–visible spectrophotometry and HPLC (Benmeziane et al., 2018). Another study predicted ripening and variety of cantaloupe using extracts and Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) (Tristán et al., 2022). Moreover, carotenoid profiles in sweet orange were analyzed at different maturity stages using HPLC (Lux et al., 2019). HPLC can precisely measure carotenoids at low concentrations, but this technique is time-consuming, costly, involves toxic solvents, requires destruction of the melon, and is not suitable for field use (Bhatnagar-Panwar et al., 2015; Hara et al., 2021).

To overcome the limitations of destructive techniques, scientists are exploring the use of non-destructive techniques such as spectroscopy and imaging (Pissard et al., 2021; Qin et al., 2011). Near-infrared (NIR) spectroscopy can be used to assess the composition and quality of products but it has low spectral resolution for aqueous samples because of the very strong infrared absorption of water (Yang & Ying, 2011). Another method, hyperspectral imaging, is costly and requires samples to be stationary. Therefore these techniques are unsuitable for real-time

* Corresponding authors.
E-mail addresses: dkurouski@tamu.edu (D. Kurouski), Bhimanagouda.Patil@ag.tamu.edu (B.S. Patil).

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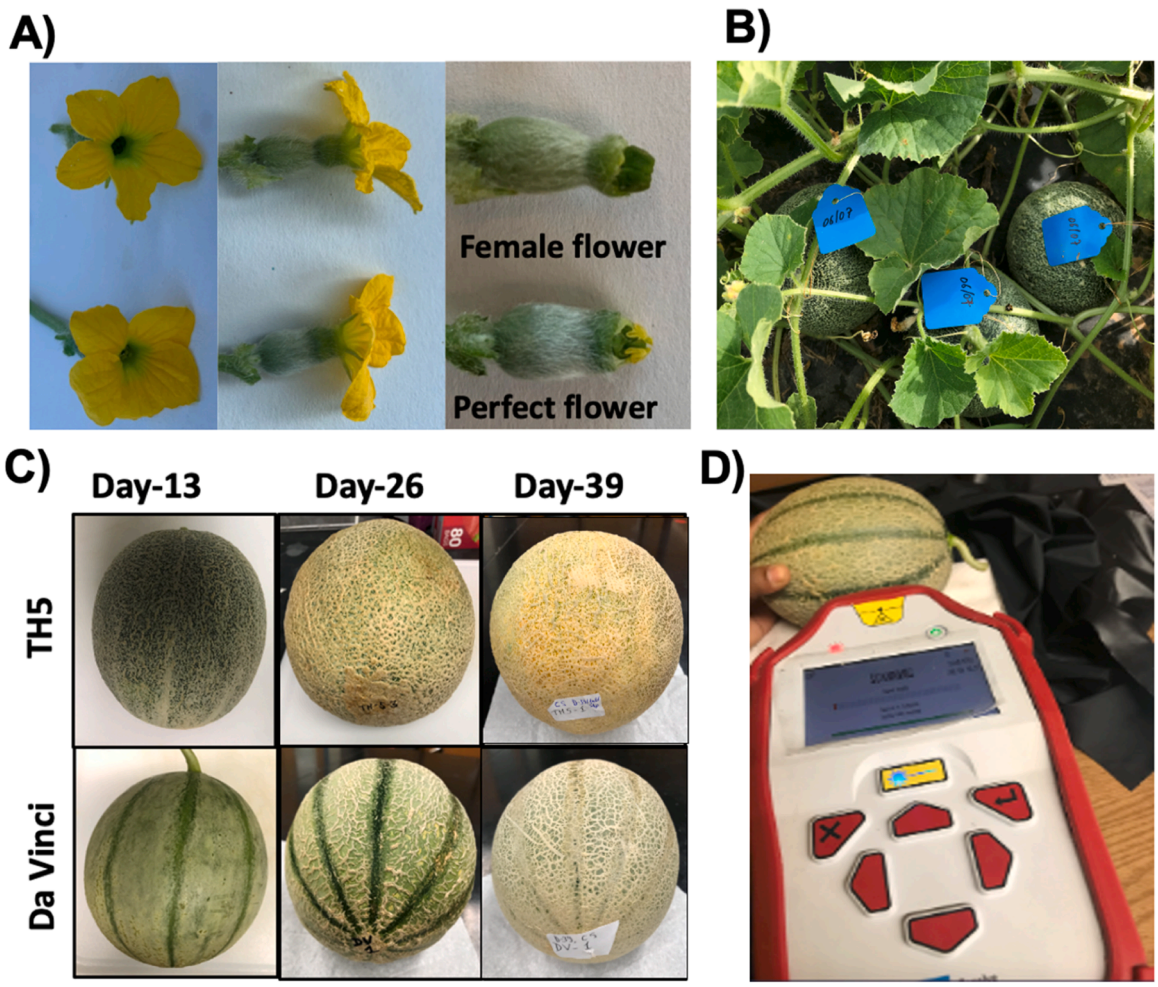

Fig. 1. A) Pictures of female and perfect flowers; **B)** Tagged fruits **C)** Harvested cantaloupes from hybrid (TH5) and commercial variety (Da Vinci) at 13, 26, and 39 days after anthesis (DAA); **D)** Surface scan of Da Vinci using a handheld Raman spectrometer.

in-field analysis (Manley, 2014). By contrast, Raman spectroscopy (RS) has been recently used by researchers as a non-destructive technique for evaluating the maturity of fruits and vegetables, identifying physiological conditions, measuring crop quality, and detecting biotic and abiotic stresses in plants (Dhanani et al., 2022; Farber et al., 2020; Goff et al., 2022).

RS has the potential to identify carotenoids based on their resonance or pre-resonance effects, and has been used to determine maturity in watermelon, differentiate olive fruit cultivars, and monitoring ripening of tomato fruits (Dhanani et al., 2022; Gouvinhas et al., 2015; Trebolazabala et al., 2017). RS was used to detect changes in carotenoids on the surface of watermelon at five different maturity stages and showed a decrease of carotenoids with maturity (Dhanani et al., 2022). Limited research has been conducted in fruits and vegetables with thick rinds (Arendse et al., 2018). For instance, non-destructive maturity detection using RS in pomegranate resulted in 100 % classification accuracy using SIMCA to discriminate immature and mature samples (Khodabakhshian & Abbaspour-Fard, 2020). Due to the strong electron-photon coupling in carotenoids, two bands in the 1100–1200 and 1500–1600 cm^{-1} regions are strongly enhanced in the Raman spectra (Withnall et al., 2003). However, until now RS has not been used to determine maturity of intact cantaloupes. Therefore, we hypothesized that a portable handheld Raman spectrometer using carotenoids as a biomarker can rapidly predict the maturity of cantaloupe. RS is a non-invasive and non-destructive technique that can be used to probe the chemical composition of analyzed specimens. It also has no interference with water, unlike FTIR. Finally, several companies make excellent hand-held Raman spectrometers that can be used to measure fruit quality directly in the field.

The present study aimed to (i) apply non-destructive Raman spectroscopy using carotenoids as a biomarker to determine the maturity of cantaloupe, and ii) compare the results obtained from RS with the HPLC results of carotenoids from the rind of cantaloupe. To our knowledge, this is the first report to employ a portable Raman spectrometer for non-destructive measurement of cantaloupe maturity. Since RS is portable, handheld, cost-effective, user and environment friendly, it has the potential to overcome the limitations of other destructive techniques. The approach used in this study will advance efforts to predict maturity in thick-rind fruits as cantaloupe, which can help in reducing fruit loss during harvesting.

2. Materials and methods

2.1. Plant materials

Five cultivars of cantaloupe consisting of three experimental hybrids (TH5, TH6, and TH16) from the breeding program of Texas A&M University, and two commercial varieties [Tuscan type Da Vinci (DV) and Harper type Infinite Gold (IG)] were cultivated in the research field in Snook, College Station, Texas. Seeds were sown in April 2021, and female and perfect flowers (Fig. 1 A) were tagged on the day of anthesis and tagged fruits (Fig. 1 B) were harvested. Four fruits from each cultivar were harvested at three different maturity stages, i.e., 13, 26, and 39 days after anthesis (DAA) (Fig. 1 C). In total 60 cantaloupes were

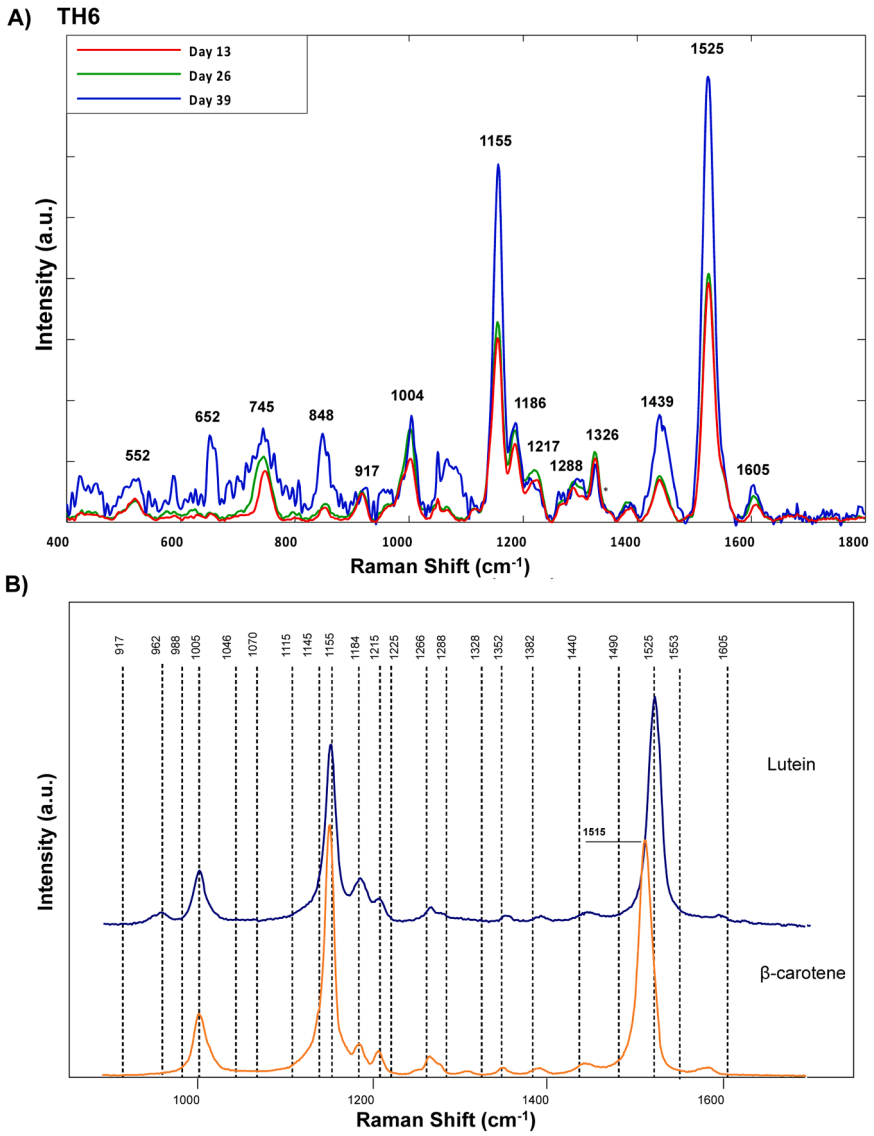

Fig. 2. Raman spectra in solid form **A)** Normalized averaged surface scan spectra of TH6 intact cantaloupe rind **B)** Lutein and β carotene standard.

harvested, with 20 fruits harvested at each maturity stage. Fruit was harvested in the morning and transported to the Vegetable and Fruit Improvement Center (VFIC), Texas A&M University, College Station, Texas, scanned on the same day, and then processed for metabolite analyses.

2.2. Chemicals and reagents

Analytical grade acetone, chloroform, methanol, tert-butyl methyl ether (TBME), β-carotene, and lutein, were acquired from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Raman spectral acquisition of cantaloupe

Raman spectroscopy is a non-destructive analytical technique, that is based on the phenomenon of inelastic light scattering (Zeng et al., 2021). A Handheld Resolve Raman Spectrometer (Agilent, USA) equipped with a 475 mW laser with a wavelength of 830 nm, and 1-sec integration time was used (Dhanani et al., 2022). It measures Raman shifts in the range of 200–2000 cm^{-1} and was used for the acquisition of Raman spectra of cantaloupe cultivars at different maturity stages using surface scan mode. Fruits were washed with distilled water to remove dirt on the surface and dried using paper towels. For spectral acquisition, fruits were held close to the nose cone of the Raman spectrometer to allow the laser light to hit on the fruit surface, with care to avoid the netted area. Fruits were scanned using the surface scan mode available in the instrument (Fig. 1 D). Each fruit was scanned to get 8–10 clean spectra. The spectra were taken from the top, bottom, and middle surface of the fruit. Same method was used to scan β-carotene and lutein standards purchased from Sigma-Aldrich.

2.4. Raman spectral data preprocessing

The Raman spectrometer used for this study has built-in software for automatic baseline correction and background subtraction of the acquired spectra. The spectral data were exported from the handheld instrument in comma-separated value (CSV) format and coupled with chemometrics and machine learning approaches for further preprocessing and analysis in the PLS_Toolbox in MATLAB 2020a. Raman spectra along with the chemical information of sample may also contain background and noise signals from sources such as instrument itself and the experimental operating environment. Therefore, to eliminate the interfering signals on the sample signal, the original data needs to be preprocessed (Zeng et al., 2021). Data normalization also referred as

data preprocessing which is very crucial as each normalization strategy has a significant impact on the data quality and distribution, and thus influences the biomarker detection for any biological study. Without proper normalization, the spectroscopic data can provide erroneous, sub-optimal data, which can lead to misleading and confusing biological results and thereby result in failed application of biological research (Misra, 2020). The spectral data in the present study was normalized at peak 1382 cm^{-1}, which originates from CH_2 vibrations. Since this chemical group is present in nearly all classes of biological molecules, normalization on this peak is minimally biased for the assessment of the changes in intensities of other vibrational bands in the acquired Raman spectra.

Optimal preprocessing for each cultivar was determined using the model optimizer tool. After pre-processing, partial least-squares discriminant analysis (PLS-DA) was performed to differentiate between ripening stages for each of the five cultivars of cantaloupe. A confusion matrix was created from the PLS-DA classification model and used for the prediction of outcomes in the machine learning algorithm.

2.5. Extraction and quantification of carotenoids from cantaloupe rind samples using HPLC

Cantaloupe rinds were separated from the pulp using an Oxo vegetable peeler, and the thickness of the rind was about 3 mm. The rinds were cut into small pieces and blended for about 1 min (Oster blender with 12 speeds, 450 W). Carotenoids were extracted from the rind sample (3.0 gm) using 6 mL of chloroform ($CHCl_3$): acetone [1:3 (v/v)] solvent. Two technical replicates per fruit were prepared (Blainey et al., 2014). The mixture was homogenized, sonicated, vortexed, centrifuged, and then the filtrate was transferred into a 15-mL tube. The extraction process was repeated with the addition of 6 mL solvent to the sample residue to ensure complete extraction of the carotenoids, as described previously (Singh et al., 2021). The second filtrate was collected in the same 15-mL tube, 800 μL pooled filtrate was centrifuged and the clear supernatant was transferred into amber vials and subjected to HPLC analysis.

Carotenoids were quantified using a YMC carotenoid C_{30} (250 × 4.6 mm) column (YMC Co., Ltd. Japan) with a guard cartridge (Phenomenex, Torrance, CA, USA) on an Agilent 1200 Series HPLC (Foster City, CA, USA). The mobile phase consisted of TBME (A), and methanol (B) and was used at the flow rate of 0.8 mL/min with 20 μL injection volume. External standards were used to quantify β-carotene and lutein at 450 nm wavelength as described previously with slight modifications (Singh et al., 2022). Gradient mode elution for the rind sample was carried out as follows: 0–3 min: 90 % B; 5 min: 85 % B; 14 min: 65 % B; 16 min: 40 % B; 18 min: 20 % B; and 21–23 min: 90 % B bringing it back to initial condition. The column was equilibrated for 2 min before the next injection. Carotenoid results were expressed as μg/g fresh weight (FW) of the sample. The concentration of β-carotene and lutein was calculated by using standard calibration, and total carotenoid (TC) concentration was estimated by adding lutein and β-carotene. HPLC graphs were created by GraphPad Prism Version 9.5.0 and statistical analysis was performed using JMP software (JMP Pro 16 for Mac, SAS Institute, Cary, NC, USA) with one way ANOVA at the alpha level of 0.05. Mean comparison for all pairs was done using Tukey-Kramer HSD test.

3. Results and discussion

3.1. Spectroscopic analysis of cantaloupe rind

RS can specifically detect carotenoids in fruits and vegetables (Hara et al., 2021; Jehlička et al., 2014) which can be advantageous for assessing crop maturity (Saletnik et al., 2022). In the Raman spectra acquired from cantaloupe rind, vibrational bands were observed that can be assigned to carotenoids at 1002, 1155, 1186, 1217, and 1525 cm^{-1}. There was an increase in the intensity of the carotenoid spectral peak from day 13 to day 39 in all cultivars, as shown by TH6 presented in Fig. 2 A and other cultivars in Supplementary Figure 1 (A-D). The carotenoid concentration was also reported to increase during the maturity period from green to yellow stage in jamun fruit and melon (Sharma et al., 2022; Vanoli et al., 2023). Our previous study of watermelon showed that carotenoid concentrations in the rind decreased as the fruits ripened, showing the importance of generating custom of RS profiles for individual fruits.

The strongest carotenoid peak intensity observed at 1525 cm^{-1} originates from the C═C stretching vibration of the carotenoid molecule, Supplementary Table.1. The second strongest peak at 1155 cm^{-1} originates from C—C stretching vibration, and the third, medium-strength peak at 1002 cm^{-1} originates from C—CH_3 in-plane rocking vibration (Jehlička et al., 2014; Schulz et al., 2005). Finally, the last two carotenoid peaks located at 1186 and 1217 cm^{-1} originate from C–C stretching vibration coupled either to the C—H in-plane bending or to the CH—CH_3 stretching modes (Grudzinski et al., 2016). We also observed vibrational bands that originate from CH and CH_2 vibrations at 1326 and 1439 cm^{-1}. These chemical groups are present in nearly all classes of biological molecules and therefore cannot be assigned to the particular class of molecular analytes.

In this study, two unique spectral bands were observed at 652 cm^{-1} and 848 cm^{-1}, which had higher intensity at day 39 as compared to day 13 and day 26 in all the cultivars. Moreover, the spectral bands at 745, 917, and 1326 cm^{-1} present in all the cultivars of cantaloupe were reported as chlorophyll by other studies (Němečková et al., 2022; Trebolazabala et al., 2017; Vítek et al., 2010).

Table 1
Confusion matrix computed from the PLS-DA model of Raman spectra collected from five cantaloupe cultivars at three different stages of maturity.

Cultivar	Ripening stage	Total spectra	Actual day 13	Actual day 26	Actual day 39	Accuracy of prediction (%)
TH5	Predicted as Day 13	41	**40**	2	0	97.5
	Predicted as Day 26	39	1	**37**	0	94.8
	Predicted as Day 39	25	0	0	**25**	100
TH6	Predicted as Day 13	42	**40**	2	0	95.2
	Predicted as Day 26	40	2	**38**	0	95.0
	Predicted as Day 39	26	0	0	**26**	100
TH16	Predicted as Day 13	39	**34**	2	0	87.1
	Predicted as Day 26	41	5	**39**	0	95.1
	Predicted as Day 39	30	0	0	**30**	100
Da Vinci	Predicted as Day 13	24	**24**	2	0	100
	Predicted as Day 26	40	0	**37**	5	92.5
	Predicted as Day 39	29	0	1	**24**	82.7
Infinite Gold	Predicted as Day 13	26	**26**	2	1	100
	Predicted as Day 26	40	0	**37**	1	92.5
	Predicted as Day 39	24	0	1	**22**	91.6

Bold numbers are the highest number of spectra predicted according to their ripening stage.

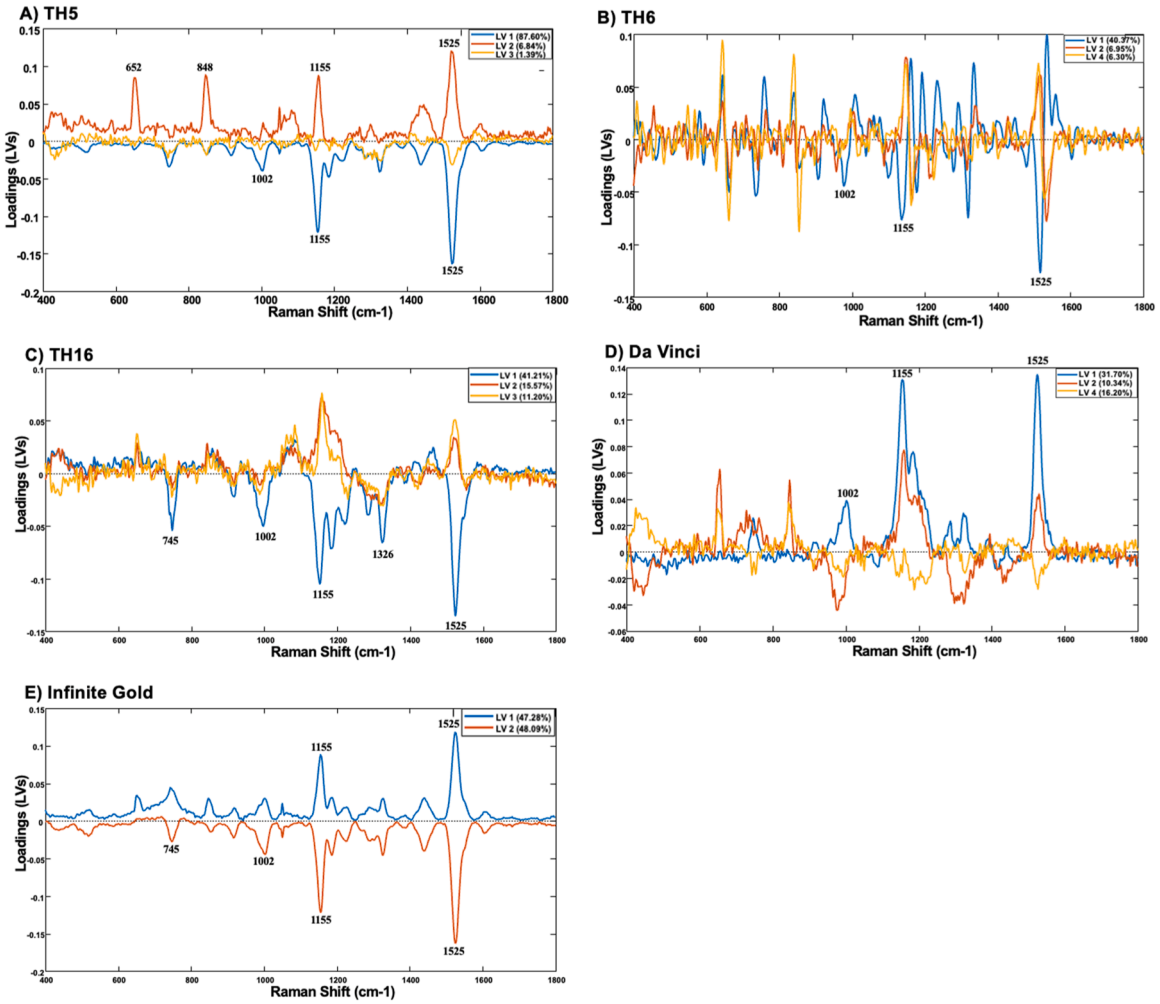

Fig. 3. Loading plots for the first three latent variables (LVs) obtained from the partial least square discriminant analysis (PLS-DA) model. LV1 (blue), LV2 (orange), and LV3 (yellow) represents the wavenumbers having the highest contribution to the maturity prediction model developed from the Raman spectra acquired from five cantaloupe cultivars **A)** TH5, **B)** TH6, **C)** TH16, **D)** Da Vinci, and **E)** Infinite Gold.

3.2. Raman spectra of standard carotenoids

To confirm the carotenoid peak obtained from cantaloupe rind, Raman spectra were acquired from lutein and β-carotene standards. The Raman spectra acquired from lutein exhibited distinct carotenoid peaks at 1005, 1155, 1184, 1215, and 1525 cm^{-1} (Fig. 2 B top). Raman spectra acquired from standard β-carotene exhibited distinct carotenoid peaks at 1005, 1154, 1184, 1215 and 1515 cm^{-1} (Fig. 2 B bottom). In the acquired Raman spectra from the cantaloup rind, the carotenoid vibrations were centered at 1005, 1155, 1184, 1215, and 1525 cm^{-1}, which suggests that RS primarily detects changes in the concentration of lutein at 1525 rather than β-carotene at 1515 in all cultivars of cantaloupe rind.

3.3. Chemometric analysis of Raman spectra for predicting cantaloupe maturity

PLS-DA was used to investigate the accuracy of differentiation among the maturity stages of cantaloupe fruits based on the acquired Raman spectra. Confusion matrix from the PLS-DA model revealed that RS can be used to predict the maturity of all varieties of cantaloupes with 82–100 % accuracy, Table 1. Results from the present study indicate that Raman spectra can be used to differentiate the fully mature stage from the immature stages with 82.7 % accuracy for DV, 91.6 % for IG, and 100 % for all the experimental hybrids (TH5, TH6 and TH16). It should be noted that similar results of ripeness prediction was reported for watermelons (Dhanani et al., 2022). Also, Raman spectra of potatoes grown in two different locations were differentiated using the PLS-DA model with an accuracy of 84.3 % and 90.9 %, respectively (Morey et al., 2020). The present study used ripeness stages that show clear, visible differences; future studies will examine fruits that are nearly ripe, perfectly ripe, and over-ripe to provide commercially useful data.

Vibrational bands corresponding to specific chemicals present in the rind of cantaloupes are labeled and discussed in Supplementary Table 1. In Fig. 3, the loading plots for the main latent variables (LVs) represent the bands that mainly contributed towards the prediction of maturity in the classification models. In TH5, the LV1 alone explained 87.60 % of the variation among the maturity stages with the highest contribution of bands at 1002, 1155, and 1525 cm^{-1} which correspond to carotenoid pigments. However, LV2 has the highest influence of bands at 652, 848, assigned to the aromatic ring, and 1155, and 1525 cm^{-1}. Similarly, in the other four cultivars (TH6, TH16, DV, and IG), the LV1 has maximum influence at 1002, 1155, and 1525 cm^{-1} however, in TH16 the bands at 745 and 1326 cm^{-1} also made some contribution to explaining the variation towards the prediction of classification models. In IG, only two LVs explained most of the variation (LV1 47.28 % and LV2 48.09 %). Overall carotenoid bands mostly at 1002, 1155, and 1525 cm^{-1} had higher contributions for the maturity prediction models, which is in line with the findings in watermelon and peanut (Dhanani et al., 2022; Farber et al., 2020).

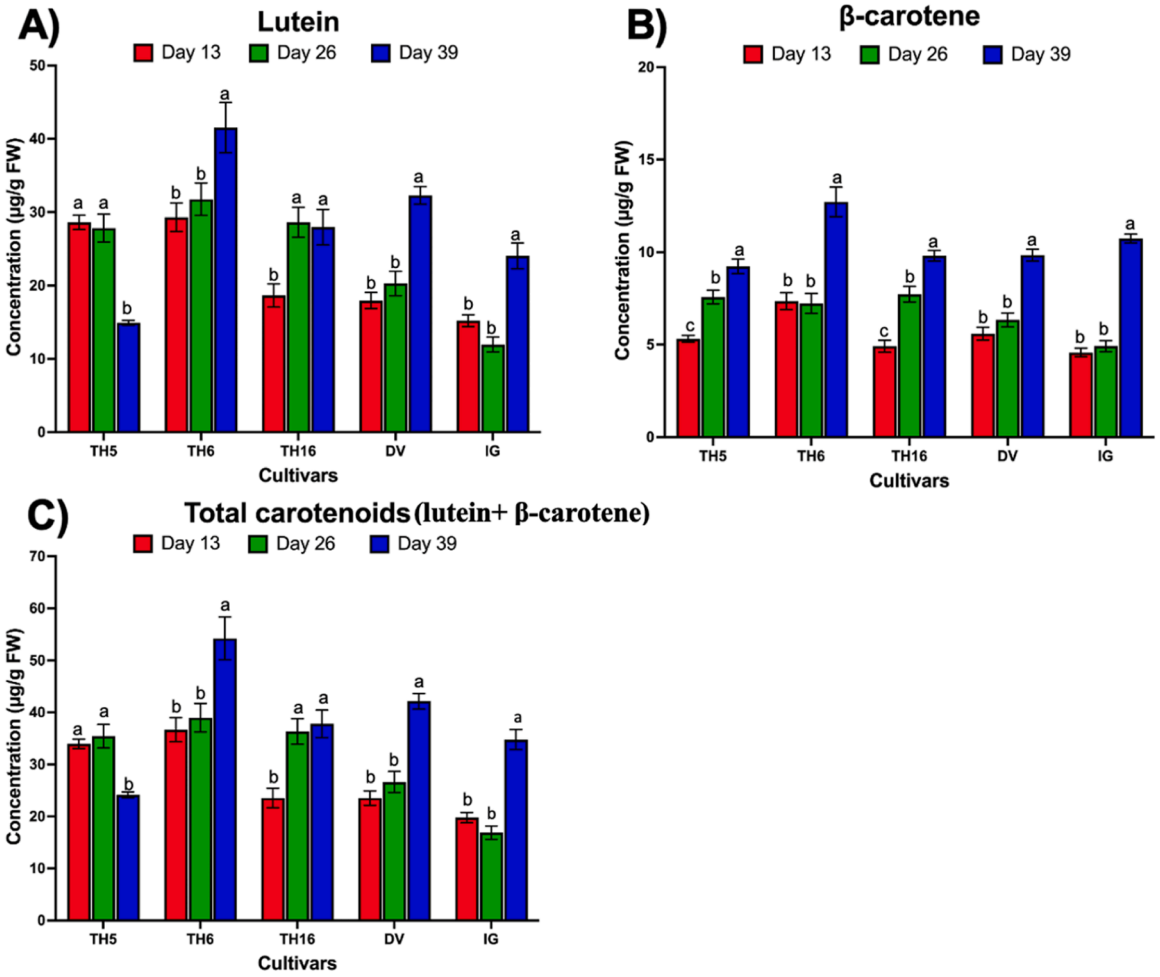

Fig. 4. Concentration of individual pigments **A)** Lutein (µg/g FW); **B)** β-carotene (µg/g FW); **C)** Total carotenoid (lutein+ β-carotene) detected at different stages of maturity from the rinds of five different cultivars of cantaloupe using HPLC. Error bars represent the standard error from the average ($n = 16$).

3.4. Quantification of carotenoids in cantaloupe rind using HPLC

Carotenoids were quantified from cantaloupe rind samples using an optimized HPLC method. Lutein and β-carotene were the major carotenoids present in the cantaloupe rind samples which ranged from 11.96 to 41.51 µg/g FW and 4.58–12.72 µg/g FW, respectively (Fig. 4. A, and Fig. 4 B), and TC (lutein + β-carotene) (Fig. 4 C) ranging from 16.88 to 54.23 µg/g FW, however traces of other carotenoids were below quantifiable level. The highest concentration of lutein, β-carotene as well as TC was observed in TH6 rind at day 39 while lutein and TC were lowest in IG at day 26 and β-carotene at day 13. Overall, an increasing trend of β-carotene, lutein, and TC was observed from day 13 to day 39, indicating an increase in carotenoids from the immature to mature stage, except for TH5, which had a lower concentration of lutein at day 39 as compared to day 13 and day 26 resulting in lower TC at day 39. This significantly lower TC in TH5 at day 39 as compared to other cultivars which can be due to varietal differences or environmental factors (Carvalho et al., 2013; Tadmor et al., 2010). As previously reported, accumulation of detectable amounts of carotenoids was also observed in melon rinds ranging from 17 µg/g FW in "'Noy Amid' hybrid to 180 µg/g FW in 'Tendral Verde Tardio' hybrid (Tadmor et al., 2010). Carotenoids such as lutein and beta carotene in oriental melon rind has been analyzed and reported using HPLC (Tuan et al., 2019). Similarly, carotenoids in cantaloupe pulp have been previously analyzed and reported from our lab (Singh et al., 2021, 2022).

As was discussed above, RS detected vibrational bands that originated from carotenoids. HPLC results showed that the concentration of carotenoids in cantaloupe increased with maturity. Expanding upon this, one can expect that RS could be used to track changes in the intensities of carotenoid vibrations, which, in turn, could be used to monitor fruit ripeness. The increasing trend of carotenoids peak in Raman spectra in all cultivars followed the trend of β-carotene, lutein, and TC concentration from HPLC except for the lutein and TC in TH5.

4. Conclusion

This study demonstrates the potential of RS as a rapid non-destructive technique to determine changes in carotenoids as a biomarker to predict maturity in cantaloupe fruit. The results indicate that RS coupled with chemometrics can predict maturity in cantaloupe with 82–100 % accuracy. There is a good increasing trend from day 13 to day 39 for carotenoids in Raman spectra and the total carotenoids determined using HPLC which is true for all cultivars except TH5. Further testing of this technique at a large scale, and different environmental conditions is warranted to enhance the validity of this instrument to determine maturity in cantaloupes. Raman spectra in combination with chemometrics may have practical applications in industry, grocery stores, research, and at the field level for carotenoid determination and maturity prediction in cantaloupes.

CRediT authorship contribution statement

Ganga K. Sah: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Nicolas Goff:** Writing – review &

editing, Data curation. **Jashbir Singh:** Writing – review & editing, Methodology. **Kevin M. Crosby:** Writing – review & editing. **Dmitry Kurouski:** Writing – review & editing, Validation. **Bhimanagouda S. Patil:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

The data from this study are incorporated into the article and its online supplementary article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2024.100698.

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